mycoses
Diagnosis, Therapy and Prophylaxis of Fungal Diseases

Poster Presentations

P001

Susceptibility of *Candida albicans* biofilms to caspofungin and anidulafungin is not affected by metabolic activity

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Objectives Biofilm formation allows *Candida albicans* to attach to plastic surfaces such as catheters and bioprosthetic devices, thus complicating the management of infected patients. Caspofungin, anidulafungin, and micafungin have potent antibiofilm activity *in vitro*. We previously observed that the antibiofilm activity of micafungin against *C. albicans* clinical isolates was dependent on the metabolic activity of the biofilm and that biofilm with high metabolic activity was more susceptible to micafungin than biofilm with low metabolic activity. It remains unknown whether this observation applies to caspofungin and anidulafungin. We compared the antifungal activity of anidulafungin, caspofungin, and micafungin against preformed *Candida albicans* biofilms with different degrees of metabolic activity from 301 isolates causing fungemia in patients admitted to Gregorio Marañon Hospital (January 2007 to September 2014).
Methods Biofilm was formed and, based on our previously reported score, each strain was tested in triplicate using the XTT reduction assay and classified as forming biofilm with low metabolic activity (LMA), moderate metabolic activity (MMA), or high metabolic activity (HMA). Echinocandin MICs for planktonic and sessile cells were measured using the EUCAST E.Def 7.2 procedure and XTT reduction assay, respectively. Preformed biofilms were treated with increasing concentrations of the three drugs (0.015 mg L^{-1} to 16 mg L^{-1}); after incubation at 37°C for 24 h, the metabolic activity was measured by the XTT reduction assay. The sessile MIC ($SMIC_{80}$) was defined as 80% reduction in the metabolic activity of the biofilm treated with the antifungal compared with the control well. Three *C. albicans* isolates were selected for scanning electronic microscopy (one each for LMA, MMA, and HMA biofilms) to study the effect of caspofungin, anidulafungin, and micafungin against the biofilm structure.
Results The three echinocandins were uniformly active against the isolates in planktonic form, regardless of the metabolic activity of their respective sessile form. All three drugs were active against the preformed *C. albicans* biofilms with micafungin showing the highest activity ($P < 0.001$). HMA biofilms were significantly more susceptible to micafungin than MMA and LMA biofilms ($P < 0.001$); anidulafungin was slightly more active against HMA biofilms than LMA and MMA, and caspofungin showed the lowest activity (Table). The activity of caspofungin and anidulafungin was not dependent on the metabolic activity of the biofilm. These observations were confirmed by scanning electron microscopy (Figure).
Conclusions Micafungin was the most active echinocandin against *C. albicans* biofilm. In contrast to that observed for micafungin, the antifungal activity of anidulafungin and caspofungin against *C. albicans* biofilms *in vitro* was not dependent on metabolic activity.

Table 1

Metabolic Activity	n	Caspofungin (mg/L)			Anidulafungin (mg/L)			Micafungin (mg/L)		
		$SMIC_{80}$			$SMIC_{80}$			$SMIC_{80}$		
		GM	MIC_{50}	MIC_{90}	GM	MIC_{50}	MIC_{90}	GM	MIC_{50}	MIC_{90}
LMA	73	27.2	≥16	≥16	29.3	≥16	≥16	21.2	≥16	≥16
MMA	127	26.8	≥16	≥16	29.3	≥16	≥16	18.8	≥16	≥16
HMA	101	26.1	≥16	≥16	26.1	≥16	≥16	12.2	2	≥16
Overall	301	26.6	≥16	≥16	28.3	≥16	≥16	17.2	≥16	≥16

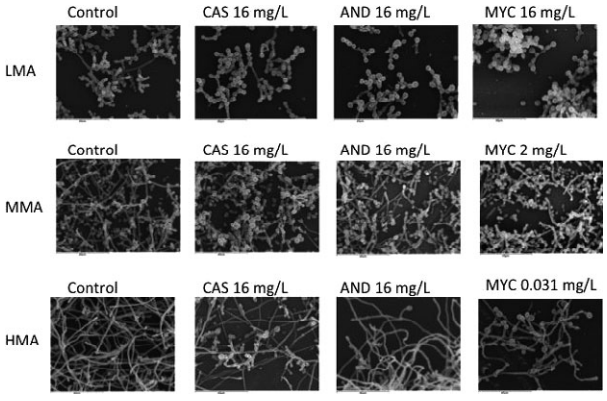

Figure 1

P002

Trailing effect of fluconazole against *Candida spp.* Which is the frequency when using EUCAST?

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Objectives Trailing is a well-known phenomenon defined as a reduced but persistent visible growth of *Candida* spp. at fluconazole concentrations above the MIC obtained by broth microdilution procedures. Although the clinical impact of trailing is unkown it may interfere with the MIC endpoint determination. Trailing has been commonly detected when using CLSI M27-A3 method but its frequency when using EUCAST EDef 7.2. method is unkown. We assesed the frequency and degree of fluconazole trailing observed after studying the antifungal susceptibility to fluconazole by EUCAST against a large number of *Candida* spp. isolates from patients with candidemia.
Methods We studied 639 fluconazole-susceptible non-krusei *Candida* spp. isolates from patients with candidemia ($n = 570$) admitted to Gregorio Marañón Hospital (January 2007 to March 2015). Isolates were identified after amplification and sequencing of the ITS1-5.8S-ITS2 region and further tested for *in vitro* fluconazole susceptibility according to the EUCAST EDef 7.2 procedure. The MIC was defined as a reduction ≥50% compared to the growth control after 24 hours of incubation; trailing was defined as the presence of residual growth (0.1–49%) in wells containing two-fold fluconazole concentrations above MIC compared to growth control. For each isolate, mean trailing was defined as the mean percentage of residual growth observed in the wells after the MIC. The percentage of isolates showing different degrees of trailing [0.1–5% (residual trailing), 6–10%, 11–15%, and >15% (prominent trailing)] was studied.
Results The species distribution of isolates, the mean fluconazole MICs, and the mean trailing is shown in the Table. The mean percentage of trailing of all isolates was 6.79%. Differences reaching statistical significance were found among species: *C. albicans* and *C. tropicalis* showed the highest trailing (9.75% and 9.29%, respectively; $P < 0.001$). Figure shows the proportion of isolates within a species showing different degree of trailing. Most of *C. albicans* and *C. tropicalis* showed trailing >5% (90% and 81% of isolates, respectively),

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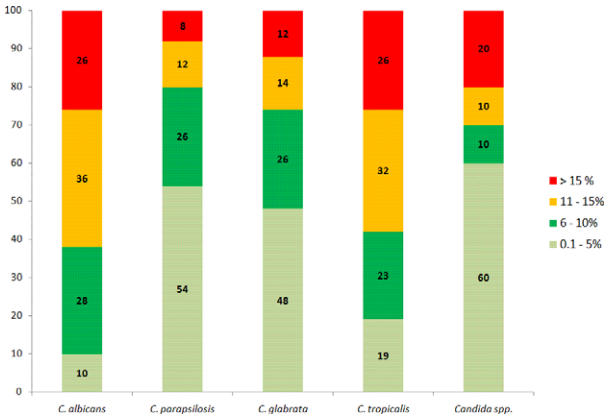

Figure 1

Table 1

Species	No. of isolates	Mean fluconazole MIC and range (in µg/ml)	Mean percentage of trailing (%)
C. albicans	313	0.15 (0.125 - 1)	9.75
C. parapsilosis	188	0.35 (0.125 - 2)	3.71
C. glabrata	65	7.51 (2 - 32)	6.06
C. tropicalis	53	0.26 (0.125 - 1)	9.29
Candida spp.	20	0.78 (0.125 - 32)	3.36
Overall	639	0.32 (0.125 - 32)	6.79

whereas less than a half of *C. parapsilosis* and *Candida* spp. exhibit trailing greater than 5%. *C. albicans* and *C. tropicalis* were the species with the highest percentage of isolates producing prominent trailing (>15%).

Conclusions *C. albicans* and *C. tropicalis* were the species showing the highest production of fluconazole trailing. The clinical impact of this finding should be evaluated in future studies.

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P003

Antifungal susceptibility of *Cryptococcus neoformans* clinical isolates from Italy

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Objectives to determine the *in vitro* antifungal susceptibility of *Cryptococcus neoformans* clinical isolates collected during a prospective survey carried out in Italy from 2010 to 2014 and to calculate the epidemiological cutoff values (ECVs).

Methods Eighty-three *C. neoformans* isolates (37 VNI, 14 VNIV and 32 intervarietal hybrids VNIII), each representative of a single case, were tested for susceptibility to fluconazole (FLZ), itraconazole (ITZ), voriconazole (VRZ) and flucytosine (FC) using the broth microdilution method previously described (Zaragoza O., Antimicrob. Agents Chemother. 2011): growth medium yeast nitrogen base (YNB) added of 0.5% glucose, pH 7.0, final inoculum of 10^4 CFU ml^{-1}, shaking (14 000 rpm) every 30 min incubation at 30°C. FC was tested in YNB 2% glucose, pH 5.6 (Viviani M.A., J. Chemother. 2003). Reading was performed spectrophotometrically at 492 nm after 48 h of incubation. The MIC was defined as the lowest drug concentration giving 50% growth inhibition compared to growth in control well. The geometric mean (GM) and the ECV, defined as the MIC (mg/L) that encompassed at least 95% of isolates in the distribution (Pfaller M.A., Drug Resist Update 2010), were calculated.

Results The MIC distribution of the tested antifungals had the following ranges: 0.12–16 for FLZ, 0.03–0.5 for ITZ, 0.03–0.25 for VRZ and 0.12–64 for FC. The ECVs and the % of isolates that had MIC≤ECV were the following: FLZ 8, 97.7%, ITZ 0.25, 98.8%, VRZ 0.12, 96.5%, FC 1, 96.5%. Different patterns of susceptibility have been observed in the three genotypes. VNIV isolates had a pattern of susceptibility to all azoles lower than the other two genotypes: FLZ GM 1 vs 2.5 for VNIII and 2.2 for VNI, VRZ GM 0.03 vs 0.06 for VNIII and 0.07 for VNI, and ITZ GM 0.04 vs 0.11 for VNIII and 0.12 for VNI. The two isolates with FLZ MIC values higher than ECV were both VNIII hybrids, the same two isolates plus one VNI isolate had also VRZ MIC>ECV. Only one isolate (VNI genotype) had a MIC value one twofold-dilution above the ECV for ITZ. All the three isolates with FC MIC>ECV were VNI genotype.

Conclusions The results of this study on *C. neoformans* wild-type isolates confirm the low frequency of reduced antifungal susceptibility. The establishment of MIC distributions of wild type *C. neoformans* isolates and ECVs will be useful in resistance surveillance and in the development of clinical breakpoints for the antifungals agents and *C. neoformans*.

P004

Pulmonary infection due to an *Aspergillus fumigatus* with a peculiar pattern of azole susceptibility

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Objective To report a case of aspergillosis caused by *Aspergillus fumigatus* isolates with a peculiar pattern of antifungal susceptibility.

Case report The patient with a history of tuberculosis presented a cavity with an inclusion in the apical right lung and a nodulation widespread the upper field of the left lung. Alternation of resolution and progression of the infection at the left lung required a prolonged antifungal treatment with different drugs (itraconazole, voriconazole, posaconazole, caspofungin) to obtain a complete resolution of the infection before the right pneumonectomy.

From a fine-needle biopsy several colonies of *A. fumigatus* showing different features grew in culture: some colonies had the typical heavily sporulated pattern and other, characterized by slower growth, a flocculent lightly sporulated Appearance The typical isolates were susceptible to azole compounds when tested with E-test, namely MICs of itraconazole, posaconazole and voriconazole were 0.25, 0.12 and 0.12 mg L^{-1}, respectively. On the contrary the lightly sporulated isolates showed a peculiar pattern of susceptibility with absence of growth at the lower concentrations of azoles and growth only in presence of the higher antifungal concentrations (paradoxical effect with azole requirement to grow). Tubulin and cyp51A genes of the anomalous isolates were sequenced. The isolates were identified as *A. fumigatus* sensu stricto and M220I mutation was found in cyp51A gene. Six isolates were genotyped by microsatellite analysis and two different allelic profiles were identified, one of the typical isolates and another of the lightly sporulated isolates.

Clinical improvement and radiological clearance of the left lung, obtained with a 3-month voriconazole treatment (200 mg × 2 day^{-1}), allowed the right pneumonectomy. Unfortunately, only fixed tissues were available avoiding culture and antifungal susceptibility testing. However molecular methods performed on the bioptic sample allowed the detection of the M220I mutation.

Conclusions The patient resulted infected by two *A. fumigatus* genotypes with different patterns of azole susceptibility. Probably the azole susceptible isolates were responsible of the infection in the left lung that resolved with voriconazole treatment and the resistant ones of the aspergilloma of the right lung as demonstrated by the detection of the mutation M220I in the cyp51A gene directly in the bioptic sample.

Probably other mutations than that found in the cyp51A gene could be responsible of the inability to grow in absence of azoles shown by these last isolates.

P005

Trends in candidaemia and antifungal consumption in Belgium

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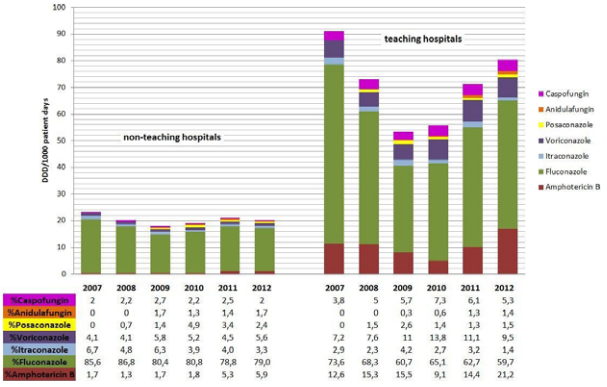
Objectives The incidence of *Candida* species causing bloodstream infection was investigated in the University Hospitals Leuven (Belgium) over the period 2004–2014. Antifungal resistance in *Candida glabrata* was also evaluated as this species is known to display decreased susceptibilities to azoles and echinocandins. Clustering between resistant *C. glabrata* isolates was analysed by strain typing and the molecular mechanisms underlying the resistance were identified. In addition, antifungal consumption in hospitals over the period 2007–2012 was analysed on a national level.

Methods *Candida* blodstream isolates of the University Hospitals Leuven were collected and identified between 2004 and 2014. The number of patient days was used to calculate incidence rates. Sequencing and qRT-PCR analyses were performed on selected genes implicated in the azole or echinocandin resistance. Antifungal consumption for the period 2007–2012 in all Belgian acute and chronic care hospitals (>150 beds) was provided by the national surveillance of antimicrobial consumption, stratified by teaching activities. Results were calculated at the ATC-level 5 (chemical substance, WHO classification system) and displayed in defined daily dose (DDD)/1000 patient days.

Results Between 2004 and 2014, 734 episodes of candidaemia occurred in the University Hospitals Leuven. The highest incidence was caused by *C. albicans* while *C. glabrata* and *C. parapsilosis* ranked respectively the 2nd and the 3rd most common agent each year (Figure 1). Analysis of the antifungal resistance in 172 *C. glabrata* isolates revealed resistance to fluconazole and echinocandins in 12.2% and 1.2%, respectively. A minimum-spanning tree did not show any correlation between resistant strains, nor within hospital units.

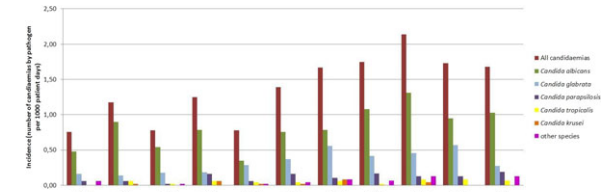
Antifungal consumption at the national level was much lower in non-teaching hospitals compared to teaching hospitals (Figure 2). Fluconazole was the drug prescribed most frequently but its usage declined between 2007 and 2012 (from 73.6% to 59.7% and from 85.6% to 79% in teaching and non-teaching hospitals, respectively). Among the mould active triazoles, DDDs were in general highest for voriconazole, followed by itraconazole and posaconazole. Amphotericin B was the *2nd* most common antifungal drug prescribed in teaching hospitals (21.2% of all antifungals in 2012). The consumption of caspofungin showed a peak in 2010 while anidulafungin remained below 2% since its introduction in 2006 (Figure 2).

Conclusion Results indicate that *C. albicans* is the most common cause of candidaemia in the University Hospitals Leuven, followed by *C. glabrata* and *C. parapsilosis.* This distribution is similar to other Northwestern European centres although an increase in non-*albicans*



	2007	2008	2009	2010	2011	2012	2007	2008	2009	2010	2011	2012
%Caspofungin	2	2,2	2,7	2,2	2,5	2	3,8	5	5,7	7,3	6,1	5,3
%Anidulafungin	0	0	1,7	1,3	1,4	1,7	0	0	0,3	0,6	1,3	1,4
%Posaconazole	0	0,7	1,4	4,9	3,4	2,4	0	1,5	2,6	1,4	1,3	1,5
%Voriconazole	4,1	4,1	5,8	5,2	4,5	5,6	7,2	7,6	11	13,8	11,1	9,5
%Itraconazole	6,7	4,8	6,3	3,9	4,0	3,3	2,9	2,3	4,2	2,7	3,2	1,4
%Fluconazole	85,6	86,8	80,4	80,8	78,8	79,0	73,6	68,3	60,7	65,1	62,7	59,7
%Amphotericin B	1,7	1,3	1,7	1,8	5,3	5,9	12,6	15,3	15,5	9,1	14,4	21,2

Figure 1. The incidence of candidaemias in the University Hospitals Leuven for the period 2004–2014



	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014
% C. albicans	63,2	76,3	69,2	62,9	44,7	54,4	46,9	61,9	61,2	54,9	61,3
% C. glabrata	21,1	11,9	23,1	14,5	36,8	26,5	33,3	23,8	21,4	32,9	16,3
% C. parapsilosis	7,9	5,1	2,6	12,9	7,9	11,8	6,2	9,5	5,8	7,3	11,3
% C. tropicalis	0	5,1	2,6	4,8	5,3	2,9	3,7	1,2	3,9	4,9	3,8
% C. krusei	0	1,7	0	4,8	2,6	1,5	4,9	0	1,9	0	0
% other species	7,9	0	2,6	0	2,6	2,9	4,9	3,6	5,8	0	7,5

Figure 2. Antifungal consumption in Belgian hospitals for the period 2007–2012

species was not observed in our study. In addition, fluconazole and echinocandin resistance remained relatively low in *C. glabrata* in comparison with other centres in Europe.

Fluconazole consumption in Belgian hospitals decreased but remained the most prescribed antifungal drug. Antifungal consumption is much higher and less stable in teaching hospitals compared to non-teaching hospitals. In teaching hospitals, amphotericin B, voriconazole and caspofungin are relatively more prescribed, which could be related to the more specialized cares provided in these settings.

P006

Detection of clinical *Aspergillus fumigatus* isolates resistant to triazoles

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Objectives The aim of this study was to investigate the presence and molecular mechanisms of resistance to triazoles within clinical *Aspergillus fumigatus* isolates collected in Wrocław Medical University, Poland.

Methods The study included 89 clinical isolates of *A. fumigatus* collected during routine diagnostic procedures from several Wroclaw hospitals in the period of 2006–2014. The isolates were identified

based on classical morphological criteria followed by molecular confirmation of the species identification (sequencing of ITS1, 5,8S, ITS2 region). Minimal Inhibitory Concentration (MIC) was tested for itraconazole (ITR), voriconazole (VOR) and posaconazole (POS) using broth microdilution methods, according to European Committee for Antimicrobial Susceptibility Testing E.DEF 1.9. The *cyp*51A gene with promoter region of all isolates was sequenced.

Results The sequencing results of the fragment of rDNA region confirmed identification of all examined isolates as *A. fumigatus*. According to the EUCAST breakpoints for the interpretation of antifungal susceptibility testing 87/89 (97.75%) isolates were susceptible to ITR, VOR, and POS with median MIC values of 0.5 mg L^{-1} (ITR), 0.25 mg L^{-1} (VOR), and 0.03 mg L^{-1} (POS). Two isolates were resistant to ITR with MIC values 32 mg L^{-1}, one of which were cross resistant to VOR, and POS (4 mg L^{-1} and 0.5 mg L^{-1} respectively), whereas the second were classified as intermediate susceptible (MIC for VOR: 2 mg L^{-1} and POS: 0.25 mg L^{-1}). The sequencing of the *cyp*51A with the promoter region revealed mutation TR34/L98H in both resistant strains.

Conclusion Triazole resistant *A. fumigatus* strains harboring mutation TR34/L98H are the emerging pathogens in many European and Asian countries. The presented study shows that such a resistance exist also in Poland. The prevalence of triazole resistance among clinical *A. fumigatus* isolates collected in Wrocław was low (2.25%), nevertheless it indicates the necessity to include susceptibility testing in routine diagnostic procedures.

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P007

Increased efficacy of fluconazol combined with lactoferrin against azole-resistant *Candida* isolates

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Candidiasis caused by fluconazole-resistant *Candida* are related to increased morbidity and mortality. The use of new antifungal drugs and/or the combination of fluconazole with different compounds, such as lactoferrin, with antimicrobial and antiinflammatory properties, can help to solve this important clinical problem.

Objective To assess the *in vitro* synergistic effect of fluconazole and lactoferrin combinations against fluconazole-resistant *Candida* clinical isolates.

Methods Seven fluconazole-resistant *Candida albicans*, three *Candida glabrata*, two *Candida tropicalis*, one *Candida krusei* and one *Candida parapsilosis* clinical isolates and the quality control *Candida krusei* ATCC 6258 were tested. Different fluconazole and lactoferrin combinations were studied: fluconazole concentrations (from 1 to 64 μg ml^{-1}) were selected according to the minimum inhibitory concentration (MIC) determined by Clinical and Laboratory Standards Institute (CLSI) M27-A3 document. MIC was defined as the lowest drug concentration showing 50% growth reduction after 24 h incubation. Lactoferrin concentrations ranged from 0.0625 to 32 μg ml^{-1}. The interaction between fluconazole and lactoferrin was assessed by a checkerboard microdilution method. The fractional inhibitory concentration index (FICI) classified drug interaction as synergistic (FICI ≤0.5), indifferent (0.5< FICI ≤4), and antagonistic (FICI >4).

Results Table 1 summarizes MICs for lactoferrin and fluconazole treatment alone and in combination against different species of *Candida*. Lactoferrin MIC range was6- > 32 μg ml^{-1} and fluconazole range8- > 64 μg ml^{-1}. However, combination of both drugs reduced the concentrations necessary for inducing 50% reduction in the growth of fluconazole-resistant isolates. This combination fluconazole-lactoferrin was synergistic in most of cases. No antagonism was found.

Table 1 Combination of antifungal activities of lactoferrin (LF) with fluconazol(FLC) againts *Candida* strains

	Alone		In combination			
Strain	FLC	LF	FLC	LF	FICI	Interpretation
CA UPV 06-100	16	>32	1	0.0625	0.07813	SYN
CA UPV 06-114	>64	>32	1	8	0.13281	SYN
CA UPV 10-166						
CA UPV 10-168	>64	>32	1	8	0.13281	SYN
CA UPV 10-169	>64	>32	1	4	0.07031	SYN
CA UPV 10-170	>64	>32	1	8	0.13281	SYN
CA UPV 10-171	>64	>32	1	8	0.13281	SYN
CG ATCC 90030	8	8	2	2	0.5	SYN
CG UPV 03-282	16	>32	1	8	0.1875	SYN
CG UPV 07-185	16	6	8	0.0625	0.5078	IND
CK ATCC 6258	32	8	1	8	1.0312	IND
CK NCPF 3321	64	8	32	0.0625	0.5078	IND
CP NCPF 3104	>64	8	1	8	1.00781	IND
CT UPV 05-014	>64	>32	1	8	0.13281	SYN
CT UPV 07-201	>64	8	1	8	1.00781	IND

SYN: synergism. IND: indifference. CA: *Candida albicans*; CG: *Candida glabrata*; CK: *Candida krusei*; CP: *Candida parapsilosis*; CT: *Candida tropicalis*

Conclusions Combination of fluconazole and lactoferrin has a potent synergistic effect against fluconazole-resistant *Candida albicans* and *Candida glabrata*.

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P008

Synergistic antifungal activity of fluconazole combined with cyclosporine A

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Objective To evaluate the *in vitro* activity of fluconazole in combination with the calcineurin inhibitorcyclosporine A against fluconazole-resistant *Candida.*

Methods A total of 15 clinical azole resistant isolates of *Candida* were included in this study: 7 *Candida albicans*, 3 *Candida glabrata*, 2 *Candida tropicalis*, 1 *Candida krusei and* 1 strain of *Candida parapsilosis*. *Candida krusei* ATCC 6258 was the quality control. The minimum inhibitory concentrations (MIC) were determined according to Clinical and Laboratory Standards Institute guidelines for yeasts (M27-A3), and defined as the lowest drug concentrations that produced a 50% inhibition of visible fungal growth after 24 h of incubation. Drug interactions were assessed by the checkerboard microdilution method. Different fluconazole and cyclosporin A combinations were studied: the final concentration of fluconazole ranged from 1 to 64 μg ml^{-1} and of cyclosporine A ranged from 0.0625 to

Table 1 Combination of antifungal activities of Cyclosporin A (CSA) with fluconazol(FLC) againts *Candida* strains

	Alone		In combination			
Strain	FLC	CSA	FLC	CSA	FICI	Interpretation
CA UPV 06-100	16	>128	1	0.25	0.0634	SYN
CA UPV 06-114	>64	>128	1	2	0.0156	SYN
CA UPV 10-166	32	>128	1	0.5	0.0332	SYN
CA UPV 10-168	>64	>128	1	2	0.0156	SYN
CA UPV 10-169	>64	>128	1	2	0.0156	SYN
CA UPV 10-170	>64	>128	1	2	0.0156	SYN
CA UPV 10-171	>64	>128	1	8	0.0156	SYN
CG ATCC 90030	8	16	1	4	0.3750	SYN
CG UPV 03-282	16	>128	8	0.25	0.5009	IND
CG UPV 07-185	16	16	2	4	0.3750	SYN
			4	2	0.3750	SYN
CK ATCC 6258	32	>128	32	0.25	1.0009	IND
CK NCPF 3321	64	>128	32	0.25	0.5009	IND
CP NCPF 3104	>64	16	1	2	0.1406	SYN
CT UPV 05-014	>64	>128	1	2	0.0156	SYN
CT UPV 07-201	>64	>128	1	2	0.0156	SYN

SYN: synergism. IND: indifference. CA: *Candida albicans*; CG: *Candida glabrata*; CK: *Candida krusei*; CP: *Candida parapsilosis*; CT: *Candida tropicalis*

32 μg ml^{-1}. The *in vitro* interaction of the drug combination was interpreted in terms of the fractional inhibitory concentration index (FICI) as follows: FICI ≤ 0.5, synergistic; 0.5 < FICI ≤ 4, indifferent; and FICI >4, antagonistic.
Results Results of the checkerboard analysis are summarized in the table. The addition of cyclosporine A resulted in a high decrease in the MIC of fluconazole from 16–64 to 1 μg ml^{-1} against all isolates of *Candida albicans, Candida tropicalis* and *Candida parapsilosis*. Although this combination was less effective against *Candida glabrata*, the effect was synergistic against two out of three isolates.
Conclusions Cyclosporine A enhanced the effect of fluconazole against fluconazole-resistant isolates. The current results underline the potential use of calcineurin inhibitors as strengtheners of antifungal therapy for recalcitrant candidiasis.
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P009

Evaluation of Cyp51A Mechanisms of Azole Resistance in *Aspergillus fumigatus* Isolates from the United States

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Objective The azole antifungals itraconazole, posaconazole, voriconazole, and isavuconazole are important therapeutic agents against invasive aspergillosis. However, increased resistance in *Aspergillus fumigatus* to these clinically available antifungal agents has been reported, especially in parts of Europe. Resistance to these agents in clinical isolates has primarily been associated with mutations within the *CYP51A* gene that lead to amino acid substitutions within the Cyp51A enzyme, as well as tandem repeats (TR) in the promoter region of *CYP51A*. Our objective was to evaluate the Cyp51A associated mechanisms of azole resistance in a U.S. collection of *A. fumigatus* isolates.
Methods The antifungal susceptibility database in the Fungus Testing Laboratory at the UT Health Science Center San Antonio was queried for itraconazole, voriconazole, and posaconazole MIC data against *A. fumigatus* isolates from 2001 through 2014. This database is populated with antifungal MIC data against fungal isolates sent to our mycology reference laboratory from institutions across the U.S. Susceptibility testing was performed according to methods in the CLSI M38-A2 standard. Isolates were classified as resistant based on the EUCAST clinical breakpoints (voriconazole & itraconazole >4 mg/L, posaconazole >0.5 mg/L). Isolates with elevated azole MICs were confirmed to be *A. fumigatus* by morphology and growth at 50°C. Isavuconazole MICs were also measured against the confirmed *A. fumigatus* isolates. The *CYP51A* gene and its promoter region were sequenced to evaluate for mutations associated with azole resistance.
Results A total of 220 isolates sent to our laboratory with elevated azole MICs and a preliminary identification of *A. fumigatus* were screened, and 37 were confirmed to be this species by morphology and growth at 50°C. Of the 37 isolates, 16 had mutations in the *CYP51A* gene resulting in amino acid substitutions in the Cyp51A enzyme associated with azole resistance. One isolate was found to have the TR_{34}/L98H resistance mechanism not previously found within the U.S. and was pan-azole resistant. Two additional isolates contained the TR_{46}/Y121F/T289A resistance mechanism. Both of these isolates were highly resistant to voriconazole and isavuconazole (MIC >16 mg/L) but had lower itraconazole and posaconazole MICs (range 1–4 mg/L). No *CYP51A* mutations or tandem repeats in the promoter region of this gene were found in the remaining 18 isolates. Strong correlations were found between itraconazole and posaconazole MICs (Spearman r-value 0.73) and between voriconazole and isavuconazole MICs (Spearman r-value 0.93).
Conclusions Point mutations previously associated with azole resistance were present in many of the *A. fumigatus* isolates in this U.S. collection. In addition, 1 TR_{34}/L98H isolate and 2 with TR_{46}/Y121F/T289A mutations were found, which have not previously described in the U.S. However, approximately half of these azole resistant isolates did not have Cyp51A associated mechanisms of resistance.

P010

Influence of microtitre tray plastic type on azole minimum inhibitory concentrations against *Candida* and *Aspergillus* Species

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Objective Antifungal susceptibility testing provides evidence of *in vitro* activity that is used to classify fungal isolates as susceptible or resistant to individual drugs. Clinicians may then use this information to make treatment decisions in patients with invasive fungal infections. The CLSI and EUCAST broth microdilution reference methods for antifungal susceptibility testing both use cell culture microtitre plates for *in vitro* susceptibility testing. However, the type of plastic that is used (treated vs. untreated) is not specified. Our objective was to evaluate the influence of the plastic type on the *in vitro* activity of azole antifungals.
Methods *Candida* spp. (*C. albicans* and *C. glabrata*; $N = 46$ total isolates) and *Aspergillus* spp. (*A. flavus*, *A. fumigatus*, *A. niger*, and *A. terreus*; $N = 50$ total isolates) received by our clinical reference laboratory were utilized. Antifungal susceptibility testing was performed with fluconazole, itraconazole, posaconazole, and voriconazole according to the methods in the CLSI M27-A3 and M38-A2 standards. Testing was performed using both cell culture treated and untreated plastic microtitre plates from the same manufacturer. Geometric mean (GM) MIC values between treated and untreated plastic for each drug between were compared by paired *t*-test.
Results The type of plastic in the microtitre trays significantly influenced the *in vitro* potencies of itraconazole and posaconazole, while the activities of fluconazole and voriconazole appeared to be less affected. Against *C. glabrata*, the GM MIC values for itraconazole and posaconazole were both 2.5 times higher when measured using untreated plastic compared to treated plastic plates ($P < 0.0001$ for each comparison). Similarly, against *C. albicans*, the posaconazole GM MIC value was 2 times higher when *in vitro* activity was measured using untreated versus treated plastic. Against *Aspergillus* spp., the type of plastic affected the *in vitro* activity of posaconazole the most. Posaconazole GM MICs measured using untreated plastic were significantly higher against all *Aspergillus* spp. compared to treated plastic (fold increase GM MIC of 2.7–3.3 times; $P < 0.001$ for all comparisons). In addition, significantly more posaconazole MICs were >0.5 mg l^{-1} (EUCAST clinical breakpoint for posaconazole resistance against *A. fumigatus* and *A. terreus*) when measured using untreated plastic compared to treated plastic (33 of 50 vs. 5 of 50, respectively; $P < 0.0001$ by Fisher's exact test). In contrast, the *in vitro* activities of itraconazole and voriconazole against *Aspergillus* spp. were less affected by the type of plastic.
Conclusions The type of plastic used for broth microdilution susceptibility testing may influence the results of these assays, and this may be drug specific. Against *Candida* and *Aspergillus* spp., the *in vitro* potency of posaconazole was significantly reduced when untreated plastic was used. This may have clinical implications in terms of the classification of isolates as being susceptible or resistant to this antifungal agent.

P011

Characterization of *Aspergillus fumigatus* in COPD patients and their homes reveals frequent coexistence of isolates with various azole-resistance profiles

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Objectives Increasing reports on *Aspergillus fumigatus* azole-resistance make detection of resistant isolates in respiratory samples a challenging issue in clinical practice. Moreover, detection in environmental reservoirs could be helpful to identify risk factors for acquisition of *A. fumigatus* resistant isolates and to improve the prevention of aspergillosis. In this study, *A. fumigatus* clinical and environmental isolates obtained from azole-naive COPD patients and their homes were characterized in order to determine the prevalence of azole-resistance and *cyp51A* mutations, and to clarify the circulation of *A. fumigatus* azole-resistant or mutated isolates between clinical and environmental reservoirs.
Methods Seventy-five respiratory samples from 41 COPD patients (56 sputa and 19 oropharyngeal washes) and environmental samples from 36 homes of these patients (mainly electrostatic dust collectors exposed for 10 weeks in the patient's bedroom) were prospectively collected in the Lille University Hospital between August 2011 and February 2015. Clinical and environmental *A. fumigatus* isolates obtained by culture on Sabouraud agar medium were further grown in medium containing itraconazole (ITZ) for selection of azole-resistant isolates. *A. fumigatus* identification was confirmed by culture at 50°C, as well as ITS rDNA regions and beta-tubulin genes sequencing. Then, the detection of mutations in the *cyp51A* gene was performed by sequencingfor all *A. fumigatus* isolates. Finally, ITZ, voriconazole and posaconazole Minimum Inhibitory Concentrations (MIC) were determined for isolates with positive growth on ITZ-medium and/or *cyp51A* mutation.
Results *A. fumigatus* detection was positive in clinical samples for 11/41 patients (26.8%) and in 15/36 patient's homes (41.7%), yielding 68 clinical and 48 environmental isolates. Growth on ITZ medium was positive for 4 clinical isolates from 2/41 patients (4.9%) and 3 environmental isolates from 2/36 patient's home (5.6%). Among clinical isolates, 1 had no *cyp51A* mutation and 3 others from 1 patient had A284T mutation. Two environmental isolates from 2 different patients had TR_{34}/L98H mutation, and 1 had the H285Y mutation, which was found to be localized by 3D modeling near the heme-binding domain of CYP51A, at the entry of channel 1. Growth on ITZ medium was negative for the remaining 109 isolates, but 4 environmental isolates had the F46Y/M172V/N248T/D255E/E427K (n = 3) or the F46Y/M172V/E427K (n = 1) mutation. Coexistence of different *cyp51A* genotypes and/or azole-resistance profiles was detected in 3/8 respiratory and 2/10 environmental samples where more than 1 *A. fumigatus* isolate had been detected (<i.*e.* 37.5% and 20.0%, respectively). Both clinical and environmental isolates were obtained from 3 patients, but all were azole-sensitive and had no *cyp51A* mutation.
Conclusion The high frequency of azole-resistant *A. fumigatus* isolates in COPD patient's homes suggests that domestic exposure could play a key role in the emergence and spread of azole-resistance in patients. Coexistence of azole-sensitive and resistant isolates in clinical samples indicates that picking a single colony for MIC determination is not sufficient to exclude the presence of azole-resistant isolates in clinical practice, and confirms that MIC determination from all colonies or screening using azole-supplemented medium should be performed when patients are to be treated.

P012

Patients with *Candida glabrata* candidemia due to caspofungin-susceptible and -intermediate strains have superior outcomes to patients infected with caspofungin-resistant strains

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Background In a recent multi-center study, we showed that caspofungin (CSP) minimum inhibitory concentrations (MICs) against *Candida glabrata* (Cg) measured by Sensititre YeastOne (SYO) cluster tightly, suggesting this method overcomes inter-laboratory variability seen with broth microdilution reference methods. Overall, 18% of Cg strains were defined as intermediate-resistant (I) to caspofungin (CSP) by CLSI criteria (susceptible (S)≤0.12 μg ml^{-1}, I = 0.25, resistant (R)≥0.5). Almost all of these strains were S to other echinocandins (EC), indicating that breakpoints (BPs) may over-call CSP non-susceptibility. In this study, we evaluated correlations between CSP MICs and outcomes of patients (pts) with Cg candidemia.
Methods We performed a retrospective cohort study of pts with Cg candidemia treated with an EC for ≥3 days as initial therapy. Pts with candidemia due to >1 Candida spp. were excluded. Treatment (Tx) success was defined as survival and sterilization of blood cultures at 14 days. EC MICs were determined by SYO panels, which we previously showed to be more sensitive than broth microdilution. PCR and DNA sequencing were used to detect mutations in hot spots of *FKS1* and *FKS2.*
Results 106 cases were included. 46% of pts were men; median age was 59 years (range: 21–89). 56% of pts were in the ICU and 25% had septic shock. Median CSP MIC was 0.12 (range: 0.03–16). 6% of strains harbored *FKS* mutations. 54%, 35%, and 11% of strains were CSP-S, I and R, respectively. All CSP-I strains were S to anidula (MIC≤0.12) and micafungin (MIC≤0.06), and none harbored *FKS* mutations. 29% of strains were collected from pts with prior EC exposure (≥3 days of EC before candidemia). Rates of EC exposure were similar for S and I strains (28% *vs.* 19%; P = 0.34), but higher for R strains (67%; P = 0.005). The overall rate of Tx success was 69%. Success rates for candidemia due to S, I and R strains were 68%, 81%, and 33%, respectively (I *vs.* R, P = 0.004; I *vs.* S, P = 0.23). The combined success rate for candidemia due to S and I strains was 74%, compared to only 33% for R (P = 0.008). Likewise, mortality rates at 30 days were 22%, 25%, and 42% for CSP-S, I and R Cg strains. Mortality rates were higher among pts infected with CSP-R strains than CSP-S or -I strains (23%; P = 0.004).
Conclusions Identification ofCg strains as CSP-R by SYO and CLSI criteria is predictive of Tx failures and death among pts with Cg candidemia. CSP-I strains are not associated with *FKS* mutations, prior EC exposure, or worse pt outcomes, suggesting that they should be re-classified as CSP-S.

P013

Epidemiology of FKS mutations among *Candida* strains at high-risk for echinocandin resistance

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Background Echinocandin (EC) breakpoints (BP) proposed by CLSI for Candida spp. are not validated and may overstate caspofungin (CSP) resistance among *C. glabrata* (Cg). The significance of discrepant EC susceptibility is unknown, as are *FKS* mutation rates among various spp. The objective of this study was to determine *FKS* mutation rates by systematic screening of high-risk Candida strains, including those with discrepant EC susceptibility.
Methods EC MICs were determined by YeastOne among consecutive strains causing candidemia from 2009 to 14. Hot spots in *FKS1* and

FKS2 (Cg only) were sequenced for strains from patients receiving ≥ 3 days of prior EC therapy or those non-susceptible (NS) to any EC by CLSI criteria.
Results 453 strains from 384 pts were included. *C. albicans* (Ca, 37%), *Cg* (37%), *C. parapsilosis* (*Cp*, 16%) and *C. tropicalis* (*Ct*, 8%) were most common. 16% of strains were EC NS. NS rates were higher for CSP (15%) than anidula (ANF; 2%) or micafungin (MCF; 2%, $P < 0.0001$ for both). EC NS was higher for *Cg* (34%) than other spp (5%, $P < 0.001$). 27% of EC NS strains were resistant to fluconazole. 21% of strains were from pts with prior EC exposure, including 3% classified as breakthrough (BT) during EC therapy. Prior exposure was more common among *Cp* (40%) and *Cg* (25%) than *Ca* (12%; $P < 0.001$ and 0.003, respectively), and among NS strains (24% vs 13%; $P = 0.02$). 32% of strains met criteria for sequencing, among which the *FKS* mutation rate was 6% (overall 2% [8/453]). Mutations occurred only in prior exposure *Ca* (2) and *Cg* (6), for spp.-specific rates of 10% and 15%, respectively. *FKS* mutation rates were 71%, 33%, 2%, and 0.3% in strains NS to 3, 2, 1, and 0 ECs, respectively. 67% of BT *Cg* were mutants. No *Cg* with CSP MIC = 0.25 μg ml^{-1} (i.e., intermediate) harbored a mutation. Using a NS cutoff ≥0.5 μg ml^{-1}, the rate of CSP NS *Cg* was reduced from 34% to 8% and the number of high-risk strains from 32% to 25% ($P < 0.001$ for both). With the revised cutoff, 58% of NS strains from pts with prior exposure harbored mutations.
Conclusions *FKS* mutations are only encountered among high-risk Cg and Ca, but are rare with the exception of BT Cg strains. Strains NS to a single EC are almost never FKS mutants, suggesting such classifications are CLSI BP artifacts and not biologically-driven. Based on our cumulative experience, CSP-intermediate Cg should be considered susceptible and the NS breakpoint increased to ≥0.5 μg ml^{-1}.

P014

Prior Azole or Echinocandin (EC) Exposure Increases the Risk for EC Resistance and Antifungal (AF) Treatment (Tx) Failure among Patients (pts) with Candidemia

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Background Prior EC exposure is an important determinant of EC resistance among Candida spp. We hypothesized that azole exposure may also influence EC resistance. Our objective was to determine the impact of prior AF exposure on candidemia epidemiology, AF susceptibility, and tx outcomes.
Methods Consecutive pts with candidemia from 2009 to 14, including up to 1 recurrent episode per pt were evaluated. Prior AF exposure was defined as ≥ 3 days (d) of AFs. Tx success was defined as survival and sterilization of blood cultures at 14 d. Fluconazole (FLC) and caspofungin (CSP) MICs were determined by YeastOne.
Results 444 strains from 395 pts were included; *C. albicans* (*Ca*; 37%) and *C. glabrata* (*Cg*; 36.5%) were most common, followed by *C. parapsilosis* (*Cp;* 14%), *C. tropicalis* (*Ct;* 8.5%), and other spp (4%). *Cg* (32 to 45%, $P = 0.02$) and *Cp* (10 to 21%, $P = 0.003$) were more common among pts with prior AF exposure, and *Ca* was less common(45–21%, $P < 0.001$); data were consistent for prior azole and EC exposure individually. CSP and FLC MICs varied by exposure for *Cg*, but not other spp. Geometric mean CSP MICs increased stepwise among *Cg* strains not exposed to AFs (0.11 μg ml^{-1}), exposed to azole alone (0.15, $P = 0.02$), EC alone (0.22, $P = 0.04$), or both (0.30, $P = 0.0002$). FLC MICs against *Cg* were increased if exposed to azoles ($P = 0.03$), but not ECs ($P = 0.21$). 357 cases of candidemia were treated w/≥3 days of AFs. Rates of tx success were higher for pts receiving an EC (70%) vs azole (58%, $P = 0.02$), and those not exposed to AFs (71%) vs exposed (52%, $P = 0.0005$). Among pts exposed to an AF, success was significantly lower when treated with an EC (52 vs 76%, $P = 0.007$) or azole (32 vs 66%, $P = 0.0003$). Excluding breakthrough candidemia (defined as failure *a priori*), rates of success were 65%, 76%, and 87% for pts exposed to AFs w/in 30 d, 31–100 d, or >100 d prior to candidemia, respectively. Rates were inversely related to total d of AF exposure; success rates were 44%, 58%, and 79% among pts exposed to >100, 31–100, or <30 d of AFs, respectively.
Conclusion Prior AF exposure is associated with a shift from *Ca* to *Cg* and *Cp* as causes of candidemia. Our data suggest that stress imposed by AF exposure, regardless of specific agent, lowers the threshold for development of EC resistance among *Cg*. Total and timing of AF exposure predict tx responses, underscoring the importance of prior exposure when treating pts with candidemia.

P015

FKS Mutation Rates of *Candida glabrata* (Cg) Vary by Echinocandin (EC)

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Background EC resistance in Cg is mediated by point mutations in *FKS1* and *FKS2* hot spots (HS). The objective of this study was to measure breakthrough mutant frequencies (MF) and *FKS* mutation rates among Cg exposed to anidulafungin (ANF), caspofungin (CSP), and micafungin (MCF).
Methods 20 *FKS* wild-type (WT) Cg with varying EC MICs from pts with or without prior EC exposure were selected. MF was calculated as ratio of CFU on SDA plates with or without ECs at 3× MIC. Breakthrough mutant prevention concentration (MPC) was the lowest EC concentration inhibiting >99% growth on SDA agar w/vs w/o drug. MICs and *FKS* genotypes were determined for colonies growing at ≥ MPC for each EC.
Results 50% of strains had CSP MICs > or ≤ the CLSI breakpoint (≤0.12 μg ml^{-1}), of which 50% in each group were from pts with prior EC exposure. Overall median ANF, CSP, and MCF MICs were 0.06 (range: 0.03–0.12), 0.12 (0.03–1), and 0.03 (0.015–0.03), respectively. Corresponding median MPCs were 0.5 (0.25–4), 2 (1 to >8), and 0.25 (0.25–1), respectively. CSP MPCs were higher than other ECs ($P < 0.0001$ for each). Median Cg MF rates in ascending order were 2.8E-9 (0 to 1.6E-7), 4.2E-7 (1.2E-8 to 5.9E-6), and 4.8E-6 (1.2E-7 to 5.2E-3) for MCF, ANF, and CSP, respectively ($P < 0.0001$ for each 2-way comparison). MF rates did not vary by CSP MIC or prior EC exposure. 81, 17, and 7 strains growing at ≥ MPC of CSP, ANF, and MCF, respectively, were collected. Compared to parent strains, CSP, ANF, and MCF MICs against 63%, 29%, and 25% of MPC mutants, respectively, were increased by ≥2-fold. Overall, 28% had *FKS* mutations (90% were in FKS2, HS2). 88% of strains with ≥2-fold MIC increase to all ECs had *FKS* mutations (vs 14% w/MIC increase to 1 EC; $P < 0.0001$). Rate of *FKS* mutations among strains recovered from CSP, ANF, and MCF-containing agar were 20%, 47%, and 71% ($P = 0.03$ and 0.008, comparing CSP to ANF and MCF, respectively). 76% of mutations occurred at position F659 (88% of CSP-associated mutants vs 38% of ANF/MCF mutations; $P = 0.19$). Mutations at S645 (FKS1) or S663 (FKS2) occurred only among strains exposed to ANF or MCF (31% of mutants compared to 0% for CSP, $P = 0.03$).
Conclusions MF and *FKS* mutation rates are inversely related for each EC against Cg. Specific *FKS* mutations also differ by agent. In rank order, MF rates are CSP>ANF>MCF, but *FKS* mutation rates are MCF>ANF>CSP. Mutations at F659 are more common for CSP, whereas S645 and S663 mutations are more likely when Cg are exposed to ANF or MCF.

P016

Activity of Isavuconazole and other antifungal agents against clinical strains of mucorales

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Objectives Isavuconazole (ISAV) is a new triazole antifungal agent that was granted approval by the U.S. Food and Drug Administration on March 6, 2015 for the treatment of invasive aspergillosis and mucormycosis. The aim of this study was to evaluate the activity of isavuconazole and other antifungal agents against mucorales isolated from clinical samples.

Methods Eighty-two mucorales strains were tested for antifungal susceptibility following EUCAST and CLSI methodologies. All strains were obtained from clinical samples and identified to species level by sequencing the Internal Transcribed Spacer Region of the rDNA. Species included in the study were: *Lichtheimia corymbifera, Lichtheimia ramosa, Mucor circinelloides, Rhizomucor miehei, Rhizomucor pusillus, Rhizopus arrhizus* and *Rhizopus microsporus.* The antifungal susceptibility testing was performed following EUCAST reference method 9.1 and CLSI M38-A2. The antifungals used in the study were: amphotericin B (AMB), ISAV, voriconazole (VCZ) and posaconazole (PCZ). All antifungals tested ranged from 0.03 to 16 mg l^{-1}. *Aspergillus flavus* ATCC 204304 and *Aspergillus fumigatus* ATCC 204305 were used as quality control strains in all test performed for both methods. Minimal Inhibitory Concentrations (MICs) were read after 24 and 48 hours of incubation.

Results With CLSI methodology over 23% of the strains did not grow at 24 h (the recommended incubation time) in contrast with EUCAST that showed good growth at 24 h for all mucorales strains. Comparable results were found between CLSI 48 h and EUCAST 24 h. Amphotericin B was the most active compound against all species with $MICs_{90}$ (MICs causing the inhibition of 90% of the isolates) < 2 mg l^{-1} for all species but *M. circinelloides* with CLSI methodology (MIC_{90} = 16 mg l^{-1} at 48 h). Isavuconazole showed good activity, with geometric means of MICs (GM) and $MICs_{50}$ < 2 mg/L to all species but *M. circinelloides* (GM = 3.49 for EUCAST 24 h and 2.08 for CLSI 48 h) and moderate activity to *Rh. pusillus* with $MICs_{50}$< 2 mg/L but MIC_{90} = 4 mg l^{-1}. Voriconazole showed no activity against the species tested. Posaconazole was active (MIC_{50}< 0.5 mg l^{-1}) for *L. corymbifera, L. ramosa, R. arrhizus* and *R. microsporus*, but *M. circinelloides* and *Rh. pusillus* showed high MICs to this compound ($MICs_{90}$ > 2 mg l^{-1}).

Conclusions CLSI showed poor growth at 24 h. Amphotericin B was the most active antifungal. Voriconazole showed no activity against the species tested. Isavuconazole and posaconazole showed good activity to all species but *M. circinelloides* and *Rh. pusillus.*

P017

Candida glabrata mutator phenotype promotes resistance to multiple antifungal drugs

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Objectives Both the incidence of invasive fungal infections and rates of multi-drug resistance associated with *Candida glabrata* have increased in recent years. The cellular mechanisms involved in the proclivity of *C. glabrata* to rapidly develop resistance to multiple drug classes, specifically triazoles and echinocandins, are unknown. Others have shown that defects in bacterial DNA repair can cause resistance to multiple antibiotics. Here, we investigate DNA repair in relation to *C. glabrata* antifungal resistance.

Methods DNA mismatch repair genes, *MSH2* and *PMS1*, and double-strand break repair gene, *RAD50*, were disrupted and deletion strains selected on multiple antifungals to determine mutational frequencies. Drug target genes, e.g. *FKS1/2*, were sequenced in resistant mutants. *MSH2* was subsequently sequenced in 234 diverse, clinical strains with varying susceptibility profiles. Identified mutations were expressed in the *msh2* deletion strain and frequencies of 5-fluoroanthranilic acid (5-FAA)- and echinocandin-resistant mutants measured. To determine if altered mismatch repair activity also promotes the development of mutations *in vivo*, we developed a mouse model of gastrointestinal (GI) colonization. Mice were orally inoculated with either a wild-type or *msh2Δ* strain followed by daily, sub-inhibitory caspofungin (0.5 mg kg^{-1} i.p.) or vehicle (PBS i.p.) treatments for 21 days. GI burdens were measured for 30 days through fecal yeast colony counts and resistance was examined through replica plating on caspofungin and Fks1/2 hotspot sequencing.

Results Disruption of *MSH2* or *PMS1*, but not *RAD50*, led to a hyper-mutable phenotype and a significant increase in the emergence of echinocandin-, triazole-, and amphotericin B-resistant mutants. As expected, the echinocandin-resistant mutants contained Fks1 or Fks2 hotspot mutations. Out of the 234 clinical strains analyzed, 48% (77/161) of susceptible isolates demonstrated a nonsynonymous mutation within Msh2, while a mutation was discovered in 67% (24/36; $P < 0.05$ vs. susceptible) of fluconazole-resistant isolates and 68% (15/22; $P = 0.07$ vs. susceptible) of multi-drug resistant isolates. When transferred to a lab strain, the *msh2* mutations from clinical isolates maintained the hyper-mutable phenotype. Mice were effectively colonized ($2–3 \times 10^6$ CFU/fecal pellet) with both the wild-type and *msh2Δ* strains for 30 days. Fks1 mutations (625delF; S629P) were identified in yeast recovered from caspofungin-exposed, *msh2Δ*-colonized mice (mutants recovered from 3 of 3 mice analyzed). No Fks1/2 mutants were recovered from caspofungin-exposed, wild-type-inoculated mice (4 mice analyzed).

Conclusion Overall, we show that strains containing loss-of-function *msh2* mutations exhibit a higher propensity to breakthrough antifungal treatment *in vitro* and *in vivo*, and that such strains are recovered at high frequencies from patients. These data suggest that defects in mismatch repair represent a key, underlying cellular mechanism that facilitates emergence of resistance to multiple antifungals in *C. glabrata.*

P018

In vitro killing activities of two major antifungals against clinically relevant Mucorales using minimal fungicidal concentrations of amphotericin B and posaconazole.

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Objectives Mucorales are filamentous fungi causing invasive infections, which are particularly aggressive and often lethal in immunocompromised hosts. There is a rising interest in antifungal susceptibility testing (AST) of amphotericin B (AMB) and posaconazole (PSC), mainly due to the increasing incidence of breakthrough invasive mucormycosis (IM). AMB has a good *in vitro* activity against Mucorales, and a minor efficacy for PSC is commonly observed. An antifungal agent with cidal activity is therapeutically more promising than an agent with fungistatic activity. Hence, the main goal of the present work was to evaluate a) *in vitro* killing activities of AMB and PSC by using minimum fungicidal concentrations (MFCs), and b) differences in MFCs on both standard medium (RPMI) and alternative supplementary minimal medium (SUP).
Methods A strain collection comprising 131 Mucorales strains was catalogued for species identification using the internal transcribed spacer region (ITS) direct sequencing analysis. *In vitro* antifungal susceptibilities against AMB and PSC were tested according to EUCAST standard method. In parallel, a modified EUCAST was performed, where RPMI was replaced by SUP to provide optimal growth conditions for Mucorales (1). The MFCs were determined as previously described (2) and evaluated using the EUCAST method with either RPMI or with SUP medium. MFC: MIC ratio was calculated for each species and antifungal compound. Both antifungals were categorized as either cidal or static.
Results A clear killing activity of AMB was observed in *Rhizopus arrhizus* and *Mucor* genus. However, in all other four studied species (*Lichtheimia* species, *R. microsporus* and *Rhizomucor pusillus*), the drug appeared to be fungistatic, as MFCs were consistently higher than respective MICs ($\geq$ 4 dilution steps). When assessing AMB MFCs in SUP, a static effect of the drug was seen in all species. For PSC MFCs in standard RPMI, the drug was *in vitro* fungistatic against all Mucorales. The same effect was seen when the drug was tested using SUP medium, with the exception of *L. corymbifera*.
Conclusion The killing activity of AMB is species- and medium-dependent, ranging from static to cidal. Posaconazole was clearly fungistatic under standard procedure. Higher MICs were obtained when both drugs were tested in modified methods using SUP. This may be associated to their generally higher growth ability in this medium. The exception was *L. corymbifera* since the species was able to grow better in RPMI than in SUP.

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P019

First description of fungicide-driven alterations in azole-resistant *Aspergillus fumigatus* in Colombia, South America

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Objective *Aspergillus fumigatus* causes a variety of diseases in humans leading to high morbidity and mortality. Recently, resistance to triazoles, the first line therapy, has been reported in azole naive patients. This resistance has been linked to fungicide-driven mutations in the CYP51A gene and its promoter (TR34/L98H, TR46/Y121F/T289A). This particular mechanism of resistance has been reported in several European countries, Asia, Africa and the United States. By contrast, no resistance was demonstrated in the few strains investigated in South America (Lockart et al., AAC, 2011; Van de Linden et al., EID, 2015). Colombia is the fourth country in the world in use of pesticides (14.5 tons/1000 hectares) (http://www.fao.org), of which 30% are fungicides. The main objective of our study was to investigate the existence of azole-resistant *Aspergillus fumigatus* in soil samples related to the use of fungicides.
Materials After analysis of the fungicides used in Colombia as well as their use in agriculture, the horticulture sector was selected. Soil samples were collected in ornamental plant beds and flower fields from the outskirts, suburbs and downtown areas of the city of Bogotá. The primary culture isolates were sent to Nantes University for molecular identification and resistance study. To detect azole-resistant *Aspergillus fumigatus*, all the strains isolated on Sabouraud agar at 43 °c were screened on agar supplemented with itraconazole or voriconazole. For resistant strains, the CYP51A gene and its promoter were PCR amplified and subsequently DNA sequenced.
Results From the 60 soil samples, 12 were positive for *Aspergillus fumigatus* and 6 exhibited strains (n = 38) that grew on agar supplemented with itraconazole or voriconazole. Most triazole-resistant strains were isolated in soil samples from the flower fields and from greenhouses. Of the 38 resistant strains, 20 were studied for resistance mechanisms. Sequence analysis of the CYP51 gene and its promoter indicated great polymorphism with the presence of TR46/Y121F/T289A (n = 17 isolates), TR34/L98H (n = 1), TR53 (n = 1) and no mutations (n = 1). Difenoconazole and tebuconazole were the azole fungicides found to be use in both places, flowers fields and greenhouses.
Conclusion This is the first study describing *A. fumigatus* harboring fungicide-driven alterations in Colombia and South America. These results underline the need for extensive monitoring of *Aspergillus* resistance in azole exposed as well as naive patients and the importance of continuing research of the environmental factors in other regions of South America.

P020

Patient's home: a clinical relevant source of *Aspergillus fumigatus* harboring the TR46/Y121F/T289A alteration

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Azole resistance in *Aspergillus fumigatus* is an emerging worrisome problem. Environmental resistance has been highlighted relatively recently in the Netherlands (Verweij, N Engl J Med 2007; Mellado, Antimicrob Agents Chemother 2007). While strains bearing the TR34/L98H alteration have been found in many countries, TR46/Y121F/T289A strains are currently less frequently isolated. We recently described the first TR46/Y121F/T289A clinical strains in France in a cystic fibrosis patient (Lavergne, Antimicrob Agents Chemother 2015). However, environmental investigations failed to detect TR46/Y121F/T289A strains near the patient's residence. Here, we report the case of a 66-year-old patient, treated for a rheumatoid arthritis (methotrexate and infliximab) who was admitted in intensive care united because of bilateral pneumopathy. BAL fluid grew with *A. fumigatus* with antifungal susceptibility testing using E-test® method evidencing high resistance to voriconazole (>32 μg ml^{-1}) together with resistance to itraconazole (6 μg ml^{-1}) and posaconazole (1 μg ml^{-1}). Seventeen days after admission, the patient died because of cerebral hemorrhage and multivisceral failure in the context of probable invasive aspergillosis.

Objectives We investigated the *A. fumigatus* isolate from the patient and looked for the presence of azole-resistant *A. fumigatus* strains bearing environmental molecular alterations at the patient's home and environment.
Methods A total of thirty-four surface or soil samples were performed: home ($n = 10$), garden ($n = 11$) and cultivated fields near the home of our patient ($n = 13$). All samples that yield *A. fumigatus* were screened on both 4 mg l^{-1} itraconazole and voriconazole-containing media. Azole-resistant strains were subjected to nucleotide sequencing of the *cyp51A* gene and its promoter.
Results As expected from MIC testing, the clinical isolate harbored the TR46/Y121F/T289A alteration. Samples from the home ($n = 3$), the garden ($n = 3$) and one sample of a field crops grew on azole-containing media. Thirty-seven representative isolates from these samples were sequenced. Thirty-two isolates from the home and the garden harbored the TR46/Y121F/T289A alteration while the 5 isolates from the soil of field crops only harbored the TR34/L98H alteration. Microsatellite genotyping using a panel of nine short tandem repeats is ongoing.
Conclusion This observation confirms the presence of TR46/Y121F/T289A strains in France and is the first description of this fungicide-driven mutation in a patient's home in our country. Taken together, this study highlights the need for regular antifungal susceptibility testing of *A. fumigatus* clinical isolates and underlines the importance of indoor and outdoor environment as a potential source of azole-resistant *A. fumigatus* isolates.

Correction added on 19 October 2015, after online publication. Author V. Letsher Bru was replaced with P. le Pape and his corresponding affiliation to CHU de Nantes, Nantes, France

P021

Multicenter surveillance of azole resistant *Aspergillus fumigatus* isolates in non-academic hospitals in the Netherlands

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Objectives *Aspergillus fumigatus* is a common cause of invasive and allergic diseases. Limited data are available on the prevalence of azole resistance outside university medical centers in the Netherlands. We aimed to determine the burden of azole resistance in medical centers outside universities.
Methods Investigators from 10 non-academic community hospitals in the Netherlands prospectively collected up to 50 *Aspergillus fumigatus* isolates. Isolates were identified morphologically and by growth at 48°C and were subsequently tested for azole resistance on screening agar plates with itraconazole, voriconazole and posaconazole. Isolates which grew on azole containing agar where molecularly identified with b- tubulin and ITS sequencing. Antifungal susceptibility testing was performed with both CLSI and EUCAST reference methods. Resistant isolates were further analyzed by sequence analysis of the *Cyp51A* gene. All isolates were typed with microsatellite typing. Origin of isolates, age of patients, and prior azole use was documented.
Results Over a 1–4 month period, each center aimed to collected 50 *A. fumigatus* isolates ($n = 470$). The number of azole-resistant isolates was $n = 76$ (16%). Resistance frequency varied from 0% to 49% per center and the time to collect isolates varied from 1 month to 4 months. The majority of resistant isolates were recovered from respiratory material ($n = 444$). The remaining isolates originated from ear swabs. All except one were from patients without previous azole exposure. Of the cyp51A mutations ($n = 35$), TR34/L98H was the most common ($n = 28$) followed by TR46/Y121F/T289A ($n = 4$), TR46/Y121F/T289A/M172I/G448S ($n = 1$), F46Y/M172V/E427K ($n = 1$) and N248K ($n = 1$). The remaining resistant isolates had no mutation in the *Cyp51A* ($n = 41$). In two centers we found clusters of 4 and 5 patients with similar isolates.
Conclusions The prevalence of resistance increased significantly compared to a previous surveillance study in 2006. The number of non-*cyp51A* mediated resistant isolates was higher than found in Dutch University Centers and comparable to the level found in the UK. Regular surveillance is warranted to monitor trends in azole resistance at a national scale.

P022

Effect of haloperidol, promethazine and cyclosporine A on the fluconazole susceptibility of *Malassezia* spp.

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Objectives *Malassezia pachydermatis* and *Malassezia furfur* cause various forms of dermatitis in both animals and humans, as well systemic infections in immunocompromised patients (Velegraki et al., PLoS Pathog 2015). Clinical data as well as the *in vitro* antifungal susceptibility studies suggest that *M. pachydermatis* and *M. furfur* are low susceptible or resistant to fluconazole (FLZ) (Iatta et al., J Med Microbiol 2014; Cafarchia et al., Med Mycol 2015). The defence mechanisms against azoles as well as the mechanisms of azole resistance have never been investigated in *Malassezia* spp. The increased expression of drug efflux pumps in the cell plasma membrane is widely recognized to be the major cause of high-level minimal inhibitory concentration (MIC) values of azoles in *Candida* spp. (Pfaller et al., Am J Med 2012). It has been shown that haloperidol (HAL), promethazine (PTZ) and cyclosporine A (CYS) act as modulators or inhibitors of drug efflux pumps in *Candida* spp., therefore causing a significant azoles MIC reductions in *Candida* spp. (Castelo-Branco et al., Med Mycol 2013; Stylianou et al., Antimicrob Agents Chemother 2014; Wibawa et al., Trop Biomed 2015). This study aims to evaluate the effects of HAL, PTZ and CYS on FLZ MIC values of *M. furfur* and *M. pachydermatis* strains, in order to assess the role of drug efflux pumps in the defence mechanism or in resistance phenomena against FLZ in *Malassezia* spp.
Methods A total of 33 *Malassezia* strains, 21 *M. furfur* from humans with bloodstream infections and 12 *M. pachydermatis* from dogs with skin lesions, were tested. The *vitro* efficacy of FLZ in combination with HAL or PTZ or CYS was performed using a broth microdilution checkerboard analysis. Data were analysed using the model-fractional inhibitory concentration index (FICI) method (Odds, J Antimicrob Chemother 2003).
Results The MIC values of FLZ of *M. furfur* and *M. pachydermatis* decreased in presence of sub-inhibitory concentrations of HAL (from 4-fold to >16-fold reduction) and PTZ (from 0 to >4-fold reduction) but not of CYS. Synergism (FICI <0.5) was observed only with HAL and FLZ in 12 *M. furfur* and 8 *M. pachydermatis* strains presenting high FLZ MIC value (i.e., MIC≥128 μg ml^{-1} and ≥64 μg ml^{-1}, respectively).
Conclusion The present study demonstrated for the first time that HAL and PTZ could enhance the antifungal effect of FLC in *M. furfur* and *M. pachydermatis* strains, thus indicating that drug efflux pumps are involved in defence mechanism to azole drugs. The synergistic effect of FLZ and HAL in *Malassezia* strains with high FLZ MIC values might be related to an increased expression of efflux pumps genes, eventually resulting in a possible FLZ resistance as in *Candida* spp. (in Pfaller et al., Am J Med 2012). The FLZ MIC≥128 μg ml^{-1} for

M. furfur and MIC≥64 μg ml^{-1} for *M. pachydermatis* might be considered as cut-off values for separating susceptible and resistant strains, respectively. Further efflux pumps expression analyses could be performed in order to confirm our results.

P023

Aspergillus spp. antifungal susceptibility to triazoles and Amphotericin B

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Objectives The emergence of resistance to antifungal agents among *Aspergillus* species isolated from different sources is dramatically increasing. The aim of this study was to evaluatethe *in vitro* antifungal susceptibility to itraconazole (ITZ), posaconazole (POS), voriconazole (VOR) and amphotericin B (AMB) against *Aspergillus* spp. isolated from different sources.

Methods A total of 102 *Aspergillus* strains (63 of *A. fumigatus*, 19 of *A. oryzae*,16 of *A. tubingensis* and 4 of *A. niger*), identified phenotypically and molecularly by sequencing the β-tubulin gene, were tested. Isolates were divided into three groups: group I consisting of 29 strains from animals (i.e., 23 of *A. fumigatus* and 6 of *A. tubingensis*); group II comprising 37 isolates from nosocomial environment (i.e., 20 of *A. fumigatus*, 10 of *A. oryzae*, 4 of *A. tubingensis* and 3 of *A. niger*); and group III comprising 36 isolates from laying hen farms environment (i.e., 20 of *A. fumigatus*, 9 of *A. oryzae*, 6 of *A. tubingensis* and 1 of *A. niger*). The minimum inhibitory concentrations (MICs) were determined by the broth microdilution method according to the CLSI M38-A2 protocol and the isolates were considered resistant if they showed a MIC above the breakpoints proposed (>2 μg ml^{-1} for ITZ and VOR; >0.5 μg ml^{-1} for POS; and >2 μg ml^{-1} for AMB - Verweij et al., Drug Resist Updat 2009; Arendrup et al., Clin Microbiol Infect 2012).

Results VOR, ITZ and POS showed the highest activity against *Aspergillus* spp. strains, whereas AMB the lowest. Two strains of *A.* section *Nigri* (1 of *A. tubingensis* and 1 of *A. niger*) isolated from nosocomial environment scored resistant to ITZ (MIC = 64 μg ml^{-1}) and POS (MIC=1 μg ml^{-1}) and *A. tubingensis* also to AMB (MIC = 4 μg ml^{-1}). Resistance to AMB was detected in 19% (12/63) of *A. fumigatus* strains, in 25% (5/20) of *A.* section *Nigri* (i.e., 25% *A. tubingensis* and 25% *A. niger*) and in 89% (17/19) of *A. oryzae* regardless of the source of isolation.

Conclusion Based on these findings, the *Aspergillus* spp. herein tested were highly susceptible to azoles and less to AMB. The cross resistance to azoles registered in 1 *A. tubingensis* and 1 *A. niger* suggests that these strains may circulate in the nosocomial environment, whereas *Aspergillus* spp. strains, considering their low activity to AMB, might be widespread. The molecular mechanisms of azole or AMB resistance, which are poorly investigated in these *Aspergillus* species, need to be evaluated in future studies.

P024

Emergency resistance *in vitro* to anidulafungin for *C. parapsilosis* strains

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Objectives The incidence of *C. parapsilosis* complex has increased significantly in the last decades. Some studies have shown that it is the second *Candida* species most commonly isolated from blood cultures in Latin America. *C. parapsilosis* has emerged as an important nosocomial pathogen, causing invasive candidiasis in hospitalized patients in critical condition and in low-weight newborns, as well as, in immunocompromised individuals, transplant and patients receiving parenteral nutrition. These diseases show high rates of morbidity and mortality and are of difficult prevention and treatment. The echinocandins are recommended as the first-line treatment for invasive candidemia, however, these drugs have shown reduced activity *in vitro* and treatment failures against other *Candida* species. This study aimed to evaluate the *in vitro* induction of resistance to anidulafungin of different clinical isolates of *C. parapsilosis* considered susceptible to anidulafungin.

Table 1. Susceptibility *in vitro* of Candida parapsilosis against anidulafungin before and after induction of resistance.

Groups	*C. parapsilosis* (n)	Susceptibility Range (μg/ml)	Geometric Mean	N° (%) Isolates Into Categories[a]		
				S	I	R
ANF-S	30	0,03-2.0	0.64	30 (100)	-	-
ANF-R	30	0.06-8.0	2.4	12 (40.0)	4 (13.3)	14 (46.6)

[a]S=susceptible; I = Intermediate; R= resistant.

Methods The resistance was induced in the 30 clinical isolates of *C. parapsilosis* susceptible to anidulafungin. The cultures were seeded in tubes containing medium Yeast Nitrogen Base (YNB) and incubated at 30°C overnight. The inoculum was standardized in new crops YNB based on the turbidity measured using a spectrophotometer for a final absorbance of 0.1 (λ = 640 nm). The standardized cultures were incubated at 30°C and, after 10 hours anidulafungin was added to start the process of induction of resistance. Each isolate was challenged to grow at increasing concentrations of anidulafungin, and the initial concentration of drugs was 0.03 μg/ml and finished with 8 μg ml^{-1}. These tests were repeated doubling the concentration of the drug until the final concentration was eight times higher than the initial concentration. From the final culture, the cells were plated on Sabouraud dextrose agar and a single colony was considered resistant isolated. The isolates were maintained on distilled water with 10% glycerol under anidulafungin concentration of resistance (≥8 μg ml^{-1}) at −80°C.

Results From 30 isolates of *C. parapsilosis* which were initially susceptible, after exposition to anidulafungin, resulted at the end of the process analysis two groups: anidulafungin-susceptible (ANF-S) and anidulafungin-resistant (ANF-R). After 25 exposures to increasing concentrations of anidulafungin (63 days), we detected the first case of resistance. It is important to note, that from the concentration of 0.12 μg ml^{-1} the strains had a delay in their growth requiring more time for visualization of growth. After 60 exposures, 265 days after the start of induction of resistance, the susceptibility of anidulafungin was evaluated resulting in 14 (46.6%) resistant strains. Based on minimum inhibitory concentration (MIC) range, breakpoints and geometric mean of MICs, the resistance was significant, (MG = 2.4 μg ml^{-1}). On the other hand, when considering non-susceptible strains (intermediated or resistant) the increase was of 18 (59.9%) to ANF-R (Table 1). Significant statistical differences were observed by comparison between the ANF-S group against ANF-R ($P < 0.05$).

Conclusion This study shows that *C. paraspilosis* acquires resistance against anidulafungin after *in vitro* exposure; the impact of resistance of *C. parapsilosis* to anudulafungin requires attention to the susceptibility tests with this species.

P025

Identification and antifungal susceptibility of *Curvularia* strains isolated from human eye infections

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Objectives Four closely related *Curvularia* species, *C. australiensis*, *C. hawaiiensis*, *C. geniculata* and *C. spicifera* are emerging causative agents of human phaeohyphomycoses. The classical morphological examination and the increasingly used ITS-based molecular identification proved to be insufficient to discern these species, especially *C. australiensis* and *C. spicifera*. Aims of the study were to develop a reliable molecular identification method for these species and to examine their antifungal susceptibility.
Methods Thirty two strains originating from human infections, plants and soil were involved in the studies. In addition to the ITS region, applicability of the calmodulin, β-tubulin and translation elongation factor 1-α (*tef*) genes, as well as the nuclear ribosomal intergenic spacer (IGS) were tested to identify the four *Curvularia* species. The *in vitro* effect of 10 antifungal agents against the *Curvularia* isolates was also tested and the MIC_{50} and MIC_{90} values were determined in each case.
Results The ITS region was suitable only to discern *C. hawaiiensis* from the other species. Calmodulin, β-tubulin and *tef* genes were not enough variable for reliable identification. At the same time, the IGS region proved to be a good choice to distinguish all tested *Curvularia* species. Isolates of *C. spicifera* proved to be less susceptible to the tested antifungals than the other strains.
Conclusion Among the tested molecular markers, the nuclear ribosomal IGS region contained clearly distinctive species-specific motives. These discriminating motifs were determined using appropriate reference strains. Majority of the strains isolated from keratomycoses belonged to *C. spicifera* and *C. hawaiiensis*. Results of the *in vitro* antifungal susceptibility tests underline the importance of the correct species identification.

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P026

In vitro activity of echinocandins and azoles against of invasive isolates of *Candida* spp: multicenter study in Russia

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Objectives We investigated the *in vitro* activity of echinocandins and azoles against invasive isolates of Candida spp.
Methods Susceptibility testing of 421 Candida spp. isolated from blood and other sterile specimens to antifungal agent was performed by the CLSI the broth microdilution method (CLSI 2012). Isolates were collected from 9 hematological (Hem) centers (2003–2014) and 11 ICU (2009–2014).

Table. Comparative activity of antifungal agents against *Candida* spp.

Candida spp.	n	MIC50/MIC90/S (%)			
		Anidulafungin	Caspofungin	Fluconazole	Voriconazole
C. albicans	160	0.016/0.032/99.4	0.016/0.064/100	0.25/2/93.1	0.032/0.125/91.2
C. parapsilosis	108	0.125/0.5/97.2	0.25/0.5/97.2	0.5/16/82.4	0.032/0.25/88
C. tropicalis	35	0.016/0.032/100	0.008/0.064/100	0.125/2/97.1	0.032/0.064/97.1
C. krusei	34	0.032/0.5/88.2	0.125/1/73.5	16/>64/n.a.	0.125/1/79.4
C. glabrata	28	0.016/0.125/92.9	0.016/0.25/89.3	8/64/0	1/2/n.a.
C. guilliermondii	19	1/8/73.7	1/16/57.9	1/2/100	0.064/0.25/100
C. pelliculosa	15	0.016/0.032/100	0.016/0.064/100	1/2/100	0.125/0.125/100
C. lusitaniae	12	0.016/0.125/100	0.25/2/91.7	0.25/1/91.7	0.016/0.064/100
C. kefyr	6	0.008/0.032/100	0.008/0.125/100	0.25/1/100	0.032/0.064/100
C. lypolitica	4	0.032/0.5/100	0.016/0.25/100	0.5/1/100	0.032/0.5/100
All Candida spp	421	0.016/0.25/95.7	0.032/0.5/93.6	0.5/16/n.a.	0.032/0.25/n.a.

Results A total of 421 *Candida* spp. (374 from blood and 47 from other sterile specimens; 191 from Hem pts and 230 from ICU pts) were included. Species distribution was as follows: 38% (160) *C. albicans*, 26% (108) *C. parapsilosis*, 8% (35) *C. tropicalis*, 8% (34) *C. krusei*, 7% (28) *C. glabrata*, 4.5% (19) *C. guilliermondii*, 3.5% (15) *C. pelliculosa*, 3% (12) *C. lusitaniae*, 2% (10) other species. Prevalence of *C. albicans* was in ICU pts (44% in ICU versus 30% in HEM, $P = 0.003$), *C.krusei* in HEM pts (11.5% versus 5%, $P = 0.02$).

Comparative activity of anidulafungin, caspofungin, voriconazole and fluconazole (MIC50/MIC90/susceptible (S) %) against all Candida spp.is presented in table.
Conclusion *C. albicans* was the predominant *Candida* spp. especially in ICU pts. Among the predominant isolates, echinocandins had reduced activity against *C.guilliermondii* and some isolates of *C.parapsilosis*. Acquired resistance to azoles was for *C.parapsilosis* and *C. albicans*. Activity of azoles was less than echinocandins for *C.parapsilosis*. In all cases of invasive cansidiasis the susceptibility to azoles should be performed before the beginning of the therapy with these agents.

P027

Antifungal susceptibility patterns of cryptic species of *Candida glabrata* and *Candida parapsilosis* clades isolated from patients suffering from oral candidiasis

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Objectives To evaluate the antifungal susceptibility patterns of oral isolates of *Candida glabrata*,*Candida bracarensis* and *Candida nivariensis* (*Candida glabrata*clade), and *Candida parapsilosis*, *Candida orthopsilosis* and *Candida metapsilosis* (*Candida parapsilosis* clade) from patients suffering from oral candidiasis.
Methods A total of 212 clinical isolates recovered from patients suffering from oral candidiasis attending the Odontology clinics at UPV/EHU from 2003 to 2013 were analyzed. Isolates previously identified by conventional mycological methods as *Candida glabrata* (114 isolates) and *Candida parapsilosis* (98 isolates) were re-identified by multiplex PCR and PCR-RFLP, respectively. Some identifications were confirmed by the D1/D2 region of 26S rDNA gene sequencing. *In vitro* antifungal susceptibility testing were performed by disk diffussion method, using the CLSI M44-A protocol, for nystatin, miconazole, fluconazole and itraconazole against all the isolates. Microdilution method (CLSI M27-A3 document) was carried out for fluconazole.
Results The molecular techniques identified the 114 clinical isolates of *Candida glabrata* clade as *Candida glabrata sensu stricto*, and the 98

isolates of*Candida parapsilosis* clade as 95 *Candida parapsilosis sensu stricto*, one *Candida metapsilosis*, one *Candida orthopsilosis* and other one as *Candida pararugosa*. *Candida metapsilosis, Candida orthopsilosis* and*Candida pararugosa* were susceptible to all antifungal agents tested. None *Candida glabrata* showed resistance to miconazole or nystatin. However, seven and eight isolates were susceptible-dose-dependent to fluconazol and itraconazol, respectively. Moreover, 14 and three isolates were resistant to fluconazol and itraconazol, respectively. Only one fluconazole-resistant isolate was confirmed by microdilution method. There were no resistance to fluconazol, nystatin or itraconazole in *Candida parapsilosis*, nevertheless, four isolates presented dose-dependent-susceptibility to itraconazol. Moreover, 46 isolates were intermediate susceptible to miconazole, and one isolate was resistant to this drug.Six and three isolates with azole cross resistance were detected in *Candida glabrata* and *Candida parapsilosis*, respectively.

Conclusion Cryptic species *Candida bracarensis* and *Candida nivariensis* were not isolated from oral candidiasis samples. The presence of *Candida metapsilosis* and *Candida orthopsilosis* was very low. Nystatin was the most active antifungal agent. Fluconazole was very effective against *Candida parapsilosis* but its activity was poor against *Candida glabrata*.

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P028

In vitro activities of six antifungal drugs against *Exophiala* spp. isolated from dishwashers and railway sleepers in Turkey

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Background *Exophiala* spp. may cause cutaneous, subcutaneous or systemic infections in immunocompetent as well as immunocompromised individuals. Source of infections is thought to be environmental in most cases. Data on *in vitro* antifungal susceptibility profiles of *Exophiala* strains are rather limited. This study was undertaken to investigate the *in vitro* activities of antifungal drugs against *Exophiala* strains isolated from environmental sources in Turkey.

Methods A total of 81 *Exophiala* strains isolated from dishwashers and railway sleepers and identified at species level by internal transcribed spacer region (ITS) sequencing were included the study. *In vitro* antifungal susceptibility testing for fluconazole (FLU), voriconazole (VOR), posaconazole (POS), itraconazole (ITR), amphotericin B (AMB) and terbinafine (TER) was performed according to CLSI M38-A2 microdilution method with modifications. The microdilution plates were incubated at 25 °C and the incubation period was extended as needed for sufficient growth. Minimum inhibitory concentration (MIC, $\mu g\ ml^{-1}$) values were determined visually using MIC-2 endpoint (~50% inhibition compared to drug-free growth control) for azoles and terbinafine, and MIC-0 endpoint (complete inhibition of growth) for AMB. For each individual isolate, MICs were interpreted on two consecutive days after sufficient growth was achieved.

Results No growth was detected for any of the isolates at 24 h in microdilution plates. Sufficient growth was observed at 48 h for all strains tested and MIC readings were performed at 48 and 72 h, accordingly. MIC_{50} and MIC_{90} values, MIC ranges and geometric mean (GM) MICs at 48 and 72 h are shown in Tables 1 and 2, respectively.

Conclusions (1) MIC readings were interpretable at 48 h for all *Exophiala* strains tested.

(2) MIC values at 48 h were similar to those at 72 h for all species and drugs.

Table 1. MIC_{50}, MIC_{90}, MIC range and geometric mean (GM) MIC values at 48h

Species (n)	MIC (μg/ml)			
E. dermatitidis (n=45)	MIC_{50}	MIC_{90}	MIC range	GM MIC
Amphotericin B	1	2	0.06-2	0.86
Fluconazole	32	64	8->64	25.01
Itraconazole	0.25	1	≤0.015-1	0.22
Voriconazole	0.03	0.06	≤0.015-0.5	0.03
Posaconazole	0.06	0.5	≤0.03-0.5	0.09
Terbinafine	≤0.03	≤0.03	≤0.03-0.125	0.03
E. phaeomuriformis (n=27)				
Amphotericin B	0.5	1	0.03-2	0.53
Fluconazole	8	32	2->64	8.64
Itraconazole	0.125	0.5	≤0.015-0.5	0.13
Voriconazole	≤0.015	0.03	≤0.015-0.125	0.02
Posaconazole	≤0.03	0.125	≤0.03-0.25	0.06
Terbinafine	≤0.03	≤0.03	≤0.03-0.125	0.03
E. crusticola (n=8)				
Amphotericin B	-	-	0.125-1	0.55
Fluconazole	-	-	8->64	20.75
Itraconazole	-	-	≤0.015-0.25	0.05
Voriconazole	-	-	≤0.015-0.125	0.02
Posaconazole	-	-	≤0.03-0.125	0.04
Terbinafine	-	-	≤0.03	0.03
E. heteromorpha (n=1)				
Amphotericin B	-	-	2	-
Fluconazole	-	-	32	-
Itraconazole	-	-	0.25	-
Voriconazole	-	-	0.25	-
Posaconazole	-	-	0.06	-
Terbinafine	-	-	≤0.03	-
All strains (n=81)				
Amphotericin B	1	2	0.03-2	0.7
Fluconazole	16	32	2->64	17.28
Itraconazole	0.25	0.5	≤0.015-1	0.16
Voriconazole	≤0.015	0.06	≤0.015-0.25	0.03
Posaconazole	0.06	0.25	≤0.03-0.5	0.07
Terbinafine	≤0.03	≤0.03	≤0.03-0.125	0.03

Table 2. MIC_{50}, MIC_{90}, MIC range and geometric mean (GM) MIC values at 72h

Species (n)	MIC (μg/ml)			
E. dermatitidis (n=45)	MIC_{50}	MIC_{90}	MIC range	GM MIC
Amphotericin B	1	2	0.5-4	1.20
Fluconazole	32	64	8->64	37.91
Itraconazole	0.5	1	≤0.015-1	0.34
Voriconazole	0.06	0.125	≤0.015-0.5	0.06
Posaconazole	0.125	0.5	≤0.03-1	0.14
Terbinafine	≤0.03	≤0.03	≤0.03-0.5	0.03
E. phaeomuriformis (n=27)				
Amphotericin B	1	2	0.125-2	0.90
Fluconazole	8	>64	4->64	12.38
Itraconazole	0.25	1	≤0.015-1	0.24
Voriconazole	≤0.015	0.06	≤0.015-0.125	0.02
Posaconazole	0.125	0.5	≤0.03-0.5	0.11
Terbinafine	≤0.03	0.125	≤0.03-0.25	0.04
E. crusticola (n=8)				
Amphotericin B	-	-	0.125-2	0.77
Fluconazole	-	-	8->64	26.91
Itraconazole	-	-	≤0.015-0.5	0.11
Voriconazole	-	-	≤0.015-0.125	0.03
Posaconazole	-	-	≤0.03-0.125	0.07
Terbinafine	-	-	≤0.03	0.03
E. heteromorpha (n=1)				
Amphotericin B	-	-	2	-
Fluconazole	-	-	64	-
Itraconazole	-	-	0.25	-
Voriconazole	-	-	0.125	-
Posaconazole	-	-	0.25	-
Terbinafine	-	-	≤0.03	-
All strains (n=81)				
Amphotericin B	1	2	0.125-4	1.05
Fluconazole	32	64	4->64	25.40
Itraconazole	0.25	1	≤0.015-2	0.27
Voriconazole	0.03	0.125	≤0.015-0.5	0.04
Posaconazole	0.125	0.5	≤0.03-1	0.12
Terbinafine	≤0.03	≤0.03	≤0.03-0.5	0.03

(3) Among the tested antifungal drugs and in general, TER and VOR yielded highest *in vitro* activities, followed by POS, ITR, and AMB against all *Exophiala* spp. tested. These five antifungal agents were favorably active *in vitro* against tested strains.

(4) In contrast, FLU showed highest MICs with no adequate *in vitro* activity in general. Of specific note, FLU GM MICs against *E. phaeomuriformis* strains were relatively lower as compared to those against other *Exophiala* spp.

(5) Further studies are required to determine correlation of these *in vitro* results with clinical outcome.

P029

Correlation between broth microdilution and disk diffusion methods for antifungal susceptibility testing of voriconazole and fluconazole against *Candida* species

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Objectives Several yeast infections, especially candida infections, represent a significant health problem in patients at high risk of infection, leading to increased morbidity and mortality, greater healthcare costs and increased duration of hospitalisation.Antifungal drugs have been used extensively in prophylaxis, empirical therapy and treatment of such infections, studies demonstrate that antifungal resistance is relatively rare. *Candida* species have varying degrees of susceptibility to the frequently used antifungal drugs. For example, while *Candida krusei* is intrinsically resistant to fluconazole, *Candida glabrata* is less susceptible or has higher MICs than other *Candida* species, which makes the correct species identification and susceptibility tests really necessary. In this study, our aim was dual: (i) to evaluate the *in vitro* activities of two drugs with different mechanisms (voriconazole and fluconazole) of action against clinical *Candida* isolates and (ii) to assess the suitability of results of disk diffusion test for use as a screening test in mycology laboratories.
Methods A total of 210 *Candida* isolates were isolated from 89 blood, 82 urine, 28 respiratory tract and 11 soft tissue samples of patients in various departments of Istanbul University, Cerrahpasa Medical Faculty, between November 2011-November 2013 Isolates were identified by using conventional methods (germ tube formation, microscopic morphology in corn meal-Tween 80 agar) and additionally, through a commercial kit API 20C (Biomeriux, France). Isolates are prepared for MALDI-TOF analysis by using a direct on target extraction method as well. The spectra were analysed by the IVD VITEK MS V.2.0 and SARAMIS 4.12 RUO database Antifungal susceptibility testing was performed by reference broth microdilution (CLSI M27-A3) and disk diffusion methods (CLSI M44- A2).
Results Atotal of 210 species of *Candida* were identified and the distribution was as follows: 100 *C. albicans*, 43 *C. parapsilosis*, 25 *C. tropicalis, 23 C. glabrata, 7 C. krusei,* 6 *C kefyr,*5 *C lusitaniae,*1 *C. guilliermondii.* Non-Candida albicans species showed higher MICs for the two antifungal agents when compared with *C. albicans* isolates.For fluconazole, the categorical agreement between broth microdilution and disk diffusion was 91%, with 2 very major error (VMEs), 4 major error(MEs), and 12 minor errors. In addition, the best agreeement was determined for voriconazole with five VMEs. For VORI, MEs and minör errors were not observed,
Conclusion Disk diffusion is technically an easier method than broth microdilution and this method shows very good agreement with the reference method for fluconazole and voriconazole against All *Candida* isolates (91% and 97%, respectively)

P030

In vitro activity of amphotericin B by timed-kill curves against *Cryptococcus neoformans* isolated from HIV-infected patients with cryptococcal meningitis and implications in clinical practice

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Introduction Infections due to *Cryptococcus neoformans* cause severe disease, mostly in AIDS patients. Availablesusceptibility tests for *C. neoformans* are not useful to detect isolates that are not susceptible to antifungal agents such as amphotericin B. The usefulness of timed-kill curve (TKC) is to estimate amphotericin B (AMB) fungicidal activity. Although this method has been explored for *Cryptococcus* spp. isolates, few studies have evaluated the correlation between laboratory findings and clinical outcome.
Objectives The purpose of this study was to correlate AMB TKC for 27 isolates of *C. neoformans* with clinical aspects, prognosis and death in 27 HIV-infected patients with cryptococcal meningitis.
Methods Retrospective study, conducted at the Instituto de Infectologia Emílio Ribas and Instituto Adolfo Lutz with 27 HIV-infected patients with cryptococcal meningitis. All of them had cerebrospinal fluid (CSF)-positve culture for *C. neoformans* that was processed through the TKC procedures up to 72 hours of exposition time to 1 mg of amphotericin B. Strains were divided into two groups according to TKC results: Group A (TKC≤24 h) and Group B (TKC>24 h and/or regrowth). The clinical records of all patients were reviewed and the following variables were analyzed: presence of severe symptoms related to central nervous system (CNS) disease; fungal burdens in the first and 2 weeks CSF sample; failure in sterilization of CSF during induction phase; increased intracranial pressure (ICP); extra-neural Cryptococcosis and outcome. Statistical testing between groups was assessed by Mann-Whitney U test for continuous variables after verification of non-normal distribution by Shapiro-Wilk test. Fischer's exact test was used for categorical variables. For survival analysis, a Kaplan-Meier curve with Long-Rank test was used to determine the influence of variable. We assumed an IC of 95% and $\alpha < 0.05$.
Results Group A comprised 20 (74.1%) patients and Group B the remaining 7 (25.9%), including 1 patient whose strain was not inhibited by amphotericin B under the test conditions. We observed

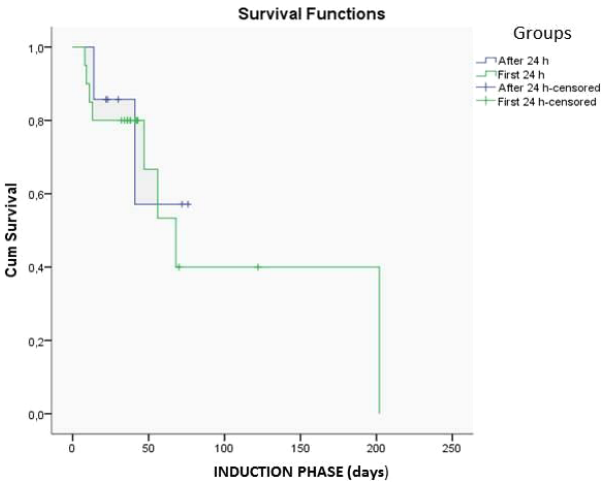

Figure 1

Table 1

Variables		Group A (TKc≤4h) N = 20	Group B (TKc>24h and/or regrowth) N = 7 (*)	P-value (IC 95% and α < 0,05)
Severe symptoms of CNS disease	Yes	6 (30%)	4 (57,1%)	0,305
	No	14 (70%)	3 (42,9%)	
Cryptococcal yeast counts (median and range) (cells/mL)	Initial	618 (1-11.520)	1.048 (0-3.680)	0,476
	2 weeks	84,5 (1-2.680)	420 (0-2.080)	-
Sterilization of CSF during induction treatment (days)	Yes	15 (75%)	4 (57,1%)	
	No	5 (25%)	3 (42,9%)	0,172
	Median	17	20	
	Range	(4-62)	(13-52)	
Increased Intracranial Pressure (cmH$_2$O)	Yes	16 (80%)	6 (100%) (**)	
	No	4 (20%)	0	0,234
	Median	39,15 (16.2-135)	74,25 (59.4-135)	
	Range			
Extra-neural Cryptococcosis	Yes	5 (25%)	3 (42,9%)	0,373
	No	15 (75%)	4 (57,1%)	
Induction phase (days)		33 (8-92)	40(7-76)	0,331
Shunt during hospitalization	Yes	5 (25%)	3 (42,9%)	0,373
	No	15 (75%)	4 (57,1%)	
Outcome	Death	8 (40%)	2 (28,6%)	0,590
	Clinical improvement	12 (60%)	5 (71,4%)	

*Includes one patient with strain not inhibited by TKmethod
**Missing data(1)

that patients from Group B presented more severe symptoms, ICP, and failure to sterilized CSF compared to Group A. Moreover, despite Group B presented a higher median of Cryptococcal yeast counts in the first CSF and with 2 weeks of induction treatment, there were no significant clinical finding correlated with TKC in the present study (Table 1). Despite patients from Group B presented worst clinical findings, they had a lower mortality. Overall, none of these aspects was significant and TKC >24 h was not associated with increased mortality in HIV-infected patients with cryptococcal meningitis (Fig. 1).

Conclusion We emphasized the difficulties in interpreting the results obtained from the susceptibility tests against *C. neoformans* and their real clinical meaning when trying to correlate them with the outcome. The TKC for amphotericin B is a promising tool for the prognosis of HIV-infected patients with cryptococcal meningitis, warrant future studies encompassing large number of patients.

P031

Species distribution and antifungal resistance profiles among Candida species isolated from invasive *Candida* infections in a Turkish university hospital

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Objectives Early and accurate diagnosis and appropriate antifungal therapy are essential for a successful treatment for patients with invasive *Candida* infections. Therefore, fast and reliable identification of pathogenic species is critical, since the sensitivity to antifungal agents varies depending on the species. In this study, our objective was to evaluate the distribution and the antifungal susceptibility profile of invasive *Candida* infections agents isolated from various clinical samples.

Methods A total of 210 *Candida* isolates were isolated from 89 blood, 82 urine, 28 respiratory tract and 11 soft tissue samples of patients in various departments of Istanbul University, Cerrahpasa Medical Faculty, between November 2011-November 2013. Isolates were identified by using conventional methods (germ tube formation, microscopic morphology in corn meal-Tween 80 agar) and additionally, through a commercial kit API 20C (Biomeriux, France) and matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF) Vitek MS system (bioMérieux, France). Isolates are prepared for MALDI-TOF MS system (bioMérieux, France) analysis by using a direct on target extraction method. The spectra were analyzed by the IVD VITEK MS V.2.0 database. *In vitro* antifungal susceptibility testing was determined using the CLSI microbroth dilution method M27 A3-S4. The tested drugs included; fluconazole (FLZ), voriconazole (VCZ), Itraconazole (ITZ), miconazole (MCZ), ketoconazole (KZ), flucytosine (FLU), caspofungin (CAS) and Amfoterisin B (AMB). *C. albicans* ATCC 10231, *C. parapsilosis* ATCC 22019 and *C. krusei* ATCC 6258 were included as control reference strains.

Table 1. Antifungal Susceptibility Testing Results of *Candida* strains

Candida species		*C. albicans* (n:100)	*C. parapsilosis* (n:43)	*C. tropicalis* (n: 25)	*C. glabrata* (n:23)	*C. krusei* (n: 7)	*C. lusitaniae* (n:5)	*C. kefyr* (n:6)	*C. guilliermondi* (n:1)
FLZ	MIC RANGE	0,06-1	0,06-8	0,06-64	0,125-64	-	0,125-8	0,06-0,5	8
	MIC50-MIC90	0,125-0,25	0,5-2	0,25-0,5	2-4		0,25*	0,06-0,25	
VCZ	MIC RANGE	0,06-0,25	0,06-0,25	0,06-16	0,06-1	0,06- 0,125	0,06-0,125	All 0,06	0,5
	MIC50-MIC90	0,06*	0,06-0,125	0,06-0,125	0,06-0,25	0,125*	0,06*		
ITZ	MIC RANGE	0,06-4	0,06-1	0,06-4	0,06-8	0,25-1	0,06-0,5	All 0,06	4
	MIC50-MIC90	0,125-2	0,25-0,25	0,125-0,5	0,5-2	0,5*	0,125-0,25		
KZ	MIC RANGE	0,06-1	0,06-0,5	0,06-2	0,06-2	0,125-0,5	0,06-0,125	All 0,06	1
	MIC50-MIC90	0,06-0,25	0,06-0,125	0,06-0,25	0,125-0,5	0,,5*	0,06*		
MCZ	MIC RANGE	0,06-4	0,06-8	0,06-4	0,06-0,25	0,25-1	0,06-0,5	All 0,06	8
	MIC50-MIC90	0,06-0,25	0,25-2	0,125-0,5	0,06-0,25	0,5*	0,06*		
AMB	MIC RANGE	0,125-4	0,25-4	0,25-4	0,25-4	0,5-2	0,125-1	1-32	0,5
	MIC50-MIC90	0,5-1	1-2	1-4	2-4	1-2	0,5-1	2-32	
FLU	MIC RANGE	0,06-0,5	0,06-0,5	0,06-0,5	0,06-0,25	0,125-2	0,06-8	0,06-1	0,06
	MIC50-MIC90	0,06-0,125	0,06-0,125	0,06-0,125	0,06-0,125	0,5-1	0,5-8	0,06-0,5	
CAS	MIC RANGE	0,06-0,125	0,06-2	0,06-0,125	0,06-0,125	0,06-0,125	0,06-0,25	All 0,06	0,06
	MIC50-MIC90	0,06*	0,06-1	0,06*	0,06-0,125	0,06*	0,06*		

* : MIC50=MIC90, FLZ: fluconazole,VCZ: voriconazole, ITZ: Itraconazole, MCZ: miconazole, KZ: ketoconazole, FLU:flucytosine, CAS: caspofungin, AMB: Amfoterisin B

Results Species distribution was as follows: 100 *C. albicans*, 43 *C. parapsilosis*, 25 *C. tropicalis*, *23 C. glabrata*, *7 C. krusei*, 6 *C kefyr*, 5C *lusitaniae*, 1 *C. guilliermondii*. The minimum inhibitory concentrations (MIC) distribution range, MIC50 and MIC 90 values of isolated *Candida* species are shown in Table 1. All of *C. albicans*, *C. tropicalis*, *C. glabrata*, *C. krusei*, and *C. guilliermondii* isolates are susceptible to caspofungin, whereas all *C. albicans* isolates were determined as susceptible to fluconazole. Resistance to fluconazole was determined in 2 *C. glabrata*, 1 *C. tropicalis* and 2 *C. parapsilosis* isolates, as resistance to voriconazole was detected in only 1 *C. tropicalis* isolate. All *C. glabrata* isolates except two were dose-dependent susceptible to fluconazole.

Conclusion *C. parapsilosis* was the most isolated species among non-*albicans Candida* species. Non- *albicans Candida* species showed higher MICs for the antifungal agents when compared with *C. albicans* isolates. Voriconazole and caspofungin were the most active antifungal in all *Candida* species.

P032

Antifungal and cytotoxicity activities of *Hamamelis virginiana* glycolic extract for *Candida* species.

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Objectives The aim of this study was to evaluate the antifungal activity and cytotoxicity of *Hamamelis virginiana* glycolic extract for *Candida* spp. standard strains in planktonic growth and monotypic biofilm.

Methods The species tested were: *C. albicans* (ATCC 36801), *C. dubliniensis* (ATCC MYA 646), *C. glabrata* (ATCC 9030), *C.*

Table 1: MIC and MMC values (mg/mL) of *Hamamelis virginiana* glycolic extract for *Candida* spp.

Planktonic growth		
Species	MIC (mg/mL)	MFC (mg/mL)
C. albicans	1.56	6.25
C. glabrata	1.56	6.25
C. dubliniensis	0.78	3.12
C. guilliermondii	0.39	3.12
C. tropicalis	0.39	3.12
C. krusei	0.19	3.12

MIC = minimum inhibitory concentration MFC = minimum fungicidal concentration

Table 2: Extract concentration (mg/mL), reduction percentage and *p* value after treatment with *Hamamelis virginiana* glycolic extract for 5 minutes.

Biofilm growth			
Species	Extract concentration (mg/mL)	Reduction percentage	*p* value
C. albicans	50	63.13%	<0.0001
C. glabrata	25	84.81%	<0.0001
C. dubliniensis	50	99.35%	<0.0001
C. guilliermondii	50	41.77%	0.0305
C. tropicalis	25	85.37%	<0.0001
C. krusei	50	54.81%	0.0064

guilliermondii (ATCC 6260), *C. krusei* (ATCC 6258) and *C. tropicalis* (ATCC 13803). Antimicrobial activity of *Hamamelis virginiana* glycolic extract was evaluated by microdilution method based on Clinical and Laboratory Standards Institute (M7-A6 and M27-A2) in order to obtain the minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC). Biofilms were formed in microtiter plates with suspensions of 10^7 cells ml^{-1} and incubation at 37°C under stirring for 48 hours. Then the biofilms were treated with the extract for 5 min with different concentrations for each species. Saline was used as control. Biofilms were disaggregated and the decimal dilutions were plated on Sabouraud Dextrose agar by drop-plate technique, and incubated at 37 °C for 48 hours. Next, the counting of colony forming units (CFU ml^{-1}) was performed. Data were compared by ANOVA and Tukey test ($p \leq 0.05$). Cytotoxicity analyses was performed using the MTT colorimetric method after contact between the extract and mouse macrophages cultures (RAW 264.7) for 5 min. The absorbance of the wells was read on microplate reader with wavelength of 570 nm. The optical densities (OD) obtained were converted into percentage of cell viability.

Results MIC and MFC values demonstrated greater resistance for *C. albicans* and *C. glabrata* to the concentrations tested in planktonic growth, MIC = 1.5 mg ml^{-1} and CFM = 6.2 mg ml^{-1} for both species (Table 1). For biofilm growth, the extract was required in higher concentrations to obtain a statistically significant reduction (Table 2). The extract was not cytotoxic until the concentration of 5%, when the cell viability was 50.58%.

Conclusions It was concluded that *H. virginiana* glycolic extract showed antifungal activity against the strains tested, however higher concentrations were required against biofilm growth. The extract was not cytotoxic until the highest concentrations, that showed fungicidal activity against biofilm growth.

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Aspergillus fumigatus susceptibility testing by CLSI and EUCAST Guidelines

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Introduction Fungal infections caused by filamentous fungi, especially *Aspergillus fumigatus,* are rising due to the increased number of patients with immunosuppression or affected by disorders like AIDS, diabetes, immunological diseases and COPD. It can cause invasive, chronic and saprophytic forms of disease. While invasive aspergillosis (IA), the most common and feared clinical presentation, affects immunosuppressed patients, chronic and saprophytic aspergillosis complicates those with pre-existing pulmonary or sinus disease. For their treatment, azoles are used and voriconazole is the first-line drug. However, in recent years, *Aspergillus fumigatus* acquired azoles resistance has been increasingly reported, especially in some European countries, Asia and Middle East. In contrast the resistance seems not to affect the USA.

On both sides of the Atlantic Ocean, two antifungal susceptibility testing guidelines for molds, using broth methodology, are used. In USA the M38-A2 protocol, proposed by the Clinical and Laboratory Standard Institute (CLSI) and in Europe the Antifungal Subcommittee of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) protocol. The objective of our work was to test *Aspergillus fumigatus* strains isolated in patients from Centro Hospitalar S. João, Porto, Portugal employing both methods and verify their susceptibilities.

Material and methods A total 49 *Aspergillus fumigatus* strains, isolated mostly from respiratory samples, between 2010 and 2014, belonging to 47 patients (21 men and 26 women) with a mean age of 55.7 years old (6 months-83 years-old), were tested using the M38-A2 and EUCAST protocols. The azoles itraconozale, voriconazole and posaconazole, the echinocandins, caspofungin and anidulafungin and the polyenes amphotericin B and liposomal amphotericin B were the antifungal drugs used. We strictly followed the methodological recommendations: for the azoles and amphotericin we read the minimal inhibitory concentration (MIC) and for the echinocandins the minimum effective concentration (MEC). Also to establish the presence or absence of non-wild-type strains among our isolates we considered the proposed epidemiological cutoff values (ECV).

Results MICs and MECs ranges for each drug/protocol combination, the range of the essential agreement (EA) between the methods and the EA, considering one/two/three dilutions, are depicted in the table 1. While for most antifungals the EAs were high, for posaconazole and caspofungin they were quite lower.

Considering the ECV, only one strain of *Aspergillus fumigatus* exhibited a MIC of 4 mg l^{-1} for both amphotericin formulations using EUCAST protocol. However, for the same strain, the MIC was 2 mg l^{-1} employing CLSI guidelines. Another strain revealed high MICs to the azoles by both methods (itra/CLSI>16 mg l^{-1}; itra/EUCAST>8 mg l^{-1}; vori/CLSI = 4 mg/L; vori/EUCAST = 4 mg l^{-1}; posa/CLSI = 2 mg l^{-1}; posa/EUCAST = 1 mg l^{-1}).

Conclusions Both methods exhibited good performance, except for posaconazole and caspofungin EAs assessment. More studies are likely to provide insights on this point. Furthermore, the difference of MIC value for amphotericin between EUCAST and CLSI protocols should be investigated. For the azoles resistant strain, both methods

Table1. MIC/MEC and EA ranges for each drug/protocol combination

	Amphotericin B		Liposomal Amphotericin B		Itraconazole		Voriconazole		Posaconazole		Anidulafungin		Caspofungin	
	CLSI	EUCAST	CLSI	EUCAST	CLSI	EUCAST	CLSI	EUCAST	CLSI	EUCAST	CLSI	EUCAST	CLSI	EUCAST
MIC/MEC Ranges (mg/L)	0.5-2	0.5-4	0.25-1	0.25-4	0.25->16	0.25->8	0.12-4	0.12->4	0.031-2	0.031-1	0.031->0.063	0.031->0.125	0.12->0.25	0.063->0.25
EA Ranges	(1+) to (2+)		(1+) to (3+)		(1+) to (3+)		(1+) to (2+)		(1+) to (3+)		(1+) to (1+)		(1+) to (1+)	
EA% n (mg/L)	98		90		100		98		92		100		81	
EA% n (mg/L)	2		8		0		2		18		0		19	
EA% n (mg/L)	0		2		0		0		10		0		0	

performed well. The mechanism of resistance involved in this strain will be investigated.

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P034

In vitro susceptibility profiles of eight antifungal drugs against clinical and environmental species of Phaeoacremonium

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Background Laboratory diagnosis and treatment of *Phaeoacremonium* infection is problematic and not well defined. The purpose of this study was *in vitro* testing of a large collection of *Phaeoacremonium* strains to eight antifungal drugs, including the novel triazole isavuconazole.

Methods *Phaeoacremonium* strains ($n = 43$) from different geographical regions were obtained from the collection of the CBS-KNAW Fungal Biodiversity Centre, Utrecht, the Netherlands, comprising *P. parasiticum* ($n = 16$), *P. krajdenii* ($n = 11$), *P. venezuelense* ($n = 6$), *P. inflatipes* ($n = 3$), *P. griseorubrum* ($n = 3$), *P. rubrigenum* ($n = 2$), and *P. alvesii* ($n = 2$). The fungi were identified to the species level by sequencing of the internal transcribed spacer regions of the rDNA region and partial β-tubulin gene (19, 30–32). *In vitro* susceptibility was determined as described in CLSI document M38-A2 for eight drugs.

Results All strains had low MICs of amphotericin B, voriconazole, posaconazole and isavuconazole. Less active drugs were fluconazole, itraconazole, anidulafungin, and caspofungin. The widest ranges and the highest MICs were seen for fluconazole and itraconazole (range16- > 64 mg l^{-1} and 4–16 mg l, respectively). The echinocandins had poor activity against both clinical and environmental strains. The geometric mean MICs were as follow in increasing order; voriconazole (0.35 mg l^{-1}), posaconazole (0.37 mg l^{-1}), amphotericin B (0.4 mg l^{-1}), isavuconazole (1.1 mg l^{-1}, anidulafungin (4.8 mg l^{-1}), caspofungin (6.9 mg l^{-1}), itraconazole (14.8 mg l^{-1}), and fluconazole (43.5 mg l^{-1}).

Conclusions Amphotericin B, voriconazole, posaconazole and isavuconazole were active against *Phaeoacremonium*. In contrast all isolates were resistant to itraconazole and fluconazole. This susceptibility profile is similar to other melanized fungi.

P035

Inhibition of *Pythium insidiosum* growth by methylene blue

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Pythium insidiosum is an oomycete pathogenic which causes pythiosis, a life-threatening disease. The infection usually occurs via direct contact with contaminated fresh water. Although the animal cases have been reported in various countries, the vast majority of human cases were described only in Thailand. Treatment using antimycotic agents is ineffective due to the fact that *P. insidiosum* is not true fungi. Radical surgery debridement is the most used. However, the high rate of re-occurrence is seen. Methylene blue is an aromatic chemical compound. Because of low toxicity toward human, it has been used for various clinical purposes including methemoglobinemia, malaria treatment as well as inactivation of some microorganisms. This study was initiated to assess the *in vitro* inhibitory effect of methylene blue on the hyphae growth of 10 clinically isolated *P. insidiosum* comparing with growth rate on media without methylene blue. Various concentrations of methylene blue ranging from 0.1 to 20 μg ml^{-1} were tested. The result revealed that inhibition rate was dose-dependent. Significant inhibition rates (85.6 ± 3.9 and 76.5 ± 8.9%) were observed in TSA media supplemented with methylene blue at concentration of 20 and 10 μg ml^{-1}, respectively. In contrast, 0.1 μg ml^{-1} of methylene blue concentrations showed minimal effect on the growth rate while approximately 30% inhibition was observed in 1 and 2 μg ml^{-1} of methylene blue. In addition, slightly decrease of inhibition effect was observed when tested on blood agar. The results demonstrated that methylene blue had promising inhibitory effect to the growth of *P. insidiosum*. This agent may be a good candidate for treatment either alone or in combination with surgical therapy.

P036

Environmental isolates of *Hortaea werneckii*: enzymatic activity and antifungal susceptibility profile

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Objectives To evaluate the antifungal activity and virulence factors through the enzymatic activity of four environmental isolates of *Hortaea werneckii* that were isolated from different substrates in Brazil due to the pathogenic nature of the fungus. *Hortaea werneckii* is the etiologic agent of *Tinea nigra*, an uncommon superficial dermatomycosis.

Methods Two strains were isolated from the salt marsh macrophyte *Spartina alterniflora* in Southern Brazil and two were isolated in Northern Brazil, one from the bromeliad *Portea leptantha* and another from the marine zoanthid *Palythoa caribaeorum*. The identification of the strains was confirmed by sequencing of the ITS region or D1/D2 domains of the LSU rRNA gene using the universal primers ITS1/ITS4 and NL1/NL4, respectively. The activities of the following enzymes were tested: proteinase (gelatinase, albuminase and keratinase), esterase, lipase, phospholipase, DNase and urease. The samples were inoculated into tubes (for evaluation of keratinase and urease) or at the center of a Petri dish (for the other enzymes). Tests were performed in triplicate and incubated at 30°C for 14 days. The enzymatic activity (Pz) in the plates was determined by the evaluation of the degradation halo, and the strains were classified as: non-producers (Pz = 1.0) and producers (PZ<1).The minimal inhibitory concentration (MIC) of 8 antifungal agents was evaluated in microdilution methods in 98 well plates according to the CLSI protocol M27-A3 adapted for *H. werneckii* (30°C/6 days) and the minimal fungicidal concentration (MFC) were evaluated in Sabouraud dextrose broth medium in tubes from the wells with no growth.

Results Lipase (Pz medium = 0.365 ± 0.070), esterase (Pz medium = 0.695 ± 0.074) and urease were positive for all isolates, while one strain was positive for gelatinase (Pz = 0.63). All isolates were negative for albuminase, keratinase, phospholipase and DNase. The MIC/MFC medium obtained were (μg ml^{-1}): tioconazole (0.073/0.210), posaconazole (0.088/0.105), ketoconazole (0.148/0.210), voriconazole (0.176/0.297), terbinafine (0.177/0.177), itraconazole (0.25/1.0), amphotericin B (2.0/4.0) and fluconazole (>64/>64). All isolates were considered resistant to fluconazole.

Conclusion The environmental isolates of *Hortaea werneckii* produced some enzymes important as potential virulence factors. The isolates were resistant to fluconazole, and presented low sensitivity to amphotericin B, showing that resistance to these antifungal agents can be found in environmental isolates of *H. werneckii*, therefore clinical administration of fluconazole should be avoided, and the use of amphotericin B in severe cases of infection by this fungus should be viewed with care.

P037

Antifungal susceptibility testing by micro-broth dilution of rare *Candida* species isolated from blood.-a study from a tertiary care center in South India

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Objective Candida species that are closely related to *C. haemulonii* are emerging sources of infection in India. These species show variable patterns of susceptibility to amphotericin B,echinocandins and azole antifungal agents. We performed the antifungal susceptibility testing to see the resistance pattern of these unusual candida species and to compare the pattern among the common species.
Methods The microbroth dilution testing was done using CLSI guidelines M27-A3. The list of drugs were amphotericin B, caspofungin, micafungin, anidulafungin, fluconazole, voriconazole, posaconazole and,itraconazole.
Results We got 8 rare blood Candida isolates (apart from the common isolates *C.albicans* 9, *C.tropicalis* 20, *and C.parapsilosis* 9) of which *C.haemulonii* was 3 *C. famata* was 3 which were identified by Bio-merieux VITEK -2 compaq and 2 isolates were confirmed by gene sequencing as *C. auris*. All cases of fungemia occurred in patients with severe underlying diseases who had central venous catheters. Four patients had undergone surgery during the hospital stay. One patient was on steroids, one was on chemotherapy for laryngeal carcinoma. Other patients were on long term broad spectrum antibiotics for various bacterial infections.

Among the eight patients from whom rare Candida was isolated four patients improved on treatment while four succumbed to the infection.

The MIC ranges for the rare candida isolates were 32–64 μg ml^{-1} for fluconazole,0.06–0.5 μg ml^{-1} for voriconazole, 0.125–1.0 μg ml^{-1} for Itraconazole, .06–0.25 μg ml^{-1} for posaconazole, 0.5–4 μg ml^{-1} for AmphotericinB, 0.125–0.25 μg ml^{-1} for Caspofungin, 0.03–0.125 μg ml^{-1} for Micafungin and 0.06–0.125 μg ml^{-1} for anidulafungin
Conclusion It is very important to speciate the Candida isolated from blood due to the emergence of newer Candia species which have high MIC's to fluconazole and amphotericin B.

The treatment should be started with one of the echinocandins in order to prevent mortality from fungemia in the ICU setting.

P038

Clinical, Epidemiological and laboratory findings amoung women with recurrent vulvovaginal candidiasis (RVVC) in an university hospital, Brazil

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Introduction Vulvovaginal candidiasis (VVC) is a frequent disease affecting more than 75% of all women at least once in their lifetime. Approximately 50% of these women will also suffer a single recurrence. A minority of women, 5–8% experience recurrent vulvovaginal candidiasis (RVVC) which is defined as >3 episodes per year. Several risk factors are known, such as pregnancy, immunosuppression, antibiotic use, AIDS, Innate immunodeficiency and diabetes may increase the susceptibility to VVC.
Objective The study undertaken to evaluate clinical, epidemiological and laboratory findings among patients with RVVC, and to analyze the frequency of different *Candida* species and their susceptibility profile.
Methods Fifty five women with RVVC from the infectious diseases service from Hospital Universitário do Oeste do Paraná (Cascavel/PR/Brazil) were studied from January 2012- December 2013. Vaginal swabs were collected and cultured on Sabouraud's dextrose agar at 35°C and isolates were identified by morphological and biochemical tests. Antifungal susceptibility of the *Candida spp*. isolates were determined by E-test against amphotericin B (AMB), itraconazole (TTZ), fluconazole (FCZ), and voriconazole (VCZ), according to CLSI M27 A2 and M27 A3 breakpoints. The following risk factors were studied: pregnancy, hormonal contraception, corticosteroids, sexually transmitted diseases (STD), HIV infection, diabetes and the previous use of antibiotics and antifungal agents. Probable external risk factors such as vaginal douches, use of female intimate soap and use of synthetic clothes were also investigated. Statistical analysis was performed using the x^2 test and $p < 0.05$ was considered statically significant.
Results Fifty five women were selected with possibility to collect vaginal specimens although only 24 had positive cultures. The mean age was 32 years (range 15–51 year) and 64, 8% were married and 90, 7% live in urban area. Only 29% of the women in this study gain >3 minimum wage (U$738). The following risk factors were statistic significant by x^2 test: vaginal douche ($P < 0.0001$); pregnancy ($P < 0.0001$), DST ($P < 0.0001$), corticosteroids ($P < 0.0001$) and recent antibiotic use ($P = 0.0029$). The most common species isolated in this cohort was *C. albicans* (67, 85%), *C. tropicalis* (10, 71%), *C. parapsilosis* (7, 14%), *C. guillermondi* (7, 14%) and *C. glabrata* (7, 14%). All the strains were susceptible to AMB, 92, 5% susceptible to FCZ, 76, 9% susceptible to ITZ and 87, 5% to VCZ.

P039

Invasive candidiasis in Pakistan: Predominant Species and Antifungal Resistance (2010–2014)

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Objectives This study reports descriptive epidemiologic data of 753 *Candida spp* isolated from blood and other sterile specimens in Pakistan (2010–2014), including species identification and antifungal susceptibility against fluconazole, itraconazole, voriconazole, caspofungin, micafungin, anidulafungin and amphotericin. Antifungal resistance data was compared with a previous invasive candidiasis survey (2006–2009) published from same center.
Methods The study was conducted at the Aga Khan Hospital Clinical Laboratories from Jan 2010 to Oct 2014. The isolates were identified by biochemical characteristics (API 20C AUX® BioMérieux, France) and microscopic morphology. Antifungal susceptibilities were determined by disc diffusion and MICs on broth microdilution (BMD) as recommended by CLSI M44-A2 (2009) and M27-A4 (2012), respectively.
Results *Candida tropicalis* remained the most common species (36%), followed by *C. albicans* (25%) and *C. parapsilosis* (22%) respectively. 11% of the infections were due to uncommon *Candida* species. Isolation rate of C. glabrata and C. krusei increased from 12.7% (2006–2009) to 15.2%. Resistance to fluconazole emerged in *C. albicans* (8.0%), *C. tropicalis* (3.1%)and *C. parapsilosis* (4.6%). When compared to previous survey (2006–2009), overall fluconazole resistance increased from 2% to 7.7%.
Conclusion Increasing rates of fluconazole resistant species as well as emergence of fluconazole resistance in previously sensitive *Candida*

species is concerning. There is an urgent need to develop expertise in both identification and susceptibility testing in laboratories in Pakistan. Ongoing surveillance is essential to monitor resistance and any emerging species.

P040

Species distribution and In Vitro and Antifungal Susceptibility in Clinically Important *Candida* Isolated from Oral Cavity

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Objectives Aims of this study were: (a) To evaluated species distribution of isolates of *Candida* sampled from the oral cavity; (b) To determined the *in vitro* susceptibilities to four antifungal agents (nystatin, fluconazole, itraconazole, voriconazole) in Brazilian strains of *Candida* spp. isolated from oral cavity.
Methods The species-level identification was performed according to standard procedures, i.e., germ tube test, colony morphology on Sabouraud Dextrose Agar (Difco, France) and Chromogenic CHROMagar *Candida* medium (Difco, USA)**.** The *in vitro* activities of nystatin, fluconazol, itraconazol, voriconazol against 28 oral *Candida* were tested by disk diffusion. Reference antifungal susceptibility testing was performed exactly as described in Clinical and Laboratory Standards Institute Guidelines (CLSI), document M44-A2. Two quality control (QC) strains, *C. albicans* (ATTCC 90028) and *C. parapsilosis* (ATCC 22019) were tested each time a set of clinical isolates was evaluated. Isolates were classified by zone interpretative criteria according to current CLSI Guidelines, document M44-S2. Collected data were analyzed using GraphPad 5.0 for Windows. *Candida* species identification and *in vitro* susceptibilities were done by the Oral Microbiology and Pathology Testing Service Laboratory at the School of Dentistry of the Serra Gaúcha Faculty. This study was conducted according to the principles expressed in the Declaration of Helsinki. It was approved by CONEP (National Committee for Research Ethics).
Results In this group study, *C. albicans* was isolated with the highest prevalence followed by *C. tropicalis* and *C. krusei* with isolation rates of 72%, 25% and 3% respectively. All clinical isolates *Candida albicans* were fully susceptible for all antifungals. Of the non-albicans *Candida* species isolates, all *C. tropicalis* strains showed an intermediate susceptibility to fluconazole. This isolates were recovered from patients with prior antifungal exposure to nystatin and triazole-derive agent (fluconazole). As expected, resistance to fluconazole was displayed in *C. krusei* isolate. For nystatin, eight clinical isolates were resistant, including one *C. krusei* and seven *C. tropicalis*. None of the *Candida* species in our study showed cross-resistance to itraconazole and voriconazole.
Conclusion As expected, we found that the resistance rates for *Candida* non-*albicans* were higher than those for *C. albicans,* in particular *C. tropicalis* and *C. krusei*, which displayed resistance rates of 25% and 3%, respectively. Our study indicates that despite the widespread use of fluconazole in Brazil, resistance to this antifungal drug among yeasts isolates from oral cavity is still a fairly restricted phenomenon (3%) with 97% of all yeasts isolated being susceptible or susceptible dose-dependent.

P041

Susceptibility testing of *Malassezia furfur, Malassezia globosa* and *Malassezia sympodialis*. Comparison of disk diffusion and broth microdilution methods

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Objectives The aims of this study were to evaluate the antifungal susceptibility of three *Malassezia* species against fluconazole, voriconazole, ketoconazole, itraconazole, miconazole, amphotericin B and terbinafine using both broth microdilution and agar diffusion methods, and to compare both methodologies using a model of linear regression analysis and categorical agreement.
Methods A total of 50 *Malassezia* isolates, including 30 *M. furfur*, 10 *M. globosa* and 10 *M. sympodialis*, were studied. Yeasts were identified by PCR-RFLP. The broth microdilution method was performed as described in CLSI M27-A3 reference document and disk diffusion test was performed on Mueller-Hinton agar using tablets and disks. To support optimal growth of these yeasts, culture media were supplemented. In order to correlate both methods, linear regression analysis was performed and categorical agreement was determined.
Results Both supplemented media allowed optimal growth of *Malassezia* yeasts and the results were read at 72 h of incubation at 32°C. Results of broth microdilution and agar diffusion testsobteined for the 50 isolates studied are summarized in Table 1.

Malassezia yeasts were highly susceptible to itraconazole, ketoconazole and voriconazole. In contrast, a significant dispersion of values was observed for fluconazole, miconazol and terbinafine.

The strongest linear association was observed for FLC. The relationship between variables for KTZ and ITZ was not linear. For the other drugs, coefficients obtained were not enough to conclude that a linear relationship exists between MIC and inhibition zone diameters variables.

The highest categorical agreement between both methods was observed for itraconazole and voriconazole and the lowest for amphotericin and fluconazole.

Table 2 indicates results of linear correlation analysis and categorical agreements when comparing both methodologies.
Conclusions Susceptibility profiles of *Malassezia* yeasts against FLC, AMB and TRB were variable and this is very important considering that are commonly used drugs. On the other hand, isolates were inhibited at low concentrations of ITZ, KTZ and VCZ, showing to be highly effective drugs.

Although modifications made to both methodologies allowed obtaining susceptibility data for *Malassezia* yeasts, both methods cannot be associated through linear correlation model. When analysis

Table 1. Susceptibility results obtained by broth microdilution and disk diffusion methods for the 50 isolates tested.

Table 1.	Fluconazole	Ketoconazole	Itraconazole	Miconazole	Voriconazole	Amphotericin B	Terbinafine
Broth microdilution (µg/ml) MIC range	0.5-128	0.03-0.125	0.03-0.125	0.125-32	0.03-0.05	0.5-16	0.125-32
GM	5.74	0.03	0.03	2.55	0.07	1.94	1.04
Mode	4	0.03	0.03	4	0.03	2	0.25
MIC_{50}	4	0.03	0.03	2	0.06	2	1
MIC_{90}	32	0.06	0.03	16	0.25	8	16
Disk diffusion (mm)							
AM tablet/disk	27.1/ 22.8	32.1	27.8	14.7	41.2	18.7	20.3
SD tablet/disk	10.6 / 11.5	3.89	3.28	4.21	5.74	1.85	3.04
Range tablet/disk	9-52 / 7-46	24-46	20-42	9-32	29-55	15-26	13-33

MIC: minimal inhibitory concentration; GM: geometric mean, MIC_{50} and MIC_{90}: MICs values at which 50% and 90% of the isolates were inhibited, mm: millimeters; AM: arithmetic mean; SD: standard deviation.

Table 2. Linear correlation coefficients and categorical agreements obtained from comparing broth microdilution and disk diffusion methods

	Linear regression analysis			
Antifungal drug	Pearson correlation coefficient (ρ)	*p*-value	Coefficient of *determination* R^2	Categorical agreement (%)
Fluconazole disk	-0.71	< 0,0001	0.48	40.0
Fluconazole tablet	-0.70	< 0,0001	0.47	58.0
Ketoconazole	-0.2	0.1627	N/A	N/A
Itraconazole	-0.02	0.8785	N/A	89.6
Miconazole	-0.52	0.0001	0.27	N/A
Voriconazole	-0.47	0.0016	0.2	87.5
Amphotericin B	-0.45	0.0012	0.21	35.0
Terbinafine	-0.34	0.022	0.12	N/A

N/A: no applicable

was applied, the determination coefficient values obtained were very low for all drugs test, indicating that based on the diameter of the inhibition zone there is a low probability of minimal concentration inhibitory prediction using a linear model.

The agar diffusion method is a low-cost, fast and simple technique that is easily applicable in routine clinical laboratories. The diameters of the inhibition zones were clearly defined when modifications were made in agar diffusion method for *Malassezia* susceptibility study. However, not a good correlation between this technique and the CLSI reference method was obtained for evaluating antifungal susceptibility of *Malassezia* yeasts. This highlights the need for to develop a reference protocol for *Malassezia* susceptibility testing in order to obtain comparable results by reproducible methods.

P042

Agreement of the direct antifungal susceptibility testing from positive blood culture bottles with conventional method for *Candida* species

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Objective Early availability of antifungal susceptibilities can ensure early institution of targeted therapy in candidemia which can improve patient outcomes. This study prospectively determines agreement between direct testing of antifungal susceptibilities from blood culture bottles by disk diffusion and E-test strips compared to standardized susceptibility testing methods allowing susceptibility results to be available 1–2 days earlier.

Methods A total of 104 blood cultures with different *Candida* species (28% *C. albicans*, 27% *C. parapsilosis*, 26% *C. tropicalis*, etc.) between Jan 2012 and May 2013 were evaluated for agreement of fluconazole, voriconazole and amphotericin susceptibility results by disk diffusion. Agreement in MICs by E-test was determined for fluconazole (21 isolates), voriconazole (28 isolates), amphotericin (29 isolates) and caspofungin (29 isolates).

Results Kappa scores for categorical agreement were highest for fluconazole by disk (0.902, SE = 0.076) and E-test (1.00, SE = 0.218) and amphotericin by disk (1.00, SE = 0.098). Pearson correlation (r) of zone diameters was strongest for fluconazole (0.69) and amphotericin (0.70), moderate for voriconazole (0.60); and strongest for fluconazole (0.94) and caspofungin (0.88) MICs. However moderate correlation of amphotericin MICs with zone diameters (-0.42) precludes the use of amphotericin disk for susceptibility testing. There were no very major errors; however there was 1 (1%) major and 5 (4.8%) minor errors on disk diffusion and 4 (13.3%) minor errors on E-test.

Conclusion Thus, antifungal disk diffusion directly from blood culture bottles is a rapid and easy method for fluconazole and voriconazole susceptibility testing for timely tailoring of candidemia therapy.

P044

Absence of FKS1 and FKS2 mutations in Caspofungin resistant *C. glabrata* isolates from candidemia patients in Delhi, India

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Objectives *Candida glabrata* is the second leading cause of candidemia in the India. Due to the decreased susceptibility of *C. glabrata* to fluconazole and cross-resistance to other azoles, echinocandins are introduced for therapy in patients with *C. glabrata* candidemia. Recently, many reports have emerged with increasing number of echinocandin resistant *C. glabrata* isolates. Resistance to echinocandins is attributed to alteration in the subunits of 1,3-β-D glucan synthase enzyme encoded by three genes (*FKS1*, *FKS2* and *FKS3)*. The present study aimed to screen the *FKS1* and *FKS2* hotspot region for mutation in the caspofungin resistant (MIC ≥0.5 μg ml^{-1}) Indian *C. glabrata* isolates.

Methods A total of 36 *Candida glabrata* isolates originating mainly from candidemia (n = 33, 92%) and bronchoalveolar lavage (n = 3, 8%) of individual patients in Delhi, India were analyzed. All isolates were preliminarily identified based on colony colour on CHROMagar™ Candida medium and morphology on rice Tween 80 agar. Further all of the isolates were molecularly characterized by amplification and sequencing of ITS and D1/D2 regions and by Matrix Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS). Antifungal susceptibility testing (AFST) was performed for azoles, amphotericin B and echinocandins with broth microdilution method (CLSI M27-A3). Isolates with elevated caspofungin MICs ≥ 0.5 μg ml^{-1} were subjected to sequencing of *FKS1* and *FKS2* hotspot regions for mutation analysis.

Results All isolates developed a pink color on CHROMagar[TRADEMARK] Candida medium and showed ovoid to elongated budding yeast cells occurring singly or in pairs. Further ITS and D1/D2 sequences of all the isolates showed ≥99% homology with *C. glabrata* in GenBank. The MALDI-TOF yielded a score values of >2 for all *C. glabrata*. AFST showed that among azoles, posaconazole had excellent activity (MIC_{90}, 0.25 μg ml^{-1}) followed by itraconazole and voriconazole (MIC_{90}, 0.5 μg ml^{-1}). No resistance to AMB (MIC range, 0.25–1 μg ml^{-1}) was observed. Among echinocandins, 24 (66.6%) blood isolates were found to be resistant to caspofungin (MIC, ≥0.5 μg ml^{-1}), whereas 3 fell in susceptible dose dependent (MIC, 0.25 μg ml^{-1}). The remaing 9 isolates had caspofungin MIC range 0.06–0.125 μg ml^{-1}. However, all isolates were highly susceptible to other two echinocandins, micafungin (MIC_{90}, 0.015 μg ml^{-1}) and anidulafungin (MIC_{90}, 0.125 μg ml^{-1}). No mutation was seen in both *FKS1* and *FKS2* hotspot regions in any of the resistant (≥0.5 μg ml^{-1}) *C. glabrata* isolates.

Conclusions The study is first to report the screening of *FKS1* and *FKS2* hotspot regions in Indian *C. glabrata* isolates. No mutation was observed in any of the hotspot region in caspofungin resistant *C. glabrata* isolates (0.5–1 μg ml^{-1}) warranting studies on larger number of *C. glabrata* isolates to determine true prevalence of caspofungin resistant isolates harboring *FKS1* and *FKS2* mutations.

P045

Aspergillus fumigatus resistance surveillance in a tertiary teaching Hospital

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Aspergillus spp. are opportunistic fungal pathogens responsible for high mortality rates, especially in immunocompromised patients, mainly neutropenic hosts who become infected after inhaling conidia dispersed in the air. Primary therapy for invasive aspergillosis has been largely limited to amphotericin B (AMB) and the triazole compounds itraconazole (ITZ) and voriconazole (VRZ). As the use of AMB has been limited by its toxic side effects, the azole derivate VRZ is been considered the first line therapy on aspergillosis treatment. *A.fumigatus*, the most common specie causing aspergillosis, has been investigated in several countries because of the emergence of azole resistant strains. The University Hospital of Campinas (HC-UNICAMP) is a tertiary care teaching institution where many high-risk patients are susceptible to opportunistic infectious agents such as the *Aspergillus* genus. In our institution, until now, there are no reports of *A.fumigatus* multi azole resistance. However, the monitoring of the possible emergence of resistant strains is needed. In this context, this study aimed to assess the profile of susceptibility to antifungal agents for clinical *A.fumigatus* isolated from patients with aspergillosis assisted at the HC-UNICAMP. Hundred and seventy-two clinical isolates, from 75 patients (2 to 6 from each patient) were evaluated searching for resistant isolates. Minimal Inhibitory Concentration (MIC) for amphotericin B (AMB); itraconazole (ITZ); voriconazole (VRZ); 5-flucytosine (5FC); miconazole (MCZ), fluconazole (FCZ) and the Minimal Effective Concentration (MEC) for micafungin (MCF); and caspofungin (CPF) were determined by microdilution method as recommended by the *Clinical and Laboratory Standards Institute* (CLSI, 2008) document M38-A2, with minor changes. Molecular analyses allowed to confirm the identification of all *A.fumigatus* isolates. The MIC ranges (in $\mu g\ ml^{-1}$) were: AMB: 0.25 to 8; 5FC:0.25 to 64; FCZ: 2 to >64; ITZ: 0.25 to 4; VRZ: 0.25 to 8 and MCZ: 0.06 to 4. The MEC ranges (in $\mu g\ ml^{-1}$) were $\leq$ 0.015 to 0.06 for MCF and 0.06 to 0.5 for CPF. Ten isolates from 9 patients showed high MICs for VRZ and; or ITZ (1 only for ITZ, 7 only for VRZ and 2 for ITZ and VRZ). Now, additional studies involving the search of mutations are being performed using the *cyp*51A gene sequencing method. Accurate and reliable species identification are important for appropriate patient treatment, management, and development of hospital infection control policies. Besides that, monitoring of the possible emergence of resistant strains is extremely important due to high mortality of azole-resistant aspergillosis that can approach 90%.

P046

Antifungal activity of aldimines (Schiff bases) against *Histoplasma capsulatum*

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Objectives *Histoplasma capsulatum* var. *capsulatum* is the causative agent of histoplasmosis, a systemic mycosis endemic in extensive areas of the Americas. Clinical manifestations of histoplasmosis are influenced on both by the intensity of exposure to fungal microconidia, as well by the host immunity, and range from asymptomatic to disseminated infections. Treatment depends on clinical manifestations, and intolerance to treatment and drug-drug interactions may occur and lead to failure in antifungal therapy. The restricted alternatives and the therapeutic complications for treating histoplasmosis have encouraged the search for new compounds with activity against *H. capsulatum*. Aldimines, also known as Schiff bases, are compounds containing an azomethine group (-CH=N-) formed by the condensation of a primary amine with a carbonyl compound. These compounds have been shown to exhibit a broad range of biological activities, including antifungal, antibacterial, antimalarial, antiproliferative, anti-inflammatory, antiviral, and antipyretic properties. This research aimed to determine the minimal inhibitory concentration of aldiminines against *Histoplasma capsulatum*, the causative agent of histoplasmosis.
Methods It was determined the minimum inhibitory concentration (MIC) of twenty-four aldimines against five strains of *H. capsulatum* var. *capsulatum*. The tests were performed according modifications of the M 38-A2 protocol drawn up by the Clinical and Laboratory Standards Institute (CLSI). Results: There was wide variation in the antifungal activity of the tested aldimines. The geometric means of the MIC varied from 15.06 $\mu g\ ml^{-1}$ (3976 strain) to 8.23 $\mu g\ ml^{-1}$ (3962 strain). In general, the aldimines 9, 13, 15 and 20 were the most activite against the tested strains, with MIC values that ranged from 2 and 8 $\mu g\ ml^{-1}$.
Conclusion Some of the tested aldimines have exhibited excellent *in vitro* activity against *H. capsulatum*, making them potential candidates to future studies on their *in vivo* activity.
Financial support: CAPES, CNPq, FAPEMIG

P047

Antifungal activity of allylimines alone and in combination with amphotericin B against *Cryptococcus gattii* strains

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Objectives Cryptococcosis is an invasive mycosis of global occurrence, mainly caused by *Cryptococcus neoformans* and *C. gattii* and affects patients both immunocompromised as immunocompetent. Its treatment may be complicated by the toxicity of the drugs used and the emergence of resistant strains. The discovery of new antifungal compounds and the combination therapy are important tools for the effective treatment of fungal infections. The allylimines exhibit similar structures to the antifungal group of allylamines, containing one double bond next to the nitrogen atom present in the molecule.
Methods In the present work we evaluated the *in vitro* antifungal activity of three allylimines (A1, A2, A3) alone and in combination with amphotericin B (AMB) against 12 *Cryptococcus gattii* strains. The compounds were evaluated for minimum inhibitory concentration (MIC), minimal fungicidal concentration (MFC) and the checkerboard assay was used to determinate the fractional inhibitory concentration index (FIC).
Results All strains were susceptible to the tested compounds with MIC values between 2.3 and 7.5 $\mu g\ ml^{-1}$ and MFC values between 4.8 and 65.8 $\mu g\ ml^{-1}$. For most of the tested strains the interaction of A1 and A2 with AMB was indifferent ($0.5 > FIC \leq 4$). The compound A3, however, was able to interact synergistically with AMB ($FIC << \leq 0.5$) for one-third of the *C. gattii* strains tested. Although the allylimines A1 and A2 had the best MIC and MFC values, A3 showed better interaction with AMB against the strains tested.
Conclusions Considering the results obtained in this study these allylimines may be considered promising antifungal agents and further studies will be done in our research group.
Financial support: CAPES, CNPq, FAPEMIG.

P048

Comparative genomic and transcriptomic analysis unveil novel features of antifungal resistance in the human pathogen *Candida glabrata*

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Background and objectives Fungal infections caused by *Candida glabrata* are associated to high rates of morbidity and mortality. An alarming increase in the incidence of infections caused by *C.glabrata* has been reported in the last years, in part, due to the emergence of strains resistant to azoles and echinocandins. In this work the genome sequences of a *C.glabrata* clinical isolate (named FFUL887) resistant to voriconazole and fluconazole (MICS of 4 and >64 mg l^{-1}, respectively) and tolerant to caspofungin (MIC of 0.5 mg l^{-1}) and of the reference strain CBS182, more susceptible to the above-referred drugs (MICs of 1, 16 and 0.06 mg l^{-1}, respectively), were compared. Although much work has been gathered on the elucidation of the molecular mechanisms of resistance to antifungals in *C.glabrata*, little is still known on the genetic adaptive responses that occur at the genomic level, a knowledge which is crucial to fully understand how this pathogenic yeast develops acquired antifungal resistance. The results herein obtained also contributed to further elucidate mechanisms of adaptation of *C. glabrata* to the human host advancing current understanding of the pathophysiology of this yeast.
Results and Conclusions The genomic sequence determined for the FFUL887 isolate includes 12.29 Mb, corresponding to 99.1% of the total genome size estimated by flow cytometry. Around 80 000 SNPs were identified, 10 000 of them corresponding to missense mutations occurring in the coding sequence of 3200 genes, corresponding to roughly 60% of the predicted *C.glabrata* ORFeome. No mutations had been found in the sequence of FFUL887 *ERG11* gene indicating that the mechanism by which this isolate acquired resistance to azoles is, apparently, not related with target modification. Around 115 proteins previously associated with drug resistance in *C.glabrata* were found to harbour mutations in the FFUL887 genome. Among these was the CgPDR1 gene, encoding the most important transcriptional regulator involved in the control of drug resistance in *C. glabrata*, which harboured a mutation not previously described. Using a transcriptomic analysis it was possible to confirm that the FFUL887 isolate over-expresses several described targets of the CgPdr1 transcription factor including the drug efflux pumps CgCdr1, CgPhd1, CgQdr2 and CgTpo3, all previously demonstrated to contribute for azole resistance in C. glabrata. These observations, together with phenotypic data, suggest that the herein uncovered mutation is a new gain-of-function mutation of CgPdr1. The influence exerted by this GOF mutation in the overall C. glabrata transcriptional regulatory network of controlled by CgPdr1 will be discussed, combining the information gathered in the genomic and in the transcriptomic analyses performed. The evolution of the non-coding genome of C. glabrata that was observed to occur between the CBS182 and FFUL887 isolates will also be discussed. Results from genomics and transcriptomics show extensive alteration of the expression and coding sequence of genes related with adhesion, metabolism of alternative carbon sources and stress response, confirming that these functions, essential for maximal adaptation to the human host, are under a strong selective pressure. These results are consistent with the increased adhesion, stress resilience and diversified metabolic repertoire of the FFUL887 strain.

P049

Identification and determination of Drug Resistant *Candida* species isolated from hospital acquired infections

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Objectives Nowadays use of antifungal drugs group Azoles is crescent as well as the number of drug resistant yeast and also frequency of Candida infections. The aim of this study is isolation and identification of Candida species causing hospital acquired infections and study of antifungal resistance.
Methods Specimens were collected from patients with approved nosocomial infections in the *Urmia* educational hospital, *Urmia, Iran*. Primary tests including direct examination and culture were performed. Differential cultures and molecular test: A molecular diagnostic method was used for differentiation and identification. Antifungal sensitivities were studied by using the Disk diffusion and Micro dilution methods. For the analysis of Candida drug resistance we focused on erg 3 gene.
Results Total of sixty isolates (23.4%) were obtained including *Candida albicans* 37 (61.6%), *C. krusei* and *C. glabrata* 7 (11.6%) each, *C. dubliniensis* 5 (8.3%) and *C. tropicalis* 4 (6.6%). Antifungal sensitivity analysis resulted *C. albicans* was not considerably resistant to amphotericin B comparing other Candida species in Disk diffusion method. Our findings of Micro dilution method confirmed the above results.
Conclusion In spite of our findings that show no considerable drug resistance in Candida isolates, monitoring of antifungal resistant Candidaspecies causing hospital acquired infections can be important.

P050

Modified response to antifungals in chromoblastomycosis agents with the inhibition of melanin biosynthesis

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Objectives To evaluate the correlation between the presence of melanin in chromoblastomycosis agents and their *in vitro* response to antifungals.
Methods Five isolates (3 *Fonsecaea* sp., 1 *Phialophora verrucosa* and 1 *Cladophialophora carrionii*) were inoculated in potato dextrose medium with and without the addition of 16 μg ml^{-1} of tricyclazole (melanin biosynthesis inhibitor). The tubes were incubated at 30°C for 14 days, and the conidia suspension were counted in a Neubauer chamber, and the final inoculums were standardized to 3–8 x 10^4 conidia ml^{-1}. The minimal inhibitory concentration (MIC) of six antifungal agents (itraconazole, ketoconazole, posaconazole, voriconazole, terbinafine and amphotericin B) were evaluated by the microdilution method in 98-well plates according to the CLSI protocol M38-A2, and the minimal fungicidal concentration (MFC) were evaluated in Sabouraud dextrose broth medium in tubes from the wells with no growth. The MIC and MFC tests were also done in media with or without tricyclazole. The MIC and MFC of isolates were analyzed in relation to the biological difference of their values between tests with or without the addition of tricyclazole, considering that there was a biological difference whenever the difference was at least two wells up or down.
Results The geometric mean of MIC (with - without tricyclazole)/MFC (with - without tricyclazole) for each species was (μg ml^{-1}): *F. pedrosoi* (0.25–0.25/2.24 to >16); *P. verrucosa* (0.097–0.28/0.70–1.59); *C. carrionii* (0.17–0.25/4–0.39). The MFCs for *F. pedrosoi* strains were higher in media without tricyclazole. For *C. carrionii*, the azolic antifungals obtained higher MFCs for the strain cultivated in medium with tricyclazole. *P. verrucosa* had higher MFCs of voriconazole and terbinafine in media without tricyclazole, but MFCs of itraconazole and ketoconazole were higher in media with tricyclazole.

Conclusion There was no difference between MICs of strains with their natural melanin and the same strains with melanin biosynthesis inhibited by the addition of tricyclazole in the media. On the other hand, MFCs showed differences. The presence of melanin in *F. pedrosoi* reduced the efficacy of the antifungal agents, with the melanized strains less sensitive. The non-melanized *C. carrionii* was less sensitive, while results for *P. verrucosa* depended on the antifungal agent. These results suggest that the correlation between melanin production and the *in vitro* response to antifungal agents depends on the chromoblastomycosis agent. More strains have to be tested to confirm this hypothesis.

P051

Antifungal susceptibility of the *Aspergillus* species by CLSI reference methods

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Objectives *Aspergillus* species cause many infections especially in immunocompromised patients. Generally, *Aspergillus* infections cannot be diagnosed early due to the lack of reliable and easy diagnostic tests. However, early diagnosis and initiation of an effective treatment is required in order to get good clinical outcomes. Since resistance to these drugs has been seen in patients, susceptibility testing may assist with determining the appropriate drug for treatment and respective epidemiology. Antifungal susceptibility tests should be performed and interpreted for an effective treatment. The present study evaluated species distribution of Aspergillus isolates which were isolated in our hospital and their antifungal susceptibility results obtained by microdilution method.
Methods A total of 33 *Aspergillus* isolates were recovered from clinical specimens received at Cerrahpasa medical faculty. Identification of each strain was performed using conventional mycological techniques. Minimum inhibitory concentration (MIC) of various antifungal agents (amphotericin B, posaconazole, itraconazole and voriconazole) were determined by CLSI M38-A2 document. In brief to induce conidium the isolates were grown on potato dextrose agar (PDA) slants at 35 °C for 2–7 days before testing. The suspensions of conidial inocula were prepared from 2 to 7 days old cultures grown on PDA slants. The conidia were mixed in sterile saline with 0.05% tween 20. The turbidity of the cell suspension was adjusted by spectrophotometry to an optical density of 0.09 to 0.13 for *Aspergillus* spp. Microplates were prepared as described in document and they were incubated at 35 °C for 48 h. Two CLSI quality control strains, *C. parapsilosis* ATCC 22019 and *C. krusei* ATCC 6258, were tested in the same manner.

Table 1.Antifungal Susceptibilities Testing Results of *Aspergillus* strains

Aspergillus spp (n)	Amphotericin B(µg/ml) MIC range	Vorikonazol (µg/ml) MIC range	Posaconazole(µg/ml) MIC range	itraconazole µg/ml) MIC range
A. fumigatus (n=16)	32-0,5	0,125-1	0,06-2	0,125-2
A. flavus (n=11)	1-4	0,06-0,5	0,06-1	0,125-0,5
A. niger (n=3)	0,125-2	0,125-0,5	0,125-0,5	0,125-0,25
A. terreus (n=1)	2	0,25	0,5	0,5
A.candidus(n=1)	4	0,125	0.06	1
A.sydowii(n=1)	0,5	0,125	0,5	0,25

MIC ran: minimum inhibitory concentration range

Results A total of 33 *Aspergillus* isolates were identified as 16 *A. fumigatus*, 11 *A. flavus*, 3 *A. niger*, 1 *A. terreus*, 1 *A.candidus*,1 *A sydowii*. We determined that voriconazole was the most effective antifungal agent againist all isolates. While 33 *Aspergillus* isolates (100%) were sensitive to posaconazole, itraconazole and voriconazole with the microdilution method, one *A.fumigatus* was resistant to Amphotericin B. In vitroantifungal susceptibility results of voriconazole,itraconazole, amphotericin B, and posaconazole were shown Table 1.
Conclusion Among four antifungals tested, the MIC levels obtained for voriconazole were lower compared to posaconazole, itraconazole, amphotericin B. In conclusion, given the toxic effects of amphotericin B, voriconazole may be alternative antifungals that can be used by the clinicians. Well-designed clinical studies are necessary to assist clinicians in choosing the best antifungal agents.

P052

Genotypic variability and antifungal susceptibility of *Candida* spp. isolated from hospital surfaces and hands of healthcare professionals

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Objectives *Candida spp.* are responsible for 90–95% of hematogenous fungal infections. In Brazil and Latin America, *C. albicans* is the most common specie, followed by *C. parapsilosis* and *C. tropicalis*. Infections caused by *Candida spp*. may have their origin in exogenous sources, transmitted to patients via contaminated infusions, biomedical devices or even by the hands of the hospital staff members. Molecular biology techniques such as Randomly Amplified Polymorphic DNA (RAPD) can show that the strains found in anatomical sites or abiotic surfaces have the same pattern genome.Moreover, in the last decades it has been observed increasing the number of yeasts isolated from hospital environment resistant to antifungals. Thus, the aim of this study was to determine the susceptibility to antifungals and intraspecies similarity among isolates of different hospital surfaces and hands of healthcare professionals.
Methods The study was conducted with 25 isolates of *Candida* spp.: 5 strains of *C. albicans* and 5 strains of *C. parapsilosis* isolated from hospital surfaces. 5 strains of *C. albicans*, 5 strains of *C. parapsilosis* and 5 strains of *C. tropicalis* isolated from hands of healthcare professionals. Professionals and surfaces belonged to intensive care units. The minimal inhibitory concentration (MIC) was determined to voriconazole (VOR), fluconazole (FLZ), amphotericin B (AMB) and micafungin (MFG) according to M27-A3 of the Clinical and Laboratory Standards Institute (CLSI). To determine the intra-species similarity, 3 primers were used: P4 (5′-AAGAGCCCGT-3′), OPA-18

Table 1. Antifungal susceptibility of Candida spp. isolated from hospital surface and hands of healthcare professionals by the CLSI method

Species		Antifungals									
		Voriconazole			Fluconazole			Micafungin		Amphotericin B	
		S (%)	DDS (%)	R (%)	S (%)	DDS (%)	R (%)	S (%)	R (%)	S (%)	R (%)
C. albicans	Hospital surface	100	-	-	100	-	-	100	-	80	20
	Hands	60	20	20	80	20	-	80	20	80	20
C. parapsilosis	Hospital surface	100	-	-	100	-	-	20	80	100	-
	Hands	100	-	-	100	-	-	20	80	100	-
C. tropicalis	Hands	-	40	60	100	-	0	80	20	100	-

S: susceptible; DDS: dose-dependent susceptible; R: resistant

(5′AGCTGACCGT3′) and OPE-18 (5′GGACTGCAGA 3′′). RAPD profiles were analyzed using BioNumerics software version 4.6. The study was approved by the Ethics in research involving human subjects, CAAE 0448.0.093.000-11 protocol.

Results In relation to susceptibility testing (Table 1), it is important to highlight that *C. parapsilosis* showed 80% of MFG resistance. *C. albicans* and *C. tropicalis* showed reduced susceptibility to VOR, and resistence of the AMB was observed for *C. albicans* (20%). All amplifications revealed distinct polymorphic bands. Genetic distances between each of the isolates were calculated and cluster analysis was used to generate a dendrogram showing relationships between them. The analysis of all primers showed similarity greater than 80% between strains of hands and hospital surfaces for intraspecies.

Conclusion Our work shows that, healthy people and hospital surfaces may be colonized by different species yeast. Furthermore, the strains studied had relative resistance to antifungal drugs most frequently used in clinical practice. Finally, there was a high similarity between samples from hands (hospital staff members) and surfaces, providing an infection risk to susceptible individuals. Healthy people working in hospitals can carry yeasts on their hands with the same potential virulence, and which therefore offer the same risk of infection. This information should be considered when preventive measures are established. Attention to the colonization of hands and surfaces should not be restricted to high-risk units such as NICUs, but should also include other sections of hospitals.

P053

Molecular genetic analysis of the cyp51a gene in azole resistant *Aspergillus* spp

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Azole drugs are the first line of therapy against *Aspergillus* spp. Resistance to azole antifungal drugs has been associated with treatment failure and deaths in patients with aspergillosis. Multi-azole resistance increases mortality rate of patients with invasive aspergillosis to 88% in comparison with 30–50% mortality of patients infected by susceptible strains. Reduction of the susceptibility to azole is mainly triggered by point mutation in the 14α-sterol demethylase (*cyp51A*) gene, which is the target enzyme for the drug. Nowadays, about 40 point mutations in *cyp51A* are identified. However, not all of them are accounted for the drug-resistant phenotype.

The aim of the study is a molecular genetic analysis of the *cyp51A* nucleotide sequence for azole-resistant pathogenic strains *Aspergillus* spp.

Materials and methods The nucleotide sequences of pathogenic *Aspergillus* spp.*cyp51A* were aligned with reference sequence from GenBank database (http://www.ncbi.nlm.nih.gov/genbank/). Optimal oligonucleotides sequences were selected for amplification and sequencing of *Aspergillus* spp.*cyp51A*. Specificity of the oligonucleotides was verified by BLAST algorithm (http://www.ncbi.nlm.nih.gov/blast/).

All *Aspergillus* isolates were identified by sequencing of internal transcribed spacer region (ITS) and fraction of the β-tubulin gene. Antifungal susceptibility testing was carried out according to the EUCAST microdilution method.

Results To date, we obtained 6 resistant clinical *Aspergillus* spp isolates. The molecular idenification of the isolates by sequencing of ITS and β-tubulin gene revealed that isolates belong to the following species *A.terreus, A.flavus, A.niger* and*A.calidoustus*. According to the EUCAST, *A.terreus* and *A.flavus* were resistant to voriconazole, *A.niger* and *A.flavus* were resistant to ketoconazole, *A.flavus* and *A.calidoustus* were resistant to voriconazole and itraconazole. The nucleotide sequence analysis of the *cyp51A* gene showed some point mutations in azole-resistant strains.

Conclusion Azole resistance has been described worldwise. Itraconazole resistance is common in developed countries (Canada, India, China and the United States are considered to be the leading countries). However, currently used antifungal susceptibility tests are laborious and not sensitive enough. Therefore, implementation of modern molecular biology techniques may allow identification of antimycotics-resistant *Aspergillus* spp. directly from the sample. The study of the mutations in *cyp51A*will help to determine molecular genetic characteristics of azole resistant *Aspergillus*isolates that may lead to development of original system for testing antimycotics-resistant *Aspergillus* spp.

P054

Virulence factors and antifungal susceptibilities of *Candida* species isolated from urinary system

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Objectives Although improvements have been made in many diseases in paralel with medical field in recent years, many fungi kinds particularly *Candida* type yeasts have become opportunist pathogens that could be encountered frequently as an infection agent; they have become most frequently isolated fungal pathogens in particularly nosocomial urinary tract infections (UTI). Moreover, the importance of virulence factors concerning *Candida* spp. as well as defence systems of host is reported in studies to clarify the pathogenesis of *Candida* infections and develop new drugs against *Candida* spp. In this study, it was aimed to research the susceptibilities of antifungal and virulence factors of *Candida* spp. isolated from UTI.

Methods A total of 83 *Candida* isolates were isolated from urine samples of patients in various departments of Istanbul University, Cerrahpasa Medical Faculty, between November 2011-November 2013. Isolates were identified by using conventional methods and additionally, through a commercial kit API 20C (Biomeriux, France) and MALDI-TOF [Vitek MS system (bioMérieux, France)] was utilised for the non-albicans *Candida* spp.While agar plage technique with egg yolk was used to determine phospholipase activity, medium containing 1%bovine serum albumin was employed to determine acid proteinase activity from virulence factors and biofilm presence was analyzed with modified microplate method. *In vitro* antifungal susceptibility testing was determined using the CLSI broth microdilution method M27 A3-S4.

Results The origins were described as following: 50 *C. albicans*, 10 *C. glabrata*, 10 *C. tropicalis*, 5 *C. parapsilosis*, 5 *C. lusitaniae*, 2 *C. kefyr*, 1 *C. krusei*. The number of strains with positive proteolytic activity was 51, *C. albicans* was determined in 41(50%), *C. tropicalis* in 5 (6%), *C. parapsilosis* in 4 (5%) and *C. kefyr* in 1. Phospholipase activity was determined as negative in all strains except for *C. albicans* whereas it was identified as positive in 34 (41%) *C. albicans*. Biofilm formations were established in 11 strains in total as 8 (9.6%) *C. tropicalis*, 2 (2.5%) *C. glabrata* and 1 *C. lusitaniae*. When examined in terms of antifungal susceptibility, it was found that all *C. albicans* and *C. parapsilosis* strains were susceptible to fluconazole, voriconazole and caspofungin. One of the strain of *C. tropicalis* was resistance to fluconazole and voriconazole, on the other hand, other all *C. tropicalis* were determined to be susceptible to fluconazole, voriconazole and caspofungin. All *C. glabrata* were susceptible to caspofungins and their MIK interval was 0.5–32 μg ml^{-1} and MIK_{50} ve MIK_{90} values were identified as 4.

Conclusion The presence of virulence factors has gained importance in studies conducted to clarify pathogenesis of infections developed by *Candida* and develop new drugs. In the study, higher proteinase and phospholipase enzyme determination compared to other species

in *C. albicans* made us think that these two hydrolytic enzymes play an important role in the pathogenesis of *C. albicans* and they could be virulence factors. In addition, the necessity has been understood that antifungal susceptibility tests should be performed to define the type for proper treatment of UTIs caused by *Candida* types and resistance ratios should be determined.

P055

Azole susceptibility and resistance in *Fusarium* spp

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Objectives *Fusarium* species are among the emerging agents of superficial, locally invasive and systemic infections in humans.. The large genus *Fusarium* contains many species with either saprophytic or plant pathogenic life styles and is capable of forming a plethora of mycotoxins. An important problem in dealing with *Fusarium* is its high intrinsic resistance to most antifungal compounds applied either in clinical or agricultural settings. In this paper we address the antifungal susceptibility of clinically relevant *Fusarium* species to current antifungal drugs and examine the role of the target genes, cytochrome P450 sterol 14a-demethylase (CYP51) paralogs, in azole resistance.

Methods Many species within the genus *Fusarium* that were recognized based on morphological characters, have proved to be species complexes, with little to no morphological differences, rather than single species. Most of the identified opportunistic *Fusarium* pathogens belong to the *F. solani* species complex (FSSC), the *F. oxysporum* species complex (FOSC), or the *F. fujikuroi* species complex (FFSC). Less frequently encountered are members of the *F. incarnatum-equiseti* (FIESC), *F. dimerum* (FDSC), or *F. chlamydosporum* species complexes (FCSC) or species like *F. sporotrichioides*. Based on multi-locus sequencing, isolates can be identified to the species level. We used published antifungal susceptibility (AFST) as well as AFST data from strains identified within our laboratory to create an overview of susceptibility and resistance.

Polyene antifungals like amphotericin B and azoles like voriconazole act by targeting the ergosterol pathway. Whole genome sequencing data and specific sequencing of the CYP51 paralogs and promoter regions in a set of *F. proliferatum* and *F. verticillioides* strains and several relatives with known AFST profiles are compared. Experimental evolution under low and high levels of the agricultural azole Carbendazim yields additional mutant strains with increased resistance.

Results and Conclusions The AFST data confirm the high level of antifungal resistance in *Fusarium* species to most available antifungal drugs, but also visualizes that for many species we lack solid data. *Fusarium species* prove to exceptionally contain three CYP51 paralogs and these genes have a high level of variation between and within species, suggesting selection for variation and high levels of resistance. Differences in the AFST profiles emphasize the need for at least species level identification for adequate treatment.

P056

In vitro antifungal activity of echinocandins against 162 invasive isolates of *Candida* spp

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Objectives The echinocandins anidulafungin (ANF), caspofungin (CSF), micafungin (MCF) represent a new antifungal group with potent activity against *Candida* species. As non-competitive inhibitors of beta-1,3-glucan synthase they have distinct mechanisms of action and target the fungal cell wall and thus present an alternative for the tratment of candidiasis. A commercially prepared dried colorimetric microdilution panel (Sensititre Yeast One, TREK Diagnostic Systems, Cleveland, OH, USA) is able to determine the susceptibility of echinocandins and demonstrated excellent corelation with the reference method M27-A3 from the Clinical Laboratory Standards Institute (CLSI). Resistance rates were determined by recently revised CLSI antifungal breakpoints. The aim of this study is to evaluate the *in vitro* activity of ANF, CSF and MCF against 162 clinically significant *Candida* strains isolated between June 2012 and December 2014 by Yeast One colorimetric microdilution panel.

Methods A total of 162 *Candida* strains were isolated from bloodcultures ($n = 69$), cultures of urine samples of symptomatic patients ($n = 55$), cerebrospinal fluid ($n = 1$), corneal abscess ($n = 1$), bronchoalveolar lavage ($n = 2$) and sputum from pediatric patients with cystic fibrosis ($n = 24$), abscess ($n = 8$) and eusophageal biopsy ($n = 2$) samples. Isolated strains were checked of purity and were identified by classical morphological tests including germ tube formation in human serum at 37 °C for 3 h, blastoconidia, pseudohyphae, true hyphae and chlamydoconidia formation on corn meal agar-Tween 80, integrating with the results of chromogenic agar (CHROMagar, HiMedia, India) and of API 20C AUX kit (Bio Mérieux, France). Identification kit procedure and colorimetric microdilution tests were performed according to the manufacturer's instructions. Quality control was ensured by testing CLSI-recommended strains *C. parapsilosis* ATCC 22019 and *C. krusei* ATCC 6258. Recently described species-specific clinical breakpoint (CBP) values were used to categorize the minimum inhibitory concentrations (MICs) of echinocandins as susceptible (S), intermediate (I) and resistant (R) against the strains of *Candida* (Pfaller Diekema 2012).

Results Among 162 *Candida* isolates, 83 were *C. albicans*, 7 were *C. glabrata*, 45 were *C. parapsilosis*, 13 were *C. tropicalis*, three of each *C. kefyr* and *C. lusitaniae*, two of each *C. guilliermondii* and *Rhodotorula* sp, and one of each was *C. kefyr, C. krusei, C. pelluculosa* and *Trichosporon* sp. Regarding to the MIC values obtained, resistance to ANF was observed in one *C. albicans*, two *C. tropicalis*, five *C. parapsilosis* strains; resistance to CSF was observed in one *C. albicans* and one *C. parapsilosis* strains; and resistance to MCF was observed in two *C. albican*, one *C. tropicalis* and five *C. parapsilosis* strains. Resistance to all three echinocandins were observed in two Rhodotorula sp, one *Trichosporon* sp and only in one *C. albicans* strain which showed multidrug resistance including to azoles and amphotericin B. The remaining strains were *in vitro* susceptible to all echinocandins tested.

Conclusion Based on newly established breakpoints for species-specific interpretive criteria, the results of this study affirmed that echinocandins exhibit excellent activity against the *Candida* species most frequently involved in human infections.

P057

Candida haemulonii complex: the true scenario by sequencing and MALDI-TOF among clinical isolates in India

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Objectives *Candida haemulonii* complex is unique in being inherently resistant to the two most commonly used antifungals, namely, amphotericin B and fluconazole. Due to increasing reports of *C. haemulonii* and inconsistencies of automated yeast identification systems, a study was undertaken to molecularly characterize these isolates from cases of candidemia and deep seated infections. We aimed to report the first molecularly confirmed case series due to *C. haemulonii* complex from India and also evaluate their antifungal susceptibility profiles.

Methods A total of 103 clinical isolates initially identified as *C. haemulonii* by VITEK2 system were subjected for their correct identification by ITS sequencing and matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF). Antifungal susceptibility testing (AFST) was performed using Clinical and Laboratory Standards Institute (CLSI) brothmicrodilution method (M27-A3/M27-S3) and VITEK2 AST card.
Results Of the 104 *C. haemulonii*, only 15 (13.6%) isolates were confirmed as belonging to *Candida haemulonii* complex whereas the remaining 89 were *C. auris*. The *Candida haemulonii* complex included isolates *viz., C. duobushaemulonii* ($n = 8$), *C. haemulonii* ($n = 6$) and *C. haemulonii* var. *vulnera* ($n = 1$) identified by both sequencing and MALDI-TOF. Phylogenetic analysis of ITS sequence and spectra by MALDI-TOF clearly found different clades for respective species. Of the 15 isolates, 9 (60%) originated from patients with diabetic foot lesions, 5 (33%) fungemia and a solitary isolate from bronchoalveolar lavage. All of the 15 isolates showed resistance to amphotericin B (MIC_{90} 16 μg ml^{-1}) by both the methods. Fluconazole had variable MIC range (1 to >64 μg ml^{-1}) by both CLSI and VITEK methods. Low geometric mean MICs of posaconazole (0.05 μg ml^{-1}) and itraconazole (0.31 μg ml^{-1}) were observed by CLSI. Barring a solitary isolate of *C. haemulonii*, which had high voriconazole MIC (4 μg ml^{-1}), all were highly susceptible (MIC range, 0.03–0.5 μg ml^{-1}) by CLSI. All isolates barring two exhibited low MICs (range, 0.06–0.5 μg ml^{-1}) against echinocandins by CLSI. Further, a solitary isolate of *C. haemulonii* var. *vulnera* exhibited high MIC (1 μg ml^{-1}) against all echinocandins and one isolate of *C. duobushaemulonii* had high MIC (1 μg ml^{-1}) for anidulafungin and micafungin. All patients that harbored *C. haemulonii* complex in the tissue underwent foot amputation, which was the definitive treatment for the gangrenous lesions. Among 5 candidemia patients three were treated with voriconazole and remaining two expired before institution of antifungal therapy.
Conclusions The present study reports the large case series of candidiasis due to molecularly characterized *C. haemulonii* complex in India. The study reports the isolation of *C. haemulonii* complex for the first time from tissue of patients with diabetic foot lesions. It highlights the usefulness of molecular characterization to determine the true prevalence of this rare multi-drug resistant species.

P058

Fungicide activity of a derivative of propolis against *Aspergillus* spp. isolated from otomycosis

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Objective Otomycosis is a kind of external otitis that can be caused by various species of fungi, amongst which species of *Aspergillus* are the most common. To use the appropriate treatment, it is necessary to identify the causal agent of otomycosis. Treatment recommendations have included local debridement, local and systemic antifungal agents and discontinuation of topical antibiotics. Sometimes otomycosis presents as a challenging disease for its long term treatment and follow up, yet its recurrence rate remains high. Thus, the purpose of this study was to identify fungi isolates from otomycosis and evaluate the antifungal susceptibility *in vitro*. In addition we also evaluated the fungicide activity of a derivative of propolis as a possible alternative for otomycosis treatment. Propolis is a natural product collected from hives of *Apis mellifera* L.. Although the antifungal activity of propolis is well known, the new derivative product of propolis is still under study.
Methods Samples were collected from patients who attended the Teaching and Research Laboratory of Clinical Analysis (LEPAC), Division of Mycology, Universidade Estadual de Maringá (UEM), Brazil, from January 2014 to May 2015. This study was approved by the Research Ethics Committee of UEM (approval no. 615.643/2014). The species isolated from ear secretion were identified by the classical method. *In vitro* antifungal susceptibility testing was performed using a microdilution method according to the Clinical and Laboratory Standards Institute (CLSI; protocol no. M38-A2). The agents tested included voriconazole (0.03–16 μg ml^{-1}), amphotericin B (0.03–16 μg ml^{-1}) and subproduct of propolis extractive solution (SPES; 11.14 to 5707.4 μg ml^{-1} of total phenol content expressed in gallic acid). The minimum inhibitory concentrations (MICs) were visually determined after 48 h incubation at 35 °C. Then, the minimum fungicide concentration (MFC) of SPES was also determined by inoculating each concentration from the MIC test into plates containing Sabouraud Dextrose Agar, that were then incubated at 35 °C for 24 and 48 h.
Results In the period of this study, two samples of ear secretion contained species of *Aspergillus,* one was identified as *A. fumigatus* and the other as *A. flavus*. *A. flavus* presented high MIC value against amphotericin B (4 μg ml^{-1}) and it was highly resistant to voriconazole (≥16 μg ml^{-1}). SPES showed fungicide activity against both species, mainly against *A. flavus* that had higher MIC values for common antifungal agents (Table 1).
Conclusion Since SPES showedfungicide activity, it has potential to be an alternative topical treatment in cases of otomycosis due to *Aspergillus* spp. More studies are necessary to investigate the activity of this derivative product and validate its use *in vivo*.

Table 1. Minimum inhibitory concentration (MIC) of antifungal agents and minimum fungicide concentration (MFC) against clinical isolates of *Aspergillus* spp.

	MIC(μg/mL)	
Drug	*A. fumigatus*	*A. flavus*
Voriconazole	4	≥16
Amphotericin B	2	4
	MFC(μg/mL)	
SPES[a]	713.42	356.71

[a] Subproduct of propolis extractive solution, total phenol content expressed in gallic acid

P059

Molecular identification and antifungal susceptibility profile of clinically significant *Aspergillus* species in a referral Chest hospital, Delhi, India

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Objectives The major *Aspergillus* species known to cause disease in humans are included in five *Aspergillus* sections: *Fumigati, Flavi, Nidulantes, Nigri* and *Terrei*. The most common species causing invasive disease is *A. fumigatus* in section *Fumigati*. Molecular diagnostic approaches have revealed that there are at least 11 species that could easily be misdiagnosed as *A. fumigatus* based on their conidial morphology. Despite being clinically relevant, the identification and therapeutic approaches of rarely reported or cryptic *Aspergillus* species remains ill defined. The accurate identification of species is critical given that different species may present peculiarities in terms of reservoir, virulence factors, natural history of infection and *in vitro* susceptibility to antifungal drugs. The aim of this study was to molecularly characterize and to determine *in vitro* antifungal susceptibility profile of rare cryptic species in various *Aspergillus* sections originating from clinical samples in a referral chest hospital, Delhi, India.
Methods A total of 25 *Aspergillus* species cultured from respiratory and CNS specimens of patients with suspected mycotic infections during 2011–14 were preliminarily identified based on colony colour and morphology of the isolates on Czapek dox agar plates incubated at 28 °C for 7 days. All of the *Aspergillus* species were

identified by amplification and sequencing of β-*tubulin* and *calmodulin* gene. Antifungal susceptibility testing was performed for azoles, amphotericin B and echinocandins with broth microdilution method (CLSI M38-A2).

Results During a 4-year survey period, 15.5% *Aspergillus* species originated from 2117 clinical samples processed. Of these clinically important 25 isolates were investigated. β-*tubulin* and *Calmodulin* sequencing identified 3 (12%) isolates each of *Eurotium amstelodami* and *A. sydowii*, followed by two each of *A. fijiensis, A. ochraceus, A. niveus* var. *indicus, A. hortai, A. aculeatus* and *A. terreus*. Further, solitary isolates of *A. clavatus, A. wentii, A. chevalieri, A. melleus and A. tritici* were identified. Also, the teleomorphic stage of *A. nidulans*, i.e., *Emericella nidulans* and its variety, *E. dentata* originated from bronchoalveolar lavage of individual patients of aspergilloma. All the *Aspergillus* species excepting *Aspergillus aculeatus* exhibited elevated MICs of amphotericin B (range 2–16 μg ml^{-1}). Also, 24% ($n = 6$) isolates had high voriconazole MICs of 2 μg ml^{-1}. The species represented were *A. fijiensis, A. niveus* var. *indicus* and *A. ochraceus*. Excellent antifungal activity against all the species tested was exhibited by posaconazole (range 0.03–0.25 μg ml^{-1}) followed by isavuconazole (range 0.06–1 μg ml^{-1}) and itraconazole (range 0.03–0.5 μg ml^{-1}). All the echinocandins revealed good activity against all the species tested. The clinical profile of the patients yielding *Aspergillus* species ranged from allergic bronchopulmonary aspergillosis, chronic pulmonary aspergillosis, invasive pulmonary and a solitary case of brain abscess. Further, in 16% cases these species were isolated from patients with pulmonary tuberculosis with damaged lungs.

Conclusions The present study reports the extension of spectrum of *Aspergillus* species involved in aspergillosis. Accurate molecular identification is not only important taxonomically, but clinically relevant as many of these newly characterized species exhibit resistance to antifungal agents thus posing serious implications for the successful therapy of aspergillosis.

P060

PCR-based detection of A. fumigatus cyp51A mutations on bronchoalveolar lavage can readily predict azole treatment failure. A multi-center validation study in 201 hematology patients with suspected invasive aspergillosis

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Objectives Azole resistance in *Aspergillus fumigatus* is increasingly reported. However, the majority of cultures from patients with invasive aspergillosis (IA) remain negative and hence azole resistance remains undetected. The AsperGenius®PCR is a multiplex real-time PCR consisting of 2 PCRs: one identifies the clinically relevant *Aspergillus* species, the other detects 4 resistance associated mutations (RAM:TR34/L98H/ T289A/Y121F) in cyp51A associated with azole resistance. In a recent single-center study the diagnostic performance of AsperGenius®PCR on bronchoalveolar lavage (BAL) was excellent with sensitivity, specificity, positive and negative predictive values (PPV, NPV) of 89%, 89%, 73% and 96% (Chong et al. 2015). The purpose of this multicenter study was to (1)confirm these findings in large patient population, and (2) evaluate if the molecular detection of the above-mentioned RAM correlates with treatment failure.

Methods Leftover BAL samples from hematology patients with suspected IA from 5 Dutch and Belgian centers were used. BAL galactomannan ≥1.0 or a positive culture was considered the gold standard for the presence of *Aspergillus*. Supernatant and pellet were tested separately and the lowest cyclic threshold (Ct) value was used. Patients *without* the EORTC/MSG clinical criteria for possible IA but with galactomannan ≥ 1.0 were defined as non-classifiable. Azole treatment failure and mortality in patients with *A.fumigatus* containing a PCR-detected RAM was compared with those without RAM. Given the rarity of azole resistance, the 2 cases of PCR-detected azole resistance from the previous study were pooled with those detected in the current study in the azole treatment failure analysis. Patients were excluded from this analysis if they received < 5 day of azole therapy or had a mixed fungal infection.

Results 228 BAL samples from 201 patients were available. 9 patients had proven, 43 probable, 32 possible and 43 non-classifiable IA. 91 of the 228 BAL samples were gold-standard positive. The optimal Ct cut-off for the *Aspergillus* spp PCR was <36. With this cut-off, the PCR was positive in 64 of 91 BAL samples (Fig. 1). 34 of these 64 were culture negative. The sensitivity, specificity, PPV and NPV for the detection of *Aspergillus* spp was 70%, 94%, 89% and 83%. 4 of 72 BAL samples with Ct<36 were only positive in the pellet. Therefore, PCR testing on pellet had little additional value. The resistance PCR demonstrated that 65 patients were infected with a wildtype *A.fumigatus* and 13 were infected with *A. fumigatus* with a RAM (8 TR34/L98H, 3 TR34/L98H + wildtype, 2 TR46/T298A/Y121F). 27 patients were excluded from the azole treatment failure analysis. Azole treatment failure was observed in 13/43 patients with wildtype compared to 6/8 patients with RAM (P=0.04). Mortality at 6 weeks was 29% higher in patients with detected RAM (21% without versus 50%, P=0.18).

Conclusion This multicenter study confirms that the AsperGenius®PCR has a good diagnostic performance on BAL. The assay differentiated wildtype from *A. fumigatus* with RAM and is particularly of added value because most BAL samples of patients with IA are culture negative. Most importantly, 75% of the patients with RAM failed azole therapy. Therefore, PCR-based RAM detection can predict azole treatment failure as soon as IA is diagnosed.

P061

Combination antifungal therapy for patients with galactomannan (GM) positive probable invasive aspergillosis (IA)

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Objectives To assess the efficacy of voriconazole (vori) and anidulafungin (anid) in combination (combo) versus vori monotherapy in treating patients with GM-positive IA.

Methods In a randomized double-blind placebo-controlled trial, patients were assigned in a 1:1 ratio to receive vori with either anid or placebo for a minimum of 2 weeks; vori monotherapy was continued to complete 6 weeks of treatment. Patients were stratified at entry according to variables known to have an independent effect on mortality: allogeneic (allo-) Stem Cell Transplant (SCT) versus none and pulmonary versus disseminated IA. This analysis is based on a modified intention-to-treat (mITT) population, including all patients with confirmed (by an independent DRC) diagnosis of GM-positive probable IA and who received therapy. GM-positivity was defined by two serum samples or a single bronchoalveolar lavage (BAL) sample positive for GM, using the U.S. Food and Drug Administration-approved index cutoff. The primary analysis was a comparison of all-

cause mortality at 6 weeks. Mortality based on enrollment stratification was also analyzed.

Results 108 patients in the combo and 110 in the vori monotherapy were identified. Patients had comparable baseline characteristics including age (mean 51.8 years, range 18-79 vs 50.5 years, 18–83), gender (males 56% vs 56%) and race (white 71% vs 67%, Asian 25% vs 28%, and black 2% vs 1%) in the combo vs monotherapy arms, respectively.

All-cause mortality at Week 6 was 15.9% (17/108) in the combo and 27.5% (30/110) in vori alone arm [estimated treatment difference of −11.5% (95% confidence interval [CI], −22.41 to −0.65%), *P*-value 0.0189]. Interestingly mortality at Week 6 in allo-SCT patients was 22.2% (8/36) in the combo and 22.6% (7/31) in vori alone [difference of −0.4% (95% CI, −20.4 to 19.7%)]; in the non-allo-SCT patients, mortality at Week 6 was 12.7% (9/72) in the combo and 29.4% (23/79) in vori alone [difference of -16.7% (95% CI, −29.5 to −3.9%)]. Baseline characteristics including host factors, site of infection and underlying conditions did not identify specific differences in non-allo-HSCT patients that may explain the mortality rate finding.

Conclusions In patients with probable GM-positive IA, combination antifungal therapy with voriconazole and anidulafungin is associated with improved mortality rate compared to voriconazole monotherapy, especially in non-allo-SCT patients.

P070

Fluconazole versus Voriconazole: *Candida glabrata* biofilms response to different azoles

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Candida glabrata is the second most prevalent yeast in fungal infections, especially in immunocompromised and/or hospitalized patients. The azole resistance within this species is very well-known and results in a low therapeutic response of *C. glabrata* infections, particularly when associated with biofilms. So, the main goal of this work was to understand the different efficacies of two azoles against *C. glabrata* biofilms: fluconazole (Flu), a long time used drug, and voriconazole (Vcz), a recent drug used only in hospitals.

Antifungal (Flu and Vcz) susceptibilities were determined in preformed 24-h-biofilms of different strains of *C. glabrata* (clinical isolates and a reference strain). The *ERG* genes expression profiles of *C. glabrata* biofilms cells were determined. Additionally biofilms' matrices composition and the retention of the two azoles within the biofilm matrix were evaluated.

The results showed that *C. glabrata* biofilms are more susceptible to Vcz than Flu. Quantitative Real-Time-PCR results revealed an overexpression of the three *ERG* genes in the presence of both azoles. However, the *ERG* expression was more dependent on the strain than on the agent. The matrix content was analyzed following biofilm exposure to antifungal agents and it was noticed a decrease in proteins, an increase in carbohydrates (also β 1,3-glucans) and ergosterol was also found. A further evaluation was made with the determination of the concentration of the agents diffusing through the biofilm, which showed a remarkable difference between the two drugs, with Vcz reaching more the cells than Flu, which could explain the differences in biofilms susceptibilities.

To conclude, this study showed that the better performance of Vcz in *C. glabrata* biofilms maybe due to its smaller molecule and therefore to its better absorption within the biofilm.

P071

Comparison of biofilm production, hydrophobicity and proteomic analysis in clinical isolated strains of *Candida albicans*

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Objectives Study biofilm production and hydrophobicity in 448 strains of *Candida albicans* from patients with fungemia from Hospital Universitario la Fe (Valencia, Spain). Classify the *C. albicans* strains according to the degree of biofilm production comparing with the strain *C. albicans* SC5314 [1] using the Crystal Violet (CV) procedure. Proteomic analysis of the extracellular matrix (EM) from biofilms of different categories of *C. albicans.*

Methods Biofilm formation. Biofilm was formed as described [2] with some modifications. Cells were counted using a Bio Rad T20 Automated Cell Counter. 10^5 cells suspended in 100 µl of RPMI medium were plated in wells of 96-well microtiter plates and then incubated 24 h at 37 °C.

Biofilm quantification by the CV method. Biofilms were quantified as described [3] but 0.05% crystal violet was used instead 0.1%. The $OD_{580\ nm}$ values were referred at the $OD_{580\ nm}$ of *C. albicans* SC5314.

Cell surface hydrophobicity. Cell surface hidrophobicity (CSH) was measured by the biphasic separation method [4].

Analysis of tryptic peptides. The tryptic peptide mixtures from EM of biofilms were subjected to LC-MS/MS. Database search was performed of CAlbicans database.

Results We have used 448 strains of *C. albicans*. The distribution of strains based on CV results was into quartiles to stablish the cut-offs, comparing with the CV values of the strain *C. albicans* SC5314, as follows: Non Adherent (NA) = 0.0–0.2; Weakly Adherent (WA) >0.2–0.5; Moderately Adherent (MA) >0.5–0.7 and Strongly Adherent (SA) >0.7–1.0. The 448 strains were distributed in this way: 15.6% SA; 14.5% MA; 30.6% WA and 39.3% NA. The CSH was also analyzed. A relative CSH of around 20% was presented by 402 strains (53 SA; 54 MA; 126 WA and 169 NA). Amongst strains with a CSH >20% the highest percentage was found in the SA strains. The proteomic profiles of the EM from three strains of each category, WA, MA and SA were obtained. We identified 1790 proteins in SA strains, 600 proteins in MA strains and 220 proteins in WA. Of all the proteins identified only 74 were presents in the nine strains analyzed.

Conclusions We can conclude that there is not a direct relationship between the capacity of biofilm formation and the pathogenicity-virulence of *C. albicans* clinical isolate and that there is not a positive correlation between the cell surface hydrophobicity and the capacity to form biofilm structures. From the proteomic results we can deduce that the SA strains secrete larger amount of proteins than the other CV groups. Those proteins could help to maintain the cellular components of the biofilm strongly fixed to each other, and to the surface where they grow, in the SA strains. Finally, we can conclude that at least 70 proteins could be needed to form a biofilm so they are common to all strains analyzed.

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P072

Antifungal activity of caspofungin, anidulafungin, and micafungin against biofilms formed by yeast isolates showing intrinsic or acquired echinocandin resistance

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Objectives Biofilm formation promotes catheter-related fungemia and other forms of fungal disease. Echinocandins have shown potent anti-biofilm activity, although resistance in yeasts causing fungemia is being increasingly reported. Acquired echinocandin *Candida* spp. resistance is a result of mutations in *FKS* genes whereas non-Candida species commonly show intrinsic resistance. Data regarding biofilm production of isolates showing echinocandin resistance and their antifungal susceptibility are very scarce. We studied the biofilm production of *fks*-mutant *Candida* and non-*Candida*isolates showing intrinsic echinocandin resistance. Furthermore, the antifungal susceptibility of biofilms to caspofungin, micafungin, and anidulafungin was studied.

Methods We studied the biofilm production of the following isolates from patients with fungemia: *Candida* spp. strains with *fks* genes mutations [*C. albicans*, $n = 1$; *C. glabrata*, $n = 1$; and *C. tropicalis*, $n = 3$; Table 1]; intrinsically echinocandin-resistant non-*Candida*s trains [*Rhodotorula mucilaginosa*, $n = 7$; *Trichosporon asahii*, $n = 2$; *Trichosporon japonicum*, $n = 1$; *Trichosporon dermatis*, $n = 1$; and *Arxula adeninivorans*, $n = 1$]; and wild-type *Candida* isolates [*C. tropicalis*, $n = 6$; *C. albicans*, $n = 2$; and *C. glabrata*, $n = 2$]. Biofilm was measured to classify strains as low (LBF), moderate (MBF) and high biofilm-forming (HBF) (cristal violet assay), or low (LMA), moderate (MMA), and high metabolic activity (HMA) (XTT reduction assay). Preformed biofilm were treated with concentrations of caspofungin, micafungin, and anidulafungin ranging from 0.015 mg l^{-1} to 16 mg l^{-1} and incubated at 37 °C for 24 h; the metabolic activity was measured by the XTT reduction assay and the sessile MIC ($SMIC_{50}$) was defined as the antifungal concentration yielding a 50% reduction in the metabolic activity of the treated biofilm compared to the growth control. The $SMIC_{50}$ for the three groups of isolates was studied and compared (Kruskall-Wallis test).

Results The *Candida* resistant isolates formed biofilms (Table 1) and most of them were MMA. The three drugs showed the highest activity against biofilms formed by wild-type *Candida* isolates followed by *Candida fks* mutant isolates and by non-Candida isolates ($P < 0.001$) (Table 2). Micafungin was the drug showing the highest activity against biofilms formed by *fks*-mutant or wild-type *Candida* isolates although the differences did not reach statistical significance. Non-*Candida* biofilms were highly resistant to the three echinocandins.

Conclusions Yeast strains isolated from patients with fungemia and showing acquired or intrinsic echinocandin resistance were able to form biofilms with moderate or low metabolic activity. The activity of the three echinocandins against *Candida fks*-mutant biofilms was higher than the activity against intrinsically-resistant isolates; micafungin showed the greatest activity against wild-type or *fks*-mutant *Candida* biofilms.

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Table 1

	Geometric $SMIC_{50}$ mean (Range)		
	Candida with *fks* mutations	non-Candida isolates	WT
Micafungin	0.99 (≤ 0.015-16)	11.28 (0.031-≥32)	0.02 (≤ 0.015-0.125)
Anidulafungin	1.74 (0.062-≥32)	15.05 (0.062-≥32)	0.04 (≤ 0.015-0.5)
Caspofungin	1.51 (0.062 ≥32)	6.70 (0.125 ≥32)	0.14 (≤ 0.015 ≥32)

Table 2

Species	FKS mutation	Biofilm production (Cristal violet assay)	Biofilm Metabolic Activity (XTT assay)
Candida glabrata	Δ649 FKS2	0.03 (LBF)	0.20 (HMA)
Candida tropicalis	S645F FKS1	1.84 (HBF)	0.17 (MMA)
Candida albicans	F641S FKS1	1.16 (MBF)	0.19 (MMA)
Candida tropicalis	F641L FKS1	0.77 (MBF)	0.15 (MMA)
Candida tropicalis	R647G FKS1	2.33 (HBF)	0.14 (MMA)

P073

Effect of caspofungin and micafungin in combination with farnesol against *Candida parapsilosis* biofilms

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Objectives The aim of our study was to compare the *in vitro* anti-biofilm activity of caspofungin (CAS) and micafungin (MICA) combined with farnesol in RPMI-1640 against five *Candida parapsilosis* clinical isolates. Farnesol is a quorum-sensing molecule, which inhibits the yeast-hyphae transition.

Methods Drug interactions were examined by broth microdilution checkerboard method and an XTT-based colorimetric assay to measure metabolic activity of cells. The tested concentrations were 300–1.17 μM, 256–4 mg l^{-1} and 512–2 mg l^{-1} for farnesol, CAS and MICA, respectively. Fractional inhibitory concentration index (FICI) was used to assess the drug interactions: $\Sigma FIC = FICA + FICB = C_A^{comb}/MIC_A^{alone} + C_B^{comb}/MIC_B^{alone}$, where MIC_A^{alone} and MIC_B^{alone} are the MIC values of agents A and B using alone and C_A^{comb} and C_B^{comb} are the MICs of agents A and B when acting in combination, respectively. FICI was defined as the lowest ΣFIC. Synergy was specified if the FICI was ≤0.5, between >0.5 and 4 was indifferent and as antagonistic if the FICI was >4. MICs alone and at all of the isoeffective combinations were determined as at least 50% reduction of metabolic activity compared with control.

The results observed in the checkerboard 96-well plate were confirmed by time-kill experiments. Three concentrations (4, 8, 16 mg l^{-1}) were chosen and examined their anti-biofilm effect alone and in combination with 75 μM farnesol. Metabolic activity of biofilms was determined at 0, 3, 6, 9, 12 and 24 h. The XTT reaction was measured spectrophotometrically at 492/620 nm.

Table 1. *In vitro* interactions of caspofungin and micafungin in combination with farnesol

Drug	Strain	Median (range) of FICI	Type of interaction
CAS	16641	0,5 (0,375-0,5)	Synergistic
	17432	0,28 (0,156-0,28)	Synergistic
	17818	0,155 (0,141-0,155)	Synergistic
	10252	0,28	Synergistic
	9613	0,25 (0,185-0,25)	Synergistic
	ATCC 22019	0,502 (0,5-0,531)	Indifferent
MICA	16641	0,5 (0,375-0,5)	Synergistic
	17432	0,188 (0,157-0,281)	Synergistic
	17818	0,14 (0,125-0,156)	Synergistic
	10252	0,093 (0,077-0,125)	Synergistic
	9613	0,155	Synergistic
	ATCC 22019	0,625 (0,563-1)	Indifferent

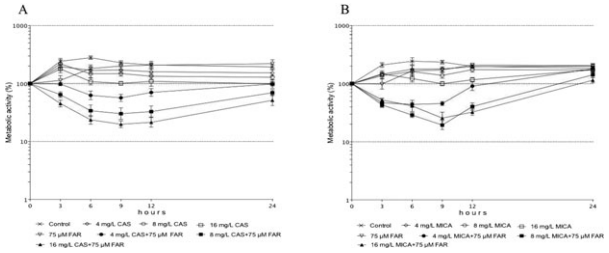

Figure 1. Time-kill curves of caspofungin (A) and micafungin (B) alone and in combination with farnesol against five Candida parapsilosis biofilms.

One-way ANOVA with Dunnett's post-testing was used to analyze the metabolic activity reduction exerted by drugs alone and in combination compared with control.

Results The median MIC values of five sessile *C. parapsilosis* clinical isolates were 32–256 mg l^{-1}, 16–512 mg l^{-1} and ≥300 μM for CAS, MICA and farnesol, respectively. However, in combination with farnesol these values reduced to 4 mg l^{-1} and 4–8 mg l^{-1} for CAS and MICA, respectively. Lower median MICs were detected for ATCC 22019 alone and in combination (CAS: 2 and 1 mg l^{-1}; MICA: 1 and 0.5 mg l^{-1}).

Synergistic interactions were observed both for CAS and MICA combine with farnesol for all of tested clinical isolates. Indifferent interaction was experienced for ATCC 22019 both in case of CAS and MICA. Antagonism was never observed. FICIs were shown in Table 1.

Our results were confirmed by time-kill investigations (Figure 1). Both tested echinocandins without farnesol demonstrated typical concentration-dependent activity against biofilms. The metabolic activity of fungal cells significantly decreased for CAS at all of three combinations (4 mg l^{-1} + 75 μM, 8 mg l^{-1} + 75 μM, 16 mg l^{-1} + 75 μM) between 3 and 24 h compared with control ($P < 0.05$–0.001). Significantly inhibition was observed for MICA with farnesol between 3 and 12 h ($P < 0.001$) but not at 24 h ($P > 0.05$).

Conclusion Farnesol exerted synergistic interaction in combination with CAS and MICA against biofilms of *C. parapsilosis* clinical isolates. Based on time-kill investigations CAS was effective longer time both alone and in combination with farnesol than MICA. Based on our results farnesol may be a potential adjuvant against biofilms for the treatment of catheter-associated infections caused by *C. parapsilosis*.

P074

Impact of biofilm forming ability on persistent *Candida tropicalis* fungemia

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Objectives From clinical studies, proportions of persistent candidemia varied from 11% to 45% with different definitions. *Candida tropicalis* is the most common non-*albicans Candida* species causing candiemia in Asia and Latin America. Little is known about biofilm forming ability of *C. tropicalis* and its impact on clinical outcomes. This case-control study aims to explore the association of biofilm forming ability and persistent *C. tropicalis* fungemia.

Methods Case patients were adult (≥18 years) patients with persistent candidemia (blood cultures positive of *C. tropicalis* for 5 days or longer) hospitalized at a 2200-bed teaching hospital in Taiwan during July 2011 and June 2013. Control patients were those with positive blood cultures less than 5 days and matched for age, gender, intensive care unit stay at onset, and month with 1:1 ratio. The minimal inhibitory concentrations (MICs) of antifungals were determined using the YeastOne® microdilution test (Trek Diagnostic Systems; Thermo Scientific, USA) and interpreted based on the Clinical Laboratory Standard Institute (CLSI) species-specific clinical break points. We determined dynamic biofilm forming ability using a 96-well plate-based, 2,3-bis (2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino) carbonyl]-2H-tetrazolium hydroxide (XTT) reduction assay, and high biofilm forming ability was categorized as the value above the median one.

Results A total of 106 and 42 isolates collected from 25 case patients and 25 matched control patients, respectively, were evaluated. For case patients, a median of 4 isolates per patient (range 2–8 isolates) were evaluated with a median 15 days of persistence (range 8–60 days). The demographics, underlying diseases/conditions, healthcare factors within 30 days prior to onset, infection focus, and fluconazole non-susceptible rate were not significantly different between case and control patients. Compared with control patients, case patients were less likely to remove central lines within 48 h after candidemia onset (28.0% vs. 56.0%, $P = 0.085$), more likely to be infected by high biofilm forming isolates (64.0% vs. 36.0%, $P = 0.089$) and more likely to receive highly active anti-biofilm agents as the definitive therapy (60% vs. 20%, $P = 0.009$). Multivariate analysis showed that removal of central lines within 48 h (adjusted odds ratio [aOR], 0.21; 95% confidence interval [CI], 0.06–0.81; $P = 0.023$) and high biofilm forming isolates (adjusted odds ratio [aOR], 4.55; 95% confidence interval [CI], 1.22–16.95; $P = 0.024$) were independently associated with persistent candidemia. The 28-day mortality in case patients was 40.0% and 52.0% in control patients ($P = 0.571$). Length of stay after candidemia onset was longer for case patients (37 days vs. 12 days, $P = 0.002$).

Conclusions Not only prompt removal of central lines but also *Candida* with high biofilm formation contributed to persistent *C. tropicalis* fungemia.

P075

Kinetic modeling of Aspergillus fumigatus biofilm developed *in vitro* using digital image analysis

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Objectives Detection of biofilm forming fungi is clinically very important but simple and effective methods for its analysis are lacking, so the aim of this study was to perform quantitative analysis of *Aspergillus fumigatus* biofilm *in vitro* by digital analysis of biofilm photomicrographs. Biofilm size and complexity during time were quantified and obtained data were used for development of kinetic model for description and prediction of the biofilm dynamics.

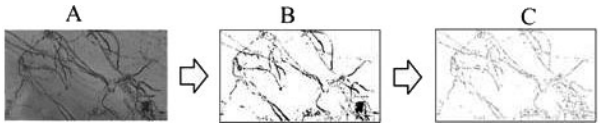

Figure 1. Image processing procedure: the grayscale image (A), the binary image (B), the outline image (C).

Methods *A. fumigatus* biofilm was developed *in vitro* on plastic cover slips (CS). CS were placed in ϕ55 Petri dishes and statically incubated with *A. fumigatus* spore suspension (10^6 colony forming units per ml) in Sabouraud dextrose broth with 8% of glucose (Sigma-Aldrich, St Louis, MO, USA) at 28 °C. In each of the six time points (after 19, 20, 21, 22, 23 and 24 h of incubation), three CS were removed from incubator, washed two times with sterile distilled water and stained with lactophenol cotton blue (Sigma-Aldrich, St Louis, MO) for 30 min at 37 °C. After washing and air-drying, CS were mounted on a microscopic slide and the biofilms were photographed using a Nikon Camera (total magnification of 400X). Total of 60 digital images were acquired and stored in JPEG format. Experiment was performed two times in triplicate.

The image analysis was done in software 'Image J' in three steps: (i) conversion of RGB images into grayscale images (ii) obtaining binary images by adaptive threshold selection and (iii) procession by image outline (Figure 1). Two biofilm parameters were quantitatively evaluated: the size (A) and the complexity of biofilm (D). Size was evaluated by measuring the area, while complexity of biofilm was done by fractal analysis of image using the box-counting method to obtain fractal dimension of contour image which estimated the level of two-dimensional (2D) geometrical complexity of the biofilm. Finally, A and D values obtained during time were used to develop a kinetic model of *A. fumigatus* biofilm growth.

Results Logistic kinetic model was used to describe the increase of the two parameters of 2D biofilm image during its growth. The experimental data successfully fitted exponential equation. The agreement between the predicted and experimental values was observed which proved that the kinetic model was appropriate for the description and prediction of A and D parameters. Kinetic model provided rate constants for both parameters (α_{size} and α_{comp} for A and D, respectively) where α_{size} had a higher value than α_{comp} (5.968 ± 0.062 and 0.409 ± 0.024, respectively). A positive linear correlation was observed between A and D ($r = 0.9$, $P < 0.05$).

Conclusion Logistic kinetic model was successfully applied for description of *A. fumigatus* biofilm growth by increase of the biofilm size and complexity. Biofilm images used for modeling were obtained and analyzed using novel, cost-effective and simple approach which can provide detection and prediction of the biofilm dynamics. This can be useful for further studies on kinetics of fungal biofilm since the prediction of biofilm dynamics can contribute to better clinical management of invasive fungal infections.

P076

Pre-exposure of *C. albicans* biofilms to sub-MIC concentrations of micafungin augments efficacy of human neutrophils, but concurrent combined treatment exerts no additive effect against biofilms

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Objectives *Candida albicans* (CA) has been associated with catheter-related fungemia leading to high mortality. CA forms biofilms (BF) on the surface of intravenous central catheters and indwelling medical devices. Human neutrophils (PMN) are critical component of innate immune response against fungi. The objective of this study was: 1) to investigate whether pre-exposure of *C. albicans* to sub-MIC concentrations of micafungin (MFG) could subsequently modify the activity of PMN against *C. albicans* mature BF and 2) to assess the effect of the simultaneous combination of PMN with MFG against intact mature BF.

Methods 10^6 blastoconidia/mL CA-M61, a well-documented BF-producing CA strain and two BF-forming *C. albicans* bloodstream isolates, were grown in RPMI at 37 °C for 24 h in the presence of MFG at 0.5xMIC, 0.25xMIC and 0.125xMIC concentrations. Pretreated and untreated *C. albicans* isolates were then grown in fresh RPMI at 37 °C for 48 h to produce mature BF. PMN were isolated from healthy donors by dextran sedimentation/ficoll centrifugation. After a brief washing step of *C. albicans* mature biofilms, they were incubated with PMN for an additional 24 h at effector to target ratio (E:T) of 5:1. In another set of experiments, intact 48 h mature BF were exposed to the simultaneous action of PMN (5:1) and MFG (0.007–4 mg l^{-1}) for 24 h. The PMN-induced BF damage measured as % reduction of metabolic activity was assessed by the XTT assay. The MIC of MFG for BF was determined as the minimum concentration that caused ≥50% fungal damage compared to that for the untreated controls. Statistical analysis was performed by ANOVA with Dunnett's post-test. Four independent experiments were performed.

Results The MIC of MFG for mature BF of the three *C. albicans* isolates was 0.25 mg l^{-1}. MFG used at 0.5xMIC, 0.25xMIC and 0.125xMIC concentrations caused 18%±3.7% ± 2.9 and 9.8% ± 2.6 BF damage, respectively. When PMN were added to drug-pretreated mature BF there was a significant augmentation of PMN-induced % damage against *C. albicans* exposed to 0.5xMIC compared to drug-untreated isolates (81% ± 1.8 vs. 60% ± 3.5; $P < 0.01$). PMN exhibited similar % damage against *C. albicans* pretreated with 0.25 × MIC or 0.125 × MIC compared to drug-untreated isolates, respectively (64% ± 2 and 67% ± 2.2 vs. 60% ± 3.5; p = ns). The BF damage induced by the concurrent combined treatment of PMN with MFG (0.007 mg l^{-1}-4 mg l^{-1}; 54–65% BF damage) against intact BF was not significantly different from that of PMN alone (48%±5.6).

Conclusion Pre-exposure of *C. albicans* to micafungin at sub-MIC concentrations increases biofilm susceptibility to the antifungal activity of PMN. However, the concurrent combined treatment of mature biofilms by MFG and PMN does not exert additive biofilm damage. These findings may be clinically important in the *Candida* biofilm-associated infections and need further study in animal models.

P077

Development of P-113-derived peptides as novel inhibitors for drug-resistant *Candida* spp. and biofilm formation

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Objectives *Candida* spp., as major opportunistic pathogens, infects immunocompromised people and is responsible for a number of life-threatening infections. The individuals commonly affected include those with oral candidiasis of human immunodeficiency virus (HIV)-positive patients, of cancer patients undergoing chemotherapy and radiotherapy, of diabetes and xerostoma patients. Moreover, *Candida* spp. can develop resistance to multiple antifungal drugs by over-expression of drug resistance-related targets and other mechanisms. *Candida* spp. can also form biofilms that are highly resistant to antibiotics treatment on host tissues or indwelling medical devices. In this study, we developed a patented anti-fungal peptide P-113 and P-113-derived peptides to overcome the emerging issue of drug-resistance in *Candida* infections and reduce the resistance of biofilms.

Methods The P-113, derived from human histidine-rich histatin 5 protein, retains a full antibacterial activity and exhibits a wide spectrum of activity *in vitro* against both bacteria and fungi. Several drug-(azoles-) resistant clinical strains including *C. albicans*, *C. krusei*, *C. glabrata*, and *C. tropicalis* were isolated from oral candidiasis patients. We modified P-113 by tandemly repeating sequences (P-113-dimer and P-113-trimer) to improve the candidacidal efficiency. Furthermore, the candidacidal activity of P-113 and its derivatives against biofilm cells of *C. albicans* was also performed. Biofilm was formed in a 96-well microplate in YPD medium for 24 h. The biofilm cells were then treated with or without various concentrations of P-113, P113-dimer, and P113-trimer at 37 °C for 1 h followed by XTT assay.

Results The results showed that P-113 was able to kill these drug-resistant strains, suggesting a promising candidacidal activity of P-113 toward drug-resistant *Candida* spp. To improve the candidacidal

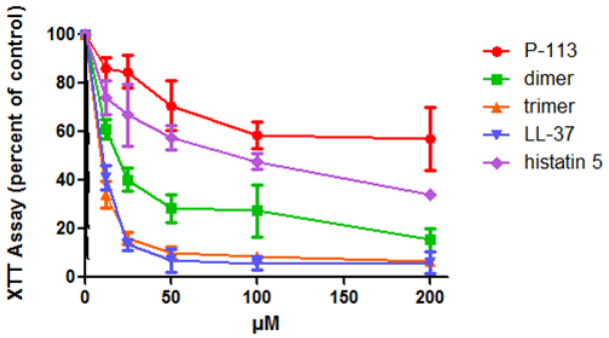

Figure 1. The efficiency of P113-dimer and P113-trimer against C. albicans biofilm cells

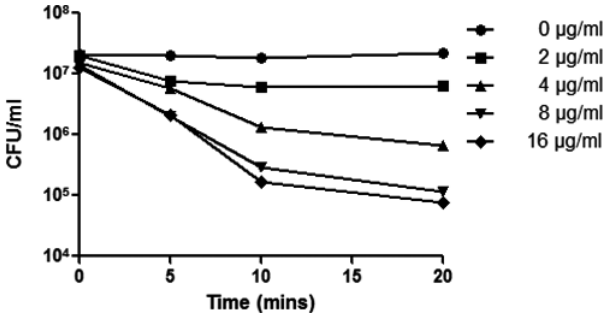

Figure 2. The candidacidal activity of P-113 is time- & dose-dependent.

activity, we designed and synthesized various P-113-derived dimeric and trimeric peptides which contain 2- and 3-time tandem repeats. Among them, the two tandem repeats improved the candidacidal potency of the peptides, and the dimer and trimer have IC_{50} values against *C. albicans* of 0.5 and 0.4 µM, respectively, compared with the monomer P-113 (IC_{50} = 2.3 µM). Moreover, we also found that both dimer and trimer form of P113 can efficiently inhibit the viability of *C. albicans* biofilm cells, showing the 50% RMA (reduction in the metabolic activity) of dimer and trimer are 20 µM and 8.3 µM, respectively.

Conclusion In this study, we showed that P-113 can kill drug-resistant clinical isolates efficiently. Studies with P-113 derivatives further revealed that P113-trimer can be folded as an α-helix structure (using by Circular dichroism), suggesting the reason that P113-trimer can effective against both planktonic and biofilm cells of *C. albicans*. P-113 and the derivatives show potential for the development of novel therapeutics against infections with drug-resistant *Candida* spp. and inhibit the biofilm formation.

P078

How do *Candida glabrata's* biofilms respond to antifungal drugs?

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Objectives *Candida* species are responsible for recurrent human infections, mostly in immunocompromised patients, due to their high vulnerability. *Candida glabrata* has been shown to have a major role in these infections being the second most prevalent species involved in human fungemia. This work aims to understand the effect of antifungal agents in *C. glabrata*'s biofilm formation, specially their role on matrix composition.

Methods Three antifungal agents, belonging to different classes, azoles, polienes and echinocandins, were selected for this work, namely fluconazole (Flu), Amphotericin B (AmB) and Caspofungin (Csf), respectively. Three strains of *C. glabrata* were used along this study. The effect of the agents on *C. glabrata* biofilm formation was assessed by Colony Forming Units (CFU) and Crystal Violet (CV) assays. Matrices' composition evaluation included the determination of polysaccharides (phenol-sulfuric acid method), proteins (BCA®-Kit) and β-1,3-glucans (Glucatell® Kit) concentrations.

Results Observing the effect of the three drugs on the biofilms, it was noticed that AmB and Csf showed the best performance in the reduction of biofilms formed by the three *Candida glabrata* strains, in opposition to Flu. However, the effect of Csf was the most notorious, achieving a biofilm reduction around 85%.

Analyzing the biofilm matrices, it was possible to observe a significative change in their composition, when biofilms were formed in the presence of the three antifungal agents, both in terms of polysaccharides and proteins. In fact, in the presence of the different antifungal agents two opposite effects were noticed, the amount of polysaccharides increased, in opposition to the profound reduction in terms of proteins. For AmB and Csf this decrease was very sgnificative, being below the minimum detected value range of the BCA® proteins Kit.

Interestingly, the amount of β-glucans on the matrices did not show important differences in the presence of the drugs, with the exception of Csf, which induced an increase of 20% of these compounds.

Conclusion As expected, the three agents had different effects on *C. glabrata*'s biofilm formation.

Moreover, matrices' composition display dissimilarities when exposed to different antifungal agents, and these differences depend on each drug is used.

Therefore, AmB, and especially Csf, were confirmed, in this study, to be the most effective pharmacoterapies for eradication of *C. glabrata* infections associated to biofilms.

P079

The farnesol influence on characteristics of *Candida albicans* biofilm formation *in vitro*

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Candida albicans is a dimorphic fungus that exists in a number of morphological forms under different environmental **conditions** budding yeast cells, pseudohyphae, true hyphae and chlamydospores. During human candidiasis, especially superficial, this microorganism forms biofilms. *C. albicans* biofilms usually consist both yeast cells and hyphae covered with a thick exopolysaccharide layer. The biofilm itself is generally resistant to a variety of antifungal agents, including amphotericin B and fluconazole, and will frequently persist as a reservoir of infection. The *C. albicans* biofilm structure, in particular the component ratio, can be different depending on the substrate type: artificial medical devices, skin or mucous membranes. But any environmental factor cannot act alone and under all conditions to provoke the *C. albicans* growth in only one morphological form. While some quorum-sensing molecules, for example farnesol (3,7,11-trimethyl-2,6,10-dodecatriene-1-ol), block the morphological shift from yeasts to mycelia and suppress filamentation.

The aim of this work was to characterize the *C. albicans* biofilm formation in the farnesol presence under different conditions *in vitro*.

The work was carried out at Biotechnological Research and Educational Center using *Candida albicans* ATCC 18804. It was cultivated in two liquid medium variants: Sabouraud (standard nutrient medium for yeast like fungus cultivation) and Spider (rich medium for

phenotype growth and colony wrinkling in *C. albicans* observation). The farnesol concentration range was 0–500 μM.

In our study it was found that the formation of mature *C. albicans* biofilm accompanied by an increase of the cell as well as exopolysaccharide number. At low temperature biofilm maturation was less intensive compared with 37 °C. But in both cases, only yeast cells were observed as part of the biofilm. Such structure of *C. albicans* biofilms was noted at urological catheter surface.

The intensity of *C. albicans* biofilm formation in Spider medium was slightly lower than in Sabouraud one but only during the last phase. However at the process beginning the significant number of hyphae that gradually associated into a continuous system was noted only in the second nutrient medium. Given the fact that hyphae production is considered as *C. albicans* pathogenicity increasing this biofilm structure was a model for the skin candidiasis – the most aggressive and common form in humans.

It was found in our study that farnesol addition in the cultivation media did not result in significant changes in the biofilm formation rate, but considerable modifications of its structure. In the case of Spider medium cultivation substantially filamentous free biofilm was detected. The most active farnesol influence was in low concentrations – up to 150 μM.

So, the nutrient medium composition and conditions effect on the *Candida albicans* biofilm formation. These factors cause manifestation of the ability to switch between different forms of microorganism morphology and overall speed of group production.

P080

Assessment of *in vitro* biofilm formation and antifungal susceptibility of *Candida albicans* isolates from vulvovaginal candidiasis

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Objectives Vulvovaginal candidiasis (VVC) is an inflammation of the genital mucosa, which mainly affects the vulva and vagina. *Candida* spp. are considered commensal fungus, however, when there is imbalance in the microbiota or the host immune system is compromised, these can become pathogenic. *C. albicans* is responsible for most cases of VVC and is able of expressing mechanisms which allow the colonization or infection in the host. These factors related yeasts, including the growth of strains resistant to antifungal agents and virulence attributes (such as biofilm formation) are important in the development of VVC. In this sense, the objective of this study was to evaluate the *in vitro* biofilm formation and susceptibility to antifungal of *C. albicans* isolates from patients with vulvovaginal candidiasis.
Methods For the study were analyzed 30 clinical isolates of *Candida albicans*. The clinical isolates were separated in groups of 10 samples of the according to symptoms presented by the patients: asymptomatic (AS), vulvovaginal candidiasis (VVC) and recurrent vulvovaginal candidiasis (RVVC). For all isolates were analyzed biofilm formation and minimal inhibitory concentration (MIC) for fluconazole and nystatin. The MIC was performed according to M27-A3 protocol of the Clinical Laboratory Standards Institute. Biofilm forming ability was assessed through quantification of total biomass by crystal violet (CV) staining, performed on 96-well microplates containing a cellular suspension of 1×10^7 cells ml^{-1} and incubated for 24 h at 37°C.
Results Antifungal susceptibility testing is showed in table 1. The isolates were tested to the two antifungals. The MIC raging from 0.125 to 2 μg ml^{-1} for fluconazole and 1 to 4 μg ml^{-1} to nystatin. The figure 1 show the quantification of the total biomass. It was evident that all the *C. albicans* isolates were able to form biofilm, although differences occurred depending on the isolated and consequently the group. Importantly it was noted that, in general, VVC and RVVC groups had similar capacity biofilm formation. On the other hand, these groups had less total biomass (average Abs = 1,091 ± 0.88) compared with AS group (average Abs = 1,521 ± 1.32).
Conclusion Although all the samples analyzed are sensitive to antifungals tested research of resistant strains is relevant, since recurrences are related to cases of VVC. Nystatin and fluconazole were effective in small concentrations for the isolates analysed. All samples were able to form biofilm and the average of the group of asymptomatic patients greater than the others. Thus, the capacity to forming biofilm is an important virulence factor in the persistence of microorganisms in infectious processes and represent an increase in resistance to antifungal and host defense.

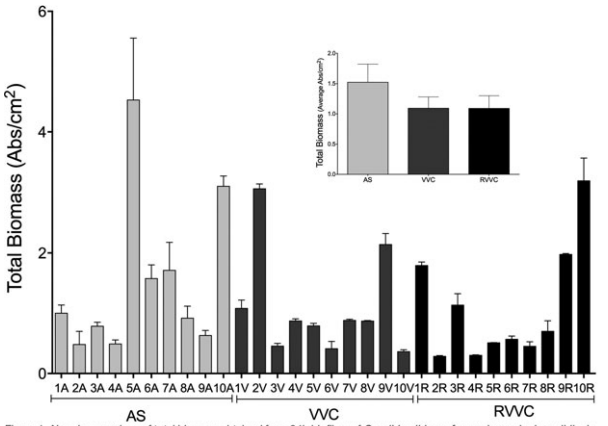

Figure 1. Absorbance values of total biomass obtained from 24h biofilms of *Candida albicans* from vulvovaginal candidiasis. AS: asymptomatic; VVC: vulvovaginal candidiasis; RVVC: recurrent vulvovaginal candidiasis. The inset represents the mean absorbance for all analyzed groups

Table 1. Range of MIC and MIC50, MIC90 of 30 clinical isolates of patients with VVC front of the antifungal fluconazole and nystatin.

	MIC (μg/mL)					
	Fluconazole			Nystatin		
Clinical groups	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range
AS	0,25	0,5	0,125 - 0,25	4	4	1 - 4
VVC	0,25	0,5	0,25 - 0,5	4	4	4
RVVC	0,25	4	0,25 - 2	2	4	2 - 4

MIC_{50} and MIC_{90} were defined as capable of inhibiting 50% and 90% growth of the isolates, respectively. The range values correspond respectively to lower and higher MIC of each drug.
VVC - vulvovaginal candidiasis. RVVC - recurrent vulvovaginal candidiasis. AS - asymptomatic.

P081

Candida albicans biofilm development in various biomaterials using the CDC Biofilm Reactor.

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Candida biofilms are important and growing problems for the treatment and outcome of catheter-associated candidiasis. There are not

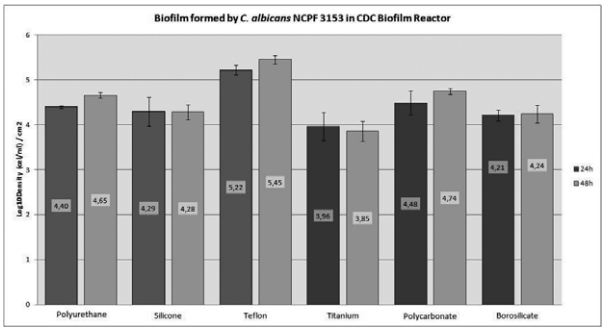

Figure 1. Biofilm mean log density formed by C. albicans NCPF 3153 on polyurethane, silicone, teflon, titanium, polycarbonate and borosilicate coupons.

standardized methods for the study of *Candida* biofilms and the effect of antifungal drugs against them.

Objective To evaluate a model of *Candida* biofilm in coupons of different biomaterials used in catheters and other medical devices by means of the CDC Biofilm Reactor.

Methods *Candida albicans* NCPF 3153 was cultured overnight at 35 °C and adjusted to a concentration of 3×10^6 cells ml^{-1}. One millilitre of the inoculum was transferred to the CDC Biofilm Reactor (BioSurfaces Technologies, USA) that incorporated the removable materials tested (polyurethane, silicone, teflon, titanium, polycarbonate and borosilicate). The reactor was filled with 500 ml of yeast extract peptone dextrose (YEPD) and *Candida albicans* was cultured onto the coupons for 48 h at 35 °C with an agitation of 120 rpm. Each coupon was rinsed in PBS to remove planktonic cells. Finally, biofilms were detached from the coupons by sonication (50% amplitude, 3 cycles of 20 s) and colony forming units (CFUs) counted in Sabouraud glucose agar plates. All test were done in quadruplicate and repeated in three different days. Analysis of variance (ANOVA) and Games-Howell *post hoc* test was performed in order to find differences between biofilms developed in the different materials after 48 h.

Results *Candida albicans* developed sound biofilms on the surface of all coupon tested. Results were repeatable and reproducible. Biofilm on teflon coupons were significantly higher (5.45 ± 0.09) compared with the rest of coupons ($P < 0.001$, Figure). Conversely, titanium coupons (3.85 ± 0.22) showed the lowest density ($P < 0.01$). Borosilicate coupons (4.24 ± 0.19) featured less biofilm density than teflon, polyurethane and polycarbonate coupons ($P < 0.0001$). *There were no*statistically significant*differences between biofilm formed on polyurethane coupons* (4.65 ± 0.05) and *biofilm formed on polycarbonate coupons* (4.74 ± 0.07) ($P = 0.724$). Likewise, there were not significant differences between biofilms developed onto silicone (4.28 ± 0.16) and borosilicate coupons ($P = 0.990$).

Conclusions This model on different biomaterial coupons using the CDC Biofilm Reactor is valid for the study of *Candida* biofilm development as it is reproducible and repeatable. Greater biofilms were formed onto Teflon coupons while the biofilm on titanium were the less dense.

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P082

Bioluminescence Imaging of *Candida glabrata* adhesion and biofilm formation

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Introduction *Candida glabrata* is an emerging human fungal pathogen causing mucosal and deep tissue infections. Majority of these infections are associated with biofilm formation on medical implants. Biofilms are difficult to treat as they are tolerant to most antifungals. Fungal load in biofilms is traditionally analyzed *post mortem*, requiring host sacrifice and enumeration of microorganisms from individual biofilms to evaluate the efficacy of antifungal treatment. In this study, we aimed to use bioluminescence imaging (BLI) to detect *C. glabrata* adhesion and biofilm development *in vitro* and *in vivo*.

Methods Wild-type bioluminescent strain based on a *C. glabrata*-codon optimized firefly luciferase was engineered. *In vitro C. glabrata* adhesion and mature biofilms were developed inside serum-coated polyurethane catheters and in 96-well polystyrene plates. *In vivo* biofilms were studied in catheters implanted on the back of Sprague Dawley rats and biofilm formation was imaged with BLI at time of implantation and followed up after 2 and 6 days. Bioluminescent signal was measured and correlated with colony forming units (CFUs).

Results We report significant bioluminescent signal from *Candida* cells *in vitro*, which were in agreement with CFU counts at different stages of biofilm maturation. Data from *in vivo* BLI signal formed in live animals at different time points after catheter implantations will be presented at the conference.

Conclusions We demonstrate the feasibility to non-invasively image and quantify *C. glabrata* adhesion and biofilms making dynamic monitoring of biofilm development possible.

P083

Efficacy of Anidulafungin plus clarithromycin against *Candida tropicalis* biofilms growing on teflon and titanium

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Objectives Up to 60% of all human infections are caused by biofilms, causing a significant clinical problem. Candida biofilm-associated infections are difficult to eradicate with antifungal treatment as biofilms cells are more resistant than planktonic cells because the exopolysaccharide matrix reduces the diffusion of antifungals inside the biofilm. Previous studies have shown that C. tropicalis biofilms are resistant to echinocandins. Here we studied the kinetics of biofilm formation on teflon and titanium of C. tropicalis and evaluated the ability of the combination of anidulafungin (AND) plus clarithromycin (CLA) to eradicate biofilm formed on these biomaterials.

Methods The selected strain of C. tropicalis was isolated in blood culture and has been demonstrated to be strongly adherent. The kinetics of biofilm formation on teflon and titanium was performed using 1.27 cm discs in the CDC Reactor, using an initial inoculum of 107 cfu ml^{-1} in YNB-100 mM glucose medium. The number of attached cells was determined at 24, 48, 72 and 96 h. The results are expressed as Log cfu/cm2. The study of biofilm eradication was also performed using the CDC Reactor. AND (16 mg l^{-1}) and AND+CLA (16 + 20 mg l^{-1}) combination was added to the biofilm of 24 h of maturation formed on titanium or teflon in the CDC

reactor. At 24, 48 and 72 h, the number of viable cells were determined using the same experimental protocol as in the kinetics studies of growth.
Results A significant difference ($P = 0.03$) was observed in the amount of C. tropicalis biofilm formed on teflon and titanium. The average count on teflon was 4.60 log cfu/cm2 and 2.17 log cfu/cm2 on titanium. The biofilm exposed to AND had an initial adhesion of 4.79 log cfu/cm2 on teflon and 4.23 log CFU/cm2 on titanium. At 72 h, adhered cells were reduced 48.49% (2.47 log cfu/cm2) on teflon and 41.06% (2.50 log cfu/cm2) on titanium. The biofilm exposed to AND+CLA had an initial adhesion of 4.86 log cfu/cm2 on teflon and 4.13 log cfu/cm2 on titanium. At 72 h, adhered cells were reduced 61.92% (1.85 log cfu/cm2) on teflon and 54.14% (1.89 log cfu/cm2) on titanium.
Conclusion C. tropicalis formed stable biofilms after 24 h of contact with the biomaterial and persistently adhered to the surface during the 96 h of the experiment. Teflon was the biomaterial on which the greatest amount of biofilm formed. AND alone was capable of eradicating 48% of biofilm formed on teflon and 41% of that formed on titanium. The combination with clarithromycin was capable of eradicating 62% of biofilm formed on teflon and 54% of the biofilm formed on titanium. A combination of AND plus CLA could be an effective strategy for the eradication of C. tropicalis biofilm.

P084

Standardization of a method to evaluate biofilm formation by *Candida* species

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Background *Candida* cells can form biofilms that frequently are sources of infections and are less susceptible to antifungal drugs. For this reason, is very important to have a method to evaluate biofilm formation by *Candida* spp. isolated of clinical samples. The main aim of this study was standardize a method to evaluate biofilm formation in yeast. So, we studied what *Candida* spp. were more biofilm producers.
Material and method We isolated 48 *Candida* spp.: *Candida albicans* ($N = 24$) and *Candida glabrata* ($N = 24$) from different human reservoirs (bloodstream, respiratory and vaginal tract). *C. albicans* was used to standardize this method. Quantitative measurement of biofilm formation was assessed by XTT reduction assay. We used different incubation time (24 vs. 48 h) and glucose concentration (2%, 5% and 10%). Finally, we compared the biofilm formation between *C. albicans* versus *C. glabrata*. We studied if the biofilm formation could be affected by the site of isolation of *Candida* spp. All experiments were repeated three times and the results were expressed as average values of absorbance.
Results The best conditions for biofilm formation by *C. albicans* were 48 h of time of incubation and 2% glucose concentration. *C. albicans* strains were more biofilm producer than *C. glabrata* strains (0.519 vs. 0.214) ($P = 0.007$). In both, biofilm formation was higher in *Candida* spp. isolated from bloodstream than respiratory and vaginal tract, respectively, although this was not statistical significance difference ($P > 0.05$).
Conclusions This method has demonstrated to be repeatable and reproducible in all strains studied. *C. albicans* strains are strong biofilm producers and they could be used as model of biofilm formation in yeast to study the antibiofilm activity of new agents. No association was found between strain's site of isolation and biofilm formation.

P085

The ability of oral *Candida albicans* and *Candida dubliniensis* isolates to form biofilm *in vitro*

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Objectives The aim of this study was to determine the frequency of oral candidal colonisation in healthy adults and to evaluate the ability of isolated *Candida albicans* and *Candida dubliniensis*strains to produce biofilm *iv vitro*.
Methods Oral swab samples were taken from 120 healthy voluntaries, 79 women and 41 man, aged 19–56 years (mean, 24). Material were cultured on chromID Candida and Sabouraud Gentamicin Chloramphenicol 2 agars (bioMerieux) and incubated 7 days, first in 37 °C (24 h) and then in 25 °C. Cultured strains were identified on the base of morphological and metabolic characteristics with the use of ID32C panels (bioMerieux). Biofilm production was tested on 96-wells microplates using YNB medium and crystal violet staining method.
Results Positive cultures were obtained from oral samples from 85/120 (71%) voluntaries, including 27/120 (22.5%) with heavy (>10 colonies) and 58/120 (48%) with scant (<10 colonies) fungal growth. The most prevalent species was *C. albicans* (81 isolates), followed by *C. dubliniensis* (3), *C. krusei* (1) and *C. kefyr* (1). Biofilm produced 23/81 (26%) of *C. albicans* and 3/3 *C. dubliniensis* isolates. No relationship between biofilm production and intensity of candidal growth in sample cultures was found.
Conclusion *Candida albicans* represent a frequent element of oral microbiota in healthy adults. A quarter part of oral *Candida albicans* isolates produced biofilm *in vitro*. The link between biofilm production and the ability to induce infection should be elucidated in future studies.

P086

Anti-*Candida* effect of *Matricaria recutita* essential oil

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Objectives The aim of this study was to evaluate the *anti-Candida* effect of *Matricaria recutita* essential oil.
Methods *Matricaria recutita* essential oil was chemically characterized by gas chromatography mass spectrometry (GC/MS). The effects of *Matricaria recutita* essential oil on *Candida albicans* (ATCC 18804), *C. glabrata* (ATCC 90030), *C. parapsilosis* (ATCC 22019), *C. krusei* (ATCC 6258), *C. tropicalis* (ATCC 13803) and *C. dubliniensis* (NCPF 3108) were assessed. Antifungal activity was screened by agar diffusion test. After, the minimal inhibitory concentration values (MIC) were determined by microdilution tests in RPMI broth buffered with MOPS. Values of minimal fungicide concentration (MFC) were obtained by subculture in Sabouraud dextrose agar. The activity on 24 h pre-formed biofilms was also evaluated. Biofilms were formed in RPMI + 2% glucose at 37°C for 24 hours. Then, they were exposed for 5 min to concentrations 2 and 4 times MIC. The cell viability after exposition was determined by serial dilutions and plating. All the experiments were performed in duplicate in three different occasions. Nystatin was included as positive control in all experiments. The results were compared by ANOVA and post hot Tukey's test, at level of significance of 5%. Results: A total of 33 compounds were detected by GC/MS. Bisabolol oxide was the majority compound, followed by β-farnesene. Agar diffusion tests revealed inhibitory activity against all the tested species. MIC values varied largely among the tested species (0.1–12.5% v/v). The most susceptible species was *C.*

glabrata and the less susceptible was *C. krusei*. Fungicide effect was also observed for all the species, with CFM values ranging from 0.30 to 25% v/v. Exposition to 2 and 4 times MIC significantly reduced cell viability of *C. tropicalis*, *C. parapsilosis* and *C. krusei* pre-formed biofilms when compared to non-exposed control ($P < 0.01$). For *C. tropicalis* and *C. parapsilosis*, final cell counts were similar to those observed after treatment with nystatin ($P > 0.05$). Conclusion: *Matricaria recutita* essential oil showed promising anti-*Candida* activity, in particular against *C. tropicalis* and *C. parapsilosis*.

P090

Echinocandin resistant *Candida parapsilosis* bioprosthetic valve endocarditis successfully treated with combined medical and surgical treatment

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Objectives *Candida parapsilosis* is a rare cause of infectious endocarditis, associated with high mortality rate, occurring usually in prosthetic valve recipients and intravenous-drug abusers. A rare case of echinocandin resistant *Candida parapsilosis* aortic valve endocarditis, successfully treated with valve replacement in combination with liposomal amphotericin B (L-AMB) and micafungin regimen is described.

Case presentation A 54-year-old man with bioprosthetic aortic valve presented with fever and pleuritic chest pain. Three months earlier he suffered acute gallstone pancreatitis and underwent cholecystectomy, complicated by cholorrhea and fever. Stents were placed at the pancreatic and the common bile duct, while two sets of blood cultures grew *Candida parapsilosis*. He was treated with fluconazole until 2 weeks after fever's resolution. A transthoracic echocardiogram at that time did not reveal signs of infectious endocarditis (IE). The patient had history of systemic lupus erythematosus treated with hydroxychloroquine, hypertension treated with metoprolol and quinapril and epilepsy treated with oxcarbazepine. Upon the present admission he was febrile (38 °C) and examination revealed a systolic murmur of 3/6. An echinocandin resistant *Candida parapsilosis* has been isolated from 4 sets of blood cultures and he was commenced on L-AMB and fluconazole. A 9 mm aortic valve abscess was detected at the transesophageal echocardiogram, causing severe valve dysfunction, while a computed tomography of the abdomen revealed multiple splenic emboli. Thus, by Duke's criteria, the patient was suffering candidal endocarditis of the aortic valve. Due to the severity of the disease and the persistence of fungemia, he underwent aortic valve replacement, and was commenced on L-AMB (5 mg kg^{-1} od) and micafungin (150 mg od) for a total duration of 3 months. Furthermore, biliary and pancreatic stents were removed and a biliary sphincterotomy was performed. He was discharged on a continuing regimen of oral fluconazole (400 mg d^{-1}). During a 24 months follow-up the infection did not recur.

Conclusions The present case emphasizes the importance of *Candida parapsilosis* as nosocomial pathogen for endocarditis in prosthetic valve recipients. Since optimal therapy has not been established yet, a combination of surgical and prolonged antifungal treatment is suggested.

P091

Experimental study to assess the virulence of clinical strains of *Candida-psilosis* complex, through the use of 'eggs model' as an animal model

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Objectives *Candida spp* is a commensal yeast in the healthy human being but could be pathogen in human being with a reduced or modified level defenses disorder. In the study of these pathogens, the animal models play an important role, embryonated eggs have been used to study features virulence various bacteria and viruses, instead of in this study, we have investigated the pathogenesis of *Candida-psilosis* complex infections in chicken embryos infected via the chorio-allontoic membrane (CAM) and we have analyzed the virulence and pathogenicity.

Methods The embryoneted eggs of chicken are an animal model used also for the growth of other pathogens. In this study we realize an inoculum *Candida parapsilosis* ATCC, *Candida orthopsilosis* ATCC, *Candica metapsilosis* ATCC in order to provoke an infection in the CAM. The CAM is composed with allantoide and corion, envelops completely the embryo.

For each experiment the eggs have been placed in incubator at 37 °C and 60% of humidity, daily have been supervised the quality and vitality of the embryos. Considering the life cycle of embryo, lasts 21 days before arriving to the inception, in the 8th has been carried out the inoculum. *Candida-psilosis* complex has been placed in YPD at 37 °C over night. The day after has been done the inoculum with 10^8 and 10^7 CFU of each strains. In order to realize the inoculum, two holes have been made in the shell, one in the air chamber and the other at 90 grade compared to the first. In the second hole the eggs have been infected, these eggs have been placed in incubator monitoring the temperature, the humidity and the vitality. From the eggs no longer vital have been removed the CAM, and have been done culture on agar plates and histologic examination.

Results From the data obtained it is evident that some factors negatively affect embryo viability and the survival rate drops considerably. Infection on the developmental day 8 with 10^8 cfu/egg leds to a rapid higher mortality. This depends on the development of the embryo and the infectious dose. Infection does of 10^8 resulted high mortality rate. While the percentage of survival increases considerably with a concentration of 10^7. The less virulent strain are *Candida orthopisilosis*.

Conclusions Through the use of the eggs model, in every experiments that we have been made, all the pathogens analyzed have been noticed by culture and histologic assay. our results suggest that embryonated eggs can be a very useful alternative infection model to study *Candida spp* virulence and pathogenicity.

P092

Fluconazole treatment response in a *Galleria melonella* infection model using wild-type and trailing *C. tropicalis* isolates- a cause for concern?

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Objective Despite being generally fluconazole susceptible some *C. tropicalis* isolates display 'trailing' growth. Trailing growth was originally defined for isolates classified as susceptible after 24 h of incubation but resistant after 48 h using the 80% growth inhibition endpoint recommended for the CLSI M27A methodology. Such isolates were found susceptible *in vitro* if using a 50% growth inhibition endpoint and *in vivo* in a mouse model of disseminated candidiasis.

On this background the CLSI methodology was subsequently revised in 2011 with recommendation of an endpoint reading after 24 h using a 50% inhibition.

Using the EUCAST EDef 7.2 susceptibility testing a noticeable proportion of *C. tropicalis* isolates display only approx. 50% growth inhibition over a broad concentration range already at 24 h, complicating MIC determination. How these isolates respond to standard doses of fluconazole is unclear, and recommendations for treatment is lacking. We therefore tested *C. tropicalis* isolates with various fluconazole susceptibility patterns in an *in vivo* larvae model in order to ascertain whether trailing and wild-type (wt) isolates respond equally well to fluconazole treatment.

Method Four clinical isolates of *C. tropicalis* (one wt (MIC: 0.5 mg l^{-1}), two trailing (T1 and T2), one resistant MIC: >16 mg l^{-1}) (R)) and one type strain (ATCC750, MIC 1 mg l^{-1}) were selected. Last instar *Galleria mellonella* larvae weighing approximately 278 mg (range: 250–325) were chosen. Groups of 15 larvae were injected with 10 µL aliquots of each *Candida* suspension (~ 5*10^7 CFU ml^{-1}) at the last left proleg (giving a 100% mortality for all isolates), followed after 30 min with 10 µL of PBS (control) or fluconazole (adjusted to 0.25, 1, 5, and 20 mg kg^{-1} larvae) injected in the last right proleg. 20 mg kg^{-1} provides an AUC of approximately 408 mg*h l^{-1} corresponding to that achieved in humans on standard dosing. Uninfected larvae receiving only PBS or fluconazole were included as control (100% survival). Larvae were kept in the dark at 37 °C. Survival was monitored daily for 5 days. Target inoculum concentration was confirmed by CFU determination using the spot technique (20 µl spots of 10-fold dilutions).

Survival proportions; fluconazole 20 mg/kg larvae

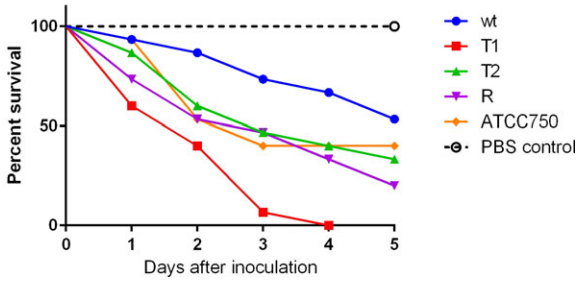

Figure 1

Table 1

Isolate	Inoculum (CFU/mL)	Fluconazole (mg/kg)	Surviving larvae (% of total) Day 1	Day3	Day5
wt	6.5*10^7	PBS	0 (0)	0 (0)	0 (0)
		0.25	7 (47)	1 (7)	1 (7)
		1	11 (73)	7 (47)	6 (40)
		5	12 (80)	5 (33)	3 (20)
		20	14 (93)	11 (73)	8 (53)
ATCC750	1.7*10^7	PBS	1 (7)	0 (0)	0 (0)
		0.25	2 (13)	1 (7)	1 (7)
		1	10 (67)	1 (7)	0 (0)
		5	12 (80)	6 (40)	6 (40)
		20	14 (93)	6 (40)	5 (33)
T1	9.3*10^7	PBS	2 (13)	0 (0)	0 (0)
		0.25	4 (27)	0 (0)	0 (0)
		1	10 (67)	1 (7)	0 (0)
		5	13 (87)	0 (0)	0 (0)
		20	9 (60)	1 (7)	0 (0)
T2	3.3*10^7	PBS	0 (0)	0 (0)	0 (0)
		0.25	14 (93)	3 (20)	2 (13)
		1	9 (60)	3 (20)	2 (13)
		5	10 (67)	2 (13)	1 (7)
		20	13 (87)	7 (47)	5 (33)
R	5.8*10^7	PBS	5 (33)	0 (0)	0 (0)
		0.25	12 (80)	1 (7)	0 (0)
		1	14 (93)	5 (33)	2 (13)
		5	14 (93)	4 (27)	2 (13)
		20	11 (73)	7 (47)	3 (20)
None		PBS	15 (100)	15 (100)	15 (100)
		0.25	8 (100)	8 (100)	8 (100)
		20	8 (100)	8 (100)	8 (100)

Light grey marking: survival 30-50%. Dark grey marking: survival below 30 %.

Results A dose dependent response was observed for all isolates (table). Only the highest dose of 20 mg kg^{-1} larvae was able to protect >50% of the larvae (table). For this dose mortality was significantly different comparing larvae infected with the five strains [$P = 0.002$ log-rank (Mantel Cox)]. Mortality among larvae challenged with the trailing isolates (20 mg kg^{-1} group) was similar to that for larvae challenged with the resistant isolate and statistically higher than for larvae challenged with the susceptible ($P \leq 0.0001$) [log rank (Mantel-Cox)] for T1 whereas only numerically for T2 ($P = 0.18$) (figure).

Conclusion The two trailing *C. tropicalis* isolates were both less susceptible to fluconazole *in vivo* when compared with the wt isolate. Mortality rates for the trailing isolates were similar to those for the resistant isolate. For T1 this difference reached statistical significance. This observation questions if fluconazole is an appropriate choice for infections due to such isolates and strongly suggests further studies on this issue are warranted.

P093

Efficacy of caspofungin, fluconazole and micafungin for the treatment of the infection caused by *Candida glabrata* complex in the non-conventional model *Caenorhabditis elegans*

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Objectives To study the virulence of *Candida glabrata*, *Candida bracarensis* and *Candida nivariensis* in a *Caenorhabditis elegans* model of invasive candidiasis and to evaluate the efficacy of caspofungin, fluconazole and micafungin for the treatment of these infections.

Methods Ten thousand nematodes (AU37 mutant strain with increased susceptibility to various pathogens) were distributed in groups of 1700 individuals and infected with each of the following reference strains: *Candida glabrata* NCPF 3203, *Candida glabrata* ATCC 90030, *Candida nivariensis* CBS 9984, *Candida nivariensis* CECT 11998, *Candida bracarensis* NCYC 3397 and *Candida bracarensis* NCYC 3133. The nematodes were distributed onto agar plates with the different species of *Candida* and allowed to feed for 2 h at 25 °C. Afterwards, 20 individuals were dispensed into each well of a microtitre plate that contained M9 buffer, 10 µg ml^{-1} of cholesterol in ethanol, and 90 µg ml^{-1} of kanamycin. For the evaluation of treatment efficacy, the nematodes were treated with 4 and 8 µg ml^{-1} of caspofungin and micafungin, and 32, 64 and 128 µg ml^{-1} of fluconazole. The plate was incubated at 25 °C overnight and a visually scoring of live and dead worms was done at 24 h for 6 days. The survival obtained with each treatment was analyzed with Kaplan-Meier survival curves and the differences between them were estimated by the log-rank test with SPSS 15.0 software.

Results The infection by *Candida glabrata* and *Candida nivariensis* strains reduced significantly the survival of *Caenorhabditis elegans* in comparison to non-infected nematodes. However, no differences in survival were obtained among nematodes infected with *Candida bracarensis* and uninfected nematodes. *Candida nivariensis* was less virulent than *Candida glabrata* ($P < 0.05$). Survival of the nematodes infected with *Candida glabrata* and *Candida nivariensis* was significantly higher after treatment with candins at 120 h ($P < 0.05$). Whereas the higher survival of the nematodes infected with *Candida glabrata* and *Candida nivariensis* CBS 9984 was after treatment with 8 µg ml^{-1} of micafungin, the highest survival of the nematodes infected with *Candida nivariensis* CECT 11998 was reached with 8 µg ml^{-1} of caspofungin.

Conclusions *Candida glabrata* was the most virulent species. This *Caenorhabditis elegans* model was not useful for assessing *Candida bracarensis* infection. Candins were effective for the treatment of candidiasis caused by *Candida glabrata* and *Candida nivariensis*.

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P094

Clinical significance and results of fungal cultures in surgical patients treated with antifungals due to intra-abdominal infections

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Objectives Definitions of candidemia, invasive candidiasis and candida peritonitis confuse the minds of physicians in Surgical Patients with health-care associated infections. This confusion is caused by overuse of antifungal agents. We aimed to determine the rate of antifungal use and to evaluate the results of fungal cultures and clinical management together about fungal intra-abdominal infections in general surgery patients treated with various antifungals.

Methods The list of patients to whom antifungals were given was obtained from the hospital pharmacy between January 1, 2012 and December 31, 2014. Mycology laboratory results and patients' records were examined for mycological and clinical data of adult (>18 years) patients, retrospectively. Patients were seperated into two groups according to the fungal culture positivity. The data was analysed statistically. Multivariate, logistic regression analysis was performed for the determination of independent risk factors for culture positivity.

Results During the study period, 114 patients had used systemic antifungal treatment. None of the patients received antifungal prophylaxis. Of the 114 patients, 59 (51.7%) had positive fungal cultures, 55 (48.3%) had negative fungal cultures. The demographic characteristics of the patients and risk factors are presented in the table 1 and table 2. There were more than one culture positivity in different clinical specimen of 12 (20.4%) patients. Of culture positive patients, 31 (52.6%) had *C. albicans*. Laparoscopic procedures were performed in only two patients in culture positivite group. In multivariate logistic regression analysis, the significant risk factors for fungal culture positivity were determined to be parenteral nutrition ($P = 0.04$), previous beta lactam and beta lactamase inhibitory antibiotic usage ($P = 0.005$), postoperative stay in ICU ($P = 0.05$) and the initiation of antibiotics in the early period of hospitalization ($P = 0.05$). In the first week of hospitalization, the number of patients used beta lactam and beta-lactamase inhibitory antibiotics was 26 (44%) in culture positive group and 10 (18.1%) in culture negative group ($P = 0.002$). The most common antifungal used was fluconazole (94 patients 82.5%) followed by anidulafungin (5 patient 4.4%) and caspofungin (4 patient 3.5%), also in 11 (9.7%) patients antifungal drugs had been changed.

Conclusion Indications for use of antifungal drugs are not clear in intra-abdominal infections, In our study, especially in immunocompromised patients regardless of culture positivity use of antifungal agents was more common. Significant risk factors detected in the culture positive group were not different from those for all other candida infections. In addition, beta lactam and beta-lactamases inhibitory antibiotic usage in early periods of hospitalization increased fungal culture positivity. Antimicrobial stewardship strategies will reduce fungal infection.

Table 1: Demographic characteristics and risk factors of the patients

	Culture positive n= 59 (%)	Culture negative n = 55 (%)	Chi-square p value
Age (mean ±SD)*	58.95 ± 16.7	58.40 ± 14.7	0.85
Gender (Male)	33 (55.9)	31 (56.4)	0.96
Primary diseases			
Upper gastrointestinal	12 (19.0)	6 (11.8)	0.16
Lower gastrointestinal	27 (42.9)	16 (31.4)	0.06
Hepato-pancreatico-biliary	13 (20.6)	19(37.3)	0.13
Others	11 (17.5)	10 (19.6)	0.94
Diabetes mellitus	10 (16.9)	5 (9.1)	0.21
Previously immunosuppression	4 (6.8)	12 (21.8)	**0.021**
Malignite	33 (52.4)	30(54.5)	0.88
Previous antibiotic usage (One month)	56 (94.5)	46 (83.6)	**0.05**
Beta lactam and lactamases inhibitory	39 (66.1)	22 (40.0)	**0.005**
First generation cephalosporin	9 (15.3)	8 (14.5)	0.91
Third generation cephalosporin	19 (32.2)	15 (27.3)	0.56
Metronidazole	22 (37.3)	13 (23.6)	0.11
Quinolone	20 (33.9)	11 (20.0)	0.09
Other Carbapenem/Ertapenem	31 (52.5)/8 (13.6)	24(43.6)/11 (20.0)	0.34/0.35
Tigecycline	15 (25.4)	12 (21.8)	0.65
Glycopeptide	7 (11.9)	10 (18.7)	0.34
Previous azole usage (one month)	5 (8.8)	6 (10.9)	0.66

*Independent groups t test were used

Table 2: Demographic characteristics and risk factors of the patients

	Culture positive n= 59 (%)	Culture negative n = 55 (%)	Chi-square p value
Number of abdominal intervention			
No intervention	10 (16.9)	11 (20.0)	0.36
One time	15 (25.4)	17 (30.9)	
One more than	34 (57.7)	27 (49.1)	
Hospitalization days before antibiotic started (min- max)*	5.22 ± 5.65 (1-31)	7.25 ± 9.28 (1-45)	**0.15**
Hospitalization days before antifungal started (min- max)*	24.76 ± 19.72 (1-109)	21.35 ± 17.81 (1-90)	0.33
Recurrent gastrointestinal perforation	18 (30.5)	16 (29.1)	0.86
Anastomotic leakages	20 (33.9)	11 (20.0)	0.09
Central venous catheter	41 (69.5)	33 (60.0)	0.28
Parenteral nutrition	47 (79.7)	30 (54.5)	**0.004**
Dialyses	10 (17.0)	7 (12.7)	0.52
Length of stay intensive care unit (day) * (min- max)	19.51 ± 25.95 (0 - 88)	9.27 ± 14.00 (0 - 81)	**0.011**
Postoperative length of hospital stay(day)* (min- max)	63.80 ± 34.64 (11 - 128)	49.75 ± 30.49 (6 - 125)	**0.024**
On the beginning day of antifungals,			
Fever (> 38 °C)	35 (59.3)	33 (60.0)	0.94
White Blood Cell (<4.000 ->10.000 µL)*	11 671 ± 6241	13 734 ± 8236	0.13
Candida scores (≥ 3)	23 (39.0)	23 (41.8)	0.75
Crude mortality	26 (45.6)	23 (41.8)	0.80

*Independent groups t test were used

P100

Fungal Rhinossinusitis: a 8 year review study

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Objectives Fungi have a controversial role in rhinossinusitis and there are still some difficulties in the categorization of fungal rhinossinusitis, according to its clinical presentation, and to associated immune and pathological responses. We present a review of patients with fungal rhinossinusitis diagnosed and treated in our institution in the past 8 years.

Methods Case review of patients diagnosed with fungal rhinossinusitis. Clinical charts were reviewed, and data collected concerning clinical presentation, surgical and medical treatment, results of histophatologic examination, direct examination and culture of samples.

Results 9 patients were enrolled in this study, including two cases of invasive disease with accurate diagnosis of the involved fungus, and 7 cases of non-invasive disease. The later included 4 cases of fungus ball, in which the agent could be detected from histopathologic examination, but there was no isolation from samples. In the other 3 cases of non-invasive disease, there was a high index of suspicion of a fungal rhinossinusitis from the imaging exams, surgery findings and the presence of an eosinophilic mucin at histopathologic examination, but no fungi was found on culture, and they were subsequently classified as eosinophilic mucin rhinossinusitis.

Conclusion Fungi are a relatively rare etiology of rhinossinusitis and a controversial topic that includes the unclear role of fungi in

diseases such as eosinophilic mucin rhinossinusitis. Culture and direct examination are sometimes difficult to obtain, however they are essential for a correct classification of fungal rhinossinusitis. Ultimately it allows the choice of targeted therapy.

P101

Rare cause of the diabetic foot: invasive fungal infections

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Objectives Diabetic foot infections compromise an important part of complicated skin and soft tissue infections and are expected to increase even more in the future. Diabetic foot infection complication rate in diabetes is increasing variety of factors; inefficient treatment of osteomyelitis and its chronic trend, resistant organisms commonly seen in diabetic foot infection. Most of the infections in diabetic foot are of aerobic and anaerobic bacterial origin, and in most cases polymicrobial, which has been characterized in detail although a few studies have reported some filamentous fungi and yeasts as etiological agents of diabetic foot infections. Fungal infections can appear as superficial or invasive tissue infections. Here, it was aimed to evaluate fungal etiology of diabetic foot infections between June 2012–April 2015 prospectively.

Methods Diabetic foot infection cases that were admitted between June 2012 to April 2015 and followed up by Ege University Faculty of Medicine, Department of Infectious Diseases and Clinical Microbiology were recorded. Demographic data of patients, diabetes characteristics, wound characteristics, laboratory findings, physical examination findings, presence of osteomyelitis, presence of significant vascular stenosis, culture results, PEDIS infection classification scores, operation types if necessary were recorded. Bone or deep tissue samples were cultured on two SDA medium which were incubated 26 and 35 °C at 10 days. The yeast colonies were identified by conventional methods (germ tube and their micromorphological appearance on corn meal agar) and ID32C (bioMérieux-France) carbohydrate assimilation features. Filamentous fungi were evaluated depending on their appearance on media and microscopy using lactophenol cotton blue.

Results Bone or deep tissue cultures were positive in eleven of 531 cases who were followed with the diagnosis of diabetic foot infection (8 female, 3 male, mean age 59.7 ± 9 years). Age, gender, diabetes type, wound characteristics, treatment types and results, bacteriology and mycology of the ulcers were summarized in table 1. Four patients have history of prior diabetic foot infection and arterial narrowing causing decreased blood flow on their lower extremities. In four of the cases antifungal therapy was also given besides the surgical treatment. Antifungal therapy was not given to two patients because they died before the culture results. Five patients were found to be positive for fungal culture after the amputation procedure. Since surgical wound boundaries were clean, antifungal theraphy was not given to these cases.

	Sex/age[1]	Type of DM[2], year of diagnosis	Osteomyelitis	Response to antibacterial therapy	Surgery, type of surgery	Site of ulcer(s)	Bacteriology of ulcer	Mycology of ulcer	Result of the treatment
1	F 79	Type 2, 10	Ys	No	Yes, below knee amputation		*Hafnia alvei*	*Candida lipolytica*	Although fluconazole therapy rapidly progressed to amputation
2	F 53	Type 2, 11	Yes	Yes, partially	Yes, Transmetatarsal amputation		*P aeruginosa.*	*C. glabrata*	Although amphotericin B therapy rapidly progressed to amputation
3	M 63	Type 2, 10	Yes	No	Yes, Above-knee amputation		*P.aeruginosa*	*C. albicans*	Amputation performed without resulting culture
4	M 49	Type 2, newly diagnosed	Yes	No	Yes, Finger amputation		*P.aeruginosa*	*C. albicans*	Amputation performed without resulting culture
5	M 64	Type 2, 16	Yes	Yes, partially	Yes, Ray's amputation		-	*C. albicans*	Amputation performed without resulting culture

Figure 1. Patients demographic data and wound characteristics-1

	Sex/age[1]	Type of DM[2], year of diagnosis	Osteomyelitis	Response to antibacterial therapy	Surgery, type of surgery	Site of ulcer(s)	Bacteriology of ulcer	Mycology of ulcer	Result of the treatment
6	M 61	Type 2, 12	Yes	No	Yes, Transmetatarsal amputation		*P. aeruginosa*	*Eucarium spp.*	Amputation performed without resulting culture
7	M 68	Type 2, 19	Yes	Yes, partially	Yes, Transmetatarsal amputation		-	*C. parapsilosis*	Patient died before culture results
8	F 50	Type 2, 8	Yes	No	No		*S. agalactiae*	*C. glabrata*	After amphotericin B therapy, Healed
9	M 63	Type 2, 3	No	Yes, partially	Yes, Ray's amputation		*Klebsiella oxytoca, Enterobacter cloacae*	*C. krusei*	Amputation performed without resulting culture
10	M 53	Type 2, 23	No	Yes, partially	No		-	*C. glabrata*	Patient died before culture results
11	M 54	Type 2, 15	No	Yes, partially	Yes, Only debridement		*S.aureus*	*C.albicans*	After fluconazole therapy, Healed

Figure 2. Patients demographic data and wound characteristics-2

Conclusion Although rare, invasive fungal infections can cause diabetic foot infection. Even the the superficial fungal infections are more frequent in patients with diabetes we should keep an open mind and to consider the possibility of invasive funcal infections are also the causative pathogen in deep tissue infections. Because of that we should think fungal pathogens in cases these do not respond the long-term antibiotic therapy. Treatment must include surgical debridement and antifungal therapy. Fluconazole may be a reasonable alternative to amphotericin B in fungal osteomyelitis. Also osteomyelitis caused by fungal pathogens in literature are very few.

This study is one of the large series in the literature regarding invasive fungal infections in patients with diabetic foot infection.

P102/M6.1

An uncommon cause of primary adrenal insufficiency in Central Europe

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Objectives In Western countries autoimmune processes are the most common reason for primary adrenal insufficiency (Addison's disease), however in countries of high prevalence disseminated tuberculosis or systemic mycosis are major causes. Aim of our case presentation is to bear in mind the similarity of tuberculosis and systemic fungal infections in a patient presenting with bilateral adrenal enlargement and primary adrenal insufficiency.

Methods We report a single case of a systemic fungal infection associated with primary adrenal insufficiency caused by the dimorphic fungus Paracoccidioides brasiliensis.

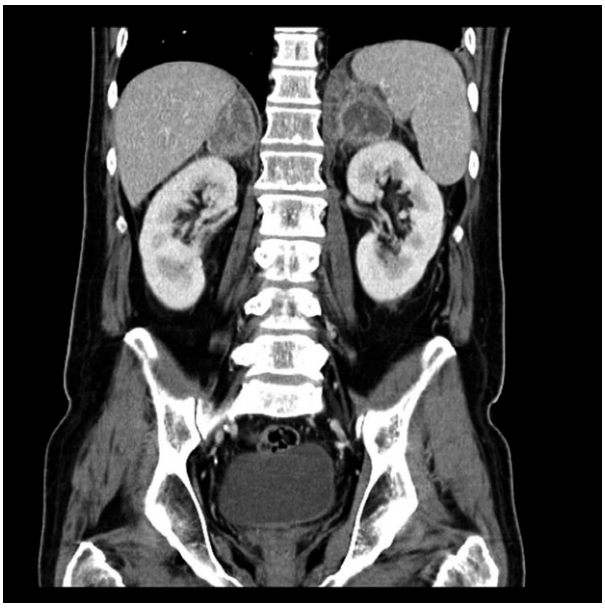

Figure 1. Abdominal CT scan

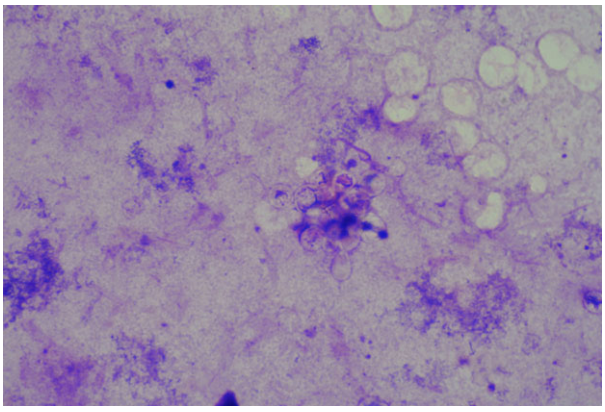

Figure 2. Giemsa stain

Results A 62-year-old man was admitted to our hospital with generalized weakness, left sided chest and abdominal pain, weight loss and night sweats. Past medical history revealed several episodes of pulmonary tuberculosis, initially diagnosed at the age of 7, followed by several reactivations, recurrently treated with tuberculostatic therapy. Five years prior to admission tuberculosis of adrenal glands was diagnosed by CT guided biopsy at another hospital based on histopathological finding of epitheloid cell granuloma with central necrosis. The patient is currently retired. More than 10 years ago he lived for several years in a rural area of Peru located next to the Amazonas to build a hospital. He had a 45 pack-year history of smoking. CT scan showed an increasing inhomogeneous enlargement of both adrenal glands and alteration of the upper lobe of the left lung, both suspicious for reactivation of tuberculosis. Afterwards performed PET scan revealed an increased metabolic activity of the enlarged adrenal glands, hypermetabolism in cervical lymph nodes but not the lung. Ziehl-Neelsen smear of the sputum showed no acid-fast bacteria and PCR as well as culture were negative for Mycobacterium tuberculosis. Serology for HIV, lues and adrenal autoantibodies were negative. Cerebral imaging due to neurologic symptoms revealed a cortical temporo-parietal located lesion, suspicious for infection or metastasis. Liquor fluid of lumbar puncture was unremarkable. Serum levels of basal ACTH and cortisol confirmed primary adrenal insufficiency, considering bilateral adrenal gland enlargement and medical history highly consistent with reactivation of tuberculosis. However, all available reports of prior hospitalizations did not reveal any microbiological confirmation of mycobacterial infection. Subsequently CT guided biopsy of the left adrenal gland and extirpation of a right cervical lymph node were performed, in neither tissue nor fluid Mycobacterium tuberculosis was detectable. Fludrocortisone and hydrocortisone were commenced for adrenal insufficiency. Histology of left adrenal gland biopsy showed granulomas with central necrosis. PAS, McManus and Grocott stain revealed evidence of fungal pathogens. Paracoccidioides brasiliensis was identified by microscopy (Giemsa stain), culture (Sabouraud agar) and PCR. Due to evidence of cerebral involvement the patient was commenced on intravenous liposomal amphotericin B followed by oral itraconazol.

Conclusion Paracoccidiomycosis (South American blastomycosis), a systemic fungal infection, should be considered for differential diagnosis in patients travelled to or lived in South or Central American countries. Furthermore we want to emphasize the clinical and histopathological similarities with tuberculosis to avoid misdiagnosis.

P103

Virulence of *Trichoderma longibrachiatum* and experimental treatment of invasive infection

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Objective To assess the virulence of *Trichoderma longibrachiatum*, and to evaluate the efficacy of Amphotericin B deoxycolate (AMB), Liposomal Amphotericin B (LAMB), Voriconazole (VRC) and Micafungin (MFC) in an immunosuppressed murine model of invasive infection.

Methods Cyclophosphamide-immunosuppressed male OF-1 mice with a mean weight of 30 g were used. Virulence was evaluated through survival (8 animals/group) and fungal burden reduction in kidney, spleen, liver, lung and brain (8 animals/group), after i.v. infection with 2 strains of *T. longibrachiatum* (UTHSC 07-3636, 09-2900) at 1×10^4, 1×10^5, 1×10^6 or 1×10^7 CFU/animal. For treatment, mice challenged with 1×10^7 CFU/animal of *T. longibrachiatum* received AMB at 0.8 mg kg^{-1} i.v., once a day (QD); LAMB at 20 mg kg^{-1} i.v., QD; VRC at 25 mg kg^{-1} p.o, by gavage QD; or MCF at 10 mg kg^{-1} i.p., QD. Efficacy was evaluated through survival rates and of fungal load reduction in liver and spleen.

Results The virulence study showed an inoculum size-dependent response with 25%, 25% and 60% mortality 15 days after infection with 1×10^4, 1×10^5 and 1×10^6 CFUs, respectively, while the highest inoculum showed a mortality rate of 100%.

On day 6 post infection, fungal cells were recovered from all the organs in mice infected with higher inocula. The number of CFUs recovered correlated with the inoculum size. Significantly higher counts were found in animals receiving 1×10^6 or 1×10^7 CFU/animal compared with those challenged with lower inocula. Spleen and liver were the most affected organs regardless of the strain and the inoculum size assayed.

AMB, LAMB, VRC and MCF were unable to prolong survival of mice compared with the controls, irrespective of the infective strain. VRC and MCF were not able to reduce fungal loads in spleen or liver of mice challenged with any of the strains; however, AMB reduced the burden in liver of mice infected with the strain UTHSC 07-3636, (AMB MIC of 0.13 μg ml^{-1}) and LAMB showed a significant reduction over controls and other therapies in both organs of animals infected with this strain ($P \leq 0.0002$).

Conclusion This is the first study to develop an immunosuppressed murine model of disseminated infection by *T. longibrachiatum*. Only

higher inocula were able to invade and proliferate in all organs tested.

No therapies improved survival and, with the exception of LAMB, no therapy was effective in reducing fungal loads. LAMB was effective against the strain with the lowest MIC for AMB (0.13 μg ml^{-1}), while no reduction was observed in mice infected with the strain UTHSC 09-2900 (MIC 2 μg ml^{-1}).

The high level of resistance shown in our study is of concern. Further studies should be carried out with more isolates that represent a wider range of AMB MICs in order to assess whether there is any relationship between MIC values and efficacy of LAMB.

P104

A cerebral aspergillosis case whom treated with intrathecal amphotericin B

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Objective Early diagnosis of aspergillosis of central neural system with non-typically clinical and cerebrospinal fluid findings is so important. The disease is dramatically progressive, its treatment is quite difficult, and it has high mortality rates. We represented our experience with cerebral aspergillosis patient who treated with non-clasical.

Method CASE: 47 years old, male patient who had chronic lymphocytic leukaemia (CLL) since 2011 and treated with 11-dose chemotherapy and additionally he had been followed for type 2 diabetes mellitus for 10 years. In October 2014 ibrutinib, a tyrosine kinase inhibitor, treatment were given to him. Weakness in left lower and upper extremities and speech disorder were developed at the tenth day of his treatment and he was hospitalized with early diagnosis of cerebral mass or bleeding.

Multiple lesions with peripheric contrast enhancement were seen in fronto-parietal cortex and sub-cortex in his magnetic resonance imaging (MRI) examination (image 1). Upon this lomber punction were done and non-specific meningitis treatment and trimetoprim-sulfamethoxasol (TMP-SMZ) medication were started for cerebral toxoplasmosis and cerebral abscess. However on the fourth day of the treatment, *Aspergillus niger* were detected in his cerebrospinal fluid culture. Upon this, non-specific meningitis treatment and TMP-SMZ treatment were terminated and voriconasol treatment was started as 4 mg kg^{-1} in every 12 h after 6 mg kg^{-1} bolus infusion. No fever and confusion were seen in his follow-up but anti-epileptic treatment was started for local convulsions, which were seen on his left arm. New lesions were seen in control MRI examination after 4 weeks voriconasol treatment and upon this control cerebrospinal fluid culture were done. Voriconasol treatment was prolonged due to negative cerebrospinal fluid culture. Occasionally diazepam infusion was done because of the on going local convulsions and a generalized convulsion. Regression on the contrast and the shapes of the lesions and a new lesion on right frontal lob were found in control MRI of the voriconasol treatment on the 52nd day. Due to lack of histopathologic diagnosis and new lesions that occurred despite treatment brain biopsy were planned to the patient. 'Abscess, necrosis and fungal hypha's in necrotic areas'were found as the result of the biopsy, which were done from frontal lob (image 2). Due to new lessions and confirmation of histopathologic diagnosis, 0.1 mg intrathecal conventional amphotericin B treatment were added two times in a week to treatment. Regression with no new lessions was found in the cerebral MRI with contrast after 8 dose of intrathecal amphotericin B. After additionaly 2 dose intrathecal amphotericin B, patient was discharged with oral voriconasol treatment.

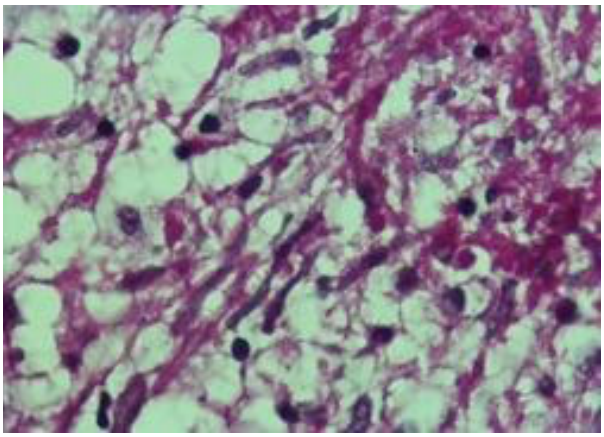

Figure 1. Multiple lesions with peripheral contrast enhancement in fronto-parietal cortex and sub-cortex in cerebral magnetic resonance imaging with contrast

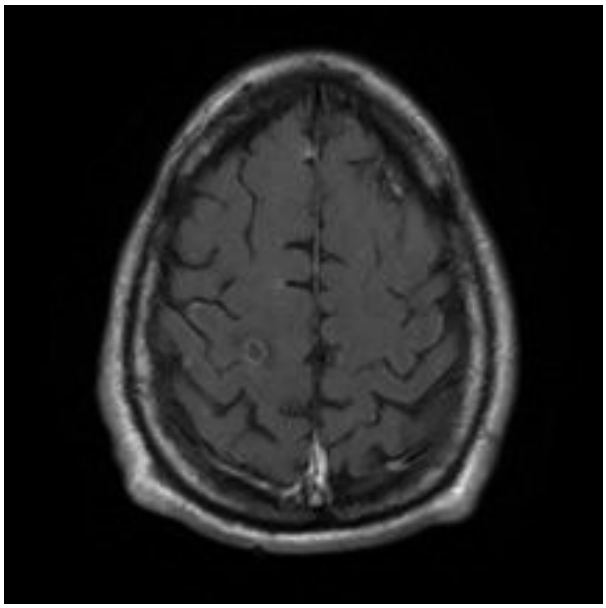

Figure 2. Fungal hypha's which detected with PAS (periodic acid shift) dye

Conclusion Our experience with this case showed us intrathecal amphotericin B could use for treatment of the patients who had no response to voriconasol. This case could be the first case in literature that shows the usage of intrathecal amphotericin B for this indication.

P105

A randomised study on the safety of daily, intermittent, or weekly administration of 1, 3 or 10 mg kg^{-1} of AmBisome as prophylaxis in acute leukaemics

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Background Antifungal prophylaxis is effective in reducing invasive fungal disease (IFD) incidence and mortality in patients undergoing

induction-consolidation (IC) chemotherapy for acute leukaemia. Spectrum of activity, bioavailability, drug-drug interactions, and toxicity mean that no one antifungal agent is universally applicable. High dose liposomal amphotericin B (L-AmB) administered intermittently can achieve adequate tissue levels for effective prophylaxis, is safe and well tolerated. To date, the safety and efficacy of different dosing regimens of L-AmB as antifungal prophylaxis has not been directly compared in the acute leukaemic setting.

Methods In this open-label, parallel-group randomised controlled trial patients ≥18 years receiving IC chemotherapy for acute myeloid (AML) or acute lymphoblastic leukaemia (ALL), with no history of IFD were randomised (1:1:1:1) to either 1 mg kg^{-1} daily, 3 mg kg^{-1} twice-weekly, 3 mg kg^{-1} three times-weekly or 10 mg kg^{-1} once-weekly of L-AmB. Patients commenced their respective dosing regimen of L-AmB within 96 h of commencement of each course of chemotherapy and were followed up for 52 weeks or until death, if earlier. The primary endpoint was the rate of all study drug-related adverse events (AE) by completion of all L-AmB prophylaxis. Secondary end-points included incidence of proven or probable IFD according to the EORTC/MSG (European Organization for the Research and Treatment of Cancer/Mycoses Study Group) criteria and overall and IFD-related mortality. Analysis was by intention-to-treat and included all enrolled patients who received at least one dose of L-AmB. This trial is registered with ClinicalTrial.gov as NCT00451711.

Results 53 eligible patients were recruited from two Australian centres between Aug 2008 and Mar 2014. Seventeen, 3, 18 and 15 were randomly assigned to 1 mg kg^{-1} daily, 3 mg kg^{-1} twice-weekly, 3 mg kg^{-1} three times-weekly or 10 mg kg^{-1} once-weekly of L-AmB, respectively. One patient randomised to 1 mg kg^{-1} daily arm did not receive a single dose of L-AmB. Fifty-one patients had AML and 2 had ALL. Of the 52 patients who received at least one dose of L-AmB 6/16 (37.5%), 2/3 (66.7%), 10/18 (55.6%) and 9/15 (60%) of patient receiving 1 mg kg^{-1} daily, 3 mg kg^{-1} twice-weekly, 3 mg kg^{-1} three times-weekly or 10 mg kg^{-1} once-weekly of L-AmB, respectively had a study drug-related AE ($P = 0.67$) with a serious AE recorded in 1/16 (6.3%), 0/3 (0%), 2/18 (11.1%) and 2/15 (13.3%), respectively ($P = 0.85$). Proven and probable IFD were diagnosed in 1/16 (6.3%), 0/3 (0%), 2/18 (11.1%) and 1/15 (6.7%) of those assigned to 1 mg kg^{-1} daily, 3 mg kg^{-1} twice-weekly, 3 mg kg^{-1} three times-weekly or 10 mg kg^{-1} once-weekly of L-AmB, respectively ($P = 0.89$). Overall mortality was similar between the groups: 5/16 (31.3%), 1/3 (33.3%), 1/18 (5.6%) and 4/15 (26.7%), respectively ($P = 0.25$). There were 2 IFD-related deaths in the1 mg kg^{-1} daily arm but no IFD-related deaths occurred in those assigned to either of the other 3 arms (i.e. 3 mg kg^{-1} twice-weekly, 3 mg kg^{-1} three times-weekly or 10 mg kg^{-1} once-weekly of L-AmB) ($P = 0.19$).

Conclusions Intermittent high-dose L-AmB is safe, feasible and effective as antifungal prophylaxis in high-risk patients receiving chemotherapy for acute leukaemia. Such a strategy is likely to have utility in the ambulatory setting, particularly for those intolerant of other antifungal agents. However, further studies are required for confirmation, including a comparison to broad-spectrum mould-active azoles.

P106

Evaluation of the *in vitro* activity of voriconazole as predictor of the *in vivo* outcome in a murine model of scedosporiosis

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Objectives To determine the possible correlation between *in vitro* activity and *in vivo* efficacy of voriconazole against *Scedosporium apiospermum* in a murine model of disseminated infection.

Methods TheMICs of voriconazole (VRC) against 40 strains of *S. apiospermum* was determined using a microdilution broth method (CLSI). Fifteen *S. apiospermum* strains showing different MICs ranging from 0.12 to 8 μg ml^{-1} were selected for efficacy studies. Groups of 16 OF-1 male mice (8 for survival studies and 8 for fungal load determination) were immunosuppressed with 200 mg kg^{-1} of cyclophosphamide every 5 days, starting 2 days before infection. Animals were challenged intravenously with $5x10^3$ CFU via the lateral tail vein and received VRC at 40 mg kg^{-1}, starting 1 day after infection and for 7 days, or no treatment (control group). When control animals began to die, animals were euthanased and kidneys and brain were aseptically removed to determine CFU g^{-1}. Efficacy was evaluated by fungal burden in brain and kidney and by survival.

Results In those animals infected with the strains showing the highest MIC values (4–8 μg ml^{-1}), VRC was not able to increase survival or to decrease fungal burden while for those with MICs of 0.12–0.5 μg ml^{-1} the drug showed significant efficacy. In those mice challenged with strains showing MICs of 1–2 μg ml^{-1}, we observed no correlation between survival and fungal burden and efficacy seems to be intermediate.

Conclusion Our results reveal a correlation between *in vitro* data and *in vivo* outcome. Although more strains will be tested to determine more accurately the relationship between MIC values and outcome, our preliminary results seem to indicate that VRC MICs ≥ 4 are indicative of resistance while ≤ 0.5 are indicative of susceptibility.

P107

Successful treatment of a proven *Aspergillus flavus* rhinosinusitis in a leukemia patient - Feasibility of intravenous 24 h continuous infusion of Amphotericin B deoxycholate

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Objectives 24 h continuous infusion of Amphotericin B Deoxycholate is a way of minimizing side effects of amphotericin B while maintaining efficacy in invasive aspergillosis as shown previously (BMJ 2001;322:579). The number of proven aspergillus infection, though, is low. Therefore it is of interest if a proven infection can successfully be treated by this approach.

Methods Intravenous 24 h continuous infusion of amphotericin B deoxycholate (Fungizone®) in a dose of 1 mg kg^{-1} body weight was administered as follows: Administration via a dedicated lumen of a quadruple central venous catheter. Coadministration of >1000 mL normal saline (NaCl 0.9%)/day plus intravenous KCl up to 160 mmol day^{-1} and sodium bicarbonate via separate lumina. Daily control of urine output (>2000 ml day^{-1}) or body weight, serum potassium levels (>4.0 mmol l^{-1}), serum bicarbonate (> 20 mmol l^{-1}) and changes of serum creatinine (preferably <100 umol l^{-1}).

Results A 50-year-old female with no relevant comorbidities was diagnosed with an acute myeloid leukemia, NOS (WHO 2008) in 2010 with a WHO (ECOG) performance status of 1.

She was treated with araC 200 mg/m2 (d1-7) and Idarubicin 12 mg/m2 d1-3. On day 16 the bone marrow was free of leukemia and the patient reached complete remission on day 30.

She initially presented with neutropenic fever. She was treated empirically with cefepime and acyclovir. On day 4 a CT scan of the head showed pansinusitis. Amphotericin B deoxycholate was started as continuous infusion and the dose escalated when creatinine was stable, up to 1.7 mg kg^{-1} of body weight (see Methods). The neutrophil count recovered on day 25 (>0.5 G/) but fever and signs of sinusitis continued. On day 29 the sinuses were operated. Histopathology showed invasive aspergillosis, cultures grew *Aspergillus flavus*.

On day 45 the second chemotherapy cycle was started.

On day 59 Amphotericin B dose was tapered down and stopped on day 64 because the fever had resolved. The patient successfully entered allogeneic bone marrow transplantation on day 100 with secondary antifungal prophyaxis with voriconazole and caspofungin. She is free of leukemia and fungal infection 5 years after treatment.
Conclusion This patient successfully recovered from a proven invasive *Aspergillus flavus* rhinosinusitis. Amphotericin B deoxycholate was administered as a 24 h continuous infusion over >50 days at a dose of 1–1.7 mg kg^{-1} body weight. Serum creatinine never raised to more than 2× the baseline levels. Potassium was checked daily and replaced rigorously. There were no acute side effects as chills. Efficacy in this patient was demonstrated and depended on several positive co-factors such as type of infection (rhinosinusitis), fungal agent (*Aspergillus flavus*), successful surgery, and most importantly cure of acute leukemia.

P108

Candida peritonitis unrelated with continuous ambulatory peritonea dialysis (CAPD) in adult patients in a Turkey hospital

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Objective We aimed to display that the epidemiology, prognosis and therapy of *Candida* peritonitis unrelated with CAPD in our hospital.
Methods A retrospective analysis of adult patients with *Candida* peritonitis between April 2011 and February 2014 was done. All of the peritoneal fluid samples, which yielded Candida species in microbial culture, were included to the study. Patient's characteristics, microbiological feature, prognosis and treatment were reviewed.
Results Thirteen patients with *Candida* spp. isolated from peritoneal fluid were examined. None of the patients were on peritoneal dialysis. All of them were considered to have *Candida* peritonitis. The data on these 13 patients were reviewed in the table. Seven of them were died in average 5 days. Most of the cultured *Candida* species in peritoneal samples were *C.albicans* but *C.krusei* yielded in three samples and *C.famata* in two. And also *Candida* spp. yielded with bacterial pathogens together such as *Enterobacteriacea* and *Enterococcus* species in seven patients. Surgical interventions seemed to be an important predisposing factor. Only in one patient, *Candida* yielded also in blood culture. No antifungal resistance was found in *C.albicans*, but *C.glabrata* was resistant to voriconazole and intermediate resistant to fluconazole. *C.krusei*, which yielded in three patients, was known to be, intrinsically resistant to fluconazole and also intermediate resistant to flucytosine. Mortality even in treated patients, were found very high. Mortality was found higher in patients with infections of non-albicans *Candida* (4/6) than *C.albicans* (3/7).
Conclusion *Candida* peritonitis is still associated with poor prognosis. Antifungal therapy can be suggested in critically ill patients with nosocomial peritonitis where *Candida* is diagnosed based on perioperative sampled peritoneal fluid. Rapid detection of *Candida* might be beneficial in this regard.

Table 1

Patient No	Age/Sex	Underlying Diseases	Intervention	Candida spp.	ICU	Antifungal Treatment	Mortality	Duration between diagnose and death*
1	69/M	Stomach cancer	Gastrectomy	C.albicans	Yes	Fluconazole	No	
2	62/M	Necrotizing pancreatitis	Mass extraction and gastro-jejunostomy	C.albicans	Yes	Fluconazole	Yes	5 days
3	66/M	Peptic ulcus perforation	Primer suturation	C.krusei	Yes	No treatment	Yes	6 days
4	63/M	Chronic liver disease+ Esophageal varices bleeding	Esophageal varices band ligation	C.albicans	Yes	Flukonazole	Yes	3 days
5	67/M	Sclerosing cholangitis	ERCP	C.albicans	No	Fluconazole	No	
6	17/W	Wilson Disease+ Hepatic transplantation	PTK	C.famata	Yes		No	
7	55/W	Choledocolithiasis	ERCP	C.albicans	Yes		Yes	7 days
8	72/W	Acute pancreatitis	Peritoneal lavage	C.parapsilosis	No		No	
9	48/M	Intestinal lymphoma	PTC	C.krusei+ C.glabrata	Yes	Caspofungin	Yes	14 days
10	82/W	Chronic liver disease+RCC	PTC	C.famata	Yes		Yes	4 days
11	83/M	Prostate carcinoma+ intestinal perforation	Resection+ ileostomy	C.albicans	Yes		No	
12	83/M	Diabetes mellitus+ Gastro-intestinal hemoragiae+renal failure	No intervention	C.albicans+ C.krusei	Yes	No treatment	Yes	1 day
13	85/W	Peptic ulcer perforation+ cardiac failure	Primer suturation	C.albicans	Yes		No	

ERCP=Endoscopic retrograde cholangio-pancreaticography, PTC=Percutane transhepatic cholangiography, RCC= Renal cell carsinoma

P109/M11.1

Cerebral Aspergillosis: Report of 2 cases with favorable response to voriconazole and neurosurgery

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Introduction and Objectives Central nervous system aspergillosis (CNSA) is an often fatal disease that usually affects immunosuppressed patients. We report two cases of proved CNSA in immunocompromised and immunocompetent patients with favorable response to voriconazole (VCZ).
Case Report Case 1. Caucasian 26 years old female, recently diagnosed with autoimmune hepatitis, taking azathioprine (50 mg day^{-1}) and prednisone (60 mg day^{-1}) with drowsiness, mental confusion, hemiplegia, upper right limb spasticity and positive Babinski reflex on right. Neuroimaging were compatible with expansive hypodense lesions with perilesional edema requiring decompressive craniotomy. The freezing biopsy was compatible with hyalohyphomycosis and culture revealed *Aspergillus flavus*. The patient received VCZ (400 mg day^{-1}) for 60 days and underwent abscess drainage with good clinical evolution but with physical sequel. Case 2. A Caucasian 67 years old male, farmer, previously healthy, presented with daily excruciating headache evolving with palpebral ptosis and mydriasis on right. Neuroimaging were consistent with expansive lesion on the right sphenoid region. Microsurgery was performed with partial resection of the lesion and revealed an invasion of the cavernous sinus and right orbit, encompassing carotid with mass effect. Histopathology was consistent with cerebral hyalohyphomycosis but culture depicted *A. fumigatus*. Chest and face sinus images did not show abnormalities. The *Galactomannan* antigen detection (GM) in serum and CSF was 0.246, 0.544 respectively. He was treated with VCZ (400 mg day^{-1} for 62 days with good clinical and neuroimaging responses. The GM index decreased in the CSF (0.201 and 0.173) at 40 and 60 days, respectively.
Conclusions Although CNSA is disease for most of the affected patients, our patients survived after neurosurgery procedures associated to VCZ therapy. Because VCZ is the smallest molecule (349 Da) with activity against *Aspergillus spp*, this compound presents sufficient penetration across the blood-brain-barrier to attain fungicidal activity in the CNS. The determination of seriated GM in the CNF was helpful to evaluate therapy in our second patient.

P120

Rapid identification of filamentous fungi by direct MALDI-TOF MS

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Objectives Identification of filamentous fungi using classical means is time-consuming and requires highly skilled technicians. MALDI TOF MS has the potential to enhance the accuracy and reduce the identification time, however, it is not routinely applied for the diagnosis of molds. In order to implement MALDI TOF MS in our routine workflow in the mycology section, we evaluated the BRUKER MALDI TOF MS platform testing our mold reference strain collection as well as human clinical samples in a prospective manner.
Methods 111 reference strains of filamentous fungi representing 30 clinically relevant species were analyzed using the toothpick method (protocol A) and the Sabouraud broth subculture approach (B). In protocol A, primary growth on Sabouraud agar after 24 to 72 h of incubation was collected by means of a wooden toothpick. The biomass was deposited on an unpolished target plate, air dried, and overlaid with 1 µl alpha-cyano-4-hydroxycinnamic acid. In protocol B, sediments of overnight subcultures in Sabouraud broth were thoroughly washed, extracted with acetonitrile-formic acid, and further processed as above. MALDI-TOF MS was performed using a BRUKER Microflex LT instrument and the BRUKER Biotyper software in conjunction with the Filamentous Fungi library version 1.0. Identification scores were interpreted as follows: Score ≥ 1.7, confident identification at species or species group level; score <1.7, no reliable identification (1, 2). We then prospectively applied protocol A on primary growth derived from 49 clinical samples which included respiratory specimens ($N = 36$), swabs ($N = 10$), and miscellaneous ($N = 3$).
Results Testing the reference strain collection, the following species and species groups gave confident and comparable identification scores using either protocol Aor B, and were correctly identified (designation according BRUKER): Aspergillusflavus ($N = 8$) A. fumigatus ($N = 37$), A. nidulans ($N = 4$), A. niger ($N = 13$), A. ochraceus ($N = 1$), A. terreus($N = 5$), A. ustus($N = 2$) A. versicolor($N = 1$), Cladosporiumherbarum($N = 1$), Exophiladermatidis($N = 1$), Fusariumdimerum($N = 2$), F. oxysporum($N = 1$), F. solani($N = 4$), Lichtheimiacorymbifera ($N = 4$),Mucorcircinelloides($N = 2$)Paecilomyceslilacinus($N = 1$), P. variotii($N = 1$), Penicilliumchrysogenum($N = 4$), P. citrinum($N = 1$), Rhizomucorpusillus($N = 1$), Rhizopusmicrosporus($N = 2$) R. oryzae($N = 1$), Scedosporiumapiospermum($N = 6$), S. prolificans($N = 1$), S. brevicaulis ($N = 1$).

The prospective study on clinical specimens showed that 44 in 49 clinical samples exhibited a score >1.7 and were correctly identified by MALDI TOF MS. For four out of the five remaining specimens without reliable score the identification suggested by MALDI TOF MS (A. niger, A. unguis, S. apiospermum and R. oryzae) was confirmed by internal transcribed spacer sequence analysis. Only one specimen without reliable score could not be identified by MALDI TOF because of the lack of reference data for Uncinocarpusqueenslandicus, the agent present.
Conclusions In conclusion, confident identification of filamentous fungi by MALDI-TOF MS is feasible without subculturing of primary growth. Our findings support the implementation of MALDI-TOF MS in the clinical setting in order to promote rapid and reliable diagnosis of fungal agents.

Table 1

		Protocol A	Protocol B
Species	Number of strains	Correct identification at species/group level (%)	
Rhizomucor pusillus	1	100	100
Rhizopus microsporus	2	100	100
Rhizopus oryzae	1	0	100
Mucor circinelloides	2	0	0
Lichtheimia corymbifera	4	100	100
Aspergillus flavus / orizae	8	100.0	85.7
Aspergillus fumigatus	37	94.6	100
Aspergillus nidulans	4	100	100
Aspergillus niger	13	100	92.3
Aspergillus ochraceus	1	100	100
Aspergillus terreus	5	100	100
Aspergillus ustus	2	100	50
Aspergillus versicolor	1	100	100
Penicillium chrysogenum	4	100	75
Penicillium citrinum	1	100	100
Paecilomyces lilacinus	1	100	100
Paecilomyces variotii	2	100	50
Fusarium dimerum	2	100	100
Fusarium oxysporum	1	100	100
Fusarium solani	4	100	100
Cladosporium herbarum	1	100	100
Exophiala dermatidis	1	100	100
Scedosporium agiospermum	6	100	100
Scedosporium prolificans	1	100	100
Scopulariopsis brevicaulis	1	100	100

P121

Induction of sporulation in dematiaceous mould by Burmese sal bark

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Conventional slide culture technique using various artificial culture media has long been a main method for fungal identification based on conidia structure of the organisms. However, many strains of mould may not produce conidia on regular culture media which lead to delay or unable to identify. Therefore, the aim of this study was to establish alternative method which mimicked natural habitat by using bark as a culture based. Seven dematiaceous moulds including *Curvularia* spp., *Bipolaris* spp., *Alternaria* spp., *Nigrospora* spp., *Chaetomium* spp., *Lasiodiplodia theobromae* and *Pyremochaeta remoroi* were used. The organisms grew on Sabouraud dextrose agar (SDA) for 5–7 days and small pieces of fungal colony were placed onto sterile bark of *Shorea siamensis* (Burmese sal). The fungal inoculated barks were then incubated in moisture chamber at 25 °C. The observation of conidia production was performed on 3-days interval using tease mount technique. Conidia production was observed in all tested isolates. Most of the mould produced conidia within 3–6 days. In contrast, *Pyremochaeta remoroi* and *Lasiodiplodia theobromae* required 15 and 30 days of incubation. The conventional slide culture method of these two organisms using SDA and potato dextrose agar (PDA) failed to induce sporulation after 30 days of incubation. This simple approach may become an alternative method for induction of sporulation and could be applied to other groups of mould.

P122/M6.3

Oral paracoccidiodomycosis mimicking lip carcinoma

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Introduction Paracoccidioidomycosis (PCM) is a systemic mycosis characterized by acute or chronic tissue inflammation caused by *Paracoccidioides brasiliensis* (Pb), a pathogenic thermally dimorphic fungus that is endemic to Latin America. Patients with chronic PCM are mostly middle-aged men with smoking and/or drinking habits and who are actively engaged in farming activities. The mucocutaneous lesions caused by Pb are easily mistaken for carcinomatous lesions of the head and neck owing to similar risk factors and clinical manifestations. In this study, we report a case of PCM with lip manifestation, referred to our department because of suspected neoplasia.
Case Report A 65-year-old man, actively engaged in farming activities, with a 90 pack-year smoking history, presented with a painless ulcerated lesion of the left labial comissure. He was referred to our department because of suspected neoplasia. The lesion progressed slowly through a 2-year period. Oral examination revealed an extensive ulcer, although flat and with no signs of orbicularis oris muscle infiltration, or involvement of mandibular division of facial nerve, or suspicious neck lymph nodes metastasis. A representative incisional biopy was performed. Histopathological examination exhibited chronic granulomatous inflammation on conventional hematoxylin-eosin staining. Grocott-Gomori staining showed fungal structures. Clinical and pathological evidences supported the diagnosis of paracoccidioidomycosis. Itraconazole was maintained for 6 months with satisfactory response to proposed therapy.
Discussion Active agricultural laborers or those who have a history of agricultural labor are at greater risk for paracoccidiodomycosis. Masons and civil construction workers rank second in terms of risk. Men, on average, have a ninefold increased risk of being affected than do women. The cutaneous and mucous lesions result from hematogenous dissemination, either by contiguity or, very rarely, by direct inoculation under the tissue. Signs and symptoms are related to the affected site, and usually appear as visible lesions or non-specific symptoms. In cases of mucocutaneous disease, the oral and nasal cavities are the most commonly affected sites.

Similarly to PCM, head and neck carcinomas usually affect middle-aged and elderly white men, mostly those with smoking and/or drinking habits, and are characterized by exophytic, infiltrative or ulcerative lesions. As risk factors and clinical manifestations are similar to those of head and neck carcinomas, a differential diagnosis is necessary.

P123

Comparison of the pre-commercial Invasive candidiasis (CAGTA) VirClia IgG Monotest with the CAGTA *C. albicans* IFA for candidaemia detection in patients admitted to the ICU.

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Objectives Evaluation of the pre-commercial 'Invasive candidiasis (CAGTA) VirClia IgG Monotest' in comparison with the standardized CAGTA *C. albicans*IFA in a selected non-neutropenic critically ill adult patients colonized and infected by *Candida*spp., with an expected ICU stay of at least 7 days.
Methods A total of 180 serum samples from 72 non-neutropenic patients, admitted to 12 ICUs Spanish tertiary hospitals from October 2012 to February 2014, were analyzed in a prospective cohort, observational, multicenter study. Demographic data, type of patients, APACHE II and SOFA scores (on ICU admission), underlying disease, comorbidities, risk factors, antifungal treatment and outcomes were recorded. Clinical assessment, APACHE II, SOFA and Candida score, Candida colonization cultures (rectal swabs, tracheal aspirates, gastric or pharyngeal aspirates and urine) were performed twice a week (other samples were taken as clinically indicated). All samples were processed by conventional methods. Patients were classified into proven invasive candidiasis (IC): 10 patients (25 serum samples) with candidaemia and 17 patients (48 samples) with intra-abdominal infection, Candida colonization (CC): 31 patients (80 samples) and neither colonized nor infected (NCNI) 14 patients with 27 serum samples analyzed.

These samples were analyzed retrospective with the VirClia assay® (Vircell Spain, SL) following manufacturer's instructions (positive values ≥1.1). Results have been compared with a standardized CAGTA *C. albicans*IFA ® (Vircell Spain, SL) (positive values ≥1/160).
Results For IC patients with candidaemia, sensitivity, specificity, positive and negative predictive values and kappa value for kit comparison were: 87.5, 81.3, 70.0, 92.9, and 0.65, respectively.

For IC patients with intra-abdominal infection were: 88.9, 77.3, 61.5, 94.4, and 0.58, respectively. For CC group were: 89.5, 85.2, 81.0, 92.0 and 0.74, respectively. For global comparison were: 88.9, 83.3, 84.4, 88.2 and 0.72, respectively. Overall agreement for both techniques was 86.1%.
Conclusions VirClia assay (CAGTA enzyme immunoassay) can be used in the diagnosis of invasive candidiasis with similar results than CAGTA.

This assay, a semiautomatic method, is easier to perform than CAGTA IFA. Furthermore, results are semicuantitative and objectively interpretable.

P124

Bis(methyl)gliotoxin as diagnostic and management tool of Invasive Aspergillosis: report of a proven case with negative galactomannan in an immunocompetent patient with mycotic aneurism

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Objectives Bis(methyl)gliotoxin (bmGT) is a derivative of gliotoxin produced by the mould *Aspergillus* spp that can be detected in serum from patients at risk of invasive aspergillosis (IA). The aim of this study was to assess its utility for IA diagnosis in a patient with proven IA and negative galactomannan (GM). The effect of antifungal treatment on bmGT kinetics was also evaluated.

Methods A 70-years-old patient presented to the emergency department (ED) with blurred vision. Endogen endophtalmitis was diagnosed and vitrectomy was planned. In the intervention, vitreous was sent for microbiological examination. During his admission for the surgery, he suffered an ischemia of the right low member. Computed tomography (CT) angiography of peripheral arteries and thorax objectified right pedis artery occlusion and a thrombus in aortic arch. He received intraarterial fibrinolytic treatment, and aortic lesion study continued. Infectious endocarditis was then discharged by transesophageal echocardiography. A thoracic CT revealed mycotic aneurysm of the ascending thoracic aorta. Graft replacement was carried out and samples were sent for histological and microbiological examination. Microbiological report revealed hyphal vision on Gram stain and caspofungin (70 mg d^{-1} on day 1, followed by 50 mg d^{-1}) was initiated. The next day, *A. fumigatus* was isolated and voriconazole (6 mg kg^{-1} every 12 h on the first day, followed by 4 mg kg^{-1} every 12 h) was added to the treatment. After surgery, patient was stable and extubation was possible. Later he developed severe respiratory failure and intubation was needed again. He was gradually getting worse, with severe renal impairment requiring continuous venovenous hemodiafiltration and vasoactive drugs. Finally, he developed a septic shock with respiratory, renal and hepatic failure and died.

We detected bmGT, galactomannan (GM) and voriconazole in four sera:(i) a serum obtained during endophtalmitis study, (ii) a serum obtained prior to graft replacement, (iii) a serum obtained after 14 days of correct antifungal treatment and (iv) a serum obtained after 22 days of treatment.

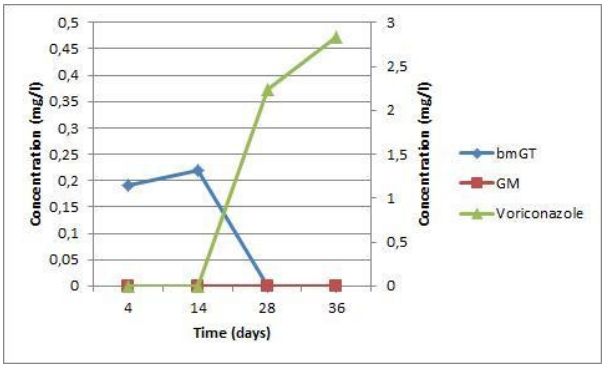

Figure 1. Serum bmGT, GM and voriconazole concentration during time

Serum GM levels were measured using the Platelia Aspergillus enzyme immunoassay kit (BioRad, France). BmGT and voriconazole were quantified by High Performance Thin Layer Chromatography (HPTLC).

Results Serum GM detection was negative in all the four sera collected. BmGT was detected in the two samples obtained prior to antifungal treatment (i and ii). The bmGT concentration during ED admission was 0.19 μg ml^{-1} and increased to 0.22 μg ml^{-1} on the day of surgery. The two samples obtained after 14 (iii) and 22 days (iv) of correct antifungal treatment did not show detectable bmGT. Voriconazole trough levels in serum iii and iv were 2.2 μg ml^{-1} and 2.8 μg ml^{-1}, respectively.

Histopathological examination of the aorta demonstrated fungal structures. *A. fumigatus* grew in both vitreous and aorta cultures.

Conclusion BmGT followed an increasing kinetic until antifungal treatment and it became undetectable after antifungal initiation. This findings support the utility of bmGT detection as a diagnostic and management tool of IA. GM was falsely negative, showing a real need of reliable biomarkers for IA diagnosis.

P125

Abdominal actinomycosis in Saint-Petersburg, Russia

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Objectives To study risk factors, clinical features, and treatment efficiency of abdominal actinomycosis in Saint Petersburg, Russia.

Materials and methods Prospective, single-center study of 157 patients with different clinical forms of actinomycosis (2005–2013 yy.). Abdominal actinomycosis was in 44% cases. Women −88%, age 27–81 year (median - 47 ± 3). The diagnosis was based on histology of postoperative material. The control group was made by patients with nonspecific inflammatory process of abdominal organs on 20 people.

Results Predisposing factor for abdominal actinomycosis were acute appendicitis, particularly with perforation (OR = 5.04 [1.07–23.72], $P < 0.05$). Pelvic actinomycosis is predominantly associated with IUCDS (OR = 7.7[2.5–23.8], $P < 0.05$). Patients usually present with a history of prolonged use IUCDS (median 7.6 + /-2.4 years).

At 67% patients abdominal and pelvic organs were involved in inflammatory process. Ovaries (43%), appendix (42%), colon (25%), cecum (22%), uterus (16%) and fallopian tubes (12%) are the most common abdominal sites of actinomycosis. Peritonitis was in 33% patients.

Patients may present with non-specific symptoms such as abdominal pain (94%), fever (80%), and weight loss (39%). In all cases the diagnosis was based on histology of operational material. The culture of *Actinomyces* spp. from 'sterile'site confirms the diagnosis (*A. israelii* - 75%, *A. naeslundii* - 25%).

Surgery was used in all patients, as well as treatment with benzylpenicillin (12–24 million units a day) for 2–4 weeks, then amoxicillin 1.5–2 g day^{-1} for 6–12 months. The median duration of antibiotic treatment was 9 ± 2 months. Efficiency of treatment was 92%.

Conclusion Abdominal involvement was in 44% cases of actinomycosis. Surgical resection of infected tissue and long-term antibiotic therapy was effective in the treatment of abdominal actinomycosis.

P126

Rapid identification of the main Candida Species isolated from blood and other clinically significant cultures by Multiplex Real-time PCR

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The frequency of systemic infections caused by *Candida* has increased considerably in the last few years, especially among hospitalized patients. It has being considered the fourth agent isolated of bloodstream and is associated with significant morbidity and mortality. Although *Candida albicans* still remains as the most common fungal isolate from clinical specimens, longitudinal studies have detected a shift towards non-albicans *Candida* (NAC) species. The non-*albicans Candida* species are associated with the increase of antifungal drug resistance, and hence, species differentiation became very important. The aim of this study was to standardize a molecular technique to rapidly differentiate the most important *Candida* species isolated from the blood cultures. In order to differentiate the *Candida* species, a set of specific primers were designed based on variability in the internal transcribed spacer (ITS) region of ribosomal DNA, whose sequences were aligned using ClustalW. After sequence alignment, a common sense primer was selected together with specific anti-sense primer for each *Candida* species detected by this system. This molecular technique was initially tested against several know *Candida* isolates and the reactions were confirmed to be specific for each species being detected. The reaction consisted of a PCR mix containing SYBR green as a fluorescent agent, a mixture of primers and fungal DNA in a total volume of 20 microliters. Reactions were run on the LightCycler instrument (Roche) and *Candida* identification could be obtained by analyzing the different melting points of the different species. Two separated reactions were used to identify: *C. albicans, C. dubliniensis, C. tropicalis, C. parapsilosis, C. krusei, C. guillermondii* and *C. glabrata.*The advantage of this methodology is, determine different *Candida* species directly from the blood.With molecular detection directly from the blood, the culture is avoided reducing the releasing time of result. Also, sometimes with the traditional methods used in yeast identification is not possible define property the species. So, it methodology helps to improving the better choice for treatment. Still, this specific method showed good sensibility and specifity in identify the *Candida* species. In conclusion, this is a rapid and possible method to apply in laboratory routine especially that attend hospitalized patients improving the epidemiology characterization and treatment of *Candida.*

P127

Development of a reference database for the identification of Histoplasma capsulatum by MALDI-ToF Mass Spectrometry

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Objectives The diagnosis of histoplasmosis is based on the isolation of the fungus in cultures. However, it is time-consuming and biosafety level 3 containment is needed. MALDI-ToF technology has been established as a suitable tool for microbial identification although there are no entries of *H. capsulatum* spectra in most used commercial MALDI databases. The main objectives of this work are: i) Construction of a reference database for the identification of *Histoplasma capsulatum* by MALDI-ToF technology. ii) Validation of this database with isolates belonging to the Collection of the Spanish National Centre for Microbiology.

Methods A reference database was created using high quality spectra from six strains of *H. capsulatum* previously identified by molecular methods. Three strains were included in both morphological states (mycelia and yeast) and also the three varieties recognized for the fungus *(var. capsulatum, var. duboisii* and *var. farciminosum*) and the type strain were represented. To construct the database, the fungal isolates were grown for 3 weeks and subjected to protein extraction following the method recommended by the manufacturer (Bruker Daltonics, Germany). Each sample was assessed eight times and MS-measurement was performed in triplicate. For validating the new database, 89 fungal isolates were selected from the Collection of the Spanish National Centre for Microbiology: 35 strains belonging to different fungal species and a total of 54 strains of *H. capsulatum.* Protein extraction was performed after 7 days of culture and all protein extracts were analysed in duplicate against the MS new reference database created plus other commercial and in-house MALDI databases.

Results Globally, 81 strains were correctly identified with variable scores ranging from 1.42 to 2.4 and 66 of them (81.5%) had a score above manufacturer cut-off for reliability at genus level (score $\geq$ 1.7). All *H. capsulatum* isolates were properly identified with scores ranging from 1.46 to 2.2. The reliability score $\geq$ 1.7 was reached by 41 of them (76%), 30 in mycelial state (86% of the total of *H. capsulatum* strains in mycelial state) and 11 in yeast form (58% of the total of *H. capsulatum* strains in yeast state). However, when this cut-off was lowered to score $\geq$ 1.6, the number of strains properly identified rose to 49 (91%), including 33 mycelial forms (94% of mycelial-form isolates) and 16 yeast forms (84% of yeast-form isolates).

Conclusion i) The database created provided a suitable identification for 76% of *H. capsulatum* isolates when the cut-off score was set at 1.7 and 91% when this cut-off was 1.6. ii) The database created was able to identify both morphological phases of the fungus and the three varieties. iii) MALDI-ToF technology yields a prompt and simple identification from *H. capsulatum* yeast forms or early mycelial cultures. It allows for reducing the time of response and decreasing the risk in manipulating the fungus.

P128

Combination of galactomannan detection and computed tomographic findings for diagnosis of invasive pulmonary aspergillosis

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Objectives To evaluate the benefit of the combination of galactomannan (GM) assay and thoracic computed tomography (CT) images for the diagnosis of invasive pulmonary aspergillosis (IPA).

Methods This retrospective study was performed at the 900-bed tertiary-care teaching hospital in Konya/Turkey using the medical records of the 400 patients between the years 2011 and 2015. The Platelia Aspergillus Ag EIA assay (Bio-Rad) was used to detect GM antigen in serum and bronchoalveolar lavage (BAL) fluid samples. CT evaluation was performed by radiologists who were unaware of patients' GM results.

Results Thorasic CT images of 400 patients with probable or possible IPA were evaluated with respect to the presence of typical radiological findings defined for IPA.The imaging findings were compared with the serum or BAL fluids samples' GM test results of the patients. Ground glass parenchymal opacities, cavitations, fungus balls and patchy *consolidations* with predominantly subpleural and/or *peribronchial* distribution were significantly more frequent in GM positive group ($P < 0.05$). On the other hand presence of consolidation with or without air bronchogram sign, nodule with or without *tree-in-bud* appearance, unilateral or bilateral pleural effusion were seen with similar frequency in both GM positive and negative groups.

Conclusion It is important to diagnose and differentiate the most frequent CT findings of IPA that is associated with positive serum or

BAL fluid positive GM tests. Together, these findings may help physicians to initiate Aspergillus-active antifungal treatment.

P129

Occurrence of fungal DNA contamination in PCR reagents: Approaches to control and decontamination

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Objectives Contamination with fungal DNA is a serious threat for the validity of fungal detection assays based on nucleic acid amplification. Here we report the presence of fungal DNA contaminants in critical reagents for real-time PCR obtained from commercial providers. Traces of fungal DNA were detected in lyophilized primers, TaqMan probes, and master mix solutions, and resulted in a considerable rate of false-positive test results in panfungal real-time PCR assays. Controlling this problem is therefore of paramount importance for molecular diagnosis of fungal infections.
Methods Identification of PCR reagent contamination was accomplished by panfungal real-time PCR screening. A decontamination method for PCR components based on the activity of a double-strand specific DNase was established. DNA-spiking experiments using quantified *Candida albicans* DNA were performed to test the capability of the DNase to digest typical quantities of contaminating fungal DNA found in PCR reagents. Individual decontamination protocols were established for each PCR reagent and reduction of false-positive test results after decontamination was evaluated. Sequencing of a PCR product obtained from a false-positive reaction was performed to identify the biological source of contaminating DNA.
Results We established rigorous handling conditions to avoid false-positive test results from exogenous sources, and found that fungal DNA contamination was limited to PCR reagents. We showed that DNase mediated decontamination was capable of digesting a much higher DNA amount than typically found as contaminants in PCR reagents. DNase treatment of contaminated oligonucleotides led to more than 10-fold reduction in the frequency of false-positive test results without compromising the overall test performance. The enzyme was also effective in decontaminating the master mix solution used in our assay, albeit with deterioration of the assay performance. Sequencing of the contaminated PCR products revealed sequences showing 100% identity with a conserved region of 28S rRNA gene found in representatives of different yeast genera indicating that contaminations observed originated from traces of yeast-derived DNA.
Conclusion Based on our findings, we strongly recommend routine monitoring of all reagents used in fungal PCR assays for the presence of relevant contaminants. As long as fungal-grade reagents are not readily available, pretreatment methods facilitating elimination of fungal DNA are instrumental for reducing the risk of false-positive results in highly sensitive molecular fungal detection assays.

P130

Non-invasive detection of mucorales DNA in serum by mucorales specific real-time PCR assay

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Purpose Invasive fungal infections (IFI) are life-threatening complications in immunocompromised patients. Due to improved diagnosis and treatment options, infections caused by *Candida spp.* and *Aspergillus spp.* are decreasing, while other rare fungal pathogens like Mucorales are on the rise. Mucormycosis is a very severe fungal infection and difficult to diagnose. Standard culture is insensitive and microscopy as well as radiological imaging unspecific. Most diagnostic procedures are invasive and thus cannot be used for screening. DNA detection from serum as described for *Aspergillus* or Mucorales (Yamakami *et al.*, 1996; Millon *et al.*, 2013) by polymerase chain reaction (PCR) is a rapid and very sensitive tool to identify pathogens and can improve diagnosis and treatment.

Table 1

patient ID	EORTC classification	available serum #	PCR positive serum	sequencing of PCR product
1	probable IFI	-153,-150,-137 to -119,-90 to -26,-1, 3	-1, 3	*Lichtheimia sp.*
2	proven IFI	-21,-17,-14,-10, 0	0	*Rhizopus sp.*
3	proven IFI	-24,-17,-10,-3	-3	*Lichtheimia sp.*
4	unclassified	n=7	-	
5	unclassified	n=13	-	

indicated by days to EORTC class.; minus indicates days before

Methods Forty-six serum specimens from 5 hematological patients were collected from 2008 to 2012 at Medical University Hospitals of Innsbruck and Wuerzburg. Sera were drawn twice weekly. According to EORTC/MSG criteria (DePauw *et al.*, 2008), 3 patients were classified having proven/probable IFI either by histology or positive culture from bronchoalveolar lavage (BAL). Sera were available up to 127 days before EORTC classification date.

DNA from 1 ml of serum was manually extracted by using a commercially available kit (UltraSens Virus Kit, Qiagen). Extracts were analysed by real-time PCR assay (qPCR) amplifying the 18S rDNA region. This assay was modified using the primers published by Bialek *et al.* (2005) comprising in addition a Mucorales specific probe and degenerated nucleotides. A single round PCR cycling was used. Amplicons of positive PCR reactions were sequenced and aligned with reference sequences using BLAST analysis search.
Results DNA extracts of serum samples were analysed by a Mucorales-specific qPCR assay within 4 h. Four samples of the 3 probable/proven Mucorales IFI cases were positive. Sequencing revealed 2 different Mucorales strains (*Lichtheimia sp., Rhizopus sp.)*confirming pathogen identification by conventional methods. PCR from serum detected Mucorales DNA up to 3 days earlier than BAL or biopsy. Day 'zero' was defined as time point of EORTC classification (positive BAL culture, biopsy). None of the unclassified control samples were positive.
Conclusion Using qPCR allowed Mucorales specific detection of DNA in serum. This probe-based assay detecting a broad spectrum of Mucorales was used as an add-on tool to confirm mucormycosis in serum. Due to easy availability of serum even in severely ill patients, qPCR can be used as a screening tool in high-risk patients with suspected mould infection. The method used is faster and more sensitive than standard methods. It can help to distinguish between infections with *Aspergillus* and Mucorales, which is relevant for antimycotic regimens and patients' outcome.

P131

Chromoblastomycosis and sporotrichosis in Madagascar: epidemiology, molecular diagnostic and perspectives

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Chromoblastomycosis (CBM) and sporotrichosis (SP) are endemic mycosis in Madagascar that occurred following injury and telluric contamination. CBM is mostly due to *Fonsecaea pedrosoi* or *Cladophialophora carrionii* and affects usually the subcutaneous tissue, whereas SP is caused by *Sporothrix schenckii* by invading the lymphatic system of the arms and legs. These fungal infections are considered neglected diseases because of poor resources allocated to their diagnosis, monitoring or prevention.

Objectives The general objective was to assess the prevalence of these mycosis in Madagascar. The specific objectives were to characterize the causative fungal species and their habitat in order to prevent contamination, and; to set up a sustainable clinical and laboratory network to allow proper case management and to provide a molecular-based species identification.

Methods The study comprised a prospective clinical study that started in March 2013. Patients were recruited during field investigations and consultations provided in a dermatology department. Pus, biopsy and squamous were sampled from the lesions. Informed consent from the patients and ethical approval from the Ministry of Health were obtained. Histopathological and mycological analysis (direct examination and culture on Sabouraud-Cycloheximide) were performed. We have developed a PCR-based strategy that was validated on reference strains provided by BCCM/IHEM (Belgian Coordinated Collections of Microorganism). First, two sets of universal primers (NL-1/NL4 and ITS5/ITS4) were used to confirm the fungal origin of the lesions. Then, specific primers (SSHF31/SSHR97, Fon-F/Fon-R, EdF/EdR) were used for fungal species identification.

Results Fifty-five patients were enrolled. Mean age of the patients was 38.8 years and men were prevalent with 69.1% of cases. Overall, 41.8% were farmers, 34.5% self-employed, 16.4% students and 7.3% unemployed. Clinically, 36.4% of cases were suspected having CBM and presented with crusted, verrucous and tumoral lesions;

Table. Clinical suspicion, mycological and molecular diagnosis of mycosis cases in Madagascar.

						Mycological diagnosis		Molecular diagnosis by PCR of DNAs from Primary Samples *vs.* Colonies Culture (PS/CC)					
LabID	Age (yr.)	Sex	Profession	Clinical suspicion	Samples	Fumagoïd cell	Sabouraud Chloramphenicol	Identification	NL-1 NL-4	ITS5 ITS4	Fon-F Fon R	EdF EdR	SSHF31 SSHF97	Species
MYC04004	44	M	Farmer	CBM	bps	NA	+	*Fonsecaea* sp or *Cladosporium* sp	+/-	+/-	+/+	-/-	-/-	*Fonsecaea* sp
MYC08005	38	M	Schoolteacher	SP	bps	NA	+	*Sporothrix* spp	+/+	-/+	-/-	-/-	-/+	*Sporothrix* sp
MYC08006	43	M	Farmer	CBM	bps	NA	+	*Fonsecaea* sp	+/+	+/+	/+	-/-	-/-	*Fonsecaea* sp
					sqs	+	+	*Scopulariopsis or acremonium*	/+	/+	/+	/-	/-	*Fonsecaea* sp
MYC08007	44	M	Farmer	CBM	bps	NA	+	*Sporothrix* sp	+/+	-/-	-/-	-/-	-/+	*Sporothrix* sp
MYC10008	9	M	Student	SP	bps	NA	+	*Sporothrix* sp	/+	/-	/-	/-	/+	*Sporothrix* sp
MYC10010	50	M	Farmer	CBM	bps	NA	+	*Absidia*	+/	+/	+/	-/	-/	*Fonsecaea* sp
MYC10011	64	M	Farmer	UN	sqs	+	+	Contaminant	+/	+/	+/	-/	-/	*Fonsecaea* sp
MYC10012	25	M	Mason	SP	bps	NA	+	*Sporothrix* sp	+/+	+/+	-/-	-/-	-/+	*Sporothrix* sp
MYC10014	55	M	Unemployed	CBM	sqs	+	+	*Cladosporium* sp or *Fonsecaea* sp	+/+	+/+	-/-	-/-	-/-	*Cladophialophora* sp
MYC11015	10	F	Student	SP	bps	NA	+	*Sporothrix* sp	+/+	+/+	-/-	-/-	-/+	*Sporothrix* sp
MYC01018	41	F	Farmer	UN	bps	NA	+	UN	/+	/+	/-	/-	/-	ongoing
					sqs	NA	+	UN	/+	/+	/-	/-	/-	ongoing
MYC02019	47	F	Merchant	SP	bps	NA	+	*Sporothrix* sp	/+	/+	/-	/-	/+	*Sporothrix* sp
MYC02020	6	M	Student	UN	bps	NA	+	*Sporothrix* sp	-/+	/+	/-	/-	/+	*Sporothrix* sp
					pus	NA	+	*Sporothrix* sp	-/	/+	/-	/-	/+	*Sporothrix* sp
MYC03021	59	M	Unemployed	SP	bps	NA	+	*Sporothrix* sp	/+	/+	/-	/-	/+	*Sporothrix* sp
MYC03023	16	M	Student	SP	bps	NA	+	*Sporothrix* sp	/+	/-	/-	/-	/+	*Sporothrix* sp
MYC04026	58	M	Farmer	CBM	bps	NA	+	*Fonsecaea* sp	/+	/+	/+	/-	/-	*Fonsecaea* sp
MYC05030	43	M	Mason	SP	bps	NA	+	*Sporothrix* sp	/+	/+	/-	/-	/+	*Sporothrix* sp
MYC06034	3	M	Unemployed	SP	bps	NA	+	*Sporothrix* sp	/+	/+	/-	/-	/+	*Sporothrix* sp
MYC06035	41	F	Seamstress	SP	bps	NA	+	*Sporothrix* sp	/+	/+	/-	/-	/+	*Sporothrix* sp
MYC06036	10	F	Student	SP	bps	NA	+	*Sporothrix* sp	/+	/+	/-	/-	/+	*Sporothrix* sp
MYC07037	78	M	Farmer	CBM	bps	NA	+	*Sporothrix* sp	/+	/+	/-	/-	/+	*Sporothrix* sp
MYC08039	26	F	Farmer	SP	bps	NA	+	*Sporothrix* sp	/+	/+	/-	/-	/+	*Sporothrix* sp
MYC08040	33	M	Farmer	CBM	bps	NA	+	*Cladosporium* sp or *Fonsecaea* sp	/+	/+	/+	/-	/-	*Fonsecaea* sp
MYC01045	18	M	Farmer	SP	bps	NA	+	*Sporothrix* sp	+/+	+/+	-/-	-/-	/+	*Sporothrix* sp
					pus	-	+	*Sporothrix* sp	-/+	/+	-/-	-/-	-/+	*Sporothrix* sp
MYC02046	64	F	Teacher	SP	bps	NA	+	*Sporothrix* sp	-/+	-/+	-/-	-/-	-/-	ongoing
MYC04050	30	M	Farmer	SP	bps	NA	+	*Sporothrix* sp	+/+	/+	-/-	-/-	-/+	*Sporothrix* sp

CBM: chromoblastomycosis; SP: sporotrichosis; bps: biopsy; sqs: squamous; UN: unidentified; NA: non applicable

40.0% of cases were SP characterized mainly by ulcerative and nodular lesions of the lymphatic system of the lower limbs; and for 23.6% the diagnosis was ambiguous. Mycological analysis revealed 18 cases of SP and 8 cases of CBM from which the PCR identified 17 strains of *Sporothrix* sp., 1 *Cladophialophora* sp. and 6 *Fonsecaea* sp. One mycetoma probably of bacterial origin was also found. CBM cases were predominant in the north-east, east and south part of the island, whereas SP cases were located mainly in the central highlands.

Conclusion These results confirmed that CBM and SP persist at a high frequency in Madagascar. The availability of a reliable PCR tool in routine and the clinical expertise gained during this study will help the national authorities to set up a proper control and prevention program. An environmental survey is planned to describe the spread of the causative agents in the environment in an attempt to prevent the contamination.

P132

Rapid and specific detection of *Aspergillus* spp. at section or species levels by multiplex q-PCR

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Invasive aspergillosis (IA) are opportunistic infections caused by *Aspergillus spp.* PCR has not been yet accepted as a mycological evidence of infection by the EORTC, but it is a rapid and highly sensitive technique that can be used to detect fungi DNA and help clinicians to obtain quicker diagnosis.

Objective The aim of this study is to develop a variety of probes that could be combined in multiplex real time PCRs (qPCR) to practical, quick and specific detection and identification at different levels within *Aspergillus* (species, section or genus).

Methods Three multiplex qPCR were developed based on ITS regions and *benA* gene (β-tubuline protein). Primers and specific Taqman probes were designed to detect and discriminate members of section *Fumigati*, *Flavi* and *Nigri* (Sections-qPCR); *A. fumigatus*, *A. flavus*, *A. niger* and *A. terreus* species (4SP-qPCR); or *A. fumigatus*-section *Fumigati* species (Fum2). Two DNA extractions methods were assayed. We also designed internal controls to detect inhibition of the reactions. Finally, several PCR facilitators were added in PCR mixtures to avoid false negatives due to PCR inhibitions. The sensitivity and efficiency of the multiplex real time PCR assay was tested with DNA extracted from hyphae and conidia of *Aspergillus* spp.

Results The Sections-qPCR demonstrated a high sensitivity with limits of detection of 20 fg and a mean efficiency about 93% for the three probes. On the other hand, 4SP-qPCR showed sensitivity and a mean efficiency of 50 fg and 100%, respectively in the detection of *A. niger* and *A. terreus*, and 100 fg and 105% in the detection of *A. fumigatus* and *A. flavus*. Moreover, the method also permitted the detection of only 1 germinated conidium, except for *A. terreus* (10 conidia), vs. 10^2–10^3 non-germinated-conidia. Finally, the Fum2-qPCR showed sensitivity and a mean efficiency of 20 fg and 82.16%, respectively, in the detection of section *Fumigati*; and 50 fg and 101.23% in the specific detection of *A. fumigatus*. Furthermore, the method also allowed the detection of 1 germinated conidium versus 10^3 non-germinated conidia.

Conclusions The ITS region allows detecting not only genus and section level, but also at species level in the case of *A. terreus* and *Neosartorya pseudofischeri*. The high inter-specific variability of the *benA* gene has allowed the identification of *A. fumigatus*, *A. niger* and *A. terreus*, but not the discrimination of *A. flavus* from their cryptic species. We also confirmed the detection of hyphae and even a single germinated conidium, but not non-germinated conidia ($<10^2$). The use of internal control together with strategies to avoid PCR inhibitions would allow the application of these techniques on complex samples.

Financial support Supported by the UPV/EHU grants (PES13/03, GIU12/44 and UFI11/25), the Government of the Basque Country grant (S-PC11UN007), and the Ministry of Economy and Competitiveness grant (MICINN CSD2009-00006). Monica Sueiro Olivares and Xabier Guruceaga have been supported by Pre-doctoral Research Grants of the Government of the Basque Country and Jesús Gangoiti Barrera Foundation, respectively. Jimena V. Fernandez Molina has been supported by Post-doctoral Research Grants of the UPV/EHU.

P133

Yeasts identification by mass spectrometry: a comparative study

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Objectives The technology MALDI-TOF MS (matrix-assisted laser desorption/ionization-time of flight mass spectrometry) represents a significant advantage for the timely identification of microrganisms in clinical laboratories. The objective of the study was to evaluate the performance of two mass spectrometry MALDI-TOF systems, namely VITEK MS BioMerieux and Bruker Daltonics MALDI BioTyper, in the identification of clinical yeasts isolates.

Methods 136 yeasts isolated from clinical specimens were tested: 28 *Candida albicans*, 5 *C. dubliniensis*, 29 *C. glabrata*, 2 *C. guilliermondii*, 1 *C. haemulonii*, 2 *C. krusei*, 1 *C. lipolytica*, 7 *C. lusitaniae*, 2 *C. norvegensis*, 15 *C.parapsilosis*, 14 *C. tropicalis*, 21 *Cryptococcus neoformans*, 3 *Saccharomyces cerevisiae*, 1 *Trichosporon ashaii*, 1 *T. asteroides*, 4 *T. mucoides*, 1 *Rhodotorula spp.* Yeast identification on the VITEK MS system (BioMerieux) and on the instrument Microflex LT (Bruker Daltonics BioTyper), was performed as according to the manufacturer's protocol using a short on-plate extraction method. The back-up routine identification methods consisted in the germ tube test, chromagar medium, API C AUX and the automated Vitek2 system.

Results Overall, Vitek MS correctly identified, 90.4% of the yeasts to the genus level, with 88% identifications to the species level; no identification results were provided in 8.8% of the strains and misidentifications in 0.7%. Bruker Biotyper results were: 89% correct identifications to the genus and species level, no identification results in 11% of the samples and there were no misidentifications results. The *Candida* strains identification analysis revealed improved results: 96.2% for genus in both systems, with species identification level of 93.3% in Vitek MS and 96.2% in the Bruker system. No identification results in 2.9% and 3.8% respectively and misidentifications 1% in Vitek MS.

In both cases no identification results were found in a higher percentage when testing *Cryptococcus neoformans* strains. (Vitek MS 38% and Bruker 43%).

Conclusions We concluded that regardless the system used, the technique is very easy to perform and very good results can be obtained very rapidly, contributing to the timely management of patients. For both systems *Candida* strains identification results were very good while overall results were inferior. This inferiority was related to the, already reported, lower efficacy of this technique in identifying mucoid strains. Although not significant we found a better performance in Bruker Biotyper in the identification of *Candida* to the species level.

P134

Determination of the detection limit of the polymerase chain reaction in the Cryptococosis

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Introduction Cryptococcosis is a fungal infection, with a large epidemiological distribution worldwide caused by encapsulated yeasts of the genus *Cryptococcus*. The disease affects about one-third of carriers of human immunodeficiency virus (HIV) presenting an opportunistic infection. Rapid and specific diagnostic tests for fungal infections are extremely important to the effectiveness in treating infected patients. With the advancement of Molecular Biology has been possible to develop new techniques for diagnosis and identification of *Cryptococcus* sp.
Objectives This study aimed to determine the detection range of the genome of *Cryptococcus* sp by PCR (Polymerase Chain Reaction), in biological samples of patients with suspect of criptococose.
Methods For the PCR reactions, we used specific primers for *Cryptococcus* sp (CN4-CN5) and global primers for fungi rDNA (ribosomal DNA - ITS1-ITS4). To determine the detection limit in culture of *Cryptococcus* neoformans, we used an ATCC sample, and to determine detection limit in cerebrospinal fluid, we used a sample from a patient with criptococose diagnosis confirmed by examining with India ink, culture and clinical findings.
Results According our results, PCR was capable to detect 30.1 DNA femtograms in culture of *Cryptococcus* sp and 26.5 DNA picograms in cerebrospinal fluid.
Conclusion According to the low level of detection presented, we can suggest that PCR may help in diagnosis of cryptococcosis, increasing life expectancy of patients and preventing disease recurrence, since it can follow the treatment for the doctor can know the right to stop the drug administration time. The next steps will be to determine the sensitivity and specificity of the technique, using cerebrospinal fluids of patients diagnosed with cryptococcosis and other diseases.

P135

Impact of systemic antifungal therapy on the detection of *Candida* spp. in blood cultures with resins or selective media, in the clinical setting of candidemia

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Objectives Despite a relatively low sensitivity, gold standard for candidemia diagnostic and follow-up still relies on blood culture (BC). The use of BC specific media is not clearly addressed in guidelines. *In vitro* studies showed that the presence of an antifungal can significantly modify the rate and the time to positivity of BC not containing adsorbing agents. This can have a direct impact on the diagnostic and on the monitoring of candidemia. Our objective was to study in clinical practice the impact of systemic antifungal therapy (SAT) on the diagnostic performance of each medium and to highlight their added value in the management of candidemia.
Methods All patients experiencing candidemia, with at least one collected blood sample, in a 3 years period (2010–2013) were included in a retrospective study at the Grenoble University Hospital, France. Vials comprising adsorbent resins (Bactec aerobic PLUS and anaerobic PLUS, BD Diagnostics, Sparks, USA) and selective vials without resins (Bactec MYCOSIS IC/F) were considered. We analyzed: 1) the positivity rate during candidemia; 2) time to positivity (TTP), and 3) agreement between vials. The presence of SAT was recorded. Univariate analysis and multivariate hierarchical model for repeated measures were used.

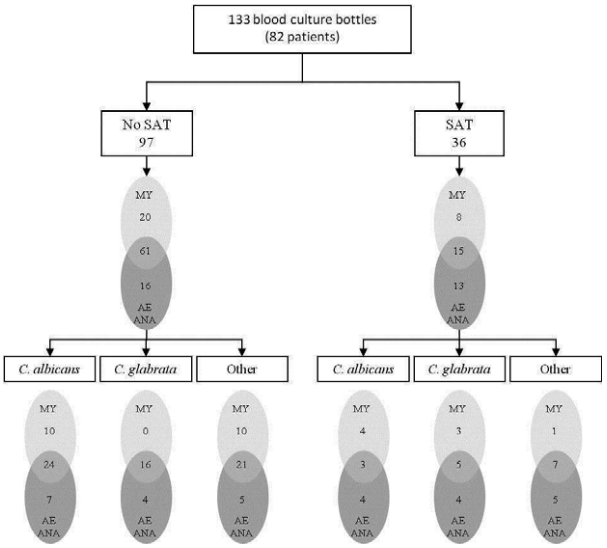

Figure 1. Venn diagrams-number of agreement or disagreement result between pairs composed of one Mycosis flask (MY) and one flask with resins (AE and/or ANA).

Results A total of 125 patients with candidemia and a median age of 61.5 years were considered. Overall, positivity rate was similar in vials with resins and in selective vials. In the presence of SAT, the positivity rate decreased significantly (OR: 0.16 (95% CI: [0.08; 0.32] $P = 0.01$), but this decrease was not linked to the presence or the absence of resins in the flask. Overall, TTP was longer in vials with resins than in selective vials. SAT prolonged TTP by a factor 1.76 ([1.30; 2.40]; $P < 0.01$). The impact of SAT on the TTP was more marked in the selective vials than in those with resin.

The agreement study showed that when the sampling was done the same day, on a same patient, on both resins and selective BC, in the absence of SAT, 16 (16%) and 20 (21%) vials were positive only on resins or only on selective vials respectively, and in the presence of SAT, 13 (36%) and 8 (22%) vials were positive only on resins or only on selective vials respectively (fig. 1).
Conclusion In this clinical study, we showed that SAT influences significantly the result of the blood culture, whether adsorbing resins are present or not in the vials. We also demonstrated that there is a clear gain in sensitivity to collect concomitantly resins and specific vials, especially when SAT is present. Concerning BC sampling, new recommendations on candidemia diagnostic and follow-up should be clarified in a near future to help clinicians in their everyday practice.

P136

Case of mycetoma by *Scedosporium* sp.

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Introduction Mycetoma is a chronic progressive granulomatous infection of the subcutaneous tissue. It may affect muscles, bones,

cartilage and joints, most often affecting the lower extremities, usually the foot. The disease is caused by either fungi or bacteria, giving rise to eumycetomas and actinomycetomas, respectively. It has a classic triad of soft tissue swelling, draining sinus tracts, and extrusion of grains.

Case Report A male, 66 years old, farmer, immunocompetent, searched medical assistance in a private dermatology office of Porto Alegre city, Brazil, in march 2015, with lesions typical of mycetoma. According to the patient, the disease has progressed for 25 years. In direct mycological examination, hyaline septated hyphae were observed in the grains, representing an eumycetoma. In cultural mycological examination, there was growth of a colony in Sabouraud's medium showing grayish pigment. In the optical microscopic observation of colonies, there were hyaline septated hyphae with ovoid conidia in simple conidiophores, indicating eumycetoma by *Scedosporium* sp. The molecular analysis for identification of the species is being performed. The antifungal activity was performed and the minimal inhibitory concentration (MIC) of seven antifungal agents was evaluated by the microdilution method in 98 well plates according to the CLSI protocol M38-A2 standardized for *Scedosporium* spp., and the minimal fungicidal concentration (MFC) were evaluated in Sabouraud dextrose broth medium in tubes from the wells with no growth. The tubes were maintained for 14 days to 30 °C until reading. The concentrations tested for fluconazole ranged from 0.125 to 64 μg/mL, and for the other antifungals, the concentrations ranged from 0.0312 to 16 μg ml^{-1}. The MIC/MFC obtained were (μg ml^{-1}): voriconazole (0.25/8), ketoconazole (0.5/8), posaconazole (1/>16), itraconazole (2/>16), amphotericin B (4/>16) fluconazole (16/>64), terbinafine (>16/>16). Therefore, the isolate was considered sensitive to voriconazol, ketoconazol and posaconazole; intermediate to itraconazole; resistant to amphotericin B, fluconazole and terbinafine. The patient is being treated with 400 mg itraconazole by day and is being monitored.

Discussion Scedosporiosis is reported infrequently. In Brazil, less than 50 cases were reported. The antifungal susceptibility in infections caused by the genus *Scedosporium* is important because of the multi-resistance of the isolates. The MFCs obtained from the isolate of this patient were very high, indicating that a successful treatment of an immunocompromised patient infected by this strain should be difficult. In the present case, in which the patient was immunocompetent, the choice of treatment was based on the outcome of the antifungal susceptibility tests, the adverse effects of the drugs, mainly because the patients is elderly, and the availability of medication publicly distributed by the SUS (Sistema Único de Saúde) in Brazil, since the patient belongs to a low income class. As there are ineffectiveness reports using itraconazole in certain cases in the literature, the monitoring of this patient is crucial to obtain the cure.

P137

PNA-FISH YTL and culture-based MALDI-TOF MS identification of etiologic agents of candidaemia in University Hospital Center Zagreb

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Objectives Candidaemia is very serious illness with high mortality. The outcome of patients with candidemia highly depends on early introduction of appropriate antifungal therapy. The choice of appropriated antifungal therapy is different according the Candida species causing the candidaemia since non-albicans species still represent a significant burden globally. Peptide Nucleic Acid *in Situ* Hybridisation Yeast Traffic Light system (PNA-FISH YTL) is molecular test designed for identification of 5 most common Candida that are the etiologic agent of candidiasis. *C.albicans*/*C.parapsilosis* shows green fluorescence, *C.glabrata*/*C. krusei* shows red fluorescence and *C. tropicalis* show yellow fluorescence. Identification with PNA-FISH YTL is done in 90 min directly from yeast positive blood culture. Matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) is very powerful tool for precise identification of bacteria, as well as yeasts.

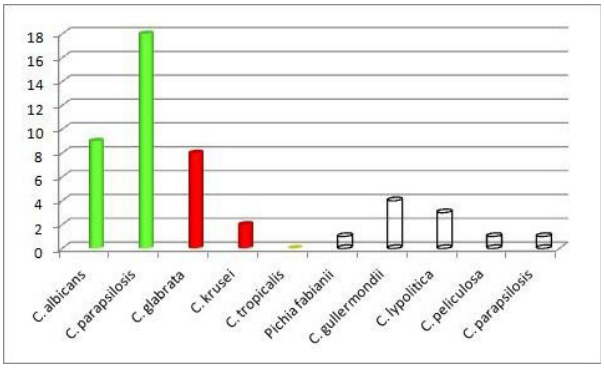

Figure 1

Methods In this study PNA-FISH YTL (AdvanDX) for rapid identification from yeast positive blood culture and culture based MALDI-TOF MS (Bruker) for identification of yeast cells from blood cultures. 47 yeast positive blood cultures from patients hospitalized in University Hospital Center Zagreb during 1 year were analyzed. PNA-FISH YTL identification was routinely performed for every yeast positive blood culture in gram stain. After cultivation conventional methods for identification and culture based MALDI-TOF MS were performed. Results from PNA-FISH YTL were compared with results of identification with MALDI-TOF MS and convential methods.

Results From 27 positive blood cultures with green fluorescence MALDI-TOF MS identification reveal 9 *C. albicans* and 18 *C. parapsilosis*. One isolate from patient hospitalized in pediatric intensive care unit did not show any fluorescence and MALDI-TOF, as well as with conventional methods was identified as *C. parapsilosis*. From 10 positive blood cultures with red fluorescence MALDI-TOF MS identification reveal 8 *C. glabrata* and 2 *C. krusei*. Isolates from yeast positive blood cultures in gram stain that did not show any fluorescence MALDI-TOF MS identified as 4 isolates of *C. guillermondii*, 1 isolate of *Pichia fabianii*, 1 isolate of *C. peliculosa*, and 3 isolates of *C. lypolitica*. (Figure 1) Two of above mentioned blood cultures were mixed and showed red and green fluorescence. From one bottle MALDI-TOF MS identified *C. parapsilosis and C. glabrata* and from other *C. albicans* and *C. glabrata*.

Conclusion PNA-FISH YTL enables clinical microbiologist to report very accurate results of Candida identification sooner (even few days) than with conventional methods and help clinicians to select the appropriate antifungal drug in order to improve the patients outcome, as well as report mixed cultures of etiologic agents causing candidaema especially presence of non-albicans species with intrinsic resistance or reduce susceptibility to some of the antifungal drugs.

P138

Towards non-invasive differential diagnosis of cryptococcosis: magnetic resonance spectroscopy reveals marker metabolites of cerebral cryptococcomas and cell viability

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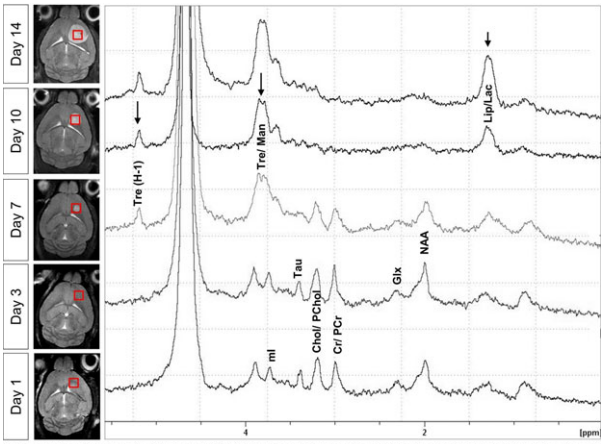
Objectives Central nervous system (CNS) cryptococcosis is associated with significant morbidity and mortality, in part due to the often late diagnosis and associated delay in initiation of antifungal



Data is shown for an animal injected with *C. neoformans* H99. The red square on the MR image indicates the position of the voxel of interest for MRS. Abbreviations: Tre (H-1): trehalose signal originating from the anomeric protons; Tre: trehalose; Man: mannitol; mI: myo-inositol; Tau: taurine; Chol/PChol: (phospho)choline; Cr/PCr: (phospho)creatine; Glx: glutamate/glutamine; NAA: N-acetylaspartate; Lip/Lac: lipids/lactate.

Figure 1. Localized MRS spectra obtained at different time points post injection identified marker metabolites.

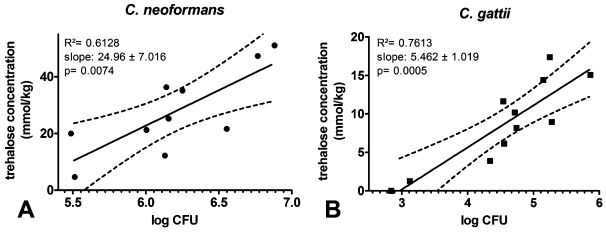


Figure 2. Linear regression shows relationship between log-transformed CFU counts and trehalose concentrations.

treatment. *Cryptococcus neoformans* and *C. gattii* can cause brain lesions difficult to distinguish from other pathologies, like cystic glioblastoma or bacterial abscesses, by means of conventional radiological examinations such as computed tomography (CT) or magnetic resonance imaging (MRI). In addition, it is currently not possible to distinguish between active and sterile microbial lesions *in vivo*. We aimed at the identification of biomarkers present in cryptococcal lesions by use of localized, *in vivo* magnetic resonance spectroscopy (MRS) in a preclinical mouse model of CNS cryptococcosis. We have tested those *in vivo* biomarkers on their ability to quantify microbial load non-invasively.

Methods *Cryptococcus* strains (*C. neoformans* H99 and *C. gattii* R265, GFP+, 10 000 CFUs, 1 µl) were stereotactically injected into the right striatum of female BALB/c mice (n = 2–4 animals per group). Mice were scanned repeatedly over 2 weeks or at a single time point using a 9.4 T MRI scanner (Bruker Biospin). We acquired anatomical (T2-weighted) and diffusion-weighted MRI as well as single-voxel ^{1}H MR spectra of the lesions (voxel size 2 × 2 × 2 mm^3). MRS results were quantified by use of jMRUI to determine absolute metabolite concentrations. Afterwards, brains were isolated for validation with *ex vivo* NMR spectroscopy, histology and fungal load quantification (CFU counts).

Results Disease progression could be monitored longitudinally with MRI whereby increases in lesion size correlated with increased fungal load (Figure 1). Diffusion-weighted MRI showed increased diffusion inside the lesion compared to normal brain tissue (data not shown). MRS detected the presence of typical fungal metabolites like trehalose and mannitol but also lipids inside the cryptococcal lesions (Figure 1), similar to what has been observed in spectra obtained from cell cultures or *ex vivo* lesion material. Comparison with MRS-based metabolite profiles from cystic glioblastoma or bacterial abscesses allows non-invasive distinction between those pathologies. Trehalose concentrations correlated with the number of colony forming units and a potentially higher trehalose production by *C. gattii* compared to *C. neoformans* was revealed (Figure 2). These results were confirmed by *ex vivo* NMR spectroscopy of lesion material but also by fibered confocal fluorescence microscopy (FCFM) (data not shown). The latter showed a higher cell density in *C. neoformans* lesions compared to *C. gattii* lesions.

Conclusion For the first time, this study presents a method for non-invasive follow-up and characterization of cerebral cryptococcomas in a mouse model. The combination of these imaging modalities will contribute to increased understanding of the pathogenesis and dynamics of cryptococcosis including the *in vivo* assessment of novel treatment strategies. MRS identified trehalose as a characteristic metabolite that can be used for diagnostic purposes and as a quantitative biomarker for *in vivo* assessment of the fungal load. These findings have great potential to assist in the differential diagnosis of brain lesions in patients, whereby MRS is a safe, non-invasive and rapid method in comparison to traditional methods such as CSF sampling or biopsies.

Correction added on 19 October 2015, after online publication. Affiliation of author Liesbeth Vanherp was changed from University Hospital Leuven, Leuven, Belgium to KU Leuven, Leuven, Belgium.

P139

TRIADx: A comprehensive and rapid invasive aspergillosis diagnostic testing service for real-time patient management

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Objective Invasive Aspergillosis (IA) remains a major concern for clinicians managing immuno-compromised patients. The Public Health Laboratory London, in collaboration with Queen Mary University of London and Barts Health NHS Trust, have recently implemented a 'triple-test' approach for IA (TRIADx) comprising a commercially available galactomannan (GM) test, a novel sero-diagnostic lateral flow device (LFD) test and a novel (in-house) PCR assay. The rapid, next day turn around time (TAT) provides an IA diagnostic service for real-time patient management. Our aim was to evaluate the performance of the TRIADx service during the set-up phase in high-risk haemato-oncology patients.

Methods This prospective study comprised 92 clinical samples received for TRIADx testing between July 2014 and March 2015. During the evaluation period, requests were received from outside of the Haemato-Oncology setting, including patients on monoclonal antibody treatment for rheumatoid arthiritis, HIV and chronic respiratory disease patients. Samples were also received from external users. Samples were tested using the 'triple-test' approach, as previously described (1). The correlation between biomarker test results was evaluated, as was TRIADx service TAT.

Results Ninety-two samples were received for TRIADx testing (7 bronchoalveolar lavage fluids (BALF), 76 sera, 5 EDTA, 1 sputum, 3 plasma). Five samples were excluded, as EDTA samples are not accepted for the TRIADx service. Fifty-two samples were successfully TRIADx 'triple tested' (56.5% of requests). Due to LFD manufacturing issues, 32 samples were GM/PCR tested only. Due to insufficient sample volume received, 2 samples were PCR tested only and 1 GM only. All samples were tested and reported within 48 h of receipt. Out of

52 TRIADx sample 'triple tests', agreement across all 3 biomarker tests was recorded in 45 samples (86.5%) and between 2 of 3 tests in 7 samples (13.5%). Five samples (from 3 patients) were positive across all 3 biomarker tests; a single patient was triple test positive from a BALF only, a second patient from both a BALF and a serum sample and a third patient from 2 consecutive serum samples. For all 3 cases, TRIADx results influenced real-time patient management. Out of 32 samples that were GM/PCR tested only, agreement between the 2 biomarker tests was 100% (2 positive and 30 negative). For both positive cases, TRIADx results influenced real-time patient management. All five (100%) IA positive cases were outside the Haemato-Oncology setting.

Conclusion Initial results from use of the TRIADx 'triple test' suggest that it impacts positively on patient management. The rapid TAT is essential to supporting real-time clinical management. Samples from both local and distant centres were processed within the self-imposed TAT. This service has improved quality and safety of patient care whilst reducing antifungal drug spend. We are currently setting up an online requesting and reporting system, to assist our external users. All service users are invited to join the Mycology Consortium for the Development of Diagnostics (MyCo D^2) to allow data collection and prospective evaluation of the service. Appropriate recognition will be given to all collaborators.

1. Johnson *et al.* (2015). JCM.00110–15; Epub 2015/04/24

P140

Serum 1,3-Beta-D-Glucan levels are reliable in patients undergoing hemodialysis or hemodiafiltration

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Aim 1,3-beta-d-glucan (BDG) is cell wall component of most pathogen fungi (e.g. Candida spp., Aspergillus spp.) and is used in daily clinical routine as serum marker for diagnosis of invasive fungal infections (IFIs). In the absence of IFIs serum BDG may be elevated in patients undergoing hemodialysis (HD) and hemodiafiltration (HDF). As data about BDG in HD/HDF patients are contradictory we evaluated the influence HD and HDF using synthetic dialyzer membranes on serum BDG levels.

Materials and Methods During March and April 2015 we prospectively included 22 patients with chronic kidney disease (CKD) without signs and symptoms of invasive fungal infections (IFIs). Ten patients did not have HD/HDF and 12 patients had HD/HDF using synthetic membranes (see Table 1). Serum BDG was measured once in patients without HD/HDF to exclude CKD causing falsely elevated serum BDG levels. Serum sampling scheme for BDG testing in patients undergoing HD/HDF is displayed in Figure 1. Additionally, we determined BDG in dialysis fluid as potential source of BDG contamination.

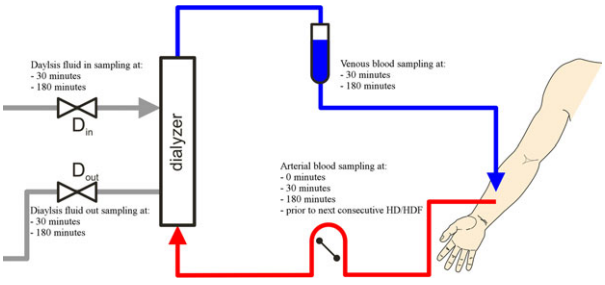

Figure 1. Sampling scheme for patients undergoing hemodialysis or hemodiafiltration

BDG was determined using the automated Fungitell® assay (Associates of Cape Cod, Falmouth, MA) and interpreted as following: <60 pg ml^{-1} negative, 60–80 pg ml^{-1} intermediate and >80 pg ml^{-1} positive. 15.4 pg ml^{-1} is the lower limit of BDG detection in our setting.

Results All patients without HD/HDF treatment had negative serum BDG levels. In patients undergoing HD/HDF 1/72 samples (1.4%) yielded a positive result in the arterial control sample at visit2, prior to the next consecutive HD/HDF session (146 pg ml^{-1}). This particular patient (patient 12) did not receive any kind of medication suspected to cause false positive BDG levels (e.g. beta-lactam antibiotics or blood derivates) or developed signs of IFI without antifungal medication. All other samples remained negative throughout HD/HDF treatment, resulting in a specificity of 0.986 (95% confidence interval 0.925–0.998).

All tested dialysis fluid samples yielded negative results (range <15.4–53.7).

Conclusion Our results suggest that serum BDG testing is a reliable biomarker for IFIs in patients with HD/HDF using synthetic membranes.

P141

Falsely elevated serum 1,3-Beta-D-Glucan levels in patients with gram-negative bacteremia

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Background In absence of invasive fungal infections (IFIs) gram-negative bacteremia was postulated as potential cause of false positive serum 1,3-beta-d-glucan (BDG) levels. However, previous studies were limited due to small sample sizes and contradictory results. Thus, we evaluated serum BDG levels in a cohort of patients with gram-negative bacteremia.

Methods In our NOBIS cohort, over 1000 blood samples were simultaneously obtained with blood cultures from patients presenting to the emergency department at the Medical University Hospital of Graz, Austria. Out of this cohort 103 samples with positive monobacterial blood culture with growth of *Pseudomonas spp.* ($n = 35$), *Enterococci* ($n = 38$), and *E. coli* ($n = 30$) were tested for BDG retrospectively. BDG was evaluated using the automated Fungitell® assay and

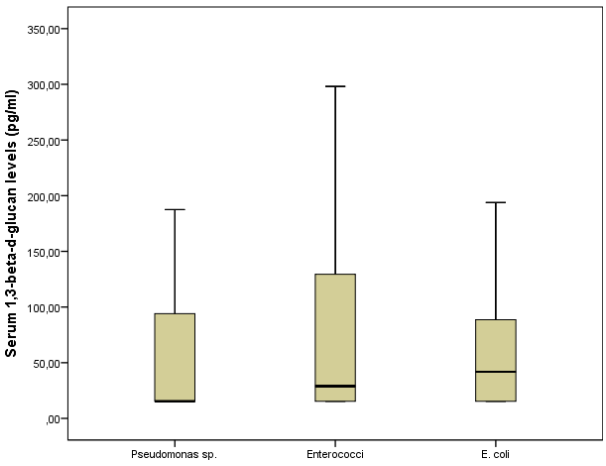

Figure 1. BDG levels in gram-negative bacteremia.

interpreted as following: <60 pg ml^{-1} as negative, 60-80 pg ml^{-1} as intermediate and >80 pg ml^{-1} as positive. 15.4 pg ml^{-1} is the lower limit of detection in our setting.

Results 25.7% of serum samples with *Pseudomonas sp*. bacteremia yielded a positive BDG results, 31.6% of samples with *Enterococci* and 26.7% of samples with *E. coli* bacteremia. Chi-quadrat test yielded no difference regarding the amount of BDG positive sera within our three groups ($P = 0.858$).

Median BDG levels plus interquartile range (IQR) are displayed in Figure 1. Kruskal-Wallis analysis yielded no difference of median BDG levels within the three groups ($P = 0.507$).

Conclusion Gram-negative bacteremia may cause false positive BDG levels in up to 31% of cases. Positive BDG levels in these patients therefore need to be interpreted with caution.

P142

Differentiation of *A. lentulus* and *A. felis* in two clinical cases using the AsperGenius assay

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Objectives The most frequent cause of invasive aspergillosis (IA) is *Aspergillus fumigatus*, followed by *A. flavus*, *A. terreus*, and *A. niger*, but many other *Aspergillus* sibling species have been associated with this infection. Limited data is available on the prevalence of *Aspergillus* sibling species in a clinical setting, which is of concern because these species show intrinsic resistance to antifungal agents. Culturing *Aspergillus* has a low success rate and no commercial tests are able to differentiate sibling species within section *Fumigati*. We describe two cases with proven IA caused by section *Fumigati* siblings and the contribution of the AsperGenius assay in the diagnosis.

Case 1 A 68-year-old man known with allogeneic stem cell transplantation for myelodysplastic syndrome, was admitted to the hospital because of possible IA. Because there was no improvement despite treatment with voriconazole and liposomal amphotericin-B (L-AmB), a lung biopsy was performed that showed fungal hyphae and *A. fumigatus* complex was cultured. The AsperGenius assay performed on this culture was compatible with *A. lentulus*. Therapy was switched to posaconazole for proven IA. Patient died of severe Graft-versus-Host disease. Post-mortem, sequencing confirmed *A. lentulus* and MIC's were 2 μg ml^{-1} for amphotericin-B, 2 μg ml^{-1} for voriconazole, and 0.125 μg ml^{-1} for posaconazole, respectively.

Case 2 A 54-year-old man with progressive chronic lymphocytic leukemia was admitted because of probable IA. Under voriconazole the lung abnormalities were progressive and a lung biopsy was performed. The culture combined with sequencing showed *A. felis* (no MIC's available). Therapy was switched to L-AmB. However, treatment was discontinued because there were no therapeutic options for his disease and patient died shortly thereafter. AsperGenius was performed on material obtained post-mortem and was compatible with *A. felis*.

Methods The AsperGenius multiplex assay was used for the diagnosis of two patients suspected of IA. The assay can detect and differentiate *A. fumigatus* and *A. terreus* directly from bronchoalveolar lavage (BAL) fluid and simultaneously identifies the most prevalent CYP51A mutations using melting curve analysis. Moreover, other clinical relevant *Aspergillus* species can be detected, including members of *Aspergillus* section *Fumigati*.

Results Analysis of both BAL samples resulted in signals for *A. fumigatus* and *Aspergillus* species with the species multiplex assay. No amplification curves or melting peaks were observed for the tandem repeat of 34 bp (TR34), but identical melting peaks for T289A were observed. Remarkably, L98H and Y121F resistance markers showed different melting peaks for both samples. To confirm these findings, sequencing of the calmodulin, beta tubulin, ITS and CYP51A gene was performed. This resulted in identification of *A. lentulus* and *A. felis* (both in section *Fumigati*) harbouring both specific melting profiles for Y121F and L98H resistance markers. These findings were confirmed using additional clinical *A. lentulus* and *A. felis* strains.

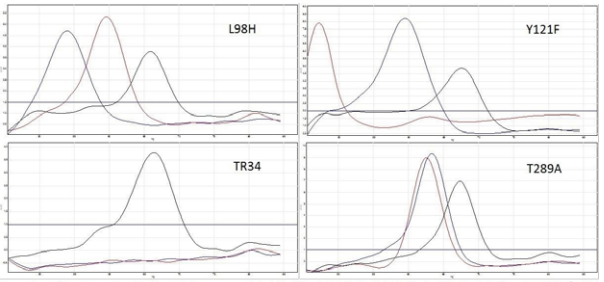

Figure 1: melting curves AsperGenius resistance assay; *A.lentulus* (blue), *A.felis* (red), *A.fumigatus* (black) mutant control (L98H/TR34)

Conclusion The AsperGenius multiplex assay enables detection and differentiation of *Aspergillus* species including members of the *Fumigati* complex using the azole resistance markers Y121F, TR34 and L98H. The ability to identify *Aspergillus* on species/complex level by molecular methods (directly on clinical material) and resistance mutations improvess correct management of these infections.

P144

Diagnostic performance of galactomannan antigen in cerebral spinal fluid in patients with cerebral aspergillosis

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Objectives Cerebral aspergillosis (CA) is a rare but often fatal infection. The diagnosis can be difficult as radiological signs are diverse, obtaining a biopsy may not be feasible, and cerebrospinal fluid (CSF) culture is only positive for *Aspergillus* species in about one-third of the cases. The use of galactomannan (GM) antigen testing on CSF has not been well validated for the diagnosis of CA. Based on 3 small studies ($n = 25$ in total) the test seems promising. In this retrospective study, we evaluated the diagnostic performance of GM antigen test in CSF in patients with suspected CA.

Methods Patients from 2004 to 2014 with suspected CA in whom CSF GM was determined, were selected. Invasive aspergillosis (IA) was defined according to the revised EORTC/MSG criteria. To avoid inclusion of the test that we wanted to validate (= CSF GM) into the gold-standard to which the CSF galactomannan test had to be compared, we removed CSF GM from the microbiology criteria in the EORTC/MSG definition. To avoid overlap between the definition of CA and IA by EORTC/MSG, we excluded central nervous system radiology from the clinical criteria. We defined probable CA when on top of a probable IA, cerebral radiological signs compatible with invasive fungal infections were present. Proven CA was diagnosed when on top of these criteria cerebral pathological evidence of IA or a positive CSF *Aspergillus* culture was present.

A CSF GM ≥1.0 was used as cutoff to calculate the sensitivity, specificity, positive and negative predictive values (PPV, NPV). CSF GM was correlated with serum GM.

Results In total, there were 38 patients with suspected CA. In these patients, there were 6 with proven IA, 11 with probable IA and 21 without IA according to the revised EORTC/MSG criteria. In the proven IA group, there were 4 patients with proven CA and 2 with probable CA (these two patients had histopathological evidence of IA

outside the cerebrum). Fifteen of the 17 proven or probable CA had a positive GM (all of them ≥2.0). Twenty of the 21 without CA had a negative GM. Therefore, the sensitivity, specificity, PPV and NPV was 88.24%, 95.24%, 93.75% and 90.91%, respectively.

Serum GM was available in 16 of the 17 patients with probable or proven CA. CSF GM was higher than serum GM in 8 of these 16 patients and was lower in 1 patient with probable CA. In patients with CA, the mean GM in CSF was higher than in serum (4.89 vs. 3.72), which illustrates that leakage from serum to CSF does not explain the CSF galactomannan levels as this would result in lower CSF galactomannan levels than in serum.

In the 17 patients with CA, there were two patients who had a positive culture in the CSF and one who had positive culture on cerebral biopsy. *A. fumigatus* was cultured in these three patients.

Conclusion GM detection in CSF shows high diagnostic performance for CA. CA can be diagnosed or ruled out based on this assay without the need for cerebral biopsy.

P145

Diagnosis of Pneumocystis pneumonia: switching from DNA quantification to RNA expression analysis

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Context Pneumocystis pneumonia (PCP) is one of the most frequent fungal infections occurring in HIV-positive and HIV-negative immunocompromised patients. PCP diagnosis still relies on microscopic visualization of the microorganism using classical or immunofluorescence stains. However, these classical means fail to detect low fungal burden associated with PCP in non-HIV immunocompromised patients in contrast to quantitative PCR (qPCR) targeting DNA which has become a valuable tool for PCP diagnosis (ECIL-5 guidelines, http://www.kobe.fr/ecil/telechargements2013/ECIL5%20-%20PjP%20guidelines-%20Biology.pdf). Nevertheless, detection of DNA is possible in patients with PCP carriage rather than active infection. As a consequence, the distinction between these two situations has relied until now on quantification of the fungal burden in the respiratory specimens: high fungal burden in active PCP and low burden in carriers (Alanio et al. CMI 2011). However, large overlaps of quantification between carriage and infection were observed. Consequently, qPCR is not fully accepted as a reference diagnostic method despite its ease, high reproducibility and quantitative results.

To overcome this limit, we developed a new assay based on the detection and quantification of the expression of two fungal genes (RNA targets) with calculation of the expression ratio between those genes. We then compared the results to those obtained with our DNA qPCR assay (Alanio et al., CMI 2011).

Methods RNA from 200 consecutive bronchoalveolar lavages (BALs) was extracted using RNA minikit (Qiagen). The expression of two genes (GENE1 and GENE2) was quantified on a LightCycler 480 platform and the ratio between genes was calculated as already described (Pfaffl et al. NAR 2001). Patients with positive DNA and RNA qPCR results were classified by two skilled clinicians as "PCP"(n = 19) or "no PCP"(n = 36) based on clinical, radiological and immunofluorescence findings. Patients with negative DNA and RNA qPCR results were assigned to the "no PCP"group.

Results From the 200 samples, 34 (17%) were both DNA and RNA qPCR positive and 148 (74%) were GENE2 RNA and DNA qPCR negative (Table 2). In 5 (2.5%) samples, GENE2 DNA but not RNA was detected, whereas in 13 samples, GENE2 RNA was detected but DNA was not (Table 2). Sixteen samples with positive GENE2 RNA but negative GENE1 RNA were associated with "no PCP"patients.

The ROC curve analysis of the 30 samples in which GENE1/GENE2 ratio was calculated showed that a threshold between 1.27 and 1.66 had the highest likelihood ratio (LR: 13.13; sensitivity, 0.94; specificity 0.93) for distinguishing infection from carriage.

Based on the whole dataset, 18/19 (95%) "PCP"samples had a positive test whereas 182/184, (99%) "no PCP"samples had a negative test. Specificity and LR of this new assay were higher than that of DNA qPCR (sensitivity, 0.86 vs. 1.00; specificity, 0.99 vs. 0.87; LR, 78.9 vs. 8.0)

Conclusion RNA expression analysis allowed a better discrimination between PCP and carriage based on the detection of different physiological states using only two mitochondrial genes. This assay paves the road for the implementation of new tests to diagnose fungal or parasitic infectious diseases.

P146

Fungal isolates from diabetic amputations: histopathologic spectrum and correlation with culture.

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Objectives Fungal infections are difficult diagnostic and therapeutic problems, serious cause of morbidity or mortality in diabetes. Literature references on fungal infections of diabetic foot ulcers are very scarce and differ significantly. In diabetic patients, mycotic infection can open the door to secondary bacterial infections promoting foot ulcers and gangrene. Because of non-specific nature of the clinical findings in fungal foot ulcer infection, the diagnosis depends on two basic laboratory approaches, mycologic and histopathologic examination.

Methods Twenty five diabetic patients undergoing limb amputation due to gangrene were included in study from a tertiary care teaching hospital over a period of 2 years. During surgery specimens from gangrenous tissue was taken for culture and KOH examination. Amputation specimens were subjected to histopathology examination. Special stains PAS and Grocott were used in all cases.

Results Age of the patients varied from 30 to 90 years with 16 males 9 females. On culture/KOH fungi were isolated in 11 cases, Candida species 8 cases, Trichophyton rubrum 3 cases, Fusarium 1 case. On histopathology with special stains fungi were seen in 4 cases, Candida species 3 cases, Trichosporon 1 case. In one case Candida was seen on histopathology while culture/KOH was negative. 14 cases were negative for fungal culture after 1-month incubation.

Conclusion Because of their typical form and size, fungal elements can be visualized in histopathologic preparations of ulcer using different staining procedures. The most common pathogens are: yeast and dermatophytes and the most frequent dermatophyte is Trichophyton rubrum. Candida infections can be first presenting sign of undiagnosed diabetes mellitus. The knowledge of fungi strain allows using the best agent, dose and length of management and greatly improving the success in treatment.

P147

Galactomannan determination in biological samples of non-hematological patients with invasive aspergillosis caused by different Aspergillus spp.

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Objectives To evaluate the sensitivity of "Platelia *Aspergillus* EIA"test for galactomannan (GM) determination in non-hematological patients with invasive aspergillosis caused by different *Aspergillus* spp.
Methods The investigation included 115 clinical samples collected during 1999–2014 years. from 60 non-hematological patients with diagnosis of invasive aspergillosis (IA) confirmed by classical mycological methods according to EORTC/MSG, 2008. The age of patients varied from 7 months to 83 years (median –36 year). The main underlying diseases were: malignancies (22.4%), chronic sinusitis (15.5%), chronic pyelonephritis (12.5%), chronic obstructive pulmonary disease (8.6%), pneumonia (6.9%), pulmonary tuberculosis (6.9%), systemic diseases with pulmonary involvement (5.8%), chronic bronchitis (9.8%), vasculitis (1.7%), osteomyelitis (1.7$); no underlying disease – 3.5%. Totally 60 serum samples and 55 samples of broncho-alveolar lavage (BAL) were examined for GM *Aspergillus* spp. antigen by means of the "Platelia *Aspergillus* EIA"test (Bio-Rad Laboratories, USA) with calculating of the optical density index (ODI) of the antigen. The serum sample was considered as positive if ODI ≥ 0.5 and BAL sample – if ODI ≥ 1.0. All BAL samples were investigated mycologically including direct fluorescent microscopy with calcofluor white and culture on Sabouraud agar.
Results Cultures of 55 BAL samples from 60 patients with IA revealed various *Aspergillus* spp.: *A. fumigatus* (31%), *A. niger* (31%), *A. flavus* (11%), *A. ochraceus* (1%). From 20 patients (34%) two or more different species were isolated including rare species *A. ustus* (6%) and *A. versicolor* (3%). *Aspergillus* spp. often caused lung disease (68%), more rarely – sinusitis (22%), in 10% of patients > 2 sites of infection. GM test was positive in 45% of serum samples and 60% of BAL samples from patients with mycologically confirmed IA. In cases with *A. fumigatus* as the causative agent of IA sensitivity of GM test in serum was 47%, in BAL – 66%. In patients with IA caused by *A. niger* or *A. flavus* sensitivity of the tests was lower: 36% ($P = 0.02$) and 53% ($P = 0.03$) for *A. niger;* 16% ($P = 0.001$) and 40% ($P = 0.002$) for *A. flavus*. In biological samples of patients with IA caused by several species of *Aspergillus* GM test was positive in 42% (blood serum) and 70% (BAL) of cases. The results show species specificity of *Aspergillus* GM metabolism during the course of infection.
Conclusion The sensitivity of GM test in blood serum and BAL samples from non-hematological patients with invasive aspergillosis is lower than in hematological patients and depends on the type of biological sample and the species of the etiological agent.

P148/M11.3

Brain Abcess due to *Cladophialophora bantiana*: first case in Portugal

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Objectives Cladophialophora bantiana is a highly neurotropic dematiaceous mold that is a rare cause of cerebral abscesses, particularly in immunocompetent patients, and is associated with a high mortality (up to 70%). The aim of the authors is to present a rare with a successful outcome case of a brain abscess caused by this species. Few cases have been reported in the literature world-wide, being this one the first reported in Portugal.
Methods and Results A 56 year-old male, with chronic hepatic disease (HCV and alcohol/drug abuse), HIV negative, referred to the neurosurgery department of our hospital with multiple neurological deficits and a space-occupying lesion in the left frontal lobe. The patient underwent multiple surgical interventions to remove an abscess and specimens were sent to the microbiology laboratory. The first abscess material sent for examination was "dura mater"and

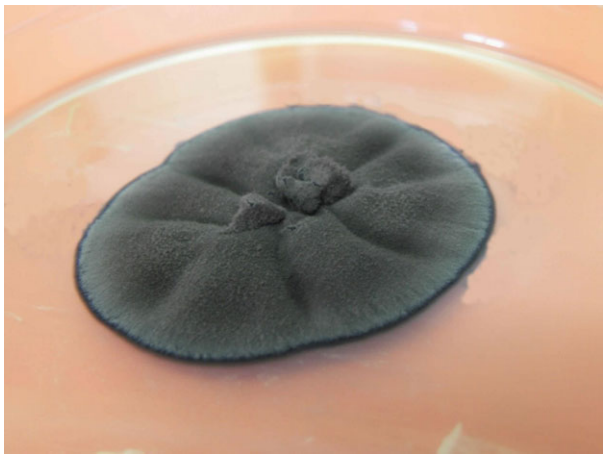

Figure 1

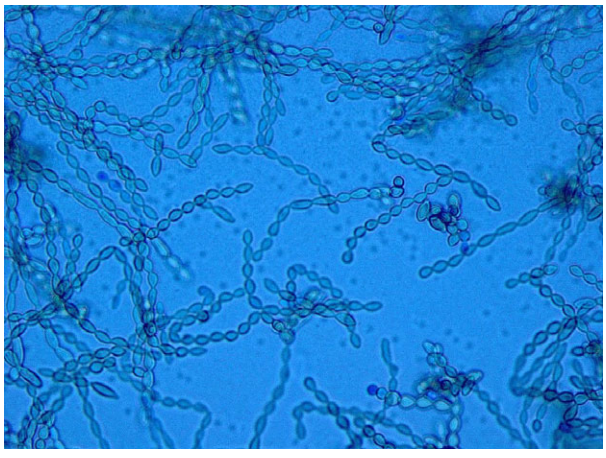

Figure 2

cultures revealed *Cladophialophora bantiana* and *Nocardia nova* (Maldi-TOF score 1.946) but in subsequent abscess material examined (pus) *C. bantiana* was the one persistently isolated. Samples were cultured on Sabouraud dextrose agar (SDA), SDA with gentamicin/chloramphenicol and SDA with actidione. After incubation velvety dark colonies were obtained with surface and reverse olive-gray to black color. Microscopic examination of a lactophenol blue mount preparation showed brown septate hyphae with long, sparsely branched conidiophores bearing wavy chains of smooth oval conidia without pigmented scars. The isolate was identified as *Cladophialophora bantiana* based on its morphological features growth at 42°C and cycloheximide resistance. Total genomic DNA was extracted. The internal transcribed spacer (ITS) region of ribosomal DNA (rDNA) of these isolates was amplified using the primer set ITS1 and ITS4. Sequencing of both strands was performed and nucleotide sequences were edited using the program Chromas2 and aligned using the program CLUSTAL X2. The obtained sequences were compared with sequences deposited in the GenBank and CBS-KNAW Fungal Biodiversity Centre databases: the isolate was identified as C. bantiana with 99% homology. Molecular results confirmed the microbiological diagnosis. Susceptibility testing was not performed. The patient was initially treated with vancomicin, meropenem and liposomal amphotericin B for 28 weeks but the poor clinical and imaging response lead to a switch to amoxicillin/clavulanic acid and voriconazole. After 16 months of the first brain abscess excision and after 5 months under therapy with voriconazol, the patient improved clinical and imagiologicaly, maintaining only minimal neurological deficits.

Conclusion New and emerging pathogens have renewed concern about the diagnosis and treatment of brain abscess and equally pose a challenge to clinical microbiologists. Given the fact that early and accurate diagnosis saves lives, such specimens should promptly be sent for microbiological analysis. Brain abscess is not only a neurosurgical emergency but also a microbiological emergency and a diagnostic challenge to both disciplines.

P149

Association of recalcitrant chronic rhinosinusitis with nasal polyposis and fungal finding in polyps single-cell suspension

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Objectives In recent years fungi are favoured as origin of recalcitrant chronic rhinosinusitis (CRS), especially with nasal polyps (wNP). The omnipresence of fungal spores makes the role of fungi in development of CRSwNP important, but difficult to determine. Data proving the hypothesis that fungi are involved in the pathogenesis of NP are limited mostly because of the difficulty to properly determine fungi and/or to interpret their finding from upper respiratory tract. Sensitive methods for fungal detection are still absent, therefore we used NP single-cell suspension for mycology investigations in aim to find out association of recalcitrant CRSwNP and fungal finding in patients that underwent functional endoscopic sinus surgery (FESS).

Methods A prospective case-series study and culture-based mycological examination was conducted in patients who underwent FESS for the first time (ft-FESS) and those with repeated FESS (re-FESS). The study was conducted in a tertiary Otorhinolaryngology Unit of Clinical Centre of Serbia and National Laboratory for Medical Mycology, Faculty of Medicine, University of Belgrade. A total of 43 consecutive patients with CRSwNP underwent FESS and 55 NP samples were analyzed by culture-based mycological examination. Samples were prepared as single-cell suspensions of NP tissue in Petri dish using a special mesh for the treatment of lymphatic tissue, under sterile conditions (Figure 1). Patient's co-morbidity data were collected.

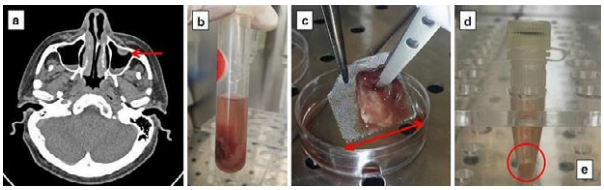

Figure 1. NP tissue processing algorithm for fungal detection

Results Our analyses found: (1) almost half of CRSwNP patients (19/43; 44%) underwent FESS more than once (re-FESS group); (2) fungi were detected (wF) in 10/43 (23.3%) patients (FESSwF group), representing 13/55 culture positive NP tissue (23.6%); (3) re-FESS group is the most often associated with asthma and aspirin intolerance (79; 58%, respectively) compared to ft-FESS group (25; 12%, respectively) ($P = 0.000$, $P = 0.002$; respectively); (4) mycology evaluation of single-cell suspension of NP tissue showed significant higher percentage of fungal finding in re-FESSwF than in ft-FESSsF group (42; 8%, respectively; $P = 0.01$); (5) we observed significantly longer duration of CRSwNP in FESSwF than in fungal negative patients (FESSsF group) (17.4 ± 10.9; 10.4 ± 7.0 years, respectively; $P = 0.033$) and (6) predominate strain was *Aspergillus flavus* detected in 6/10 patients.

Conclusion This is the first study which analysed association of fungi in single-cell suspension of NP tissue and recurrent CRSwNP. We demonstrate significantly higher percentage of positive fungal finding in re-FESSwF than in ft-FESSwF group. Although the role of fungi in CRSwNP is still controversial, we gave new insight in this field showing correlation between fungi and prolonged duration of CRSwNP and fungi and relapse of NP after FESS. Further studies are needed to determine if the nasal lavage with commercial or natural antifungals may have negative impact on the fungal growth in upper respiratory tract and benefit for patients with CRSwNP.

P150

Construction of a new diagnostic tool for the human pathogenic members of the *Fusarium fujikuroi* species complex

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Objectives Construct a new fast, accurate and reliable diagnostic tool for members of the *Fusarium fujikuroi* species complex: *Fusarium* species are emerging agents of superficial, locally invasive and systemic infections in humans. Especially members of the *Fusarium fujikuroi* species complex (FFSC) can cause disseminated fusariosis in immunocompromised – particularly leukemic – patients. Such disseminated fusarioses result in a high mortality rate of patients but patient survival may increase with rapid diagnosis. While most *Fusarium* species have high levels of resistance to antifungal compounds, they vary in their susceptibility towards commonly used antifungal drugs, like amphotericin B, voriconazole and posaconazole.

Methods The DNA-based Luminex assay we selected as platform is a microsphere phase array-based analysis, that combines a multiplex PCR with hybridization to probes linked to fluorescent microspheres, followed by analysis with a flow cytometer (Luminex Molecular Diagnostics, Toronto, Canada). This technique can detect up to 500-1000 different microsphere-linked probes simultaneously, making it possible to screen for a wide set of pathogens in one reaction, while enabling the build-in of safeguards and redundancy.

The FFSC harbours notorious plant pathogens of crops, like maize, rice and sugar cane. At least 13 species of the 34 species within the

FFSC where shown to cause also human infections. As barcoding region for species identification of members in the FFSC complex we chose one of the known more variable gene regions in the translation elongation factor 1-α gene (*tef-1α*). Sequence data of all species were collected from specialized curated databases like the FusariumID and FusariumMLST databases or were generated from well described isolates in this study. The sequence data of 2-14 strains from each species were used to construct consensus sequences for each species. Species-specific single nucleotide polymorphisms (SNPs) were selected for the production of species specific probes.

Results and Conclusions The *tef-1α* gene is one of the known most variable regions within the genus *Fusarium*, and based on SNPs between sibling species it is possible to distinguish between all species – clinical and agricultural – of the FFSC, whether known human pathogen or not. We are currently in the process of testing all constructed probes and testing the sensitivity and reproducibility of the system. To enhance the robustness of the technique even further and not to rely on a single gene detection we will expand the set with probes based on other barcode genes like the RNA polymerase II second largest subunit *rpb2*.

P151

Proteomic and molecular identification of *Aspergillus spp.* in clinical samples isolated from the South of Spain (FUNGAE-IFI Study)

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Objectives To analize the distribution of *Aspergillus* spp. in Andalucía and Extremadura (South of Spain), through a multicenter prospective study involving 16 hospitals, and to determine the performance of MALDI-TOF for routine identification of*Aspergillus* spp. using molecular sequencing as gold standard.

Methods The study was conducted prospectively from July 2014 to March 2015. All the patients who were culture positive for *Aspergillus* spp. in the basis of respiratory samples, blood cultures or biopsy specimens were included. The strains were sent to a reference laboratory included in the list of the participants that made proteomic and genetic identification. For proteomic profile the strains were identified by on-plate extraction method adding 1 μl of formic acid. The acquisition and analysis of mass spectra were performed by a Microflex LT mass spectrometer (Bruker Daltonics) using the MALDI Biotyper software package (version 3.0) with the Filamentous Fungi Library 1.0 (Bruker Daltonics) and default parameter settings and a species cutoff of 1.7.Genomic DNA was isolated and molecular identification was performed by sequencing a portion of the β-tubulin gene. All of the sequences were compared with reference sequences from the GenBank.Different results between MALDI-TOF MS and molecular identification were categorized as erroneous identifications.

Results A total of 179 strains were included. The most frequent specieswas *Aspergillusfumigatus* (55.8%), followed by *A. terreus* (13.9%), *A.flavus* (11.2%), *A.tubingensis* (10.0%) and *A.niger* (3.3%). Other cryptic-sibling*Aspergillus* species accounted for 2.2%, and their distribution was as follow: *A. calidoustus* (1 strain), *A. awamori* (1 strain), *A.carbonarius* (1 strain) and*A.carneus* (1 strain). MALDI-TOF identified 175 isolates, with concordant results compared to molecular identification in 88.3%. Erroneous identification mainly involved cryptic species. *A. tubingensis* and *A. awamori*were identified by MALDI as *A. niger*, and *A. carneus* as *A. terreus*. *A. carbonarius* cannot be identified by MALDI as they are not included in the database. Finally, *A. ustus* and *A. calidoustus* have identical sequence and MALDI identifies as *A. ustus*. The MALDI score median for the most prevalent species confirmed by sequencing was 2281 (*A. fumigatus*), 2227 (*A. flavus*), 2094 (*A. niger*/*A. tubingensis*), and 2068 (*A. terreus*). We observed a geographicaldistributionnot homogeneous.

Conclusion *A.fumigatus* is the most common species of *Aspergillus* isolated. Cryptic species of *Aspergillus* have significant prevalence in clinical specimens, and *A. tubingensis* (section nigri) was the most prevalent. MALDI TOF is very useful in routine identification of *Aspergillus* spp. However, the database would benefit from additional species entries to elucidate the capacity of MALDI-TOF MS to differentiate between phylogenetically closely related species and to decrease the rate of nonidentified isolates particularly its database should be expanded to include cryptic species that could be increase the identification accuracy.

P152

Polymerase chain reaction-sequencing based identification of fungal pathogens in formalin fixed and paraffin embedded tissues

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Objectives To standardize and evaluate polymerase chain reaction (PCR) followed by sequencing for the detection and identification of fungal pathogens in formalin fixed and paraffin embedded (FFPE) tissue biopsies.

Methods Archived FFPE biopsies with proven fungal infections as reported by experienced histopathologists were used for the study. For optimization of DNA extraction from FFPE tissues, we compared the performance of a commercial kit and the conventional phenol chloroform isoamyl alcohol (P-C-I) method on 40 μm sections from 29 FFPE biopsies. For amplifying fungal DNA, the multicopy nuclear ribosomal DNA was selected for amplification using pan fungal primers. Whole Internal Transcribed Spacer (ITS) region, ITS 1 region, ITS 2 region and divergent DI/D2 domains of large ribosomal subunit (28S rDNA) were compared for their amplification from FFPE DNA. To check the specificity of the ITS1 target gene, we correlated FFPE tissue sequence results with culture results for 32 samples. Tissue samples for less common hyalohyphomycosis agents such as *Fusarium, Scedosporium* and mucoralean agents such as *Mucor, Lichtheimia, Cunninghamella* were obtained through disseminated murine infection. Also a mucorale specific PCR was performed for biopsies with aseptate hyphae.

Results The yield of total DNA was higher by the P-C-I method [mean 298.5 ng μl^{-1}] than the commercial kit [mean 121.7 ng μl^{-1}] with the same amount of starting material. The quality of DNA extracted by the two methods showed no significant difference as evidenced by 260/280 ratio: commercial kit [mean 1.92, SD 0.15] and P-C-I method [mean 1.79, SD 0.12]. Out of the different targets tested, ITS1 region showed higher sensitivity (60.9%) in PCR, followed by ITS 2 region (52.2%). The whole ITS (ITS1 + 5.8S+ITS2) region showed poor amplification results (17.4%) and the amplification in D1/D2 domains was 34.8%. ITS1 region sequencing showed good concordance with culture results and also in tissue biopsies obtained from murine infection. Mucorale specific semi nested PCR increased the detection of mucoralean DNA from mucormycosis implicated biopsies (90.2%), than the broad range ITS1 PCR (70.7%). More than one PCR amplicon by pan fungal PCR was frequently seen in tissue biopsies from nasal polyps, nasal scrapings and palatal biopsies indicating the co-amplification of commensal fungal DNA from these sites which included *Malassezia* clones, uncultured ascomycota fungi and *Candida* spp, whereas biopsies from

deep tissues yielded single amplicons. Extraneous fungal DNA contamination was observed in 11.7% samples.
Conclusion In our study, conventional P-C-I method proved better than commercial kit for extracting DNA from FFPE biopsies for fungal PCR. Individual ITS regions, but not whole ITS region is a promising target for broad range PCR-sequencing based detection of fungal signatures in FFPE tissue biopsies. Potential environmental fungal DNA contamination should be prevented by following strict precautions.

P153

Bronchoalveolar Lavage 1,3-Beta-D-Glucan Testing for Prediction of Overall 30-day Mortality

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Background 1,3-beta-d-glucan (BDG) is a cell wall component of most pathogenic fungi. The objective of the study was to investigate the prognostic value of 1,3-beta-D-Glucan (BDG) levels in bronchoalveolar lavage fluid (BALF) samples and to correlate BALF BDG levels with Candida colonization.
Methods A total of 300 BALF samples were collected in 272 patients between 2012 and 2014 at the Medical University Hospital of Graz, Austria, and tested for BDG (using the Fungitell® assay) and growth of *Candida* spp. in fungal culture. Kaplan Meier analysis was performed for three different BDG-level groups: ≤100 pg ml^{-1} (group 1), 101-400 pg ml^{-1} (group 2), >400 pg ml^{-1} (group 3). Hazard ratios for 30- day mortality were determined by COX regression analysis for BALF BDG levels, age at time of BALF and Candida colonization.
Results A significant difference regarding 30-day cumulative survival was observed between *group 1* (17/134, 12.7% died) and *group 3* [22/90, 24.4% died; Log Rank analysis: $P = 0.049$]. Median BDG levels were also found to be significantly higher in patients with *Candida* spp. colonization ($n = 83$; median 289.1 pg ml^{-1}; IQR: 95.1-921.1 pg ml^{-1}) compared to patients without colonization ($n = 215$; median 85.9 pg ml^{-1}; IQR: 15.4-325.4 mg ml^{-1}; $P < 0.001$). In the multivariate COX regression model BDG was the most important predictor of 30-day mortality. The risk of dying within 30 days after bronchoscopy increased by 0.5% with every 10 pg ml^{-1} higher BALF BDG level.
Conclusions BALF BDG was the most important predictive variable in the COX regression analysis and testing of BDG levels in BALF may allow for cut-off dependent prediction of 30-day mortality.

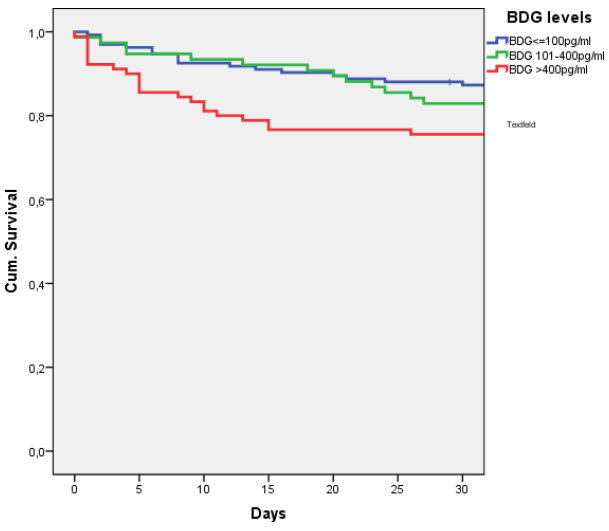

Figure 1. Kaplan-Meier curves, showing 30-days cumulative survival depending on BDG cut-off in BALF samples

P154

Calculation of the Galactomannan-Creatinine Index improves the Diagnostic Performance of Galactomannan testing from Urine Samples

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Background Recent studies on urine Galactomannan (GM) determination did not take into account urine dilution. The aim of this study was to evaluate if an index of urine GM divided by urine creatinine could improve the diagnostic performance of urine GM determination.
Methods This prospective cohort study was conducted between 09/2014 and 05/2015 at the Medical University of Graz, Austria. GM and creatinine levels were determined in 109 urine samples from 18 hematological patients with possible/probable or proven invasive aspergillosis (IA) according to EORTC/MSG, criteria and in 49 urine samples from 49 outpatients without evidence for IA (validation cohort (VC)). We calculated the GM/creatinine index by dividing the optical density index (ODI) value of the urine GM ELISA test (multiplied by 100) by the urine creatinine level. We evaluated the new GM/creatinine index clinically by comparing the index to conventional GM urine measurement.
Results 96 samples from 17 patients with possible IA and 13 samples from 3 patients with probable/proven IA were included in the IA cohort. Conventional urine GM levels were slightly higher in the IA cohort when compared to the VC (median 0.07 ODI vs. median 0.06 ODI, $P = 0.024$), while the GM/creatinine index was markedly higher in the IA cohort when compared to the VC (median 0.17, IQR 0.11-0.31 vs. median 0.07, IQR 0.03-0.14, $P < 0.001$).A GM/creatinine index cut-off of 0.12 was determined by Youdens index and displayed a sensitivity of 69% and a specificity of 73% for IA.
Conclusions Our data indicate that calculation of the GM/creatinine index may improve diagnostic performance of urine GM determination.

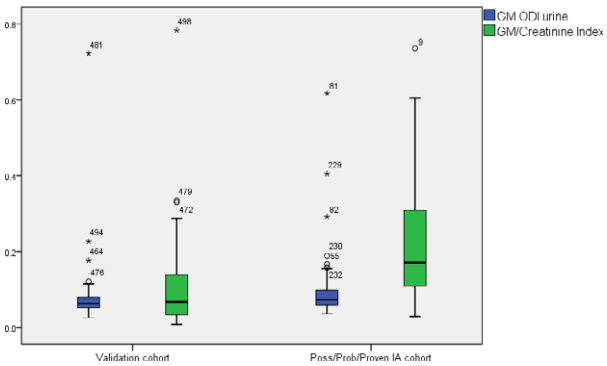

Figure 1

P155

The regional *Paracoccidioides spp.* exoantigen for Double Immunodifusion Radial (IDR) test in Midwest of Brazil.

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Objective Paracoccidioidomycosis (**PCM**) is one of neglected tropical disease(**NTDs**), caused by fungal infection, that's one most prevalent systemic mycosis in Latin America, specially in Brazil. In the central-west region of Brazil the studies with regional exoantigen show greater serological reactivity than exoantigen B-339. The new biological specie named *Paracoccidoides lutzii. (Teixeira et al. 2009)*, is present in this central region of Brazil. The *Paracoccidiodes brasiliensis* complex include species criptics groups as S1, PS2, PS3 e PS4. S1 group is predominant in Latin American and S1 has an predominance in the central-west region of Brazil, therefore studies show relevant results about this serology to monitorate the treatment of Paracoccidoidomycosis. The objective is to study serological (double immunodiffusion technique) of paracoccidioidomycosis patients from Midwest Brazil using regional exoantigen (Exo-MT) that reacts well with most sera from patients living in the state of Mato Grosso – Brazil. **Methods** Tests performed using regional exoantigen (Exo-MT) were reactive with sera from patients with suspected of paracoccidoidomycosis attended by Julio Muller Hospital (HUJM) and MT Laboratory (Lacen-MT) from 2011 to 2015 (until the month of may).
Results The sera of **319** patients were analyzed. This serology was performed by double immunodiffusion technique in agarose gel and data were obtained by lifting medical records and/or patient files. 288 (90.3%) patients were male, raging in age from 1 to 84 years old, with a mean age of **47.4**. The results of serological analysis showed reactive in **134 (42.0%)** and non reactive in **166 (58.0%)**. Of the 134 reactive sera, **99 (73.8%)** were reactive with a regional exoantigen (Exoantigen-MT), and only **35 (26.1%)** sera reacted with exoantigen B-339.
Conclusion False negative results were previously released to patients with PCM. Currently the use of regional exoantigen enables improved sensitivity and specificity in IDR tests used in monitoring the treatment of patients with PCM. If we compare the positivity of Regional Exoantigen against positivity the Exoantigen-MT, we observed a sensitive improvement (48%) in serological reactions by IDR. This results in a thoughtful and careful look at the exoantigens this region of Brazil.

P156

Profiling the diversity of the humoral immune response in feline sporotrichosis due to *Sporothrix brasiliensis* toward discovery of potential diagnostic and vaccine antigens

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Sporotrichosis is a neglected infectious disease that is remarkable in the fungal kingdom due to its occurrence as zoonotic outbreaks or sapronoses around the temperate warm regions. Over the last few decades sporotrichosis has changed from a relatively obscure endemic infection with scattered cases to an emerging health problem, achieving epidemic proportions. *Sporothrix schenckii* and allied species are the agents of human and animal sporotrichosis. The global burden of sporotrichosis stress *Sporothrix* spp. as an important threat the health of the warm blooded host. Epidemiological surveillances highlight an overwhelming presence of the highly pathogenic offshoot *Sporothrix brasiliensis* during feline outbreaks. With a few exceptions, contamination of warm-blooded hosts occurs after traumatic inoculation of propagules of *Sporothrix* spp. into subcutaneous tissue, usually from an environmental source such as soil or plants, but zoonotic transmission from the scratches or bites of a diseased cat is a highly efficient route of disease spread. Upon infection, naturally and acquired immunity triggered by *Sporothrix* antigens may balance the host-parasite interplay, the development of clinical symptoms and control fungal invasion.
Objectives Using an immunoproteomic approach we characterized proteins of potential significance in pathogenesis and invasion that trigger the humoral response during feline sporotrichosis.
Methods We explored the presence and diversity of serum-derived antibodies (IgG) in naturally infected cats against *S. brasiliensis* antigens and its potential use for serodiagnosis. For antigenic profiling, we included yeast cell proteins extracts from the closest known relatives *S. brasiliensis* and *S. schenckii*. Our analyses used ELISA assay and Western blotting, probed with sera from sporotrichotic cats ($n = 49$), healthy feral cats ($n = 19$), and cats with other diseases ($n = 20$). In addition, IgG-seroreactive spots (pooled sera; $n = 10$) were investigated by two-dimensional (2D) gel electrophoresis.
Results ELISA-based quantitation of anti-*Sporothrix* IgG has high sensitivity and specificity among sporotrichotic cats (AUC: 1.0; 95% CI: 0.94-1000; $P < 0.0001$) when compared with controls. A remarkable cross-reactivity was found using both *Sporothrix* antigens, supporting the hypothesis that antigenic epitopes may be conserved among close related agents. Our findings based on 1D immunoblot support 3-carboxymuconate cyclase (60-kDa protein in *S. brasiliensis* and 70-kDa protein in *S. schenckii*) as the immunodomintant antigen in feline sporotrichosis. In addition, 2D immunoblot revealed six IgG-reactive isoforms of gp60 in the *S. brasiliensis* proteome, similar to the response in human sporotrichosis. Although a primary environmental function related to the benzoate degradation pathway of aromatic polymers has been attributed to orthologs of this molecule, our findings support the hypothesis that gp60-70 are important for pathogenesis and invasion in feline sporotrichosis.
Conclusion A convergent IgG-response from different hosts (mice, cats and humans) has important implications for our understanding of the coevolution of the warm blooded host and *Sporothrix*. Moreover, we propose 3-carboxymuconate cyclase as the major target for the serological diagnosis as for vaccine development.

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P157

Development of RFLP-PCR method for the identification of medically important Aspergillus species using single restriction enzyme MwoI

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Objectives In this study we attempted to modify the PCR-RFLP method using restriction enzyme *Mwo*I for the identification of medically important *Aspergillus* species.
Methods Our subjects included 9 standard *Aspergillus* species and 205 *Aspergillus* isolates of approved hospital acquired infections and hospital indoor sources. First of all, *Aspergillus* isolates were identified in the level of species by using morphologic method. A twenty-four

hours culture was performed for each isolates to harvest Aspergillus mycelia and then genomic DNA was extracted using Phenol-Chloroform method. PCR-RFLP using single restriction enzyme *Mwo*I was performed in ITS regions of rDNA gene. The electrophoresis data were analyzed and compared with those of morphologic identifications.
Results Total of 205 *Aspergillus* isolates included 153 (75%) environmental and 52 (25%) clinical isolates. *A. flavus* was the most frequently isolate in our study (55%), followed by *A. niger* 65 (31.7%), *A. fumigatus* 18(8.7%), *A. nidulans* and *A. parasiticus* 2(1% each).
Conclusion *Mwo*Ienabled us to discriminate eight medically important *Aspergillus* species including *A. fumigatus, A. niger, A. flavus* as the most common isolated species. PCR-RFLP method using the restriction enzyme *Mwo*I is a rapid and reliable test for identification of at least the most medically important *Aspergillus* species.

P158

Presence of (1-3) β-D-Glucan in patients at risk for invasive fungal infections

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Introduction Invasive fungal infections (IFIs) occur in up to 10% of immunosuppressed patients causing high morbidity and mortality, in part due to the difficult and delayed diagnosis; improved diagnostic strategies are needed. (1-3) β-D-Glucan (BDG) is a panfungal noninvasive seric marker that has shown promise for the diagnosis of commonly encountered IFIs such as: invasive aspergillosis (IA), invasive candidiasis (IC), *Pneumocystis* pneumonia (PCP) and invasive histoplasmosis (IH). The use of BDG has been validated as an adjunct diagnostic tool in different high-risk populations.
Objectives To evaluate the diagnostic utility of serum BDG for the diagnosis of IFIs in an unselected population of high-risk patients. Describe the incidence of BDG according to the different microorganisms causing IFIs.
Methods We performed an observational, prospective study between November 2013 and May 2014, patients were enrolled from 6 medical institutions. All patients were at risk of IFIs according to the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) criteria, serum BDG was performed to assess its utility for the diagnosis IFIs.
Results We collected the serum of 40 patients at risk for IFIs with proven/probable infections according to the EORTC/MSG criteria. 27 of the patients were male. The most common infections were: IC 27.5% ($n = 11$), invasive histoplasmosis 17.5% ($n = 7$), IA 15% ($n = 6$), *Cryptococcus* infection 12.5% ($n = 5$), PCP 10% ($n = 4$), paracoccidioidomycosis 7.5% ($n = 3$), histoplasmas/criptococcus 5% ($n = 2$), *candida/aspergillus* 2.5% ($n = 1$), and trichosporiasis 2.5% ($n = 1$). The most common organs affected were lung 52% ($n = 21$), central nervous system 15% ($n = 6$), skin 12.5% ($n = 5$), and 40% ($n = 16$) of the IFIs were disseminated involving more than one organ. The BDG test was positive in 67.5% ($n = 27$), negative in 25% ($n = 10$), and indeterminate in 7.5% ($n = 10$). The overall sensitivity was 65.8%, and the specific sensitivity by microorganism was: PCP: 100% (4/4), IA 83.3% (5/6), IC 81.8% (9/11), IH 71.4% (5/7), *Criptococcus* 0% (0/5), *Paracoccidiodes* 30% (1/3), *Tricosporum*: 0% (0/1), IC/IA 100% (1/1), *Histo/cripto* 50% (1/2). The corrected sensitivity excluding 9 patients (5 *Cryptococcus*, 3 *Paracoccidiodes*, 1 *Tricosporum*) is 84%. Serum BDG showed good sensitivity for the diagnosis of PCP, IA, IC, and histoplasmosis but was insensitive for the diagnosis of cryptococal infections, and the less frequent infections caused like paraccocidiodomycosis and trichosporiasis.
Conclusion Serum BDG is a useful test for the diagnosis of IFIs in immunosuppressed patients, in agreement with previous reports. The BDG test has a good performance for the diagnosis of IC, IA, PCP and histoplasmosis in our cohort. The BDG test does not detect infections caused by *criptococcus* and other infrequently found fungal infections.
Keywords Invasive Fungal Infections (IFIs), 1-3 β-D-Glucan (BDG), Invasive aspergillosis (IA), Invasive candidiasis (IC), *Pneumocystis* pneumonia (PCP).

P159

Evaluation of mueller hinton agar for germ tube test

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Objectives To aim of this study is to evaluate Mueller Hinton Agar (MHA) as a simple medium for the determination of germ tube test (GTT).
Methods A total of 86 *Candida* species isolated from different clinical specimens were included. All isolates were cultured onto Sabouraud Dextrose Agar (Salubris, Turkey) and incubated at 37°C for 24–48 h. They were identified by VITEK MS (Biomérieux, Frence). For serum GTT, a single *Candida* colony was inoculated into 0.5 ml freshly obtained human serum. For MHA GTT, an inoculum of single colony was streaked by Dalmau technique onto MHA and covered by a sterile coverslip. After incubation at 37°C for 1.5 h and 3 h, all sera and MHA plates were examined using a light microscope at ×40. Serum GTT was accepted as gold standard method for the determination of sensitivity and specificity values.
Results The identification results were as follows: 51 *Candida albicans*, one *C. dubliniensis*, 12 *C. parapsilosis*, 12 *C. glabrata*, 4 *C. kefyr*, 3 *C. tropicalis*, 3 *C. krusei*. Serum GTT was positive in 30 (58.8%) and 36 (70.6%) of *C. albicans* isolates at 1.5 h and 3 h, respectively. MHA GTT was positive in 21 (41.2%) and 40 (78.4%) of *C. albicans* isolates at 1.5 h and 3 h, respectively. All non-albicans species except *C. dubliniensis* were found as negative using both tests. Both GTTs were positive for *C. dubliniensis* at 1.5 h and 3 h. MHA sensitivity and specificity was determined as 58% and 92% at 1.5 h; 86% and 81% at 3 h, respectively.
Conclusions MHA is safer and easier medium for GTT that may be used as alternative to serum GTT. The evaluation of GTT on MHA after 3 h incubation is recommended since it has higher sensitivity compared to 1.5 h.

P160

Possible impact of beta-glucan assay results on the length of antifungal therapy

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Objectives To evaluate sensitivity, specificity, positive and negative predictive value of beta-glucan (BG) assay and to estimate the impact of this test on the length of antifungal therapy (AF-Tx) in patients with proven candidemia or for which an empirical antifungal therapy was started.

Methods Prospective, monocentric, cohort study. An ID consultation team was implemented in November 2012 in a 1100-bed university hospital in Rome, Italy, to optimize anti-infective treatment. Data for every consultation were prospectively collected by the team using a standardized database. In the present study, we included patients with proven candidemia, patients for which an AF-Tx was started but without candidemia, and patients who never started an AF-Tx but for which a BG test was done. A BG result was considered evaluable when it was performed ± 48 h from the positive blood culture or from starting an empiric AF-Tx. Positive BG was defined when >80 pg ml^{-1}.

Results At April 2014, an evaluable BG result was available for 1365 patients. Of these patients, 251 (111 BG positive and 140 BG negative) started AF-Tx and 1114 (150 BG positive and 964 BG negative) did not start AF-Tx. Sensitivity, specificity, positive predictive value and negative predictive value were 44.2%, 86.5%, 42.5%, and 87.3%, respectively. Including only patients with proven candidemia, the sensitivity, specificity, positive predictive value and negative predictive value were 86%, 86.5%, 22.7%, and 99.2%, respectively.

Overall, there were 538 (124 with proven candidemia and 414 without proven candidemia) patients, including the 251 with an evaluable BG result, started an AF-Tx. For this group of patients, median age was 67 years (IQR 52–77), 52% were males, 53.7% had a CVC, and 35.1% received an antibiotic therapy within the previous 30 days. Mean length of antifungal therapy was 12.7 days (SD 11.0) in patients with a negative BG result, 20.2 days (SD 17.5) in patients with positive BG and 15.5 days (SD 12.5) in patients without an evaluable BG result (P at ANOVA <0.0001). Similar results were found excluding patients who died during the study period.

Conclusions As expected, BG assay showed a very high negative predictive value. When used in a real-life setting, BG assay allowed to reduce the mean antifungal treatment length of 3 days.

P161

Aspergillus antibody detection: results of the French Society for Medical Mycology (SFMM) poll among French experts on the use of technical tools

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Objectives The detection of *Aspergillus* antibody is an essential biological argument for the diagnosis of allergic and chronic pulmonary aspergillosis. For this purpose, numerous commercial and in-house techniques are available. The French nomenclature recommends a two-steps strategy consisting in a screening test followed in case of positivity by a confirmatory one. This situation leads to a huge number of serological practices. In order to better appreciate the usage of and the confidence in these techniques, the SFMM initiated a poll among a panel of French experts.

Methods In January 2015, forty mycologists were asked to answer an on-line questionnaire. For each of the 37 questions, an answer was mandatory, using a score from 1 to 9: from full disagreement (1) to full agreement (9). Based on information from a nationwide French EEQ program, questions investigated eight different techniques: indirect hemagglutination, ELISA (without other precision), electrosyneresis, double immunodiffusion, immunoelectrophoresis, coelectrosyneresis and western blot. Descriptive statistics was performed using box plots to determine the median value and the dispersion of the values. In addition, the coefficient of variation (CV) was calculated for each item.

Results 36 questionnaires were completed. CV ranged from 10% to 75%, 38% of them being superior to 50%. Differentiation between screening and confirmatory techniques was considered overall relevant (median = 7). A median ≥ 8 with a CV <30% was only observed for ELISA as a screening test, and for immunoelectrophoresis and western-blot as confirmation test. Hemagglutination was not recommended neither for screening nor for confirmation. The use of a confirmation technique was strongly recommended when the screening test is positive (median = 9; CV = 14%). This is also recommended even in the case of a negative screening, depending on the clinical context (median = 8; CV = 31%) or during follow-up of patients (median = 8; CV = 33%). When the screening and the confirmation techniques are discordant, the experts recommended to interpret the results giving priority to the confirmation technique and integrating the clinical data of the patient (median = 8; CV = 28%).

Conclusion This first investigation on serological tests for *Aspergillus* antibody detection points out the diversity of the practices in France. Only, very few items received a clear response allowing to edict recommendations among which, *Aspergillus* antibody detection should include an ELISA testing as screening and precipitin detection using immunoelectrophoresis as confirmation. This poll however underlines the lack of standardization of several methods, including the immunoelectrophoresis still considered as the reference method. These results warrant further studies to better evaluate the performance of those techniques for diagnosis and follow-up.

P162

Molecular diagnosIs of *Pneumocystis jirovecii* pneumonia: is the dilution factor of bronchoalveolar lavage a confounding factor for the interpretation of real time PCR results?

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Objectives PCR-based methods can improve the microbiological diagnosis of *Pneumocystis jirovecii* (PJ) pneumonia (PJP) especially in HIV-immunocompromised patients in which low fungal loads are found in cases of PJP. Semiquantitative information obtained by real time PCR (PCRrt) applied to bronchoalveolar lavage (BAL) may allow differentiation between PJP and PJ colonization status but there is range of positive values that cannot discern between the two entities. One possible hypothesis is that the variable dilution factor of BAL could interfere with the interpretation of PCRrt results.

The aim of this study is to assess the effect of the dilution factor in BAL samples on PCRrt values by comparing their values with the concentration of human DNA present in the sample in order to normalise PCRrt results.

Methods Between November 2013 and November 2014, we received 68 BAL from 68 immunocompromised patients (including HIV+ and HIV- patients) for PJ investigation. A PCRrt which targets

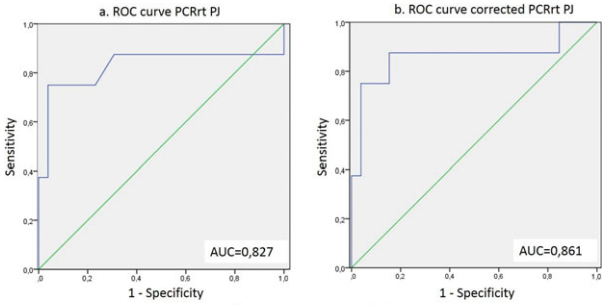

Figure 1. ROC curves analysis

Figure 1

the dihidropteroate synthase gene (DHPS) was performed to detect and semiquantify PJ. Human DNA present in samples was semiquantified in 63 samples using a PCRrt, which targets human RNAase P. For further analysis, only the 63 samples having results for PJ and human DNA were taken into account. Results were expressed in cycle threshold (Ct) values.

Demographics and clinical variables of patients were recorded. According to clinical, radiological and laboratory findings, patients were classified as PJP, PJ's colonization and PJ-unrelated clinical process.

The accuracy of PCRrt and corrected PCRrt for human DNA was measured by the area under the Receiver Operating Characteristic (ROC) curve (AUC).

Results 8 out of 63 patients were classified as PJP; additional 26 patients were categorized as colonisations. Mean and 95% confidence interval (95%CI) of PCRrt Ct values were 27.9 (23.8-32) and 33,9 (32.7-35.1) for PJP and PJ's colonisation respectively. Mean and 95%CI of PCRrt Ct values corrected for human DNA were 27.9 (23.9-31.2) and 34.0 (32.4-35.6) for PJP and PJ's colonisation respectively. ROC curve analysis showed an AUC = 0.827 for PCRrt values and AUC = 0.861 for PCRrt corrected for human DNA values. Cutoff Ct values were 28.3 for PCRRrt and 28.7 for corrected PCRrt. Both PCRrts (corrected and not corrected) showed identical values: sensitivity = 75%, specificity=96.2%, PPV = 85.7% and NPV = 92.6%.

Conclusion Introducing a correction for the variable dilution factor of BAL. marginally improves the results obtained with the PJ PCRrt studied. Nevertheless, the slight improvement introduced in the AUC adds robustness to the Ct values. Further studies aimed to increase the number of cases included in our series are warranted to assess the usefulness of this approach.

P164

Molecular diagnosis based on species-specific nucleic acid detection of the pathogenic agents of sporotrichosis

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Sporotrichosis is a chronic infection of the cutaneous and subcutaneous tissues caused by thermodimorphic fungi embedded in the plant-associated order Ophiostomatales. With major differences between routes of transmission and virulence traits, *Sporothrix* are emerging as new threat to the vertebrate host, particularly in form of outbreaks. Large zoonoses and sapronoses are ongoing in South America and Asia, respectively. Currently diagnosis methods based only on morphological and biochemical features are inaccurate due to the overlap of phenotypes; nevertheless with the introduction of dissimilar species with differences in epidemiology, host predilection, virulence and antifungal susceptibilities there is a critical need for developing new diagnostic tools that are sensitive and cost effective.

Objectives In the present study, we have successfully applied species-specific primers to: (1) Identify etiological agents of human and animal sporotrichosis circumscribed in the *S. schenckii* complex; (2) perform large scale screening of *Sporothrix* in epidemiological studies; (3) detect *Sporothrix* spp. DNA in fresh-tissue and biological samples from experimentally infect animals; and (4) we highlight the utility of *Sporothrix* conidia as a simple, fast-processing source material for genotyping.

Methods We developed a panel of novel markers based on calmodulin (*CAL*) gene sequences with potential applications to perform large scale diagnosis and epidemiology of members of the *Sporothrix-Ophiostoma* complex including *S. brasiliensis*, *S. schenckii sensu stricto*, *S. globosa*, *S. mexicana*, *S. pallida* and *Ophiostoma stenoceras*. *CAL* sequences, covering a worldwide array of haplotypes were aligned and primers were designed to target species-specific regions.

Results *In silico* primer-BLAST searches revealed that candidate sequences were conserved intraspecifically through a genetically diverse group of *Sporothrix*. Moreover, primers were highly sensible being able to detect as much as 1 pg of gDNA in a single round PCR. A simple assay using conidia directly in PCR as a source of DNA was effective for the rapid and low-cost genotyping of *Sporothrix*. We explored a murine model of disseminated sporotrichosis and successfully detected *S. brasiliensis* and *S. schenckii* DNA from a variety of fresh-tissue samples including the spleen (100%), liver (100%), lungs (100%), heart (100%), brain (100%), kidneys (100%), tail (100%) and feces (60%) of the diseased animals, but not from the control group (healthy animals). Our results suggest that animal feces may be valuable samples for ecology and epidemiology of sporotrichosis. The knowledge that feces from diseased animals may infect adjacent soil and increase the focus and risk of outbreaks in endemic areas are important to implement measures to contain the progress of the epidemic.

Conclusion The singleplex PCR-based method was successfully used for the detection and identification down to species level from both culture and clinical samples. Thus, the feasibility of the species-specific primer lies on its simplicity, high throughput, and accurate diagnosis of sporotrichosis. Improvements in diagnosis and surveillance systems may help to identify and tackle future outbreaks.

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P165

Genus/species-specific versus panfungal PCR assays for detection and quantification of fungal pathogens in clinical samples from immunocompromised patients

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Introduction Molecular methods, the most important of which is PCR, are routinely used in every day clinical microbiology practice. Concerning fungal pathogens, molecular methods have their advantages in great sensitivity and possibility to identify fungal species very accurately. The major point of criticism of its general use is lack of standardization, since most of the leading laboratories successfully use in-house PCR assays and widely validated commercial assays are missing.

Material and methods For PCR detection of fungi in clinical samples either genus/species-specific or panfungal assays can be used. Since lungs are the most common organ affected, bronchoalveolar samples are frequently tested for presence of fungal pathogens. In our institution we routinely screen BAL samples from patients at high risk of IFD or symptoms suspicious of IFD (CT findings, positive serum galactomannan etc.) with two methods – commercial real-time PCR assay for detection of 4 most common *Aspergillus* species and in-house PCR followed by high resolution melt analysis (HRMA) for detection of mucormycetes. If results of both assays are negative and there is still clinical, epidemiological and/or histological suspicion of fungal infection, samples are further tested by panfungal PCR followed by sequencing or HRMA.

Results Advantages or disadvantages of both approaches must be considered. Species-specific primers might miss infections caused by untargeted fungi; conversely, panfungal primers can provide false positive results due to environmental fungal DNA contamination. Results of species-specific methods are usually available within 24 h, while when panfungal PCR assays are done verification of the results by sequencing is necessary. Due to above raised issues, use of panfungal PCR has been so far focused mainly on sterile samples (serum, blood, cerebrospinal fluid) or for identification of fungal pathogens in

fresh or formalin-fixed paraffin embedded tissue specimens from patients with culture or histologically proven IFDs.
Conclusions The aim of this presentation is to summarize our experience and published data concerning testing of various clinical samples (BAL, cerebrospinal fluid, serum, tissue) from immunocompromised patients using specific and panfungal PCR assays.

P166

Investigation of a Semi-automated DNA Extraction Procedure from Stool Specimen with a Quantitative Real-time PCR Assay for the Detection and Identification of Microsporidial Infections

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Objectives Microsporidia are fungi-related pathogens mainly responsible for diarrhoea in immunocompromised patients. However, the spectrum of patients at risk has been recently enlarged making the screening for microsporidial infection more frequently indicated. A number of species-specific PCR and probe-based real time PCR have been developed but either fail to cover the diversity of species involved or represent a significant financial effort if used in a screening strategy. In addition, the isolation and amplification of DNA from stool samples is challenging, due technical barriers and the frequency of polymerase inhibitors in stool. In this study, we investigated (i) a simple semi-automated extraction procedure involving a simple chemical lysis of pathogens, and (ii) a novel quantitative PCR based on a fluorescent dye and including a melting-curve analysis for species identification.
Methods Ten stool specimens previously found positive by microscopic examination were processed in parallel using ParaGENIE® DNA Stool Extraction kit (Ademtech) and QIAamp DNA Stool kit (Qiagen). In addition, quantified DNA extracted from culture spores of different species were tested. qPCR was set up to specifically amplify rDNA SSU of all microsporidia species. A touch-down amplification of 40 cycles was performed using an EvaGreen mix on a Stratagen MX3005 thermal cycler, followed by a final denaturation step for melting temperature determination. An internal positive control (IPC) was also added to the assay. A prospective study was then initiated on clinical samples collected from diarrheic patients over a 4 months period.
Results qPCR assay produced amplification with all the microscopy-positive samples with a 100% (range 95-105%) efficiency and a dynamic range of at least six orders of magnitude. The specificity of the assay was validated against various microorganisms, especially protozoan. Three different melting temperatures, all different from that of IPC, allowed the differentiation between *Enterocytozoon bieneusi*/*Encephalytozon intestinalis*, *Encephalitozoon cuniculi* and *Encephalitozoon hellem*. Comparative results between the two extraction methods showed a benefit of a mean of 5 cycles threshold using the ParaGENIE® DNA Stool Extraction kit. Besides, this new extraction procedure allowed to process twelve specimens in parallel and took half the time compared to the QIAamp DNA miniStool kit. During the prospective study, among fifty-two tested patients, two cases of microsporidial infections were diagnosed. One of them was found in an immunocompetent patient who had not been initially identified by microscopic examination.
Conclusion The method set up in this study allows a simple, rapid and sensitive screening for microsporidial infection in diarrheic patients. Differentiation of species is partially possible and can be confirmed with an additional molecular method. ParaGENIE® DNA Stool Extraction kit, which has been shown to efficiently extract DNA from *Giardia intestinalis*, *Amoebae*, and *Cryptosporidium spp*, leads to higher microsporidial DNA recovery, increasing thus the sensitivity of detection.

P170

Saprolegnia parasitica qPCR Quantification in water: since the areas of high fish mortality in river to tap water

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Objectives In the river Loue (Franche-Comté region, East of France), the death of large numbers of fish is observed for several years. An aquatic oomycete, *Saprolegnia parasitica*, could contribute this important mortality cases observed in *Salmo trutta fario* and *Thymallus thymallus*. *S. parasitica* is a secondary pathogen and only colonize unhealthy and morbid individuals, being the cause of skin mycosis, respiration trouble and death.

Besançon city is partly supplied by two pumping water station on the river Loue. The biggest pumping water station deliver drinking water in 39 000 inhabitants of the city of Besançon. The presence of *S. parasitica* in the Loue river therefore raises the question of its presence in drinking water and this potential risk for the human health, even if no link to health problems has ever been described. Health authorities in the region have requested to develop a specific technique to quantify *S. parasitica* in the Loue river and use this tool to verify its absence in drinking tap water.
Methods A species-specific quantification assay using real time quantitative PCR (qPCR) was developed to analyze the abundance variation of *S. parasitica* in Loue river, during spawn period of *Salmo trutta fario* and *Thymallus thymallus*, and in the drinking water. Water samples (10L) and baiting samples (with hemp seeds) were performed in the Loue river, once a week during 12 weeks, between late January and early April 2015. Six stations were defined along the river, from the river spring to the interest area (water pumping station temporally closed for renovation). Devices with hemp seeds, which are baits for the pathogen, were used to verify its presence.

Tap water samples (10L) were performed in two dwellings too: one connected to the little pumping station in the headwater of the river, and another pumping station.
Results *S. parasitica* DNA was detected in 64% of river samples ($n = 72$), with quantity between 0.03 to 68 equivalent spores per liter. Time and space related variations were observed in the river. Hydrologic data have been considered. A dilution effect seems to occur during high flow periods not allowing detecting the pathogen with qPCR, whereas baiting samplings prove its presence. Greater quantities seem to be observed at the sampling station near the big pumping station. No *S. parasitica* DNA was detected in the tap water.
Conclusion This research work led us to suggest a standardized detection tool to quantify *S. parasitica* in the water. Larger quantities observed near to the big pumping station showed that at the opening of the station after renovation, the absence of *S. parasitica* should be tested in tap water from this station.

P171

Evaluation of the susceptibility of phlebotomine sand flies to *Beauveria bassiana* and to the essential oil of *Eucalyptus globulus*

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Leishmaniases are diseases which affects several animal species including humans, caused by protozoa of the genus *Leishmania* and transmitted through the bite of infected female phlebotomine sand flies. *Beauveria bassiana* is an entomopathogenic fungus, which can act as a biological control agent against important vectors of

pathogens. A few studies have demonstrated that *Phlebotomus papatasi*, the vector of *Leishmania major* in the Old World, and *Lutzomyia longipalpis*, the main vector of *Leishmania infantum* in the New World, are susceptible to *B. bassiana*. Besides that, the essential oils of *Eucalyptus* trees are natural insecticides for controlling *Lu. longipalpis*. The biological control of these vectors would reduce the use of chemical insecticides, resulting in benefits for the human beings and for the environment.

Objective Therefore, the aim of the present study was to evaluate the effect of the association of *B. bassiana* with the essential oil of *E. globulus* for the biological control of *Lu. longipalpis.*

Methods Phlebotomine sand flies of the species *Lu. longipalpis* were collected using CDC light traps in the municipality of Passira, Pernambuco State, Brazil and separated into five groups comprising 10 specimens per group. Treatments were carried out using *B. bassiana* (10^7 spores ml^{-1}), *E. globulus* (0.1 mg ml^{-1}), *E. globulus* plus *B. bassiana*, distilled water as negative control, and cypermethrin (0.1 mg ml^{-1}) as positive control. The formulations were sprayed onto a filter paper. Each experiment was performed twice, and *Lu. longipalpis* survival rates were recorded.

Results The groups treated with *B. bassiana*, alone or in association with *E. globulus* essential oil presented a reduced survival time. In particular, the negative control group survived for 13 days approximately, while the treated groups presented an average survival time of about 6 days (i.e., half of its lifespan). Positive control group survived for around 24 h. No significant difference was observed between the groups treated or not with *E. globulus* essential oil.

Conclusion These results show that the entomopathogenic fungus is a good tool to be used in the biological control of *Lu. longipalpis*. On the other hand, the use of *E. globulus* essential oil did not potentiate its pathogenic effect on *Lu. longipalpis*. Further studies are needed to test a larger number of phlebotomine sand flies and to assess the efficacy of this biological control agent under environmental conditions. The development of low-cost, biologic control tools against vectors should be encouraged for controlling vector-borne tropical diseases such as leishmaniasis.

P172

Characterization of the fungal Microbiome in dolphins of the coast of Portugal

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Objectives Marine mammals are excellent sentinels reflecting the effects of natural and anthropogenic threats on marine ecosystem. However, data on changes in marine mammal health are quite limited in animals inhabiting Portuguese and northern Spanish waters. The information available regarding microbiological aspects of marine mammals describes mainly pathogenic bacteria and, despite reports of new or re-emerging fungal infections with epizootic and potential zoonotic consequences, studies on fungal species are lacking. The new emerging diseases elucidate the need for detailed epidemiological studies to predict outbreaks and facilitate their control.

The main objective of the present study was to investigate whether dolphins might serve as potential reservoirs for known and emerging human and marine mammals fungal pathogens.

Methods A total of eight stranded dolphins were studied. Samples were collected during necropsies by authorized personal of Sociedade Portuguesa de Vida Selvagem and Coordinadora para o Estudio dos Mamiferos Mariños, using sampling protocols already established.

For the mycobiome analysis, total DNA of tissue samples of oral cavity and vagina was extracted using JetQuick DNA purification Kit and the Internal Transcribed Spacer (ITS) region was sequenced with Illumia MiSeq. The data analysis was done using the QIIME pipeline and the R package.

For fungal culture and isolation, swab samples from the oral cavity and blowhole were collected. The culture was performed using PDA and Sabouraud-Dextrose agar and the incubation was performed at 30°C and 18°C. Fungal identification was performed by macro and microscopic observation, and by sequencing the ITS regions. Fungal ITS sequences obtained were compared against NCBI nucleotide database for fungal identification.

Results The metagenomic analysis demonstrated the presence of 29 fungal genera across all samples studied. Among the genera, 6 were present only in oral cavity and 12 genera were present only in vaginal tissue. *Malassezia* species were the most frequent, followed by *Candida, Debaryomyces, Cladosporium, Aspergillus, Penicillium and Rhodotorula*; and all these genera were found both in oral and vaginal tissues.

The fungal culture and isolation using different temperatures and media resulted in a total of 92 isolates, including 68 yeasts (74%) and 24 filamentous fungi (26%). The oral cavity presented a higher number of isolates (45 yeast isolates and 9 filamentous fungi), when compared with the blowhole (23 yeast isolates and 15 filamentous fungi). No significant differences between the use of different media and temperatures were observed. *Candida* species were the most frequently found, followed by *Rhodotorula* sp., *Aureobasidium sp., Debaryomyces* sp., *Penicillium* sp. and *Cladosporium* sp.

Conclusion This study is the first systematic work for evaluation of dolphin fungal microbiome in different body sites. The preliminary results demonstrated a high fungal diversity in all body locations studied and highlight variation in the their distribution according to organ type. Furthermore, a high variety of known fungal pathogens were detected, such as *Candida tropicalis*, *C. guilliermondii*, *Fusarium oxysporum*, *Scedosporium apiospermum*, *Cladosporium cladosporioides* and different *Malassezia* species, confirming these marine mammals as potential reservoirs.

P175

Rhodotorula glutinis catheter-related fungemia in University of Santo Tomas Hospital: A Case Report

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Objective To describe a case of *Rhodotorula glutinis* catheter-related fungemia in an immunocompromised patient with sigmoid adenocarcinoma who was successfully treated with caspofungin and removal of central venous access.

Method We describe the risk factors, course and treatment of a patient who acquired Rhodoturolosis after undergoing abdominal surgery and receiving multiple antibiotics and total parenteral nutrition through her central vascular access.

Results The use of a central venous catheter, exposure to broad-spectrum antibiotics and total parenteral nutrition, recent abdominal surgery, and the presence of malignancy are the known risk factors found in the patient to develop *Rhodotorula* fungemia. Although echinocandins are shown to have high minimum inhibitory concentrations against *Rhodotorula spp*., it was succesfully used in eradicating this pathogen with concomittant removal of the central vascular device.

Conclusion *Rhodotorula* is an emerging opportunistic fungus reported to cause infection in immunocompromised patients. Most common risk factors for developing infection with this organism are malignancy, use of cytotoxic drugs, and central venous catheters. It is most effectively treated with amphotericin B or flucytosine. This case is the first reported documentation of *Rhodotorula glutinis* catheter-related fungemia with clinical and microbiologic improvement after treatment with echinocandin and catheter removal.

P176

Gastrointestinal Basidiobolomycosis; a new common entity in the southern region of Saudi Arabia

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Background Basidiobolous ranrum is a fungus that is known to cause subcutaneous infection. Invasive form is a rare entity and poorly recognized. Clinical presentation is wide, non-specific symptoms masquerading inflammatory bowel disease, Tuberculosis or Malignancy.
Case Report We report a 33-year-old Saudi male suffered from gastrointestinal basidiobolomycosis. He was misdiagnosed as malignancy and then as intestinal Tuberculosis. He presented with 4 month-history of abdominal pain, vomiting, diarrhea, recurrent melena, weight loss and shortness of breath. Clinical examination and laboratory investigations showed unilateral pleural effusion, minimal ascites, anemia and peripheral eosinophilia. Endoscopic and radiological examinations showed diffuse thickening of the whole gastrointestinal wall from the upper esophageal sphincter up to the right colon with multiple intramural lesions and stenosis. Treatment with Antituberculosis drugs failed to prompt a response compelling an exploratory laparotomy with right hemicolectomy. Histopathology revealed a granulomatous inflammation with Splendore-Hoeppli Phenomenon. Patient improved clinically and endoscopically with itraconazole.
Conclusion Gastrointestinal basidiobolomycosis appears to be endemic in the Southern region of Saudi Arabia. It should be considered as differential diagnosis in patient resining in this part of the country with suspicion of intestinal TB or Inflammatory Bowel Disease.

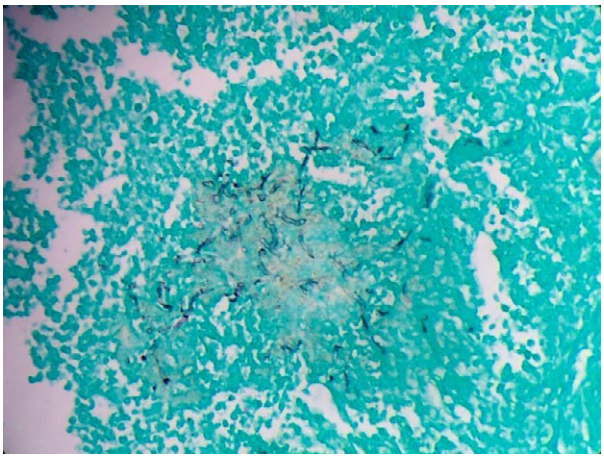

Figure 1. A scarcely septated broad hyphae surrounded by inflammatory cell infiltrates (Grocott's methenamine Silver GMS stain).

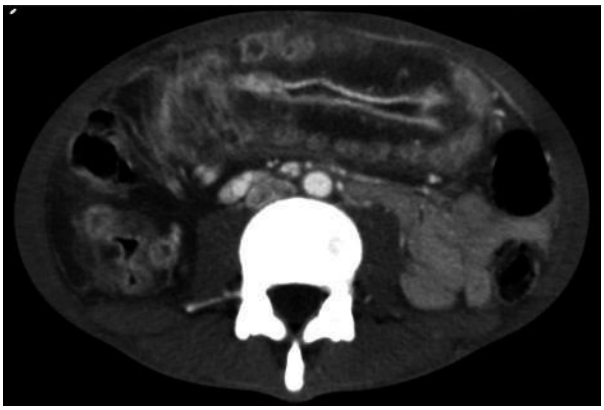

Figure 2. Markedly thickened gastric wall more in the antrum with multiple ring enhancing lesions. Suggestive of intramural abcesses.

P177

Phylogenetic diversity of human pathogenic *Fusarium* and occurrence of uncommon virulent species in Qatar

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Objectives *Fusarium* species cause a broad spectrum of life-threatening invasive infections especially in immune-compromised patients. Cutaneous infections, such as keratitis, endophtalmitis or onychomycosis are frequently manifested in immunocompetent hosts and may be associated with trauma. A phylogenetic study was carried out to characterize the most common invasive and cutaneous infecting *Fusarium* species observed in Qatar.
Methods A set of 44 *Fusarium* isolates from human clinical specimens diagnosed during the period 2003 to 2014 at Hamad General Hospital, Qatar were collected. The isolates were molecularly verified using two molecular markers, the second largest subunit of the RNA polymerase gene (RPB2) and the translation elongation factor 1 alpha (TEF-1α) that were amplified directly from the genomic DNA for multi-locus sequence typing.
Results Phylogenetic analysis revealed that the etiological agents belonged to four speciescomplexes (sc); *F.* solani sc (FFSC) (75%), *F.* oxysporum sc (4.5%), *F.* fujikuroi sc (13.6%), and *F.* dimerum sc (6.8%). The identified species were *Fusarium keratoplasticum* (n = 14, 31.8%), *F. falciforme* (n = 9, 20.5%), *F. solani* (n = 5, 11.4%), *F. acutatum* (n = 4, 9%), *F. petroliphilum* (n = 3, 6.8%), *F. lichenicola* (n = 2, 4.5%), *F. proliferatum* (n = 2, 4.5%),*F. oxysporum* (n = 2, 4.5%), *F. dimerum* (n = 2, 4.5%) and *F. delphinoides* (n = 1, 2.27%). Sites of infection were nails (n = 19, 43.2%), skin (n = 7, 15.9%), cornea (n = 6, 13.6%),blood (n = 3, 9%), wound (n = 4, 6.8%), burn (n = 2, 4.5%), and urine (n = 1, 2.27%). Comorbidities associated with invasive infections were hematological malignancy and autoimmune disorders. Mortality was 75% for the blood infections irrespective of antifungal chemotherapy and was associated with *F. petroliphilum* and *F. solani*.
Conclusion The study indicates that *Fusarium* is an uncommon agent of invasive fungal infections in Qatar, recalcitrant to antifungal treatment, and a potentially lethal opportunistic pathogen particularly in immunocompromised patients. Phylogenetic diverse members of the FSSC, predominatly cause cornea, nail and bloodstream infections. Fast and more accurate molecular diagnostic tests to species level may contribute to an earlier and more precise diagnosis and subsequently optimized treatment.

P178

A case of subcutaneous and gastrointestinal basidiobolomycosis in Singapore

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Objectives Entomophthorale is ubiquitous in nature. Entomophthoromycosis due to Basidiobolus is a known rare cause of chronic

subcutaneous zygomycosis affecting the limbs and trunk. It occasionally causes disseminated or invasive disease involving the gastrointestinal tract and lungs. Although widely prevalent in tropical and subtropical regions, it is infrequently seen in a developed country like Singapore. However, as residents travel and volunteer abroad more frequently, infections not seen locally are increasingly observed.

We describe the first case of entomopthoromycosis in Singapore to increase awareness of this entity. This will in turn facilitate early diagnosis and allow prompt institution of treatment.

Methods We discuss the case of a 67 year old Singapore Chinese male with proven subcutaneous entomophthoromycosis at initial presentation, who subsequently developed gastrointestinal symptoms, due to presumptive gastrointestinal basidiobolomycosis.

Results Our patient presented with a 3 week history of left lower limb swelling in July 2012. He had travelled frequently to rural areas of Thailand and Indonesia where he was involved in voluntary construction work. There were no other signs and symptoms. Blood investigations were unremarkable. Of note, he did not have eosinophilia. Imaging confirmed a soft tissue mass in the left inguinal region. At that time, workup for malignancy, bacteria, fungal, parasitic and tuberculous infections was negative. In view of the lack of a definitive diagnosis, he sought alternative treatment. He was readmitted to our institute in May 2013 with a progressively enlarging left inguinal mass with intra-abdominal extension, causing compression of the ureters with resulting hydronephroses. He underwent multiple investigations to ascertain the cause of the mass and to rule out malignancy. These were unyielding. His clinical condition continued to worsen and he developed gastrointestinal distension and persistent ileus in July 2013. Repeat imaging showed dilated large and small bowel loops with no intra or extraluminal obstructing mass. There was also nonspecific mucosal thickening extending from the descending colon to the rectum. He underwent a colonoscopy where corresponding nodular mucosal thickening was seen. Histology revealed tubular adenoma with low grade dysplasia. Due to the absence of malignancy and based on his clinical presentation, he was treated as for entomopthoromycosis. Unfortunately, he responded poorly to treatment and passed away 4 months after initiation of therapy. With the help of our counterparts in India, we eventually proved our clinical suspicion by polymerase chain reaction on the tissue specimen.

Conclusion Basidiobolomycosis is an emerging disease. This is especially so with increased international travel which has progressively led to sporadic cases of mycotic infections outside their previously restricted areas of endemicity. Diagnosis of basidiobolomycosis is often delayed due to its nonspecific presentation and myriad of differentials. It is rarely encountered in Singapore, hence collaborative effort is necessary to establish the diagnosis via histopathology or molecular techniques. This case highlights the importance of increased awareness, early diagnosis and prompt treatment of the disease, particularly in travellers returning from regions of the world where these infections are endemic. This will then translate to less morbidity and mortality.

P179

Kodamaea ohmeri: case report

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We report the first case of *Kodamaea ohmeri* fungemia in a patient undergoing treatment of peritoneal dialysis, in a northeast hospital of Rio Grande do Sul, Brasil. *K. Ohmeri* is a yeast and a teleomorph form of *Candida Guillermondii*. This is commonly used in the food industry because of its fermentation properties. *Kodamaea ohmeri* is a rare clinical isolate that has recently become known to cause various human infections as fungaemia, cellulitis, peritonitis, endocarditis and wound infection bringing high mortality in both immunocompromised and in imunodependentes patients. A 57-year-old woman who had systemic arterial hypertension, ankylosing spondylitis, terminal chronic renal failure secondary to hypertensive nephropathy, undergoing treatment of automated dialysis peritoneal was admitted to our hospital, with abdominal
pain and fever. Was performed the culture of peritoneal dialysis fluid and after the peritoneal dialysis catheter tip with revealed the fungal growth. The yeast was identified as *K. ohmeri* by Vitek 2 system (Vitek ID-YST, bioMérieux). The patient was started on Micafungin. Because of fungical peritonitis, peritoneal dialysis was stopped and submitted the patient to long catheter permanence in the femoral vein to do hemodialysis. However, despite the proposing treatment the patient died. K. ohmeri, can be identified by morphology of colonies in CHROMagar® *Candida* medium by absorption of carbon compounds (Vitek API ID32C and 2) or by molecular biology with the rDNA sequencing. The treatment is not well established in the literature due to shortage of cases, however, some reports obtained a good response with the use of Amphotericin B and Micafungin.

P180

A case of Endophthalmitis and corneal abcess caused by *Bipolaris* spp.

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A built worker's left eye injured by a piece of iron was applied to local hospital. He was diagnosed as corneal and scleral penetration injury (Zone1-2) and operated. Two weeks later, he was transferred to our University hospital for follow-up and treatment. The patient was diagnosed as corneal ulcer and endophthalmitis and hospitalized. Then, intravenous (iv) vancomycin, iv ceftazidim (CTZ), iv voriconazole (VRZ), topical vancomycin, CTZ and amphotericin B (AMB) treatment was started. Treatment was continued for 12 days. One day after hospitalization, left anterior camera lavage, vitreous tap, intravitreal vancomycin plus AMB injections were done. Two days later, intravitreal vancomycin, CTZ plus AMB treatment was repeated. Ten days later, in direct microscobic investigation of vitreal fluid by KOH, clusters of hyphae were observed. Bacterial culture was negative and there was no bacteria and leucocyte detected in gram staining of vitreal fluid. Fungal culture of vitreal fluid on Sabouraud dextrose agar showed a mycelial growth. According to investigation of mold colonies with lactophenol cotton blue under light microscope, mold culture was identified as *Bipolaris* spp. After the culture result, topical VRZ was given instead of topical AMB and oral itraconazole was given instead of iv VRZ. Three days later, iv vancomycin and CTZ treatment was stopped. This treatment was continued for twenty days. After the treatment corneal oedema, inflammation, conjunctival hyperemia, corneal ulcer and endophthalmitis regressed.

P181

Peritonitis caused by *Gymnascella hyalinospora* in a patient receiving peritoneal dialysis

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Introduction Peritoneal dialysis (PD) is a risk factor for fungal peritonitis. *Candida* spp. are the most common etiologic agents of PD-related fungal peritonitis. More rarely the disease may be caused by a number of opportunistic molds.

Objectives To present a first clinical case of peritonitis due to the rare ascomycetous mold *Gymnascella hyalinospora* in a patient with prolonged continuous ambulatory PD.
Case report A 45-year-old female with chronic renal disease had a history of PD since 23.04.2007. On 10.08.2014 she presented with fever, nausea, vomiting, abdominal pain and clouding of dialyzing solution and was admitted to the Dialysis Department of Mariinskaya City Hospital. At admission empirical antibiotic therapy (cefazolin 2 g day^{-1}; amikacin 100 mg day^{-1}) was started and cultures of blood, dialysate and feces were performed. *Klebsiella pneumonia* was recovered from feces; repeated blood cultures were negative. Antibiotic therapy was changed to tienam 1 g day^{-1} with no clinical improvement.

Culture of dialysate from 12.08.2014 grew mycelial fungus which was identified by DNA-sequencing (ITS region) as *Gymnascella hyalinospora.* The same mold was cultured repeatedly from dialysate sample taken on 22.08.2014. On 27.08.2014 peritoneal catheter was removed and visible biofilm was observed on the surface of its distal fragment. Direct microscopy of the biofilm revealed narrow hyaline septate hyphae and *G. hyalinospora* was isolated by culture.

On 30.08.2014 signs of pneumonia were found at X-ray of lungs and antifungal therapy with voriconazole was started (6 mg kg^{-1} on day 1; then 4 mg kg day^{-2}), but the patient worsened and on 07.09.2014 she died because of acute intestinal bleeding.

At post-mortem histopathological investigation narrow septate hyphae were revealed in lung tissue and peritoneum along with toxic damadge.
Mycology Cultures of *G. hyalinospora* grew moderately rapidly on Sabouraud dextrose agar at 37°C. Young colonies were thin, fluffy, pinkish, without any sporulation. After 3 weeks of growth abundant white aerial mycelium developed and the surface of colonies became pale green. Numerous asci aggregated in dense groups, hyaline to pale green ascospores were found at microscopic examination of cultures.
Conclusion We report the first case of peritonitis in a patient with chronic renal disease and prolonged peritoneal dialysis, caused by *Gymnascella hyalinospora.* This mold formed a biofilm on the surface of the peritoneal catheter. The patient died in spite of antifungal treatment and catheter removal. To our knowledge, this is the second reported case of invasive mycosis due to this fungal species (Iwen P.C. et al., 2000).

P182

A case of cutaneous mucormycosis caused by *Lichtheimia corymbifera*

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Mucormycosis is the second most frequent invasive mould infection. It is usually very aggressive due to the angioinvasive nature of the etiological agents. Cutaneous mucormycosis is the third most common clinical manifestation. If surgical debridement is done promptly, solitary cutaneous infection has a favorable prognosis and a low mortality rate. Early diagnosis of the disease is crucial for successful management.

Presented is a case report of cutaneous mucormycosis in a 38-year-old healthy male. He was involved in a motorcycle-car accident in June 2014 and both his lower limbs were amputated during subsequent surgery. Later, he developed fungal wound infection of the left stump. Fortunately, the infection was detected very early although the diagnosis was difficult to make as only a small area was affected. The infection was detected by histopathology, fluorescence microscopy and culture. The isolate was identified as *Lichtheimia corymbifera* by sequencing of the ITS region of its rDNA. The infection was successfully treated by surgical debridement followed by administration of liposomal amphotericin B.

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P183

Maxillary sinus infection due to *Schizophyllum commune*

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Schizophyllum commune is a thermotolerant basidiomycetous wood-decaying fungus which occurs on dead branches and fallen trunks of deciduous trees. Fungal infections of humans caused by this fungus are extremely rare. However, In the Czech Republic, however, maxillary sinusitis due to the fungal species was already found in 2005 [1].

Presented is maxillary sinus infection caused by *Schizophyllum commune* in a 49-year-old female patient. She suffered from recurrent sinusitis over a period of 5 years. During the last episode, the infection was suspected to be of bacterial etiology and treated with clindamycin only. As the treatment failed and a CT scan revealed almost complete opacification of the right maxillary sinus, the patient was indicated for endoscopic supraturbinal antrostomy.

In the course of the surgery, a polyp, hypertrophic mucosa, and slimy brown mass were found in the maxillary sinus. Fungal infection was detected in the surgically obtained sample by a microscopic finding of hyphae and by culture. The fungal colonies were finally identified as *Schizophyllum commune* based on sequencing of the ITS regions of the rDNA.

The infection was successfully treated by surgical debridement only and antifungal therapy was not initiated. Currently, the patient is without any signs of infection.
Reference Dobiasová S., Dobias R., Klecka P., Kubatova A., Kolarik M. [Filamentous micromycetes as agent of chronic sinusitis.] *Abstract Book of the 5th Czech and Slovak Interdisciplinary Conference of Medical Mycology*, Pardubice, Czech Republic, 2007, p. 62.

This work was supported by the Palacky University Olomouc Internal Grant Agency (project no. IGA_LF_2015_021).

P184

Genotyping of multidrug resistant Indian *Candida auris* isolates by Multi Locus Sequence Typing, Amplified Fragment Length Polymorphism and MALDI-TOF-MS and their antifungal susceptibility profile

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Objectives *Candida auris* is multidrug-resistant yeast that causes a wide range of infections, especially in intensive care settings. Recent

reports from Asia and South Africa have highlighted the increasing incidence of *Candida auris* as a nosocomial bloodstream pathogen affecting persons of all age groups. The emergence of this pathogen as an agent of fungemia highlights the concerns of elevated MICs for azoles and caspofungin in *C. auris*. Herein, we characterized a large number of *C. auris* isolates prevalent in tertiary care hospitals of India using multilocus sequence typing (MLST), amplified fragment length polymorphism (AFLP) and matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF) to evaluate the genetic relatedness among the Indian *C. auris* isolates.

Methods A total of 120 *C. auris* isolates originating mostly from fungemia and deep seated infections from various hospitals of India were analysed. All the isolates were subjected to MLST using 3 loci, namely, *ITS*, *RPB1* and *RPB2*. Also, the population structure of *C. auris* isolates was determined by AFLP and principal component analysis (PCA) with MALDI-TOF BioTyper 3.0. Antifungal susceptibility testing was performed for azoles, amphotericin B and echinocandins with broth microdilution method.

Results MLST demonstrated homogenous population of Indian *C. auris* isolates, which were highly related to each other. The AFLP and PCA analysis of *C. auris* spectra were in concordance with MLST demonstrating close relationship among the Indian *C. auris* isolates. The large number of invariable fragments observed in AFLP suggested their monophyletic origin. High MIC_{90} were observed for fluconazole (64 μg ml^{-1}), flucytosine (32 μg ml^{-1}), voriconazole (8 μg ml^{-1}), amphotericin B (4 μg ml^{-1}) and isavuconazole (2 μg ml^{-1}).

Conclusions The clonality of Indian *C. auris* isolates by all three techniques suggests their nosocomial transmission. Considering the frequent prevalence of multi-drug resistant strains of *C. auris* in the intensive care units in the present series, the accurate identification and antifungal susceptibility testing of this yeast is pertinent for guiding therapy and determining the prognosis in such settings.

P185

Trichosporon Asahii urinary tract infections in immunocompromised hosts

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Objectives Trichosporonosis is an emerging infection predominantly caused by *Trichosporon asahii*. *Trichosporon* (Beigel, 1985)speciesare ubiquitous, exclusively anamorphic, yeast like fungi belonging to Trichosporonaceae. *Trichosporon* is implicated in superficial and mucosal infections, however, systemic infections are known in immunocompromised, cancer, burns, transplant patients as well as patients on steroids, peritoneal dialysis, prolonged mechanical ventilation and those undergoing prosthetic valve surgeries. Alimentary tract, respiratory tract, broken skin and mucosa, are possible portals of entry. Persistent and disseminated infections have poor prognosis. Fatal outbreak in neonates and breakthrough trichosporonosis under antifungal therapy has been reported. Urinary tract infections (UTI) by *Trichosporon* are rare and remain scantily reported. This study was aimed at studying the characteristics of *Trichosporon* UTI at an Indian tertiary care hospital.

Figure 1. Antifungal susceptibility of Trichosporon asahii through E-test showing an MIC of 3 μg ml^{-1} for Voriconazole

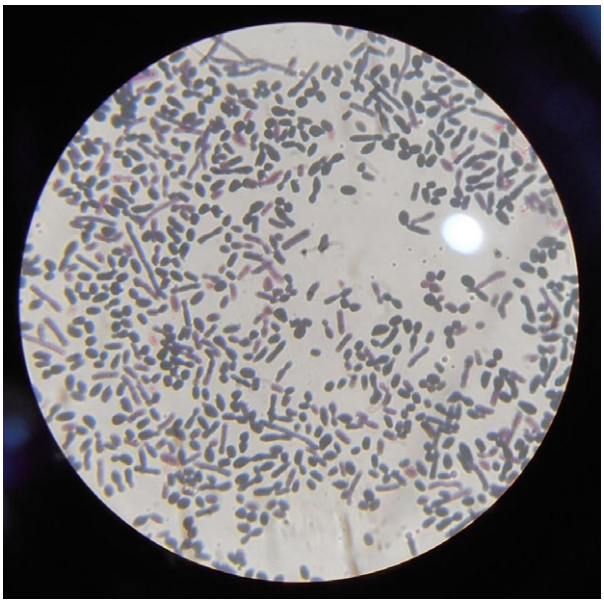

Figure 2. Gram stain of Trichosporon asahii showing budding yeasts and barrel shaped arthroconidia

Methods An active surveillance for *Trichosporon* UTI was undertaken after the occurrence of three such cases from various centres in a tertiary care apex teaching and referral hospital. *Trichosporon* was identified phenotypically by a combination of manual and automated systems. Identification through morphology on cornmeal agar and Gram stain, hydrolysis of urea, assimilation of carbon/nitrogen compounds was confirmed VITEK 2 compact automated system (bioMérieux, France). Antifungal susceptibility was tested by E-test (AB BIODISK, Sweden) for fluconazole, itraconazole, voriconazole, Amphotericin B and anidulafungin (Fig 1 and 2). Non repeat positive cultures were interpreted in conjunction with colony characteristics, cellular morphology, E-test antifungal susceptibility patterns, clinical correlates and environmental surveillance.

Results *T. asahii* and *T. inkin* were isolated from urine of 20 and two immunocompromised patients respectively who were receiving in-patient treatment for multiple comorbidities. Ten patients were confirmed HIV positive while the rest were iatrogenically immunocompromised after solid organ or bone marrow transplantation. Antifungal susceptibility done by E-test revealed multresistance with preserved susceptibility to voriconazole (MIC 3 μg ml^{-1}).

Discussion *Trichosporon* infections present diagnostic and therapeutic challenges. They are likely to surpass routine laboratory identification. The ubiquity and biofilm formationposes difficulty in establishing pathogenicity and delineating community-acquired or hospital-acquired infections. Risk factors such as prolonged multiple antimicrobials, indwelling catheter and comorbidities may be contributory to the establishment of a nosocomial opportunistic *Trichosporon* infections. Dedicated efforts targeted at infection control are needed to optimize management and control of *Trichosporon* infections.

P186

Emerging yeasts and algae in a tertiary healthcare set up

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Objectives Emerging organisms are organisms that have newly appeared in a cohort/population or have existed but are rapidly increasing in incidence, geographic or host range. One-tenth of all infectious diseases are attributable to emerging organisms. Operationally defining an organism as emerging is a subjective endeavour. As emerging organisms sporadically affect a relatively small percentage of population, they are not studied at large. This study was aimed at studying the characteristics of emerging yeasts and algae at an Indian tertiary care hospital.
Methods 33836 positive isolates obtained from 132646 processed samples during 2011-14 were included. Identification percentage >85% along with inbuilt standards for identification comparison were considered for final validation through automated systems.Non repeat positive cultures were interpreted in conjunction with colony characteristics, cellular morphology, disc-diffusion antifungal susceptibility patterns, clinical correlates and environmental surveillance. The frequency of isolation, sources, referring centres, susceptibility profiles and phenotypic characteristics. A literature search was done to identify reports on human pathogenicity and yeasts and algae reported fewer than 100 times on PubMed were defined as emerging.
Results 332 (0.98%) yeasts/algae were isolated from 33836 isolates, of which 174 isolates including 14 yeast species and one algae were found emerging. Non-albicans Candidemia was caused by 84 emerging non-albicans Candida isolates comprising ten species in multidisciplinary ICU, NICU and bone marrow transplant centre. Non-albicans *Candida* species such as *haemulonii, famata, rugosa, guilliermondii, lusitaniae, utilis, zeylanoides, sphaerica, krusei* and *intermedia* were isolated along with *Trichosporon asahii, Trichosporon inkin, Malassezia furfur* and non-noeformans *Cryptococcus*. Twelve *Prototheca wickerhamii* were isolated from blood samples from multidisciplinary Oncology centre. All non-albicans *Candida* species were multidrug resistant and led to frank sepsis in 24 patients. Environmental surveillance was not corroborative.
Conclusion Emerging yeasts and algae may infect compromised hosts and pose difficulty in management due to inadequate identification and multidrug resistance. Astute efforts directed at identification of emerging organisms and containment of infection are required.

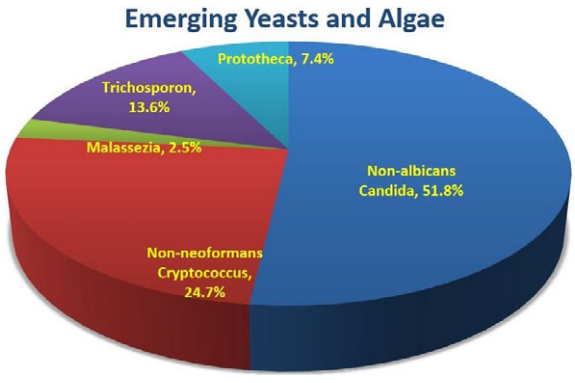

Figure 1. Distribution of emerging yeasts and algae

P187

Tinea capitis with cicatricial alopecia caused by *Trichophyton schoenleini*

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Objective Dermatophytes represent the prevailing type of fungi that cause infection of the skin and nails. Although dermatophyte infections are common disorders worldwide, some agents, like *Trichophyton schoenleini* are rarely seen in the United States and Europe and have been considered eradicated in these areas. Here we report a case of tinea capitis with cicatricial alopecia, caused by *Trichophyton schoenleini* that had been previously diagnosed and treated as psoriasis for nearly 2 years.
Methods A 14-year-old caucasian girl was referred to our consultation due to a long standing alopecia of the scalp with associated scaling. She was applying a topical salicylic acid cream since she had been diagnosed as having psoriasis. Clinical examination revealed areas of perifollicular erythema on the scalp with adherent scaling, positive traction test of the hair and frontoparietal cicatricial alopecia. A skin biopsy was performed and scales and hair were collected for mycological examination. The patient was started on oral itraconazol and topical ketoconazol shampoo.
Results Direct microscopy of the hair with potassium hydroxide 20% revealed hyphae within the hair showing characteristic channeling of *Trichophyton schoenleini*, hair infection (favic ringworm). *Trichophyton schoenleini* was isolated in culture. The patient completed treatment with oral itraconazol for 6 weeks and topical minoxidil with clinical resolution of the infection and partial recovery in hair count.
Conclusions Without adequate diagnosis and treatment, favic hair infection evolves to cicatricial alopecia as we have observed in this misdiagnosed case. Although scalp infections due to *Trichophyton schoenleini* are uncommon this was not our first favus case, as we had already detected this agent in other patients' scalp infection. Clinicians must be aware and should always workout to exclude a fungal etiology in the differential diagnosis of psoriasis and tinea amiantacea or other situations with scaling and alopecia of the scalp.

P188

Schizophyllum colonization in an orally open antral cyst (case report)

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In a 43-year old otherwise healthy man the abscessed tooth 16 (upper right molar) was extracted by a dentist. Four days later the patient was referred to our clinic because of an apparently open oroantral fistula. On radiographic examination, a residual root of tooth 14 was still in place and an antral cyst of appr. 2.5 cm diameter was found (tooth 15 had been extracted many years earlier.). There was no radiodense object detectable. 3 weeks later the root 14 was extracted, the fistula closed and the antral cyst removed. At follow-up 1 year later the area was clinically and radiographically without evidence of disease.

The histopathological report of the dark and slimy content of the cyst was identified morphologically as a "fungus ball", suspicous for aspergillus. Twice performed cultures were negative for fungi. PCR amplification and sequencing of the ITS2 region revealed *Schizophyllum commune* and excluded any of *Aspergillus* spp. as a causing pathogen. To our knowledge this is the first report of a *Schizophyllum commune* colonization in an antral cyst. The duration of the lesion, however, remains unknown: It is possible that already at the time when tooth 15 was extracted, the cyst had been opened from the

mouth. In this case the fungus colonization could have started many years ago. It is also possible that during the 3 weeks between the opening of the cyst now and the final surgery some necrotic debri (e.g. food) could have been colonized by *Schizophyllum*. As no more details of the patient's history were available we cannot arrive at a final conclusion in regard to the duration.

P200/M6.2

Paracoccidioidomycosis: a case report mimicking adrenal neoplasia

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Paracoccidioidomycosis is the most prevalent systemic mycosis in Latin America with the highest number of cases arising from Brazil. It is characterized by a polymorphism of lesions and can affect any organ, in particular, the skin, the lymph nodes, the lungs, the oral, nasal, and gastrointestinal mucous membranes; caused by dimorphic fungi *Paracoccidioides brasiliensis* and *Paracoccidioides lutzii*. A case of systemic Paracoccidioidomycosis, in a private hospital in Caxias do Sul, Rio Grande do Sul, Brazil is presented. Male patient, 66 years old, previously healthy was admitted to the hospital presenting sweat, chills, fatigue, weight loss (25 kg) and daily fever episodes. The signs and symptoms have started 2 months earlier. He was in hematological follow-up because of alteration in transaminases and in serum ferritin. Abdominal CT scan showed a bulky lesions affecting both adrenal glands, measuring 8.8 × 4 × 3.7 cm at right and 11 × 5 × 4.8 cm at left. The right lesion determined invasion of the inferior vena cava, where was observed 2.3 cm tumor thrombus. Initially, it was suspected of neoplastic disease, however, the pathological study of the left lesion aspiration revealed a high number of leukocytes, a presence of necrotic material and spores suggesting *Paracoccidioides* spp to Grocott method. The diagnosis was confirmed by culture the fungus. The treatment was started with Itraconazole 200 mg day^{-1} with gradual improvement in symptoms and weight regain. The patient still remains with antifungal therapy associated with the use of prednisone 7.5 mg day^{-1} due to the partial adrenal glands destruction. After 6 months of treatment, the CT still reveals the presence of adrenal wounds, however, with a reduction in volume (7.7 at right and 9.3 at left) compared with the previously CT. Paracoccidioidomycosis can affect locations such as pancreas, adrenal, CNS, and mimic other diseases, delaying diagnosis and leading to severe and fatal prognosis. Although the patient in question resides in urban center, he used to prune vines, a very common activity in the northeast of Rio Grande do Sul, therefore establishing connections with rural environment. As so, the main risk factors for acquiring the disease are activities involving soil management and plantations.

P201

Invasive mycoses caused by *Trichosporon* spp. in Saint-Petersburg, Russia

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Objective *Trichosporon* spp. are rare etiologic agents of invasive mycoses (IM) and publications about these diseases are limited.

Methods The prospective multicenter study in Saint-Petersburg, Russia, 2012-15 yy. Diagnosis of IM was made according to EORTC/MSG criteria, 2008.

Results We observed 1 pediatric and 3 adult patients with IM caused by *Trichosporon* spp. The median age of patients was 34.5 years (range 12–56), 3 males. Underlying conditions were: acute renal failure, chronic renal disease with peritoneal dialysis, bacterial meningitis and allogeneic haematopoietic stem cell transplantation in a pediatric patient with acute lymphoblastic leukemia.

Diagnosis of invasive infection caused by *Trichosporon* spp. was confirmed by culture of cerebrospinal fluid - 2, blood - 1, peritoneal fluid - 1, and renal biopsy - 1. The etiological agents were *T. asahii* - 2, *T. asahii* + *Candida albicans* - 1 (meningitis), and *T. inkin* - 1. The clinical variants were meningitis - 1, fungemia + meningitis - 1, nephritis -1, and peritonitis - 1 (*T. inkin*).

Antifungal therapy was used in all patients: fluconazole - 3 (adults), and voriconazole - 1 (pediatric). Duration of antifungal therapy was 10-44 days (median - 40.5). Overall survival rate in 30 days was 50%.

Conclusion Invasive mycoses caused by *Trichosporon* spp. are rare diseases with high mortality. Early identification of **yeast** isolates should be done to choose appropriate therapy.

P202

The ROCANET study: results from a prospective observational survey of candidaemia in the Rome city

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Objectives *Candida* species are leading causes of nosocomial bloodstream infections and are associated with substantial morbidity, mortality and costs. While the antifungal susceptibility pattern is closely linked to the species, it is yet greatly important to understand and monitor the local species epidemiology as well as the antifungal resistance rate. The ROCANET (Rome Candida Network) was established in December 2012 in order to perform prospective surveillance of all candidaemias among patients hospitalized at selected medical centres in the Rome city area. Its aim is to improve the knowledge of the burden of candidaemias in different groups of patients, better define patients at risk and understand the epidemiology, species distribution, antifungal susceptibility and outcomes of candidaemias from the study sites.

Methods All the patients admitted to 10 large hospital medical centres of Rome (Italy) from January 2013 to December 2014 and diagnosed with candidaemia will be studied. All the *Candida* isolates from the study patients will be collected and maintained at the Clinical Microbiology Laboratory of the Università Cattolica del Sacro Cuore of Rome. The isolates will be re-identified by the MALDI-TOF MS method and sequence identified using the ITS gene region, whereas susceptibility testing will be performed against 7 antifungal agents (anidulafungin, caspofungin, micafungin, fluconazole, itraconazole, posaconazole, voriconazole) using CLSI and EUCAST methods. In the case of antifungal drug-resistant isolates, underlying molecular resistance mechanisms will be assessed, as well all the isolates will be genotyped using multi-locus sequence typing, as appropriate.

Results From January 2013 to October 2013, a total of 668 isolates of *Candida* species were studied, including 319 *C. albicans*, 197 *C. parapsilosis* complex (including 4 *C. orthopsilosis* and 2 *C. metapsilosis*), 65 *C. glabrata* complex (including 1 *C. nivariensis*),41 *C. tropicalis*, 2 *C. guilliermondii*, 12 *C. krusei*, 7 *C. lusitaniae*, 5 *C. guilliermondii*, 5 *Rhodotorula mucilaginosa*, and other 17 belonging to other 9 species. Overall, resistance to the echinocandins was very low, with *C. albicans* (1 isolate) and *C. glabrata* (1 isolate) being resistant to anidulafungin, caspofungin or micafungin and shown to have *fks* mutations. Resistance to fluconazole was low among isolates of *C. albicans* (1 isolate) and *C. tropicalis* (1 isolate), whereas 12 isolates of *C. parapsilosis* complex (11 *C.*

parapsilosis and 1 *C. metapsilosis*)were found to be resistant to fluconazole. Surprisingly only 4 isolates were resistant to azoles. Voriconazole and posaconazole were active against all *Candida* species except *C. glabrata* and 2 isolates of *C. parapsilosis*.
Conclusion Overall, echinocandin and triazole resistance were uncommon. Although no fluconazole and echinocandin co-resistance among *C. glabrata* isolates was observed, continued close surveillance is locally warranted.

P203

An estimation of burden of serious fungal infections in France

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Introduction Our objective was to estimate the burden of serious fungal disease in France, based on epidemiological data. Epidemiology of several of them remains unknown, whereas precise data are available for invasive fungal infections such as invasive aspergillosis, candidemia, cryptococcosis and pneumocystosis. Herein, we report an estimation of the incidence and prevalence of invasive but also chronic and immunoallergic fungal diseases in France in order to provide a basis of reflection in terms of Public Health.
Methods Published epidemiology papers reporting fungal infection rates from France were identified. Where no data existed, we used specific populations at risk and fungal infection frequencies in those populations to estimate national incidence or prevalence, depending on the condition. Population statistics were derived from the Institut National de la Statistique et des Etudes Economiques (INSEE-2014). Incidences of invasive fungal infections were derived from Institut National de Veille Sanitaire (InVS) and the National Center of Reference for invasive fungal diseases (CNR-MA, Institut Pasteur)(2010).
Results 82% of the 65.8 M population (2014) are adults. The asthma and COPD prevalences in adults are 6.7% and 7.5%, respectively. In this context, the model predicts 124 678 SAFS episodes (189 cases/100 000 adults per year), 95 361 ABPA episodes (145/100 000) and 3450 chronic pulmonary aspergillosis cases (109/100 000)(Table 1). These high estimated burdens indicate that such patients should be better identified and characterized in order to improve their management. Good estimates of incidence rates of documented invasive fungal diseases are available: 3.6/100 000 for candidemia, 1.8/100 000 for invasive aspergillosis (IA), 1/100 000 for pneumocystosis, 0.2/100 000 for cryptococcosis and 0.12 for mucormycosis. However our estimate of IA complicating COPD is 1300 cases against a smaller documented number whatever the underlying condition, suggesting a major diagnostic gap., which may be also be true of pneumocystis. These severe infections have persistently high mortality rates and represent high costs due to hospitalization, diagnostic management and antifungal drugs use. Finally, estimates of *Candida* peritonitis, oesophageal candidiasis and recurrent vaginal candidiasis rates are 0.74/100 000, 13.8/100 000 and 2220/100 000 (rate for adult females only), underline the need for adjusting such potential high burdens with additional studies.
Conclusion Using local and literature data of the incidence or prevalence of fungal infections (with the exclusion of tinea capitis), approximately 1 000 000 (1.47%) people in France are estimated to suffer from fungal infections each year. Beside a good evaluation of 5000 cases/year of invasive fungal diseases, recurrent vaginitis and chronic and immunoallergic pulmonary diseases represent a serious impact on human health. Prospective or comprehensive registry surveys are required to accurately determine their epidemiology and improve the management of these disorders.

P204

Is *Aspergillus Fumigatus* tr34/l58 h prevalent among colonised cystic fibrosis patients? Experience of an Spanish cystic fibrosis reference center

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Objectives A growing number of clinical *A. fumigatus* strains carrying the TR34/L58H mutation are reported in Northern Europe (particularly The Netherlands, Germany and Northern France) and seem to be spreading to the South. However, only a few of these strains have been isolated in Spain. Moreover, its prevalence in clinical samples in our country is unknown, although it is supected to be very low. Our aim was to study the rate of *A fumigatus* TR34/L58H among strains recovered from patients diagnosed of Cystic Fibrosis (CF) and colonised with *A. fumigatus* as they may represent a sentinel population for the emergence of the TR34/L58H mutation in our area.
Methods 35 consecutive *A. fumigatus* strains isolated between January 2014 and April 2015 from sputum samples of 22 CF patients (11 pediatric and 11 adult patients, mean age 20.7 years, range 8–36 years) classified as being *A. fumigatus*-colonised attending our CF Unit were studied. The presence of the TR34/L58H mutation was determined using a commercial Real Time PCR kit with specific probes for both mutations. As positive external control, we used an *A fumigatus* strain isolated in our center from an Invasive Aspergillosis case; this strain had been previously characterised as positive for the TR34/L58H mutation. Genetic material was obtained from pure cultures of the strains after beadbeating and proteinase K pretreatment using an EasyMag apparatus. Real Time PCR was carried out in an SmartCycler termocycler.
Results None of the 35 CF strains tested positive for the TR34 or the L58H mutation, whereas the previously known positive strain isolated from a case of invasive aspergillosis gave a positive result for both probes.
Conclusions *A fumigatus* strains carrying the TR34/L58H mutation conferring pan-azole resistance seem to be absent or, at least, to be of very low prevalence among CF patients chronically colonised with *A. fumigatus* in our area, although larger and prospective studies should be carried out to confirm the observation made by our group. Nevertheless, it is in the line of previous works published by Spanish groups in non-CF related selected populations or samples. Real-time PCR seems to be a fast and useful alternative for screening/confirming the presence of the TR34/L58H mutation in *A. fumigatus* strains.

P205

Ethiological profil and epidemiology of tinea capitis in the region of Mitidja (BLIDA) in Algeria

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Introduction Tinea capitis is a mycological affection observed most of the time in children. It is due to dermatophytes:Trichophyton and Microsporum. In Algeria numerous studies in scattered regions of the country discussed the mycological and epidemiological character of this pathology. We present for the first time the results of a retrospective survey from August, 1999 till August, 2014 in the Mitidja region of Blida situated in 50 km in the southwest of Algiers.
Patients and methods It is a retrospective descriptive study based on the data of the registers of the laboratory of parasitology-mycology of the teaching hospital of Blida The majority of the patients are

from the city of Blida and from the bordering localities. The patients whose personal or clinical information was incomplete were not included.our study.

Results We took all in all 4140 patients among whom 2750 are positive 66.42% is the prevalence the mean age is of 24 years, the extremity of age is between 02 months is 83 years old, the median is of 25 years the mode is of 25 years and the standard deviation is of 17 years. The average, the mode and the median being practically equal, ca evokes a normal distribution (casting), 2/3 of the patients got their age fluctuates enter ±standard deviation between 7 years and 41 years.

The samples concerned hair the alopecic region by scratching of the lesions with a sterile scraper to collect hairs and then a culture on Sabouraud media with antibiotics at 30c is systematic. From 4140 patients, only 2750 patients are considered to have Tinea capitis. The direct examination had returned positive for 2420 sick while the culture was negative that is 58.45%. *Microsporum canis* and *Trichophyton mentagrophytes* are the most identified.

Conclusion This is the first study done in this region of Algeria, we noticed that these pathogenics fungies have zoophile and telluric origin although that the lifestyle of the patients concerned by the study is urban.in fact most Algerian living in the cities doesn't have pets.

These results could be explicated by the Lack of hygiene which remains a public health problem in our country.

P206

Molecular typization of *Arthroderma benhamiae*, a zoonotic agent of epidemic dermatophytosis in Central Europe

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Objectives The aim of this study was to find informative molecular markers to evaluate population structure of dermatophyte species *Arthroderma benhamiae* in the studied area. One of the main issues was to determine whether the current outbreak of dermatophytosis caused by *A. benhamiae* in Central Europe is caused by a new more virulent genotype which is with the high success rate transmitted to humans or whether other causes must be considered (e.g. high prevalence of the pathogen in animal husbandry).

Methods A total number of **262** *A. benhamiae* strains associated with cases of human and animal dermatophytosis from the Czech Republic (CZ), Belgium, UK, Germany, Italy, Japan and USA were used for analysis. Ten microsatellite markers were developed and used for typization of *A. benhamiae* strains together with sequence analysis of ITS region rDNA and glyceraldehyde-phosphate dehydrogenase gene. Bayesian inference analysis and distance methods were applied on the datasets to reveal the genetic variation of *A. benhamiae* population. PCR-based determination of mating idiomorphs was also performed in all strains.

Results The sequence analysis based on two genes revealed presence of five genotypes. Most common genotype (n = 195, 74.4%) was characterized by yellow colony reverse on MEA agar with the exception of isolates from dogs in North America which showed different phenotype. Only MAT1-1 idiomorph was amplified in isolates of this genotype from Europe and low variability in microsatellites data was recorded. In contrast, the isolates of this genotype from North America were highly variable by microsatellite data and isolates of both mating type genes were revealed. The second most common genotype (n = 47, 17.9%) was characterized by dominant red (mating type MAT2) or brown (MAT1) colony reverse on MEA agar. The third genotype (n = 16), closely related to second genotype, included all strains from Japan and some from the CZ and Belgium and was characterized by both red and yellow colony reverse and MAT1-1 idiomorph. The remaining two genotypes were each represented by only two isolates from the CZ.

Conclusion *A. benhamiae* is a new emerging pathogen in the CZ and some other countries in Central and Western Europe region. According to recent studies this species is the most important zoophilic dermatophyte in the CZ causing almost 23% of tinea corporis (median age of patients – 10 years, females - 70%) and 29% of tinea capitis infections. The majority of infections are transmitted from guinea pigs and other rodents. It is probable that the most common genotype of *A. benhamiae* responsible for the outbreak of infections in the Central Europe (agent of 79.7% of human infections in the CZ) spread clonally. This hypothesis is also supported by uniform phenotype and mating type in European strains. The american strains of the same genotype (can be separated by microsatellite data) however showed different phenotype and presence of both mating type genes indicating sexual reproduction. Other genotypes contribute only marginally on the outbreak.

P207

Re-emerging paracoccidioidomycosis in Argentina with peculiarities in the diagnosis and clinical manifestations

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Paracoccidioidomycosis (PCM) is a systemic endemic mycotic disease caused by the thermally dimorphic fungus *Paracoccidioides*. The fungus has a geographic distribution limited to tropical and subtropical areas from rural areas of Central and South America. PCM is the commonest systemic mycosis in Latin America without mandatory reporting and considered as a Neglected Disease.

Argentina has two endemic areas of PCM; the more extensive is located in the northeast near Paraguay and Brasil, where the chronic PCM is the historically observed clinical form.

In the last years, an increase of the PCM incidence associated with urban cases, patients with rare clinical manifestations or infrequent locations and also, cases of juvenile PCM never described before in that area was observed. In addition, a high percentage of negative serological diagnosis was detected both in chronic and juvenile clinical forms.

In order to know the status of the current clinical and epidemiological characteristics of PCM in Argentina, a multicentric study of PCM coordinated by Departamento Micología of Instituto de Medicina Regional of Universidad Nacional del Nordeste (Argentina) was started.

We present a retrospective descriptive analysis of PCM cases from 2013 to 2015.

Table 1

Clinical manifestations	Relative Frequency
Pulmonary	34%
Mucocutaneous	33%
Skin	11%
Nods	10%
Central Nervous System	5%
Disseminated PCM (deadly PCM)	2%
Osteomyelitis	2%
Genital ulcer	1%
Ocular	1%
Arteritis	1%

A total of 67patients with PCM, aged 13–89 years (median 49 years) were recorded. In 2013 were recorded 23 cases, 32 in 2014 and 12 cases only from January to April 2015, pointing the annual increasing.

A lower male/female relationship (12 : 1) than reported by others countries was observed.

Chronic form in 63 patients and juvenile PCM in 4 patients were recorded. Without classical manifestations that suggest PCM, about 15% of patients, only showed rare manifestations or infrequent locations such as osteomyelitis, cerebral and cerebellar abscesses, cutaneous lesions, that delayed the diagnosis. Clinical manifestations observed in all patients are presented in Table 1.

Serological and microbiological diagnosis was achieved in 46 patients, in 6 patients only serological and only microbiological in 15 cases. Negative serological results with microbiological diagnosis were obtainedin 9 cases (13%).

The knowledge that *Paracoccidioides* includes P. lutzii and P. brasiliensis with cryptic species can be an explanation for this finding. Although the impact of this genotypic diversity is not completely understood, data suggest that false negative results could be obtained with the standard immunodiffusion test performed using the Gp43 antigen of *P. brasiliensis* B 399 strain in patients infected with a strain *that has* different antigenic profile.

Several unexplained peculiarities have been noted in the diagnosis and clinical manifestations. The recognition of the re-emergence of PCM in Argentina with particular characteristics requires urgent research to explore specifically the circulating *Paracoccidioides* species in order to improve the diagnosis and therapeutic tools.

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Trichophyton mentagrophytes – a rare zoophilic dermatophyte isolated in Germany – morphological and molecular biological characterization of four wild strains

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Objective Based on what is known today from molecular biological analysis, *T. mentagrophytes*is now only used to refer to the formerly *T. mentagrophytes* var. *quinckeanum* (the pathogen in mouse favus),a highly uncommon pathogen in WesternEurope. *T. mentagrophytes* is predominantly isolated in the Near East,and Asia, as a zoophilic dermatophyte which has its source in camels and rodents, like mice. In humans,*T. mentagrophytes* causes tinea capitis and tinea corporis.

Patients and methods Four wild strains of *T. mentagrophytes* were isolated from skin scrapings of patientssuffering from dermatophytosis or onychomycosis. All strains were characterized based on morphological andmetabolic features. For molecular biological detection of the dermatophytes in skin scrapings a uniplex PCR-EIAwas used. Species identification was confirmed by sequencing of the internal transcribed spacer region (ITS)of the ribosomal DNA. Further, the strains were typed by MALDI TOF MS.

Results The four strains of *T. mentagrophytes* were isolated from 2 children and 2 adults. Patients sufferedfrom tinea corporis or tinea faciei. In case of a 44 year old woman, *T. mentagrophytes* was isolated from nailscrapings of onychomycosis or tinea unguium, respectively. The patient had barefooted contact to soil due togardening. A guinea pig was source of infection in one out of the four patients. The other patients had contactto cats or dogs, however, these animals were not considered as source of infection. All here describeddermatophytoses probably were acquired in Germany. All isolates had typical white to yellowish stained powderyflat colonies (Fig. 1). The strains showed small spherical microconidia arranged alongside the thin hyphae,spiral hyphae were seen. Big chlamydospores and thin-walled macroconidia could be detected. All strainswere confirmed as *T. mentagrophytes* by PCR-EIA, sequencing of the ITS rDNA, GPD gene and microsatellite typing.In MALDI TOF MS the four strains had identical spectra.

Figure 1. T. mentagrophytes var. quinckeanum

Conclusion The isolation of *T.mentagrophytes* (which is not *T.interdigitale* incurrentsense) in Germany has tobe considered as a very rare finding. In Germany, zoophilic strains of *T. interdigitale* are standing for the majorityof zoophilic dermatophytes isolated from rodents and affected patients. *T. mentagrophytes sensu strictu* is still quitecommon in some areas in Asia (in particular in China), however, not in Europe.

P209

Equine dermatophytosis due to *Trichophyton bullosum* in the Czech Republic

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Trichophyton bullosum is a zoophilic dermatophyte from *Arthroderma benhamiae* complex with poorly known distribution.

Presented is a case of dermatophytosis caused by *T. bullosum* in a 6-year-old male horse. In October 2013, he developed a weeping skin lesion approximately 10 cm in diameter in a saddle area with subsequent loss of coat. The infection spread rapidly to the upper chest and to both sides of the trunk. Fungal hyphae were revealed microscopically and, subsequently, fungal colonies grew during culture. The horse was initially treated with enilconazole solution. Afterwards, the lesions were treated with flutrimazole spray for 3 weeks. In the course of this antifungal treatment, the skin started to be covered with soft hairs and then healed completely.

The fungal colonies were suspected as dermatophyte based on their morphology. Interestingly, the isolate grew better at 35°C than at 25°C and was first identified as *Trichophyton verrucosum* in slide culture. However, sequence analysis of the ITS region of its rDNA revealed *T. bullosum*.

An epidemiological investigation showed that the horse had been placed in a racing stable with other horses at the time of infection and had never been abroad. Subsequent swabs taken from the other horses stabled in the same building yielded negative results. Before the infection occurred, the horse had not shown any signs of injury or visible grazes and had been used for racing. It had always been stabled with horses only and had never been in direct contact with cows, sheep or other livestock. Therefore, the source of the infection remains unclear.

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P210

The burden of fungal diseases in Algeria

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Objectives In Algeria superficial mycoses are very commonly diagnosed. Deep fungal infections are less often observed. Few data from Algeria are found in the literature. We report for the first time in this paper the main causes of these diseases in our country.

Methods We searched for existing data and estimated the incidence and prevalence of fungal diseases based on the population at risk and available epidemiological data. Demographic data were derived from the Service (Office) of the Statistics (ONES), World Health Organization (WHO), The Joint Nations Programme on HIV/AIDS (UNAIDS) and national published reports. When no data existed, risk populations were used to estimate frequencies of fungal infections, using described methodology by LIFE.

Results Algeria had 39.5 million inhabitants in January, 2015 and a forecast growth of >2% based on population since 2013. Males represent 50.62% of the population, 28% were under 15 years, and 16.4% were over 50 years. There are about 50 000 new cases of cancer every year among including 1500 in children, and about 200 new cases of leukaemia annually. For HIV 6472 patients are seropositive and 1422 patients have AIDS. In 2012, there were 8753 survivors of pulmonary TB, an estimated 317 762 patients with COPD of whom 20.3% were estimated to be admitted to hospital each year. Using data from Morocco for asthma, 2.84% of the adult population have asthma, a total of 807 700 people.

The incidence of invasive aspergillosis in the neutropenic patient in the center of the country is about 8%, while candidaemia occurs in 5% dominated by *Candida albicans, Candida parapsilosis* and *Candida glabrata.* Cryptococcosis is less frequent – we count 50 cases/year and pneumocystosis 30 cases annually.

Concerning the superficial mycoses, tinea capitis is by far the most infection which is culture positive, with the pathogenic agents *Microsporum canis* and *Trichophyton mentagrophytes* being common; favus has almost disappeared. We noticed that *Trichophyton rubrum*, *Trichophyton interdigitale* and *finally Trichophyton violaceum* are the most commonly incriminated species in tinea pedis and epidermatophyties. About ten cases of sporotrichosis have been identified nationally since 1999.

Conclusion Probably at least 1.34% of Algerians have a serious fungal infection each year. This is dominated by recurrent vaginal candidiasis and allergic fungal disease complication asthma. Not counted

THE BURDEN of FUNGAL DISEASES in ALGERIA.

Infection	Number of infections per underlying disorder /year					Total	Rate
	None	HIV/AIDS	Respiratory	Cancer/Tx	ICU	burden	/100K
Oesophageal candidiasis		1,508				1,508	3.8
Candidaemia				1,383	593	1,975	5.0
Recurrent vaginal candidiasis >4/times/year	474,379					474,379	2402
Allergic bronchopulmonary aspergillosis			20,192			20,192	51
Severe asthma with fungal sensitisation (SAFS)			26,654			26,654	67
Chronic pulmonary aspergillosis (CPA)			3,642			3.642	9.2
Invasive aspergillosis				238	434	672	1.7
Mucormycosis				79		79	0.2
Cryptococcal meningitis		34		16		50	0.13
Pneumocystis pneumonia		115				115	0.3
Total burden estimated						**529,266**	

here are the most frequent mycoses – superficial. Invasive mycoses are dominated by aspergillosis and candidosis.

P211

The burden of serious fungal infections in Portugal

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Objectives We here attempt the first estimate of the burden of serious fungal disease in Portugal using deterministic scenario modelling and published incidence and prevalence data.
Methods Published epidemiology papers reporting fungal infection rates from Portugal were identified. Where no data existed, we used specific populations at risk and fungal infection frequencies in those populations to estimate national incidence or prevalence, depending on the condition. Population statistics were derived from the Statistics Portugal and the population census (2011). The incidence and prevalence of fungal infections affecting HIV patients were obtained from the report on HIV/AIDS 2013 published by the National Health Institute Doutor Ricardo Jorge, I.P. The total number of transplants was obtained from the Authority for Blood Services and Transplantation (data from 2011). Data on respiratory infections were recovered and inferred from the WHO data on TB.
Results The population of Portugal in 2011 was 10.56M, with 75% >15 years of age. An estimated 150 000 women (15–50) suffer from recurrent vulvovaginal candidiasis each year. We have not estimated oral or oesohphageal candidiasis rates. Candidaemia affects 0.88/1000 hospital admissions or approximately 8.1/100 000 patients, a total of 856 cases nationally. An estimated 150 patient develop intra-abdominal candidiasis, post-operatively. Invasive aspergillosis is less common than other countries as COPD is uncommon in Portugal, a total of 180 cases annually. An estimated 69 patients develop chronic pulmonary aspergillosis after TB each yearSo, assuming a 15% annual mortality and surgical resection rate, the prevalence is 218 cases following TB, whereas the prevalence of chronic pulmonary aspergillosis for all underlying pulmonary conditions was 654 patients. In contrast, asthma rates are high and so an estimated 23 198 SAFS episodes (220 cases/100 000), 17 586 ABPA episodes (167/100 000). 81 patients developed pneumocystis pneumonia in AIDS and 16 cryptococcosis. Data on dermatophytosis and fungal keratitis were not included in this study.
Conclusion Using published data, we were able to estimate the incidence or prevalence of the above referred fungal infections and ~194 293 (1.8%) people in Portugal suffer from those fungal infections each year. Vaginal candidiasis was the most frequent fungal infection detected in this study. Further inclusion of dermatomycosis data will largely increase the incidence and prevalence of fungal infections. This is a preliminary study but constitutes the first report on the global burden of fungal infections in Portugal. Further studies based on local surveys are required to obtain more precise and complete data.

Table 1

Infection	Number of infections per underlying disorder per year					Rate/100K	Total burden
	None	HIV/AIDS	Respiratory	Cancer/Tx	ICU		
Oesophageal candidiasis	-	-	-	-	-	-	-
Candidaemia	-	-	-	556	300	8.1	856
Candida peritonitis	-	-	-	-	150	1.4	150
Recurrent vaginal candidiasis (1x/year +)	149,751	-	-	-	-	2.856*	149,751
ABPA	-	-	17,586	-	-	167	17,586
SAFS	-	-	23,198			220	23,198
Chronic pulmonary aspergillosis	-	-	654	-		6.7	654
Invasive aspergillosis	-	-	-	97	33	1.2	130
Mucormycosis	-	-	-	21	-	0.2	21
Cryptococcosis	-	16	-	-	-	0.2	16
Pneumocystis pneumonia	-	81	-	-	-	0.8	81
Total burden estimated	149,751	97	41,438	674	533		194,293

* rate for females only.

ABPA = Allergic bronchopulonary aspergillosis SAFS =Severe asthma with fungal sensitisation

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The burden of serious fungal infections in Venezuela

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Introduction Venezuela is just north of the equator and has high rates of endemic fungal infections, HIV and hospital infections. The burden of fungal infection in Venezuela is not known, despite some epidemiological studies. This is the first estimate of the burden of serious fungal disease in Venezuela.
Methods We have used deterministic scenario modelling and published incidence and prevalence data on both underlying diseases and fungal infection rates from Venezuela to estimate burden. Where no data existed, we used specific populations at risk and fungal infection frequencies in those populations to estimate burden. Population statistics were derived from the Instituto Nacional de Estadística (http://www.ine.gov.ve). Data on respiratory infections were obtained and inferred from the WHO data on TB.
Results In 2011, the population of Venezuela was 29.44M, with 71% >15 years of age, and about 9% >60 years of age. An estimated 474 720 women (15-50) suffer from recurrent vulvovaginal candidiasis each year. Of the 110 000 HIV positive patients, an estimated 59 000 have CD4 counts <350, and 14 700 are at high risk of infection. We estimate that 2699 develop PCP *Pneumocystis* pneumonia in AIDS, 693 cryptococcosis and 1746 disseminated histoplasmosis, in AIDS. There are an estimated 3800 deaths from AIDS annually. Candidaemia is relatively common with affects 1.72/1000 hospital admissions or approximately 16/100 000 patients, a total of 4798 cases nationally. An estimated 824 patient develop intra-abdominal candidiasis post-operatively. Invasive aspergillosis is thought to affect 1143 people each year. An estimated 226 patients develop chronic pulmonary aspergillosis after TB each year, a prevalence of 711 cases and overall 1422 patients. Assuming ABPA affects 2.5% of adult asthmatics, 33 440 cases are likely and 44 141 SAFS cases. Data on dermatophytosis were not included in this study and there are few data on fungal keratitis, mucormycosis, coccidioidomycosis and paracoccidioidomycosis.
Conclusion Over 565 685 Venezuleans are affected by serious fungal disease annually, with many deaths. Further studies based on local surveys are required to obtain more precise and complete data.

Table 1

Infection	Number of infections per underlying disorder per year					Rate/100K	Total burden
	None	HIV/AIDS	Respiratory	Cancer/Tx	ICU		
Oesophageal candidiasis	-	-	-	-	-	-	-
Candidaemia	-	-	-	3150	1648	16	4,798
Candida peritonitis	-	-	-	-	824	3	824
Recurrent vaginal candidiasis (1x/year +)	474,720	-	-	-	-	3,225¥	474,720
ABPA	-	-	33,440	-	-	114	33,440
SAFS	-	-	44,141			150	44,141
Chronic pulmonary aspergillosis	-	-	1422	-	-	5	1422
Invasive aspergillosis	-	-	-	190	963	4	1143
Mucormycosis	-	-	-	59	-	0,2	59
Cryptococcosis	-	693	-	-	-	2	693
Pneumocystis pneumonia	-	2,699	-	-	-	9	2,699
Disseminated histoplasmosis		1,746	-	-	-	6	1,746
Total burden estimated	474,720	5,138	77,581	3,389	3,435		565,685

P213

Clinical, epidemiological and geographic characteristics of patients with paracoccidioidomycosis in Southern Brazil: A cross-sectional analysis

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Objectives Aims of this study were: (a) (b) To describe the key clinical-epidemiological features on the paracoccidioidomicose (PCM) cases in north-eastern part of the Rio Grande do Sul, (Southern Brazil); (c) To investigate the cities of origin for the patients with paracoccidioidomycosis, including its distribution in time and space.
Methods We conducted a cross-sectional analysis from PCM cases of a pathology laboratory in the city of Caxias do Sul, RS, Brazil. This city is located in north-eastern part of the Rio Grande do Sul, (Southern Brazil). Descriptive analysis of PCM cases covering the period January 2012 to March 2015. The study was carried out retrospectively by evaluating the databases of this laboratory. These cases were confirmed by the detection of multiple budding yeast cells typical of *P. brasiliensis* in tissue fragments. All of the cases confirmed by histopathology were included in this study. The following information was collected for this study using the biopsy data sheets and laboratory evaluations: age, sex, city of origin, professional activity, course of the disease and location of lesions. The data were compiled and descriptively analyzed using the Graph Pad Prism 5.0 (GraphPad Software INc. La Jola, CA, USA). This study was conducted according to the principles expressed in the Declaration of Helsinki. The study was approved by CONEP (National Committee for Research Ethics).
Results A total of 50 patients diagnosed with PCM by histopathological examination were registered between January 2012 and March 2015. Ages ranged from 15 to 99 years (average = 57 years). Patients ≥ 40 years represented 92% of all diagnostics and 96% were men. Information on the area of origin (rural or urban) and occupation/profession was obtained for 45 (90%) patients. Of these patients, 30 (89%) of them resided in rural areas, and their occupation involved land-related activities, especially agriculture. The affected anatomical regions were the lower respiratory tract, lymph nodes, oropharyngeal mucosa and skin. All cases diagnosed from oropharyngeal mucosa lesions had associated pulmonary involvement.This type of information, however, was not available for the remaining 13 patients. The city of origin was available for 39 patients. Approximately 60% of these patients were from Caxias do Sul. The remaining patients were from Vacaria, Campestre da Serra, Bento Gonçalves, Pinto Bandeira, Nova Prata, Veranópolis, Antonio Prado, Vila Ipê, Flores da Cunha, Nova Petrópolis, Gramado, Carlos Barbosa and Garibaldi.
Conclusion We found that the Southern of Brazil is particularly affected by tropical neglected infectious diseases, and PCM is part of this list. Paracoccidioidomycosis is a mycosis with an important number of reported incidences in cities of the north-eastern part of the Rio Grande do Sul. This study highlights the need to include PCM as a differential diagnosis of respiratory infection, especially in patients with oropharyngeal lesions and in rural males.

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Burden of serious fungal infections in Serbia

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Objective To estimate the annual burden of serious fungal infections (SFIs) in Serbia based on a size of population at risk and limited available epidemiological databases.
Methods National population data were obtained from Statistical Office of the Republic of Serbia, Institute of Public Health of Serbia, National Reference Medical Mycology Laboratory database as well as from National register for cystic fibrosis. Number of serious fungal infections among all underlying disease groups was estimated based on the number of risk patients using previously described methodology by Leading International Fungal Education (LIFE) and Global Action Fund for Fungal Infections (GAFFI).
Results Of the 7.2M population (48.7% male and 51.3% female), 14.3% are children (0–14 years), 67.8% are adults (15–65 years) and 17.8% of population are >65 years old. The current annual burden of SFIs in Serbia was estimated to be 109 283 (1517.8/100 000 population, Table 1).

The most common was recurrent vulvovaginal candidiasis (RVVC) with 95 907 cases per year assuming a literature rate 6% in women between 15 and 50 years old (1.6 M population). This is followed by severe asthma with fungal sensitisation (SAFS) and allergic bronchopulmonary aspergillosis (ABPA) that together comprised 10.6% of SFIs in Serbia. Chronic obstructive pulmonary disease (COPD) was common with 320 000 cases, contributing to the total 236 cases of invasive aspergillosis (IA; 0.2% of all SFIs) together with patients with cancer, transplantation (Tx) and stay in intensive care unit (ICU). Using low average literature data for candidemia rate (5/100 000) we estimated 360 candidemia cases annually in Serbia.

Based on 1124 cases of pulmonary tuberculosis, the annual incidence of new chronic pulmonary aspergillosis (CPA) cases was estimated at 371 cases, and prevalence 856 cases. Out of 1692 HIV patients, *Pneumocystis* pneumonia was estimated at 26, while oesophageal candidiasis and cryptococcal meningitis were estimated at 212 and 2 cases, respectively. Data on fungal keratitis and tinea capitis was not available.
Conclusion Based on these estimates, approximately 1.52% (n = 109 283) of the population suffer from a SFIs every year in Serbia. Crude rate for SFIs in Serbia is 1517.8 new cases per 100 000 population per year. This study serves as a basis but further epidemiological studies are necessary to better categorize, validate and extend this estimation.

Table 1. The annual burden of serious fungal infections (SFIs) in Serbia

Serious fungal infection (SFI)	Number of SFIs per underlying disorder per year					Total number of cases	% of SFIs	Crude rate/ 100,000
	None	HIV/AIDS	Respiratory	Cancer/Tx	ICU			
Oesophagial candidiasis		212				212	0.2	2.90
Candidaemia				252	108	360	0.3	5.00
Candida peritonitis					54	54	0.05	0.75
Recurrent vulvovaginal candidiasis	95,907					95,907	87.8	1332.0
Invasive aspergillosis			196	34*	6	236	0.2	3.28
ABPA			5,030			5,030	4.6	69.80
SAFS			6,600			6,600	6.0	91.60
Chronic pulmonary aspergillosis			856			856	0.8	11.90
Cryptococcus meningitis		1**			1**	2**	0.002	0.03
Pneumocystis pneumonia		26				26	0.02	0.36
Total burden estimated	**95,907**	**239**	**12,682**	**286**	**169**	**109,283**	**100.00**	**1517.8**

Abbreviations: ABPA - allergic bronchopulmonary aspergillosis; HIV/AIDS – human immunedeficiency virus/acquired immune deficiency syndrome; ICU- intensive care unit; SAFS - severe asthma with fungal sensitization; SFIs – serious fungal infections; Tx – transplantation. * - Some of the transplantations were done in Italy; ** - Data from patient database from National Reference Medical Mycology Laboratory of Serbia; no other data available

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Molecular epidemiological investigation of clustered *Pneumocystis jirovecii* pneumonias in four recently-liver transplant patients

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Objectives The main purpose of this study was to look for epidemiological links between four cases of Pneumocystis jirovecii pneumonia (PCP) in four recently-liver transplant patients, which occurred over a 5-month period at Tours University Hospital, France.
Method Bronchial-alveolar lavage fluids (BALFs), which had previously been found positive for P. jirovecii were processed for Multi Loci Sequence Typing (MLST) analysis. DNA was extracted from each sample using the Qiagen DNA mini kit® (Qiagen). Molecular typing was performed at three loci (SOD, CYB and mt26s) as previously described (Maitte et al., 2013). Sequences were analyzed using the Codon Code Aligner®software. Nine P. jirovecii positive BALFs, collected from unrelated patients, five from HIV-positive individuals, and four from other immunocompromised patients, were investigated by the same method. Phylogenetic relationships between the isolates were assessed using the MEGA 6.06® software (Center for Evolutionary Medicine and Informatics).
Results Our MLST approach resulted in allowing a reliable discrimination of all the P. jirovecii strains found in unrelated immunocompromised patients (one single genotype per patient). The allelic profiles were shown to be the same as those previously reported, except for two strains that, to our knowledge, exhibited new Multi-Nucleotide Polymorphism (MNP) resulting from previously unknown combinations of mutations in position 279-516-838 of the CYB gene. Three of the four P. jirovecii strains hosted in the liver transplant patients (14116074, 14113359 and 14111271) were successfully sequenced and found to be closely related (bootstrap-score: 100%, sequence similitude: 100%) (Figure 1).
Conclusion Our results highlighted the importance of genotyping during investigation of PCP outbreaks. They confirmed high phylogenetic diversity in P. jirovecii strains amongst the unrelated immunocompromised patients in the cohort. Besides, we have described for the first time a very rare example of clustered PCP in liver transplant patients. Epidemiological investigation showed that concerned patients were found to be hospitalized at concomitant days, with several overlapping dates of outpatient consultations. These findings support the evidence of nosocomial PCP outbreak.

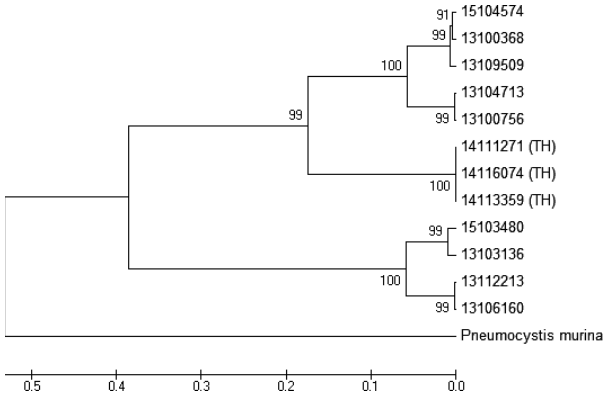

Figure 1. Phylogenetic dendrogram

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Tinea capitis epidemiology in Mali

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Objectives Dermatophytosis, and particularly the subtype tinea capitis, is common among African children; however, the risk factors associated with this condition are poorly understood. This study aimed at testing whether climate influences the epidemiology of dermatophytosis.
Methods Cross-sectional surveys were conducted in public primary schools located in three distinct eco-climatic zones, namely the Sahelian, Sudanian and Sudano-Guinean eco-climatic zones in Mali.
Results Among 590 children (mean age 9.7 years) the overall clinical prevalence of tinea capitis was 39.3%. Tinea capitis prevalence was 59.5% in the Sudano-Guinean zone, 41.6% in the Sudanian zone and 17% in the Sahelian eco-climatic zone. Two anthropophilic dermatophytes species, *Trichophyton soudanense* and *Microsporum audouinii*, were the most frequent species associated with tinea capitis among primary schoolchildren in Mali. As expected, *Microsporum audouinii* was isolated primarily from large and/or microsporic lesions, while *Trichophyton soudanense* was primarily isolated from trichophytic lesions. Multivariate logistic regression analysis showed that male gender (OR = 2.51; 95%CI [1.74–3.61], $P<10^{-4}$) and residing in the Sudano-Guinean eco-climatic zone (OR = 7.45; 95%CI [4.63–11.99], $P<10^{-4}$) were independently associated with an increased tinea capitis risk.
Conclusion In this homogenous schoolchild population, our findings highlight an increased tinea capitis risk with increasing climate humidity. Further studies should aim at investigating the role of climatic factors in the geographic distribution of dermatophyte species.

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Outcomes from In Utero, Neonatal and Pediatric Environmental Mold Exposure

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Objectives Both visible mold growth and malodorous vapors and are recognized health hazard markers for indoor contamination with toxin-producing, infectious fungi. Molds such as Aspergillus/Penicillium, Chaetomium and Stachybotrys produce myriad harmful products including carcinogenic, neurotoxic, immunosuppressant mycotoxins (MCTs), toxic debris, particulates, & volatile compounds. Despite disinfection, environmental products may remain hazardous.

Both cell-mediated immunity and metabolic detoxification are poorly developed in early life when hazardous microbial and toxin exposures are expected to cause more harm.
Methods Environmental spore air trap, microscopy, fungal-specific PCR, ERMI analysis, MCT measurement, medical examination, analysis: exposure timing/severity, mold spp, obstetrical/pediatric symptom chronology outcomes, fungal quantitative IGG titers, MCT excretion.
Results Of 66 children (25M, 38 F, 3 embryos. exposed 1st trimester - 18 yrs.), disease severity was inversely related to age exposed: 3/9 (30%) 1st-trimester died, 3 life-threatening, 17 severe, 28 moderate, 14 mild outcomes with later exposure-ages.

Visible and malodorous microbial exposures contributed the most: 50% total to visible mold and 38% to odors, paralleling disease

severity (44% for mild, 46% moderately ill, 38% severe life-threatening). Chemical and pesticide exposures appeared to contribute to life-threatening outcomes.

Of 18 Inutero exposures (9 1st-trimester, 1 2nd-trimester, 2 3 d, 6 indeterminate), 3 miscarried, 9 were complicated by prematurity or organ damage (2 hydronephrosis, 2 congenital heart abnormalities, 1 microcephalus), 3 had abnormal placentas, 10 birth complications and 5 neonatal complications. All 6 (100%) breast-fed developed problems: 4 projectile vomiting, 4 unexplained body dermatitis, 1 intussusception with refractory painful anorectal rash, 2 choking (one apneic requiring ICU treatment for respiratory arrest/pulmonary hemorrhage), 3 black oro-nasal drainage, 1 neurologically-impaired oropharyngeal function from ingesting Ochratoxin(Ochra)/Trichothecene (Trichos) contaminated breastmilk.

The most affected neonates were from 2 mothers in different homes with severe 1st-trimester exposures: both developed congenital heart disease (PDO, VSD), cognitive/neurological impairment, projectile vomiting and choking breastfeeding. One was hospitalized within 2 weeks with respiratory arrest & pulmonary hemorrhage. The other developed black oronasal discharge and lost oropharyngeal motor function lactating Ochra/Trichos contaminated milk. Vomiting and discharge resolved when lactation was stopped.

All breastfed by mother #1 from the Trichos-contaminated home developed rashes. Of 2 not breastfed, one developed chronic fatigue, speech delay, and cognitive, matching fungal IGGs, urinary Ochra/Trichos excretion impairment after playing extensively in the basement. The other, minimally-exposed to that home was normal.

Overall, skin rashes 42(67%), throat inflammation 42 (67%), nasal inflammation 40 (63%) (30 PND, 17 nosebleeds), pulmonary 35 (56%), cough 31 (49%), 25 neurologic & GI (nausea/vomiting 19 (30%)) cognitive impairment (memory, ADD, Apraxia, learning disability) 22 (33%), developmental delay 9 (14%).

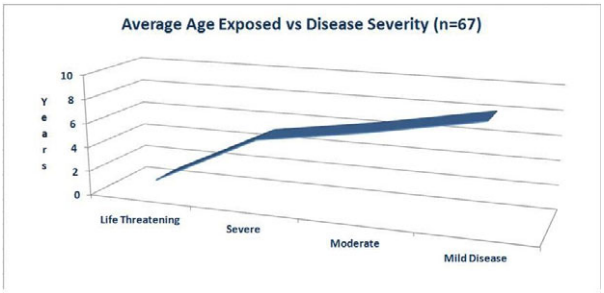

Figure 1. Average Age exposed versus Disease Severity.

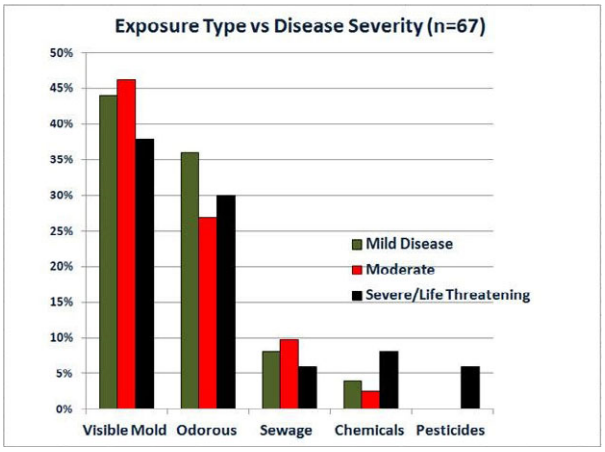

Figure 2. Exposure Type versus Disease Severity.

Conclusions Outcome severity correlated with early hazardous exposure.

Visible mold and odorous exposures predominated in all disease severity levels.

Chemicals and pesticides appear to contribute to severe/life-threatening outcomes.

Environmental in utero exposure to toxin-producing, infectious molds and/or hazardous products appears to cause in-utero/congenital defects, placental abnormalities.

Exposure timing, severity correlated with pregnancy complications, congenital/placental defects, postnatal & lactation outcomes.

Epidemiological studies are urgently needed.

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Candida spp. causing vulvovaginal candidiasis (VVC) in an outpatient clinic in Bilbao, Spain

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Objective To identify the species of *Candida* isolated in a population of women suffering VVC and (2) to evaluate their susceptibility to Fluconazole (FLC) and Clotrimazole (CLT).

Materials And Methods A total of 121 vaginal swabs were analyzed from women with symptoms resembling *Candida* vulvovaginitis. Specimens were inoculated onto Candida CHROMogenic Agar (Conda) to ensure detection of mixed infections. Yeasts were identified based on carbohydrate assimilation (ID32C-Biomerieux) and/or by PCR. Susceptibility tests to CLT and FCL, used normally to treat VVC in our clinic, were performed using the broth microdilution method according to CLSI guidelines. Since there are no MIC limit ranges established for CLT microdilution test, we followed the recommendations of other authors [1, 2].

Results Seventy-five (62%) patients were diagnosed with VVC: acute VVC (17/75; 22.7%) and Recurrent VVC (RVVC) (58/75–77.3%). Among the VVC patients 42 were pregnant (56%), and 11 (14.66%) of them had previous treatment episodes with antibacterials, 7 (9.33%) had allergic rhinitis, 6 (8%) were taking hormonal contraceptives and 1 was participating in an experimental vaccination trial against VVC. There were 46 (38%) negative cases for VVC, and among them 19 (41.3%) were diagnosed with bacterial vaginosis: *Gardnerella vaginallis* (13/19–68.42%), *Streptococcus agalactiae* (3/19–15.78%) and enterobacteria (3/19–15.78%) were the aetiological agents. One additional patient was diagnosed with *Trichomonas vaginalis*.

With respect to species distribution *Candida albicans* was the most frequently isolated yeast (94.66%), followed by *Candida parapsilosis* (2.66%), *Candida tropicalis* (1.33%) and *Candida glabrata* (1.33%). Only one patient with VVC showed a mixed fungal infection.

Both azoles tested showed good in vitro activity, only 7 strains showed MIC values for CLT $\geq$ 0.5 μg ml^{-1} (resistance breakpoint value according to Pelletier et al. [2]), and 3 of them also showed high FLC MIC (>64 μg ml^{-1}).

Conclusions Although many authors claim that there is a trend towards the emergence of non-*Candida albicans Candida* spp., in our group of study we still found that *C. albicans* was the most commonly isolated yeast, followed by far by *C. parapsilosis*. Regarding antifungal susceptibility, CLT remains a good topical therapy option.

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P219

Evaluation of *A. fumigatus* aerocontamination dispersion indoor and outdoor hospital blocks during demolition works at hospital

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Objectives A large University Hospital, Edouard Herriot composed of 32 blocks (France) is involved in a large modernization program which consisted in the demolition of an entire central medical block. Spores may be suspended specially during demolition near immunocompromised patients who are at risk to develop severe invasive aspergillosis (IA). The aim of the study was to evaluate the variability of Airborne *A. fumigatus* conidia contamination indoor and outdoor hospital ward during demolition work and to better understand conidia spread.

Methods Since the beginning of the demolition site in February 2015, we started a daily environmental survey of fungal loads in 8 wards of the hospital located all around the demolition site: 4 intensive care units (ICU), 1 unit of kidney and liver transplantation and 3 other medical wards. We choose to present data from one ICU (G1) located very closely at south of demolition work site and the unit of kidney and liver transplantation (G2) located further at north of the site (Figure 1). The environmental survey consisted in sampling air twice a day, indoor and outdoor, by impaction onto Sabouraud Chloramphenicol agar supplemented with antibiotic chloramphenicol plates (Biomerieux) using a an agar impact sampler (Air-Ideal 90 mm). Plates were incubated 48 h at 37°C. After incubation *A. fumigatus* were identified. Data were entered into an Excel database. Pairwise associations between presence of *A. fumigatus* aerocontamination and binary variables were evaluated by Fisher exact test. $P < 0.05$ was considered statistically significant.

Results Between February and May 2015, a mean of 112 air samples were realised weekly. *A. fumigatus* was detected outside and inside of the 8 wards with a mean load of respectively 9.24 CFU/m3 ($n = 278$, SD: 23.27) and of 3.94 CFU/m3 ($n = 365$, SD: 27.99). Outside each monitored wards, *A. fumigatus* was more frequent outside when major intensive construction work (ICW) were taking place with a relative risk (RR) of 1.22 ($n = 206$, $P = 0.29$). Inside sampled wards same trends was observed, *A. fumigatus* was more present inside during ICW with a RR of 1.29 ($n = 269$, $P = 0.24$). For G1, the RR of *A. fumigatus* was of 0.37 ($n = 40$, $P = 0.04$) into corridors and rooms when ICW occurred. For G2, the RR of *A. fumigatus* inside was of 2.50 ($N = 39$, $P = 0.26$). When windows and door of wards were opened, *A. fumigatus* was more present in G2 with a RR of 1.86 ($N = 51$, $P = 0.30$). As openings were caulked, no RR could be calculated for *A. fumigatus* colonisation in G1.

Conclusion Although G1 was located just in front of ICW, it was the less contaminated by *A. fumigatus*. On contrary, G2 unit which was further of the ICW has highest A. fumigation colonization. These preliminary findings have already permitted to applied corrective actions to improve professional practices and develop relevant methods to control and evaluate the microbiological risk during construction works in health institutions.

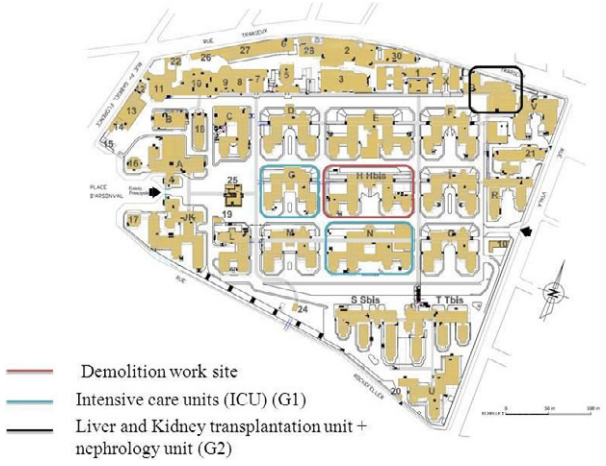

Figure 1. Map of Edouard Herriot Hospital, France

P220

Prevalence and population structure of buccal and cutaneous *Candida* spp. in a pediatric population

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Objectives *Candida* spp are the most frequent fungal commensals on the buccal mucosa and according to some authors their overall prevalence in healthy carriers may be as high as 50%. Recent investigations in our labs have suggested that this may be an overestimate with large geographic variations. This study was thus initiated to investigate the prevalence, population structure and antifungal resistance profile of *Candida* spp. on the buccal mucosae and on the skin of healthy children.

Methods 100 healthy children who attended the paediatric department of the local hospital for routine checks were included in this investigation. Samples from the oral cavity and from skin were collected using sterile swabs and incubated at 30°C during 48 h on chromID Candida Agar medium. *Candida* species were identified by MALDI-TOF MS and when needed the identification was confirmed by sequencing of the ITS region. Population structure analysis was carried out by microsatellite-based typing using sets of genetic markers with various discriminatory powers. The *in vitro* susceptibility profile to azoles, polyenes, and echinocandins was done by microdilution.

Results and Conclusion *C. albicans* was the most common species isolated. *C. parapsilosis* was also present at lower frequency. The populations were predominantly clonal with few or no genetic variation within each idividual and we could not detect any single widespread genotype.

P221

Estimated burden of serious fungal infections in Pakistan

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Introduction Due to absence of population based surveillance, true-burden of fungal infection in Pakistan is unknown. Laboratory and institutional based reports from the country highlight the existence of these infections in many patient settings. Similarly high risk populations for fungal infections (TB, diabetes, chronic respiratory diseases, asthma, cancer, transplant, HIV) in the country are also prevalent. The situation is more complicated due to poor diagnostic capabilities of most laboratories in Pakistan, lack of antimicrobial

stewardship and poor infection control practices. So we estimate here the burden of fungal infections in Pakistan to highlight the public health significance of these infections.

Methods Population estimates was referred from WHO population statistics 2013 data. Morbidity data of conditions at risk were determined by reviewing national and global data. TB rates were taken from WHO TB global report 2014. Asthma prevalence data was taken from global asthma prevalence rates. COPD rates were taken from BREATHE study. HIV rates were taken from UNAIDS 2013 report. Cancer rates were determined from GLOBOCAN 2012. Diabetes rates were taken from national data on NCD by Heartfile. Published data from Pakistan reporting fungal infections rates in general and specific populations was reviewed and used when applicable. Burden of cryptococcal and *Pneumocystis* infection in HIV was determined using data from Baqi S et al. and Luxmi S et al. Estimations for allergic bronchopulmonay aspergillosis (ABPA), severa asthma with fungal sensitization (SAFS) and chronic pulmonary aspergillosis (CPA) in asthma, sarcoidosis and TB patients were done using methodologies by Denning DW et al. and Agarwal R et al. Mucormycosis and candidaemia rates were calculated using estimated burden in India by Chakrabarti A et al. Mycetoma burden was computed using Indian data from global burden of mycetoma by van de Sande et al. Recurrent vulvovaginitis burden was estimated using assumption that it affects 6% of women between 15 and 55 years. Invasive pulmonary aspergillosis (IPA) rates were determined in COPD patients assuming it affects 3.9% of admitted COPD patients, as in China (Xu et al.).

Results In the 182 million people in Pakistan, 34% are children (<15 years) and 6% are over the age of 60 years. An estimated 3 216 331 (1.77%) people are affected by a serious fungal infection, omitting all cutaneous infection, oral candidiasis and allergic fungal sinusitis, which we could not estimate. Compared with other countries the rates of candidaemia (21/100 000) and mucormycosis (14/100 000) are estimated to be very high, and based on data from India. Chronic pulmonary aspergillosis rates are estimated to be high (25.6/100 000) because of the high TB burden, with only 20% of cases not related to TB (i.e. 500 due to sarcoidosis). Fungal keratitis is also problematic in Pakistan with an estimated 24 260 cases (13.3/100 000). Large numbers of COPD patients probably develop invasive aspergillosis (using the 3.9% rate in hospitalizedpatient from China) (9846 annually).

Conclusions Pakistan probably has a high rate of certain life or sight-threatening fungal infections. The unavailability of lipid preparation of amphotericin, flucytosine and posacozole and limited availability of voriconazole prevents good outcomes from many of these infections.

Table 1. Burden of serious fungal infections in Pakistan

Infection	Number of infections per underlying disorder per year					Rate/100K	Total burden
	None	HIV/AIDS	Respiratory	Cancer/Tx	ICU		
Cryptococcal meningitis	?	263	-	4	-	0.15	267
Pneumocystis pneumonia	-	840	-	?	-	0.46	840
Oesophageal candidiasis	-	7,500	-	-	-	4.1	7,500
Candidaemia	-	-	-	25,500	12,750	21	38,250
Invasive aspergillosis	-	?	-	1,093	9,846	6.0	10,938
Mucormycosis	20,000			5,500		14	25,500
Recurrent vaginal candidiasis (4x/year +)	2,820,518	-	-	-	-	3,097*	2,820,518
ABPA	-	-	99,177	-	-	54	99,177
SAFS	-	-	130,913			72	130,913
Chronic pulmonary aspergillosis	-	-	46,667	-	-	26	46,667
Fungal keratitis	24,261	-	-	-	-	13	24,261
Mycetoma	18 182					0.05	100
Total burden estimated	2,844,879	8,601	276,857	26,633	22,596		8,216,331

P222

The burden of serious fungal diseases in Madagascar

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Background In Africa, few data on the burden of fungal disease are published, but Madagascar is an exception. We have estimated the burden of serious fungal infections in Madagscar.

Methods We searched for existing data and estimated the incidence and prevalence of fungal diseases based on the populations at risk and available epidemiological data. Data were derived from the World Health Organization (WHO), The Joint United Nations Programme on HIV/AIDS (UNAIDS) and national and regional published reports. When no data existed, risk populations were used to estimate frequencies of fungal infections, using previously described methodology by LIFE.

Results The population of Madagascar is around to 19 625 000; 43% are children, and 5% are women >60. Recurrent vulvovaginal candidiasis (>4 episodes/year) is estimated to occur in 2 703/100 000 females. Oesophageal candidiasis is estimated to affect 6.700 HIV patients, given the ~25 000 with <350 CD4 cells not on ART. An estimated 875 and 1250 patients develop cryptococcosis and PCP each year, with an overall HIV mortality of 3900 people. An estimated 2.625 have chronic pulmonary aspergillosis after pulmonary tuberculosis 67% of the total burden. ABPA and SAFS were estimated in 66.6/100 000 and 87.8/100 000 respectively, in ~522 000 adult asthmatics. A population estimate of chromoblastomycosis was completed 1996 and an rate of 14/100 000 was found (2745 cases). Tinea capitis is estimated to affect a minimum of 10% of children, over 840 000 cases.

Conclusions The present study indicates that around to 6% (1 155 796) of the population is affected by a serious fungal infection, predominently recurrent VVC in women and tinea capitis in children. Further epidemiological studies are needed to validate and extend these estimates.

Table 1

Infection	Number of infections per underlying disorder per year					Total burden	Rate /100K
	None	HIV/AIDS	Respiratory	Cancer/Tx	ICU		
Oesophageal candidiasis	-	6,700	-	-	-	4,150	34.1
Candidaemia	-	-	-	687	294	981	5
Recurrent vaginal candidiasis (4x/year +)	265,248	-	-	-	-	265,248	2,703
ABPA	-	-	13,060	-	-	13,060	66.7
SAFS	-	-	17,239			17,239	87.8
Chronic pulmonary aspergillosis	-	-	3,975	-	-	3,978	90
Invasive aspergillosis	-	-	??	118	?	297	
Mucormycosis	?	?	?	39	?	39	0.2
Cryptococcal meningitis	-	875	-	-	-	875	4.5
Pneumocystis pneumonia	-	938	-	-	-	938	4.8
Histoplasmosis	-	??	?	-	-	?	-
Fungal keratitis	?	-	-	-	-	?	
Chromoblastomycosis	2,745	-	-	-	-	2,745	14
Tinea capitis	843,875	-	-	-	-	843,875	4,300
Total burden estimated	1,111,868	8,513	34,277	844	294	1,155,796	

P223

Epidemiological profile of candidemias in a university hospital in the Midwest Region of Brazil: 2006–2014

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Objectives This study aimed to determine the epidemiology and risk factors associated bloodstream infection caused by *Candida* spp. in a university hospital in the city of Cuiabá.

Methods For this, we used a cross-sectional study with laboratorial exams and secondary data analysis between 2006 and 2014. Eighty-seven episodes of candidemia were identified.

Results The prevalence of candidemia among patients hospitalized in the period was 2.5 per 1000 admissions (87/33975) and the attributed mortality rate was 1.3 per 1000 admissions (44/33975). The prevalence of candidemia among patients with hospital infection was 30.5 per 1000 admissions (87/2850) and the attributed mortality rate was 15.4 per 1000 admissions (44/2850). The coefficient of lethality was 50.6% (44/87). Most patients were females (n = 51; 58.6%). The average age was 30 years (SD = 29; 95%CI = 24–37) and the mean hospital stay was 53 days (SD = 45; 95%CI = 43–62). Overall, 83.9% of patients were prescribed antifungal drugs: amphotericin B (53.0%) and fluconazole (38.5%) were the most common. *Candida parapsilosis* was the most common etiological agent (n = 36; 41.4%), followed by *C. albicans* (n = 34; 39.1%); *C. tropicalis* (n = 13; 14.9%), *C. glabrata* (n = 2; 2.3%) and *C. guilliermondii* (n = 2; 2.3%). The distribution of cases by hospital sector showed higher concentrations in adult and neonatal intensive care units (75.9%). The following risk factors were identified: central venous catheter (n = 73; 83.9%); ventilatory support (n = 64; 73.6%); parenteral nutrition (n = 62; 71.3%); H2 blockers (n = 47; 54.0%); corticosteroids (n = 41; 47.1%); previous surgery (n = 36; 41.4%) and neutropenia (n = 24; 27.6%). In newborns, low birth weight was detected in 51.5% (17/33) and prematurity in 48.5% (16/33). The following risk factors significantly associated with the incidence of death in the univariate analysis: ventilatory support (OR = 5.6; 95% CI=1.8–17.0; P = 0.001); parenteral nutrition (OR = 6.8; 95% CI = 2.2–20.5; P = 0.0003); central venous catheter (OR = 4.7; 95% CI = 1.2–18.2; P = 0.02).

Conclusion Candida non-albicans accounted for 60.9% of the causative agents of candidemia in this population, this fact reinforces the importance of considering local mycological profile of bloodstream infections for the therapeutic management.

P224

An estimation of the burden of serious fungal infections in Mozambique

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Introduction Mozambique is a Portuguese-speaking, sub-Saharan African country with a high HIV and high TB burden. Few studies have reported on the burden of fungal disease in Mozambique and we have attempted to summarise these and estimate the total burden of the serious fungal diseases there, to assist determine public health and research priorities.

Methods Published epidemiology papers reporting fungal infection rates from Mozambique were identified. Where no data existed, we used specific populations at risk and fungal infection frequencies in those populations to estimate national incidence or prevalence, depending on the condition. Population statistics (2011), pulmonary TB rates (2013) and HIV data were derived from WHO and UNAIDS. We assumed the rate of asthma was the same as Malawi (4.67% in adults) (Yo et al., 2012). It was assumed that no transplantation was done and COPD data was not available.

Table 1

Infection	Number of infections per underlying disorder per year					Rate/100K	Total burden
	None	HIV/AIDS	Respiratory	Cancer/Tx	ICU		
Oesophageal candidiasis	-	84,250	-	?	-	368	84,250
Candidaemia	-	-	-	801	343	5.0	1,145
Recurrent vaginal candidiasis (4x/year +)	303,976	-	-	-	-	2,656*.	303,976
ABPA	-	-	14,967	-	-	65	14,967
SAFS	-	-	19,756	-	-	86	19,756
Chronic pulmonary aspergillosis	-	-	10,227&	-	-	45	10,227
Invasive aspergillosis	-	?	-	137	?	0.6	137
Mucormycosis	-	-	-	46	-	0.2	46
Cryptococcosis	?	14,815	-	?	-	65	14,815
Pneumocystis pneumonia	-	22,145	?	?	-	97	22,145
Tinea capitis	1,007,248	?	-	-	-	4,400	1,007,248
Total burden estimated	1,311,224	121,190	44,960	984	515		1,744,364

* rate for females only. & assumes 80% of cases are related to TB, 20% other pulmonary conditions.

Results 56% of the 22.98 million population (2011) are adults, of whom only 5% are over 60 years. The estimated HIV prevalence is estimated at 1.6 million, with 590 000 with CD4 counts <350. Cryptococcal meningitis incidence was estimated to be estimated 14 815 cases, *Pneumocystis* pneumonia 22 125 cases and oesophageal candidiasis cases 84 250 annually. Following the approximately 40 000 pulmonary TB survivors annually, the annual incidence of chronic pulmonary aspergillosis was estimated at 2596 and the prevalence at 8181, assuming a 15% annual mortality. ABPA complicating asthma is estimated at 14 967 (2.5% rate) and severe asthma with fungal sensitisation at 19 756, both in adults only. In 2006/7 an estimated 10% of children had tinea capiitis - an estimated 1 007 250 children affected. Among fertile women (15–50 years), an estimated 303 976 suffer from recurrent vulvovaginal candidiasis. No data on fungal keratitis or histoplasmosis were available.

Conclusion Approximately 1 744 000 (7.6%) people in Mozambique are estimated to suffer from a serious fungal infections in any year. Tinea capitis in children recurrent *Candida* vulvovaginitis are the most common, with HIV fungal complications being the next more common. Prospective epidemiology studies are required to validate these estimates.

P225

Burden of serious fungal infections in Greece

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Introduction Data on the epidemiology of serious fungal infection in Greece are scarce. The aim of this work is to calculate the burden of serious fungal infections in Greece.

Methods A thorough literature search for papers reporting epidemiological data on serious fungal infections in Greece was performed. For fungal infections where no Greek data existed, we used a structured set of assumptions to estimate their burden, based on specific population with risk factors for fungal infection, such as immunosuppression, chronic disease, and surgical procedures. Population statistics were derived from the Greek Statistics Authority and the latest (2011) population census. Data on HIV/AIDS (2014) were obtained from the Hellenic Centre for Disease Control and Prevention; data for transplantations (2012) from the National Organization for Transplantation; data for tuberculosis from the World Health Organization (2012); data on COPD, cystic fibrosis, asthma, abdominal surgeries form the relevant scientific Greek societies; data on the number of critical care beds and hospital admissions from the Greek Ministry of Health.

Results 85.5% of the 10.8 M population are adults, 53% are women, 27.4% women are over 60 years and 40.3% over 50; 27% of population are ≥60 years old. Estimates are: 243 567 Greek

Infection	Number of infections per underlying disorder per year					Rate/100K	Total burden
	None	HIV/AIDS	Respiratory	Cancer/Tx	ICU		
Oesophageal candidiasis	-	353	-	-	-	0.59	353
Candidaemia	-	-	-	379	162	5.0	541
Candida peritonitis	-	-	-	-	81	0.75	81
Recurrent vaginal candidiasis (4x/year +)	263.567	-	-	-	-	5.0	243.567
ABPA	-	-	20.805	-	-	192	20.805
SAFS	-	-	26.500			253	26.500
Chronic pulmonary aspergillosis	-	-	1.388	-	-	3.21	1.388
Invasive aspergillosis	-	-		1040	85	10.4	1.125
Mucormycosis				12		0.11	12
Cryptococcal meningitis	-	2	-	-	-	0.02	2
Pneumocystis pneumonia	-	28		-	-	0.26	28
Histoplasmosis	?	?	?	?	?	?	?
Fungal keratitis	?	-	-	-	-	?	?
Tinea capitis	56	-		-	-	0.52	56
Total burden estimated	263.623	383	48.693	1.431	328		294.458

Figure 1. Fungal infections in Greece

women get recurrent vaginal thrush (≥4x/year). Of 14 434 HIV positive patients, 1732 are not receiving ARVs. Oral candidiasis is estimated to occur at least once in 90% of patients with CD4 cell counts <200 μL and oesophageal candidiasis in 20%. We estimated that there are 1.296 cases of oral candidiasis and 353 cases of esophageal candidiasis. 2 (2%) of 107 new AIDS cases each year develop cryptococcal meningitis. Annual incidence of Pneumocystis pneumonia is 0.26 cases/100 000 in HIV+ patients, 28 cases. Of the 500 cases of TB in 2012, 45 with AIDS, it is estimated that 22 new cases of chronic pulmonary aspergillosis (CPA) occurred and that the 5-year period prevalence is 347 cases (assuming 15% annual mortality). As CPA occurs in multiple other conditions including COPD a prevalence of 1 388 cases is estimated. Estimates of asthma prevalence in adults are about 9% and assuming 2.5% of asthmatics have ABPA, 20 805 patients with ABPA are likely and 26 500 with SAFS. Assuming the rate of candidemia in Greece is 5.0/100 000 population there are 541 cases. We have estimated 81 cases of post-surgical candida peritonitis (~60 000 abdominal surgeries/year). Invasive aspergillosis in immunocompromised patients is estimated at 1125 patients annually including 85 cases in intensive care. For mucormycosis, there were 12 cases annually, and 56 cases of tinea capitis.

Conclusion According to our calculations ~ 300 000 (2.72%) people in Greece suffer from fungal infections each year. This is the first attempt to determine the burden of fungal disease in Greece and provides a crude estimate on its impact on public health.

P226

Epidemiology of fungi isolated in blood cultures after identification by MALDI-ToF implementation: Brazilian experience in 2014

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Objective Matrix-assisted laser desorption/ionization - Time of Flight (MALDI-Tof) technique has changed microbiology laboratory's routine, due to its improved accuracy and faster performance to identify microorganisms. Many authors have validated its usefulness to fungi identification. In order to provide additional epidemiologic data about fungemia in Brazil, once no data about private hospitals is currently available, we analyzed the results of all blood cultures performed in a large private laboratory of São Paulo after MALDI-Tof full routine implementation.

Methods We retrospectively analyzed the results data bank of a Brazilian private laboratory located in the Southeastern city of São Paulo, the largest and most populated city of Latin America. This lab analyzes samples from more than 10 hospitals in the main capitals of Brazil (about 2000 beds). All blood cultures performed during 2014 were included, and their results were evaluated regarding positivity, specie identification, site of sample collection, type of positive vial and, when available, isolate susceptibility. All isolates were identified using MALDI-Tof, and the method for susceptibility tests was Etest®, interpreted according to CLSI breakpoints. Samples from a single patient, positive for the same fungi, in an interval of up to 30 days, were considered as belonging to the same fungemia episode.

Results Our lab has performed 206 007 blood cultures in 2014, 20 751 (10.07%) of which were positive. A total of 867 vials were positive for fungi, which corresponded to 357 fungemia episodes. More than 56% (490) of these samples were collected from peripheral sites, while the others were collected from different types of vascular catheters and devices. Regarding the identified fungi, 97% (347) of them were yeasts. Most episodes were related to *Candida* species: 104 *C. albicans* (29.1%), 87 *C. parapsilosis* complex (24.3%), 61 *C. tropicalis* (17.1%), 42 *C. glabrata* (11.8%); 25 were due to other *Candida* species. Of notice, there were 9 episodes of *Saccharomyces cerevisiae* and 8 of *Trichosporon* sp fungemia, all of them in critically ill and/or immunosuppressed patients. Mean time of detection was 38.5 h, ranging from 25 (*C. tropicalis*) to 55 (*C. glabrata*) among most frequent species. Thirty-two episodes were diagnosed only by means of specific fungi blood culture (BACTEC Myco/F lytic vial), and they corresponded to 25% of episodes related to *Trichosporon* sp, 16.6% of *C. krusei*, 10.5% of *C. albicans*, 8.1% of *C. tropicalis*, 7.1% of *C. glabrata* and 6.9% of *C. parapsilosis* complex episodes. Regarding susceptibility, *C. parapsilosis* and *C. tropicalis* exhibited the largest proportion of Fluconazole resistant isolates (7 each, 8.0% and 11.4%, respectively).

Conclusion Fungi are important agents of blood stream infection, especially in critically ill and immunosuppressed patients, a large proportion of which using invasive vascular devices. Although *Candida albicans* remains the most frequent yeast when species are considered separetly (29.1%), altogether, non-*albicans* species were the most frequent cause of fungemia (60.2%). Aerobic blood culture vials have good sensitivity for most yeasts, but associated use of fungi specific vials may improve positivity for some species, mainly *C. krusei* and non-*Candida* yeasts. Resistance to azoles requires attention in non-*albicans Candida*.

P227

Changing epidemiology of the Non-albicans *Candida*-group in clinical specimen in a 10 year- single centre survey in Austria 2005 to 2014

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Objectives and background Non-albicans species are reported to being emerging yeasts in clincal relevant specimen within the Candida-group over the last years. Therefore we set up a 10-survey with the aim to identify the most emerging Non-albicans- species.

Methods We did a retrospective data analysis of our surveillance data comparing two time intervalls: 2005–2009 versus 2010–2014. We screened all relevant clinical isolates at species - level according to routine diagnostic procedures of our clinical microbiology laboratory. We ranked the top 10 species of each time period and compared the proven species-frequency.

Results 5685 single patient isolates from the first period were compared to 6977 isolates of the second period both including C. albicans and Non-albicans species.

Top 5 non- albicans species were Candida glabrata, C. tropicalis, C. parapsilosis, C. krusei and C. lusitaniae in both groups and time periods. But there was a move of Candida parapsilosis from the third to the second most frequent position (197/3.5%–238/3.4%) with a stable overall percentage. A significant rise was found in Candida glabrata (485/8.5% to 777/11.2%) and a decrease in Candida tropicalis (278/4.5% to 236/3.4%).

Conclusion The emergence of Candida glabrata and furthermore of Candida parapsilosis which became the second frequent species within the non-albicans-group needs further analysis regarding risk-factors of patients and antifungal therapeutic consequences.

P228

A 3-year survey of dermatophytosis in Belgium

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Objectives Dermatophytosis refers to superficial fungal infections of keratinized tissues caused by keratinophilic dermatophytes. They are the most common cause of superficial fungal infections worldwide. Epidemiological studies regarding dermatophyte infections have been conducted in several countries and differences in the incidence and in etiological agents have been reported for different geographical areas. That is why national surveillance of circulating strains causing dermatophytosis is crucial. The Belgian National Reference Center (NRC) for Mycoses conducted a survey on dermatophytes strains circulating from 2012 to 2014. The present study was performed to assess the profile of dermatophytosis and to identify the species involved.

Methods The Belgian NRC for Mycosis collected 9138 strains between January 2012 and December 2014. The isolates were cultured from patients clinically suspected for fungal infections of skin, hair and nails. Isolates were sent by Belgian laboratories to the two labs of the Belgian NRC (UZ Leuven and CHU of Liège) in order to identify the fungus or to confirm the identification. All isolates cultured from patients of UZ Leuven and CHU of Liège were also included. Fungal identification was performed by microscopy after subculture and in case of doubtful identifications, by ITS sequencing.

Results Among the 9138 samples, 3587 were identified as dermatophytes. *Trichophyton. rubrum* (*T. rubrum*) was the most prevalent species accounting for 56 17% ($n = 2015$) of the infections from all sources, followed by *T. mentagrophytes complex* (2183%, $n = 783$). The other main etiological agents of dermatophytosis recorded in this study in descending order of prevalence were *M. audouinii* ($n = 303$), *M. canis* ($n = 120$), *T. violaceum* ($n = 112$), *T. tonsurans* ($n = 95$), *T. soudanense* ($n = 66$), *M. praecox* ($n = 59$) and *E. floccosum* ($n = 14$) Our data also reveal the predominance of anthropophilic species causing tinea capitis especially *M. audouinii* responsible for 36 49% ($n = 163/448$) of hair/scalp infection. *Trichophyton violaceum,* rarely observed in our country, is increasing in frequency these last years as 12.8% ($n = 57$) of the reported cases of tinea capitis are due to this species. The retrospective evaluation of data collected also shows that zoophilic strains as *M. canis* well represented in the past epidemiology of tinea capitis, is decreasing in frequency accounting for only 7.2% ($n = 32$) of clinical cases. Finally, our data confirm the high prevalence of *T. rubrum* commonly observed in Europe as causal agent of onychomycosis (70.9%, $n = 1603$) followed by *T. mentagrophytes complex* (20.9%, $n = 455$). *T. rubrum* and *T. mentagrophytes complex* are also responsible for the majority of skin infections as they represent respectively 40% ($n = 386$) and 24.75% ($n = 239$) of skin dermatophytosis during the study period.

Conclusions The present work has provided recent data on the prevalence of several dermatophytes species circulating in Belgium. Such data is critical for the establishment of therapeutic strategies and measures for prevention and control of dermatophytes infections. Our study confirms the predominance of *T. rubrum* followed by *T. mentagrophytes* in the Belgian population but also highlights the emergence of new anthropophilic species such as *M. audouinii* and *T. violaceum* as causative agents of tinea capitis in children, in relation with African immigration.

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Fungal contamination in one hotel room: Does carpet coating in the floor enhance fungal contamination?

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Objectives Several materials used indoors can contribute to enhance fungal contamination inside the building and taking this in consideration was developed a study intending to know the carpet influence when used in the floor of a hotel room.

Methods Twelve air samples of 250L (six in a room with carpet and six more in a room with wood floor) were collected through an impaction method with a flow rate of 140 L/min onto malt extract agar (MEA) supplemented with chloramphenicol (0.05%), using the Millipore air Tester (Millipore), during cleaning activities. Outdoor sample was also performed to be used as a reference. Surface samples from floor and desks, taken at the same time, were collected by the swabbing method. All the collected samples were incubated at 27°C for 5 to 7 days. After laboratory processing and incubation of the collected samples, quantitative (colony-forming units - CFU/m^3) results were obtained. Besides fungal contamination, we also assessed particulate matter contamination in both rooms during the same cleaning tasks. Two metrics were considered: particle mass concentration (PMC) - measured in 5 different sizes (PM0.5; PM1; PM2.5; PM5; PM10) - and particle number concentration (PNC) based on results given in six different diameters sizes, namely; 0.3 μm, 0.5 μm, 1 μm, 2.5 μm, 5 μm and 10 μm.

Results The air fungal load in the room with carpet ranged from 1 CFU.m-3 to 68 CFU.m-3 and in the room without carpet from 1 CFU.m-3 to 112 CFU.m-3. From both rooms, only one air sample of the one without carpet presented higher counts than the outdoors. Regarding surfaces, the room with carpet presented contamination in only one sample ($1x10^4$ CFU.m-2) and the room without carpet presented statistically significant differences from the carpeted room, with the first one having higher counts that ranged from $10x10^4$ CFU.m-2 to $115x10^4$ CFU.m-2. The most prevalent fungal genera were the same in the air of both rooms (*Penicillium* sp. 40.7% - 12.3% and *Cladosporium* sp. 43.5% - 55.4%). In the analyzed surfaces, isolates belonging to *Aspergillus fumigatus* complex were the only fungi found in the carpeted room, whereas in the other room we found *Penicllium* sp. (63.6%) and *Aspergillus* sp. (13.6%) as the most frequent genera. In the case of particles the room with carpet obtained significant higher values for both metrics (PMC and PNC), showing that carpet may has influence on particles' contamination of the room.

Conclusion Taking in account the obtained results, and contrarily to what was initially expected, carpeted floor does not seem to harbor higher fungal contamination. Nevertheless, when different particles parameters are analyzed, an increase in PMC and PNC was observed in room with carpet, compared to the room without carpet. More research had to be made to describe cleaning measures and characterize cleaning products since it seems that can have an influence in fungal contamination, besides carpet presence.

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Identification of *Aspergillus* cryptic species in hospital environment

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Objectives Invasive aspergillosis is a fungal infection caused by *Aspergillus* spp. affecting mainly immunocompromised patients. The mortality rate can reach 85%. *Aspergillus* identification should be based on molecular methods as there are species morphologically similar but distinct at the molecular level (cryptic species), with variable antifungal susceptibility profiles. Recent studies have shown that cryptic *Aspergillus* species can cause approximately 10% of the cases of invasive aspergillosis. Since *Aspergillus* infections in immunocompromised patients are mainly nosocomial, knowledge of the fungal epidemiology found in hospital environments would have an important role in controlling the development of aspergillosis. Therefore, selected hospital wards, housing patients at higher risk to develop invasive fungal infections, were screened in order to understand the epidemiology and distribution of *Aspergillus*, especially regarding the presence of cryptic species.
Methods During a 1-year period, four seasonal samplings, i.e., air and hard surface, were performed. A total of 101 air samples and 99 surface samples were collected from the Hematology, Oncology, and Intensive Care Unit (ICU) wards of a Portuguese Central Hospital. *Aspergillus* isolates were plated for growth as single colonies on malt extract agar with chloramphenicol to check the colony purity. These isolates were identified on the basis of microscopic morphology and through the use of molecular tools. Genomic DNA was prepared from each isolate and the sequencing of the Internal Transcribed Spacers (ITS) regions, specifically the ITS1 and ITS2 non-coding regions flanking the 5.8S rDNA was used to determine the species complex, whereas β-tubulin and calmodulin sequencing was done to achieve the correct species identification.
Results 548 environmental fungal isolates were obtained. Of these, *Aspergillus* was the most frequently isolated genus (19.7%) and from the total of *Aspergillus* isolates, 75 were screened for cryptic species detection. The remaining *Aspergillus* isolates were not speciated either because viability was lost, contaminants were impossible to eliminate or amplification remained unsuccessful. Six misidentifications at the species-complex level (based on morphology) were resolved by ITS sequencing. This methodology allowed the identification of ten different sections within the *Aspergillus* genus: *Versicolores* ($N = 20$), *Nigri* ($N = 11$), *Flavi* ($N = 10$), *Circumdati* ($N = 10$), *Fumigati* ($N = 8$), *Usti* ($N = 4$), *Terrei* ($N = 4$), *Nidulantes* ($N = 4$), *Aspergilli* ($N = 3$) and *Cremei* ($N = 1$). From those, 25 different *Aspergillus* species were identified by β-tubulin and calmodulin sequencing, and a high percentage of cryptic species (i.e., not *sensu stricto*) was found (59%). Sections *Usti*, *Versicolores* and *Circumdati* harbored the highest proportion of cryptic species [100% (4/4), 95% (19/20) and 90% (9/10), respectively].
Conclusion The high number of cryptic species found raises concerns about the possible reduced susceptibility to antifungals of hospital environmental *Aspergillus* isolates. These data reinforce the importance of hospital air and surface monitoring, mainly in immunocompromised patients' wards. The knowledge of the *Aspergillus* epidemiology in hospital settings and the use of routine susceptibility testing will allow the monitoring of the rate of resistance in environmental strains and its potential impact on initial antifungal choices and therapeutic outcome.

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Epidemiology of *Candida pelliculosa*, *Candida utilis* and *Candida fabianii* in the Czech Republic

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Objectives Clinical yeast isolates belonging to *Candida pelliculosa, Candida utilis* and *Candida fabianii* are difficult to differentiate in a routine mycology laboratory using standard commercial biochemical kits. During the past decade, the use of invasive procedures and administration of antimicrobial agents and new technologies such as bone marrow transplants or chemotherapy have resulted in an increase in the incidence of non-*albicans* infections such as these three species. The aims of this study were (1) to determine the prevalence of *C. pelliculosa*, *C. utilis* and *C. fabianii* in clinical samples collected from 10 Czech hospitals using the biochemical kit ID 32C (bioMérieux) and MALDI-TOF mass spectrometry (Bruker Daltonics) and (2) to compare their minimum inhibitory concentrations (MICs) for 9 antifungals from various aspects.
Methods Two hundred and fifty-seven clinical yeast isolates were included in this study. Type strains of *C. pelliculosa* (CBS 605), *C. utilis* (CBS 841) and *C. fabianii* (CBS 5481) were added as controls. The whole group was first identified using ID 32C and then by the MALDI-TOF MS system. Identification of each strain was repeated in triplicate by both methods. In case of questionable identification, a sequencing analysis was performed. MICs of the systemic antifungals amphotericin B, fluconazole, itraconazole, voriconazole, posaconazole, anidulafungin, micafungin, caspofungin and flucytosine were determined using the commercially available colorimetric broth dilution panels Sensititre YeastOne (TREK Diagnostic Systems). The results were compared with respect to patients' age, gender and site of infection and location of the hospital.
Results From a total number of 257 clinical isolates, 179 were biochemically identified as *C. pelliculosa*, 77 as *C. utilis*and 1 as *Williopsis saturnus*. The type strain of *C. fabianii* was determined as *C. pelliculosa.* Using MALDI-TOF MS confirmed with sequencing, 228 isolates were identified as *C. fabianii* (88.7%), 21 as *C. pelliculosa* (8.2%), 6 as *C. utilis* (2.3%) and 2 as *Ogataea polymorpha* (0.8%). The mean MICs ($\mu g\ ml^{-1}$) after 48 h were as follows: amphotericin B 0.77 (range, 0.12–2.0), anidulafungin 0.14 (0.015–2.0), micafungin 0.08 (0.008–1.0),caspofungin 1.17 (0.03–8.0), 5-flucytosine 7.61 (0.06–64.0),posaconazole 1.20 (0.03–8.0),voriconazole 0.21 (0.008–8.0), itraconazole 1.0 (0.03–16.0)and fluconazole 8.57 (0.5–256.0).The highest mean MICs were found in yeasts isolated from blood cultures and central venous catheters. No significant differences in MICs between genders were found.
Conclusion This study showed that, unlike routine biochemical identification, MALDI-TOF MS found *C. fabianii* to be most prevalent in clinical samples as compared with the other studied species. The absence of *C. fabianii* in databases of commonly used commercial biochemical kits for yeasts including ID 32C leads to misidentification of this species. In addition, some strains resistant to two or more antifungals were detected.
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Occupational exposure to fungi and particles in poultry and bovine slaughterhouses

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Objectives Dust released due to slaughterhouses activities is largely organic and can be contaminated with bacteria, fungi and endotoxin which may represent a health hazard upon inhalation. In addition several respiratory symptoms have been reported among the exposed workers. Taking this information in consideration a study was performed intending to assess the fungal contamination and particles exposure in poultry and bovine slaughterhouses.

Methods Air samples between 100 and 250 L were collected through an impaction method, while surface samples, taken at the same time, were collected by the swabbing method and subject to further macro and microscopic observations. Besides fungal contamination, we also assessed to exposure particles. Two metrics were considered: particle mass concentration (PMC) - measured in 5 different sizes (PM0.5; PM1; PM2.5; PM5; PM10) - and particle number concentration (PNC) based on results given in six different diameters sizes, namely; 0.3 μm, 0.5 μm, 1 μm, 2.5 μm, 5 μm and 10 μm.

Results Fungal quantification in the poultry slaughterhouse air ranged from 10 to 970 CFU.m-3 while in bovine slaughterhouse ranged from 10 to 36 CFU.m-3. Surfaces presented results that ranged from 0 to $10x10^4$ CFU.m-2 in the poultry slaughterhouse and from 0 to $90x10^4$ CFU.m-2 in the other slaughterhouse. In poultry slaughterhouse were detected eight species/genera in the air, being the most found *Scopulariopsis candida* (59.5%) and *Penicillium* sp. (32.8%). Only *Mucor* genera was isolated in surface. In the bovine slaughterhouse were found eight species/genera in the air being the most isolated *Penicillium* sp. (80.8%). Regarding surfaces, five different fungal species/genera were detected and the most prevalent were *Scopulariopsis brumpti* (40.0%) and *A. terreus* complex (30.0%).

It was possible to detect the task in each slaughterhouse that was responsible for higher particles exposure and with priority for preventive and protective actions: in poultry slaughterhouse (4.669 mg.m-3) was the handling of the live birds when hanging them in the line for slaughtering and in bovine slaughterhouse was the animal cutting without skin (0.456 mg.m-3). Results showed that exposure occurs in both units but with significantly higher values in poultry slaughterhouse ($P < 0.0001$).

Conclusion Air fungal load was higher in poultry slaughterhouse and this can represent higher risk since particle have an important role as a carrier of fungi and their metabolites for workers respiratory system. Due to species/genera identification we have to consider co-exposure not only to fungi and particles, but also to several mycotoxins, since species with toxigenic potential were isolated.

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Mucormycosis in a large Spanish hospital from 2007 to 2015: incidence, epidemiology, and etiological agents

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Objectives Mucormycosis, although sporadic, is a devastating disease with a purportedly increasing incidence and changing epidemiology. We reviewed all cases of mucormycosis diagnosed in patients admitted to our hospital during an 8.5 year-period in order to describe its incidence and epidemiology. We also obtained an accurate knowledge of the etiological agents by performing molecular identification and antifungal susceptibility testing with the inclusion of Ambisome in the panel of antifungal agents to study its activity against Mucorales for the first time.

Table 1

Species	No. isolates infection/colonisation	Geometric mean MIC and range (in μg/ml)				
		AmB	Ambisome	VOR	ITC	POS
Lichtheimia ramosa	13 / 8	0.50	0.07	16	1.26	0.22
Lichtheimia corymbifera	5 / 2	1.10	0.11	16	1	0.25
Rhizomucor pusillus	4 / 3	0.37	0.09	16	0.09	0.45
Mucor circinelloides	9 / 5	1.1	0.09	16	16	1.22
Cunninghamella bertholletiae	6 / 0	2	16	16	1	0.5
Rhizopus arrhizus	2 / 11	0.85	0.10	9.90	2.48	0.22
Rhizopus microsporus	0 / 5	0.76	0.66	1.15	8	0.33
Overall	73	0.77 (0.062-2)	0.15 (0.031->8)	14.00 (2->8)	2.14 (0.5->8)	0.37 (0.062-2)

Methods From January 2007 to May 2015, 17 patients had proven ($n = 10$) or probable ($n = 7$) mucormycosis according to the EORTC/MSG revised definitions. The available colonies morphologically identified as Mucorales were stored and revived to perform molecular identification and antifungal susceptibility testing to amphotericin B, Ambisome, itraconazole, and posaconazole by using CLSI M38-A2 methodology. The incidence of the infection during the study period was calculated and compared with that found in the same hospital from 1988 to 2006 (*M. Torres-Narbona. Med Mycol 2008, 46, 225–230*).

Results The patients with mucormycosis were mainly male (82%) and mostly admitted to onco/haematology (47%) or ICUs (35%); they had hematological malignancies (59%), or surgical/other invasive iatrogenic wounds (41%). In 8 patients the lower respiratory tract was affected and the remaining 9 patients had skin and soft tissue infections [3 and 4 patients with surgical or other iatrogenic wound infections, respectively]. Antifungal agents within the month previous to diagnosis were administered in 65% of patients, most of them without activity against Mucorales. Patients with proven mucormycosis had almost exclusively skin and superficial involvement whereas patients with probable mucormycosis had lower respiratory tract infection (microbiological findings and CT scan). The species found were *Lichtheimia ramosa* ($n = 4$), *Lichtheimia corymbifera* ($n = 3$), *Cunninghamella bertholletiae* ($n = 3$), *Rhizomucor pusillus* ($n = 2$), *Mucor circinelloides* ($n = 2$), *Rhizopus arrhizus* ($n = 2$), and *Saksenaea vasiformis* ($n = 1$). In patients in whom several isolates were studied only one species was found. Isolates from patients with mucormycosis or colonization ($n = 39/34$, respectively) were studied for antifungal susceptibility testing. Ambisome showed the highest activity followed by posaconazole, amphotericin B, and itraconazole (Table, $P < 0.001$). Ambisome was more active than amphotericin B for all species with the exception of *Cunningamella bertholletiae* ($P < 0.05$). All patients received antifungal treatment, mainly Ambisome (88%). In patients in whom surgery was part of the therapy (59%) mortality was significantly lower (20% vs. 68%, $P = 0.015$). The incidence of the infection, in cases per 100 000 hospital admissions, increased from 1.2 (1988–2006) to 3.7 (2007–2015) ($P = 0.004$).

Conclusions We detected an increase in the incidence of mucormycosis during the study period compared to that reported from 1988 to 2006. Only half of cased involved the respiratory tract and no cases of rhino cerebral mucormycosis were detected. None of the patients had diabetes as underlying condition and although hematological malignancy was the most common underlying disease, iatrogenic skin trauma was a major predisposing condition. *Lichtheimia* spp. and *Cunninghamella bertholletiae* were the main species involved. Ambisome was the agent showing the highest antifungal activity against Mucorales, supporting the need to start early treatment with agents showing anti-Mucoralean activity.

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FungiScope - Global Emerging Fungal Infection Registry

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Objectives Number of rare invasive fungal infection (IFI) are rising worldwide due to increasing patient population at risk. To broaden knowledge on epidemiology of emerging IFD, FungiScope a global registry has been initiated. Currently, partners from 60 countries contribute cases that eventually help determining clinical patterns, improve diagnostic procedures and therapeutic regimens.
Methods FungiScope uses web-based data capture accessible through www.fungiscope.net. For case enrollment, cultural, histological, antigen or molecular evidence on the occurrence of infection with non-endemic fungi is required. Data collected include demographics, underlying conditions, immunosuppressive medication, clinical signs and symptoms, sites of infection, diagnostic tests, pathogen identification, antifungal treatment and outcome. Clinical isolates are collected for centralized identification, molecular analyses and exchange between collaborators.
Results To date, 429 cases have been captured. Mucorales (n = 196; 45.7%), Fusarium spp. (n = 65; 15.2%), yeasts (n = 54; 12.6%), and dematiaceae (n = 48; 11.2%) are the most frequently registered pathogens. Chemotherapy (n = 195; 45.5%) and stem cell transplantation for hematological malignancy (n = 102; 23.8%) were the predominant risk factors, followed by intensive care (n = 95; 22.1%), diabetes mellitus (n = 84; 19.6%), and chronic renal disease (n = 35; 8.2%). For 20 cases (4.7%) no risk factor was identified. Major sites of infection included lung (n = 214; 49.9%), paranasal sinuses (n = 79; 18.4%), blood stream (n = 77; 17.9%), and deep soft tissue (n = 65; 15.2%). Disseminated infection (n = 72, 16.8%) was mostly associated with lung (n = 51, 70.8%), blood stream (n = 31, 43.1%) and CNS involvement (n = 24, 33.3%). For 212 (52.6%) patients, complete or partial response to treatment of IFD was documented. All-cause-mortality and mortality attributable to IFD was 45.2% and 34%, respectively.
Conclusion The clinical relevance of emerging IFD is increasing. FungiScope is a vividly expanding network that attained increasing interest throughout the years. In a short time period, a wide variety of cases has been collected that provide a comprehensive view on the epidemiology and clinical presentation of rare IFI.

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Presence of dermatophytes in infected pets and their household environment

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Dermatophytes are important fungi for public health because they are transmitted between animals and humans, causing zoonoses. These fungi have been reported in dogs, cats, guinea pigs and rabbits, among other pets. They can be isolated from animals with or without lesions, which represent sources of infection for other animals and humans. However, researches about their presence in homes where animals live with humans are still scarce. The purpose of this study was to diagnose dermatophytosis in pets with lesions clinically consistent with this infection, and investigate the presence of dermatophytes in their home environment. Samples from haircoat were collected from 54 pets of both genders and varying ages. Of these samples, 36 were from dogs, 15 from cats, 2 from guinea pigs and 1 from a rabbit. There were visited 20 homes of animals that had the disease confirmed by mycological culture. There were collected 160 samples in the household environments, selecting objects/sites with the help of owners. Many of these sites were of human-animal common use, but they were categorized as follows: 58 samples from sites that were of predominant use by the owners (sofas, chairs, beds, sheets); 48 samples from sites of predominant use by the animals (animals' houses, cribs, toys, feeders); 40 samples from general flooring (carpet, wood) and 14 samples from other animals that were in contact with the infected animals.Samples from the animals and the environment were seeded on *Mycosel* agar(BBL-BD) and incubated at 25° C for up to 4 weeks. Colonies were submitted to microculture technique and identified by their macro-and-microscopic characteristics. Dermatophytes were found in 37% (20/54) of the samples originated in sick animals. *Microsporum canis* was the most prevalent dermatophyte (17/20–85%) and it was isolated from dogs and cats; *Trichophyton quinckeanum* (2/20–10%) was isolated from guinea pigs, and *Microsporum gypseum* from a dog (1/20–5%). Dermatophytes were found in 65% (13/20) of the surveyed homes. Among the samples taken from household environments, 29% (47/160) were positive, with dermatophytes isolated in 24% (14/58) of the sites/objects of predominant use by owners, 37.5% (18/48) from sites/objects of prevalent animal use, 25% (10/40) from floors and 35%(5/14) from animals that had some contact with infected animals. The same species of dermatophytes previously detected in animals with lesions were isolated in homes: *M. canis*, *T. quinckeanum* and *M. gypseum*. Viable fungal spores were found in the home environment weeks after sick animals began treatment with antifungal drugs and presented healing of the lesions. Sick animals were sources of infection for other animals and humans, and it was possible verify intra- and inter-species cross-infection in the same residence. Animals that showed no lesions, but from which dermatophytes had been isolated, were considered asymptomatic carriers, and they also represent sources of infection, helping to spread the fungus in the household environment. Nowadays the contact between humans and pets is becoming more intimate, with greater sharing of the same space in a home, making dermatophytosis important in medical/veterinary clinics and in public health as a whole.

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A multiple gene genealogical strain typing approach for assessing the genetic diversity of a clinical collection of *Aspergillus flavus* and its antifungal drug susceptibility

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Objective To determine the genetic diversity and antifungal drug susceptibility of a clinical collection of *Aspergillus flavus*.
Methods Six housekeeping genes (Acetamidase, Polygalacturonase, *O*-Methyltransferase, b-Tubulin, Acetate regulation and Calmodulin) of 44 clinical strains of *A. flavus* were sequenced. Allele types (AT) were determined by alignment of individual loci of each strain in Clustal X2. AT was assigned on the basis of single nucleotide polymorphism found in each locus. Sequence types (ST) were assigned to each strain based on allele type profile. The multilocus sequence data was analysed in eBURST integrated in PHYLOViZ (Phylogenetic inference and data visualization for sequence based typing methods) and minimum spanning tree (MST) was constructed at single locus variant level (SLV-1). The Discriminatory Power (D) was calculated by employing Simpson's Index of Diversity. The antifungal susceptibility to polyene amphotericin B, itraconazole, voriconazole, posaconazole, caspofungin, micafungin and anidulafungin was determined by broth microdilution as per CLSI M38-A2 guideline.
Results All the isolates were from the single centre isolated form patients with AFRS, fungal ball, invasive FRS, CNS aspergillosis, pulmonary aspergillosis and keratitis. A total of 24 STs (A-X) were found in 44 strains included in the study. The discriminatory power

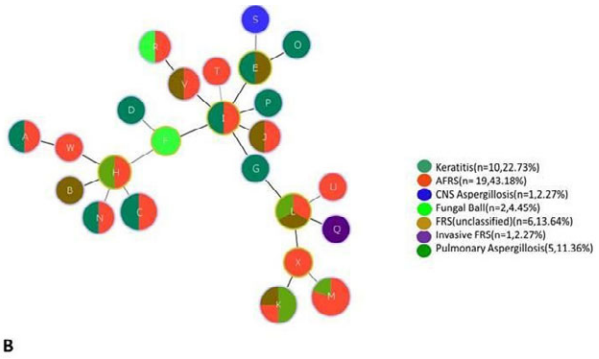

Figure 1. On the eBURST network,clinical source of the isolate is integrated with the genotypes

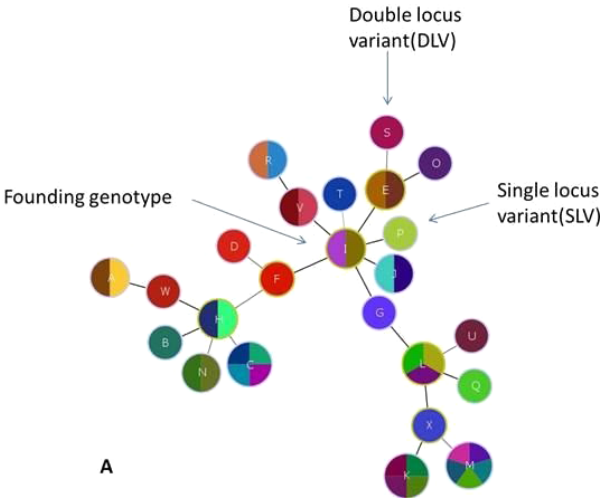

Figure 2. An eBURST tree drawn at the SLV level with lines connecting different MLST genotypes

of this typing method was 0.965. Sequence type I was assigned as the founder genotype. With the less stringent definition of 4/6 shared alleles, 3 clonal complexes could be differentiated in the eBURST diagram (Figure A) The most prevalent ST was the 'M' type which was found in 5 strains. Four of these isolates were from AFRS patients and one from pulmonary infection. However, no discernible difference between isolates on the basis of clinical manifestations was observed (Figure B). ST 'S' and 'Q' were the unique genotypes found only in invasive fungal rhinosinusitis and CNS aspergillosis, respectively.

For amphotericin B, most of the isolates exhibited elevated MICs of 8 μg/mL (4.08%) and 4 μg/mL (46.93%) with MIC_{50} and MIC_{90} of 2.0 $\mu g/ml^{-1}$ and 4.0 respectively. All the triazoles and echinocandins exhibited good in *vitro* activity against *Aspergillus flavus*. The MIC_{50}, MIC_{90} andgeometric mean MICs of itraconazole, voriconazole and posaconazole were 0.25, 0.5, 0.22; 0.5, 1.0, 0.31 and 0.12, 0.25, 0.12 $\mu g\ ml^{-1}$ respectively. The MEC_{50}, MEC_{90} andgeometric mean MECs of caspofungin, micafungin and anidulafungin were 0.06, 0.12, 0.06; 0.015, 0.02, 0.02 and 0.25, 0.25, 0.22 $\mu g\ ml^{-1}$ respectively.

Conclusion The present study is an attempt to develop multi locus sequencing approach to type *A. flavus* isolates. There was considerable genetic diversity in the clinical collection of *A. flavus* isolates. The technique is highly discriminatory for typing of *Aspergillus flavus and* can be applied for epidemiological investigations of outbreaks caused due to this agent. The high MIC of amphotericin to *A. flavus* should be considered before using this antifungal for treatment of infections due to *A. flavus*.

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Study of consumption of antifungal drugs in a tertiary hospital during 7 years

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Objectives Over the past years, the consumption of systemic antifungal agents has been increased worldwide resulting in developing antifungal drug resistance and causing a significant pharmaco economic burden. Antifungal drugs are widely used not only for treatment but for prevention (prophylaxis and empiric therapy) of fungal infections. The role of appropriate use of antifungal drugs is very important in this difficult time of economic crisis, in order to achieve the best cilinical results in conjuction with the best handling of the economic budget. The purpose of this study is the evaluation of the total cost of the antifungal treatment and the study of the progressive role of the usage of the antifungal drugs during the last 7 years in our tertiary hospital.

Methods Consumption data for systemic antifungal drugs were obtained through the hospital pharmacy from 2008 to 2014regarding all the medical, surgical wards, intensive care and transplantation units (931 beds). The consumption of the antifungal agents was estimated in accordance to the cost and the number of DDDs (Defined Daily Doses). The consumption of antifungal agents was also estimated during the period of 3 months. As well as the consumption and the cost in each ward independently (internal medicine, surgical, ongology, haematology, intensive care units) was calculated. We used the WHO-ATC/DDD Index 13 for the calculation of DDDs for antifungal drugs with systemic effect (ATC J02). The quantitation of results was performed in the level of the active substance of the drug and the conclusion was expressed in DDD/100 beds-days (DDDs/100BD).

Results According to the data, the finding results declared that the cost of antifungal treatment is almost the half of the total cost of the antimicrobial therapy and the 1/10 of the total pharmaceutical treatment in our tertiary hospital. The use of antifungal agents was increased during the last 7 years as these data were calculated with DDDs, even the decrease of the cost of the drugs (reduction of the price due to financial crisis). The most often used antifungal drugs were triazoles. There was not observed seasonal variation in antifungal drug therapy. The antifungal medication was greater in haematological wards and intensive care units.

Conclusion The presented study gives an update status of the antifungal consumption in a teriary Greek hospital during the past years. We report an increased utilization of antifungal drugs despite the reduction in the cost of these drugs. The presented pharmaco-epidemiological data are very important for the initiation and implementation of effective antifungal stewardship programmes, despite the variability of the prescribing drugs between the different medical wards. They might serve as important initiating information for other hospitals with similar structures in order to make practice the appropriate use of antifungal agents.

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Aspergillosis of the nose and paranasal sinuses: A review of 54 cases

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Introduction *Aspergillus* species are considered opportunistic fungi of increasing clinical importance. Information regarding extrapulmonary involvement is scarce.
Objective The aim of this study was to isolate the different species of *Aspergillus* in patients with rhinosinusitis.
Methods A retrospective study was conducted in a university hospital in Porto Alegre, Brazil (1986–2014). For mycological diagnoses, paranasal tissue obtained at surgery was subjected to histopathology examination and sent for fungal cultures.
Results Of the 54 samples analyzed, 34 the diagnosis was made by direct examination and culture and in 19 patients, the diagnosis was made exclusively by histology with the visualization of the *Aspergillus* conidiophore. In one patient, the diagnosis was by direct fluorescent antibody staining (*Aspergillus* and *Mucor*). The underlying causes of immunodeficiency were: six with transplantation (bone marrow, three; lung, two; kidney, one) and two with hematological disease (bone narrow neoplasia, one; leukemia, two). In the present study, the clinical manifestations of rhinosinusitis aspergillosis were: allergic, 20; fungus balls, 20; and acute invasive, 14. The strains isolated were: *Aspergillus fumigatus*, 14; *A. flavus*, six; *A. niger*, two; *A. terreus*, one; *A. fischeri*, one; and *Aspergillus* sp., three. Two concomitant species of *Aspergillus* were observed in two patients: *A. fumigatus* and *A. flavus*; and *A. fumigatus* and *A. niger*. In four patients, *Aspergillus* was associated with other fungi: *A. flavus* and *Fusarium*, one; *A. fumigatus* and *Rhyzopus, one; A. flavus* and *Mucorales, one; and Aspergillus sp. and Mucorales*, one. The most common strains of *Aspergillus* that are responsible for paranasal sinus infections are *A. fumigatus, A. flavus*, and *A. niger*. **Conclusions** Fungal infection of the nose and paranasal sinuses is rare, although it has been reported more frequently in recent years, it is important to report this vast series on the theme, highlighting the main clinical, etiological and diagnostics findings, to alert clinicians as this pathological condition.

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Fungal infections of eye and ear sites in referral patients to medical mycology lab of special clinic of Kermanshah University of medical sciences

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Objectives Keratomycoses and otomycoses have different risk factors. This research designed to study of epidemiological parameters of fungal infections of eye and ear in referral patients to Kermanshah medical mycology lab during 1993 - 2011.
Methods this research is a descriptive study on referral infected patients to medical mycology lab of special clinic of Kermanshah University of medical sciences. In these study epidemiological parameters such as age, sex, job, infected season, anatomical site of infection, habitant place and diseases in all infected fungal infections of eye and ear were collected.
Results in all admitted patients, 54 cases have mycoses in ear site, that more frequent risk are age group 0–9, student. And most isolated agents were Aspergillus and Dermatophytes.

38 persons were infected to mycoses of eye site that more frequent risk are 0 -9 age group, student and most isolated agents were Candid and Fusarium.
Conclusion Child age groups have most fungal infections of eye and ear canal sites. That care of eye and ear canal for prevention of illness for this age group strongly recommended.
Key words keratomycosis, otomycosis, Epidemiology, Kermanshah

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Accessing occupational exposure to fungi in a cork industry

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Objectives Different forms of fungal diseases affecting the nose and paranasal sinuses are recognized, including invasive and non-invasive fungal rhinosinusitis. *Penicillium glabrum* complex is associated with respiratory diseases such as suberosis, a typical disease of cork industry workers. In addition, *Chrysonilia sitophila* has been described as causing occupational asthma, associated to prolonged exposure to high counts of spores. In this study we aimed to access fungal exposure in workers from one cork industry through the mycological analysis of their nasal exudate and the environmental fungal contamination of their surroundings as well.
Methods Nasal mucous samples from 127 workers were taken with sterilized cotton swabs. Parallel samples were taken from one nostril. The swabs were rotated against the internal anterior walls of the nostril and then placed in the provided transport tube. The obtained swabs were then plated onto malt extract agar (MEA) supplemented with chloramphenicol (0.05%), and onto screening media to detect azole-resistant *Aspergillus* isolates. Regarding environmental sampling, collections for conventional-based culture studies, were made through the collection of 50–100 L air samples from 5 indoor sampling sites by the use of an impaction method. All the collected samples were incubated at 27 °C for 5 to 7 days and fungal obtained in positive samples were identified according their morphological characteristics. In addition to cultural methods, four environmental samples collected from 250L were used to specifically identify the *Penicillium glabrum* complex, by Real Time PCR.
Results Eighty workers (63.0%) presented contamination of their nose nostrilwith *Chrysonilia sitophila*, which number of colonies was countless. *Talaromyces* sp. was another species that also presented a countless number of colonies in 3 of the workers. The third most frequently found species/genus with very high colony forming units was *Penicillium* sp. (42.7%). Within the *Aspergillus* genus, the complexes *Fumigati*, *Circumdati*, *Versicolores* and *Candidi* were isolated. No azole-resistant *Aspergillus* isolates grew in the selective media used (screened itraconazole and voriconazole resistance).

Regarding the environmental results obtained by culture-based methods, all samples also showed countless *Chrysonilia sitophila* colonies. DNA from the *Penicillium glabrum* complex was detected in three out of the four samples.
Conclusion The fungal species identified in the collected nose swabs were shown to be correlated with the results obtained in the environment. This approach allowed us to estimate the risk associated with these tasks performance. Moreover, the cork industry is related to high dust contamination and this can promote exposure to fungi since dust particles can act as carriers of fungi to the worker's nose. Assessment by molecular tools will ensure the specific targeting of DNA from *P. glabrum* complex in worker's nose.

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Candida lusitaniae fungemia: report of three cases

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Introduction *Candida lusitaniae* is an uncommon cause of fungemia, frequently resistant to amphotericin B, accounting for about 1% of all candidemia cases, with the majority of them being documented in patients with hematological malignancies. Three cases of *Candida lusitaniae* fungemia in non hematological patients are described.
Cases presentation *Candida lusitaniae* fungemia occurred in three patients (a 22 year-old male, a 57 year-old female and a 14 week-old infant). The male had been operated due to severe traumatic brain injury due to traffic accident and was hospitalized for more than a year, when *C.lusitaniae* was isolated from the blood. The female had been operated due to uterine sarcoma (not on chemotherapy) and was admitted to the Intensive Care Unit (ICU) due to hemorrhagic shock, while *C.lusitaniae* was isolated from blood 3 days later. The infant was admitted to the pediatric ICU due to septic shock. All three had fever, central venous catheters, were intubated, receiving total parenteral alimentation and on prolonged antimicrobial treatment. After the *C.lusitaniae* isolation all patients received micafungin (150 mg od for adults and 10 mg od for the infant) for 14 days the infant, 20 the male and 10 the female. The latter died due to hemorrhagic shock, while the two others were successfully treated and the fungus disappeared from their blood and did not recur during a long follow-up. Catheters were removed. All strains were susceptible to amphotericin B, 5- fluorocytosine, fluconazole, voriconazole and echinocandins.
Conclusion Although rare, *C. lusitaniae* is an important pathogen, even in non strictly immunocompromised but "susceptible"patients. Surgery, central venous catheters, parenteral alimentation, mechanical ventilation, ICU stay and prolonged antimicrobial therapy must increase the suspicion index for such a fungemia.

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Mycotic Keratitis by *Acremonium* spp - Case Report

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Fungal keratitis is the cornea infection with suppurative and ulcerative aspect. This can be a severe disease and difficult to be treated. More than 60 species of fungi have been recognized like keratitis agents depending on the geographic region. *Acremonium* is an uncommon cause of cornea infections. A male patient, agriculturist, with prior cornea transplantation went to a doctor because of pain and redness in the ocular region. He had an ulcerative lesion that improved even after the first medical treatment, thus cornea re-transplantation was necessary. The result of material culture was positive for *Acremonium spp*. It chosed to systemic therapy with Ketoconazole and maintenance of primary topical therapy with Amphotericin B for 2 months. Few cases were related on literature and most of them occurred after procedures like Laser *in situ* keratomileusis (LASIK). Fungal keratitis can be caused by filamentous fungi and yeast fungi and each of these needs a specific treatment based on microbiology analysis. The disease outcome depends of effective antifungals medicine.

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Malassezia japonica isolated from cutaneous microbiome of free-living Golden-headed lion tamarins (*Leontopithecus chrysomelas*)

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Malassezia sp is yeast found in the skin microbiome of domestic animals and humans. However, when an imbalance with the host occurs it can cause infections, particularly otitis and dermatitis. Data about the presence of these yeasts in wildlife is still scarce, regarding both its role in the microbiome as well as in infectious processes. The golden-headed lion tamarin (GHLT - *Leontopithecus chrysomelas*) is a species considered *EN-Endangered* by the *Red List of Threatened Species-World Conservation Union* (IUCN), and appears on the National List of Endangered Brazilian Fauna Species. A free-living exotic population of this species, which was introduced in a State Park in Rio de Janeiro, Brazil, was restrained and translocated to another area, in Bahia, where the species is endemic. The purpose of this study was to investigate *Malassezia* spp. in the external ear canal and haircoat of GHLT, before the translocation, since there is no research about the presence of these yeasts in such a broad sampling of non-human primates. There were chemically restrained (ketamine-10 mg kg^{-1} + midazolam-0.3 mg kg^{-1}) 199 animals: 102 males (51.3%) and 97 females (48.7%), 77 juveniles (38.7%) and 122 adults (61.3%). There were collected 597 clinical samples: 398 of cerumen (66.7%) and 199 of haircoat (33.3%). Otic samples were obtained by the introduction of sterile swabs in the external ear canals (right and left) and from skin surface by rubbing sterile carpets on the haircoat. Samples were seeded on Sabouraud dextrose and Dixon agar; plates were incubated at 32° C for up to 2 weeks. For molecular analysis, RFLP was carried out after DNA was extracted and 26S rDNA was amplified through PCR technique. Since all strains presented the same pattern on RFLP, four strains were randomly selected and their PCR products were purified and submitted to bidirectional sequencing. The sequences obtained were compared to mycobank.org. *Malassezia* sp was isolated from 76 animals (38.2%) and 87 samples (14.6%), with 26 from the cerumen (6.5%) and 61 from the haircoat (30.7% - statistically superior). *Malassezia* sp was isolated from 43.1% (44/102) and 33.0% (32/97) of males and females, and from 44.2% (34/77) and 34.4% (42/122) of juvenile and adult animals, respectively, and there were no statistic differences related to gender and age. All of the 87 isolates showed lipodependency. In PCR-RFLP techniques all strains showed the same pattern of bands. Four strains submitted to sequencing provided 510pb large subunit (26S) ribosomal partial sequences that were 99.8% to 100% similar to *Malassezia japonica*. Results confirmed that lipodependent *Malassezia* is part of the skin microbiome of these animals. However, it is important to notice that *M. pachydermatis*, which is considered the animals' species and the only non-lipodependent has not been isolated here. *Malassezia japonica* has been documented in humans but not in animals, until today. Perhaps because of a closer phylogenetic proximity, the fungal skin microbiome of these primates would be more similar to that of humans than other domestic or wild mammals. It does not exclude the possibility of being different species.

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Cases of dermatomycoses in seniors Open University students of Universidade Estadual de Maringá

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Dermatomycoses are fungal infections which affect the skin, hair, and nails. Epidemiological studies have showed that these dermatomycoses are the most common dermatological infections, which affect around 20% of the world's population. Immunosuppression, diabetes and advanced age may increase the risk for developing these infections that usually require extended treatments with high rate of relapse and/or reinfection. The Open University '*Universidade Aberta para a terceira idade*' (UNATI) is part of The Universidade Estadual de Maringá (UEM) that provides to senior citizens higher education opportunities. Thus, the aim of this study is related with a social project that evaluated the occurrence of dermatomycosis in senior citizens enrolled at the UNATI/UEM and performed antifungal susceptibility testing of fungal isolated from these elderly. The students with suspected dermatomycosis were referred to the Teaching and Research Laboratory of Clinical Analysis (LEPAC), Division of Mycology, UEM. The inclusion of elderly followed the rules of the Research Ethics Committee of UEM and approved (no. 615.643/2014). Fungi were isolated from skin and nail samples, using the method of scraping and skin peeling and nail. The samples were clarified with 40% potassium hydroxide (KOH) plus Evans blue and were cultivated in Sabouraud Dextrose Agar dextrose agar and Mycosel. The fungi identification was performed using classic methods, including the macro and micromorphology analysis. The isolates were maintained in a freeze-dried state in the Mycology Collection of the LEPAC/UEM. In vitro antifungal susceptibility testing was performed according to Clinical and Laboratory Standards Institute, document M27-A3 and M38-A2 to yeast and filamentous fungi, respectively. The antifungal used were fluconazole, itraconazole, voriconazole, amphotericin B and micafungin. A total of 19 senior citizens' UNATI/UEM with suspect of dermatomycosis was observed, 12 were women and seven were men, with mean age of 67 years old. It was collected 28 samples of these elderly (71.43% from skin and 28.57% from nail), 53.57% of these were positive for direct mycological examination and 28.57% for culture. Fungus isolated were *Candida parapsilosis*, *Candida tropicalis* and *Trichophyton rubrum*. Additionally, for all antifungals tested in this study showed good activity against these etiologic agents. Despite the limited number of positive samples for dermatomycosis, this group of elderly, there was a higher occurrence of yeast than dermatophyte fungi.

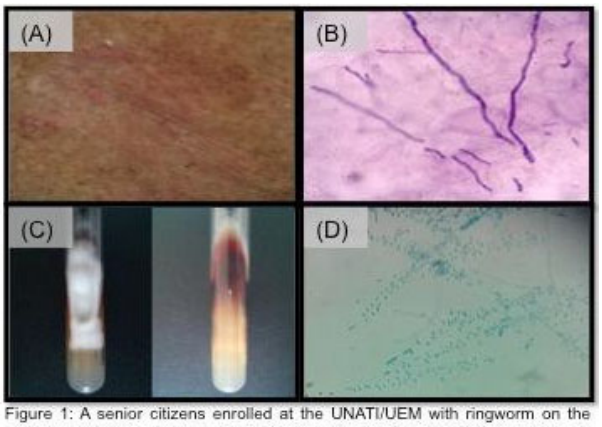

Figure 1: A senior citizens enrolled at the UNATI/UEM with ringworm on the arm due to *Trichophyton rubrum*. A) Superficial infection with bilateral scaly patches on the skin; B) Direct mycology examination of the skin showing hyaline-branched septate fungal hyphae and a few conidia (Potassium hydroxide, ×40); C) Culture on Sabouraud's dextrose agar yielded heaped and glaborous, pink colored colonies of Trichophyton rubrum within 4–5 days; D) Lactophenol cotton blue mount showed septate hyphae with typical pencil-shaped macroconidia.

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Introduction of an *Aspergillus* PCR assay to the clinical mycology service in Iran

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Objectives *Aspergillus* species are most abundant and widely distributed in soil, water, air, seed and food. These species are associated with allergic bronchopulmonary disease, mycotic keratitis, otomycosis, nasal sinusitis and invasive infection. In this study we developed a PCR-Single Strand Conformational Polymorphism method to identify the most common *Aspergillus* species.
Methods Our subjects included *Aspergillus* clinical isolates of an educational hospital in Urmia, Iran, also some *Aspergillus* standard species which obtained from Japanese Collection of Microorganisms. All *Aspergillus* isolates were identified by using the morphological (colonies and microscopic) features. For the molecular identification, the ITS2 region of rDNA gene (approximate length size: 330 bp) was amplified in PCR. The PCR product was incubated at 95°C for 5 min and then moved quickly into ice bath for an immediately quenching. A vertical electrophoresis with 6%-12% Gradient Poly Acrylamide Gel was used full time cooling at 4°C.
Results As our result, some of tested Aspergillus species including *A. nidulans*, *A. fisheri*, *A. fumigatus* and *A. niger* discriminated. SSCP assay enabled us to identify above *Aspergillus* species within 8–12 h after overnight incubation.
Conclusion It is concluded that Single Strand Conformational Polymorphism is a simple and rapid method for identification of some medically important Aspergillus but we recommend this as a compliment test with other molecular methods such as PCR-restriction fragment length polymorphism to cover identification of more Aspergillus species.

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Epidemiological characters of Tinea barbae among admitted patients of medical mycology lab of Kermanshah University of medical sciences 1994 -2011

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Background and objectives superficial mycoses have different risk factors. This research designed to study the epidemiological parameters of Tinea barbae among admitted patients of medical mycology lab of Kermanshah University of medical sciences 1994 -2011
Material and methods This research is a descriptive study on referral infected patients to medical mycology lab of special clinic of Kermanshah University of medical sciences. In this study epidemiological parameters such as age, sex, job, and infected season, anatomical site of infection, habitant place and diseases in all infected fungal infections of face were collected.
Results in 2290 admitted patients, 780(34%) case have dermatophytosis, that more frequent age group are 20–29, and student are most infected group, T. verrucosum more frequent agents of dermatophytosis.

Conclusion In this disease active age group, males, and hot month has frequent infections. So skin care for prevention of disease most recommended.
Key word Tinea barbae, Epidemiology, Kermanshah

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Dermatophytosis in Iran: clinical, molecular characterization and in vitro antifungal susceptibility of dermatophytes isolates

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Objective Dermatophytosis is a common mycotic infection of skin, nail and hair caused by dermatophytes belong to three genera, i.e., *Trichophyton*, *Microsporum* and *Epidermophyton*, associated with major public health concern worldwide. Various species of dermatophytes show significant differences in susceptibility to antifungals. In the present study we aimed the epidemiology of dermatophytosis and antifungal susceptibility of the largest series of molecularly identified dermatophyte isolates from tropical regions of South of Iran.
Methods A total of 9485 clinically suspected patients to dermatophytosis from three southern provinces of Iran during October 2012 until April 2014 were involved in the study. The scraped skin samples from all patients were evaluated with potassium hydroxide examination and culture on Sabouraud's glucose agar supplemented with chloramphenicol and cycloheximide. Restriction fragment length polymorphism (RFLP) analysis and sequencing of the ribosomal DNA (rDNA) Internal Transcribed Spacer (ITS) regions was performed to differentiate dermatophyte isolates. *In vitro* antifungal susceptibility tests were carried out according to the Clinical and Laboratory Standards Institute (CLSI) M-38A2.
Results The prevalence of dermatophytosis was 11.7%. The most common dermatophyte infections was tinea corporis (32%) followed by tinea pedis (21.2%) and tinea cruris (16.1%). Three hundred and sixteen dermatophyte isolates originating from patients with dermatophytosis. *Trichophyton interdigitale* was the most prevalent species ($n = 156$) followed by *T. rubrum* ($n = 60$), *Epidermophyton floccosum* ($n = 42$), *Microsporum canis* ($n = 29$), *Arthroderma benhamiae* ($n = 17$) and *T. tonsurans* ($n = 12$). In general, the MIC range of terbinafine (0.002 to 0.25 μg ml^{-1}) was the most potent antifungal followed by itraconazole (0.004 to 0.5 μg ml^{-1}), griseofulvin (0.125 to 8 μg ml^{-1}) and fluconazole (4 to 128 μg ml^{-1}).
Conclusion Our results in comparison to previous reports in Iran have revealed a different epidemiological trends for *Arthroderma benhamiae*, which showedthe lowest susceptibility toterbinafine.

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Evaluation of Epidemiological and diagnostic features of dermatophytosis in Selcuk University Hospital in 3 years' time

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Objectives To determine the incidence of dermatophytosis based on clinical conditions and identification of fungus by direct microscopy and culture methods.

Methods A retrospective study was conducted on samples examined in Microbiology Department of Selcuk University Faculty of Medicine in Konya/Turkey in 3 years' time. Skin scrapings and hair and nail samples were taken from clinically dermatophytosis suspected patients. The samples were subjected to potassium hydroxide (KOH) examination (direct microscopy) and were cultured in Sabouraud's dextrose and Mycobiotic agar. The isolates were identified by conventional methods.
Results During 3 years, among 3510 clinically suspected patients 1085 (31.0%) 633 (58.3%) male and 452 female (41.7%) were confirmed by direct examination and/or fungal culture. The most common clinical type of infection was tinea pedis (47.6%), followed by tinea unguium in feet (30.0%) and in hands (4.7%), tinea corporis (8.4%), tinea inguinalis (4.5%), tinea manum (4.1%), tinea capitis (0.3%).

Fungal elements were examined in 985 (28%) of the 3510 direct examination-ordered samples. Fungal culture was applied to 2060 samples and the dermatophytes were isolated from 321 (15.5%). Of the 321 culture positive samples fungal elements were examined in 221 (10.7%) by direct examination. On the other hand, in 109 (5.2%) samples fungal elements was observed but culture was negative. The most common dermatophyte was *Trichophyton rubrum* (84.5%), followed by *Trichophyton mentagrophytes* (12.7%), *Trichophyton tonsurans* (2.5%), and *Trichophyton terrestre* (0.3%).
Conclusion In our area tinea pedis was the predominant clinical type and *T. rubrum* was the most common determined dermatophyte. It is important clinically and epidemiologically to determine incidence, etiological agents and clinical types of dermatophytosis. This study also shows the need of performing both a direct examination and culture to improve sensitivity.

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Candidaemia in a Tertiary Referral London Hospital over an eighteen year period

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Objectives Candidaemia remains the commonest fungal infection worldwide and it is still one of the main causes of mortality and morbidity of all types of patients.We retrospectively reviewed all yeasts isolated from blood cultures in order to ascertain the prevalence of species and their susceptibility to antifungals, the choice of antifungal treatment and mortality rates, to compare the distribution in adults and children and identify the medical specialties with the highest rate.
Methods The medical notes of patients with candidaemia in our hospital between 1996 and 2013 were reviewed. We recorded patients' demographics and clinical details such as parental nutrition, long line *in situ*, immune status, treatment and outcome. Candida isolates were identified by germ tube test and API or MALTI TOF in the last 2 years. Susceptibility testing using the CLSI method started with five and finished with nine antifungals.
Results A total of 234 patients with 240 episodes, 157 (17–93 years) adults and 77 (1 day to 15 years) children were included. There was an even distribution for adults whereas there were 12% more male than female cases among children. The specialties rank order for adults was Intensive Care 35.7%, Surgery 27.4%, Internal Medicine 14.6% and HIV Medicine 12.1%, whereas for children Neonatal Intensive Care 71.4%, Surgery 11.7% and General Paediatrics including Oncology 11.7%. In adults *Candida albicans* accounted for 60.5%, *C. glabrata* for 19.1% and *C. parapsilosis* for 9.6% whereas for children *C. albicans* was 62.3% followed by *C. parapsilosis* 26% and *C glabrata* 6.5%. There were no isolates resistant to amphotericin B or any of the three echinocandins, anidulafungin, caspofungin and micafungin. Only *C. glabrata* and *C. krusei* were resistant to the four triazoles, fluconazole, itraconazole, posaconazole and voriconazole. Elevated MICs of the three echinocandins against

all isolates of *C. parapsilosis*, *C. lusitaniae* and *C. guilliermondii* were noticed. Flucytosine was very active against all isolates and in particular *C. glabrata*. Liposomal amphotericin was the most frequent drug used followed by fluconazole and caspofungin and the overall crude mortality in adults was found to be 27.3% and in children 21%.

Discussion The incidence of candidaemia in our hospital was found to be relatively low when compared with similar centres. The species and their sensitivity also were found to be similar to previously published data with *C. albicans* being the most frequent isolate followed by *C glabrata* in adults and *C. parapsilosis* in children. The overall mortality rate is much lower to that published by many institutions and this might be partly due to the prompt communication of results by the Medical Microbiologist, as direct germ tube was ready in less than 2 h after the blood culture became positive, to the bedside clinicians which ensured prompt and appropriate treatment. The influence of parameters, such as removal of lines on mortality rates, predisposing factors and attributable mortality will also be presented in detail.

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The evaluation of the relevance of antifungal chemotherapy prescription in *Candida* spp bloodstream infections in a French University Hospital

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Objectives *Candida spp* bloodstream infections are frequent and dreaded complications in patients' care. Anitfungal chemotherapy is very expensive and local guidelines have been put forward to guide their prescriptions particularly echinocandins in fever-driven, diagnosis-driven and targeted treatment approaches. The search for deep tissue infection and organ involvement is of the upmost importance and collaboration between the clinician and the microbiologist is crucial. We have investigated the prescriptions with an antifungal stewardship perspective.

Methods A retrospective single centre study between 2013 and 2014 was carried out. Each prescription is re assessed as per the local guidelines and those of IDSA 2009. **Inclusion criteria**: the positivity of at least one blood culture *and/or* another presumed sterile sample culture for *Candida spp.* **Exclusion criteria**: negative peripheral *and* simultaneous positive deep central catheters' blood cultures (colonization) *and/or* positive peritoneal liquid cultures after gut perforation.

Results 76 patients were included: 38 patients from ICU, 19 surgical, 13 medical, 3 oncology and 3 gynaecology. 8 were treated for solid cancer, 18 were in septic shock and 19 had deep tissue infections (endocarditis, pleuropneumonitis, peritonitis, mediastinitis, febrile neutropenia). 59 (78%) presented multiple organ failure (MOF). Fever-driven, diagnosis-driven and targeted treatment approaches were done in 12 (16%), 15 (21%), 38 (52%) respectively. Twelve (16%) had no antifungal chemotherapy. Biological samples included 64 (74%) blood, 3 deep pus (4%), 1 graft conservation medium, 7 (8%) pleural, 5 (6%) peritoneal, 4 (5%) ascites, 1 (1%) amniotic fluid, 1 (1%) native mitral valve, 1 (1%) prosthetic aortic valve and 1 (1%) lymphatic node smear cultures. *Candida albicans*, *C. glabrata* and C.

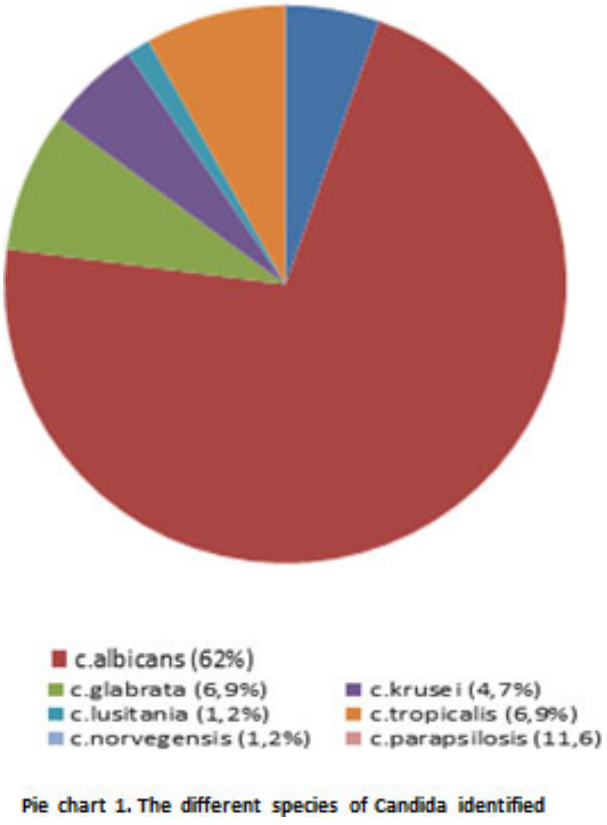

Figure 1

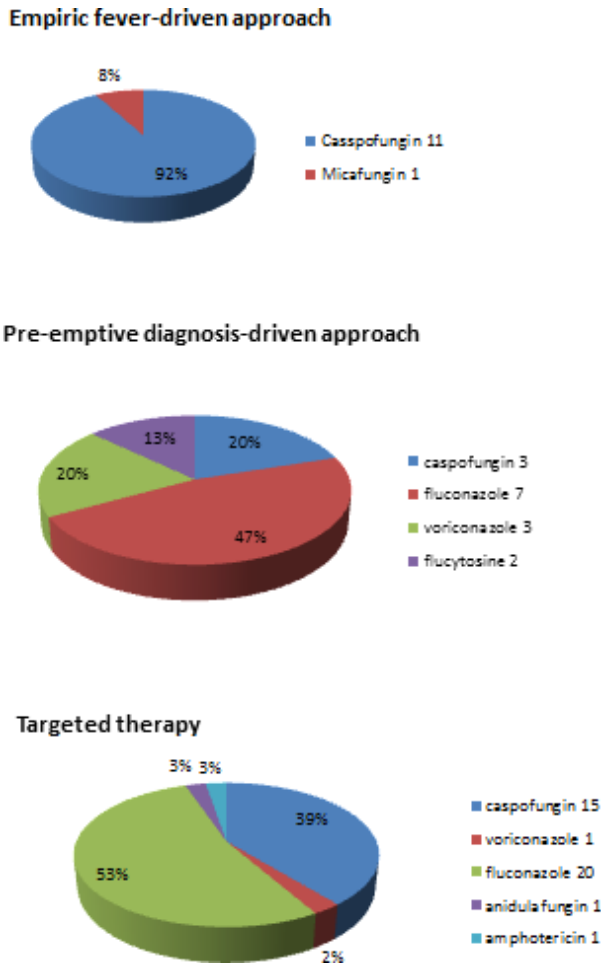

Figure 2

tropicalis were found in 62%, 7% and 7% respectively. Caspofungin is used in the front line in 26 cases of MOF of which 10 septic shocks, 2, 3 and 4 cases of endocarditis, pneumonitis and peritonitis repectively. Decrementation to oral fluconazole is observed in 19 (26%) patients where the fungal agent is susceptible (86%) and whose clinical status improved (50%). **The accordance with the guidelines is 19.2%.** Transoesophageal ultrasonics, fundus oculi and repeated skin examination are done in only 41 (56%), 13 (18%) and 19 (26%) cases respectively. The global death rate is 38%; it is about 55%, 50%, 45% and 17% among patients having bloodstream infections with *C. parapsilosis*, *C. glabrata* and *C. krusei* taken together, *C.albicans* and *C. tropicalis* respectively.

Conclusion This study confirms the extremely serious burden of invasive bloodstream *Candida spp* infections. The accordance with the guidelines is weak, not only for the search of deep-tissue involvement but also for the decrementation to oral fluconazole. Important taskforces have to be implemented on the training and guidelines' issuance among clinicians and the distribution of antifungal chemotherapy using a computerized questionnaire, duly filled by the clinician and the hospital pharmacist. The right time for oral fluconazole decrementation, the removal of deep central catheters and the search for deep tissue involvement must be done systematically. Furthermore, such actions will help to preserve the public hospital's fungal ecology, not to mention making substantial savings.

P252

Pulmonary fungal infections among outpatients in medical Mycology Laboratory, of Kermanshah University of Medical Sciences special clinic 1993- 2012

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Objectives Systemic fungal infections in immunocompromised patients have a high prevalence. the goal of this study was to determine the epidemiological characteristics of pulmonary fungal infections in client's patients' of mycology laboratory clinic of Kermanshah University of Medical Sciences.

Methods In a study using data recorded in the archives of the University Mycology Laboratory during 1993 to 2012, the data were extracted and analyzed.

Results Total admitted patients of Mycology Laboratory; Kermanshah University of Medical Sciences with pulmonary clinic during, 1993 to 2012 was 297 persons. 145 cases with diagnostic techniques: Direct smear or Culture on selective media has been positive. Lung and pulmonary Aspergillosis 8 cases, Candidiasis 35 cases and Pnumosistosis have been detected in two cases.Candida albicans and Aspergillus fumigatus more common agent were isolated in this study.

Conclusion Prevalence of fungal disease in patient's mycology laboratories in this study is of high importance. Definitely check fungal infections in specific groups eligible risk associated with a higher frequency of fungal diseases. The matter is further suspected cases of high importance.

Key words
Candidiasis, Aspergilosis, Pnumosistosis, Epidemiology, Kermanshah

P253

Clinical and epidemiological aspects of the largest epidemic of sporotrichosis in dogs: 203 cases (2004–2014)

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Sporotrichosis is a subcutaneous mycosis caused by the *Sporothrix* complex, which is rarely described in dogs. However, an epidemic of this mycosis has been occurring since 1998 in the metropolitan region of Rio de Janeiro, Brazil, affecting humans, dogs and cats. The Evandro Chagas National Institute of Infectious Diseases (INI)/ Oswaldo Cruz Foundation (Fiocruz) is a referral center for fungal diseases in Rio de Janeiro. In this institution, more than 4000 human cases and 4000 feline cases of sporotrichosis were diagnosed between 1998 and 2012. Usually canine sporotrichosis is described in hunting dogs, which acquire the infection through the classic transmission resulting from the traumatic implantation of the fungus from soil or contaminated plant material. In the epidemic in Rio de Janeiro, the transmission to dogs has been occurring through scratches, bites or contact with exudates from cutaneous lesions of sick cats, which are the main source of infection of the fungus.

Objective The purpose of this study was to describe the clinical and epidemiological aspects of the largest series to date of canine sporotrichosis.

Methods The study included dogs with isolation of *Sporothrix* spp. in fungal culture and followed up at the INI/Fiocruz, between 2004 and 2014. Retrospective data were collected from the medical records of the animals (sex, breed, age, contact with cats, origin, clinical signs, treatment regimens and outcomes).

Results Two hundred and three dogs were diagnosed with sporotrichosis in the period of the study. Among the positive dogs, 118 were males ($N = 201$; 58.7%) and 86 were mongrel dogs ($N = 193$; 44.6%). The median age was 48 months ($N = 185$) and 148 ($N = 189$; 78.3%) dogs had contact with cats. Regarding the origin, 117 dogs ($N = 200$; 58.5%) were from Rio de Janeiro city. At the first clinical examination 165 dogs ($N = 194$; 85.1%) were in good condition. Respiratory signs were observed in 134 animals ($N = 185$; 72.4%) and lymphadenopathy in 72 ($N = 158$; 36.4%).

These dogs have received different treatment regimens using ketoconazole, itraconazole or potassium iodide. The antifungal prescription varied according to the clinical picture and the availability of the drug provided free of charge by INI/Fiocruz. To date, the treatment outcome of 97 dogs was described. Seventy-six (78.4%) achieved clinical cure, eight (8.3%) died of causes unrelated to sporotrichosis and one (1.0%) was euthanized due to the severity of clinical signs.

Conclusions To date, the study with the largest number of dogs with sporotrichosis was performed by Schubach et al. (2006), which evaluated 44 dogs diagnosed at INI/Fiocruz from 1998 to 2003. In our study, 203 dogs were diagnosed over a period of 10 years, demonstrating the increasing number of cases due to the epidemic. To conclude, sporotrichosis persists as a neglected disease in Rio de Janeiro, especially in animals.

P260

Association of lectin pathway proteins with intra-abdominal *Candida* infection in high-risk surgical intensive-care unit patients. A prospective cohort study

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Objectives Intra-abdominal candidiasis (IAC) is a life-threatening complication of gastrointestinal (GI) tract perforation or acute necrotizing pancreatitis. Mannose-binding lectin (MBL) has been shown to bind to *Candida albicans* leading to enhanced complement activation and augmented opsonisation *in vitro*. However, human studies on the role of MBL in patients with invasive candidiasis have yielded conflicting results. We investigated the influence of MBL and other lectin pathway proteins on *Candida* colonization and IAC in a cohort of high-risk intensive-care unit (ICU) patients.
Methods Prospective observational cohort study (Fungal Infection Network of Switzerland) of 89 patients with recurrent GI perforation or acute necrotizing pancreatitis. Levels of lectin pathway proteins at study entry and six *MBL2* single-nucleotide polymorphisms (SNP) were analysed by ELISA and genotyping (Illumina Veracode genotyping platform or KASP[TRADEMARK] system, LGC Genomics), respectively, and correlated with 1,3-β-D-glucan levels, development of heavy *Candida* colonization (corrected colonization index (CCI) >0.4), occurrence of IAC, and mortality during a 4-week period. Patients with pre-emptive antifungal therapy were excluded from the analysis of IAC.
Results Within 4 weeks after inclusion a CCI>0.4 and IAC was observed in 42/89 (47%) and 27/69 (39%) patients, respectively, and 30-day mortality was 8%. Neither serum levels of MBL, ficolin-1,-2,-3 or MASP2 nor *MBL2* genotypes or haplotypes were associated with elevated 1,3-β-D-glucan levels, development of heavy *Candida* colonization or 30-day mortality ($P > 0.05$ for all analyses). Similarly, none of the analysed proteins was found to be significantly associated with IAC with the exception of lower MBL levels (median 0.87 (IQR 0.21–1.93) vs. 1.90 (IQR 0.19–3.44), $P = 0.03$) at study entry. However, there was no difference in MBL deficiency (<0.5 μg ml^{-1}), *MBL2* haplo- or genotypes in patients with and without IAC.
Conclusion Lectin pathway protein levels and *MBL2* SNPs investigated in this study were not associated with heavy *Candida* colonization or IAC in a small cohort of high-risk ICU patients. The lectin pathway of complement appears to have limited influence on the susceptibility to invasive *Candida* infections in high-risk surgical ICU patients.

P261

Investigation of Candiduria Cases in Intensive Care Unit: a 5-year Observational Study

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Objectives While most of the urinary infections are of bacterial origin, the incidence of fungal pathogens in urine cultures has been reported to be nearly 12%. The most common fungal pathogen isolated from urine cultures was *Candida* spp. In this study, we aimed to investigate the incidence and risk factors of candiduria in intensive care units (ICU).
Methods All patients hospitalized in one of the ICUs of our hospital for more than 3 days within 5 years period were included in the study. Candiduria was defined as isolation of *Candida* species from urine cultures of patients. Demographic and clinical data including age, gender, underlying illnesses, severity of illness and all invasive procedures applied were collected from medical reports of all patients. All statistical analyses were performed by using Statistical Package for the Social Sciences (SPSS, Version 15.0). For the variables that had significance in the chi-square tests, logistic regression analysis was applied. P of <0.05 were considered as statistically significant.
Results In 5 years period, 161 candiduria cases were identified among 1076 cases (15%). Of the cases 64 (39.8%) were male and 97 (60.2%) were female. Mean age was 59.98 ± 22.52. Most common *Candida* species isolated from urinary cultures was *Candida albicans* (103 cases-64%) followed by *Candida glabrata* (24 cases-14.9%), *Candida tropicalis* (20 cases-12.4%) and *Candida parapsilosis* (6 cases-3.7%). Older age, female gender, red blood cell transfusion, presence of central venous catheter, previous usage of antibiotics and antifungals, duration of central venous and urinary catheters were found as risk factors for candiduria. In 11 patients (6.8%), candidemia was also detected with candiduria concurrently.
Conclusion Candiduria is not a rare clinical condition in ICU patients and in some patients candiduria may be an indicator for candidemia. Risk factors associated with candiduria are very similar to which are associated with candidemia and those patients should be followed up carefully.

P262

A prospective study of biomarkers to aid diagnosis of invasive fungal disease in critical care patients

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Background and objective Invasive fungal diseases (IFD) are a major cause of morbidity and mortality in critical care patients. The majority of these are due to *Candida* spp., however there are reports of a recent increase in the incidence of pulmonary aspergillosis in this cohort. Because the diagnosis of IFD in critical care patients is difficult, non-culture based techniques such as assays for detection of fungal antigens and DNA are increasingly being investigated. The main objective of this study was to assess the role of biomarkers in the diagnosis of IFD in our critical care unit.
Methods prospective observational study was performed from December 2012 to January 2014 where patients who had been receiving critical care for >7 days were recruited. After informed consent, serum samples were taken twice weekly while the patient remained in the unit. The following assays were performed: detection of (1–3)- β-d-glucan (BDG), and galactomannan (GAL) using commercial kits, and *A. fumigatus* DNA by an in house rtPCR. Clinical data collected included: age, sex, reason for admission to critical care, underlying diagnosis, host risk factors for IFD, antifungal therapy, laboratory and radiology results and outcome. The patients were classified into the following groups: no IFD, colonisation, possible, probable/putative and proven IFD using clinical and laboratory criteria that excluded biomarker results and were adapted from published guidelines for invasive candidiasis or a diagnostic clinical algorithm (1) for invasive pulmonary aspergillosis (IPA). Sensitivity, specificity, negative (NPV) and positive predictive value (PPV) were calculated to assess the performance of the biomarkers.
Results One hundred patients were recruited into the study. Of these, 10 had no IFD, 43 were colonised by *Candida* or *Aspergillus* spp., 14 and 18 had possible and probable invasive candidiasis respectively, 3 had probable/putative invasive aspergillosis and 12

had proven IFD of which 9 were invasive candidiasis, 1 *P. jirovecii* pneumonia, 1 *S. cerevisiae* mediastinitis and 1 *Candida* skin and soft tissue infection. The sensitivity, specificity, PPV and NPV of a single positive BDG for the diagnosis of IFD were 63%, 84%, 66% and 83% respectively. With 2 consecutive positive results, specificity increased slightly to 86%. The area under the receiver operator characteristics curve of the serum BDG was 0.758. A further 8 probable/putative IPA cases were identified when results of the BDG, GAL and PCR were incorporated into the diagnostic algorithm (1).
Conclusion The performance of serum BDG detection in our cohort is comparable to previous reports (2). Biomarkers increase the detection of cases of invasive candidiasis and invasive aspergillosis in the critical care setting and support stewardship programs to optimise antifungal therapy.

P263

Aspergillus Monitoring Project in a large Iranian Educational Hospital

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Objectives The main object was monitoring of Aspergillus infections and epidemiological approaches in the greatest university hospital of Urmia, Iran.
Methods The subject of our study included bronchial fluid and sputum were collected from the hospitalized patients with acute respiratory symptoms. All clinical specimens were transported to the medical mycology lab, UMSU, for the diagnostic purposes and fungal identifications. For environmental detection of Aspergillus isolates, some specimens were collected from air and environment surfaces. A morphological study was firstly performed including; growth characteristics and microscopic features of Aspergillusspecies on mycological media. For the Confirmation of Aspergillusisolates which similarly found in clinical and environmental sources, PCR-RFLP using a novel restriction enzyme *Mwo* I and an additional molecular technique of RAPD were carried out.
Results A total of 102 fungal isolates, including *Candida* species 82 (80%), *Aspergillus* spp.20 (19.6%) and the other fungi 2 (0.4%). Among the clinical isolates of Aspergilli; *Aspergillus flavus* (8.4%), *Aspergillus fumigatus* (4.2%) and *Aspergillus niger* (2.8%) were identified, as well as environmental *Aspergillus* isolates *Aspergillus flavus* (19.4%), *Aspergillus niger* (6.5%) and *Aspergillus fumigatus* (6.5%).
Discussions Comparing the clinical and environmental findings, 2 of 11 clinical Aspergillus isolates were matched with environmental isolates in RAPD method and the other environmental sources were not confirmed.

P264

Invasive candidiasis in intensive care units in Serbia: preliminary results on species distribution, antifungal susceptibility profile and biofilm formation of *Candida* spp. isolates from blood

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Objective *Candida* species are an important cause of morbidity and mortality in the critically ill patients, especially those in intensive care units (ICUs). The purpose of this prospective 1-year study was to collect, identify, determine antifungal susceptibility and test biofilm formation of *Candida* spp. recovered from blood of ICU patients in 11 centers in Serbia.
Methods Study design included: 1) collection of isolates from blood; 2) identification by culturing, biochemical tests and matrix assisted laser desorption ionization time-of-flight (MALDI-TOF); 3) broth-dilution susceptibility testing to amphotericin B, fluconazole, 5-fluorocytosine, itraconazole, posaconazole, voriconazole, micafungin, caspofungin and anidulafungin using Micronaut-AM MHK-2 plates (Merlin Diagnostika, Bornheim, Germany); 4) biofilm formation test using crystal violet assay (Sigma-Aldrich, St Louis, MO) and 5) data analysis using methods of descriptive and analytical statistics.
Results A total of 32 *Candida* isolates were collected from 32 patients: *C. albicans* (18/32; 56.3%) was the predominant species followed by *C. parapsilosis* (11/32; 34.4%) and *C. tropicalis* (3/32; 9.3%). No resistance to amphotericin B, 5-fluorocytosine, micafungin, caspofungin and anidulafuingin was observed. Out of 32, 11 isolates were found to be azole-resistant (34.4%). Resistance rates to fluconazole, voriconazole, posaconazole and itraconazole were 6.25% (232), 15.6% (5/32), 15.6% (5/32) and 31.25% (10/32), respectively. Azole-resistant strains were predominantly *C. albicans* (10/11, 90.9%) and one *C. tropicalis* (1/11, 9.1%). Among *C. parapsilosis* strains we detected no resistance, but 45.5% (5/11) of isolates were susceptible-dose dependent to itraconazole. When the difference in minimum inhibitory values (MIC) between *C. albicans* strains and non-albicans *Candida* strains (NAC) was tested, *C. albicans* had significantly higher MIC values for itraconazole, voriconazole and posaconazole, while NAC had significantly higher MIC values for micafungin and caspofungin ($P < 0.05$). The ability of biofilm formation was present in 84.4% (27/32) of tested strains: 88.8% of tested *C. albicans* (16/18), 72.7% of *C. parapsilosis* (8/11) and all *C. tropicalis* strains (3/3, 100%). No association between planktonic antifungal susceptibility and biofilm formation was observed.
Conclusion *C. albicans* was the most commonly isolated species from the blood of Serbian ICU patients (56.3%). Antifungal resistance was restricted to azoles (6.25–31.25%). All isolates were susceptible to echinocandins which should be regarded as the empirical treatment of choice for candidaemia in severely ill patients or in the presence of risk factors. The majority of strains were able to form biofilm (84.4%) so the next step in this study is to correlate biofilm formation with clinical patient data on risk factors, therapy and outcome.

P265

Beta-D-glucan and *Candida albicans* germ tube antibody in ICU patients with invasive candidiasis

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Purpose To assess the value of (1→3)-ß-D-glucan (BDG) and *Candida albicans* germ tube antibody (CAGTA) for the diagnosis of invasive candidiasis (IC), their kinetics in the presence of systemic antifungal treatment, and the influence of main confounding factors on BDG levels in unselected, non-neutropenic ICU patients.
Methods Prospective cohort study of 107 ICU patients admitted ≥ 7 days. BDG (cutoff positivity ≥ 80 pg ml^{-1}) and CAGTA (cutoff positivity ≥ 1/160) assays were performed twice a week. Confounding factors included amoxicillin-clavulanate and piperacillin-tazobactam treatments, recent surgery, Gram-positive bloodstream infection, renal replacement therapy, and enteral nutrition. Patients were classified as neither colonized nor infected ($n = 29$), *Candida* spp.

colonization ($n = 63$) (low-grade, $n = 32$; high-grade, $n = 31$), and invasive candidiasis (IC) ($n = 15$).
Results A total of 465 measurements of biomarkers were performed (4.3 per patient). BDG values were higher in patients with IC and high-grade colonization than in the remaining groups ($P = 0.012$), and two consecutive measurements ≥ 80 pg ml^{-1} discriminated IC from the remaining groups (sensitivity 80%, specificity 73%). Significant changes of BDG and CAGTA kinetics in IC patients treated with antifungals were not observed. In patients neither colonized nor infected or with low-grade *Candida* spp. colonization, none of the confounding factors was associated with a significant increase in BDG positivity.
Conclusions Two consecutive BDG values ≥ 80 pg ml^{-1} allowed discrimination among IC and high-grade colonization. Systemic antifungal therapy could not be monitored with biomarker kinetics, and BDG levels were not interfered by confounding factors in neither colonized nor infected patients or with low-grade colonization.
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P266

Surveillance of Candidaemia in a Greek Intensive Care Unit from 2009 to 2014

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Objectives The aim of the present study was to investigate the local epidemiological data of candidaemia (species distribution and susceptibility profile), collected from 2009 to 2014, in a 12-bed Intensive Care Unit (ICU) of a large tertiary-care hospital in Greece.
Methods All candidaemia cases were recorded. Non-duplicate *Candida* isolates were identified to species level by using CHROMagar *Candida* Medium (Becton Dickinson) and Vitek 2 system (bioMerieux), supplemented with examination of the morphology on cornmeal Dalmau plates. All *C. parapsilosis* strains were characterized as *C. parapsilosis* sensu stricto with MALDI-TOF (Bruker). In vitro antifungal susceptibility testing was performed by broth microdilution method, according to CLSI M27-A3 guidelines. Recently revised CLSI M27-S4 species-specific clinical breakpoints were applied.
Results A total of 92 episodes of candidaemia from 83 ICU patients were registered during the study period. Ninety-six *Candida* bloodstream isolates were studied. Of these 96 isolates, 46 (47.9%) were *C. parapsilosis*, 23 (24%) were *C. albicans*, 17 (17.7%) were *C. glabrata* and 8 (8.3%) were *C. tropicalis*; there was 1(1%) isolate of *C. krusei* and 1 (1%) isolate of *C. lusitaniae*. *C. parapsilosis* was the predominant species, always first at the rank order over the study period. Decreased fluconazole susceptibility, defined as MIC >4 μg ml^{-1} was detected in 9/46 (19.5%) *C. parapsilosis* isolates (MIC range 8–32 μg ml^{-1}). Despite the finding that 50% of the *C. parapsilosis* clinical strains tended to have elevated anidulafungin MICs, clustering at 2 μg ml^{-1}, no resistance to anidulafungin (MIC ≥8 μg ml^{-1}) was demonstrated. Only 5 out of 46 *C. parapsilosis* strains were categorized as intermediately susceptible to anidulafungin (MIC of 4 μg ml^{-1}). The MIC values obtained for caspofungin were in general 1 to 2 dilution steps lower than those for anidulafungin, among *C. parapsilosis* isolates. All 23 *C. albicans* strains were fully susceptible to azoles and candins. All *C. glabrata* isolates were categorized as susceptible dose-dependent to fluconazole (MIC_{90} = 8 μg ml^{-1}) and 100% as susceptible to anidulafungin (MIC ≤0.12 μg ml^{-1}); 64.7%, (11/17) were classified as being intermediately susceptible to caspofungin (MIC of 0.25 μg ml^{-1}). A single *C. tropicalis* isolate was categorized as susceptible dose-dependent to fluconazole with a drug MIC of 4 μg ml^{-1}, while for another *C. tropicalis* strain, the MIC values were suggestive of resistance to caspofungin (MIC of 1 μg ml^{-1}) and susceptibility to anidulafungin (MIC of 0.016 μg ml^{-1}).
Conclusion In the particular ICU setting, *C. parapsilosis* has emerged as a significant bloodstream pathogen, steadily outranking *C. albicans*. The emergence of fluconazole resistant *C. parapsilosis* strains raises questions about the superiority of fluconazole as a better drug against *C. parapsilosis*. Therefore, it is of great value to monitor the local epidemiology so as to guide the timely and successful empirical anti-*Candida* treatment.

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Effectiveness and safety of anidulafungin: A real-life multicenter data in Turkey

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Objective Fungal infections are a growing global problem. The most common global mycoses are due to infections by *Candidiasis*, *Cryptococcosis*, *Aspergillosis*. More antifungal agents have reached clinical use in the past two decades than at any other time. The echinocandins have been a welcome addition to this group, with the latest being anidulafungin. Anidulafungin is a semisynthetic product of echinocandin B and it inhibits enzyme complex 1,3-β-D-glucan synthase and thereby inhibits fungal 1,3-β-D-glucan synthesis. Anidulafungin is FDA approved for the treatment of invasive candidiasis, candidemia and esophageal candidiasis in 2006. This drug is used since 2012 in our country. We aimed to examine the real-life data of the effectiveness and side effects of anidulafungin in the multicenter study,
Methods The patients whose had proven fungal infection or based on their candida colonization index/candida score or empirically anidulafungin treatment was started by the Infectious Diseases specialists from seven different centers are retrospectively screened between the dates of 1st January 2012 and 31st December 2014.
Results Total number of 262 patients (115 female, 147 male, mean age 58.65 + 19.51, min:15 max:95) from seven centers were involved in this study. 140 (53.4%) patients were hospitalized at the internal medicine departments and the other 122 (46.6%) patients

Table 1. Risk factors

Risk Factors	n	%
Ulcer prophylaxis	252	96.2
Antibiotic usage (at last month)	245	93.5
Urinary catheter	237	90.5
Central venous catheter	216	82.4
Mechanical ventilation	187	71.4
Surgical operation	120	45.8
Hearth Disease	64	24.4
Diabetes	61	23.3
Corticosteroid usage	53	20.2
Chronic renal failure	41	15.6
Neutropenia	16	6.1
Transplantation	10	3.8

were at the surgical departments. 206 patients (78.6%) were followed at the 3rd level of intensive care units. Treatment was started for culture positive 163 (62.2%) patients. Blood cultures were positive for candida spp. (67 *C.albicans*, 33 *C.parapsilosis*, 17 *C.tropicalis*, 13 *C.glabrata*, 10 others) in 140 patients. 67 patients were treated with the other antifungal agents (48 fluconazole, 12 caspofungin, 5 voriconazole, 2 amphotericin B) and their treatments were switched to anidulafungin afterwards.

In 121 (%86.4) of 140 blood culture positive patients, source was central venous catheter and 59 (48.8%) of them were removed after the culture results. There was significant difference comparing the catheter removed group and unremoved group in terms of mortality rates ($P = 0.046$; Fisher's E. Test).

In blood culture positive group mortality rate was 52.9% (79 of 140 patients). These high mortality rates due to not only candidemia but also other co-morbidities. During the course of anidulafungin treatment, 1 anaphylaxis, 1 skin eruption, 1 trombocytopenia were seen as side effects.

Conclusion In this study which has large number of patients, anidulafungin treatment was seen as effective in candidemias and low side effects. Considering invasive candida infections in clinically severe patients, we believe that anidulafungin can be preffered as initially and spectrum of treatment can be adjusted after the culture results.

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Species distribution and susceptibility testing of *Candida* spp. isolated from patients with invasive candidosis in Russia

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Objectives To study etiology of invasive candidosis and susceptibility of *Candida* spp. isolates to antifungal agents: fluconazole, voriconazole, posaconazole and caspofungin.

Methods Species identification was performed by MALDI-TOF-mass-spectrometry and DNA-sequencing. Susceptibility testing was conducted according to CLSI M27-A3 (S4, 2012) microdilution method.

Results A total of 322 *Candida* spp. isolates from normally sterile body sites of patients with invasive candidosis hospitalized in 37 medical centers of 6 Federal regions of Russia have been studied prospectively (2011–2014 yy.). Species distribution was as follows: *C. albicans* –43.2%, *C. parapsilosis* - 20.2%, *C. glabrata* - 11.5%, *C. tropicalis* -9.6%, *C. krusei* - 6.2%, *C. guilliermondii* -5.3%, *C. lusitaniae* - 1.3%, *C. pararugosa* - 0.6%, *C. dubliniensis* - 0.6%, *C. inconspicua* - 0.3%, *C. kefyr* - 0.3%, *C. utilis* - 0.3%, *C. bracarensis* - 0.3%, *C. lipolytica* - 0.3%. Prevalence of *C. albicans* decreased from 54% (2012 y.) to 32.8% (2014 y.), $P = 0.015$. Prevalence of *C. parapsilosis* increased from 16.4% (2012 year.) to 27.9% (2014 year), $P = 0.012$. Differences in etiology of invasive candidosis were revealed in various geographic regions. Proportions of *C. albicans* were significantly more in Siberian Federal District (75%) and Ural Federal District (57.8%), than in Northwestern FD - 37% and Southern FD - 35% ($P < 0.05$).

Susceptibility patterns to antifungals: fluconazole - S - 75%, SDD-13%, R- 12%; voriconazole -S - 97.5%, SDD - 0.6%, R - 1.6%; caspofungin - S - 100%. MIC_{100} of posaconazole - 0.25 mg l^{-1}.

Conclusion Among etiologic agents of invasive candidosis in Russia the proportions of *C. albicans* is decreasing and *C. parapsilosis* - increasing.

P269

Additional value of cytochrome B gene sequencing to internal transcriber spacer sequencing for the correct identification of Trichosporon species

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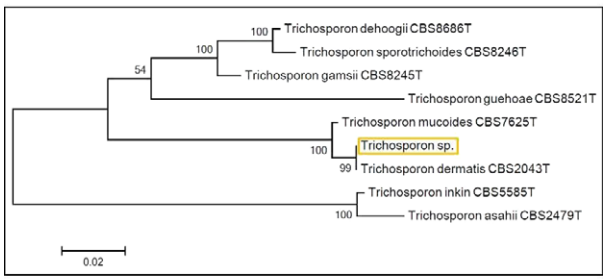
Objectives *Trichosporon* species are emerging fungal pathogens in immunocompromised patients. *Trichosporon* species are generally susceptible to triazoles and amfotericine B. Correct species identification of *Trichosporon* species is challenging. Here we show the additional value of sequencing the cytochrome B gene for identification of *Trichosporon* species of a high-level triazole resistant *Trichosporon* isolate. This strain was isolated from a recipient of a liver re-transplant admitted to the surgical intensive care unit.

Methods Species identification was based on matrix associated time of flight mass spectrometry (Maldi-TOF) and sequencing of the internal transcriber spacer (ITS) of the 18S ribosomal subunit. The addition of sequencing the cytochrome B gene lead to definitive identification. Susceptibility of the isolate was tested using a commercial microdilution test (Yeast Sensititre One™) by the national reference center.

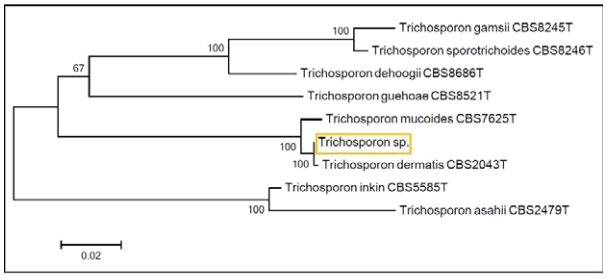
Results *Trichosporon spp* was isolated from abdominal fluid sample of recipient of a liver re-transplant receiving fluconazole and amphotericin B. Provisional susceptibility testing by e-test indicated resistance to triazoles. Microdilution testing showed MICs to amphotericine B 0.25 mg l^{-1}, fluconazole >256 mg l^{-1}, itraconazol >16 mg l^{-1}, voriconazol >16 mg l^{-1}, posaconazol >8 mg l^{-1} caspofungin >16 mg l^{-1} and 5-flucytosine >64 mg l^{-1}. The isolate was identified as *Trichoporon mucoides* with a score of 2.17 by Maldi-TOF.

Figure 1. Maximum parsimony tree based on the sequence data of ITS of the *Trichosporon* isolate.



ITS; internal transcriber spacer of the 18S ribosomal unit
Numbers above branches represent bootstrap values

Figure 2. Maximum parsimony tree based on the combined sequence data of ITS and *cytB* of the *Trichosporon* isolate.



ITS; internal transcriber spacer of the 18S ribosomal unit, cytB gene; cytochrome B gene
Numbers above branches represent bootstrap values

Identification by ITS sequencing could not differentiate between *T. mucoides* and *T. dermatis* subspecies (figure 1). Combining the data from ITS sequencing and the sequence data from the cytochrome B gene lead to the correct identification *T. dermatis* (figure 2).
Conclusion Here we present a highly triazole resistant *T. dermatis* isolate. The subspecies was misidentified as *T. mucoides* using Maldi-TOF, and by ITS PCR, now differentiation was possbible between *T. dermatis* and *T. mucoides*. The addition of cytB was needed for correct species identification of *T. dermatis*. Correct species identification is essential for epidemiology of resistance of *Trichosporon* species.

P270

The impact of a diagnostics-driven antifungal stewardship programme in a UK tertiary referral teaching hospital

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Background Candidaemia is a serious complication in critically ill patients, and associated with high mortality. The University Hospital of South Manchester, UK (UHSM) candidaemia guidelines for the ICUs recommend early initiation of antifungal therapy based on strong clinical suspicion and the presence of specific risk factors. Echinocandins are the first line therapy, and micafungin is the echinocandin on formulary. Serum β-1-3-D-glucan (BG) should be used to guide discontinuation of therapy in the absence of other microbiological evidence of candidaemia as it has a very high negative predictive value. The aim of this study was to evaluate the compliance of the 3 ICUs (including cardio-thoracic & transplant, burns, acute medical) in UHSM (total of 71 beds) with the current guideline, the impact of the UHSM antifungal stewardship programme on antifungal consumption.
Methods The usage of micafungin at UHSM between April and July 2014 was reviewed using pharmacy databases, and by reviewing patient records. All prescriptions were reviewed by the audit team and a decision made as to whether or not the use of micafungin was appropriate in each individual case. Antifungal consumption data was analysed for the general adult ICU (19 beds) for the past 24 months.
Results A total of 72 patients admitted to ICU were started on micafungin during the 4-month study period. Of these, 45 were treated for suspected or proven candidaemia, and 25 for suspected or proven aspergillosis or scedosporiosis. Of those treated for candidaemia, seven (16%) had *Candida spp.* isolated from blood cultures. Two of these were ECMO patients. In 14 cases (31%) the local guideline was not fully followed. In eight cases the documented reason for starting micafungin was isolation of *Candida spp.* in a single superficial sample such as sputum or urine. In four cases microbiological tests had not been taken as per guideline, and in two cases negative BG result was not acted upon. Of the 38 cases without evidence of invasive candidosis when the patients were reviewed by a member of the Infectious Diseases team, micafungin was discontinued in 19 cases (50%). In two further cases, discontinuation of micafungin was recommended, however the primary team (both surgical) chose not to follow the advice given. Two patients had been prescribed higher (150 mg od) dose in the absence of clinical suspicion of aspergillosis or oesophageal candidosis. There were no deaths due to candidaemia during the study period. The monthly micafungin expenditure on the AICU was some 13 000€ per month before the audit and was reduced to £9200€ during the audit and has stayed on that level until now.
Conclusion True candidaemia is a medical emergency and micafungin a well-tolerated drug. Therefore, it would be inappropriate to restrict the initiation of first-line therapy in cases of suspected candidaemia. Our study shows that with the active presence of Infectious Diseases Consultants on ICU, and with the appropriate use of diagnostics, it is possible to safely discontinue echinocandin therapy when it is not indicated. This has important implications for antifungal stewardship and cost saving in intensive care.

P271

Invasive candidiasis in ICU patients. Diagnostic usefulness of anti-mycelium antibodies, 1-3-Beta-D-Glucan, mannan/antimannan antibodies, and detection of *Candida* DNA (PCR). Cava Trem Project

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Objectives To assess the usefulness of biomarkers (BM) (Candida albicans germ tube antibodies [CAGTA], (1,3)-β-D-glucan [BDG], mannan antigen [MN], anti-mannan antibodies [AMN] and Candida DNA PCR) in the diagnosis of invasive candidiasis (IC) in critically ill adult patients with primary acute gastrointestinal injury* and ICU stay ≥7 days.
Methods Prospective, cohort, observational and multicentre study (12 ICUs/18 months). Twice a week: BM assays and Candida PCR. Techniques included CAGTA (Vircell® kit), BDG (*Fungitell®*), MN and AMN (Platelia TM Candida Ag Plus) and detection of DNA/Candida by PCR-TR multiplex. Cutoff positivities: CAGTA: ≥ 1/160, BDG ≥ 80 pg ml^{-1}, MN: ≥ 60 pg ml^{-1}; AMN: ≥ 10 UA ml^{-1}, and PCR (+) or (-). BM were considered positive in the presence of two consecutive results of CAGTA, MN and AMN, maximal geometrical mean of two consecutive observations for BDG and at least one positive isolate for PCR. For each patient, these assessments were performed at or before the episode of IC. When an episode of IC did not develop, the highest value of all recorded values was used. Patients were classified into: neither colonized/nor infected (NCI), *Candida* spp. colonization of low grade (LGCC) and high grade (HGCC), intra-abdominal candidiasis (IAC) and candidemia (C). Categorical variables were expressed as frequencies and percentages, continuous variables as mean and standard deviation (SD) or median and interquartile range (IQR, 25th-75th percentile). Data were analyzed with the chi-square test, the Fisher's exact test and the Kruskal-Wallis test as appropriate. ROC

Table 1.- Values of biomarkers and PCR according the groups of patients.

	NCI (n = 48)	LGCC (n = 131)	HGCC (n = 24)	IAC (n = 20)	Candidemia (n = 11)	P
BDG, pg/mL	73.6 (41.9 ; 233)	107.3 (53.5 ; 201)	211.6 (77.1 ; 331)	219.7 (73.4 ; 363)	500 (239 ; 2403)	< .001
CGTA +, no. (%)	11 (22.9)	46 (35.4)	17 (70.8)	5 (25.0)	7 (63.6)	< .001
MN +, no. (%)	10 (20.8)	43 (33.1)	15 (62.5)	5 (25.0)	3 (27.3)	.012
AMN +, no. (%)	6 (12.5)	14 (10.8)	4 (16.7)	5 (25.0)	2 (18.2)	.367
PCR +, no. (%)	14 (29.2)	37 (28.5)	6 (25.0)	9 (45.0)	7 (63.6)	.087

Observations before or at episode IC, no.(%): patients number and percentages

Table 2.- Diagnostic accuracy of biomarkers and PCR for the diagnosis of invasive candidiasis

	AUC (95% IC)	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)
BDG *	67 (56.1;79.1)	77.4 (58.9-90.4)	42.1 (35.2;49.2)	17.0 (14.1;20.4)	92.3 (86.1;96.0)
CAGTA +	-	38.7 (21.8;57.8)	63.4 (63.4;70.0)	14.0 (9.1;20.7)	87.1 (83.3;90.1)
MN +	-	25.8 (11.9;44.6)	66.3 (59.4;72.8)	10.5 (5.9;18.1)	85.4 (82.2;88.0)
AMN +	-	22.6 (9.6;41.1)	88.1 (82.8;92.2)	22.6 (12.1;38.2)	88.1 (86.0;90.0)
PCR +	-	51.6 (33.0;69.8)	71.8 (65.0;77.9)	21.9 (15.8;29.6)	90.6 (86.0;93.4)

*BDG ≥ 80 pg/mL

curve analysis for BDG to discriminate between IC and the remaining groups (80% sensitivity of the cutoff selected). In both cases (BDG geometric mean and maximum values), the cutoff was 80 pg mL^{-1}. Statistical significance was set at $P < 0.05$.

Results 233 patients: NCI 48, LGCC 130, HGCC 24 and IC 31 (IAC 20 and C 11). Surgical 187 (80%), medical 39, T, 7)], age: 66.7 (13.2) years and M/F: 67/33%. Hospital/ICU stay: 15 (10;25)/37 (21;57) days. Antifungal treatment: 119 (50.8%). Overall mortality: 68 (29.4%). Samples: BM: 860 (median 3.69 [1–10] per patient) and PCR 213 measurements. Table 1 shows BM and PCR in relation to the groups of patients, and Table 2 their values in the diagnosis of IC, with BDG (cutoff >80 pg/mL) showed a 77% sensitivity and 42% specificity (AUC ROC 0.67). For the diagnosis of candidemia, BDG with a cutoff of 235 pg ml^{-1} showed 82% and 74% sensitivity and specificity, respectively (AUC ROC 0.79). The remaining BM and PCR did not show relevant values. The association of BDG with other BM and PCR did not improve the diagnostic capacity of BDG for IC and specifically for candidemia.

Conclusion (1,3)-β-D-glucan was the only biomarker allowing discriminating candidemia from the remaining groups analyzed.

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P272

Invasive Candidiasis in ICU patients. Epidemiological Study. Cava Trem Project

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Objective Epidemiological study of selected non-neutropenic (primary acute gastrointestinal injury*) critically ill adult patients colonized and infected by *Candida* spp., with an expected ICU stay of at least 7 days.

Methods Prospective, cohort, observational, multicentre study carried out during 18 consecutive months in 12 Spanish ICUs from tertiary hospitals. Demographic data, type of patients, APACHE II and SOFA scores (on ICU admission), underlying disease, comorbidities, risk factors, antifungal treatment and outcomes were recorded. Clinical assessment, APACHE II, SOFA and Candida score, *Candida* colonization cultures (rectal swabs, tracheal aspirates, gastric or pharyngeal aspirates and urine) were performed twice a week (other samples were taken as clinically indicated). All samples were processed by conventional methods. Patients were classified into: neither colonized/nor infected (NCI), *Candida* spp. colonization of low grade (LGCC) and high grade (HGCC), intra-abdominal candidiasis (IAC) and candidemia (C). Categorical variables are expressed as frequencies and percentages, and continuous variables as mean and standard deviation (SD).

Results A total of 233 patients (67% men, mean [SD] age 66 [13.0] years) were included. The mean APACHE II and SOFA scores on ICU admission were 18.4 (6.3) and 7.2 (3.3), respectively; 211 (90.6%) patients underwent abdominal operations (344 surgical procedures). Risk factors included central venous catheter (99%), urinary catheter (98%), broad-spectrum antibiotics (97%), arterial catheter (88%), mechanical ventilation (84%), parenteral nutrition (82%), steroids (37%) and renal replacement therapy (25%). There were 48 patients in the NCI group, 130 in the LGCC, 24 in the HGCC and 31 (13.3%) in the IC. Positive samples for *Candida* spp. accounted for 34.7% ($n = 885$) of all 2549 cultures, with multifocal colonization in 120 (49%) patients. Pharyngeal exudates were the most frequent colonized samples (59.6%) followed by rectal and respiratory samples (51% each). The most frequently isolated *Candida* spp. were *C. albicans* (60.1%) and *C. glabrata* (11.7%). There were 31 cases of IC (20 intra-abdominal infection, 11 candidemia), with *C. albicans, C. glabrata and C. parapsilosis* isolated in 16, 7 and 3 cases, respectively. *C. tropicalis, C. krusei, C. famata, C. dubliniensis and C. albicans + C. glabrata,* in 1 case each. Two cases were catheter-associated candidemias. Twenty-three (74%) patients showed multifocal colonization. The mean time to develop IC from hospital and ICU admission was 17.4 (15.7) and 7.1 (6.0) days, respectively. A total of 119 (51%) patients received antifungal therapy, 29 and 90 patients from the IC and CC/NCI group, respectively. The overall mortality rate was 68 (29.4%).

Conclusions In this cohort, a high rate of multifocal multicolonizacion was observed. *C. albicans* followed by *C. glabrata* were the most frequently isolated species. A high percentage of patients (38.6%) without documented *Candida* infection were treated with antifungal agents.

* Blaser AR et al. ICM 2012;38:384–94

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P273

Directed/empirical antifungal treatment in non-neutropenic critically ill patients with abdominal conditions. Differential characteristics. Cava Trem Project

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Objectives To assess the characteristics of antifungal therapy (AFT) in non-neutropenic critically ill patients with surgical abdominal conditions or non-operated pancreatitis with an ICU stay of ≥ 7 days.

Methods Prospective, cohort, observational and multicentre study carried out during 18 consecutive months in 12 ICUs. Demographic data, type of patient, APACHE II and SOFA scores, comorbid conditions, risk factors, AFT and clinical outcome were recorded. Type, doses and duration of AFT, given in monotherapy, combined or following sequential regimens were registered. Patients were classified into the groups of neither colonized/nor infected (NCI) and *Candida* spp. colonization of low grade (LGCC) and high grade (HGCC), intra-abdominal candidiasis (IAC) and candidemia (C). Categorical variables were expressed as frequencies and percentages, continuous variables as mean and standard deviation (SD) or median and interquartile range (IQR, 25th-75th percentile). The Student's *t* test (means) and the Kruskal-Wallis test (medians) were used for the analysis of continuous data. Statistical significance was set at $P < 0.05$.

Results Directed treatment: 31 patients (13.2%) presented invasive candidiasis (IC), 20 IAC and 11 C (catheter-related in two cases). AFT was administered to 29 patients (93.5%). The median time elapsed from hospital and ICU admission to diagnosis was 16 (6;19)

and 7 (3;13) days, respectively. AFT was started on the day of IC diagnosis (21/29), before (6/29) and 1 day later (2/29), and was maintained for a mean of 14 (8.0) days. Initial AFT included azoles (15) and candins (14). Sequential AFT with a second (4) and third (1) antifungal agents was also recorded. Empirical treatment: 90 patients (38.4%) without IC received AFT, 81 (90%) undergoing abdominal surgery, overall mortality 31.1% (28 cases). The indication of AFT was associated with severe sepsis (66 [73.3%]) and/or multifocal colonization (39 [43.3%]). The mean time of starting AFT from hospital and ICU admission was 14.8 (15.2) and 7.08 (6.0) days, respectively. Initial AFT included candins (56) and azoles (34) with a mean duration of treatment of 12.1 (6.9) days. Eleven patients received a second course of treatment: de-escalation (5), poor clinical course (3) and other causes (3). All antifungals were administered in monotherapy or sequentially. The mean APACHE II, SOFA and *Candida* score at the beginning of AFT, directed and empirical, were 16.8 (4.9) and 17.7 (6.2); 6.1 (3.5) and 6.8); and 3.9 (1.0) and 3.0 (0.9), respectively. The comparative analysis of these variables did not reveal statistically significant differences.

Conclusion In this type of patients, neither clinical features nor severity-related scores were different between both groups (directed and empirical treatment) at the time of starting AFT. In both groups, AFT was initiated around 7 days of ICU stay. The number of empirical AFT was very high. Types of antifungals and duration of treatment seems adequate.

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P280

Imaging findings in patients after allo-HSCT with pulmonary mucormycosis

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Objectives to describe computer tomography (CT) features of pulmonary mucormycosis in patients after allogeneic hematopoietic stem cell transplantation (allo-HSCT).

Materials and methods A prospective single center study in 2010–2014 yy. We include 15 patients (age from 4 to 59 year) after allo-HSCT with probable and proven ($n = 4$) pulmonary mucormycosis according to EORTC/MCG 2008 criteria. All the patients underwent CT guided bronchoscopy with comprehensive study of broncho-alveolar lavage fluid (BAL). The samples were sent to the laboratory for microscopy, culture and galactomannan test. If the result of BAL fluid was negative, we performed further diagnostic procedures. Two patients underwent forceps biopsy and one percutaneous biopsy under ultrasonography navigation control.

Results The majority of the patients (60%) had unilateral lesion on CT scan. Main lesions were pleural effusion (47%), solitary mass with the halo sign (46%), later with the appearance of reversed the halo sign (31%), small ill-defined infiltrates (27%), and multilobar consolidation (27%).

Conclusions Pulmonary mucormycosis usually appears as a pleural effusion and unilateral mass with the halo sign followed by transformation in mass with the reversed halo sign. Multilobar consolidation and small ill-defined infiltrates are less common.

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Gastrointestinal mucormycosis in a patient with acute myeloblastic leukemia

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Objectives In patients with hematological malignancy mucormycosis is the third most common invasive mycosis after aspergillosis and candidiasis. The most expected localisation is paranasal sinuses, orbit and brain or lungs. We report a rare case of gastrointestinal mucormycosis. Most cases of gastrointestinal mucormycosis are associated with diagnostic delay and high mortality.

Methods Patient 6 month-old boy diagnosed with AML and treated with stadart chemotherapy in a regional hospital. Remission was achieved after first course of induction therapy. After consolidation course (*Cytosine arabinoside 3000 mg/m2 every 12 h day 1–4, Idarubicin 10 mg/m2 day 2–4*) the patient developed sepsis with pneumonia, enterocolitis. *Candida krusei* was recovered from blood. One week later massive GI bleeding started and gastroscopy revealed extensive duodenum ulcer with thrombotic and necrotic mass. GI bleeding was treated conservatively. Febrile neutropenia developed after second consolidation (*Cytosine arabinoside 1000 mg/m2 every 12 h day 1–3 and 8–9, mitoxantrone 10 mg/m2 day 2–3, L-asparaginase 6000 ME/m2 day 4 and 10*) and was complicated by recurrent GI bleeding. During gastroscopy perforation of duodenum ulcer was find out.

In very poor condition he was admitted to our hospital. After examination, wich revealed leakage of radiopaque substance from duodenum to the right part of abdominal cavity and necrosis of the right kidney, child was immideately taken to the operating room. Abdominal cavity revision showed perforation of duodenal ulcer, total rupture of vertical part of duodenum, perforation of cecum and total necrosis of the right kidney and paranephral tissues. Surgery included resection of horizontal part of duodenum and duodeno-jejunoanastomosis, resection of ileocecal angle and ileo-ascendoanastamosis, right nephroectomy, gastrostomy.

Results In histopathological specimen of resected duodenum, cecum and kidney the picture of invasive mycosis was seen: wide, ribbon-like mycelium with no regular septation and different angles of branching such as 90 °. All these characteristics confirm gastro-intestinal form of mucormycosis. Most probably, that kidney necrosis was secondary. Considering the complicated situation: septic condition of patient, nephroectomy in one hand and age of 9 month, gastrostasis, bad intestinal absorbtion in other hand, we decided to start with combined antifungal therapy. One month he recieved lipid formulation amphoB 5 mg/kg/every day with posaconasol 15 mg kg day^{-1} followed by 4 months posaconasol monotherapy. In a good condition with normal nutritional status in complete hematological remission he was discharged without any sign of infection.

Conclusion Despite gastro-intestinal form remain to be very rare, we should keep it in mind, that in children especially before one year old it happens 3 times more frequent than in general population. Prompt diagnosis is very important, which means minimal invasive technics with histological examination, not cultures only. Therapy should be multimodal and includes agressive surgery combined with liposomal/lipid formulations of ampho B. Posaconasol can be a complimentary/step-down therapy.

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Delphi-based study and selection of key risk factors for invasive fungal infection (IFI) in hematologic patients

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Introduction New risk factors for IFI have been identified in recent years and should be classified, in order to improve patient management and prognosis.

Objective: The purpose of this study was to identify key risk factors for the development of IFI caused by filamentous fungi (particularly of the *Aspergillus* genus) in oncohematologic patients in various clinical settings, excluding allogeneic transplants.

Methods A national Delphi-based prospective study was conducted during November 2014, in order to reach a consensus. All risk factors were identified following a literature review and then assigned to the following groups: allogeneic hematopoietic stem-cell transplantation (HSCT), acute leukemia/myelodysplastic syndrome, and lymphoma/multiple myeloma. The study was performed anonymously by e-mail contact with hematologists. A risk factor was considered key for a specific group when at least 70% of experts rated the risk as 'maximum'or 'high'in their response.

Results The panel was composed of 42 hematologists, 28 (66.7%) of whom completed the questionnaire. Risk factors associated to Allo-HSCT were presented at EBMT 2015 and therefore the following table lists the results for acute leukemia/myelodysplastic syndrome, and lymphoma/multiple myeloma

Conclusions The Delphi method is a useful tool to determine and classify key risk factors for IFI caused by filamentous fungi in patients with hematologic cancer. This identification can aid the management of patients at high or changing risk for IFI, when deciding on prophylaxis adjustments or initiation of early antifungal treatment. The method can be used to construct and validate a score with the risk factors selected, so that the most adequate strategy can be adapted according to an individual assessment of each case.

AGREEMENT INDEX (maximum and high risk)	KEY RISK FACTORS	
	ACUTE LEUKEMIA AND MYELODYSPLASIC SYNDROMES	LYMPHOMAS/MULTIPLE MYELOMA
100%	• Deep (< 100 cells/mL) or prolonged (>14 d) neutropenia • Consolidation without response, or refractory • History of IFI	
99%-90%	• Induction treatment	• Use of high-dose steroids > 1 mg/kg/d, > 2 weeks)
89%-80%		• Neutropenia
79%-70%	• Prophylaxis with broad-spectrum azoles • Proximity to construction or refurbishment areas • Rooms without HEPA filters	• Hematologic disease in progression • Anti-CD52 biologic therapies • Proximity to construction or refurbishment areas

IFI: invasive fungal infection.

Figure 1

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High frequency of HIV-seronegative patients colonized by *Pneumocystis jirovecii* in a Community Central Hospital in Lisbon, Portugal

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Pneumocystis jirovecii is an important opportunistic agent that causes one of the most severe respiratory infections in immunocompromised patients, known as *Pneumocystis* pneumonia (PcP). Although PcP is commonly associated with HIV infection, the numbers of PcP cases and *P. jirovecii* asymptomatic carriers are increasing among HIV-seronegative patients with other predisposing immunodeficiencies. In the last decades, molecular techniques have allowed the detection of very low burdens of *P. jirovecii*, usually not detectable by the classic microscopic methods.

The aim of this study was to determine the frequency of *P. jirovecii* infection in HIV-seronegative Portuguese patients from a community central hospital in Lisbon, using molecular approaches.

A total of 274 respiratory specimens were obtained from HIV-seronegative patients, underlying medical conditions included: 139 malignancies, 45 chronic obstructive pulmonary diseases (COPD), 37 pneumonias (not PcP), 17 tuberculosis (TB), 16 autoimmune diseases, nine solid-organ transplantation and 11 other causes.

All samples were analyzed by real-time PCR (RT-qPCR) targeting the *P. jirovecii* nuclear single-copy kexin-like serine protease (*KEX1*) gene and by nested-PCR directed to the *P. jirovecii* mitochondrial multicopy large-subunit rRNA (*mtLSU rRNA*) gene.

The analysis of the results, indicate that *P. jirovecii* DNA was detected in 51 (36.7%) malignancies, 20 (44.4%) COPD, nine (24.3%) pneumonias (not PcP), one (5.9%) TB, five (31.2%) autoimmune diseases, eight (88.9%) solid-organ transplantation and four (36.4%) other causes. There was a statistically significant association between solid-organ transplantation HIV-seronegative ($P = 0.003$) patients and the presence of *P. jirovecii* DNA.

This study confirms the importance of the HIV-seronegative patients, with other predisposing immunodeficiencies, colonized by *P. jirovecii* in a hospital environment. This population is at risk of developing PcP or may transmit the pathogen to other susceptible patients, and may play an important role in the circulation and transmission of *P. jirovecii* in the community.

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Non-Aspergillus fungal rhinosinusitis at a tertiary-care hospital and the first report of human infections by *Trichoderma asperellum*

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Objective Describe a cohort of 27 cases of fungal rhinosinusitis caused fungi other than *Aspergillus*, which have been diagnosed at university hospital in Porto Alegre, Brazil during a 24-year period, with a particular emphasis on their etiologic agent and the predisposing factors. In this series we also report the first case of rhinosinusitis and human infection by *T. asperellum*.

Methods A retrospective study was conducted in our institution (1989–2014). For mycological diagnoses, paranasal tissue obtained at surgery was subjected to histopathology examination and sent for fungal cultures, patients must have fulfilled the following criteria: positive nasal sinus cultures and/or biopsy specimens demonstrating fungal hyphae.
Results Fungal cultures were obtained in 20 cases and showed no growth in 07 cases. Classification of mycotic disease of the nose and paranasal sinuses in invasive (9) and non-invasive (18) based on clinical, radiological, and histopathological. A total of 14 of 27 cases were in women, and 13 cases were in men. The mean age of patients was 45.26 years. The most common pathogens were *Histoplasma capsulatum* ($n = 4$), *Scedosporium apiospermum* ($n = 2$), *Alternaria alternata* ($n = 2$), *Schizophyllum commune* ($n = 2$), *Pseudallescheria* boydii ($n = 1$), *Penicillium* sp ($n = 1$), *Absidia corymbifera* ($n = 1$), *Xylaria enteroleuca* ($n = 1$), *Trichoderma asperellum* ($n = 1$), *T. harzianum* ($n = 1$), *T. viride* ($n = 1$), *Fusarium solani* ($n = 1$), *Cladosporium sp* ($n = 1$) and *Cryptococcus neoformans* ($n = 1$). From the ones that have non-growth, four was classified as hyalohyphomycosis and three mucormycosis by the histopathological findings. In addition, we describe the first well-documented case of rhinosinusitis and human infection by *T. asperellum*, in a 44-year-old woman, with a history of asthma, chronic rhinosinusitis, and allergic rhinitis since childhood.
Conclusions Fungal infection of the paranasal sinuses is an uncommon disease. *Aspergillus* and *Mucor* are the most commonly implicated fungal organisms in invasive rhinosinusitis. Nevertheless, numerous fungi can colonize the paranasal sinuses, and it is not surprising that many them can cause symptomatic infections, but rarely documented. It is important to report this series of non-aspergillus rhinosinusitis, highlighting the main clinical, etiological and diagnostics findings, to alert clinicians.

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Fusarium infections in pediatric oncology patients: 13 years' experience in a single Brazilian institution

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Background *Fusarium* species are ubiquitous moulds that may cause severe opportunistic infections in immunosuppressed patients. In Brazil this pathogen is one of the most common molds causing invasive fungal disease in patients with hematological malignancies and/or undergoing hematopoietic stem cell transplantation (HSCT). Infections occur by inhalation of airborne conidia or skin breakdown due trauma, burns or central lines. There is scarce literature data on fusariosis in pediatrics.
Aim To describe clinical and epidemiological aspects of 15 consecutive pediatric patients with invasive fusariosis in a single institution.
Methods We reviewed all culture-proven *Fusarium* sp infections diagnosed at the Pediatric Oncology Institute (IOP/GRAACC-UNIFESP), Sao Paulo, Brazil. The case definition was based on EORTC/

Table1: Cases of bloodstream infections related to central line venous catheter caused by *F.oxysporum*

Case	Underlying disease	HSCT	Age	Gender	Neutropenia	CVC	Galactomannan	Chest CT	Abdominal Ultrassound	ECO	Fundoscopic exam	Therapy	Duration of therapy (days)	Outcome
1	Solid tumor	Auto	3 years	M	yes	Port-a-cath	negative	normal	normal	normal	normal	Voriconazole	21	Alive
2	Solid tumor	no	2 years	F	no	Port a cath	negative	normal	ND	normal	normal	Voriconazole	21	Alive
3	Solid tumor	no	1 year	M	no	Port a cath	negative	normal	ND	normal	normal	Voriconazole	21	Alive
4	Solid tumor	no	9 months	F	no	Port a cath	negative	normal	ND	normal	normal	Ampho B L	16	Alive
5	Solid tumor	Auto	1 year	F	no	Port a cath	negative	normal	ND	normal	normal	Voriconazole	21	Alive
6	Solid tumor	no	2 year	F	no	Port a cath	negative	normal	normal	normal	normal	Voriconazole	21	Alive
7	ALL in remission	no	8 years	M	no	PICC	negative	normal	ND	normal	normal	Voriconazole	14	Alive

ALL – acute lymphoblastic leukemia. Auto- Autologous. M – male. F- female. CVC- central venous catheter. PICC- peripherally inserted central catheter. ampho B L, amphotericin liposomal, ND- not done.

Table 2: Cases of disseminated invasive tissue infections by *Fusarium* spp

Case	Underlying disease	Status of the underlying disease	HSCT	Age years	Gender	CVC	Duration of neutropenia before Fusariosis (days)	Acute GVHD	Chronic GVHD	On steroids	Galactomannan	Fever	Sites of infection	Portal of entry	Prymary Therapy	*Fusarium* species	Outcome	Cause of Death
8	ALL	Active (relapsed)	Allogeneic mismatched related	5	M	Port a cath	12	No	No	Yes	ND	Yes	Blood,lungs	Airways without positive culture	Ampho B deoxicolate	*solani*	Died	Other cause
9	Solid tumor	Remission	Autologous	2	F	Port a cath	NA	No	No	No	ND	Yes	CVCtip, blood, lungs	CVC with positive culture	Voriconazole	*solani*	Alive	NA
10	ALL	Active (relapsed)	no	15	F	Port a cath	5	NA	NA	Yes	Negative	No	Lungs, sinuses	Airways with positive culture	Voriconazole	*solani*	Died	Fusariosis and underlying disease
11	ALL	Active (relapsed)	no	16	M	Port a cath	17	NA	NA	Yes	Positive	Yes	Skin, blood, lungs	Airways without positive culture	Voriconazole	*solani*	Died	Fusariosis and underlying disease
12	ALL	Active (relapsed)	no	18	F	Port a cath	50	NA	NA	Yes	Negative	No	Skin	No	Voriconazole	*solanii*	Died	Fusariosis and underlying disease
13	ALL	Complete remission	Unrelated cord blood	10	M	Port a cath	9	Grade III	NA	No	Positive	Yes	Skin, blood, lungs	No	Voriconazole and ampho B L	*oxysporum*	Died	Other cause
14	AML	Complete remission	Allogeneic matched related	9	F	Port a cath and non tunneled CVC	NA	NA	Extensive	Yes	Negative	No	Skin, lungs	Airways with positive culture	Voriconazole and ampho B L	*solani*	Died	Fusariosis
15	ALL	Active (relapsed)	no	14	M	Port a cath	49	NA	NA	Yes	ND	Yes	Skin, blood, CVC tip, lungs, liver	Airways without positive culture	Voriconazole and ampho B L	*solani*	Died	Fusariosis and underlying disease

ALL – acute lymphoblastic leukemia ; AML: acute myeloid leukemia;HSCT: hematopietic stem cell transplantation; CVC: central venous catheter; M: male; F: female; ampho B L: amphotericin liposomal; NA - not applicable, ND – not done

MSG criteria. Fungal isolates were identified by phenotypic and molecular methods.
Results From 2002 to May 2015, 15 children were diagnosed with invasive fusariosis. The mean age was 7 years (9 months–18 years) and 7 were male. *Fusarium oxysporum* caused seven catheter-related bloodstream infections (BSI) (Group 1 -Table 1). *Fusarium solani* was isolated in seven out of eight disseminated tissue infections and *F oxysporum* in one (Group 2- Table 2). Group 1 ($N = 7$) included patients with mean age of 2.5 years (9 months-8 years), 6/7 had an underlying solid tumor and only one patient was neutropenic at the time of the positive blood culture. Patients had positive cultures drawn from the central line, catheter tips and swab from the port-a-cath reservoir. All peripheral blood cultures, chest CT and serum galactomannan were negative. All patients had the central line promptly removed and voriconazole was the therapy of choice in all but one patient who received liposomal amphotericin B. All patients survived without any further complication. Group 2 included children with disseminated tissue infections ($N = 8$) with mean age of 11years, 4 were male, 7/8 had underlying acute leukemia: 5 had active disease and 2 were in remission undergoing allogeneic HSCT. Most patients (6/8) were receiving prednisone (mean dose of 2.5 mg kg day^{-1}) and neutropenia was present in 6, lasting for 5 to 50 days before the diagnosis of fusariosis. The infection involved lungs (7), skin (6), sinus (1), and liver (1). Five had tissue involvement and a positive blood culture and 3 only positive tissue biopsies. Galactomannan was tested in 5 patients and positive in two. It was possible to identify the portal of entry in 6 patients: airway in 5 and central line in one. Only one (non-neutropenic) patient survived, but Fusariosis was the immediate cause of death in just 1/8 cases.
Conclusion This is the largest case-series of invasive *Fusarium* infection in a pediatric oncology cohort. Catheter-related blood stream fusariosis caused by *F. oxysporum* in non-neutropenic patients has excellent outcome with the central line immediately removed and systemic therapy. Patients with disseminated *Fusarium* tissue infections and underlying hematologic malignancies, on steroids or undergoing HSCT had high mortality. Galactomannan presented low sensitivity to diagnose fusariosis in this pediatric setting, especially for *F. oxysporum* infection.

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Retrospective evaluation of new diagnostic methods for the detection of invasive candidiasis in paediatric haematooncology patients

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Objectives Invasive candidiasis (IC) is important cause of morbidity and mortality in immunocompromised host. The detection of invasive fungal diseases (IFD) remains a challenge and there are few data in paediatric patients published. Therefore we performed retrospective study evaluating two methods of detection: (1→3)-β-D-glucan and PCR, as they are rarely published in children.
Methods We tested 157 serum samples of 33 patients (20F/13M, age 0.7–20.1, median 6.64 years) in high and intermediate risk of IFD treated in period 07/2012 to 06/2013 in Department of Paediatric Haematology and Oncology, Motol University Hospital, Prague, Czech Republic. All cases of IFD were classified according to EORTC/MSG 2008. Serum samples were retrospectively tested for the presence of glucan (Fungitell, Associates of Cape Cod, East Falmouth, MA, USA) and *Candida* spp. DNA (RenDx Fungiplex Assay, Renishaw Diagnostics Ltd., Glasgow, UK). DNA extraction was performed by QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany). Obtained results were added to results of the standard methods used in our hospital: blood culture (Becton Dickinson, NJ, USA) and mannan antigen (Platelia Candida, Bio-Rad, California, USA). All tests were performed according to the manufacturer's instructions.
Results All 7 detected IFD cases were IC; 4 were proven and 3 probable IFDs and were observed mainly in leukaemia patients (3F/4M, 1.7–16.3 years, 5 ALL, 1 AML, 1 NHL). The invasive candidiasis was detected as candidaemia (2× *C. albicans*, 1× *C. fabianii*), hepatolienal with/without affection of other organs (3x) and pulmonary (1x). Glucan was positive in all 7 patients with IC, false positivity was observed in 13 patients (test evaluation: sensitivity 100%, specificity 50%). The onset of glucan positivity correlated with the blood culture or PCR positivity or with the development of the hepatosplenic infiltrates after restoration of haematopoiesis in pancytopenic patients. The long-term evaluation of glucan in single patient showed persistent positivity in IC cases. *Candida* PCR was positive in 4 patients (3× *Candida* spp., 1× *C. glabrata*). PCR reaction was inhibited in 1 patient in sample correlating with the onset of the disease and there were 2 false positive patients detected (test evaluation: 80%, 92%). In 2 IC cases PCR was the only proof of the pathogen. Mannan was positive in 4 IC (test evaluation: 57%, 88%).
Conclusion The study confirmed accessory value of new methods in IFD diagnostic to the conventional methods. In our cohort, we confirmed all IC cases by glucan; in one case it was the only positive technique. PCR helped identify the etiological agent in 2 additional cases.
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Risk factors and clinical outcome of breakthrough yeasts bloodstream infections in patients with hematological malignancies in the era of new antifungal agents

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Objectives In recent years, breakthrough candidemia constitutes a substantial proportion of candidemia in patients with hematological malignancies, ranging from 19% and 72%. In the same population, the rare yeasts bloodstream infections (BSIs) are increasingly seen and associated with high mortality rate. This study was performed to identify the risk factors and clinical outcome of breakthrough yeast BSIs in these patients in the era of new antifungal agents.
Methods We reviewed medical records of all consecutive patients with hematological malignancies developing yeasts BSIs at a tertiary care center from January 2009 to December 2014. Breakthrough yeasts BSIs were defined as recovery of at least one yeast by blood culture in patients receiving one or more systemic antifungal agents for ≥3 days before the first positive blood culture. We performed a case-control study comparing breakthrough BSIs with *de novo* BSIs.
Results In this population-based, prospective, observational study, 21 (43%) of 49 patients with yeasts BSIs met the definition for breakthrough yeasts BSIs. Overall, the most common yeast was *Candida tropicalis* (12 of 51 isolates, 24%). Among 4 non-*Candida* yeasts (2 *Saccharomyces cerevisiae*, 1 *Trichosporon asahii*, and 1 *Pseudozyma aphidis*) isolated from 4 patients, 3 cases were breakthrough BSIs. Patients with breakthrough BSIs were receiving various kinds of antifungal agents (fluconazole [$n = 11$], itraconazole [$n = 3$], posaconazole [$n = 1$], amphotericin B [$n = 3$], caspofungin [$n = 2$], or micafungin [$n = 1$]). In multivariate analysis, acute leukemia or myelodysplastic syndrome as underlying hematological malignancies (OR 9.735, 95% CI 1.916–49.449, $P = 0.006$) and neutropenia

within 3 days prior to yeasts BSIs (OR 11.274, 95% CI 2.062–61.633, $P = 0.005$) were significantly associated with breakthrough BSIs. The proportions of *C. krusei* and *C. tropicalis* were significantly different between breakthrough BSIs and *de novo* BSIs (32% vs. 3%, $P = 0.015$; 5% vs. 38%, $P = 0.007$, respectively). As the first-line antifungal agents, amphotericin B (57%) was most frequently administered, followed by echinocandins (22%) and triazoles (20%). The appropriateness of first-line antifungal regimen was not different between breakthrough BSIs and *de novo* BSIs (86% vs. 93%, $P = 0.639$). The 6-week and overall mortality rates of breakthrough yeasts BSIs were not different from *de novo* BSIs (33% vs. 43%, $P = 0.564$; 62% vs. 64%, $P = 1.000$). Independent predictors for 6-week mortality were refractory neutropenia (HR 64.488, 95% CI 10.488–368.852, $P < 0.001$) and Pitt bacteremia score (HR 1.886, 95% CI 1.142–3.114, $P = 0.013$).

Conclusion Breakthrough yeasts BSIs were associated with acute leukemia or myelodysplastic syndrome and neutropenia. The distribution of yeasts species in breakthrough BSIs was significantly different from *de novo* BSIs. However, breakthrough BSIs did not show a higher mortality rates compared with de novoBSIs.

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A new time dependent analytical approach for assessment of the impact of Invasive Aspergillosis on short and long-term survival

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Objectives Patients with hematological malignancies treated with chemotherapy courses and haematopoietic stem cell transplantation (HSCT) are at risk for the development ofInvasive Aspergillosis (IA). This complication has often been reported to yield a high mortality rate. However, patients with a generally unfavourable prognosis with regard to their hematological condition have a higher probability to develop IA as well as a greater risk of death due to other disease related causes. To assess the short and long term association of IA with mortality in this vulnerable patient population, full consideration of changing patient characteristics over time is necessary to obtain reliable estimates of the correlation of IA with death.

Methods Consecutive patients who initiated remission-induction therapy for AML or MDS at the Leiden Academic Medical Centre were included. During the period of study an assertive protocol for diagnosis of suspected fungal infection was executed instead of standard antifungal prophylaxis. Population characteristics and data acquisition was previously described and published. IA was classified according to the 2008 revised European Organization for Research and Treatment of Cancer criteria. We studied the effect of IA on risk of death by means of four different survival analyses, using the Cox proportional hazards model, considering (1) time from diagnosis to death before HSCT, (2) time from diagnosis to HSCT, (3) time from HSCT to death after HSCT and (4) time from diagnosis, both before and after HSCT.

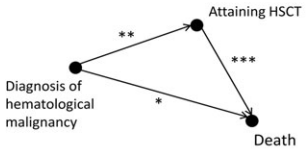
Adjustment for confounding variables was performed (the use of granulocyte colony stimulating factor (GCSF), age, sex, WHO hematologic risk category, number of chemotherapy courses needed to accomplish complete remission, previous lung disease, secondary or relapsed hematologic disease and a day-by-day appraisal of the presence of neutropenia). The presence of neutropenia was entered as time dependent covariate. The analyses were performed using STATA v 12.0.

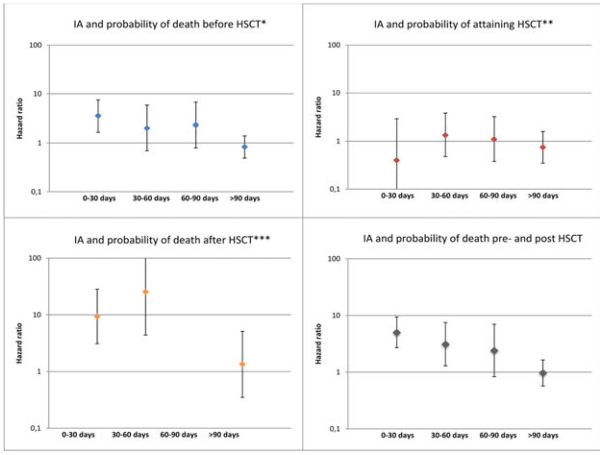
Table 1 Hazards ratios for reaching the endpoint per analysis and for probability of death pre- and post HSCT after diagnosis of IA and different co-variates

Variable	Probability of death before HSCT	Probability of attaining HSCT	Probability of death after HSCT	Probability of death pre- and post HSCT
Number of days after IA				
0 - 30 days	3.5 (1.7 - 7.5)	0.40 (0.05 - 2.9)	9.4 (3.1 - 28.3)	5.0 (2.7 - 9.3)
30 - 60 days	2.0(0.69 - 5.9)	1.3 (0.48 - 3.8)	25.5(4.4 - 146)	3.1 (1.3 - 7.6)
60 - 90 days	2.3 (0.79 - 6.8)	1.1 (0.39 - 3.2)	NA*	2.4 (0.83 - 7.0)
> 90 days	0.80 (0.49 - 1.4)	0.75 (0.35 - 1.6)	1.3 (0.35 - 5.1)	0.97 (0.57 - 1.6)
Neutropenia (per day)	1.1 (0.60 - 2.0)	0.67 (0.36 - 1.2)	12.3 (0.97 - 155)	1.1 (0.60 - 1.9)
Gender (male)	1.3 (0.92 - 1.9)	1.3 (0.86 - 2.0)	1.4 (0.79 - 2.5)	1.3 (0.90 - 1.9)
Age (per year)	1.02 (1.00-1.03)	0.98 (0.97-0.99)	1.00 (0.97-1.02)	1.02 (1.00-1.03)
Previous lung disease	1.4 (0.87 - 2.3)	0.68 (0.37 - 1.3)	1.9 (0.82 - 4.5)	1.4 (0.86 - 2.3)
Use of G-CSF	0.78 (0.48-1.3)	2.6 (1.6 - 4.1)	0.66 (0.33-1.3)	0.82 (0.50-1.3)
Secondary AML	1.6 (0.81 - 3.1)	0.68 (0.27 - 1.7)	1.9 (0.66 - 5.4)	1.4 (0.71 - 2.8)
Relapsed AML	3.0 (1.9 - 4.9)	0.49 (0.23 - 0.99)	2.4 (0.93 - 6.0)	2.7 (1.7 - 4.5)
Hematological risk classification (per category)	1.4 (1.1 - 1.9)	1.0 (0.77 - 1.4)	1.1 (0.69 - 1.7)	1.4 (1.0 - 1.9)
Number of courses before complete remission (per course)	1.6 (1.1 - 2.3)	0.56 (0.35 - 0.89)	1.3 (0.66 - 2.8)	1.6 (1.1 - 2.3)

Legend: HSCT : hematological stem cell transplantation; IA: Invasive Aspergillosis; G-CSF: Granulocyte-Colony Stimulating Factor; AML: Acute Myeloid Leukemia; *: No events in this group. Values shown are Hazard ratios with 95% Confidence Interval between brackets.

Figure 1 Phases of treatment of haematological malignancy corresponding to analytical models estimating the probability of death after diagnosis of IA.





Legend: HSCT: hematological stem cell transplantation; IA: invasive aspergillosis; bars represent 95% confidence intervals. Each of the arrows in the top figure designates a particular phase or state for which a specific model is constructed. The asterisks (*) correspond with the accompanied graphs.

Results In 55 of 167 enrolled patients, at least possible IA was diagnosed at some point during treatment. Hazard ratios for death after diagnosis of IA were calculated by using the above described models, and adjusting for the confounding variables (table 1). Hazard ratios (HR) and 95% Confidence Intervals (95%CI) on mortality after the diagnosis of IA within 30 days, between 30 and 60 days, between 60 and 90 days or more than 90 days were 5.0 (2.7–9.4), 3.1 (1.3–7.6), 2.4 (0.83–7.0) and 0.97 (0.57–1.6) respectively in model no.4. The HR to reach the point of stem cell transplantation was not significantly altered by the occurrence of IA prior to HSCT (figure 1).
Conclusion The results provide new insight in short and long-term survival of patients diagnosed with IA. By applying a new analytical approach, we found that the association of IA with mortality remained substantial. However, while an increased risk of death was significantly present in the first and second month after diagnosis of IA, no unfavourable association with mortality was observed in the later course of treatment. Other patient characteristics conferred comparable HRs for death. Furthermore, the occurrence of IA did not affect the probability of attaining HSCT in our population.

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Galactomannan Testing and *Aspergillus* PCR in same-day bronchoalveolar lavage and blood samples obtained from patients with hematological malignancies at risk for invasive mould infection

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Objectives Invasive pulmonary aspergillosis (IPA) is a live threatening disease in patients with hematologic malignancies. Early and reliable diagnosis is needed for rapid initiation of first line antifungal (AF) therapy, which is crucial in order to reduce the mortality rate. In recent years galactomannan antigen testing (GM) and also *Aspergillus* PCR have become increasingly important for IPA diagnosis. Whether or not these tests need to be performed with bronchoalveolar lavage fluid (BALF; i.e. primary site of infection), or testing of blood samples is sufficient, remains, however, a matter of debate.
Methods We evaluated the diagnostic performance of GM ELISA, Aspergillus PCR and other tests (e.g. Panfungal PCR, Aspergillus specific lateral flow device test, culture) for invasive mould infection (IMI) by using bronchoalveolar lavage fluid samples and blood samples obtained at the same day (within 0–4 h). All patients with hematologic malignancies, who received bronchoscopy and BALF sampling between April 2014 and April 2015 at the Medical University of Graz, Austria were screened for includion in this prospective study. IPA and IMI were defined according to the revised EORTC/MSG criteria.
Results 42 bronchoalveolar lavage/biopsy samples from 34 patients were included in the final analyzes. One patient had proven invasive mucormycosis (with positive panfungal PCR result from biopsy specimens, but negative PCR result from blood). Of the remaining 41 cases, 8 (20%) had probable or proven IPA, 9 (22%) possible IPA, while 24 (58%) did not fulfill IPA criteria. Sensitivity, specificity, positive predictive value, negative predictive value, and diagnostic odds ratio of the different tests for diagnosing probable/proven IPA are shown in table 1. The combination of positive GM result and/or *Aspergillus* PCR was significantly more sensitive for detection of probable and proven IPA when performed in BAL (75% sensitivity), when compared to same day blood specimens (sensitivity 13%; $P = 0.04$).

Table 1 Sensitivities, Specificities, PPVs, NPVs, and DORs (including 95% CI)for GM, LFD, BDG and conventional culture for diagnosing probable/proven IPA versus no IPA

Test methods	Sensitivity	Specificity	PPV	NPV	DOR
BAL GM >0.5 ODI	63% (5/8)	96% (23/24)	83% (5/6)	88% (23/26)	38.3 (3.3-449)
Serum GM >0.5 ODI	13% (1/8)	96% (23/24)	50% (1/2)	77% (23/30)	3.3 (0.2-60)
BAL Aspergillus PCR	25% (2/8)	96% (23/24)	67% (2/3)	79% (23/29)	7.7 (0.6-100)
Blood Aspergillus PCR	0% (0/8)	100% (24/24)	0% (0/0)	67% (24/32)	-
GM and/or Aspergillus PCR BAL	75% (6/8)	92% (22/24)	75% (6/8)	92% (22/24)	33 (3.8-286)
GM and/or Aspergillus PCR Blood	13% (1/8)	96% (23/24)	50% (1/2)	77% (23/30)	3.3 (0.2-60)
BAL LFD	38% (3/8)	96% (23/24)	75% (3/4)	82% (23/28)	13.8 (1.2-162)
Conventional BAL Culture	0% (0/8)	100% (24/24)	0% (0/0)	67% (24/32)	-
BAL BDG >80 pg/mL	38% (3/8)	38% (9/24)	17% (3/18)	64% (9/14)	0.4 (0.1-1.9)
BAL BDG >200 pg/mL	13% (1/8)	79% (19/24)	17% (1/6)	73% (19/26)	0.5 (0.1-5.5)
Serum BDG >80 pg/mL	63% (5/8)	71% (17/24)	42% (5/12)	85% (17/20)	4.1 (0.8-21.7)

Abbreviations: PPV = positive predictive value; NPV = negative predictive value; DOR = diagnostic odds ratio; 95% CI = 95% confidence interval; GM = galactomannan; LFD = lateral-flow-device; BDG = 1,3-beta-d-glucan; IPA = invasive pulmonary aspergillosis; BAL = bronchoalveolar lavage; ODI = optical density index

Conclusions Our results indicate that among patients with hematological malignancies at risk for IPA, PCR and GM testing should preferably be performed in samples from the site of infection (i.e. BAL or biopsy), as the diagnostic yield when these tests are performed in blood might be significantly lower.

P292

Cost of antifungal drugs in different haemato-oncology settings

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Objectives Invasive fungal disease (IFD) remains a difficult area in Haemato-Oncology, with significant clinical and financial impact. This study calculates the cost of antifungal medications per admission over a 5 year period, costs per type of leukaemia and transplant, and the costs during induction versus subsequent cycles of chemotherapy. In addition, the EORTC scores for each episode were correlated with the cost of antifungal drugs.
Methods A retrospective analysis was performed of 528 inpatient episodes (representing 303 patients) in the Division of Haemato-Oncology at St Bartholomews Hospital between 2004 and 2009. EORTC scores (2008) were calculated. Anti fungal prices were extracted from the local formulary (July 2014) as cost per mg of the drug. Costs were calculated per episode (admission) and statistical significance analyses applied to determine the differences between cohorts of patients. The data set includes 118 episodes where voriconazole was used principally for primary prophylaxis of acute meloid leukaemia (AML) induction chemotherapy according to local protocol.
Results Of a total 528 episodes, 408 were described in patients with AML or high-risk myelodysplastic syndrome. Acute lymphoblastic leukaemia (ALL) was the primary diagnosis in 37 episodes. Prophylactic antifungals alone were prescribed in 320 episodes, while in 157 episodes prophylactic and treatment antifungals were given. There were 137 episodes of bone marrow transplants including 111

reduced-intensity allografts (sibling and unrelated), 12 ablative allografts (unrelated and sibling) and 14 autografts.

In the whole cohort (n = 528), the mean total cost of prophylactic antifungals was £1150.80, compared to the mean total cost of treatment, which was £1762.10. The mean total cost of antifungal drugs in all 528 episodes was £2912.90.

The total antifungal cost per episode associated with AML intensive chemotherapy (n = 408) was higher (mean £3196.60) than those with ALL (n = 37; £2706.70) (P = 0.509). Moreover, antifungal therapy during induction chemotherapy for AML (n = 68) cost more (£3643.60) than during post induction regimes (n = 280; £2817.70) although this difference is not statistically significant (P = 0.123).

The antifungal costs for different transplants were a mean £3262 vs £2769.70 vs £1720.20 for ablative allografts (n = 111), reduced intensity allografts (n = 12) and autografts (n = 14), respectively.

In 118 episodes where voriconazole was used as prophylaxis, the total antifungal costs for these episodes were significantly more expensive than the mean total antifungal cost for the 353 episodes where fluconazole was used as primary prophylaxis (£3861.50 vs £2330.60) ($P \leq 0.001$). Using the 2008 EORTC/MSG scoring system, total costs for treatment and prophylaxis were: £1 351 302.60 in the 'none'category (i.e, no evidence of IFD; n = 489; mean of £2763.40 per episode); £115 410.40 with a 'possible' score (n = 28; mean £4121.80); £35 225.60 for a 'probable' score (n = 4; mean £8806.40); and £36 056.30 for a 'proven' IFD (n = 7; mean £5150.90).

Conclusion These data provide average costs for antifungal drug use for AML, ALL and different types of transplant. Mould-active prophylaxis was associated with increased total antifungal drug expenditure. Interestingly, the vast majority of antifungal drug expenditure occurs in episodes classified as unlkely to have an IFD by the EORTC/MSG criteria.

P293

Fungal infections by filamentous fungi in hematologic patients at high risk. It's useful looking for fungal DNA in serum?

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Objectives The aim of this study was to compare three different commercial available RT-PCR assays in hematological patients at high risk of fungal infection for early diagnosis of invasive aspergillosis (IA). The objective is to assay the sensitivity of each of the kits in the three methods of extraction and amplification.

Methods From January to March 2014, 86 samples of serum from patients admitted to the CTMO (Center Bone Marrow Transplant) and at the Hematology of Verona were analyzed; the patient were University of Verona, Italy selected according to seric fungal biomarkers results: 59 negative for galactomannan assay (GM), 27 positive; 58 negative for (1-3) Beta-D-Glucan (BG) assay, 28 positive.

The detection of serum DNA of filamentous fungi was performed using three diagnostic kits: Liferiver ™ fungus real time PCR kit (Shanghai ZJ Bio -Tech Co) which provides extraction and amplification reagents.

MycoGENIE Aspergillus fumigatus Real-Time PCR kit. DNA extraction was made automatically with AutoMag solution (Automated DNA Extraction-Ademtech, Pessac, France).

Myconostica Myc Assay ™ Aspergillus Cepheid Smartcycler serum. In this case extraction was performed with High PCR template preparation Kit by Roche Diagnostics (Mannaheim, Germany).

All Real time PCR were run on a Smart Cycler II, software version 3.0 (Cepheid).

We have analyzed the three systems of amplification with the three extraction kit to evaluate the efficacy

Results To analyze the limit of detection of the three assays we performed the Real-Time PCR on serial dilutions of DNA of *Aspergillus*

Table 1. RT PCR assay results obtained with three commercial kits: RT-PCR kits are listed in columns. Different extraction methods (listed in raws) were combined to amplification reagents. GM: galactomannan assay; BG: β-D-Glucan assay

	Real-Time Myconostica (number of positive results)	Real-Time MycoGENIE (number of positive results)	Real-Time Liferiver ™ (number of positive results)
GM+ BG+ (19/86)			
Extraction Roche	2	0	0
Extraction MycoGENIE	3	3	0
Extraction Liferiver ™	0	0	0
GM+ BG- (8/86)			
Extraction Roche	0	0	0
Extraction MycoGENIE	0	1	0
Extraction Liferiver ™	0	0	0
GM - BG+ (7/86)			
Extraction Roche	0	1	0
Extraction MycoGENIE	0	0	0
Extraction Liferiver ™	1	0	0
GM- BG- (52/86)			
Extraction Roche	5	1	0
Extraction MycoGENIE	4	9	0
Extraction Liferiver ™	2	0	0

fumigatus in water, starting from a concentration of 20 μg ul^{-1} to a final concentration of 2×10^{-7} pg μl^{-1}.

The amplification kit gave the following results: Real-Time PCR Myconostica Myc Assay ™ were positive up to 2×10^{-4} pg μl^{-1} of DNA, Real-Time PCR MycoGENIE 2×10^{-6} pg μl^{-1}, Real-Time PCR Liferiver ™ 5 pg μl^{-1}.

We analyzed 19 patients. For each patient multiple sera were analyzed.

The results are summarized in table 1.

Conclusion Latest guidelines for the diagnosis of IA do not recommend any molecular test for the diagnosis, neither any preferable biology sample because lack of standardization.

We compared three commercial kits using serum from at risk hematological patients. Our study showed comparable results for Myconostica and MycoGENIE kit, even with different extraction systems and even with some reinterpretation of positivity cut off. Lower sensitivity exhibit the Liferiver ™ kit, which showed an inferior performance also in the analysis of the limits of detection. This preliminary results confirm the utility of PCR in early diagnosis of IA.

P294

Reflex Cryptococcal antigen screening among children and adolescents in two districts in Gauteng province, South-Africa

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Objective To compare the number needed to test (NNT) to detect a case of new cryptococcal antigenemia for children, adolescents and adults during implementation of reflex cryptococcal antigen (CrAg) laboratory screening in South Africa. Current guidelines recommend screening for adults only; however, reflex laboratory testing was performed irrespective of age, enabling determination of NNT for children and adolescents.

Methods All persons with a CD4 count <100 cells μl^{-1} were reflexively screened for CrAg at 106 healthcare facilities in two Gauteng provincial districts from September 2012 through August 2014. Laboratory/clinical data were collected from patients with a positive plasma CrAg test at 45 facilities. Cases of incident cryptococcal meningitis (CM) were reported to national surveillance. CrAg screening and CM surveillance data were cross-matched to determine if screened patients had prior CM. Children, adolescents and adults were defined as persons aged ≤9 years, 10–19 years and ≥20 years, respectively.

Results The overall prevalence of antigenaemia was 4.5% (820/18357). Among those with recorded age, 3.7% (637/17149) were aged ≤19 years, 31 (4.9%) of whom had a positive plasma CrAg test; 24/31 (77.4%) were adolescents. The prevalence of antigenemia among adults, adolescents and children was 4.5% (735/16512), 5.0% (24/483) and 4.5% (7/154) respectively. The number needed to test (NNT) to detect a case of new antigenemia among adults, adolescents and children was 27 (16512/623), 28 (483/17) and 31 (154/5) respectively.

For 31 children/adolescents with a positive plasma CrAg test, the median CD4 count was 13 cells μl^{-1} (IQR, 8–31). Nine of these 31 (29.0%) had prior CM. Seventeen of 22 (77.3%) cases of new antigenaemia occurred among adolescents. Clinical data were available for 9 of these 22 cases; 2 were symptomatic. All 7 asymptomatic patients were prescribed fluconazole, CSF specimens were negative in all 4 where lumbar puncture was performed and no subsequent laboratory-confirmed CM was recorded for these 7 patients. Antiretroviral therapy (ART) was commenced for 5 patients; 2 were already on ART at screening.

Conclusion The NNT to detect a case of new antigenemia among children and adolescents was comparable to the NNT for adults. Children and adolescents with CD4 count <100 cells μl^{-1} could also be included in a cryptococcal screen-and-treat program.

P295

Diagnostic performance of 1,3-beta-d-glucan Serum Screening in Patients receiving Hematolopoietic Stem Cell Transplantation

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Background 1,3-beta-d-glucan (BDG) is used as a biomarker for invasive fungal infections (IFI). Patients receiving allogeneic or autologous hematopoietic stem cell transplantation (HSCT) are considered a highly vulnerable group for IFI development, therefore early detection and treatment of IFIs are crucial to improve survival. We evaluated the diagnostic performance of serum BDG screening in HSCT recipients.

Methods 33 HSCT recipients were prospectively enrolled in this study, which was performed between September 2014 and May 2015 at the Medical University hospital of Graz, Austria. Serum BDG screening was performed twice weekly (total 225 samples) by using the Fungitell® assay. All samples were classified according to the 2008 EORTC/MSG criteria (at the time the sample was obtained), with serum BDG results being not considered for classification. To evaluate the diagnostic performance of BDG testing for IFI we calculated sensitivity, specificity, negative predictive value (NPV), positive predictive value (PPV) and diagnostic odds ratio (DOR) for all samples.

Results 27 (71.1%) patients received allogeneic HSCT and 11 (28.9%) autologous HSCT. 24/38 patients were male and 14/38 female. Acute myeloid leukemia ($n = 17$) was the most frequently observed underlying disease, followed by non-hodgkins lymphoma ($n = 10$), acute lymphatic leukemia and myelodisplastic syndrome (each 4) and multiple myeloma ($n = 3$). 167/225 (74%) serum samples were collected from patients under ongoing antifungal prophylaxis. Performance of BDG screening is displayed in Table 1.

Conclusions Our data suggest that serum BDG screening in HSCT patients may be associated with a very high NPV of over 99% and therefore a helpful tool to rule out IFI.

P296/M11.2

Paecilomyces lilacinus and *Fusarium solani* central line infection in a paediatric patient with acute lymphoblastic leukaemia

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Objectives Fungaemia due to moulds is rare and the presence of more that one mould at the same time is exceedingly rare. We describe a case of central line infection caused by *Paecilomyces lilacinus* and *Fusarium solani* in a paediatric patient with acute lymphoblastic leukaemia treated with voriconazole and central line removal.

Methods *Paecilomyces* spp. and *Fusarium* spp.both cause hyalohyphomycosis. *Paecilomyces* spp. are found ubiquitously within the environment. These are uncommon pathogens with *P. lilacinus* and *P. variotii* the two species most frequently associated with human illness. Ocular and cutaneous disease are the most common presentations, however fungaemia is reported and associated with haematological malignancies and indwelling prosthetic material.

Fusarium spp. are the most common cause of fungal keratitis worldwide. In patients with immunodeficiency *Fusarium* spp. can cause disseminated disease and is second only to *Aspergillus* spp. within this population. Cutaneous manifestations of fusariosis can precede fungaemia and blood cultures may be positive in up to 40% of cases.

Both fungi were identified from the cultural characteristics and microscopic appearance as well as MALDI-TOF. Susceptibility to nine antifungals was performed using the Clinical and Laboratory Standards Institute (CSLI) recommended method.

Results A seven years old female was treated for relapsed acute lymphoblastic leukaemia with methotrexate, cytarabine and cyclophosphamide. Chemotherapy was given by central venous access via a Hickmann line. She attended our institution with neutropenic fever with no localising symptoms or signs. She was at the end of a once daily outpatient teicoplanin course for a recent line infection episode, when blood cultures via the Hickmann line had grown *Bacillus* sp. and *Rhodococcus* sp. She was commenced on piperacillin-tazobactam and vancomycin with resolution of the fever. Seventy 2 h after admission blood cultures flagged positive and filamentous fungi were identified on Gram stain. The microscopic appearance of the filaments were consistent with Paecilomyces spp. however the culture yielded *Fusarium spp.* which produce chlamydospores when they disseminate. As *Fusarium solani* sporulates faster than *Paecilomyces lilacinus*, extended incubation was required to differentiate the two moulds. The MICs of *P. lilacinus* and *F. solani* to voriconazole were 0.25 and 0.125 mg l^{-1} respectively however the MIC to amphotericin B for *P. lilacinus* was >8 mg l^{-1}. The patient received intravenous voriconazole and the central line was removed 16 days after admission. No deep seated focus was identified and she remained well and afebrile after 30 days.

Conclusion This is the first report, to our knowledge, of a mixed fungal central line infection with *P. lilacinus* and *F. solani*. This case highlights the importance of antifungal drugs susceptibility testing for moulds to target treatment. This allowed administration of single agent voriconazole. Removal of prosthetic material is critical in the successful management of fungal line infection.

P297

Use of May-Grunwald Giemsa stain for microscopic evaluation of cell culture inoculated with Microsporidia *in vitro*

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Microsporidia are obligatory intracellular spore-forming microorganisms that infect a wide range of invertebrate and vertebrate species. Traditionally, they were considered protozoa but recently have been reclassified phylogenetically as fungi. They have been recognized as human pathogen particularly in immunodeficient patients and after the advent of HIV and AIDS, interest in the *in vitro* culture of them has been increased. In our laboratory we have been used *in vitro* culture to study the genera and species *Encephalitozoon cuniculi* and *E. intestinalis* in monkey and rabbit kidney cell lines (Vero and RK-13 respectively). Objective: This study aimed to investigate the May-Grunwald Giemsa stain for microscopic evaluation of cells inoculated with *E. cuniculi in vitro*. Methods: Cell lines were incubated in sterile circular glass coverships in 24-well plates and were inoculated with spores of *E. cuniculi*. After the inoculation and incubation, the coverslips were fixed with methanol, stained with May-Grunwald Giemsa, mounted on glass slides and examined with a light microscope. Each entire coverslip was scanned at a magnification of × 1000. Results: May-Grunwald Giemsa stain permitted the visualization of parasitophorous vacuoles (PV) containing mature microsporidian spores in the cytoplasm of the infected cells. Conclusion: The stain used in this study is commonly used in hematology laboratory routine but has not been used to identify microsporidia spores. This result suggests that May-Grunwald Giemsa is adequate for visualize the PV containing spores of microsporidia inside the infected cell cultures to study this pathogenic fungi *in vitro*.

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Encephalitozoonosis in B-cell deficient XID mice

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Encephalitozoon cuniculi is an opportunistic pathogenic fungi for people with AIDS or other immune deficiencies. The adaptive immune response is essential for the elimination of *E. cuniculi*, but evidence indicates that the innate immune response is responsible for initiating and setting up the pathogen or will not survive. B-1 cells act as antigen-presenting cells, phagocytes, produce IL-10 and had other immune functions. The possible role of these cells in the dynamics of the inflammatory process of various etiologies is unknown. Objective: This study aimed to investigate the role of B-1 cells in *E. cuniculi* infection. Methods: BALB/c and BALB/c XID (deficient B-1 cells) were inoculated with fungal spores. The B-1 and B-2 cells, macrophages and $CD4^+$ and $CD8^+$ lymphocytes obtained from the spleen and peritoneal washes were quantities by flow cytometry. The Th1 cytokines and Th2 serum were quantified by flow cytometry by CBA assay. Results: We identified significant increase in macrophages population in the peritoneal cavity of infected BALB/c mice, but there was no difference in BALB/c XID infected or not. Despite the increase in the number of $CD4^+$ lymphocytes and $CD8^+$ cells in BALB/c mice XID, these animals were still susceptible to infection. Conclusion: We show that BALB/c XID mice were more susceptible to infection experimentally induced *E. cuniculi*, so these animals can be used as a biological model for encephalitozoonosis.

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The case of succesful treatment of pulmonary mycosis caused *Exophiala Dermatitidis* in a patient with acute myeloblastic leukemia (m4)

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Frequency of invasive mycoses due to rare pathogens in hematological patients has increased in last years. The number of publications about these mycoses is limited.

Methods A clinical case of successful treatment of pulmonary mycosis caused by *Exophiala dermatitidis* in a patient with acute myeloblastic leukemia (M4).

Results Male, 56 years old, was admitted to the Leningrad regional clinical hospital 31.10.2014 with mucous membranes hemorrhages, weakness, and fever up to 38.5 °C. The diagnosis was acute myeloid leukemia (M4 variant). He was treatment with '7 + 3', 'HAM' cytostatic chemotherapy. The patient's condition deteriorated on the background of the prescribed treatment (short of breath, rare cough, persisted subfebrile body temperature). Period of severe neutropenia ($<0.5 \times 10^9$) was 49 days.

CT scan of the chest was made on 16.12.2014: increased lung pattern due to the interstitial component in S6 and basal segments of the lungs. Antibacterial therapy was enhanced (imipenem, vancomycin, linezolid) with partial clinical effect, then empirical antimycotic therapy was prescribed - amphotericin B (1 mg kg day^{-1}) 19.12.2014. On control CT scan (26.12.2014), negative dynamics was identified, signs of lesions of both lungs (on the boundary of S2 and S6, S6, left in S6 and S10 - diameter 4.8 mm, 4.6 mm, 12 mm, 15 mm, respectively), small hydrothorax on the right (up to 7.2 mm). Test «Platelia *Aspergillus* EIA» (Bio-Rad) was negative in BAL. At microscopy of BAL the fungal elements not detected. Numerous colonies of black fungus were cultined. The isolate was identified as *Exophiala dermatitidis* morphologically and by ITS-sequensing. Amphotericin B were replaced by itraconazole 400 mg day^{-1}. The patient CSF. Clinical improvement of the patient and the positive dynamics of changes in the lung during a CT study (a minimum setback of infiltrative changes decrease from interstitial 08.01.2015).

Invasive fungal infection has stabilized after two months of antimycotic therapy. Due to the presence of risk factors the patient continued antifungal therapy with itraconazole.

Conclusion Onco-hematological patients with neutropenia need the fiber-optic bronchoscopy with mycological examination BAL, and mandatory identification of the pathogen. Successful treatment of fungal infections were requires a combination of adequate antifungal therapy and correction of neutropenia.

P300

A Comparison between Invasive Pulmonary Aspergillosis (IPA) caused by *Aspergillus fumigatus* and non-*fumigatus Aspergillus* species

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Background *Aspergillus fumigatus* is the most common pathogen that causes invasive pulmonary aspergillosis (IPA) in hematologic patients. However, recent studies have reported an emergence of IPA caused by non-*fumigatus aspergillus* species. The aim of this study was to evaluate the epidemiology, characteristics, and outcomes of culture-positive IPA and to compare the clinical characteristics of *A. fumigatus* and those of non-*fumigatus* IPA.

Method All consecutive cases of invasive fungal infections (IFIs) were reviewed retrospectively from January 2011 to February 2015 at the Catholic Blood and Marrow Transplantation Center. Only culture-positive IPA cases in hematologic patients were included in this study.

Results During the study period, there were 361 proven/probable IFIs, of which, proven/probable IPA and culture-positive IPA were 323 (89.5%) and 63 (17.5%) cases, respectively. The isolated pathogens consisted of 38 (60.3%) *A. fumigatus*, 20 (31.7%) non-*fumigatus*, and 5 (7.9%) of multiple species with *A. fumigatus* and non-*fumigatus Aspergillus species*. There were no significant differences in baseline patient characteristics, including age, sex, underlying hematologic diseases, treatment for hematologic diseases, severity of neutropenia, and graft-versus-host disease between *A. fumigatus* and non-*fumigatus* group. However, as an aspect regarding the manifestation of IPA, two or more organ involvement of IFI was more common in non-*fumigatus* group (2.6% vs. 20.0%, $P = 0.041$). Paranasal sinus (PNS) involvement was most common (75%) in IPA patients with ≥ 2 organs involvement. Overall and IPA-related mortality were not different between the two groups.

Conclusion Non-*fumigatus* consists one-third of culture-positive IPA in this institute. Multiple organ involvement, especially PNS, was frequent in non-*fumigatus* IPA compared to *A. fumigatus* IPA. Differences between non-*fumigatus* and *fumigatus* IPA should be the focus of future studies with *in vitro* susceptibility test of *Aspergillus* isolates with large number of patients.

P301

Outcomes of Hematologic Patients with Invasive Aspergillosis before Allogeneic Stem Cell Transplantation

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Background Previous invasive fungal infection (IFI) is one of the risk factors for recurrence of fungal infection and mortality in allogeneic stem cell transplantation (SCT) recipients. Until now, there is no report about the outcome of patients with remained invasive aspergillosis (IA) before SCT. The aim of this study was to evaluate the clinical characteristics and outcomes of patients continuing treatment of IA before the allogeneic SCT for the hematologic diseases.

Method All consecutive cases of IA were reviewed retrospectively from January 2011 to March 2015 at the Catholic Blood and Marrow Transplantation Center. Only proven or probable IA cases in adult hematologic patients were included in this study.

Results During the study period, there were 365 patients with proven/probable IAs, of which 96 patients had experienced IA before allogeneic SCT. Patients who had a history of IA that had been resolved ($n = 17$), coinfected with another fungus ($n = 13$), participated in clinical trials ($n = 1$) were excluded from this study. A total of 65 patients were included in the analysis. The median age was 49 years (interquartile range [IQR], 42 - 57). Sixty percent (39 of 65) of patients were male. Median time from the diagnosis of IA to SCT was 92 days (IQR, 57 - 130). All of the patients kept taking voriconazole per oral or intravenously according to the tolerability or severity of the mucositis. Relapse or progression of IA were observed in 8 of 65 patients (12.3%). Median time to relapse of IA was 253 days (IQR, 114 - 454). These 8 patients experienced relapse of underlying hematologic diseases or graft-versus-host disease (GVHD) that needs ≥ 2 immunosuppressant or high-dose steroid therapy. IA-related mortality and overall mortality was 9.2% (6 of 65) and 35.4% (23 of 65), respectively. Independent risk factors for death were refractory state of the underlying hematologic diseases (adjusted odds ratio [OR] 12.072, 95% confidence interval [CI] 2.058 - 70.796, $P = 0.006$) and GVHD (adjusted OR 0.134, 95% CI 0.036–0.504, $P = 0.003$).

Conclusion Performing SCT for underlying hematologic diseases might be feasible during the treatment of IA. Further criteria for the clinical conditions of IA patients or optimal timing of SCT should be studies in this group of patients.

P302

Invasive pulmonary aspergillosis in a kidney transplant recipient

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Objectives Invasive pulmonary aspergillosis is a fungal disease with high mortality rate that especially encountered in patients with

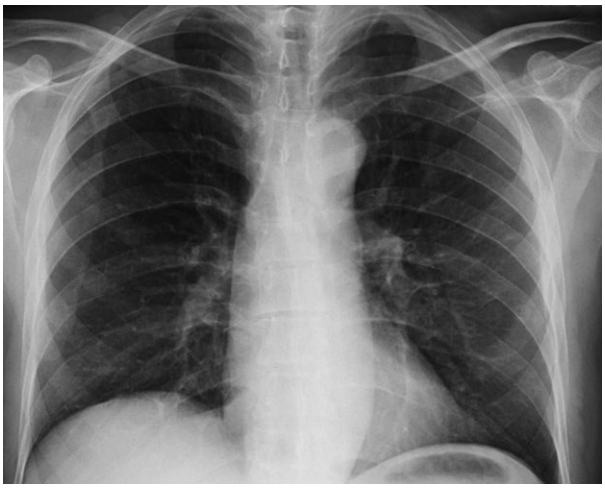

Figure 1. Chest radiography after 10 weeks of treatment

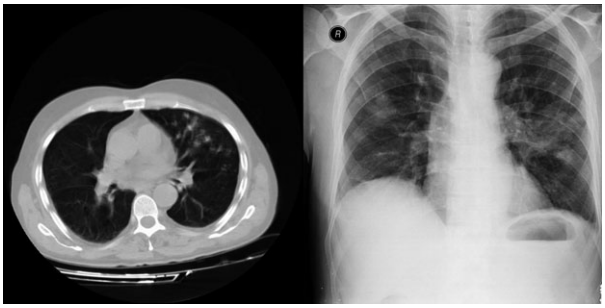

Figure 2. Chest radiography before treatment

hematological malignancies but also affect the immunosuppressive therapy patient population. We aimed to remind that invasive pulmonary aspergillosis could be seen in kidney transplant recipients.

Case A 50-year-old male, who underwent kidney transplantation a month ago, admitted to our hospital with complaints of cough, fever (38.7 °C) and poor general condition was hospitalized. The patient, who was considered as pneumonia, underwent chest x-ray and empirical antibacterial treatment was. Because of persistent fever on the fifth day of the treatment, sputum, urine and blood cultures were obtained, serum galactomannan antigen test was performed, and pulmonary HRCT (high resolution chest tomography) was obtained. The patient was on treatment of steroid and tacrolimus for solid organ transplantation. His laboratory examination was as follows: white blood cell (WBC): 14.400/mm3, CRP: 168 mg l^{-1} (0–5), creatinine: 3.3 mg dl^{-1} (0.67–1.17), BUN: 43. Serum galactomannan antigen test was positive (2.44, normal range between 0 and 1). HRCT revealed multiple pulmonary nodules bilaterally with the highest diameter of 7 mm in the left lung, which were considered in favor of fungal infection (Figure 1). With the diagnosis of invasive pulmonary aspergillosis, the patient was prescribed intravenous voriconazole. The patient was closely followed for the possibility of the accumulation of sulfobutylether-β-cyclodextrin, which is present in the intravenous form of voriconazole, in the patients with renal insufficiency. Because of a possible interaction with voriconazole, tacrolimus dose was decreased and its level was monitored. Amphotericin B treatment was initially not considered to be appropriate for this patient due to potential nephrotoxicity of the drug in a kidney transplant who had high creatinine clearance. On the ninth day of voriconazole treatment, serum galactomannan antigen level was negative. While fever decreased, WBS and CRP levels returned to normal limits. In the fourth week of intravenous voriconazole, it switched into oral voriconazole. There was a regression on follow up chest x-ray and HRCT images of the patients and no complication occurred (Figure 2).

Conclusion In kidney transplant recipients, invasive pulmonary aspergillosis should be considered in the patients with the clinical findings of pneumonia and especially in the ones who did not respond empirical antibacterial treatment. For the diagnosis, obtaining serum galactomannan antigen and HCRT should be kept in mind.

P303/M6.4

First report of the bloodstream infection associating with brain lesion by *Schizophyllum* commune in Brazil.

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Infections due *Schizophyllum commune* are rare in humans. The first report was an onychomycosis case occurred in 1950. Recently, a review of 114 cases revealed three cases presenting as brain abscess, including the first case of the brain abscess by *S.commune* from Brazil.

Objectives The objective of this study is to report the first case of bloodstream infection associated with brain lesion by *S.commune*, in a HIV-infected person, from Instituto Nacional de Infectologia Evandro Chagas, Brazil.

Methods and Results A 49 years old man, admitted in our institute with a difficult-to-treat HIV - tuberculosis (TB) coinfection. He was antiretroviral naïve, with baseline CD4 count was 106 cells μl^{-1} at that time. Aside respiratory symptoms and fever, the patient presented headache and neck stiffness. A CT-scan pointed a single round hypodense lesion in right capsular nucleus, without contrast enhancing, measuring 2.5 cm, and other smaller hypodense lesions in left cerebellar hemisphere and right parietal subcortical region. CSF analyses revealed a typical inflammatory pattern, but cultures were negative. The clinicians decided to treat as tuberculosis meningitis, adding steroids to therapy and the patient was discharged. Antiretroviral therapy was initiated in ambulatory setting, but after two weeks, the patient was admitted again, with new neurological findings. A new CT-scan showed worsening of lesions. Two days after admission, mycology laboratory reported the growing of a rare fungus, and amphotericin B deoxicolate (1 mg kg day^{-1}) was started. Culture was did and 37 °C grew a fast expanding cottony white mycelium. Culture this moth initial in medium PDA (Potato Dextrose Agar) after a week produce a distinctive fruity odor, cottony white and turned light grey with appears as hyaline, septate nondichotomously branching hyphae in direct potassium hydroxide. It was not possible to make a diagnosis using only conventional techniques of mycology being realized the partial sequencing of the region internal transcribed spacer (ITS). The sequences were edited using the Sequencher 4.9 software and compared by BLAST (Basic Local Alignment Search Tool) with sequences available from NCBI/GenBank being this 100% concordant with sequences of S. commune deposited in Genbank. After one month on HAART, antituberculous and antifungal therapy, without any other complication, the patient got better, CD4 count of 424 cells μl^{-1}. New CSF analysis showed improvements of inflammatory pattern and cultures were negatives, and important healing noted in CT-scan. Until now, this patient is well. We chose doing maintenance treatment with fluconazole, because of its better penetration through hematoencephalic barrier. HIV immunodeficiency can change the medical course of diseases, and lead rare opportunistic diseases.

Conclusion Differently from the other cases reported, we did not isolate the fungi in the CSF or in the brain tissue, but we had a positive blood culture, which was the first case described. We also believe this report is relevant, as it was the first case in the world that the patient with probably brain abscess due *S.commune* who survived.

P304

Rhino-orbital-cerebral Mucormycosis in a known diabetic patient in Nigeria

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Introduction Mucormycosis is a rare, fulminant and potentially lethal invasive opportunistic infection caused by Mucorales. The mortality rate in patients with systemic mucormycosis is as high as 50%, while in patients with cerebral involvement, it exceeds 80%; 50% of these patients have diabetes mellitus (DM).

Case Report A 60-year-old known type 2 diabetic and hypertensive male, who defaulted clinic attendance 5 years ago, presented with a 6-week history of persistent headache. He was rushed to hospital from church where he had collapsed. The headache was severe and right sided; there was an associated right sided dark pigmented necrotic facial eschar with nasal congestion and proptosis (Fig 1). He also had progressive loss of vision in his right eye and pain in the right ear. The patient also complained of fluids taken orally dripping through the nose.

There was necrosis of the palate extending from upper right second premolar to distal to the last molar.

Differential diagnoses were rhinocerebral mucormycosis, T-cell lymphoma and necrotising fasciitis. Incisional biopsy was done and specimen sent for histopathological and microbiological examinations. Histology revealed broad non-septate hyphae and cultures yielded a heavy growth of *Lichtheimia* species (Fig 2). CT scan of skull revealed hypodensity of skin and soft tissue overlying the anterior wall of the right maxilla. The right orbital content could not be delineated as the area showed a diffuse hyperdensity. Liposomal amphotericin B was initiated one week later due to the need to procure it from the US since it is not available in Nigeria. Surgical debridement of the necrotic region and right orbital exenteration were performed by a combined team of oral and maxillofacial surgeons and ophthalmologists. The extent of the lesion was confirmed intra-operatively to have extended into the optic canal and temporal and infra-temporal fossae making complete debridement very difficult. The necrotic palatal tissue was excised. His clinical condition, however, degenerated on the second postoperative day with eventual demise of the patient.

Discussion The prevalence of DM in Nigeria is 0.6–11% and it is estimated to be highest in Africa. Rhinocerebral or sino-orbital mucormycosis are common among diabetic patients, especially those who are poorly controlled, as was the case in this patient. Studies have demonstrated that DM alters the immunological response to resist mucormycosis through a reduction of the phagocytic properties of granulocytes in the presence of acidosis and hyperglycemia with an inability of diabetic serum to inhibit Mucorales *in vitro*. In addition, Mucorales thrive best in an acidic and glucose-rich environment.

Successful management of rhinocerebral or sino-orbital mucormycosis depends on early diagnosis, early intervention with ablative surgery, and intravenous Amphotericin B, as standard treatment. However in Nigeria, Amphotericin B is not readily available and voriconazole is the only licensed available parenteral antifungal available in Nigeria.

Conclusion There is dire need to increase the awareness of clinicians in Nigeria regarding Mucormycosis in diabetic patients and a critical need to provide essential antifungal agents when indicated.

P305

Sphingosine kinase inhibition for the treatment of invasive aspergillosis

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Invasive aspergillosis (IA) is an increasingly common cause of death in immunocompromised individuals. During IA, the activation of phagocytes leads to a pro-inflammatory cytokine response with excessive production of TNF-α leading to tissue destruction. The low efficacy of antifungal drugs currently available and a rising immunocompromised population has resulted in an increasing need for more effective treatments. Sphingosine kinase 1 (SphK1) is a lipid kinase that generates the bioactive sphingolipid sphingosine-1- phosphate (S1P) from sphingosine. S1P regulates many cellular functions and is recognized as a critical regulator of inflammation.

Inhibition of fungal-dependent TNF-a in the presence of FTY720 and the sphingosine kinase 1 specific inhibitor SKI-II in murine macrophages demonstrates that pro-inflammatory cytokines are SphK1 dependent. FTY720 (Gilenya) is currently used as a treatment for multiple sclerosis and has been shown to block the S1P G protein-coupled receptor $S1P_1$.

In a murine study a reduction in lung consolidation and mRNA levels of TNF-a in whole lung homogenate indicate that FTY720 reduces inflammation in response to a fungal challenge. Further murine data indicated that FTY720 directly reduces fungal burden as well as having anti-inflammatory properties. Additionally, there is a known homology between mammalian and fungal sphingosine kinases, and anti-fungal studies demonstrate FTY720 to have fungistatic effect at high doses, with SKI-II having significant anti-fungal activity at a low dose. These data suggest that the sphingolipid pathway is important in fungi for normal growth, and therefore a novel antifungal target.

Overall, my findings indicate that mammalian sphingolipid pathways are important mediators of fungal induced inflammation and that FTY720 has unexpected direct antifungal properties that lead to enhanced outcomes in animal models of aspergillosis.

P306

Cutaneous infection caused by *Scopulariopsis brevicaulis* in a renal transplant recipient

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Introduction *Scopulariopsis brevicaulis*, a filamentous fungus, is widely distributed in soil and in environment. It presents, primarily, as a fungal nail infection and, rarely, as cutaneous or disseminated infection. These situations are noticed mainly in immunosuppressed patients where the cutaneous involvement is usually secondary to disseminated pulmonary or visceral disease; rarely, it is consequent to primary skin inoculation. Here, we present a case of cutaneous infection by *Scopulariopsis brevicaulis* in a renal transplanted patient, following skin trauma [1].

Case On October 2013, a 72 years-old Caucasian female was referred to our hospital due to a ESBL positive *Escherichia coli* pyelonephritis; she began treatment with meropenem that continued for fourteen days. The patient had been kidney transplanted five years before, due to autosomal dominant polycystic kidney disease, and was medicated with tracolimus and prednisolone since. On the physical exam a nodular erythematous-exudative lesion, surrounded by smaller satellites, were observed on both legs; the patient remembered a skin trauma with a rake about a year before. A swab from

the lesion exudate was sent to the laboratory and *Scopulariopsis brevicaulis* was identified. In the following days, two additional swabs and two cutaneous biopsies from both legs lesions were performed and sent to pathological and mycological study, which revealed fungal structures and the same fungus growth. Imagiological exams to exclude systemic infection were all negative. The patient was medicated with caspofungin and voriconazole. Seven days later, cryosurgery was employed to remove the skin lesions. Treatment continued with voriconazole and caspofungin for seven days and then caspofungin was replaced by terbinafine; this dual therapy schedule lasted for four months. No relapse was observed after one year of follow-up.
Microbiology The three swabs and two biopsies sent to the laboratory were cultured on Sabouraud with gentamicin and chloramphenicol; in all, tan powdery colonies were grown; the microscopic exam evidenced branched annellides and chained round conidia with truncated base, suggestive of *Scopulariopsis brevicaulis*. The sequence of the internal transcribed sequence (ITS) region of the ribosomal RNA showed similarity with *Scopulariopsis brevicaulis* (GenBank Accession: KP117309; CBS Accession: DTO 001-F7/002-C9/012-E6/012-E7). Susceptibility testing was made following the Clinical Laboratory Standard Institute protocol M38-A2 [2] and the MIC results were amphotericin 2 mg l^{-1}, fluconazole >64 mg l^{-1}, ketoconazole 8 mg l^{-1}, itraconazole >16 mg l^{-1}, posaconazole >16 mg l^{-1}, voriconazole >8 mg l^{-1}, anidulafungin 0.25 mg l^{-1}, caspofungin 0.5 mg l^{-1} and terbinafine 8 mg l^{-1}. These high MIC values are according to the reported *in vitro* resistance of *Scopulariopsis* to antifungal agents [3].
Conclusions The case emphasizes the increased frequency of unusual fungal infections affecting immunocompromised patients. In contrast with the frequent systemic involvement, in this case, it was confined to the skin, likely to be port of entry following local trauma. *Scopulariopsis brevicaulis,* the identified fungal agent was effectively treated with local cryotherapy and commonly used antifungal drugs, despite the *in vitro* voriconazole high MIC.

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P307

Evaluation of chronic pulmonary aspergillosis (CPA) as a cause of smear negative TB and or anti-TB treatment failure in HIV infected Nigerians

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Introduction and Objectives Nigeria has an estimated 3. 2million people living with HIV/AIDS. Tuberculosis (TB) is the recognised leading cause of death in people with HIV and thousands of Nigerians are believed to be living with a multidrug-resistant strain. CPA has been linked to pulmonary tuberculosis. Our primary objective is to report the prevalence of *Aspergillus* antibodies along with the prevalence of CXR changes consistent with CPA in patients with TB. We compared the rate of CPA in the HIV positive and negative patients with tuberculosis described to establish whether HIV status is associated with a different risk of developing the disease. We also compared the rate of CPA in HIV positive patients with high and low CD4 counts to determine if advanced immunosuppression is associated with developing CPA. We then assessed any other potential risk factors.
Methods We conducted a cross-sectional survey in three centers in Nigeria. Adults HIV positive and negative consenting patients who are at the end or are in their last month of TB treatment (smear and/or Genexpert positive = documented TB) or currently being treated for 'smear negative TB' were recruited from DOTS, chest and ART clinics in Lagos and Ilorin states, Nigeria. All were assessed with clinical assessment, chest X-ray and Aspergillus IgG serology. *Aspergillus*-specific IgG was measured by ELISA using Dynamiker, which has a sensitivity of 77% and specificity of 97% for the diagnosis of CPA. Sputum was collected for fungal culture in those producing sputum. Patients' demographic and clinical data was collected using a semi-structured questionnaire. Healthy blood donors were used as control. CPA was defined as a positive Aspergillus IgG titre (>xx IU ml^{-1}), compatible chest X-ray and pulmonary or systemic symptoms, despite anti-TB therapy.
Results 209 patients were recruited between December 2013 and September 2014. 150 (75%) were HIV positive. Mean age was 39.46 (95% CI), 59.9% were female and 18.9% were unable to work. Mean CD4 (mean±S.E) was of all HIV patients was 246.20 ± 39 and 244.8 ± 45.4 in the HIV infected patients with positive *Aspergillus* IgG. One hundred and thirteen (54%) had documented TB and 70.5% had productive cough. Forty-one patients had hemoptysis, while one 8/41 had frank blood hemoptysis. Only one patient had a co-existing chronic obstructive airway disease (COPD). The prevalence of Aspergillus IGg was 30.0%; 48/149 (32.2%) in HIV positive and 17/60 (28.3%) in HIV negative. HIV co-infection had no significant impact on the frequency of CPA (p-0.645). Patients with weight loss, fatigue, ongoing or worsening respiratory symptoms + Aspergillus IgG were defined as probable CPA, pending radiological review (on going). There were 48 probable CPA HIV positive patients, 17 HIV negative CPA patients, 11 documented TB patients with probable CPA and 44 smear negative/genExpert negative probable CPA patients.
Conclusion CPA is a neglected disease and represents a significant public health challenge. CPA will fit into the WHO diagnostic criteria for 'smear-negative' tuberculosis. Accurate diagnosis of CPA in this group would avoid unnecessary and potentially toxic TB therapy

P308

Seroprevalence of cryptococcal antigenemia in antiretroviral naive and antiretroviral experienced HIV infected patients with CD4 + count below 250 cells/mm3 in Lagos, Nigeria.

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Introduction and objectives Cryptococcal meningitis accounts for high mortality in HIV infected persons in Africa. This is a preventable outcome when early screening and preemptive therapy is administered. WHO recommends screening for <100 $CD4^+$ cells/mm^3, this is presently not done in our setting and the antifungals recommended for treatment of this infections are not readily available in Nigeria. We evaluated the cryptococcal prevalence (CrAg), possible associated factors and outcomes in HIV infected patients being managed in a tertiary hospital in Lagos, Nigeria.
Methods Sera were collected from 216 consenting HIV infected participants attending PEPFAR clinics, with CD4 + counts less than 250, between November 2014 and May 2015. CrAg LFA (IMMY) was used for testing. Pertinent clinical data was from patients and their case notes.
Results Of the 214 participants, 96 (44.9%) were male and 118 (55.1%) were female. Mean age was 41.25 + /= 9.350. Majority (>95%) were ART experienced. The seroprevalence of cryptococcal antigenemia was 9.7% overall, of which 38.1% were ARV naïve and 19.04% were ART defaulters. The rate of CrAg posivity was 6/19 (38.1%) in those with CD4 counts <100, with CD4 counts 100–200 6/19 (28.6.9%) and 7/19 (33.3%) with CD4 count of 200 = 250. (38.1%) had $CD4^+$cell count below 100 cells/mm^3 and 13 (61.9%)

above CD4$^+$cell count above 100. 27 (12.1%) had associated oral thrush, comorbidities such as diabetes mellitus (1) and Tuberculosis (2). Potential meningitic symptoms were common in the studied group but was not statistically significant when both CrAg positive and CrAg negative were compared. Of the 21 participants with positive CrAg, 3 died and 10 were lost to follow up. Empirical fluconazole 200 mg thrice weekly was commonly given to those with low CD4 counts >100 cells/mm^3 (WHO guidelines) (p-0.018), unrelated to Crag positivity..

Conclusion We have reported a seroprevalence of 9.7% cyprococcal antigenemia in as setting where amphotericin B, flucytosine and intravenous fluconazole are not readily available. HIV infected patients frequently present with comorbidities. Public health officials in Nigeria should consider adding CrAg screening to existing national guidelines for HIV/AIDS care, as recommended in the WHO guidelines.

P309

An estimation of the Pneumocistose, Aspergillose and Histoplasmose diseases in Mozambique

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Introduction Pneumocystis jirovecii, Aspergillus fumigatus, and Histoplasma capsulatum are organisms with global distribution, and important opportunistic agents responsible for a high rate of morbidity and mortality of pneumonia in HIV positives patients. The widespread use of prophylaxis with trimethoprim-Sufametoxazol for pneumonia by PCP, Amphotericin B and other antifungals, as well as the Antiretroviral Treatment, caused a considerable decline in the incidence of three diseases in industrialized countries. In Africa until the early twenty-first century, the PCP was considered a rare disease, but recent studies have reported a significant increase in the frequency of this particular AIDS-associated disease, while for Aspergillus and Histoplasmas was still lack studies. In Africa, few studies was performed on the genetic characterization of identified isolates of P. jirovecii, Aspergillus fumigatus, and Histoplasma capsulatum, to determine their virulence. In Mozambique, particularly in HIV-infected patients, suspected cases of pneumocystis pneumonia, aspergillosis and histoplasmosis have been increasing, as well as case reports alone was confirmed. However, knowledge of the epidemiological behaviour of the three diseases in the country, it is essential so that you can think about and implement control measures of these opportunistic diseases.

Objectives Estimate incidence of pneumonia by P.jirovecii, A. fumigatus and H. capsulatum in adults patients infected with HIV and admitted in Maputo Central Hospital

Methods A prospective study in admitted adult's patients HIV positives with pneumonia. The patients are admitted into the study after signing inform consent. We collecting 15 ml of pulmonary samples and it is analyzing in Microbiology Laboratory. Vital data, chest X-ray and sputum induction are collected from patients. A slide is read by immunofluorescence to identify the P. Jirovenci with use of commercial kits and posteriorly we will do molecular analysis.

Results The study started in May 2015 and we expected we will recruit around 100 patient until end of September 2015. We will present the prevalence of this 3 disease in Adults HIV positives patients after analyzing data in end of September 2015.

P310

Clinical significance of immunological parameters in hematological patients with invasive aspergillosis

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Objective To study of immunological parameters dynamics and it's prognostic value in hematological patients with invasive aspergillosis (IA).

Materials and methods We observed 83 hematological patients with IA. Immunological parameters were evaluated within 2–4 weeks after IA diagnosis (median–14 days), after 2–3 months, and before the end of antifungal therapy. For the diagnosis of IA criteria EORTS/MSG, 2008 were used.

Group I included 48 patients after cytotoxic chemotherapy (CT), age 18 - 78 years, median - 46 (33 ÷ 58); males - 42%. Group II - 35 recipients of allogeneic hematopoietic stem cell transplant (allo-HSCT), age - 18 - 59 years, median - 26 (21 ÷ 44); males - 51%. Lymphocyte subsets and immunoglobulin levels were examined. Blood cell supernatants were tested for IFN-γ, IL-6, IL-10, IL-17, TNF-α and G-CSF. Receiver operating characteristic (ROC) analysis was performed to determine prediction rules for clinical outcome of IA.

Results IA was diagnosed between 5 and 50 days (median - 30) after CT course in group I. IA developed in the early post-transplant period (100-day) in 64% of patients of group II; 56% of patients had unrelated donors, HLA-matched - 31% and HLA-mismatched - 13%. In both groups, the prevailing underlying diseases were acute leukemias - 56% and 66%, respectively. All patients received antifungal therapy after diagnosis of IA, voriconazole predominantly (79%). 12th weeks overall survival was 90% and 71%, respectively. We identified significant decrease in the absolute number of lymphocyte subsets in the both groups at the early stage of IA. In allo-HSCT patients were found significant increase in T-helper cells CD4 + number ($P = 0.01$), natural killer cells CD16 + ($P = 0.001$) and a lower production of IgG and IgA ($P = 0.007$ and $P = 0.0003$), respectively, compared with CT patients. Reduction of proinflammatory cytokines production (IFN-γ, TNF-α, IL-17, G-CSF and IL-10) were detected in all hematological patients with IA, in allo-HSCT group it was the most significant. We found positive dynamics of immunological parameters in patients with a favorable outcome. In CT patients increase in TNF-α ($P = 0.02$) and G-CSF ($P = 0.001$) production, in allo-HSCT recipients - increase of absolute number CD4 + ($P = 0.03$) and the ability of leukocytes to TNF-α ($P = 0.04$), G-CSF ($P = 0.02$), IL-10 ($P = 0.03$) and IL-6 ($P = 0.04$) production were detected. Immunological parameters recovery are necessary for successful course of IA.

We found positive predictors of a 12-week survival in hematological patients with IA: the absolute T-helper CD4 + number > $0.177*10^9$/L, ($P = 0.002$), and the TNF-α level > 215 pg ml^{-1}, ($P = 0.001$).

Conclusion The absolute number of T-helper CD4 + > $0.170*10^9$/L and the blood cells ability to produce TNF-α > 215 pg ml^{-1} are significance prognostic markers of a favorable outcome of invasive aspergillosis in hematological patients.

P311

Initial Use of Combination Treatment Does Not Impact Early Survival of 106 Patients with Hematologic Malignancies and Mucormycosis: a Propensity Score Analysis

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Objectives Mucormycosis is an emerging opportunistic mold infection in patients with hematologic malignancies (HM) and hematopoietic cell transplant (HCT) recipients. In view of the poor outcomes associated with mucormycosis, antifungal combinations are frequently used, yet the value of such strategy remains unclear.
Methods We retrieved the records of patients with HM and probable or proven mucormycosis (EORTC/MSG criteria), seen at The University of Texas MD Anderson Cancer Center from 1994 to 2014. Monotherapy was defined as single agent treatment with amphotericin-B or posaconazole. Combination treatment included any combination of amphotericin-B, posaconazole and echinocandins, given as initial treatment. The primary outcome was mortality at 42 days after treatment initiation. Univariate and logistic regression analyses were used to determine predictors of mortality. A multivariate analysis with a propensity score for receiving combination treatment was performed to adjust for baseline imbalances.
Results Of the 106 patients identified, 44% received monotherapy and 56% combination treatment. Patients who received combination therapy were more likely to be on voriconazole prophylaxis (69% versus 38%; $P = 0.001$), be HCT recipients (61% versus 38%; $P = 0.02$), have active graft-versus-host disease (36% versus 17%; $P = 0.03$), or develop mucormycosis after 12/2004 (83% versus 40%; $P < 0.01$). In contrast, patients who received monotherapy were more likely to be on fluconazole prophylaxis (34% versus 14%; $P = 0.01$), when compared to those who received combination treatment. There were no differences in delayed initiation of therapy (≥ 6 days, 38% versus 41%; $P = 0.8$) or 42-day mortality (43% versus 41%; $P = 0.9$) between monotherapy and combination treatment groups. Patients who died within 42 days were more likely to have disseminated mucormycosis (30% versus 11%; $P = 0.02$), active HM (80% versus 55%; $P < 0.01$), higher APACHE II scores (median, 15 versus 13; $P < 0.01$), neutropenia (≤500 cells μl^{-1}, 48% versus 29%; $P = 0.05$), lymphopenia (≤500 cells μl^{-1}, 84% versus 50%; $P < 0.01$), or be admitted in ICU at diagnosis (39% versus 8%; $P < 0.01$). Patients who survived were more likely to have localized mucormycosis (21% versus 2%; $P < 0.01$) or receive hyperbaric oxygen therapy (16% versus 2%; $P = 0.02$). In multivariate analysis, lymphopenia (OR = 5.3; 95% CI = 1.9 - 15; $P < 0.01$) and ICU admission at diagnosis (OR = 7.7; 95% CI = 2.2 - 27.6; $P < 0.01$) were associated with increased 42-day mortality. Localized mucormycosis was associated with better outcome (OR = 0.07; 95% CI = 0.01 - 0.7; $P = 0.02$). Initial combination treatment had no impact in mortality, even after propensity score adjustment (OR = 0.8; 95% CI = 0.3 - 2.3; $P = 0.6$).
Conclusions With the current status of mucormycosis diagnosis, there was no difference in mortality in HM patients, whether they received monotherapy or combination treatment. Earlier diagnosis and immune reconstitution are unmet needs to affect outcomes.

P312

Antifungal prophylaxis in high-risk, adult hematological patients - results from multicenter, real life MIFI study

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Objective To assess the overall efficacy of antifungal prophylaxis (AFP) in patients with hematological malignancies in real life setting; to compare posaconazole and fluconazole in adult patients with acute myeloblastic leukemia (AML).
Methods Management of invasive fungal infection (MIFI) is ongoing database project in Slovakia. The aim of this project is to cumulate and analyze the data about antifungal strategies used in participating centres with the focus mainly on prophylaxis of the high-risk hematological patients. Data input is performed via web based registry. Data from six center (study period 2012–2014) were retrospectively analyzed. Withdrawn of antifungal drug regardless of reason was considered as a failure of prophylaxis. The predefined reasons for failure were non-compliance, toxicity and breakthrough invasive fungal disease (proven, probable, possible) according to EORTC/MSG criteria 2008. Comparison of primary prophylaxis of patients with AML was performed using statistical analysis.
Results 664 adult patients, 325 (49%) males and 339 (54%) females with average age 54.2 years were evaluated. The most common hematological diseases were AML (69%), malignant lymphomas (13%) and ALL (5%). 181 (27%) patients underwent hematopoietic cell transplantation with 52% of allogeneic transplants. At the start of AFP 583 (88%) patiens were neutropenic, 618 (93%) received broad-spectrum antibiotics, 504 (73%) had inserted central venous catheter and steroids were administered to 159 (24%) patients. Posaconazole was used in 353 (53%) and fluconazole in 272 (41%) patients. Average duration of prophylaxis was 23 days and 26 days in patients with successful AFP. Overall success was documented in 374 (56%) patients; posaconazole and fluconazole were successful in 220 (62%) and 135 (50%) patients, respectively. The reasons of failure were breakthrough IFD in 122 (17%) patients, but only 10 (2%) were proven. Uncompliance was presented in 136 (20%) patients and adverse effects in 32 (5%) patients. Survival at day +100 from the start of AFP was 79% in total and 90% in group with successful AFP, whereas in group with AFP failure survival was only 50%. We performed an analysis comparing patients with posaconazole and fluconazole AFP in adult patients with AML. The number of risk factors was similar in both groups with some differences in the proportion of risk factors. Average duration of AFP in posaconazole group was 24.5 days (1–416 d) and in fluconazole group 21 days (1–316 d). Comparison of posaconazole and fluconazole primary AFP in adult AML patients showed successful AFP in 59% of 246 patients in posakonazol group and in 42% of 176 patients in fluconazole group ($P = 0.001$). Survival at day + 100 was 81% and 74% in posaconazole and fluconazole group, respectively ($P = 0.072$).
Conclusion Real - life retrospective data on AFP in high-risk hematological patients is the important source of valuable information outside the clinical trials. We have confirmed the position of AFP in this population of patients - patients with successful AFP had better survival. Our analysis documented superior efficacy of posaconazole in comparison to fluconazole with trend to better survival at day +100 in adult patients with AML.

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Investigation of pulmonary fungal infections leading to hospitalization in HIV-infected adult patients

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Objectives To study the occurrence of pulmonary fungal infections in HIV-infected patients hospitalized with acute respiratory symptoms
Methods Cross-sectional study that included patients HIV-infected ≥ 18 years, hospitalized for community-acquired pneumonia (CAP) from 2012 to 2015. The diagnosis criteria of the pulmonary infections was based on the review of the cases by specialists and the results of following exams: Culture and direct microscopic examination of sputum and bronchoalveolar lavage (BAL), serum LDH, blood cultures, chest radiography and tomography, serum (1,3) β-D-glucan and galactomananan, LAMP-PCR for P. *jirovecii* in sputum, BAL and oral lavage. Risk of mortality was evaluated by the CURB-65 score.
Results Fifty-three patients were included with mean age 43.64 ± 9.99 years; 34 (65%) patients were male; median time of the diagnosis of HIV infection was 12.14 ± 7.62 years and 35(65%) patients had an intermediate mortality risk. Abdormalities in chest X-rays and in chest tomography were found in 95% of 53 patients and in 96% of the 29 patients respectively. The median CD_4^+ T lymphocytes was 87 cel/mm^3 and median LDH serum level was 443. Twelve patients (23%) had undetectable viral load and 33 (62%) had CD4 + T lymphocytes ≤ 200 cel/mm^3. Two patients had positive sputum culture for *Mycobacterium tuberculosis* and two for *Mycobacterium avium*. Blood cultures were positive in 6 patients (4 *Streptococcus pneumoniae*; 1 micrococcus; 1 *Acinetobacter baumanii*); (1,3) β-D-glucan values were > 80 pg (reference value) in 22 (44%) patients and for galactomannan, 3 (6%) patients had values >0.5. LAMP-PCR was positive for *P. jirovecii* in 1of 2 BALs, 4 (21%) of 19 samples of sputum and negative in 13 samples of oral lavage. The probable diagnoses were: pneumocystis pneumonia (PCP): 14; CAP: 15; PCP + CAP: 3; Tuberculosis: 3; Mycobacteriosis + PCP: 2; Nocardiosis: 1; Histoplasmosis: 1; Cryptococcosis: 1; Lower respiratory infection: 8; Pulmonary embolism: 1; Unconclusive: 3; Strongyloidiasis: 1. Overall mortality was 15% (8 deaths) and 21% (3 deaths) for the 14 patients with PCP. The diagnosis of PCP had a significant relationship with: late diagnosis of HIV infection ($P = 0.008$), higher values of LDH ($P = 0.002$), lower CD4 + T lymphocytes count ($P = 0.001$) and values > 80 pg of (1,3) β-D-glucan ($P = 0.0002$). Patients with undetectable viral load had significantly lower values of (1,3) β-D-glucan ($P = 0.04$). There was a significant relationship between higher values of (1,3) β-D-Glucan and the presence of diffuse infiltrate in chest radiography ($P = 0.02$). The sensitivity and specificity of (1,3) β-D-glucan in PCP cases were 82% and 80%, respectively.
Conclusion Unlike the present data from developed countries, PCP was the leading cause of lung disease in patients with AIDS, followed by CAP. Pulmonary fungal infection represented 40% of the leading causes of hospitalization in HIV/AIDS patients. The combination of chest radiography and tomography with serum biomarkers was useful in the etiological characterization of lung infections in HIV adult patients.

P314

Epidemiology of candidaemia in burn-injured patients: A Croatian nine-year study

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Objectives Advancements in burn care therapy have extended survival of seriously burned patients, exposing them to increased risk of infectious complications. According to several published reports, different *Candida* species have become increasingly common causes of bloodstream infections in these high risk patients within hospital setting. However, published data regarding these infections in Croatian burn-injured patients are scarce. Therefore the main objective of this study was to determine the annual and the overall incidence of candidaemia in adult patients hospitalized in the Reference Centre for Burns, Zagreb Clinic of Traumatology, Croatia, during a 9-year period. In addition, we have provided species identification of *Candida* bloodstream isolates together with their susceptibility profiles to amphotericin B, flucytosine, fluconazole, itraconazole, and voriconazole.
Methods Prospective single-centre cohort study had commenced in January 2006 and lasted until January 2015. Blood samples were taken in accordance with clinical indication in patients with superficial, partial-thickness or full-thickness burn injuries of ≥ 10% total body surface area. Yeast isolates from Sabouraud glucose agar were identified using an API ID 32C kit (bioMerieux, France) and on the basis of their cornmeal agar morphology. *In vitro* antifungal susceptibility testing was performed using the ATB FUNGUS 3 (bioMerieux, France) microdilution method, and the results were interpreted in compliance with the European Committee on Antimicrobial Susceptibility Testing (EUCAST) recommendations.
Results Candidaemia was determined in 16 out of 355 patients (4.5%). The lowest incidence was established in 2008 (0%), and the highest in 2014 (7.7%). During the studied period, the average incidence of candidaemia amounted to 1.09 per 100 admissions and 0.61 per 1000 patient days. It annually ranged between 0 and 4.1 per 100 admissions and between 0 and 1.77 per 1000 patient days. Species identification revealed that *C. albicans* was the most predominant (50% of isolates), followed by *C. parapsilosis* (25%), *C. glabrata* (18.8%), and *C. lusitaniae* (6.2%). No *in vitro* resistance to amphotericin B, flucytosine, fluconazole, itraconazole, and voriconazole was observed in *C. albicans* isolates. However, non-albicans *Candida* species isolates displayed a decreased susceptibility to fluconazole. The highest value of the frequency of *C. albicans* bloodstream infection never exceeded 0.75 per 1000 patient days. Opposed to that, the non-albicans *Candida* species candidaemia frequency proved to be significantly higher, reaching as much as 1.42 per 1000 patient days precisely in the final year of our study.
Conclusions Our findings demonstrated a low incidence of candidaemia in patients hospitalized at the Zagreb Reference Centre for Burns in the period from 2006 until 2015, despite the fact that this incidence was becoming more frequent toward the end of the conducted study. We furthermore established that the presence of more resistant non-albicans *Candida* species isolates from blood cultures has been on the rise, which emphasizes the need for further investigation of local candidaemia epidemiology in order to be able to properly implement systemic antifungal therapy.

P315

Virulence potential of *Candida albicans* isolated from oral cavity of patients with chronic renal failure on hemodialysis

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Objective In patients with chronic renal failure (PCRF), the frequency of colonization of the oral cavity by yeasts of genus *Candida* spp. is high compared with healthy individuals. These yeasts have virulence factors that may contribute to the persistence of colonization and the development of these infections. The aim of this study was evaluate aspects of virulence from *Candida albicans* isolated from oral cavity of PCRF on dialysis.
Methods This study was inittialy conducted with 49 clinical samples of *C. albicans*. The virulence factors assayed were produce of biofilm, germ tube, determination of adherence in oral epithelial cells and evaluation of resistance to the antimicrobial action of neutrophils and mononuclear cells.
Results All isolates were highly efficient in forming biofilms on polystyrene microplates, where 94% of the samples formed 4 + biofilm. Used as a screening test, of which three isolates were selected with different degrees of ability to form biofilm to assess other indicators of virulence. Overall, the isolates exhibited different characteristics regarding the virulence factors analyzed. It was also observed that the hypophosphorous acid (HOCl), production, one of leading inflammatory mediators with fungicidal action, also varied especially when the neutrophils, and not mononuclear cells, were stimulated with different samples. (Figure 1).
Conclusion Therefore, our results indicate that *C. albicans*, is not only the most common species in the oral cavity of CRFP on dialysis, but also it presents the main virulence attributes, which reinforces the importance of monitoring of these patients towards the prevention of fungal infections.

P316

Hyperglycemia and increased risk of vulvovaginal candidiasis (VVC)

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Objective The vulvovaginal candidiasis (VVC) is a prevalent opportunistic mucosal infection that affects a significant number of healthy women, with a peak incidence between 20 and 40 years old. The recurrent form of the disease (RVVC) is defined usually as 3 or more episodes of VVC that occur within a 12-month period. According to many authors, diabetes mellitus is a predisposing factor for vulvovaginal candidiasis, along with pregnancy, use of broad-spectrum antibiotics, high-estrogen-dose oral contraceptives, obesity and drug addiction. Others studies consider hyperglycemia as the major cause of increased susceptibility of diabetic patients to vulvovaginal candidiasis. It is known that increased glucose levels in genital tissues enhance yeast adhesion and growth and also that hyperglycemia may impair several aspects of humoral host defense, resulting in decreased random motion of neutrophils, chemotaxis, phagocytosis, and microbial killing. Despite those findings, there are few and inconclusive scientific reports concerning diabetes and VVC and RVVC. Thus, we evaluated the relationship between VVC and RVVC with diabetes as a potential risk factor for vulvovaginal candidiasis
Methods This prospective study included 254 women sexually active, aged between 15 and 50 years old (33.5 ± 8.95), with or without signs and symptoms of VVC and RVVC. Vaginal cultures were performed for yeasts isolation and identification by classical methods. Fasting blood was collected for plasmatic glycemia and insulin measurements. Insulin levels were determined employing chemiluminescent microparticle immunoassay using the Architect-Abbott analyzer. The insulin sensitivity was derived from fasting glucose and insulin data, using the homeostasis model assessment (HOMA) mathematical model. Measurement of plasma glucose was performed by enzymatic colorimetric GOD-PAP method (Diasys) using the equipment Vitalab Selectra2. Data distributions were expressed as mean ± standard deviation. Significant differences among means were identified using GraphPad Prism® 5.0 software. Bonferroni test was used to calculate the multiple comparisions and Chi-square (χ 2) test using the STATA for Statistics and Data Analysis 9.1 software was used to investigate variables between control group and positive culture group. All variables were expressed as absolute and relative frequencies, p values < .05 were considered significant.
Results Yeasts were isolated from 48 (19%) women: 22 (46%) in acute episode (VVC) and 26 (54%) with RVVC. Control group (negative culture) comprised 206 (81%) women. The average values found in fasting blood glucose was very similar in all groups. Abnormal glycemia (≥100 mg dl^{-1}) was detected in 16 (7.8%) control women and in 5(20.5%) of the positive culture group (VVC+ RVVC). The HOMA Index (HI) indicating insulin resistance were detected in 42 (20%) women of control group and in 16(64%) of the positive culture ones.Compared to control group, women with positive culture (VVC + RVVC), were significantly associated with abnormal glucose metabolism (P = .0002; OR= 4.6;CI=2.0–10.9) and insulin resistance (P = 0.0317; OR= 2.2;CI= 1.05–4.84).
Conclusion Our data suggest a significant association between abnormal blood glucose levels and the incidence of vulvovaginal candidiasis. Women with blood glucose above the reference values and with insulin resistance (HOMA IR) presented at least twice the risk to have positive culture for *Candida* spp.

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Impact of Prior Invasive Fungal Disease on the outcome of Allogeneic Hematopoietic Stem Cells Transplantation

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Objectives The objectives of the study was to estimate impact of prior IFD on overall survival (OS) after alloHSCT, the incidence of IFD event and related risk factors during alloHSCT, and evaluate the effectiveness of preventing measures against IFD event.
Methods In retrospective and prospective observational single-center study 256 HSCT recipients (303 alloHSCT, 256 first and 47 second HSCT) were included from Jan 2012 to Dec 2013 in Raisa Gorbacheva Memorial Institute of Children Oncology, Hematology and Transplantation, First Pavlov State Medical University of Saint-Petersburg. Most of patients had acute leukemia (71.6%), 162 males, and median age 44 years (1–67 years). AlloHSCT from HLA-matched unrelated donor were performed in 73.9%, HLA-matched related donor - 18.8%, haploidentical - 7.3%, predominantly with reduce

intensity conditioning regimen (76.2%) with complete remission of underlining disease in 56.1%. EORTC/MSG 2008 criteria for diagnosis IFD and response to therapy were used. 'Active IFD' is the IFD diagnosed immediately prior HSCT. OS and effectiveness of antifungals (fungal free survival (FFS) - survival without any events: relapse, progression, and new episode of IFD) estimated by the Kaplan-Meier analysis at 1 year after alloHSCT.

Results The incidence of IFD before alloHSCT was 27% (83/303): proven/probable (PP)-IFD (65), possible IFD (18). In PP-IFD group the most frequent IFD was invasive aspergillosis (IA) 85%, invasive candidiasis/candidemia (IC) - 4.5%, some of cases had two pathogens: *IA+IC* (3), *IA+Fusarium* (2). At the moment of HSCT active IFD was 30.8%, PR - 43.1%, CR- 26.2%. The main sites of infection were lungs 70.1%, other localizations were observed less frequently and mostly in combination with lung involvement - 12%. Most of patients received voriconazole (88.1%) during HSCT. Possible IFD group consist of two types: possible according EORTC/MSG with typical radiological signs and negative mycological criteria ($n = 7$) and group of patients with host factors, atypical radiological signs and negative mycological criteria but were treated with voriconazole with effect ($n = 11$) because of high risk IFD - AML/MDS, Diagnostic procedures included bronchoscopy (50%), culture of blood and respiratory substrate (68.2%), GM in BAL (50%), GM in serum (80%). Voriconazole was used as secondary prophylaxis during alloHSCT in 72% of cases, caspofungine - 17%, other - 11%. One-year FFS for patients who had prior IFD from the day of HSCT was 81.9%. Cumulative one-year incidence of IFD event was 18% (15). There was no difference in cumulative incidence of IFD event and OS between possible IFD and PP-IFD group (27.8% vs 15.6%- $P = 0.436$, 77.8% vs 56.3% $P = 0.068$, respectively). Twelve patients had progression of prior IFD after HSCT. Three patients developed the new IFD due to *Paecilomyces variottii*; *Candida krusei* and*Trichosporon*. 'Active' (relapse/progression) underlying disease in early (before Day+100) post transplant period was the only risk factor for any IFD event after alloHSCT (9.1% vs 37%, $P = 0.001$). The one-year OS after HSCT in patients with prior IFD was 61% and 63.8% in patients without history of IFD ($P = 0.870$).

Conclusion Prior IFD did not impair the outcome of alloHSCT with effective methods of diagnosis and prophylaxis being used.

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The case of successful treatment of invasive pulmonary mucormycosis (*Rhizomucor* sp.) in patient with acute myeloid leukemia

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Introduction Rare fungal infections in immunocompromised patients became actual in recent years. We report a case of invasive pulmonary mucormycosis (Rhizomucor sp.) in patient with acute myeloid leukemia.

Case report In April 2012 27 years old male with acute myeloid leukemia (FAB-M4) was treated with chemotherapy '7 + 3'(cytarabine 100 mg/m^2, mitoxantrone 12 mg/m^2). Prophylactic fluconazole 300 mg/d was used in neutropenia. On the 8th day of the chemotherapy fever was noted. A chest and paranasal sinuses x-ray was unremarkable. Antibacterial therapy (ertapenem 2 g/d, linezolid 1.2 g/d) was used, but fever persisted. Lung CT scan was unremarkable, but in paranasal sinuses CT pansinusitis was revealed. Voriconazole (800 mg d^{-1} in 1st day, then 400 mg d^{-1}) was added. Blood test for galactomannan was negative. Despite antibacterial and antifungal therapy high fever persisted (38–39 °C). Coughing and dyspnea were observed. At 28.04.2012 a lung CT revealed alveolar and interstitial infiltration with indistinct, rough contours in S1 + 2 and S6 of the right lung and an area of consolidation average and big intensity with indistinct, rough contours, roundish form, with a diameter of 8–13 mm in S3, S6 of the left lung. At 29.04.2012 the normalization of level of leukocytes and body temperature was registrated. At that time the patient refused from diagnostic bronchoscopy. At 02.05.2012 clinical and hematologic remission of AML was established. Due to the changes in lungs the next course of the chemotherapy was postponed. At 10.05.2012 control lung CT revealed cavities in S1–2 of left lung and in S3, S6 of right lung, and size of cavity in S6 increased in left lung. At 12.05.2012 bronchoscopy was done. Test for galactomannan was negative in bronchoalveolar lavage, *Rhizomucor* sp. was cultured. Treatment with amphotericin B 1 mg kg d^{-1}, then posaconazole 800 mg d^{-1} was used. At 10.06.2012 the resection of S1 + 2 and S3 of the left lung was performed. The cytostatic chemotherapy was continued successfully with antifungal secondary prophylaxis with posaconazole.

Conclusions Hematological patients have the high risk of rare fungal infections. The complex treatment with surgical debridement allow to treat acute myeloid leukemia in patient with mucormycosis.

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Cryptococcosis in and around Delhi, India: Clinical, antifungal and molecular pattern

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Objective To analyse the clinical, antifungal and molecular pattern of cryptococcosis in and around Delhi, India.

Methods Cryptococcosis was diagnosed by direct microscopy, latex agglutination and culture. Cryptococcal species and variety was confirmed by sequencing. Antifungal susceptibility was carried out by broth microdilution as per CLSI 2012 guidelines.

Results A total of 129 isolates of *C. neoformans* were isolated from 101 patients, as nineteen cases were of disseminated cryptococcosis over a period of six years. These were isolated from CSF (99), blood (11), respiratory sample (sputum/BAL/pleural fluid) (5) and urine (13), soft tissue (1). One hundred one cryptococcosis cases included 79 HIV positive cases and 22 immunocompetent. Among HIV negative patients the known risk factors were leucopenia (9), neutropenia (2), Hodgkin's lymphoma (1), lymphocytosis (2), tuberculosis (1) and apparently immunocompetent (7). Overall mortality was 29 (28.7%). Mortality (25), Relapse (4) and dissemination (18) were more in HIV positive than HIV negative group (4), (1) and (1), respectively. Co-infection with tuberculosis was seen in HIV (6) AND HIV seronegative (4) patients. HIV positivity was the most important risk factor of cryptococcosis. Predictors of mortality in HIV positive patients were CD4 + count $\leq$50 mm^3, non-usage of ART, inadequate dose of fluconazole. Predictors of mortality in HIV negative patients were control of underlying condition. HIV seronegative patients, were observed to have better survival (77.3%) as compared to HIV positive patients (72.2%). Symptoms of fever, headache, altered sensorium were observed to have better survival. Patients who exhibited any degree of neurological deficit at first examination had a higher proportion of deaths (35.5%) due to cryptococcal meningitis as opposed to those without any deficit (22.9%). Patients with normal findings on first CT (head & neck), had a higher proportion of survivors (87%) at the end of the study period, as compared to those with any abnormal finding (69.2%). Patients who were treated with a combination of antifungals and ATT/ART (as required) had maximum number of survivors (86.1%) as opposed to use of either antifungals alone (68.5%) or ART/ATT alone (55.6%). The antifungal susceptibility of the isolates to amphotericin B, fluconazole, itraconazole, voriconazole, posaconazole were in the susceptible range. MIC of all the *C. gattii* isolates was higher as compared to C. neoformans

serotype A & D isolates. A total of 40 isolates were sequenced, targeting the 26S ribosomal subunit by using ITS-1, ITS-2 and ITS-4 region. Out of these isolates 32 were *C. neoformans var. grubii*, 2 were *C. neoformans var. neoformans*, 6 were *C. gattii.*
Conclusions Cryptococcosis is fatal despite despite timely diagnosis, institution of antifungal therapy and the sensitive isolates to antifungals. It is common in HIV seronegative patients. *C. gattii* can also infect HIV population. More ecological, phenotypic, host-defense and molecular studies are warranted to study this mycoses.

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Invasive fungal infectious in HIV positive patients

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Objective Individuals with human immunodeficiency virus (HIV)/AIDS are at significant risk of invasive fungal infections (IFIs). Pulmonary and oesophageal candidiasis, *Pneumocystis jiroveci* pneumonia are AIDS-defining fungal infections. Invasive aspergillosis is relatively rare. In this sudy it is amimed to evaluate invasive fungal infections in HIV(+)/AIDS patients.
Methods The patients records hospitalized because of invasive fungal infections in infection diasease department and respiratory intensive care unit were evaluated retsospectively between 2006 and 2015. Inclusion criteria for each specific IFI were adapted from the 2002 guidelines of the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group. Data for each IFI were collected including patient demographics and clinical characteristics, comorbidities, and concomitant bacterial infections. Patient characteristics included HIV-specific information such as CD4 cell counts, and viral load. Detailed information about the IFI was collected and included the specific fungal pathogen and species, infection site.
Results The data of 11 patients (9 male, mean age 49.6 ± 13.7, min:30-max:73) had invasive fungal infections were evaluated. The data of patients were summarized in table.
Conclusion Fungal infections remain a clinical challenge in HIV patients with severe immunosuppression. Our data also suggest that HIV patients with CD4 cell counts >100 cells μl^{-1} and other underlying conditions may be susceptible to invasive fungal infections including pulmonary aspergillosis.

Table 1. The data of patients were summarized

Table: The data of patients were summarized

Patient	Gender	Age	CD4	Viral load copy/mL	PCP	The site of fungal infections except	The cause of fungal infection	Concomitant opportunistic infections	Result
1	F	30	25	100000	+	-		-	
2	M	64	10	100000	+	-		Herpes virus	
3	F	46	80	100000	+	Pulmonary*	*Aspergillus*	CMV***	Ex
4	M	52	72	100000	+	Pulmonary*	*Candida*	CMV	
5	M	60	60	100000	+	Pulmonary*+ Urinary	*Candida*	CMV	
6	M	73	119	67520	+	-	-	CMV	
7	M	57	93	75000	+	-	-	Atypic TB	Ex
8	M	52	42	25406	+	-	-	Dissemine TB	
9	M	46	40	100000	+	-	-	Atypic TB	
10	M	33	74	855125	+	Pulmonary*	Probably aspergillosis	-	Ex
11	M	33	14	1776	+	Oesophagus	*Candida*	-	

F:Female, M:Male

*Candida plaques were seen in bronchoscopic examination., *C. albicans* were growth in culture.,

** Cytomegalovirus

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Aspergillossis and Cryptococcal meningitis in HIV-infected patients in C H U Oran

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Objective The invasive fungal infections in hospital is a subject of concern both for the healthcare professionals .The infection at the hospital is a major risk for the patients the invasive acts, in first row of morbidity, mortality.
Materials and methods A retrospective study was conducted from september 2011 to Mars 2014, the fungal infections from immunocompromised patients and the occurrence of Cryptococcus neoformans admitted in unites of disease infections ,with Aspergillosis investigation serology .Cryptococcal meningo-encephalitis was diagnosed in 23 individuals. The median age of the patients under study was39.25 years With male preponderance . Typical presentations were persistent headaches (27 cases/36), neck stiffness (16/36), altered consciousness (14/36), fever (12/36) and convulsions (9/36).Cryptococcal meningitis highly contributes to mortality in HIV-infected patients. A need exists to improve strategies for clinical management of AIDS patients and systhematique Aspergillosis serology all samples sent to Laboratory for mycological analysis. A questionnaire was conducted in which there is information for each sampleInclusion criteria: The patients admitted in risk servicesExclusion criteria: - Patients under antifungal treatment430 samples from different departments .Cryptococcal meningitis in HIV-infected patients is an important fungal pathogen in immunocompromised patients. High mortality was related to delayeddiagnosis. A need exists to improve strategies for clinical management of AIDS patients.
Results prevalence of the meningo-encephalitis cryptococcose at the patients infected by the HIV was 0.4 %. frequency practically decreased while it was 2.09 % for the ten years.
Discussion the man is preferentially touched. The average time evolution of the symptomatologie before hospitalization represented 1 week. The mode of installation of the disease is progressive,fever, headache,immunosuppressed patient, with a rate of CD4 lower than 200/mm3, any suspicion of meningitis has to make look for Cryptococcus neoformans . positive in 72.7 % the death arose in 54.5 %
Conclusion The fungal infection relegated to the last rows neuromeningeal cryptococcose Aspergillosis ,necessarily in systematic study from subjects HIV + the prescription of effective systematic antifungals is primary prevention with Antiretrovirals in front of a therapeutic

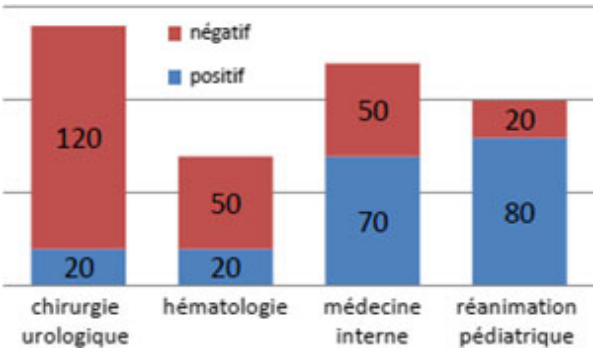

Figure 1. Parasitology and mycology CHU Oran

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Antifungal Susceptibility Testing to Predict Appropriate Initial Antifungal Therapy and Outcome in non-Aspergillus Invasive Mold Infections

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Objectives Non-*Aspergillus* invasive mold infections (NAIMI) are associated with high mortality. Mucoromycotina, *Fusarium* spp. and *Scedosporium* spp. account for the majority of NAIMI and exhibit distinct susceptibility profiles with resistance to many antifungal classes. The lack of established clinical breakpoints precludes the interpretation of minimal inhibitory concentration (MIC) values for these molds. The aim of this study was to assess the association between MIC and clinical outcomes and the possible role of antifungal susceptibility testing in guiding antifungal therapy of NAIMI.

Methods Medical charts of patients with positive cultures for the most relevant non-*Aspergillus* mold species collected at Duke University (Durham, NC, USA) between 2009 and 2013 were retrospectively screened. Patients with proven/probable NAIMI (EORTC-MSG definitions) were included in the analysis. Antifungal susceptibility testing for amphotericin B, voriconazole, posaconazole, micafungin and caspofungin was retrospectively performed according to CLSI procedure. Patient characteristics, pathogen MIC values, antifungal therapy and NAIMI outcome were analyzed.

Results 39 NAIMI episodes were included (19 mucormycosis, 12 fusariosis, 4 scedosporiosis, 3 *Paecilomyces* spp. infections and 1 *Scopulariopsis* spp. infection). Twenty-two (56%) patients had hematological malignancies and 13 (33%) were solid-organ transplant recipients. Mortality at week 12 was 59% (44% before week 4). Amphotericin B had variable *in vitro* activity against Mucoromycotina (median MIC 0.5 μg ml^{-1}, range 0.125 - 4) and *Fusarium* spp. (2 μg ml^{-1}, 1 - 4), but had minimal activity against other mold species. All *Fusarium* spp. exhibited high MICs to voriconazole (≥16 μg ml^{-1}) and 53% of Mucoromycotina had high MICs to posaconazole (>16 μg ml^{-1}). Initial antifungal therapy was found to be crucial for NAIMI outcome. Success rate at week 6 was 75% for initial appropriate antifungal therapy defined as use of an antifungal drug with a MIC cut-off ≤0.5 μg ml^{-1} during the first 72 h of treatment versus only 13% in cases of MIC >0.5 μg ml^{-1} or absence of antifungal therapy (p value = 0.001). There was no other significant association with better outcome including immunosuppressive status (neutropenic vs non-neutropenic) or underlying condition (hematologic malignancies vs others).

Conclusion NAIMI remain associated with a very high mortality rate. Because of the unpredictable susceptibility profile of Mucoromycotina and *Fusarium* spp. to amphotericin B and azole compounds, antifungal susceptibility testing is useful. A MIC cut-off of ≤0.5 μg ml^{-1} is a good indicator of appropriate initial antifungal therapy, which is associated with better outcomes.

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Invasive aspergillosis in patients with hematological malignancies in Czech, Slovak and Croatian hematooncological departments: Fungal INfection Database (FIND) analysis (2001–2014) - an update

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Objectives 'Fungal InfectioN Database'(FIND) represents international database of invasive fungal infections in Czech, Slovak and Croatian hematooncological departments. FIND – aspergillus covers all case of invasive aspergillosis (IA) in participating centers since 2001.

Methods The goal of our retrospective analysis was to evaluate incidence, early diagnostic procedures and effect of antifungal therapy in proven and probable IA that occurred in 18 institutions participating in FIND database between 2001–2014. Till 2009 we followed EORTC/MSG 2002 and from 2010 EORTC/MSG 2008 criteria in evaluation of IA diagnosis and therapy response.

Results 444 probable and 94 proven IA (87.6% isolated pulmonary IA, IPA) have been documented. Prolonged, profound neutropenia (60.4%) and long-term use of corticosteroids (28.4%) were identified as the major risk factors of IA. 58.4% pts. had consecutive positivity of serum-galactomannan (S-GM) (OD index >0.5). 82.0% pts. with IPA and bronchoalveolar lavage (BAL) had positive GM in BAL fluid (OD index ≥ 0.5). In pts. with IPA only 12.8% BAL fluids and 11.8% sputum samples had positive microscopy for filamentous fungi and 19.7% BAL fluids and 49.6% sputum samples had positive culture for Aspergillus spp. The primary antifungal therapy of IA was used in 82.2% pts. - 51.6% voriconazole (VORI), 5.9% echinocandins (ECHINO), 19.0% VORI+ECHINO, 4.5% amphotericine B deoxycholate (C-AMB), 13.1% lipid-based AMB (LBA) and 5.9% other. Overall RR to primary therapy of IA was 51.6% - VORI 57.9%, VORI+ECHINO 57.1%, C-AMB 35%, LBA 44.8%, ECHINO 23.1%. There was a statistically significant difference in overall RR to targeted

therapy in pts. with neutrophil count <0.1 and >1.0 × 10^9/l at the end of therapy (28.3% vs. 53.4%; $P = 0.004$). The overall mortality rate was 57.6%, with 30.7% attributable to IA.
Conclusion Based on our analysis we have confirmed typical risk factors for IA and critical role of S-GM and CT for early diagnosis and prompt start of antifungal therapy of IA. A reasonable treatment response was achieved using VORI, VORI+ECHINO or LBA in primary therapy of IA. We have confirmed neutropenia at the end of antifungal therapy as the major predictive factor for therapeutic response. On behalf of CELL - The Czech leukemia study group for life.

P330

Invasive Candidiasis in children with organic aciduria

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Objectives Organic acidemia or aciduria (OA) refers to a heterogenic group of inherited metabolic disorders, resulting from dysfunction of a specific step in amino-acid catabolism usually due to deficient enzyme activity. Invasive candidiasis (IC) has not been recognized to be associated with OA. On the other hand, acidotic metabolic state (as in diabetic ketoacidosis) has been previously implicated in impaired neutrophil function (impaired adherence, chemotaxis, phagocytosis, pathogen killing, and respiratory burst) and therefore in predisposition to invasive *Candida* infections. Herewith we report a case series of OA pediatric patients with significant metabolic acidosis and simultaneous presence of *Candida* bloodstream infections (CBI), enhancing the hypothesis that OA may predispose to the occurrence of IC.
Methods Children with OA and occurrence of CBI hospitalized at the University Hospital of Muenster (for the period 2006–2014) were enrolled. Demographic data, clinical features, laboratory exams, microbiology data, antifungal treatment and outcome were retrospectively collected. Statistical analysis was performed with SPSS statistical package12.
Results Five patients with OA were enrolled with median age 10.5 yrs (IQR 11) of whom 4 (80%) were males. Three children were diagnosed with methylmalonic acidemia, one patient with glutaric aciduria and one with multiple acetyl CoA dehydrogenase deficiency, respectively. All patients had a permanent central venous catheter and experienced at least one episode of bacteremia while hospitalized for metabolic deterioration. Prior to *Candida* infection, all five children presented with metabolic deterioration with significant acidosis, high ammonia levels and neutropenia with median neutrophil count 742 µl. The median duration of hospitalization was 22 days. Two weeks prior to *Candida* infection, 2 pts presented infection due to *St. epidermidis* and in one patient a *Kocuria rhizophila* infection was recorded**.** Blood cultures grew in 3/5 of patients *Candida albicans*, in 1/5 *Candida parapsilosis* and in 1/5 *Candida lusitaniae*, respectively. Initial antifungal treatment consisted of caspofungin and liposomal *amphotericin* B (*LamB*) in two cases each, and of combined treatment with flucytosine and LamB in one case. Median time for bloodstream sterilization of *Candida* spp. was 5 days. In all patients CVCs were removed with a median time of removal after *Candida* diagnosis 5 days. None of the patients died due to invasive *Candida* infection.
Conclusion The present case series suggest an increased risk of patients with OA for invasive Candida infections during metabolic decompensation with acidosis, granulocytopenia**,** high concentration glucose infusion, antibacterial treatment and presence of central venous catheters as contributing factors.

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Invasive Fungal Infections after Autologous Hematopoietic Stem Cell Transplantation in Children and Adolescents: the Münster Experience

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Background High dose chemotherapy followed by autologous hematopoietic stem cell transplantation (HSCT) carries risks of infectious morbidity. Little is known, however, about the occurrence of invasive fungal diseases (IFDs) in pediatric patients undergoing autologous HSCT. We therefore analyzed the epidemiology and management burden associated with IFDs in a cohort of children and adolescents undergoing autologous HSCT for solid tumors or lymphoma.
Patients and methods In a retrospective single center study, epidemiology and management burden associated with IFDs were analyzed in all pediatric cancer patients who underwent autologous HSCT for solid tumors or lymphoma between 2005 and 2015. Clinical, radiographic, and microbiological data were assessed until engraftment and up to 100 days post-transplant. The primary endpoint was the incidence of proven, probable, and possible IFDs according to current EORTC/MSG definitions. Further endpoints included the use of systemic antifungal agents for prevention and management of suspected or proven/probable IFDs; non-infectious and infectious co-morbidities; and survival until day +100. Patients were housed in single non-HEPA-filtered rooms and received non-absorbable polyenes and penicillin plus ciprofloxacin for antimicrobial prophylaxis. Piperacillin/tazobactam plus gentamycin was used as initial empiric regimen. Interventions for persistent or recurrent fever consisted of pulmonary computed tomography (CT) imaging and appropriate modification of antibacterial empiric therapy. The use of systemic antifungal prophylaxis and of empiric antifungal therapy were at the discretion of the respective attending physician.
Results During the ten-year observation period, 95 patients underwent 103 autologous HSCT procedures (solid tumors 92, lymphoma 11; mean age 9.9, range 0.75-20). High-dose chemotherapy included treosulfan plus melphalan (50), carboplatin based (33), and various other (20) regimens. The mean time to neutrophil engraftment and to discharge were 12 (r, 7–29) and 16 (r, 11–72) days, respectively.

Systemic antifungal prophylaxis was administered to 49 HSCT procedures (47.5%) and consisted mostly (43) of fluconazole. At least one CT scan was performed in 21 procedures. There was no single case of invasive yeast infection. Similarly, no single case of proven/probable mold infection was diagnosed. Nine cases (8.7%) fulfilled criteria of a possible pulmonary mold infection during neutropenia and received mold active antifungal therapy for a median of 14 days (r, 7–35). In an additional 12 procedures, empiric antifungal therapy with mold active agents was given for a median of 8 days (r, 3–105). Considering the total antifungal treatment burden of the cohort, systemic antifungal treatment was administered in 63 procedures (61.2%), either as systemic prophylaxis only (42; 40.8%), prophylaxis followed by empirical or directed treatment (7; 6.8%) and empirical or directed treatment only (14; 13.6%).

Grades III/IV mucositis was recorded in 51 procedures and microbiologically documented non-fungal infections in 17. Five patients were transferred to the ICU. One patient died from biopsy documented toxic endothelial damage at day 83 post-transplant, accounting for an overall mortality rate of <1%.
Conclusions Autologous HSCT for solid tumors or lymphoma was associated with low morbidity from IFDs at our institution. However, utilization of pulmonary CT imaging and use of systemic antifungal agents for prevention and management of suspected IFDs were considerable

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Successful treatment of knee Actinomycosis

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Objectives Actinomycosis of a knee is rare, with few published cases. We report here a case of successful treatment of knee actinomycosis.

Materials and methods A 8-year-old girl was admitted at March 2014 with painless swelling of the left knee after daily activities. One year before trauma of left foot (stab wound with a rusty nail) occured. The patient was diagnosed as 'infectious-allergic arthritis'. She was treated with non-steroid anti-inflammatory drugs. At August 2014 movements of the affected joint was limited. Lab tests (hemoglobin and total white counts, total protein, ALT, AST, blood sugar, rheumatologic tests) were normal. CT scan revealed inhomogeneous contents in the projection prepatelly bags, extending to the anterolateral surface with clear contours, size 50 * 8 mm. Intraoperative inspection showed edema and fibrosis of the tissue surrounding patella left knee, education dimensions of 8 * 6 * 2 cm, yellowish-brown color, with areas of necrosis. Histology showed inflammatory infiltrate comprised of neutrophils, lymphocytes and plenty of foamy macrophages around a sulphur granules.

She was treated for two weeks with IV penicillin 24 000 000 units/d with rapid clinical response and disappearance of all symptoms. Then she used oral amoxicillin 750 mg d^{-1} for 4 months. She has remained asymptomatic since this time. She has remained asymptomatic since this time. General state of health is satisfactory: no pain, full motion of the knee joint. CT imaging of left knee are normal.

Conclusion Intravenous and oral penicillin therapy for 4.5 months to which patient showed excellent response. Antimicrobial therapy with surgery is the treatment for actinomycosis.

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The role of bronchoscopy in the diagnosis of invasive pulmonary aspergillosis in children after allogeneic hematopoietic stem cells transplantation

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Background Early diagnosis of invasive pulmonary aspergillosis (IPA) in patients undergoing hematopoietic stem cell transplantation (HSCT) is one of the factors positively influencing the survival of adult patients. The role of bronchoscopy in diagnosis of IPA in children after HSCT is not clear.

Materials and methods We evaluated the safety and efficacy of bronchoscopy with bronchoalveolar lavage (BAL) fluid ($n = 222$) testing for the diagnosis of IPA in 150 children after allogeneic hematopoietic stem cells transplantation (allo-HSCT). The median of age was 12 years old. We used EVIS EXERA II Olympus endoscopic video system with an external diameter of the distal end tubes of the 3.6 mm and 4.9 mm in a specialized endoscopy room or ICU (12%). Different methods of anesthesia were performed, depending on the age and the degree of respiratory failure of patients with mandatory monitoring of vital functions of the body and oxygen levels. The obtained samples were sent immediately to the laboratory for microscopy with calcofluor white, culture, and galactomannan (GM) detection (Platelia Aspergillus, Bio-Rad).

Results No major complications occurred during bronchoscopy. According to EORTC/MCG 2008 criteria IPA was seen in 30% patients with different various lesions on computed tomography, 16% of them have been proven by biopsy. In multivariate analysis, GM test in BAL fluid showed a high sensitivity compared to microscopy and culture (83.3% and 46.3%, respectively). When the index of the optical density was 0.925, diagnostic accuracy shown in the area under the ROC curve (AUC) analysis was 0.794 (95% CI: 0.665–0.923). The most diagnostic efficiency was achieved by using all three methods - microscopy, culture, and GM test in BAL: AUG was 0.914 (95% CI: 0.853–0.975).

Conclusions - Bronchoscopy with modern video endoscopy equipment and adequate anesthesia can safely be used for diagnosis of invasive pulmonary aspergillosis in children after allo-HSCT.

- The most diagnostic efficiency is achieved by using microscopy, culture, and galactomannan test of bronchoalveolar lavage fluid.

P335

Invasive Fungal Infection (IFI):one center experience with rare filamentous fungi

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Introduction IFI has increased over years as a major cause of morbidity and mortality among hematological malignancy.In paediatric population not only candida cause IFI but filamentous fungi had been implicated due to increasing immunosuppresion and invasive procedures.

Objective We report here our center incidence of IFI and case reports of rare infection by mucomycosis and one disseminated fusariosis.

Methods Retrospective analysis of IFI from January 2014 to December 2014 in paediatric oncology ward of Paediatric Institute,Hospital Kuala Lumpur,Malaysia.

Results 10 cases of fungaemia with 7 cases of candidaemia and 3 cases involving filamentous fungi (2 *fusarium spp,1 of rhizomucor spp.)*.

1st case report of 2 year old child with meduloblastoma with persistent fever and respiratory symptoms with rhizomucor spp isolated from both central and peripheral lines. Despite chemoport removal, fever persists and examination of skin, sinus or lung and CNS were normal. Imagings show pneumonia with no sinusitis or CNS involvement. Responded to prolong couse of liposomal amphotericin B with posaconazole (off label use).

2nd case; a 9 year old girl diagnosed with precursor B ALL in October,2014.Following chemotherapy develop vesicular nodular lesion with crusting and bleeding from nose. Examination showed a febrile child with multiple skin lesions consisting of papulonodule over arms, legs with bilateral crusting of both nostrils (EUA: anterior septal perforation 2 × 2 cm with necrosis of cartilage. Lt side: anterior perforation). Other systema;revealed pneumonia with hepatosplenomegaly and normal CNS examination. Skin biopsy and nasal tissue during EUA grew *Fusarium spp*. Imaging show sinusitis; multiple lung nodules and cavity at rt upper lobe, splenic nodules with no intracranial extension. She had poor response to liposomal amphotericin B but infection resolved with combination of triazole and terbinafine.Environmental screening were negative for both mould.

Conclusion IFI by filamentous fungi is now common in children and isolation even a rare mould need prompt action from clinician to prevent mortality.

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Chronic Hepatosplenic Candidiasis with related IRIS in young child with leukaemia

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Introduction Invasive candidiasis(IC) is common among children undergoing intensive treatment of haematological malignancies. In our institute, the incidence of candidaemia is 13 cases out of 14 fungaemia in year 2012. Chronic form of IC is a rare form of disseminated fungal infection seen during neutrophil recovery seen in subset of patients with IC.
Case report We report here a case of infant who had febrile neutropenia complicated by *Candida tropicalis* infection following chemotherapy for acute lymphoblastic leukaemia. Despite clearance of candidaemia and removal of central venous catheter, fever persisted. Search for other fastidious infections including mycobacterium and filamentous fungi were negative. Repeated bone marrow showed child to be in remission.Splenectomy was performed but despite that and almost three months of amphotericin B, there was no resolution of sign and symptoms hence immune reconstitution was thought of and empiric steroid started. Symptoms finally abated after 12 weeks of steroids.
Method Retrospective analysis of episodes of candidemia in paediatric oncology ward of Paediatric Institute of Hospital Kuala Lumpur from January to December 2014 and the presence of chronic form of IC in this subgroup.
Results There were seven cases of candidaemia in children admitted to paediatric oncology ward throughout 2014.Only one child had chronic hepatosplenic candidiasis.Ultrasound abdomen showed splenic micro abscesses after 2 weeks of neutrophil recovery. Serial ultrasounds later show involvement of liver and kidney. Blood cultures grew *candida tropicalis* and serum mannan was 560 pg Ml^{-1}. Echocardiogram showed pericardial effusion, eye examination was normal and splenic tissue cultures were negative for bacteria, fungi and mycobacterium. Histopathological examination of spleen showed necrotizing granuloma with presence of fungal elements suggestive of candida. Serum galactomanan was repeatedly negative. Ultrasound after 5 months of antifungal; initial monotherapy of amphotericin B followed by combination showed resolution of micro abscesses with serum mannan turning negative.
Conclusions When dealing with chronic disseminated candidiasis, need to think of immune reconstitution inflammatory response (IRIS) and trial of steroid given after exclusion of concomitant bacteria,viral other fungal infections if symptoms did not resolve with antifungal alone.

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Dermatophytosis transmission from guinea pig to child: a case report

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Introduction Dermatophytosis or *Tineas* are superficial fungal infections of the hair, skin and nails of humans and animals. Dermatophytes are spread by direct contact with other people (anthropophilic organisms), animals (zoophilic organisms), and soil (geophilic organisms), as well as indirectly from fomites.
Case report Female patient, 11 years old, presenting erythematous and scaly plaque with vesicles and pustules in lesion on the chest. The lesion appeared four months before the medical assistance, and was associated to itching and burning. The adult guardian claims to have conducted several treatments, including antifungal medications, achieving a partial improvement. Transmission of the fungus by *Cavia porcellus* (guinea pig) was suspected, as the patient said that she frequently took her pet and put near the site of the injury. The animal had scaly lesions on the ear, which began before the appearance of lesions in the patient. Direct mycological examination of the patient's skin material revealed the presence of arthroconidia characteristic of dermatophytes. In the cultural mycological examination of the material from the patient and the guinea pig there was growth of a filamentous fungus with the same whitish and powdery aspect. The microscopic cultures showed hyphae, macro and microconidia caracteristic of *Trichophyton* sp. The urease test was positive, indicating *T. mentagrophytes* or *T. interdigitale*, which can be identified only by molecular methods that are currently being performed. The antifungal susceptibility tests were performed for both isolates, and the minimal inhibitory concentration (MIC) of eight antifungal agents were evaluated by the microdilution method in 98-well plates according to the CLSI protocol M38-A2. The MICs (μg mL^{-1}) for patient/guinea pig were: posaconazole (0.03/0.03); terbinafine (0.03/0.125); tioconazole (0.125/0.06); voriconazole (0.125/0.125); itraconazole (0.25/0.25); amphotericin B (0.5/0.5); ketoconazole (8/8); fluconazole (32/32). These results indicate that the isolates had similar MICs for all antifungals, most of them low, except ketoconazole and fluconazole. The girl and her pet were treated with terbinafine and the lesions were regressing until cure.
Discussion The anthropophilic subspecies of *T. mentagrophytes*, as well as many of the zoophilic strains, formerly differentiated as var. *mentagrophytes* or var. *granulosum*, are indistinguishable and are now designated *T. interdigitale*. The morphological differentiation between anthropophilic and zoophilic *T. interdigitale* strains by classical microscopical and biochemical methods is often problematic. Molecular identifications methods are required to differentiate between the zoophilic strains of *T. interdigitale* and *T. mentagrophytes*. Zoophilic dermatophytes produce acute severe inflammation with pustules and vesicles as described in our case. The household exposure increases the appearance of dermatophyte infection in pre-puberty population, and the extent of inflammation depends on the causal pathogen and the host immune response.

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In vitro hemolytic activity and biofilm production by isolates of yeast species from immunosuppressed pediatric host

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Introduction The ability of pathogenic organisms to acquire elemental iron has been shown to be of pivotal importance in their survival and ability to establish infection within the mammalian host. The pathogen is equipped with a mechanism that destroys the heme moiety and enables it to extract the elemental iron. The enzymes mediating such activity are broadly classified as hemolysins. Medically important biofilms can be split into two major types: those that develop on the surface of implanted devices and those that form directly on the host tissues. Yeast cells in biofilms differ greatly from their planktonic counterparts in terms of their susceptibility to antimicrobials and evasion of host defenses, and there appear to be multiple mechanisms of antimicrobial resistance.
Objective To study isolates of yeast cells species recovered from pediatrics patients for the capacity to produce biofilm and hemolytic activity.

Methods Biofilm formation was determinated by inoculating each strain of yeast species into a glass tube containing Sabouraud Dextrose Broth and other glass tube containing Brain Heart Infusion. Tubes were incubated at 37°C for 24–48 h without agitation. After incubation, the culture broth in the tube was aspirated and the tubes were washed once with distilled water. The walls of the tubes were stained with safranin after media and yeast cells were discarded. Hemolysin production was evaluated using a plate assay. Each strain of yeast species was inoculated on a Sabouraud dextrose agar supplemented with 5% sheep blood. The plates were incubated at 37°C for 48 h. The presence of a distinct translucent halo around the inoculums site, viewed with transmitted light, indicated positive hemolytic activity. Results. 228 isolates were obtained from leukemia, cancer, tuberculosis, fibrocystic disease of breast, meningocele and Fallot's tetralogy patients. The isolates were cultured from urine (n = 108) and respiratory specimens (n = 120). The yeast strains including 132 *Candida albicans,* 44 *C.tropicalis,* 21 *C.parapsilosis,* 11 *C.glabrat*a, 10 *C.lusitaniae,* 6 *Trichosporon asahii* and 4 *C.krusei*. A total of 132 (57.9%) of 228 yeast species isolates obtained were biofilm positive. Biofilm production was most frequently observed for isolates of *C.krusei* (100%, 4 of 4); *C.tropicalis* (93.2%, 41 of 44); *T.asahii* (83%, 5 of 6); and *C.parapsilosis* (71.4%, 15 of 21). At 48 h postinoculation, alpha hemolysis and beta hemolysis could be observed circumscribing the yeast colony. A total of 153 (67.1%) of 228 yeast species isolates obtained produced hemolytic activity. Hemolysin production was most frequently observed for isolates of *C.krusei, C.parapsilosis, C.glabrata* and *C.tropicalis.*

Conclusions Even though non-*albicans* species are considered less invasive and virulent than *C.albicans*, some species are inherently less susceptible to common antifungals and some species such as *tropicalis, glabrata* and *parapsilosis*; have the ability to produce biofilm and hemolysins as components of fungal virulence and cause disrupting host cell membranes.

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Galactomannan antigen detection as a biomarker for invasive mycosis in immunosuppressed pediatric host

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The diagnosis of Invasive Mycosis is based on clinical suspicion, imaging studies, and collection from affected tissue for pathologic and microbiologic examination. Because the prognosis of the disease improves with early commencement of specific therapy, diagnosis should be made as quickly as possible. The detection of galactomannan antigen in pediatric patients immunosuppressed serum samples used in conjunction with other diagnostic procedures can support the diagnosis of Invasive Mycosis.

Objective Galactomannan antigen test in serum for neutropenia leukemia and immnunosuppressed pediatric patients to support invasive mycosis diagnosis.

Method Immunosuppressed pediatric patients galactomannan antigen test was used by immunoenzymatic sandwich microplate assay in serum samples. A first serum sample was tested for galactomannan antigen as individual reference value for each patient and 72 hours after a second serum sample tested was compared with the first value due to galactofuranose in cereals foods as interference probable. Serum samples subsequent were tested weekly. Invasive Mycosis diagnosis established with related host, clinical, microbiologic and histopatologic criteria. **Results** Serum samples were studied for detection of galactomannan antigen in 121 pediatric patients; 83 (68.6%) male and 38 (31.4%) female; 98 of 121 (81%) with neutropenia in leukemia and oncosis patients; 10 of 121 (8.3%) immune-deficiency syndromes; 9 of 121 (7.4%) Fallot's tetralogy; 3 of 121 (2.5%) cystic fibrosis; and 1 of 121 (0.8%) hemolytic-uremic syndrome. Galactomannan antigen was detected in 54/121 patients (44.6%) with >0.500 ng/ml. Only 15 (27.8%) of 54 patients were true positives results in conjunction with mycological culture and clinical evidence; 39 (72.2%) of 54 patients were false positives results because sepsis evidence and presence of galactofuranose in cereals foods. *Aspergillus fumigatus* was in 11 of 15 invasive mycosis with detected galactomannan antigen 0.622-4.101 ng/ml; *Aspergillus versicolor* with 0.598 ng/ml antigen Galactomannan; *Penicillium* spp with 1.225 ng/ml, *Fusarium solannii* with 1.071 ng/ml, and *Candida tropicalis* with 0.880 ng/ml as antigen value results. The death rate was 33.3% (5 of 15) and 66.7% (10 of 15) for successful medical treatment with Voriconazole and Caspofungin acetate. *Aspergillus fumigatus* was etiological agent for invasive mycosis in 4 of 5 dead patients, 2 Fallot's tetralogy, 1 fibrocystic disease of breast, and 1 hemolytic-uremic syndrome. *Fusarium solanii* was etiological agent for invasive mycosis related to Fallot's tetralogy in 1 of 5 dead patients only.

Conclusion Utilizing biopsy to diagnose Invasive Mycosis is hampered by the physiologic condition of the patients, as most cannot tolerate invasive procedures due to thrombocytopenia, coagulation disorders and/or their critical condition. Positive test results for galactomannan antigen with no clinical signs have been reported, especially in young children; however, current data support the usefulness of the galactomannan antigen detection as a biomarker for Invasive Mycosis to be used in conjunction with other diagnosis test as radiographic procedures and clinical findings.

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Disseminated mucormycosis in a 6 month old infant following congenital heart defect corrective surgery: Case report

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Objectives (Introduction) Invasive fungal infections in children are becoming more frequent primarily due to an increased survival of children with primary or secondary immune deficiencies. Although uncommon, mucormycosis has been increasingly identified among immunocompromised patients and carries a high fatality rate. There are several risk factors specially related to an immunodeficiency state such as hematologic malignancy with prolonged neutropenia and bone marrow or solid organ transplant, illustrating the role of phagocytic capacity as well as cellular immunity in the prevention of mucosa and tissue invasion.

Methods (Clinical case) A 6 month old infant was admitted to our intensive care unit due to central cyanosis and heart failure. He had past history of prematurity (35 week and 6 days), neonatal cholestasis, atrial dysplasia with multifocal atrial tachycardia, right ventricle hypoplasia with ventricular tachycardia and inferior vena cava interruption with continuity to superior vena cava through azygos. He was submitted to medical and surgical correction and was on mechanical ventilation and central venous catheterization. During hospital stay he suffered from several nosocomial infections treated with large spectrum antibiotics. On day 48 he was diagnosed with sepsis. A *Rhizomucor pusillus* was isolated from urine and 4 days later it was also detected from several tracheal aspirates. Due to his clinical condition, tissue biopsy was not an option. He was treated with liposomal amphothericin B for 40 days. Caspofungin was added for 27 days. Also vesical irrigations with amphotericin B deoxycholate was done as initial therapy. He was on peritoneal dialysis because of acute renal injury AKIN (Acute Kidney Injury Network) III. Immunological study revealed decreased lymphocyte absolute number, mainly due to CD8 subpopulation. Renal echography showed 2 mm bilateral nodules suggestive of fungal etiology. He was extubated on day 90 with success. Due to clinical recovery but persistence of dialysis dependence, he was transferred to a pediatric nephrology unit after 122 days.

Results (Laboratory findings) A classical urine culture on blood agar revealed the presence of a filamentous non-septate fungus after 24 h incubation at 37 °C. Based on its microscopic morphology it was identified as *Rhizomucor pusillus*. A direct examination of the centrifuged urine showed broad, irregular, hyaline, non-septate hyphae. Culture of respiratory secretions yielded growth of the same fungus and on the wet mount non-septate hyphae were clearly visible. One repeated specimen of urine and two respiratory specimens collected in the following days revealed growth of the same fungus. As morphological identification may be misleading, the isolate was sent to identification by sequencing of genomic DNA fragments.
Conclusion This is a rare case of possible invasive fungal disease in an infant without known inherited or acquired immunodeficiency with positive cultures for *Rhizomucor pusillus* both in urine and tracheal aspirate. Cellular immunity defect with a decrease in TCD8 positive cells may have played a critical role in this rare clinical spectrum of mucormycosis.

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Platelets and complement in the pathogenesis of invasive fungal infections

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Objectives *Aspergillus* and mucormycetes species are leading causes for invasive fungal infections. To better understand the antifungal immune reaction and relevant pathomechanisms we studied the interaction of the fungi with platelets and the complement system, two important elements of the innate immunity. Our recent studies showed that *Aspergillus fumigatus* secretes soluble galactosaminogalactan (GAG) which induces platelet activation and complement deposition on their surface. We expanded these studies to other *Aspergillus* species as well as to different mucormycetes.
Methods Clinical isolates of different *Aspergillus* andmucormycetespecies were grown in RPMI medium for 48 h followed by filtration to obtain the fungal supernatants (SN).Activation and complement deposition on platelets derived from healthy donors were investigated by FACS analysis using specific antibodies. GAG production was detected on the hyphae *of Aspergillus* and mucormycetespp. as stained with FITC-labeled SBA lectin.
Results The culture supernatants of all tested clinical isolates of *Aspergillus fumigatus* and *Aspergillus flavus* triggered significant platelet activation as measured by quantification of the activation marker CD62P. In parallel, SN-induced deposition of complement factor C3 on the platelet surface as well as formation of the terminal complement complex (TCC) could be demonstrated. However, no or only weak induction of platelet activation or complement deposition on their surface could be achieved using the culture supernatants derived from *Aspergillus niger*, *versicolor* or *terreus* as well as from all *mucormycetes* species except one isolate of *Mucor racemosus*. These results correlated perfectly with the expression of GAG by the different fungi, as demonstrated by immunofluorescence using specific staining with SBA lectin.
Conclusion The correlation between GAG expression by the Aspergillus and mucormycete species and isolates with the capacity of the fungal supernatants to stimulate platelet activation and opsonisation underlines our hypothesis that the polysaccharide GAG might be an important fungal immunomodulatory molecule. Putative consequences of its activity are platelet-mediated antifungal attack but also the formation of platelet thrombi.

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Characterization of the *in vitro* interactions between *Aspergillus fumigatus* and the mouse macrophage cell line RAW 264.7

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Objectives The study of the virulence of *Aspergillus fumigatus* and their behavior during the first steps of an infection may involve the use of animal models. However, it requires the approval of the Ethics Committees, which recommend that animal models are replaced or limited. In this context, the use of cell cultures is increasing in importance as an alternative method to study the role of the most important cells during an infection.

That is why, the aim of this study is to standardize and characterize an *in vitro* cell culture model based on the interaction between macrophages and *A. fumigatus.*
Methods The *in vitro* model consisted of co-incubating *A. fumigatus* Af293strain conidia and the murine macrophage RAW 264.7 with a Multiplicity of infection (MOI) of 10. The study was carried out using RPMI 1640 medium containing 2 mM glutamine and penicillin-streptomycin and, in presence and absence of 10% heat-inactivated fetal bovine serum (FBS), during 8 h at 37 °C humidified 5% CO_2 atmosphere. The parameters studied were: a) Phagocytosis and germination b) Reactive oxygen species (ROS) and NO_2 production, and d) Killing by MTT reduction.
Results A progressive increase in the conidia germination was shown, being in the co-incubation higher than in the control of the fungal growth. The maximum phagocytosis rate of 73.04 ± 6.97% was reached after 4 h of infection with *A. fumigatus*. The presence of FBS in the culture medium increased the phagocytosis rate but also the conidia germination. Moreover, we observed that NO_2 production of the macrophages during the infection was a relatively quick process, showing statistical difference regarding to the control condition, whereas the production of ROS was more unstable. Finally the killing study showed a progressive reduction in the fungus viability during infection.
Conclusions The incubation of *A. fumigatus* with RAW 264.7 promotes the germination of the fungus, which is also increased by FBS addition. The presence of FBS also enhances 7 conidia uptake by RAW 264.7 phagocytes. However, at the end of the experiment there were 40% of fungal cells that remained active. On the other hand, NO_2 production by macrophages showed to be a very fast process, whereas there was not a significantly higher ROS production in contact with the fungus than cells alone.

Finally, the results of this work analyze the main parameters of the fungal-macrophage interaction, which may explain what happen during an infection by *A. fumigatus* without the use of any animal model.
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A systems genetics approach to *Candida* infection

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Objectives *Candida albicans* is an opportunistic fungal pathogen that ranks fourth among pathogenic causes of systemic bloodstream infections (candidemia). Immunocompromized patients have an increased risk of *Candida* infections, making adjunctive immunotherapies an attractive strategy to improve outcomes for these patients. Not all at-risk individuals develop *Candida* infections, implying that host genes must influence disease susceptibility, and making insight into genetic susceptibility a first step towards new treatment strategies. Unfortunately, genome wide association studies (GWAS) to identify susceptibility genes are challenging due to the necessity for large cohorts. In addition, GWAS alone cannot implicate functional genetic variants and pathways. To overcome these challenges, we applied a systems genetics approach to candidemia in which multiple layers of molecular data were integrated, and which allowed us not only to identify susceptible variants for candidemia, but also provided deeper biological insights into susceptibility genes and molecular pathways implicated in the host immune defence against *C. albicans*.
Methods We performed Immunochip-wide association (IWA) analysis by using the largest candidemia cohort to date to identify genetic variants associated with candidemia susceptibility. Using RNA sequencing data and genotype data from 629 healthy-donor blood samples, *cis*-expression quantitative trait loci (*cis*-eQTL) mapping was performed to study the effect of genetic variants identified by Immunochip analysis. The functional specificity to *Candida* infection of the *cis*-eQTL genes was further validated in peripheral blood mononuclear cells (PBMCs) upon time-dependent *Candida* stimulation. Candidemia SNPs were then correlated with cytokine levels from *Candida* stimulated PBMCs, providing further evidence for their functional role in anti-*Candida* host immune defence. Pathway enrichment analysis was performed on the candidemia genes we identified using the co-expression-based GeneNetwork database. By integrating these multiple sources of molecular data (Figure 1), we selected gene candidates for functional (knock-down) experiments to further investigate their role in host immune defence against *C. albicans*.
Results The IWA analysis revealed three genome-wide significant loci ($P < 5 \times 10^{-8}$) and 28 SNPs with suggestive, independent associations ($P < 9.99 \times 10^{-5}$) with candidemia. From eQTL mapping, we found 19 SNPs that significantly affect the gene expression levels ($P < 0.01$). We also found, for the first time, that some of the candidemia SNPs affect the expression of long intergenic non-coding RNAs (lincRNAs). Pathway analysis using these eQTL genes revealed pathways that could be implicated in anti-*Candida* host immune defence, including the well-described type I Interferon (IFN) signalling pathway, as well as novel pathways (coagulation and lipoprotein metabolism). Furthermore, our SNPs were significantly correlated with *Candida*-induced IFN-gamma, TNFa, IL-8 and IL-6. Lastly, both *in vitro* and *in vivo* gene perturbation experiments confirmed a significant role for our gene candidates in host immune defence against *Candida* infection.
Conclusions Our system genetics approach has revealed not only genetic variants associated with disease susceptibility but, importantly, molecular pathways implicated in the anti-*Candida* host immune defence. Our study also suggests a role for lincRNAs in host immune defence against *C. albicans*. Ultimately, knowledge derived from our approach could be used for identification of new immunotherapeutic targets for susceptible patients.

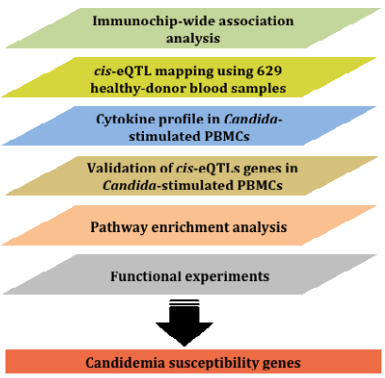

Figure 1: Systems genetics approach revealed novel genetic variants associated with susceptibility to candidemia as well as molecular pathways implicated in anti-*Candida* host immune defense by integrating multiple layers of molecular data.

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Neutrophils functionality against *Aspergillus fumigatus* in allogenic hematopoietic stem cell transplant recipient

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Background *Aspergillus fumigatus* is a major threat for immunocompromised patients, including allogenic hematopoietic stem cell transplant (HSCT) recipients. Although polymorphonuclear neutrophils cells are key effectors against the mould, little is known concerning their functionnality in HSCT recipients during the recovery and over time.
Objectives The aim of this study was to assess the functionality of neutrophils against *A. fumigatus* in allogenic HSCT recipients at several points of the graft.

Methods: the study is a prospective longitudinal study. Thirty-seven patients who benefited from a matched related donor stem cell transplantation were included. The functions of their neutrophils were compared to the functions of the neutrophils sampled from the donors. Recipients were sampled at the recovery time, 2 months, 6 months and 10 months after the transplant. At each point, neutrophils ability to inhibit the fungal growth was assessed. Production of reactive oxygen species (ROS) and expression of neutrophils major surface molecules (CD11b, CD62L, CD66, TLR-2, TLR-4, Dectin-1) were also evaluated by flow cytometry.
Results Our results indicate that the ability of the neutrophils to inhibit *A. fumigatus* hyphal growth is weakened during the recovery. We found that the impairment relies on the use of calcineurine inhibitors, such as cyclosporine. Indeed, we found that neutrophils sampled in patients with an insufficient plasma concentration of calcineurin inhibitor (without other immunosuppressive drugs) at recovery time were as efficient as neutrophils get from donors. Then we performed *in vitro* experiments that confirm this result.

Interestingly, cessation of immunosuppressive drugs 10 months after the graft leads to a retrieval of the inhibition capacity. No difference was observed concerning the ROS production and the surface molecules expression between recipients and donors.
Conclusion Innate immunity against *A. fumigatus* driven by neutrophils is impaired among allogenic HSCT recipients. During the recovery, the impairement seems to be dependant on the use of calcineurin inhibitor, such as cyclosporin. However, the mechanism responsible of this impairment is not yet elucidated. ROS production, one of the major mechanisms used by neutrophils against pathogens as well as surface molecules expression keep stable over time. Therefore, others pathways such as the neutrophil extracellular traps (NET) production have to be investigated.

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In vivo laser therapy as an adjuvant treatment in experimental paracoccidioidomycosis: effect on neutrophils

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Introduction Paracoccidioidomycosis (PCM) is a systemic mycosis caused by the thermodimorphic fungus *Paracoccidioides brasiliensis* (Pb). The presence of granulomatous inflammatory reactions highly permeated by neutrophils is typical in this infection. Neutrophils are crucial in the initial stages of PCM, participating in the innate immunity and directing the acquired immune response towards an effective response. PCM patients show decreased neutrophil activity compared to healthy individuals, suggesting that Pb has a modulating effect on these cells. The treatment of this disease includes the use of antifungals which, in addition to the prolonged administration time needed, have many side effects; so research for more efficient drugs or adjuvant treatments is needed. Low level laser therapy (LLLT) was shown to be an effective activator of immune cells. The aim of this study was to develop a treatment capable of stimulating neutrophils arriving at the injury site elicited by either infection with Pb or inoculation with Zymosan (Z) in subcutaneous air pouches.

Materials and Methods We used *in vivo* application of LLLT at two points on each hind paw of mice on alternate days focusing the bone marrow of femur, in animals either infected with the highly virulent Pb isolate or inoculated with Zymosan (Z) in subcutaneous air pouches; the control groups were infected with Pb or inoculated with Z but did not receive LLLT therapy. The highly pure neutrophils population isolated from the air pouches was analyzed after 10 days of infection or inoculation for the number and viability of neutrophils, protein production, mitochondrial activity, reactive oxygen species production (ROS) and fungicidal capacity by quantification of colony forming units.

Results LLLT increased the production of ROS, the metabolic activity and the fungicidal activity in both Pb and Z groups. Neutrophils were re-exposed to Pb18 (virulent) and Pb265 (avirulent) yeast cells and ROS and protein production were analyzed, showing a higher deactivating effect of Pb18 than of Pb265. Neutrophils submitted to LLLT and exposed to Pb18 produced similar amounts of ROS and of total proteins than PMNs exposed to Pb265. LLLT rendered PMN more active metabolically, leading to higher fungicidal activity against Pb, probably due to increased production of ROS and independently of primary or secondary exposure to Pb.

Conclusions Our results suggest that Pb exerts modulating effect on neutrophils, that this effect depends on the virulence of the fungal isolate and that *in vivo* LLLT treatment reverts this effect, rendering PMN more active metabolically, resulting in higher fungicidal activity against Pb, probably due to increased production of ROS, independently of the primary or secondary exposure to the fungus. We propose this treatment as a complementary therapy for paracoccidioidomycosis.

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Platelets and invasive *Candida* infections *in vitro* and *in vivo*

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Objectives Platelets are nowadays recognized as an important part of innate immunity. They can be activated in response to contact with fungal pathogens, which may lead to multifaceted antimicrobial effects, but also to thrombosis or excessive inflammation.

As common inducers of fungal septicaemia, *Candida* species come in close contact with platelets in the bloodstream. Putative subsequent processes such as mutual binding, activation and decrease of viability can profoundly influence the clinical outcome. For that reasons, we studied platelet-*Candida*-interactions *in vitro* as well as in the blood of patients with Candidaemia.

Methods Human platelets were isolated from the blood of healthy donors and subsequently incubated with clinical isolates of different *Candida* species. Furthermore, blood samples from patients with proven candidaemia were taken every other day. Interactions of platelets with *Candida* were studied by confocal immunofluorescence microscopy and scanning electron microscopy. Platelet activation and viability were investigated by flow cytometry. Fungal growth and viability were quantified by luminometric assay and plating.

Results *In vitro*, the adhesion rate of platelets to yeast cells and hyphae/pseudohyphae of Candida was rather low. Furthermore, no or only marginal activation of platelets could be demonstrated after co-incubation with clinical isolates of different Candida species (*albicans, glabrata, parapsilosis, tropicalis, dubliniensis, lusitaniae, rugosa*). Similarly, the presence of platelets did not affect growth or viability of the fungus. To monitor whether Candida-derived secreted compounds had a stimulatory effect, we incubated platelets with fungal-culture supernatants, but still could not detect any activation.

In a second set of experiments we mimicked the situation *in vivo* using a whole-blood-model, where the interaction between Candida and platelets occurs in the presence of other cellular and soluble elements of the immune defence. However, no platelet activation by incubation with Candida was visible in this setting.

All these *in vitro* results are in contrast to the situation *in vivo* in candidaemic patients. Our pilot study showed that platelets derived from the blood of these patients were significantly stimulated with enhanced levels of the characteristic activation markers CD62P and CD63 as well as increased numbers of circulating platelet-derived microparticles. Furthermore, a decrease of platelet membrane integrity and viability could be monitored, which are typical consequences of platelet activation. The kinetic of the activation markers and platelet viability in the course of the disease differs between the patients, presumably due to various underlying diseases and drug regimens.

Conclusions We hypothesize that the survival of Candida in blood and the establishment of sepsis is facilitated by the lack of direct platelet activation, as shown by *in vitro* experiments. However, stimulation of platelets and subsequent antimicrobial effects may be induced *in vivo* by indirect activation mechanisms, as found in patients suffering from candidemia.

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The *Candida albicans* factor H binding molecule Hgt1p - *in vivo* evidence that it functions as virulence factor

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Objectives The complement system is tightly controlled by several regulators. In particular Factor H (FH) is preferentially acquired by pathogens conveying resistance to complement attack.

The aim of the study was to determine whether the FH binding molecule 'high affinity glucose transporter 1'(CaHgt1p) of *Candida albicans*, a potentially life-threatening yeast, is a significant virulence factor *in vivo*.

Methods The gene coding for this molecule was initially identified by probing an expression library and homozygous deletion mutants of the respective gene have been constructed previously. An *in vivo* study employing the *Galleria mellonella* model has now been used to investigate whether this complement evasion molecule is a virulence factor, i.e., whether Galleria inoculated with the knock-out mutant (i.e. lacking CaHgt1) are surviving longer than those inoculated with the wild type.

Results Especially at 30 °C, but also at 37 °C, *Galleria* larvae inoculated with 10^5 homozygous *hgt1Δ/Δ* deletion mutant yeast cells per larva significantly ($P < 0.05$) lived longer than those inoculated with the restored strain, in which *HGT1* was reintegrated, or inoculated with the wild type strain.

Conclusions The multifunctional complement evasion molecule CaHgt1p is not only a complement inhibitor, but also a virulence factor, corroborating *in vitro* data.

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The role of cytokines in the regulation of inflammation in oropharyngeal candidiasis in patients with hematological malignancies

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Introduction It is clear that patients with hematological malignancies have systemic defects of immune response, which is initially caused by functional imbalance of the bone marrow and later - by therapeutic interventions. The transition from commensalism to infection in the oral mucosa is caused by changes in the local oral microenvironment, overgrowth off *C. albicans* and oropharyngeal candidiasis (OFC). However, according our data, patients with hematological malignancies are not highly prone to OFC. It will occur only in 9% of all hematological patients. Understanding of immunity to Candida may serve the development of new additional immunoregulatory therapy, which lies in their ability to generate and/or enhance the appropriate host immune response required for the control of microorganisms.

Objective This study aimed to investigate cytokine levels in oral fluid of hematological patients with OFC.

Materials and methods We observed three groups. Group I included 38 hematological patients with OFC, Group II: 85 hematological patients without OFC, Group III: 19 healthy people

Results The decrease in local levels of IFN-γ and IL-10 were detected in all hematological patients compared with healthy group. Not also detected significant changes in the levels IL-17, the main cytokine of the early stage of inflammation, which could prevent the fungi invasion into the tissues of the oral cavity mucosa. We also determined the increase of IL-6 and IL-8 in all patients with hematological malignancies compared with healthy people. It was found that the level of hemoattractant MCP-1 in oral fluid in hematological patients in groups I and II was significantly higher compared to healthy individuals (88 (44÷154) and 156 (62÷188) vs 44 (30÷53) pg ml^{-1}, $P < 0.05$, respectively), although in patients with OFC increasing of its production was weaker than in patients without infection.

Conclusion Thus, the increase in cytokines production, activating neutrophils and other cells of the innate immune system, may indicate the increase of antimicrobial protection in the conditions of T-dependent mechanisms deficiency, but can cause inflammatory mucosal damage, which in turn may facilitate its invasion by fungi. The data indicate the need to study other factors of local immunity, significant for the resistance to *C. albicans*.

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Chronic widespread dermatophytosis due to *Trichophyton rubrum*: a syndrome associated with a *Trichophyton*-specific functional defect of phagocytes

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Background and objective Dermatophytes are agents of usually benign superficial infections. However, an increasing number of severe infections in immunocompromised hosts have been reported. We aimed to understand the factors underlying the existence of a cohort of patients presenting chronic widespread dermatophytosis (CWD) due to *Trichophyton rubrum*, but no signs of immunodeficiency.

Methods Fourteen patients meeting the criteria for CWD were studied: *T. rubrum* culture-proven cutaneous lesions with ≥10 cm in at least one dimension, involving at least 3 non-contiguous localizations for >1 year duration, and no predisposing conditions. For comparison, we also studied 13 acute *Tinea pedis* (*Tp*) patients. Macrophages and neutrophils were isolated and tested for *T. rubrum* conidia phagocytic and killing activity. H_2O_2, NO, and pro- and anti-inflammatory cytokines release were measured. All experiments were run with age and sex-matched healthy donors' cells in parallel.

Results CWD's macrophages and neutrophils present reduced *T. rubrum*-phagocytic and killing abilities, and reduced H_2O_2 and NO release compared with healthy donors. CWD's macrophages secreted lower levels of the pro-inflammatory cytokines IL-1beta, IL-6, IL-8 and TNF-alpha, but enhanced levels of the anti-inflammatory cytokine IL-10. Neutrophils' secretion followed closely this unbalanced pattern. In contrast, responses to the positive controls zymosan, LPS and PMA were comparable with those of healthy donors. The same experiments were performed with *Tp*'s macrophages and neutrophils and showed no differences when compared with matched healthy donors.

Conclusion Patients with CWD and a *T. rubrum*-related deficiency of phagocytesmay represent a new specific syndrome in the complex spectrum of the *Trichophyton*-host interaction.

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Antifungal activity of anti-*Candida albicans* germ tube antibodies (CAGTA) from patients with Invasive Candidiasis

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Objective mTo characterize the antifungal activity of antibodies raised by patients with Invasive Candidiasis (IC) against *Candida albicans*. Effect on viability and metabolic activity of *C. albicans.*

Materials and methods Immune sera from 29 patients with IC from different clinical units (Hospital Severo Ochoa-Madrid and University Hospital Cruces-Barakaldo, Spain). Group I: 15 patients infected with *C. albicans*. Group II: 14 patients infected with non-albicans *Candida* spp.

- Anti-*C. albicans* blastospores antibodies (CABLA): human sera were adsorbed with heat inactivated *C. albicans* blastospores and the reacting IgG antibodies were eluted and purified with the Melon Kit (Pierce, USA).

- *C. albicans* germ tube antibodies enriched IgG fraction (CAGTA-fr) was obtained from human sera that had been previously adsorbed with *C. albicans* blastospores. IgG fraction was purified with the Melon Kit.

- CAGTA were eluted with sodium iodide from *C. albicans* germ tubes that had been incubated with immune sera previously adsorbed with heat inactivated blastospores [5]. Eluted antibodies were dialyzed against PBS.

- The effect of antibodies on the metabolic activity of *C. albicans* was estimated with a colorimetric XTT assay [2, 4].

- The viability of *C. albicans* cells was assessed by plating on Sabouraud agar plates and colony counting (CFU). Cells treated with different antibody fractions were also stained with vital fluorescent dyes CFDA and DiBAC [1, 3].

Results The CABLA fraction, mainly anti-mannan antibodies, reduced the metabolic activity of *C. albicans* by 22% at 100 μg ml^{-1}.

- The CAGTA-fr of patients infected with *C. albicans,* at concentrations ≥100 μg ml^{-1}, reduced the metabolic activity of *C. albicans* cells by at least 69%. This inhibitory effect was more effective for CAGTA-fr purified from patients with the highest titers of these antibodies than those with low titers.

- On the contrary, the CAGTA fractions of patients infected with non-*albicans Candida* strainsshowed a reduced inhibitory effect (range 38–43% at concentrations ≥100 μg ml^{-1}) that was not statistically significant with reference to the untreated cells control.

- Concomitantly, at concentrations ≥100 μg ml^{-1}, CAGTA-fr reduced *C. albicans* CFU by 58%.

- Staining with CFDA and DiBAC proved that purified CAGTA (40 and 20 μg ml^{-1}) were fungicidal for *C. albicans* blastospores and filamentous cells.

Conclusions 1. The activity of anti-blastospore antibodies, mainly anti-mannan antibodies, on the metabolic activity of *C. albicans* did not justify the effect of IgG from whole sera of patients with Invasive Candidiasis.

2. The CAGTA enriched IgG fraction is the main responsible for the *in vitro* antifungal effect of the IC immune sera on viability and metabolic activity of *C. albicans*. Thus, the antigens recognized by these antibodies could be the basis for the development of future vaccines against *Candida* invasive infection.

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P355

Microglia shows inefficient phagocytic capacity against the neurotropic fungus *Lomentospora prolificans*

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Objectives The filamentous ascomycete *Lomentospora prolificans* is an opportunistic pathogen that, when gets disseminated into the bloodstream, tends to infect the Central Nervous System (CNS), being termed as a neurotropic fungus. In order to deeply understand the underlying mechanisms of this neurotropism, the aim of this study was to analyze the interactions of fungal cells with microglia, the innate immune cells in the CNS. Moreover, in order to determine if microglial cells are inefficient against this fungus, we compared our results with the activity of monocytes.

Methods *Lomentospora prolificans* strain CECT 20842 was subcultured on potato dextrose agar at 37 °C, being conidia obtained by washing plates with sterile saline. Then, BV2 microglial and J774A.1 monocyte cell lines, and rat primary microglial cultures were used in co-incubation experiments at a multiplicity of infection (MOI) of 1 so as to measure phagocytosis of conidia, conidial germination and hyphal branching, pro-inflammatory cytokines, and reactive oxygen (ROS) and nitrogen species (RNS). Animal experimental procedures were approved by the Ethics Committee for Animal Welfare of UPV/EHU. All cell cultures and fungus-cell co-cultures were maintained in DMEM/10% FBS/2 mM L-Glutamine in a humidified atmosphere at 37 °C and 5% CO_2.

Results Phagocytosis dynamics during co-incubation showed that, while *L. prolificans* is recognized and engulfed by BV2 microglial cells, phagocytic indexes were low in comparison with previously published data concerning this fungus. Accordingly, microglia was not able to inhibit conidial germination. In fact, *L. prolificans* even increased the production of branched hyphae in the presence of microglial cells. Moreover, while microglia was able to significantly produce ROS and RNS, pro-inflammatory cytokines TNF-α and IL-6 were poorly released. All these data made us hypothesize that microglial cells may be inefficient when infected by this fungus. Hence, we performed co-cultures with a monocyte-like cell line to compare the response of both cell types, J774A.1 cells showing improved phagocytic and secreting capacities. To verify and validate our results, we used primary cultures of microglia that, in accordance with the results obtained with the BV2 cell line, showed similar features when exposed to *L. prolificans*.

Conclusions The evaluation of *L. prolificans*-microglia interactions, and their comparison with other phagocytes, has allowed us to determine microglial inefficiency during these fungal infections. In this way, we have identified a decreased phagocytic capacity, both in the cell line and primary cultures, and a poor pro-inflammatory cytokine production. In consequence, although more in depth studies should be performed in order to understand the molecular basis of microglial inefficiency, the results obtained from this work might explain the neurotropism that *L. prolificans* shows during disseminated infections.

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P356

Enolase, cyclophilines and heat shock protein 70 are the major antigens of *Lomentospora prolificans* recognized by human salivary IgA

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Objective *Lomentospora prolificans* is an opportunistic pathogenic fungus, which mainly infection route is the airways. Once inside the host, one of the most important defense mechanisms to protect the mucosa against microorganisms is salivary immunoglobulin A (IgA). These antibodies are able to inhibit pathogen adhesion and tissue invasion, and promote phagocytosis.

Thereby, the main objective of this project was to identify the most important conidial antigens of *L. prolificans* recognized by human IgA, and the study of their prevalence and localization. Moreover, the immunome of this fungus was compared to the phylogenetically related fungi *Scedosporium apiospermum* and *Scedosporium aurantiacum*.
Methods Conidia from *L. prolificans* CECT 20842, *S. aurantiacum* CBS 116910 and *S. apiospermum* CBS 93851 were grown firstly on Potato dextrose agar plates and secondly on Potato dextrose broth, both at 37 °C for 7 days. Finally, conida were recovered by centrifugation and washed with sterile saline. Then, in order to obtain total and cell wall protein extracts, conidia were disrupted by glass bead beating, or incubated during 10 min at 100 °C on extraction buffer, respectively.

The most prevalent antigens of *L. prolificans* were detected by 2-DE and immunobloting, using saliva from 20 healthy donors, and identified by LC-MS/MS. Afterwards, their presence in the salivary immunomes of *S. aurantiacum* and *S. apiospermum* was also analyzed using the same methods.
Results Enolase, cyclophilines and heat shock protein 70 (Hsp70) were the most prevalent antigens of *L. prolificans* recognized by human salivary IgA, being detected by 90%, 80% and 75% of the samples, respectively. In addition, they were studied in the cell wall extract of *L. prolificans*, being the cyclophilines the most intense. To finish, the three antigens were also recognized by IgA on *S. apiospermum* and *S. aurantiacum* immunomes.
Conclusions Enolase, cyclophilines and Hsp70 are the most prevalent antigens of *L. prolificans* recognized by human salivary IgA. Their presence on total and cell wall extracts not only from *L. prolificans* but also from *S. aurantiacum* and *S. apiospermum* has been also proved. Therefore, although it must be researched more in depth, these results and the fact that these antigens are phylogenetically very conserved in eukaryotic and prokaryotic organisms, it may be concluded that their high prevalence, is likely due to cross-reactivity with other fungi.

P357

Hypereosinophilic syndrome with pulmonary Nocardiosis

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Objectives The hypereosinophilic syndromes (HES) are a group of disorders marked by the sustained overproduction of eosinophils, in which eosinophilic infiltration and mediator release cause damage to multiple organs. Nocardiosis is an uncommon gram-positive bacterial infection caused by aerobic actinomycetes. Nocardiosis is typically regarded as an opportunistic infection especially in the patients who require prolonged glucocorticoid therapy.We report a rare case of HES with pulmonary nocardiosis and empyema due to using long term corticosteroid.
Case Report A 32 year-old male patient was admitted to a local university hospital for dyspnea, chest pain and hemoptysis 8 months ago. Laboratory examination showed white blood cell count (WBC) 5.71 × 109/L, eosinophil 63.9%. The peripheral blood smear and bone marrow biopsy showed eosinophilia. FIP1L1/PDGFRA rearrangement was positive. He was diagnosed HES and the treatment of high dose prednisolone was started. In the following 5 months prednisolone dose was reduced. Following the reoccurrence of the same symptoms, dose was increased again. He was admitted to our hospital in February 2012 with the same symptoms. Physical examination revealed both of the lower zones of the lung sounds reduced and the liver and the spleen were palpable. Laboratory examination showed WBC count 7.8 × 10 9/L, eosinophil 0.2%, erythrocyte sedimentation rate 101 mm h^{-1}. In 5 days he had become febrile. For the treatment of HES imatinib 400 mg day^{-1} was started. Pulmonary computed tomography angiography revealed no pulmonary tromboemboly. There was a large pleural effusion (3.5 cm of wideness) in the left hemithorax. There were atelectasis by the pleural effusion. There were necrotic consolidation areas at the left lung upper lobe 8 × 9 cm and 4 × 5 cm at the right lung upper lobe. The thoracentesis was performed to the left pleural effusion. The empyema was diagnosed and thorax tube was placed for the drainage. Nocardia growed in the culture of pleural fluid. For the severity of the disease trimethoprim-sulfamethoxazole 3 × 2 gr day^{-1}, imipenem 4 × 500 mg day, and linezolid 2 × 600 mg day were started. During the clinical follow-up his fever was controlled and the complaints disappeared.
Discussion Pulmonary involvement and hepatosplenomegaly are usual signs of HES. The best characterized and most frequently observed chromosomal aberration is an interstitial deletion on chromosome 4q12 resulting in the fusion of two genes, that for Fip1-like1 (FIP1L1) and PDGFRA. For all patients with the FIP1L1/PDGFRA mutation (even if asymptomatic), imatinib mesylate is recommended for initial therapy, in preference to other available agents (Grade 1B). Nocardia spp have the ability to cause localized or systemic suppurative disease in immunocompromised humans and animals. Despite the success of TMP-SMX in the treatment of nocardiosis, combination therapy with other agents is warranted in patients with severe infections. *In vitro* susceptibilities and animal models of disease have demonstrated activity against Nocardia with a variety of antibiotics, including amikacin, imipenem, meropenem, and linezolid.

P358

Influence of Laminarin in colonisation process of *Candida albicans*

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Objective *Candida albicans* is responsible for the majority of cases of vulvovaginal candidiasis (VVC), one of the most important candidal virulence factors is the ability to adhere to host surfaces. Chemotherapies that seek to improve the host immune response are an alternative to control fungal infections. β-glucans are polymeric carbohydrates that have been reported to modulate human inflammatory responses *in vitro* and *in vivo*. The aim of this study was to determine the influence of Laminarin (LAM) a β-glucan on *C. albicans* virulence, namely colonisation of HeLa cells.
Methods To assess the role of LAM in the cell colonization process, HeLa cells were previously treated or not with 3 mg mL^{-1} of LAM (β-glucan extracted from *Laminarina digitata*) for 30 min at 37 °C,

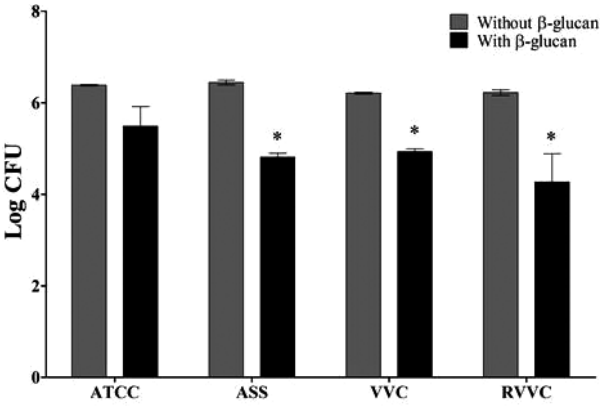

Figure 1. Adhesion of *Candida albicans* from VVC in Hela cells before and after treatment with Laminarin. *p<0.05, significance difference among treated and untreated group.

5% CO_2. Three clinical isolates (5V, 7V and 9V) obtained from female vaginal secretions and one reference strain (ATCC 90028) were used in the study. These strains were separated according to symptoms presented by the patients. Colonization assays were assessed for 2 h incubation at 37 °C, 5% CO_2, with $2x10^5$ HeLa cells mL^{-1} treated or not with LAM and $1x10^7$ yeast mL^{-1} of different clinical isolates of *C. albicans*. After colonization assays, adherent *C. albicans* cells were harvested by detaching the cervical cells monolayer to evaluation of viable cells (colony forming units).

Results In this study, LAM significantly decreased the interaction of VVC clinical isolates with Hela cells (Figure 1). For ASS and VVC isolates, there was a similar reduction in the number of viable cells during colonization process, approximately one log ($P < 0.05$). Moreover, RVVC isolate showed a reduction more expressive, approximately two log ($P < 0.05$).

Conclusion The pathogenesis of VVC involves the initial adherence of the yeast to the vaginal mucosa, followed by asymptomatic colonisation, ultimately leading to infection (symptomatic vaginitis). This study was able to show that LAM a β-glucan can negatively modulate the process of interaction between HeLa cells and *Candida albicans*. These results show that this carbohydrate might be a promising agent for preventing the first contact between yeast and vaginal epithelium, and consequently the development of VVC.

P359

The use of Shock waves for the killing of *Candida albicans*

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Introduction Unprotected sailors swimming in the vicinity of depth charges in World War II were killed with deep tissue changes and minimal external trauma. This was attributed to the unknown effects of Shockwaves (SW).

Early studies on the effects of SW on living tissues such as muscles and lung led to effective use of SW in the 1980s for the treatment of renal stones. SW therapy has been extended for a variety of orthopaedic conditions including chronic non-unions, rotator cuff problems, tennis elbow, and plantar fasciitis. Infection has been perceived as a contraindication to SW treatment in the orthopaedic context due to fears that it will disseminate an otherwise localised infection. The main group of microorganisms implicated in deep tissue, bone and prosthetic material infections are the *Staphylococci* including MRSA, however fungi such as *C. albicans* are of paramount importance as they form biofilms at the site of infection thus preventing the activities of the immune system and antifungal drug. Several studies have shown that when microorganisms are exposed to SW it damages their cell membranes thereby increasing permeability due to the disturbance of the osmotic gradient, allowing electrolyte leakage leading to cell death. The aim of this study was to determine whether SW at levels used for treating patients can kill *C. albicans* planctonic cells and to determine the effect SW exert on tissue.

Methods Four sets of duplicate experiments were performed in which *C. albicans* cells were sandwiched in animal muscle in semisolid of similar viscosity to pus, 1 mL cylinder of 0.6% agar, thus mimicking the conditions during infection. The *Candida* cells were treated with SW at various settings using the Storz Modulith SLX-F2 lithotripter. After SW treatmentthe cylinder containing the cells was transferred into physiological saline, dispersed and viable counts were estimated using decimal dilutions. The viable counts were compared to those of untreated controls as well as controls that did not come into contact with animal muscle. MICs of the SW treated and untreated cells were tested for Amphotericin B, Flucytosine, Fluconazole, Itraconazole, Voriconazole, Posaconazole, Anidulafungin, Caspofungin and Micafungin directly after treatment and after re-culturing using the CLSI recommended method.

Results Exposure of *Candida* cells to animal muscle did not affect their viability. All settings resulted in cell death with 90% killing when 4000 SW were applied at 3 Hz and energy flux density 6 and large focus. The SW also caused a physical effect on the muscle at the interface of different viscosities but did not cause any changes in susceptibility.

Conclusion This study shows that SW exert a significant killing effect on *C albicans*. We believe that the physical effect seen on both sites of the muscle at the interface between low and high viscosity, a phenomenon not normally observed in the clinical situation because of the blood flow of the surrounding tissues that can dissipate heat, will play a crucial role in clearing infections by permitting antifungal drugs and above all the immune system to penetrate the biofilms and the surrounding necrotic tissues.

P360

The absence of TLR-4 receptor *in vivo* increased the severity of *Sporothrix brasiliensis* infection

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Introduction Sporotrichosis is a subacute or chronic infection caused by fungals thermo-dimorphic *Sporothrix* complex. Recent molecular studies show that the genus *Sporothrix* comprises at least six distinct species. Among them the leading cause of epidemic outbreaks, proven virulence and lack of evaluation of immune response is the *Sporothrix brasiliensis* species.

Objectives The aim of this study was to elucidate the role of Toll-4 in the immune response against *S. brasiliensis* 5110.

Material and Methods Bone-marrow derived macrophages (BMMs) from C57BL/6 mice (wild-type and Toll-4-/-) were incubated with *S. brasiliensis* yeast for 3, 6, 12 and 24 h. The interaction was evaluated by optical microscopy and IL-6, TNF-α and IL-10 levels were measured by ELISA. Groups of 4 C57BL/6 mice (wild-type and Toll-4-/-) were infected with *S. brasiliensis* yeast intraperitoneally. Animals were maintained for 14 days before being euthanized. Liver and spleen were collected for determination of fungal burden and cytokine measurements for CBA (IL-10, IFN-γ, IL-4, TNF-α, IL-6, IL-2).

Results The *in vitro* results showed that in the absence of Toll-4 receptor, there was a minor amount of yeast phagocytosed. In *in vivo* tests, in mice Toll-4-/-we observed that after 14 days of infection, the liver and spleen showed more fungal burden. In relation the cytokines, interestingly, we observed absence in the production in wild-type and Toll-4 -/-, which needs to be further investigated.

Conclusions Therefore, it can be suggested that Toll-4 absence interferes with the course of the infection induced by the fungus *S. brasiliensis.*

P366

Detection of *Pneumocystis jirovecii* DHPS genotypes in bronchoalveolar lavages from immunocompromised patients at a University Hospital in Belgium

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Objectives Trimethoprim-sulfamethoxazole (TMP-SMX) is the recommended first line treatment and prophylaxis regimen for *Pnemocystis jirovecii* pneumonia (PCP). This combination inhibits 2 enzymes in folate metabolism: dihydropteroate synthase (DHPS) and dihydrofolate reductase (DHFR) respectively. Mutations in DHPS are associated with prophylaxis failure of TMP-SMX in PCP due to sulfa drug resistance. In this study, we aimed to determine the presence of *P. jirovecci* harbouring DHPS mutations in our hospital and its impact on treatment efficacy.
Methods Between January 2010 and September 2014, *P. jirovecii* was detected by real-time PCR (RT-PCR) in bronchoalveolar lavages (BALs) from 121 patients hospitalized in Erasme Hospital. *Pneumocystis* positive BALs were selected for DHPS analysis: extracted DNA was amplified by nested PCR to amplify a 278-bp region of DHPS gene encompassing polymorphic nucleotide positions ^{165}A/G and ^{171}C/T. The nested PCR products were sequenced using BigDye Terminator chemistry and the DNA sequences were analysed with Bionumerics 6.5. Patients' characteristics were acquired retrospectively by medical chart review.
Results Amplification of the DHPS gene was attempted in 73 of the 121 BAL samples and in these cases DHPS locus was successfully genotyped. No amplification was observed in 48 BALs probably due to low fungal burden. Sixty eight patients harboured *P. jirovecii* wild-type. Two DHPS mutation genotypes were observed in 5 BAL from four patients (6.8%): single mutation resulting in Pro57Ser amino acid substitution (M2 *P. jirovecii*) and double mutation resulting in Thr55Ala and Pro57Ser (M3 *P. jirovecii*). Patient number 1, a renal transplant patient, was treated with TMP-SMX for PCP due to M2 *P. jirovecii*, but died 15 days later of respiratory failure and septic shock. Patient number 2 had primary Sjögren's syndrome and was chronically treated with corticoids and immunosuppressive drug (azathioprine). Two populations of *P. jirovecii* were observed in this patient: M2 *P. jirovecii* and wild type. This patient was switched successfully to pentamidine after poor clinical response despite 10 days of TMP-SMX treatment. Patient number 3 had severe rheumatoid arthritis treated with corticoids and methotrexate and was hospitalized in the intensive care unit for respiratory distress due to influenza A infection. This patient harboured M3 *P. jirovecii*, considered to be colonization, and thus was not treated. These 3 patients were not receiving TMP-SMX prophylaxis before *P. jirovecii* detection by PCR. Patient number 4 was a HIV patient with low CD4 count and not on antiretroviral therapy nor on TMP-SMX prophylaxis. This patient harboured M2 *P. jiroveci* during a first episode of PCP treated successfully with TMP-SMX. Nine months later, on TMP-SMX prophylaxis, M3 *P. jirovecii* was detected during a new PCP episode not observed in the previous sample. This patient was treated successfully with pentamidine.
Conclusions As only low mutation rate of *P. jirovecii* harbouring DHPS mutations was observed in our hospital, it's difficult to attribute prophylactic and/or treatment failures. However 2 patients had failure to TMP-SMX treatment or and 1 to TMP-SMX prophylaxis, which suggests a possible correlation. Further studies are required to better understand the clinical impact of these mutations.

P367

Development and evaluation of a quantitative droplet digital PCR for the molecular diagnosis of invasive aspergillosis

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Objectives Several PCR assays have been described as a possible diagnostic tool for the diagnosis of invasive aspergillosis (IA). Droplet digital PCR (ddPCR, Biorad) is a method that uses emulsion PCR technology to divide complex samples into small, manageable and highly diluted DNA containing subunits. It provides absolute and accurate target quantification without standard curve. In this study, we have optimised and evaluated ddPCR for the diagnosis of IA.
Methods Primers and probes were specifically designed on the basis of the ITS region from *A. fumigatus* and *A. flavus*. Limit of quantification (LOQ) was defined as the amount of DNA (genomic units (GU)/μl) that generate at least 3 positive droplets. LOQ were determined on quantified DNA from *A. fumigatus* and *A. flavus*. Different strains (13 fungi, 8 yeasts, and 11 bacteria) were used to determine the analytical specificity. Diagnostic performance was evaluated on two groups of patient's samples: 1) galactomannan (GM) positive (Platelia-*Aspergillus*, Bio-Rad), composed of 15 broncoalveolar lavage fluid samples (BALs) (index ≥1) and 8 sera (index ≥0.5) and 2) GM negative, composed of 15 BALs (index <1) and 5 sera (index <0.5). Revised European Organization for Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) criteria for the diagnosis of IA were applied by an infectious disease expert to classify the patients in proven, probable, possible and non IA. DNA extraction was performed by QIAsymphony DSP virus/pathogen extraction kit (Qiagen). DdPCR was performed on QX200 Droplet Digital PCR system (Bio-Rad). An home-brew pan-*Aspergillus* real-time PCR (with excellent performance in European Aspergillus PCR Initiative (EAPCRI) evaluations) was performed to confirm ddPCR results.
Results Analytic performance results showed that LOQ of ddPCR was 0.15 GU μl^{-1} of *A. fumigatus* and *A. flavus* DNAbothdiluted in sterile water or spiked in BAL. Among all 32 strains tested for specificity, only *A. fumigatus complex* strains(*A. lentulus*)and *A. ustus* were amplified. Patient's demographics, clinical characteristics and laboratory results are shown in the following tables. Considering proven and probable IA as true positives, and absence of IA as true negatives, the diagnostic performance results showed 31% of sensitivity and 96% of specificity. Concordance with RT-PCR was good (70%).

Table 1

	Galactomannan	
	Pos (n=23)	Neg (n=20)
Age (mean)	62	59
Sex, male/female	12/11	11/9
Underlying disease		
Haematological malignancy	5	2
Solid organ transplantation	7	9
COPD* + corticoids	6	2
Other	5	7

*COPD=Chronic obstructive pulmonary disease

Invasive aspergillosis (EORTC/MSG) classification (number of patients)	Proven (n=1)	Probable (n=12)	Possible (n=4)	Non IA (n=26)
Galactomannan (pos/neg)	1/0	12/0	2/2	8/18
ddPCR (pos/neg)	1/0	3/9	0/4	1/25
RT-PCR (pos/neg)	1/0	3/9	0/4	2/24

Conclusions ddPCR is a promising new technology that allows extremely accurate quantification. However, further prospective evaluation is necessary to evaluate the real diagnostic performance of this ddPCR in the diagnosis of IA.

P369

Caspofungin elicits highly-dynamic and specific phosphatidylinositol-(4,5)-bisphosphate (PIP2) and cell wall integrity pathway (CWIP) responses in *Candida albicans*, which correlate with killing and paradoxical effects

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Background CSP exposure for as brief as 5 min mislocalizes PIP2 to aberrant plasma membrane (PM) sites, and results in sustained killing of *C. albicans* (Ca) over 24 hrs. In baker's yeast, PIP2 activates the CWIP. Our objective was to study PIP2 dynamics and CWIP activation upon exposure to CSP and other agents.
Methods We visualized and quantitated PIP2 over 3 hrs by live-cell imaging of Ca SC5314 expressing CaPH-GFP, measured CWIP activation by Mkc1p phosphorylation, and performed 24 hr time-kills.
Results CSP (0.25x-4× MIC) induced PIP2 mislocalization in the PM within 5 mins, which time-lapse imaging revealed to be highly chaotic over 3 hrs. Mislocalization increased in conjunction with PIP2 accumulation in a dose-response manner, and culminated in arrested, unidirectional septae at aberrant sites. At ≥8× MIC, these responses progressively declined. CWIP activation was transient, and maximal within 10 mins in the same dose-response manner. Likewise, CSP exerted dose-depended killing at ≤4x, but paradoxical growth occured at ≥8× MIC. PIP2 abnormalities were not evident in response to fluconazole (4× MIC), amphotericin B (4× MIC), calcofluor white (50 μg ml^{-1}) or H_2O_2 (10 μg ml^{-1}), even though the latter 3 agents were fungicidal. H_2O_2 was the only other agent to activate the CWIP.
Conclusions CSP exerts rapid and highly-dynamic PIP2 and CWIP responsesthat are specific to β-D-glucan inhibition, correlate with the extent of Ca killing and paradoxical effects, and are not induced by other agents or caused by cell death. Taken with previous studies of PIP2 and septin mutant strains from our lab, the data demonstrate that dynamic regulation of a novel PIP2-septin-CWIP network is a crucial early regulator of CSP responses. Failure to either activate or down-regulate the network increases susceptibility to CSP.

P370

High resolution melting analysis for rapid identification of a wide range of *Candida* species from culture

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Objectives Although *Candida albicans* remains the most common fungal isolate from blood, many studies have described an increasing trend in non–*albicans* infections. Correct identification of *Candida* species is important for targeted antifungal therapy and for epidemiological purposes. The aim of this study was to develop method for broad range *Candida* species identification from culture.
Methods We have tested real-time PCR followed by high resolution melting analysis (HRMA) on 25 reference *Candida* strains and validated on additional 143 clinical isolates. HRMA outcomes were compared with biochemical tests results. All reference and clinical strains revealing discrepancy in species identification between biochemical tests and HRMA were sequenced using *ITS2* region.
Results Considering reference and clinical strains together, 23 out of 27 *Candida* species could be clearly distinguished by HRMA and 4 species were grouped in 2 pairs. *Candida* species could be identified by applying the mean Tm ± 3 SD values of reference strains, the shape of the derivative melting curve and, in some cases, the normalized and temperature - shifted difference plot against *C. krusei*.
Conclusion Real-time PCR followed by HRMA is a simple, rapid and inexpensive tool to identify wide spectrum of *Candida* species. It seems to be a suitable complement to current clinical diagnostic approach based on usage of commercially available biochemical kits.
The study was supported by the grant CKTCH No. 201

P371

Candida parapsilosis clinical isolates resistant and susceptible to azoles with identical genetic profile determined by PFGE-RFLP

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Objectives to characterize the genetic profiles of *C. parapsilosis* clinical isolates presenting different patterns of susceptibility to azoles.
Methods nine *C. parapsilosis sensu stricto* isolated from sterile sites (blood, bone marrow aspirate and central venous catheter) were analyzed. These isolates were obtained from seven intensive care unit patients, one patient from the emergency unit and one from the nephrology service, at a tertiary care hospital of the Universidade Federal do Mato Grosso do Sul, Brazil from 2012 to 2015. PFGE of the *Sfi*I restriction fragments was carried out to evaluate the genetic profiles of all *C. parapsilosis* isolates. The dendrogram analysis was performed by using Bionumerics software. The results were expressed employing the position tolerance setted at 1% and optimization at 3%. The Dice coefficient was used to analyse the similarities of the band patterns and the unweighted pair group method using arithmetic averages (UPGMA) was used for cluster analysis. Susceptibility testing was performed by the standardized broth microdilution technique described by EUCAST with amphotericin B, fluconazole (FCZ) and voriconazole (VCZ), and results were interpreted according to EDef 7.2.
Results the antifungal susceptibility tests showed seven isolates with azole cross-resistance to FCZ (MIC ranging from 32 to 64 mg l^{-1}) and to VCZ (MIC ranging from 0.5 to 2 mg l^{-1}). One isolate was resistant only to FCZ (MIC 8 mg l^{-1}) and the other presented MIC 4 mg l^{-1}. All isolates were susceptible to amphotericin B. The PFGE-RFLP presented the same pattern of bands ranging from 600 to 50 kb for all isolates and the UPGMA dendrogram indicated 100% of similarity.
Conclusion PFGE-*Sfi*I was able to identify closely related clonal isolates from different patients, anatomic sites and nosocomial settings, indicating a possible common source. However, despite being closely related according to the PFGE-*Sfi*I, the isolates showed distinct patterns of susceptibility. Therefore our results suggest that the PFGE-RFLP is not accurate enough to detect possible genetic alterations associated with antifungal susceptibility.

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Typing of *Scopulariopsis* and *Microascus* fungi by Random Amplified Polymorphic DNA (RAPD)

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Objectives *Scopulariopsis* species and their teleomorphs of the genus *Microascus* are commonly isolated from soil, decaying plant material and indoor environments. Moreover, certain *Scopulariopsis* and *Microascus* species are recognised as opportunistic human pathogens. Although most species can be identified by detailed morphological study, phenotypic characters appear to overlap in several cases and morphology seems to be insufficient for establishing species limits in these fungi. Molecular typing has improved understanding of species concepts in many fungal groups, but such a study has not been performed for *Microascus* and *Scopulariopsis* genera. The aim of this study was to investigate the relationships between *Scopulariopsis* and *Microascus* isolates and evaluate the differentiation potential of six Random Amplified Polymorphic DNA (RAPD) assays towards fungi of *Scopulariopsis* and *Microascus* genera.
Methods The study included DNA samples of 38 *Scopulariopsis* and *Microascus* isolates representing 24 species, isolated with Brillowska-Dąbrowska method (*J Clin Microbiol* 2007; 45(4):1200–4). The isolates were obtained from international culture collections (CBS; BCCM/IHEM; DSMZ) and Molecular Biotechnology and Microbiology Department collection of fungi (Gdańsk University of Technology, Gdańsk, Poland). Six primers: LIAN2004-4, LIAN2004-7, CgAP50-1, $(GACA)_4$, CgRAPD-EDP4, CgRAPD-EDP8 were evaluated in RAPD assays. RAPD mixtures, of 20 μ each, consisted of 10 μl of 2× PCR Master Mix Plus High GC (A&A Biotechnology, Gdynia, Poland), 0.2 μl of primer at 100 μM, and 2 μl of DNA. RAPD time-temperature profile included initial denaturation for 3 min at 94 °C followed by 40 cycles of 45 s at 94 °C, 45 s at 45 °C, and 90 s at 72 °C. The presence of amplicons was examined electrophoretically on 2% agarose gels, stained with ethidium bromide.
Results Application of RAPD analysis of DNA extracted from *Scopulariopsis* and *Microascus* fungi revealed differences between species and even single isolates among species. We were able to recognise between 20 and 24 genotypes, depending on the primer used.
Conclusion In this study we found, that RAPD is sufficient for typing of *Scopulariopsis* and *Microascus* fungi. Obtained results support recent redefinition of *Scopulariopsis*, *Microascus* and allied genera by Sandoval-Denis *et al.* (*Persoonia* 2016; 36:1-36).
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Rapid diagnosis of candidaemia using a multiplex PCR test directly to blood from intensive care units patients with presumed sepsis

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Objectives Sepsis is a serious medical condition that requires rapidly administered and appropriate antibiotic treatment. The rapid detection of pathogens in blood is critical for a favorable outcome of patients with suspected sepsis. Especially for *Candida* bloodstream infections (BSI), although blood cultures (BC) are essential for diagnosing candidaemia, their sensitivity to detect *Candida* is 50–75%, with lower sensitivity rates reported in neutropenic patients and those undergoing antifungal treatment. In this study, we attempted to estimate the impact of a commercial available multiplex PCR system in the diagnosis of *Candida* spp. among patients with suspected sepsis in intensive care units (ICUs).
Methods Blood samples from patients with presumed sepsis were incubated into a Bactec 9240[TRADEMARK] system (Becton Dickinson, Heidelberg, Germany) and blood in EDTA from the same patients subjected to analysis with a commercial multiplex real-time PCR (LightCycler® SeptiFast assay, Roche Molecular Systems) according to the manufacturer's instructions. LightCycler® Septifast (LC-SF) assay uses a renovated technology that enables simultaneously the direct detection of a wide panel of Gram-negative, Gram-positive and fungal microorganisms (5 Candida species and Aspergillus fumigatus), commonly involved in systemic infections.
Results From 352 patients examined during 5-years period (from 2010 to 2015), 56 (16%) were detected as positive (31 patients with Gram-negative microorganisms, 16 with Gram-positive microorganisms and 9 with *Candida* spp.). Especially in patients with candidaemia: *C. albicans* was detected in 4 patients, *C. parapsilosis* in 4 patients, while mixed *C. albicans* and *C. tropicalis* was obtained from one case. In six of 9 cases, the pathogen was confirmed by a positive BC and the rest were characterized as negative by BC. In the case of mixed *Candida* bloodstream infection (BSI), only *C. tropicalis* was detected in BC. LC-SF results were available in 7–15 h, in contrast to the 48–72 h required for BC. All patients had risk factors for *Candida* BSI: use of broad-spectrum antibiotics, presence of a central vascular catheter and mechanical ventilation. According to the LC-SF results, initial therapy was inadequate in 6 patients, and antifungal treatment was added promptly in all patients with *Candida* BSI.
Conclusion The rapid multiplex pathogen detection LC-SF system complemented the traditional culture-based methods and offered some added diagnostic value for the timely detection of causative fungal pathogens having a relevant impact on clinical management for a subset of patients with clinically suspected sepsis.

P374

A new *Aspergillus fumigatus* typing method based on hypervariable tandem repeats located within exons of surface protein coding genes (TRESP)

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Objectives Many studies have shown a high genotypic diversity of *A. fumigatus* in the air. Most cases of invasive aspergillosis are caused by *A. fumigatus*, whose conidia are ubiquitous in outdoor and hospital air. *Aspergillus fumigatus* strain typing can fulfill many needs both in clinical settings and otherwise. Short tandem repeats of *A. fumigatus* (STRAf) is widely accepted as the first choice for *A. fumigatus* genotyping. However, difficulties associated with sizing of the obtained PCR, reproducibility results among laboratories and availability of the specific technology in some laboratories encourage the development of novel typing techniques. Recent research has shown the presence of hypervariable tandem repeats within exons of surface protein coding genes (TRESP). The purpose of this study was to create a panel based on these markers which would make possible to differentiate epidemiologically and geographically non-related *A. fumigatus* strains.
Methods Ninety two *A. fumigatus* strains (48 azole susceptible and 44 azole resistant), were used in this study. They belong to our own fungal collection obtained from clinical setting and from some international collaborators. These strains were characterized by PCR amplification and sequencing, with a panel of three TRESP: Afu3 g08990,(*cspA* for cell surface protein A), Afu2 g05150 (MP-2 antigenic galactomannan protein), and Afu6 g14090 (hypothetical

protein with a CFEM domain). For each strain a TRESP type was assigned according to the composition and number of repeats. The type of Afu3 g08990 (*csp*A) was assigned according to the types previously described. A final genotype for each strain was assigned combining the types obtained from the three TRESP genes.
Results A total of 64 genotypes among the 92 strains of *A. fumigatus* were identified with our method, showing a high level of discrimination (Simpson index (D) = 0.968). For Afu3 g08990,we identified 15 types, 25 types for Afu2 g05150 and in the case of Afu6 g14090, we identified 17 types. Azole susceptible isolates were distributed across a total of 43 types (D = 0.995). However, resistant isolates were distributed among only 25 genotypes (D = 0.880).
Conclusions We have developed a novel genotyping method (TRESP), based on PCR and sequencing, which has showed a high discriminatory power. In addition, it is reproducible, easy to perform, and could be specially useful for studying outbreaks; (ii) Interpretation of the sequence information does not require sophisticated algorithms or software and thus can be easily translated into the clinical microbiology laboratory; (iii) Interestingly, a lower index of diversity is obtained when resistant strains are tested compared to the index obtained from the analysis of susceptible strains, suggesting a common ancestor among resistant strains, as previously reported.

P375

Effect of microarray expression data normalization for the choice of differentially expressed genes of *Aspergillus fumigatus* during an intranasal infection

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Objective Microarray technologies are widely used for massive screening of differentially expressed genes under different experimental situations. However, the analysis of this amount of data remains a challenging issue due to changes in experimental and hybridization conditions, but also to the existence of many data preprocessing and normalization methods which attempts to compensate for such effects through use of internal controls. The aim of this abstract is to compare and assess the differences between combinations of seven methods of background correction and two methods of array data normalization in a microarray dataset obtained during an experimental animal model of *Aspergillus fumigatus* intranasal infection.
Methods After intranasal infection during 4 days in a mouse model, and taking daily samples, the transcriptome of *A.fumigatus* was studied using the AWAFUGE expression microarray (v.1) designed by our research group. After hybridization of the RNA samples extracted from the lungs of mice with the Agilent custom chip and following recommendations about pre-processing array expression dataset present in the literature, combination of different methods of background correction and dataset normalization were tested. Regarding to the background correction, exponential-normal method and convolution model were tested combined with quantiles and scale normalization methods were also studied. All these methods and their analyses were implemented using the limma package contained in the Bioconductor project.
Results After the analysis of differentially expressed target genes over the different groups only comparisons between groups 3 and 4 were statistically significant ($P < 0.01$). However, differences among the different normalization and background correction methods were recorded, and the results are on the table attached (table 1).

Based on the data contained on table 1, the normexp background correction yields to the largest number of differentially expressed genes identified regardless the method use to perform the normalization of the microarray data. Despite this appreciation, quantile method of normalization seems to have less variability and give more consistent results than the ones offered after the normalization using the scale method.

Table 1 Results of different normalization and background correction methods

Background correction method	"scale" normalization method			"quantile" normalization method		
	G1–G3	G1–G4	Common expression	G1–G3	G1–G4	Common expression
none	1	7	0	18	20	14
subtract	1	7	1	4	6	2
half	71	0	0	7	7	2
minimum	71	0	0	7	7	2
movingmin	0	3	0	9	14	6
edward	71	2	0	9	14	6
normexp	18	15	10	15	13	10

Conclusion The normalization process is essential to compare the varying conditions of microarray experiments. While there are several methods, in our experience, quantile method seems to work best in reducing all the differences in expression values for replicates samples and gives the most consistent results.
Financial support This work was funded by the UPV/EHU grants (PES13/03, GIU12/44 and UFI11/25), the Government of the Basque Country grant (S-PC11UN007), and the Ministry of Economy and Competitiveness (MICINN CSD2009-00006). Research Grants of the: Fundación Jesús Gangoiti Barrera, Government of the Basque Country and University of Basque Country (UPV/EHU), have supported Guruceaga X., Sueiro-Olivares M., and Fernandez-Molina J. respectively.

P376

Trichophyton anamorph of *Arthroderma benhamiae* - an emerging pathogen in dermatomycology - morphological and molecular biological characterization of six wild strains

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Objective In the past few years, the zoophilic dermatophyte *Trichophyton* (*T.*)anamorph of*Arthroderma* (*A.*)*benhamiae* was increasingly isolated as causative agent of often inflammatorydermatophytosis predominantly in children and adolescents, however, also in adults. Source of infectionof *T.* anamorph of *A. benhamiae* are small rodents, first of all guinea pigs.
Patients and methods Six wild strains of *T.* anamorph of *A. benhamiae* were isolated from skin scrapingsof patients suffering from dermatophytosis. All strains were characterized based on morphological and metabolicfeatures. For molecular biological detection of the dermatophytes in skin scrapings a uniplex PCR-EIA was used.Species identification was confirmed by sequencing of the internal transcribed spacer region (ITS) of the ribosomalDNA. In addition to ITS rDNA, GPD gene and microsatellite typing scheme were used for identification of the strains.Further, the strains were typed by Matrix Assisted Laser Desorption/Ionisation Time-Of-Flight MassSpectrometry (MALDI TOF MS).
Results The six strains of *T.* anamorph of *A. benhamiae* were isolated from 4 children and 2 adults suffering fromtinea corporis or tinea faciei. Guinea pigs were the source of infection in 5 out of 6 patients, the sixth patient haddifferent pets at home. 5 out of the 6 isolates had typical yellow stained colonies (Fig. 1), one isolate was the socalled white-colony-type (Fig. 2). Microscopically, the yellow strains showed few microconidia, however ring hyphaewere detected.

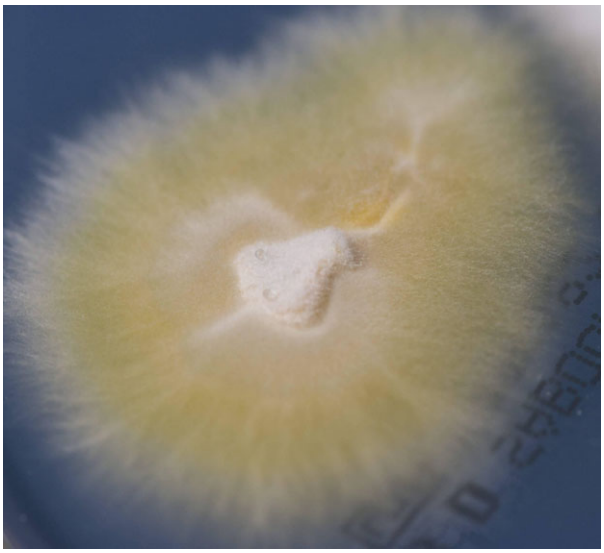

Figure 1. T. anamorph of A. benhamiae strains with yellow colonies

Figure 2. T. anamorph of A. benhamiae strains with white colonies

The white strain showed abundant microconidia. Urease test was negative in all yellow strains, thewhite strain showed strong positive urease reaction. Hair perforation test was negative in all strains. All strains wereconfirmed as *T.* anamorph of *A. benhamiae* by PCR-EIA, sequencing of the ITS rDNA, GPD gene and microsatellitetyping. In MALDI TOF MS the 5 yellow strains had identical spectra, the white strain showed marked differences.All six strains used in this study have been deposited in the Centraalbureau voor Schimmelcultures (CBS), Utrecht,The Netherlands: No. CBS 139072, 139073, 139074, 139075, 139076 and 139077.

Conclusion The dermatophyte *T.* anamorph of *A. benhamiae* represents an 'emerging pathogen' both in Germany,but also in the Czech Republic and other European countries. Within the species *T.* anamorph of *A. benhamiae* strainswith yellow colonies can be distinguished from those with white colonies. There are clear morphological and moleculardifferences between these two types.

P378

Sporothrix brasiliensis infection in cats: a mycological and molecular study of 47 clinical isolates from Rio de Janeiro, Brazil

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Sporotrichosis is a subcutaneous mycosis caused by the *Sporothrix* complex. Since 1998, in Rio de Janeiro, Brazil, it has been diagnosed a wide number of cases in humans and animals, especially cats. Based on phenotypic and molecular studies, a high intraspecific variability between morphologically isolates identified as *S. schenckii* was demonstrated, which led to proposition of a complex of six species: *S. schenckii*, *S. brasiliensis*, *S. globosa*, *S. mexicana*, *S. pallida* and *S. luriei*. *S. brasiliensis* has been described as an emerging specie, highly pathogenic for humans and animals, with a regional distribution in Brazil. Studies involving the characterization of cat isolates are scarce.

Objectives The objective of this study was to characterize 47 *Sporothrix* spp. isolates from cats obtained before start of antifungal treatment and assisted at the Evandro Chagas National Institute of Infectious Diseases/Fiocruz, Rio de Janeiro, Brazil.

Methods For characterization in species level were used characterized phenotypically, according to the identification key, including analysis of conidial morphology and the auxonogram analysis using raffinose and sucrose as carbon sources. However, identification based on this key could be inconclusive due to phenotypic variability within these species being used the genotypic identification of species by T3B PCR fingerprinting. The control strains of *S. brasiliensis*, *S. globosa*, *S. schenckii*, and a *S. mexicana* were included in this study.

Results In phenotypic identification thirty-five isolates were characterized phenotypically, according to the identification key as *S. brasiliensis*. Twelve did not show a typical phenotype with any species of the complex, being classified as *Sporothrix* spp..

Applying the molecular analysis of the T3B PCR fingerprinting all strains included in this study were identification as *S. brasiliensis*. This methodology was also able to distinguish the species of strains misidentified by the phenotypic analysis.

Conclusion *S. brasiliensis* is the only species in feline sporotrichosis in endemic area of Rio de Janeiro until now. However, the correlation between phenotypic and molecular methods are necessary for species characterization.

P379

Multilocus sequencing of the micromycetes-biodestructors *Stachybotrys* spp.

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Micromycetes *Stachybotrys*spp. are commonly found in water-damaged buildings and often considered to be an indicator organism of indoor air problems. This fungus is known to produce mycotoxins that has been associated with a number of human and veterinary health problems. Many investigators noticevariation inthe levels of toxin production among Stachybotrys individuals that can be predicted by nucleotide analysis. The most variable genes of Stachybotrys are the trichodiene synthase gene and the chitin synthase gene.

Objective of this study is intraspecies typing of *Stachybotrys*spp. by multilocus sequencing for discriminating toxic and nontoxic strains.
Methods We examined 16 strains of *Stachybotrys*spp. All isolates were tested biochemically, the quantity of mycotoxins were measured. DNA was extracted from 10-day samples using a modification of a CTAB extraction protocol. PCR was performed by the trichodiene synthase loci using primer pairs Tri5 and STOX, and by the chitin synthase loci with primer pair Chs. Sequencing was performed by Sanger method using the analyzer ABI Prizm 3500. Sequences obtained from Tri5, STOX, and Chswere aligned in the program MEGA 5.2. Isolates were analyzed by unweighted pairgroup method of arithmetic averages (UPGMA) cluster analysis.
Results The amplification program was modificated. Reaction performed with 0.5 ng of DNA and annealing temperature 52 °C. There were 10 high-toxic and 6 low-toxic strains investigated biochemically. The trichodiene synthase gene fragment presented in all samples. Phylogenetic trees that represent connections between strainswere constructed. We inwestigated several distinct groups of strains with similar sequences. This strains in each group had also similar amountof mycotoxins.
Conclusion The intraspecies typing by multilocus sequencing can be used for discriminating toxic and nontoxic strains of *Stachybotrys* spp.

P380

Characterization of the effect of anti-KRE9 monoclonal antibody against *Candida albicans*

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Objectives *Candida albicans* is an opportunistic fungus, being the main fungal pathogen responsible for the nosocomial infections. Among all its proteins, *C. albicans* KRE9 is an essential and highly mannosylated protein which is involved in beta-1,6-glucan biosynthesis. Therefore, the aim of this study was to generate an anti-KRE9 monoclonal antibody (mAb), and to test its specificity towards KRE9 and its effect on *C. albicans* growth.
Methods The anti-KRE9 mAb was produced using the hybridome technique. Among the positive cell lines generated, the most reactive one was selected to be cultured and to purify the mAb used in this work.

The specific recognition of the protein was tested by 1-D and 2-D electrophoresis and Western Blot. On the other hand, in order to localize the protein in the yeast, an indirect immunofluorescence (IIF) was also performed; using different strains of live and sodium metaperiodate (50 mM)-treated *C. albicans*. Finally, the effect of the mAb over yeast growth and endothelial cell activation was also measured.
Results The anti-KRE9 mAb recognized specifically one band, corresponding to KRE9 protein, on the extract of *C. albicans*. However, using IIF it was only detected on the cell surface of the metaperiodate treated yeast, thus, when mannoses of the cell wall had been removed. On the other hand, the mAb was able to reduce *C. albicans* growth, both of the reference strain and the clinical isolate CECT 13062, at least at a concentration of 10 ng/ml. Endothelial cell activation induced by *C. albicans* was also significantly diminished by the antibody at the same concentration.
Conclusion The anti-KRE9 mAb specifically binds to *C. albicans* KRE9. However, since this protein is highly mannosylated, the detection by IIF was unable unless *C. albicans* cells were treated to remove the mannoses present in the cell wall. Even so, the mAb was able to reduce significantly yeast growth and endothelial cell activation by *C. albicans*. Therefore, although promising results have been obtained, more studied should be carried out and in the near future other effects will be studied in depth, such as inhibition of germination or biofilm formation. In conclusion, this antibody, alone or in combination with commonly used antifungal drugs, may be of interest for the treatment of immunocompromised patients suffering from *C. albicans* infections.
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P381

Genetic adaptive mechanisms mediating response and tolerance to acetic acid stress in the human pathogen *Candida glabrata*: role of the CgHaa1-dependent signaling pathway

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Background and objectives *C. glabrata* is a commensal found in the human genitourinary tract but under certain conditions this harmless colonization evolves to a mucosal infection and, in more serious cases, to disseminated mycosis. To thrive in the acidic vaginal tract *C. glabrata* has to cope with the presence of a competing commensal microbiota that restrains the overgrowth of pathogens by producing acetic and lactic acids, among other interference effects. The persistent emergence of *C. glabrata* strains resistant to currently used antifungals demands the implementation of novel therapeutic strategies based on non-conventional targets. Genes contributing to increase *C. glabrata* competitiveness in the vaginal tract by mediating tolerance to the organic acids found therein are a cohort of interesting, and yet unexplored, set of therapeutic targets. Thus, the objective of this work is the identification of key genes/pathways/mechanisms underlying survival of *C. glabrata* to acetic and/or lactic acids. In particular, the characterization of the involvement of a new signalling system, controlled by the putative transcription factor CgHaa1, in C. glabrata tolerance and response to acetic acid is aimed.
Results and conclusions Elimination of *CgHAA1* gene from *C. glabrata* genome dramatically increased susceptibility of this pathogenic yeast to concentrations of acetic acid similar to those found in the vaginal tract. A transcriptomic analysis revealed that CgHaa1 impacted the expression of roughly 70% of the overall set of C. glabrata genes that are activated in response to acetic acid stress, confirming the crucial role of this transcriptional regulator in the control of genomic expression under these conditions. Functional clustering of the genes activated by CgHaa1 under acetic acid stress shows an enrichment of those involved in carbohydrate metabolism, transport, cell wall maintenance, regulation of internal pH and nucleic acid processing. The mechanisms by which the CgHaa1 pathway mediate tolerance to acetic acid in *C. glabrata* were further dissected, exploring a transcriptomics approach, being of notice the involvement of this regulatory system in the control of internal pH and in reducing the internal accumulation of the acid. In the presence of acetic acid CgHaa1 enhanced adhesion and colonization of reconstituted vaginal human epithelium by *C. glabrata*, an *in vitro* model of vaginal infection. Consistently, CgHaa1 expression exerted a positive effect over the expression of several adhesin-encoding genes and increased C. glabrara adherence to the extracellular matrix proteins fibronectin and vitronectin.

On the overall the results obtained show similarities, but also remarkable differences, in the way by which the ScHaa1 and

CgHaa1 pathways mediate tolerance to acetic acid in *S. cerevisiae* and in *C. glabrata*, indicating a 'functional expansion'of the network in the later species. Further details on the evolution of this network from S. cerevisae to C. glabrata will be discussed. The role of the CgHaa1-pathway in the extreme acetic acid-tolerance exhibited by vaginal *C. glabrata* isolates will also be discussed, along with other uncovered mechanistic insights that were found to be on the basis of the acid tolerance phenotype exhibited by these isolates.

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Standardization of a two step real-time PCR based method for species specific detection of medically important *Aspergillus* sp.

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Objective To standardize a two step real-time PCR based method using 28S rDNA and beta-tubulin gene, calmodulin gene specific target as a tool for rapid detection of aspergillosis from whole-blood samples.
Method Five medically important *Aspergillus* sp. i.e. three reference isolates *Asp. fumigatus* (ATCC-204305), *Asp. niger* (ATCC-16404), *Asp. flavus* (ATCC-204304) and two clinical isolates *Asp. terreus, Asp. clavatus*, were used for standardization. The inoculum size was adjusted to a concentration of 5×10^6 to 1×10^8conidia ml^{-1} by hematocytometer. Total genomic DNA was extracted by bead beating with thermal shock treatment prior to the use of commercial DNA extraction kit. Extraction yield and purity was checked by NanoDrop 2000 and gel electrophoresis. Efficiency of extraction was checked in real time PCR. A PAN fungal PCR was performed using custom designed primers and probe targeting 28S rDNA for screening (Step-I) followed by species specific primer targeting beta-tubulin gene and calmodulin gene as targets (Step-II). All positive PCR products from each of these reactions were then sequenced in duplicate or triplicate using standard BIGdye3 chemistry and methods on an ABI3100 (Applied Biosystems, CA).
Result DNA extracted was observed to have a concentration between 40 to100 μg $μl^{-1}$. In terms of purity the 260/280 ratio were between 1.2 to 2.0. Taq Man based real time-PCR showed satisfactory sensitivity and specificity for *Asp. fumigatus*, *Asp. flavus, Asp. niger, Asp. terreus*. Analytical sensitivity for PAN *Aspergillus* 28S rDNA primer and probe was found to be 10^2 cfu ml^{-1}. Species specific detection of *Aspergillus* was made for four species i.e. *Asp. fumigatus, Asp. niger, Asp. flavus, Asp.terreus* and specificity was checked against 37 different microbial species including eukaryotes and prokaryotes. All the species specific primers BLASTn, result were then carefully analyzed in graphical and statistical form. The graphical form was used to ensure that the all of the four products of the query sequence entered into the search was subsequently aligned with the high scoring database sequence returned (99–100% query coverage and 99–100% identity).
Conclusion Data from this study indicate the efficacy of the current DNA extraction method using physical sharing and it is found to be specific, sensitive, stable and easy to serve as a useful tool for rapid detection of aspergillosis from whole-blood samples.

P383

Epidemiological aspects and genotypic characterization of *T.violaceum* strains collected during a Belgian National survey on anthropophilic tinea

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Objectives The last 2 years, clinical cases of tinea capitis caused by *Trichophyton violaceum (T. violaceum)*, have been identified in Belgium. To better understand the emergence of this species in the population, the Belgian National Reference Center (NRC Liège) launched a 1-year national survey in 2013. Epidemiological aspects and genotypic characterization of the strains were included.
Methods The study was conducted from March 2013 up to February 2014. All Belgian laboratories were asked to send *M. audouinii* and *T. violaceum* strains isolated from hair to the NRC with a form to fill in including epidemiological data. The fungal strains were identified by microscopy or ITS sequencing in case of doubtful identification. The genotypic analysis was performed by the DiversiLab® system (bioMérieux) for DNA fingerprinting and analysis. Epidemiological data were analyzed with the help of a biostatistician.
Results Amongst the collected isolates, 23 strains were confirmed as *T.violaceum* (results concerning the 116 *M. audouinii* strains have already been reported). Analysis of the epidemiological characteristics of the infected population shows that the main age category concerns 0–4 year-old children ($n = 9$, 39.1%) with a sex-ratio M/F of 1.875. Data concerning the ethnical origin of the family were present in 82.6% of the cases and reveal that patients were mainly of Ethiopian origin ($n = 8$, 57.9% of known cases). One patient was also from Burundi showing that *T. violaceum* strains probably circulate mainly in East Africa. The genotypic analysis by DiversiLab® led to the distinction of 2 variants of *T. violaceum*. The major group was composed of 17 strains which were mainly collected in the North of Belgium and included also the reference strain (18/23, 83.3%). The other group (6 strains) was close to the major group but the analysis of the spectral superposition showed some differences between these two groups, defining two distinct variants of *T. violaceum* in the Belgian population. This second variant was mainly recovered from South Belgium (5/6, 83.3%). No correlation could be made between the genotypic group and a particular ethnical origin as Ethiopian subjects were found in both groups.
Conclusion The DiversiLab® system proved to be an efficient method to investigate the molecular epidemiology of dermatophytes infections as reported previously for *M. audouinii*. These results show that two distinct isolates co-exist in Belgium providing evidence of genetic heterogeneity and a possible spread of one genotypic variant in a restricted geographic area or the co-existence of two variants circulating in different African communities. However, no clear correlation could be established between the appartenance to a group and epidemiological factors, such as age or ethnical origin.

P384

Environmental isolation of *Cryptococcus gattii* molecular type VGII and *Cryptococcus neoformans* molecular type VNI from wooden poles located in the deep Amazon Rio Negro basin

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Background Primary cryptococcosis caused by molecular type VGII (serotype B, MAT alpha) prevails in immunocompetent patients in the North and Northeast of Brazil, revealing an endemic regional pattern of cryptococcosis by this molecular type.
Objective This study investigated the presence of cryptococcosis agents on wooden poles in neighborhoods located in the Upper Rio Negro, municipality of Santa Isabel do Rio Negro, Amazon, Brazil.
Methods The decaying wood was collected from the poles, and this material was diluted and plated on agar bird seed. Dark brown colonies were identified phenotypically and genotypically by URA5 polymorphism restriction fragment (RFLP) analysis.
Results The samples were positive for *C. gattii* molecular type VGII and *C. neoformans* molecular type VNI by URA5 polymorphism restriction fragment (RFLP) analysis.
Conclusions For the first time *C. gattii* molecular type VGII and *C. neoformans* molecular type VNI were isolated from wooden poles, largely used by the rural population of the mainland forest of the Amazonia basin. These findings correlate with the endemic status of cryptococcosis in Amazonia and complement previous study in the same area of Santa Isabel do Rio Negro. In this municipality, different MLST subtypes of *C. gattii* VGII were isolated from indoor dust from typical wooden houses (Brito-Santos *et al.* 2015). Besides the dust indoor, the wooden poles may act as potential sources of human infection by agents of cryptoccocosis, which are associated with a specialized niche resulting from the natural biodegradation of wood (Lazera et al. 2000).

P385

Standardization of PCR and molecular speciation of 17 cases of *Fusarium keratitis* from South India using sequences of the ITS and TEF 1a genes

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Objectives To standardize an in-house method of PCR using primers specific to ITS-1 & 2 and Translation Elongation Factor - α (TEF-1α) genes to detect and differentiate clinical *Fusarium* isolates.

Sequencing of PCR amplicons and sequence analysis and molecular identification using BLAST

To confirm if TEFα is a better target gene than ITS for unambiguous differentiation of *Fusarium* species.
Methods 17 *Fusarium* isolates of human keratitis from Tamilnadu and Andhra Pradesh in south India were studied. Growth in standard media like Sabouraud's Dextrose Agar and microscopic features of slide cultures using Oatmeal Agar were noted. Genus level identification was done but speciation wasn't possible in majority of isolates.

DNA isolation - performed using growth from Sabouraud's Dextrose Broth following Phenol-Chloroform method. No commercial kit used.

Primer sequences for ITS and TEF-1 genetic regions were obtained from Mitchell et al., 1994 & Jackson et al., 2006 respectively. *Fusarium solani* and *Fusarium oxysporum* isolates confirmed by gene sequencing were used as control.

Agarose Gel Electrophoresis was performed to visualize the amplicons.

The ITS (Internal Transcribed Spacer) region TEF1α (Translation Elongation Factor 1α) gene from all the 17 *Fusarium* isolates were sequenced (SciGenom labs, Cochin, Kerala, India). The sequences results were then used for nucleotide search using the BLAST algorithm at the NCBI website (http://www.ncbi.nlm.nih.gov/BLAST/) and Fusarium ID BLAST (http://isolate.fusariumdb.org/index.php). Hits more than 99% were considered.

Result

BLAST analysis ITS sequences - Out of the 17 isolates, 14 (A47, A68, A71, A72, A73, A97, A98, A99, A103, A109, A74, A96, A100 and A108) sequences gave definitive results and 3 out of 17 (A95, A102 and A110) gave more than one result in the NCBI blast. In Fusarium ID blast, 5 out of 17 (A71, A72, A95, A97 and A 99) gave definitive results, 3 out of 17 (A73, A102 and A110) gave two results where both belonged to the same species complex but

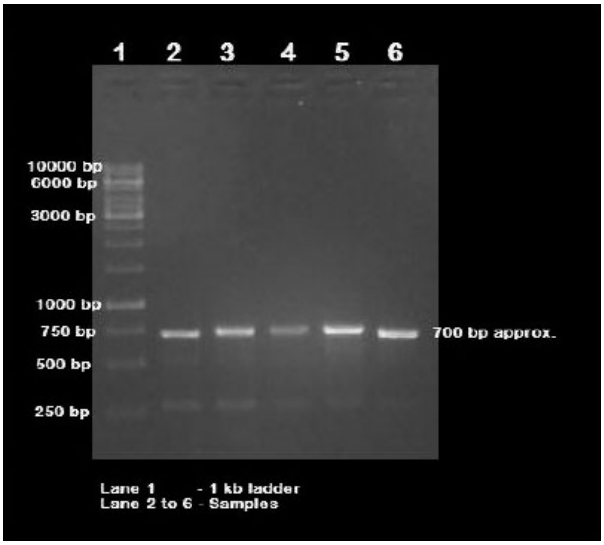

Figure 1. Gel showing amplicon of TEF region

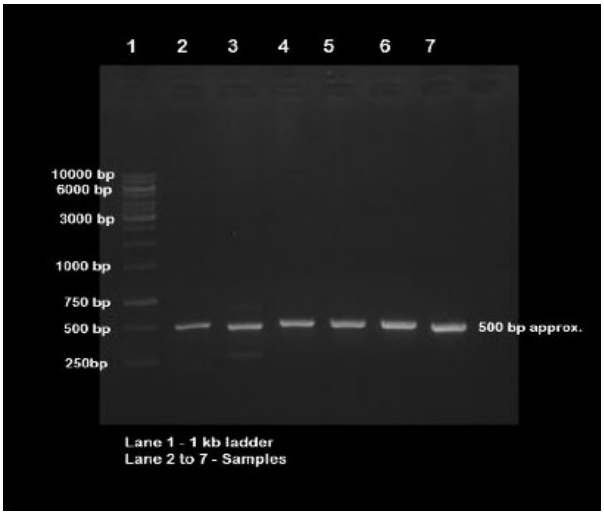

Figure 2. Gel showing amplicon of ITS region

different groups, 9 out of 17 (A47, A68, A98, A103, A109, A74, A96, A100 and A108) gave more than one result where all belonged to different species. Furthermore, the results obtained in NCBI blast and Fusarium ID blast did not correlate with each other.

TEF 1α sequences - Out of the 17 *Fusarium* isolates, 10 were *Fusarium falciforme*, which belonged to *Fusarium solani* species complex (isolate numbers-A47, A72, A74, A95, A96, A99, A100, A103, A108and A109. The remaining 7 isolates belonged to *Gibberella fujikuroi* species complex. Thevarious *Fusarium* species which were perceived in the *Gibberella fujikuroi* species complexwere 1 *Fusarium proliferatum* (A110), 1 *Fusarium sacchari* (A98), 1 *Fusarium verticilloides* (A71), 1 *Fusarium thapsinum* and 1 *Fusarium subqlutinans/Fusarium pseudocircinatum*. Isolate A102 belonged to *Fusarium oxysporum* species complex. Isolate A97 showed BLAST result as *Fusarium solani* in NCBI database and as *Giberella fujikuroi* in Fusarium ID database leaving it ambiguous.

Conclusion ITS region provides adequate genetic information to reliably detect *Fusarium* upto species level, which serves ideal for rapid molecular test for diagnosis in a clinical setting, TEF-1α is the most phylogenetically informative gene and is ideal for molecularspeciation in epidemiological studies.

Correction added on 19 October 2015, after online publication. Presenting Author was changed from S. Sharma to A. Tupaki Sreepurna.

P386

Molecular epidemiology of *Mallasezia* species in students of Sabzevar universities

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Objectives The genus *Malasezia* currently includes fourteen species that have been considerd as part of normal flora of skin in healthy and diseased human and animal skin. However, there were differences with respect to the species most commonly isolated, not only in patients with various skin diseases but also between healthy individuals. The aim of this study was to analyze the prevalence of *Malassezia* species from clinically normal skin of the scalp and trunk in students of Sabzevar universities and to examine if the range of species varies according to body site, gender and age.

Methods The study was conducted at the Department of Microbiology, Sabzevar University of Medical Sciences, Iran from September 2012 to November 2013. One hundred and eighty-nine healthy students of Sabzevar University with no skin diseases and aged from 18 to 28 years were studied. The samples were obtained by scraping the skin surface from the upper and middle part of trunk and from scalps of all subjects and then all samples were prepared with KOH 20% and methylene blue for staining and undergone microscopic examinations and also inoculated in plates containing Glucose-Neopepton-Yeast extract medium (pH 6.2) with additional olive oil (20 $mL.L^{-1}$), Tween 80 (2 $mL.L^{-1}$) and Glycerol monostrated (2.5 $mL.L^{-1}$) incubated at 31 °C for 2 weeks and examined at frequent intervals for developing colonies.

DNA extraction was performed from colonies. The ITS1 region of rDNA from isolates of Malassezia species were amplified by PCR reaction. The PCR were digested by Cfo I enzyme.

Quantitative data were analyzed by the group of t-test. The data of the students were analyzed using chi-square test. Correlation of predisposing factors with *Mallasezia species*as well as the difference between isolates from students was evaluated by Fisher exact test. A ρ-values of <0.05 were considered as significant.

Results The products of restriction digestion are shown separately in Fig. 2; the bands generated were of the predicted sizes. Using CfoI (Roche Diagnostics,Mannheim, Germany), we could distinguish four different species, including *Malassezia furfur,s, Malassezia globosa,, Malassezia restricta* and *Malassezia sympodialis.*

The prevalence of different Mallesezia species among students were as follows: Ten (5.3%) *M. restricta*, 5(2.6%) *M. sympodialis*, *M.globosa* 7(3.7%), and 3(1.5%) *M. furfur* in boys and 6 (3.1%) *M. restricta*, 3 (1.5%) *M.sympodialis*, *M. globosa* 4(2.1%), and 3(1.5%) *M. furfur* in girls. Chi-square analyses.

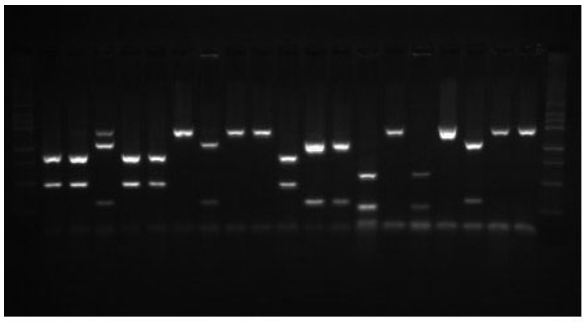

Fig 1. 26S rDNA PCR products after digestion with *CfoI*: Lanes1-21: 100 bp ladder *M. sympodialis,M: sympodialis, Mixed M.restricta* and *M.globosa ,M. sympodialis ,M. sympodialis ,M. restricta,M. glpbosa ,M. restricta ,M. restricta,M.sympodialis,M. globosa ,M. globosa ,M. furfur,M. restricta ,M. furfur,M. restricta,M. globosa , M. restricta,M.resticta* and 100 bp ladder.

Conclusion This study revealed that there is no correlation significant difference between *Malassezia Species* distribution, PV and gender and also shown that oily skin contained more *M. restricta* than dry ones and dry and normal ones contained more *M. sympodialis*, *M. globosa* and *M. furfur* compared to only ones.

P387

Spectral subtraction signal post-processing of *Candida parapsilosis* noisy microsatellite fragment data typing profiles

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Objectives Microsatellite markers have demonstrated their suitability for typing clinical isolates from different species of *Candida*. Despite the apparent simplicity of this technique, many problems and sources of error could lead us to confounding conclusions and must be considered before producing accurate genotypes. The aim of this work was to evaluate the reliability of a spectral subtraction signal analysis (SSSA) (a method which removes noise from noisy signals in the frequency domain) in a set of microsatellite data obtained from two *Candida parapsilosis sensu stricto* reference strains.

Methods Two *C. parapsilosis* reference strains, ATCC 22019 and CDC-317, were used for this study. Yeast cells were grown on Sabouraud glucose liquid medium for 24 h previous to DNA isolation using a UltraClean® Microbial DNA Isolation kit, according to the manufacturer's instructions. PCRs for all microsatellite markers were performed according to the protocols established by Sabino et al. (J. Clin. Microbiol. 2010; 48 (5): 1677–1682). Subsequent fragment size determination was performed on a 3130 Genetic Analyzer using 1 µl of a 1:10 dilution of each PCR product obtained, 13.7 µl of formamide and 0.3 µl of the internal size standard. After size determination, data were analyzed using the Peak Scanner software and the alleles obtained were designated by their sizes (in mer). The noise removal algorithm was implemented using Matlab and Sig-View software. Briefly, the corresponding spectrums of the different signals obtained after microsatellite analysis were computed using the FFT, removing the noise from the noisy spectra, and reconstructing the signal back into the time domain using the inverse Fast Fourier Transform (IFFT). The performance of the algorithm was evaluated by calculating the Signal to Noise Ratio (SNR). Frame averaging using different windows was an optional technique to improve the SNR.

Results Microsatellite analysis lead to four different sized PCR products which lengths varied from 150 to 330 bp. However, stutter effect was remarkable making sometimes the allele designation difficult. On the contrary, results from the SSSA showed better signals profiles which made the allele designation an easier task. The Signal to Noise ratio ranged from 8.1 to 12.3 depending on the windows and the frame length used. No frames averaging showed the best performance in removing noise from noisy microsatellite fragment data profiles. Despite these promising results we also observed that SSSA tend to dismiss the amplitude and wide the length of the reconstructed peaks which could lead to inaccurate fragment size determination.
Conclusion Despite microsatellite analysis is a useful technique for *C. parapsilosis* typing, careful design of primers and PCR conditions should be considered for a reliable allele determination. SSSA could be a novel post-processing technique of such data when adverse design conditions are found. However, limitations found make advisable to recommend more research in this field to assess the robustness of this typing technique.
Funding This study was financed in part by Fundación Jesús de Gangoiti Barrera and projects GIC12 210-IT-696-13, UFI 11/25 (UPV/EHU).

P388

Standardization of nested-Polimerase chain reaction for detection of *Histoplasma capsulatum* genome in batÃ?Â's guano droppings.

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Introduction Histoplasmosis is a systemic, opportunistic infectious disease caused by inhaling the fungus *Histoplasma capsulatum* microconídeos. Histoplasmosis started to gain more prominence in the 1980s and 1990s, with the advent of acquired immunodeficiency syndrome (AIDS) and other diseases that affect the immune response, as leukemic patients, transplant and taking chemotherapy. Rapid and specific diagnostic tests for fungal infections are extremely important to the effectiveness of treatment of infected patients. With the advancement of molecular biology has been possible to develop new techniques for diagnosis and identification of *Histoplasma capsulatum*.
Objectives The present study aimed to standardize the technique of nested PCR for detection of *H. capsulatum* genome in bat guano.
Methods For this purpose, 14 guano's samples were isolated from a cave near the town of Unai, Minas Gerais, Brazil. The primers pairs Fungus I and II; Histo I and II, described for the amplification of regions of ribosomal DNA (rDNA) were used, generating a fragment of approximately 231 bp. The DNA of *H. Capsulatum* was detected in 78.5% of sample analyzed ($n = 11$).
Conclusion The level of detection of *H. capsulatum* DNA by nested-PCR technique, proposed by the study, was $22.6 \times 10–2$ ng ul^{-1}. This result showed that the PCR is capable of detecting very low concentrations of DNA in an environmental sample. Therefore, the nested-PCR technique can facilitate the detection of fungu's DNA in guanosine, without the needing of fungus isolation in culture media.
Funding/support FAPEMIG - Fundação de Amparo à Pesquisa do Estado de Minas Gerais, Brazil, CNPq - Conselho Nacional de Desenvolvimento Científico e Tecnológico, Brazil.
Key words histoplasmosis, *Histoplasma capsulatum*, *PCR*, *DNA*, glass beads, rDNA

P389

Molecular epidemiology of *Candida albicans* from vulvovaginal Candidasis

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Objectives To investigate ABC genotype and mating type of *C. albicans* from vulvovaginal candidiasis (VVC). Analysis of virulence gene expression and antifungal susceptibility of the *C. albicans*.
Methods *C. albicans* were collected from the vulvavaginal of patients with VVC from January 1, 2003 to September 30, 2014. The ABC type was also determined, based on the presence or absence of an intron in DNA sequences encoding rRNA. The mating type, i.e., a/α, a/a, or α/α, was determined by PCR. HWP1, ALS3, SAP1 gene expression was also determined by using RT-PCR analysis. Antifungal susceptibility testing, performed on 658 of the isolates, performed using the broth microdilution technique. The results based on continuous measurement were analyzed using a one-way analysis of variance (ANOVA).
Results Among the 1427 strains investigated, 1319 isolates (92.4%) were found as genotype A, 77(5.39%) were genotype B and 31 (2.17%) were genotype C. There were no genotype D and genotype E. 1419 isolates were MTL locus heterozygous isolates and 8 were MTL locus homozygous isolates. Of the 8 homozygous isolates, 7 were MTLa and one was MTLα. Expression levels of virulence-related genes Sap1 was a little higher in heterozygous isolate cells than in homozygous isolate cells (1.02 ± 0.68 V.S 0.83 ± 0.31, $F = 3.55$, $P = 0.07$). Hwp1 was significantly higher in heterozygous isolate cells than in homozygous isolate cells (1.71 ± 1.42 V.S1.20 ± 0.62, $F = 5.14$, $P = 0.03$). There was no statistical difference of the expression of virulence genes HWP1, ALS3 and SAP1 in the different ABC genotypes. The resistant rate of azoles including voriconazole, fluconazole, itraconazole, clotrimazole and miconazole in genotype A was more to than those in genotype B and genotype C. Flucytosine and caspofungin were susceptible to all genotypes,
Conclusion *C. albicans* genotype A and MTL locus heterozygous isolates was the most prevalent among patients with VVC. Hwp1 was significantly higher in heterozygous isolate cells than in homozygous isolate cells. The resistant rate of azoles in genotype A was more to than those in genotype B and genotype C.

P390

Features of *Aspergillus flavus, A. oryzae* and related isolates revealed by MALDI-TOF-MS

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Objective of this study is to find out the possibility of correct species identification and strains grouping of *Aspergillus* spp. from section Flavi by MALDI-TOF-mass-spectrometry and related software.
Methods 38 isolates of *Aspergillus* spp. belonging to section Flavi were collected from sputum, bronchoalveolar lavage, paranasal sinuses washing fluids, biopsy materials, corneal scraping and another kinds of specimens. Strain were subcultivated in Eppendorf tubes with 0.5 ml of Sabouraud broth with 2% dextrose at 37 °C overnight. For extraction of proteins from cultures we performed standard method situated for MALDI-TOF-MS with using ethanol, formic acid and acetonitrile. MALDI-TOF-mass-spectrometry of protein extracts form cultures was performed in Autoflex speed TOF/TOF (Bruker Daltonics, Germany). Some strains were underwent described manipulations 2–3 times to obtain several spectra as a control of stability of the discrimination of strains in different groups. Totally 48 mass-spectra were collected. Mass-spectra were identified in Biotyper

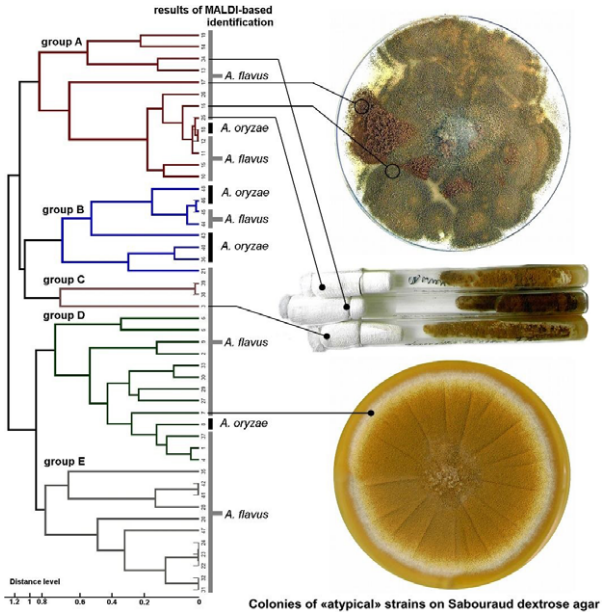

Figure 1

OC with 'Fungi Library'database. Although the difference in mass-spectra profiles (MSP) of *A. flavus* and *A. oryzae* and grouping of strains by construction of the dendrogram of hierarchical principal components analysis (hPCA) were investigated with using of the same software. Morphological features of selected strains were studied in Sabouraud dextrose agar (SDA) and Czapek yeast autolysate agar (CYA).

Results 41 mass-spectra (MS) were identified as *A. flavus* and 7 - as *A. oryzae*. 5 MS from group of '*A. flavus*'corresponded with strains, that characterized of orange-brown colorings of cultures without any yellow or green shades in CYA and SDA witch not typical neither to *A. flavus*, nor to *A. oryzae*. The result of the strains grouping by hPCA is shown in fig. 1. Strains were subdivided into 5 groups. The most of *A. oryzae* spectra are situated in group B, but this group contains some spectra of *A. flavus* too. 2 spectra of *A. oryzae* obtained from one strain were placed in another groups (A and D). The comparison of mass-spectra-profiles identified as '*A. flavus*'and '*A. oryzae*'demonstrated at least 2 ranges of M/Z in which the differences between this group of species-specific MSP are most pronounced: 4450–5800 Da and 7450–7850 Da. Both ranges include several small peaks in *A. oryzae*, witch not situated in *A. flavus*. The comparative analysis of MSP from strains with orange-brown colonies not allowed to identify of any regular differences with each other as well as with '*A. flavus*'and '*A. oryzae*'groups. Matching of positions of mass-spectra in hPCA-dendrogram allowed to reveal that spectra from identical strain are situated in different groups.

Conclusion Our study demonstrates that species identification of aspergilli from section Flavi by standard technique of MALDI-TOF-MS is very difficult. We consider, that one of the probable reason of this situation is in obtaining of poor detailed mass-spectra. For solving this problem an improved protocol of proteins extraction is needed to obtain more itemized mass-spectra from cultures of filamentous fungi.

P391

Optimization of the heterologous expression enzyme Chorismate Synthase from *Paracoccidiodies brasilensis* - focus in drug development

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The aim of this work is to optimize the main conditions necessary to express the chorismate synthase from *Paracoccidiodies brasilensis* with focus in drug development. For the heterologous expression of CS from *Paracoccidioides brasiliensis* (PbCS), the gene was chemically synthesized using preferential codon of *Escherichia coli* and cloned into the expression vector pET series (Novagen). For optimization of PbCS recombinant production many parameters were evaluated: *E. coli* host strains (BL21(DE3) and BL21(DE3) *pLysS*), induction periods (0 to 24 h), and different culture media (Luria Broth - LB, Terrifc Broth -TB, 4YT and mininal medium - M9). The IPTG concentration (1 mM) and temperature (37 °C), were maintained constant during the tests. The *Pb*CS recombinant (PbCSr) expression in different conditions was analyzed by SDS-PAGE and Western blotting. The structural characterization of enzymes involved in the metabolic processes of pathogens with selective toxicity may be an alternative to drug development. The Chorismate Synthase (CS) has been shown to be an excellent target for antifungal drug, as it is involved in a metabolic pathway that is present only in plants, fungi and bacteria. The high-level expression of *Pb*CSr was observed both in inclusion bodies and cell lysate supernatant. The maximum amount of *Pb*CSr in native form was obtained using the strain *E. coli* BL21 (λDE3) cultivated in medium TB to 4 h of induction (Figure). During the *Pb*CSr purification process, the best imidazole concentration in the washing buffer was 80 mM and in the elution buffer was 250 mM. The concentration of purified protein was 0.2 mg ml^{-1} of supernatant. **T**he *Pb*CSr purification was confirmed by Western Blotting using anti-His antiserum. In this work, we developed an efficient procedure for the *Pb*CSr production and optimizing protein purification conditions. Validation steps of enzyme activity, characterization of the structure and crystallization of the recombinant protein, may be carried out with the aim of increasing the basic knowledge of this enzyme, and, in the future, the development of antifungal drugs or vaccine directed to this target.

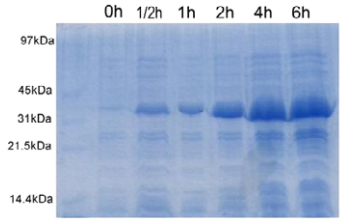

Figure 1 - Heterologous expression of PbCS recombinant protein in *Escherichia coli*. The maximum amount of PbCSr was obtained using the strain *E. coli* BL21 (λDE3) cultivated in medium TB to 4h of induction.

P392

Molecular characterization of *Aspergillus* infections of an Iranian educational hospital using RAPD-PCR

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Objectives The nosocomial infections by Aspergillus species are associated with constructions and increased dust loads in hospital

indoors. Our main object was to find the environmental sources of Aspergillus species causing hospital acquired infections.
Methods The clinical and environmental samplings were performed during 18 months from spring 2010 to summer 2011 in Imam educational hospital, Urmia, Iran. A morphological diagnosis was performed including microscopic characterization of isolated aspergillus from cultured specimens and Polymerase Chain Reaction - Restriction Fragment Length Polymorphism (PCR-RFLP) for the identification in the level of species. Random Amplified Polymorphic DNA - PCR RAPD-PCR using random primers for rDNA gene was performed to compare Aspergillus isolates of clinical cases with the relevant environmental sources.
Results Use of RAPD method resulted various differential patterns, so that some Aspergillus isolates from the clinical and hospital indoor were completely matched (matched pairs) and some other Aspergillus isolates were not matched. In the case of matched pairs, *A. niger* and *A.flavus* isolated from broncoalveolar lavage and sinus discharge were relevant to those of air conditioner and walls surfaces, respectively.
Conclusion The hospital sources for the Aspergillus clinical isolates included air condition and walls. RAPD-PCR analysis can play a trivial role to find the hospital sources of Aspergillus clinical isolates.

P393

Molecular Identification of Yeast: Gold Standard or Fool's Gold?

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Objective Traditionally, fungal identification has been considered a labor-intensive process with few automated options as compared to bacterial identification. Yeast lend themselves to automated systems allowing their identification to become more routine. In the last decade, molecular testing has shortened the time needed for species identification and is presumed by many to be the 'gold standard'. We reviewed 130 clinical yeast isolates submitted to the Fungus Testing Laboratory for identification to determine the efficiency of sequencing data alone to obtain a final identification to the species level.
Methods A total of 130 clinical yeast isolates were received for identification in 2014. All isolates were sequenced, with ITS and D1/D2 being the primary loci used for this purpose. The resulting sequences subsequently underwent BLAST searches to determine the percent identity with species available in public databases (e.g., GenBank, CBS-KNAW). In addition to sequencing, the isolates underwent extensive physiologic and morphologic testing, including carbohydrate assimilation patterns (API 20C AUX, bioMeriuex, St. Louis, MO), cycloheximide susceptibility, temperature tolerance, and microscopic morphology on Cornmeal Agar. Additional tests that were performed when appropriate included urea hydrolysis, nitrate utilization, and L-Canavanine Glycine Bromothymol Blue Medium (CGB) reactions.
Results Of the isolates tested, sequencing alone provided a clear and correct identification in only 30 isolates (23%). For 60 isolates (46%) additional testing other than solely by molecular sequence analysis was required to differentiate between two to three separate species. Furthermore, 26 isolates (23%) required additional testing to differentiate between four to five species. Interestingly, additional assays were required to discriminate between six to up to twelve species for the remaining 13 isolates (10%). Each of these isolates was documented as having a high percent identity among different yeast species. For 2 of the isolates, identification to the exact species was not possible.
Conclusions This data reveals that sequencing alone is not sufficient for yeast identification, and that a combined approach is required to obtain a correct identification to the species level. Although only yeast were tested, several strains had 100% identity with moulds such as *Aspergillus*, *Exophiala,* and *Trichophyton* spp. One strain surprisingly had 100% identity with a parasite. We contend that there is no single 'gold standard'in fungal identification testing and that a combined approach is the best approach for identifying fungi.

P400

In vitro activity ASP2397 against *Aspergillus* isolates with and without acquired resistance mechanisms

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Objectives ASP2397 is a new compound with activity against *Aspergillus* and *C. glabrata*. It is actively transported into the fungal cells via the siderophore transporter Sit1. The mode of action is novel and yet not known, but different from that of the azoles and amphotericin. The purpose of this study was to investigate the *in vitro* activity against a well characterised panel of wild type susceptible (wt) and azole resistant *A. fumigatus* and *A. terreus* isolates by the EUCAST and CLSI methodologies and to compare that with the activity of amphotericin B (AMB), itraconazole (ITC), posaconazole (PSC) and voriconazole (VRC).
Methods 34 isolates were included: *A. fumigatus*: 4 wt, 5 with TR/L98H, 9 with M220, 9 with G54 CYP51A alterations and 1 with a HapE mutation. *A. terreus*: 2 wt and 1 with an M217I CYP51A alteration. EUCAST EDEF9.2 and CLSI M38-A2 MICs were read visually day 2 at a 100% inhibition endpoint. Reference strains were: *A. fumigatus* ATCC 204305, F6919 and ATCC MYA-2636.
Results MIC_{50} (MIC range) (mg l^{-1}) determined by EUCAST and CLSI for ASP2397 were: 0.5 (0.25–1); 0.25 (0.06–0.25) against *A. fumigatus* wt isolates and similar to those against *A. fumigatus* harbouring azole resistance mutations: 0.5 (0.125 to >4); 0.125 (0.06 to >4). The similar values for the comparator drugs against wt isolates were: ITC 0.125 (0.125–0.25); 0.125 (0.06–0.25), PSC ≤0.03 (≤0.03–0.06); ≤0.03 (≤0.03–0.06), VRC 0.5 (0.5–1); 0.25 (0.25–0.5) and AMB 0.25 (0.125–0.5); 0.25 (0.125–0.25) but higher for the azoles against *A. fumigatus* harbouring azole resistance mutations:: ITC >4 (1 to >4); 4 (0.5 to >4), PSC 0.5 (0.06 to >4); 0.5 (≤0.06 to 4), VRC 0.5 (0.25 to >4); 0.5 (0.125 to 4) and not for AMB 0.25 (0.125 to 1); 0.25 (0.125 to 1). For *A. terreus* the pattern was somewhat different as both azole and ASP2397 MICs were elevated for the CYP51A mutant isolate: ASP2397 EUCAST MIC: >4 vs. 0.5–1 for wt and CLSI MIC: 2 vs. 0.5–1 for wt isolates. Similar MIC elevation was also observed for the azole compounds but not for AMB (data not shown). Further studies of ASP2397 against more isolates of *A. terreus* are needed before the implication of the findings for this species can be interpreted.
Conclusion ASP2397 displayed *in vitro* activity against *A. fumigatus* and *A. terreus* isolates. The activity was independent of the absence or presence of azole target gene resistance mutations in *A. fumigatus*. This finding holds promising implications at a time where azole resistant is emerging globally in this species.

P401

In vitro activity of Isavuconazole and Comparators against Clinical Isolates of the Mucorales Order

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Background The *in vitro* activity of isavuconazole (ISC), a new broad spectrum azole, has been studied using the EUCAST methodology against *Candida* and *Aspergillus*, however, data on *in vitro* activity against Mucorales isolates is sparse. The purpose of this study was to

investigate the *in vitro* activity against clinical isolates of the Mucorales order by the EUCAST and CLSI methodologies and to compare that with the activity of amphotericin B (AMB), posaconazole (PSC) and voriconazole (VRC).

Materials and Methods 72 clinical isolates were included: *Lichtheimia* (*L*) *corymbifera* 12, *L. ramosa* 5, *Mucor* (*M*) *circinelloides* (Group I) 5, *M. circinelloides* (Group II) 9, *Rhizomucor pusillus* 9, *Rhizopus* (R) *microsporus* 26 and *R. oryzae* 6. Species identification was confirmed by ITS DNA sequencing. MICs were read visually day 1 and 2 for EUCAST EDef 9.2 and day 2 for CLSI M38-A2 (no visible growth day 1).

Results MIC_{50} (MIC range) (mg l^{-1}) determined by EUCAST (day 1), CLSI (day 2) and EUCAST (day 2) for ISC were: 1 (0.125 to 16); 1 (0.125 to 2); 4 (0.5 to >16) across all isolates with a high agreement between the EUCAST day 1 and CLSI day 2 MICs. The similar values for the comparator drugs were: PSC 0.25 (≤0.03 to >16); 0.25 (0.06 to >16); 1 (0.06 to >16), AMB 0.06 (≤0.03–0.5); 0.06 (≤0.03–0.25); 0.125 (≤0.03–1) and VRC 16 (2 to >16); 8 (1 to >16); >16 (8 to >16) in agreement with the lack of clinical efficacy of VRC against *Mucorales*. Species dependent ISC activity was observed with the lowest MICs for: *L. corymbifera* 1 (0.5–2); 1 (1–2); 2 (1–4), *L. ramosa* 0.25 (0.125–0.5); 1 (0.5–2); 2 (0.5–4), *Rhizomucor pusillus* 0.5 (0.5–1); 1 (0.125–1); 2 (1–2), *R. microsporus* 1 (0.5–4); 0.5 (0.125–1); 4 (1–8), and *R. oryzae* 1 (0.5–4); 1 (0.125–2); 4 (0.5–8). The highest MICs were found for *M. circinelloides* Group I 8 (4–8); 4 (2–4) and 16 (2–16) and *M. circinelloides* Group II 8 (1–16); 8 (1–8); 16 (4 to >16). This pattern was also observed for the *in vitro* activity of PSC but not for AMB.

Conclusion ISC displayed *in vitro* activity against *Mucorales* isolates with exception of *M. circinelloides*. The MICs were in general 1–3 steps higher than those for PSC. However, in the clinical setting this may be compensated by the higher exposure (AUC ranges: ISC: 100 vs PSC 15–35 mg.h ml^{-1}).

P402

Identification and characterization of Hemofungin, a novel antifungal compound which inhibits the final step of heme biosynthesis

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The incidence of fungal infections such as invasive pulmonary aspergillosis (IPA) caused by *Aspergillus fumigatus* (*A. fumigatus*), has risen dramatically throughout recent years, especially among immunocompromised patients. Despite this rise in invasive infections, there is only a limited number of antifungal drugs active against fungal pathogens and even with treatment, the mortality rate remains high. Therefore, there is an urgent need to develop novel effective antifungal drugs to treat fungal infections.

The aim of this study was to evaluate CW-208/hemofungin as a novel antifungal compound. This molecule was previously identified in our laboratory by a library screening of synthetic drug-like compounds and shown to cause swelling and lysis of growing fungal cells. Hemofungin inhibited growth of pathogenic Aspergillus, Candida, Fusarium and Rhizopus isolates at micromolar concentrations, while only weakly affecting the growth of mammalian cell lines. Genetic and biochemical analyses in *A. nidulans* indicated that hemofungin primarily inhibits ferrochelatase, the last enzyme in the heme biosynthetic pathway that converts protoporphyrin IX to hemin, suggesting that its effect on the cell-wall is indirect. Hemofungin significantly reduced mortality rates of larvae infected with *A. fumigatus*, in a dose-dependent manner without signs of toxicity. Several additional findings strengthened our hypothesis that ferrochelatase is the target of hemofungin- (i) addition of the end-product hemin canceled inhibition by hemofungin in *A.fumigatus* in a dose-dependent manner. (ii) enzymes in the hemin biosynthetic pathway were strongly up-regulated in *A. fumigatus* treated with hemofungin (iii) HPLC analysis revealed higher levels of protoporphyrin in *A. fumigatus* treated with hemofungin.

In summary our approach in finding novel antifungals by screening a library of drug-like molecules has yielded a promising novel compound, hemofungin, which inhibits the process of fungal heme biosynthesis and is strongly inhibitory towards a panel of fungal strains while only weakly affecting the growth of mammalian cell lines and is active and non-toxic in an insect model of fungal infection. Further investigation of this promising compound in additional animal models of fungal infection is strongly warranted.

P403

In vivo Antifungal activity of compound selected against thioredoxin reductase from *Candida albicans*

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Objective Invasive fungal infections (IFIs) have emerged as a public health problem worldwide. The therapeutic options currently available are limited and restricted to four antifungal classes. Furthermore, the resistance and side effects caused by these therapies, indicate significant need for the new antifungal development. Therefore, the aim of this work was to select compounds by virtual screening against thioredoxin reductase from *Candida albicans*, contributing to the new antifungal development for the treatment of important fungal infections worldwide.

Methods There is no crystallographic structure presently available for pathogenic fungi thioredoxin reductase. Therefore, the 3D structures of Trrr from *Candida albicans*, in different conformation, were constructed by homology modeling based on known structures with high percentage of identity in amino acid sequences. The stability of 3D structures was analyzed by Dynamic Molecular (DM) Simulation. The program NAMD was employed in conjunction with the CHARMM22 force field to DM simulation. After that, a bank of commercially available compounds from Life Chemicals database was docked with the model by virtual screening simulations. The minimal inhibition concentration (MIC) determination was performed to *Candida*, *Cryptococcus* and *Paracoccidiodies* species, according to M27-A3 guidelines (CLSI) with modifications to new compounds. The cytotoxicity studies was performed in HeLa cells, using the CellTiter 96 assay (Promega, Madison, WI, USA), based on the reduction of MTS (3-[4,5-dimethylthiazol-2-yl]-5-[3- carboxymethoxyphenyl]-2-[4-sulfophenyl]-2H-tetrazolium). The experimental model of candidemia was used to evaluation of *in vivo* antifungal activity.

Results Three 3D structures were constructed to Trr1 from *C. albicans*, one the oxidized flavin conformation (FO) and two others in the flavin-reducing (FR) conformation (Figure A). Since these

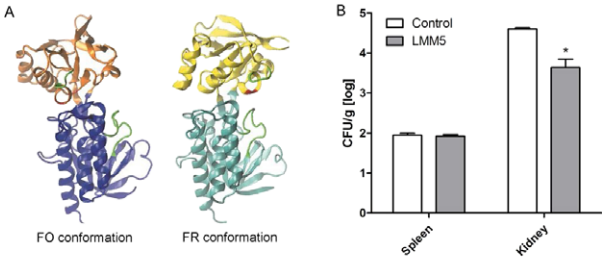

Figure: (A) The 3D structures to TRR1 from *C. albicans*. (B) Effect of LMM5 treatment on a mice model of systemic candidiasis, *p<0.05 significant difference compared with untreated and treated mice.

conformations are important in the catalytic mechanism. The DM simulations results show that systems apparently remained stable over the course of 10 ns. By docking simulation, the small molecules that interact with the models were ranked. Finally, eleven molecules were selected as putative inhibitors of Trr1. Two compounds (LMM5 and LMM11) showed antifungal activity *in vitro* against the pathogenic fungi. The MIC values of LMM5 ranging from 8 to 32 μg ml^{-1} against *Candida* species. For *Cryptococcus* species the MIC values were 32 μg ml^{-1} and to *Paracoccidiodides* species was 4 μg ml^{-1}. In other hand, the MIC values of LMM11 ranging from 32 to 64 μg ml^{-1} against *Candida* species. For *Cryptoccocus* sp. and *Paracoccidiodes* spp. the MIC values were the same observed to LMM5. These compounds not showed *in vitro* cytotoxicity on HeLa cells. The *in vivo* results are promising for LMM5 compound which showed a significant reduction in CFU in the kidneys of mice treated with this compound, when compared to animals that received only PBS control group in experimental candidemia (Figure B).

Conclusion One of compounds showed *in vitro* and *in vivo* activity antifungal against important human pathogenic fungi, thereby creating additional perspectives for innovation and technological development of antifungal drugs.

P404

Phase 3 Randomized, Double-Blind Trial Evaluating Isavuconazole vs. Voriconazole for Primary Treatment of Invasive Fungal Disease Caused by *Aspergillus* Spp. or Other Filamentous Fungi (SECURE)

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Objectives Isavuconazole is a novel triazole with broad-spectrum antifungal activity *in vitro* and is offered in an IV and bioequivalent oral formulation. The objectives of this randomised, double-blind, multinational study were to assess efficacy and safety of isavuconazole versus voriconazole in patients with invasive fungal disease (IFD) caused by *Aspergillus* spp. or other filamentous fungi.

Methods Patients meeting the EORTC criteria for proven/probable/possible disease were randomized 1:1 to isavuconazole or voriconazole. Isavuconazole patients received 200 mg IV TID for 2 days, followed by 200 mg QD (either IV or oral). Voriconazole patients received 6 mg kg^{-1} IV BID on day 1, then 4 mg kg^{-1} IV BID on day 2, then either 4 mg kg^{-1} IV BID or 200 mg PO BID. Study drug could be administered for up to 84 days. The primary efficacy endpoint was non-inferiority for day 42 All-Cause Mortality (ACM) in the ITT population based on a pre-specified non-inferiority margin (NIM) of 10%. The key secondary efficacy endpoint was Overall Response at end of treatment (EOT) as determined by an independent blinded data-review committee (DRC) in the mITT population. The mITT population included patients with proven or probable disease as determined by the DRC.

Results 527 patients were randomized, 516 (258 per group) received at least one dose of study drug and comprised the ITT population. The mITT population included 143 isavuconazole and 129 voriconazole patients (85% aspergillosis). Baseline characteristics were balanced between treatment groups; 92% pulmonary involvement, 84% haematologic malignancies, 65% neutropaenic and 20% allogeneic haemotopoetic stem-cell transplantation. ACM through day 42 for the ITT population was 18.6% (isavuconazole) and 20.2% (voriconazole). The primary objective was achieved since the upper bound of the 95% CI (-7.8, 5.7) for the adjusted treatment difference was lower than the NIM of 10%. ACM through day 42 in the mITT population was 19.6% (isavuconazole) and 23.3% (voriconazole). ACM through day 84 in the ITT population was 29.1% (isavuconazole) and 31.0% (voriconazole) and in the mITT population was 30.1% (isavuconazole) and 37.2% (voriconazole). Overall Response rates at EOT in the mITT population were 35.0% (isavuconazole) and 36.4% (voriconazole). Treatment-emergent adverse events (AE) were reported in 96.1% (isavuconazole) and 98.5% (voriconazole) of patients. The most common AEs (i.e. nausea, vomiting, pyrexia, and diarrhea) were reported at similar rates between treatment groups. Drug-related AEs were reported in 42.4% (isavuconazole) and 59.8% (voriconazole) of patients. Fewer ($P < 0.05$) AEs were reported in the isavuconazole treatment group in the System Organ Classes of Skin (33.5% v 43.5%), Eye (15.2% v 26.6%) and Hepatobiliary Disorders (8.9% v 16.2%).

Conclusions Isavuconazole is effective for the primary treatment of invasive fungal disease caused by *Aspergillus* species or other filamentous fungi. Isavuconazole was well tolerated relative to voriconazole, with fewer study drug related AEs and AEs of the Skin, Eye and Hepatobiliary system.

(Encore abstract: Previously presented at the European Congress of Clinical Microbiology and Infectious Disease [ECCMID], Barcelona, Spain; May 10–13, 2014; O230a).

P405

Outcomes in Patients with Invasive Mold Disease Caused by *Fusarium* or *Scedosporium* spp. Treated with Isavuconazole: Experience from the VITAL and SECURE Trials

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Objectives Isavuconazole is a novel, broad-spectrum, triazole antifungal developed for the treatment of invasive fungal diseases. *Fusarium* and *Scedosporium* spp. are associated with high mortality in immunocompromised patients; however, treatment options are limited. We report outcomes in a subset of patients enrolled in the VITAL and SECURE trials with invasive mold disease (IMD) caused by these pathogens.

Table 1. Patient demographics and outcomes.

	Fusarium (n=9)	*Scedosporium* (n=3)
Age (years), range	28–79	26–68
Risk factor, n	Allogeneic BMT (1)/Uncontrolled malignancy (5)/Neutropenic (6)/Hematologic malignancy (6)	Uncontrolled malignancy (1)/Neutropenic (1)/Hematologic malignancy (1)
Underlying disease, n	AML (3); ALL (1); lymphohistiocytosis (1); aplastic anemia (1); B-cell lymphoma (2); malignant tongue neoplasm (1)	CML (1); interstitial lung disease (1); cystic fibrosis (1)
Pathogen, n	*F. solani* (3); *Fusarium* NOS (4); *F. solani* + other mold (1); *Fusarium* NOS + other mold (1)	*S. apiospermum* (1); *S. prolificans* + other mold (1); *Scedosporium* NOS + other mold (1)
Infection site, n	Pulmonary (1); pulmonary + other site (6); other site (2)	Pulmonary (2); pulmonary + other site (1)
Therapy status, n	Primary (7); refractory (2)	Primary (2); refractory (1)
Treatment duration (days), range (median)	1–181 (15)	8–97 (12)
Overall response at EOT	Success (complete [2], partial [1]); failure (stable [1], progression [5])	Success (partial [1]); failure (stable [1], progression [1])
Deaths, n	5 (Days 1, 5, 7, 22, 39)	1 (Day 8)
Safety, n (of patients)	AEs (9)/drug-related AEs (5)	AEs (3)/drug-related AEs (1)

Methods VITAL and SECURE were Phase 3 trials that evaluated efficacy and safety of isavuconazole treatment in patients with IMD. Dosages were isavuconazole 200 mg TID for 2 days followed by 200 mg QD (IV or PO). Proven/probable IMD (EORTC/MSG criteria), and overall response at end of treatment (EOT) were determined by independent, data-review committees. Mortality rates, safety and tolerability were also analyzed.
Results Demographics and outcomes data are shown in Table A1.
Conclusions Isavuconazole may be a promising treatment for patients with invasive *Fusarium* or *Scedosporium* spp. infections. Data are limited for patients with these infections and require further investigation.
ClinicalTrials.gov Identifiers VITAL (NCT00634049) and SECURE (NCT00412893).

(Encore abstract: Previously presented at the 54th Interscience Conference on Antimicrobial Agents and Chemotherapy [ICAAC]; Washington, DC, USA; September 5–9, 2014; M-1760).

P406

Primary treatment of invasive mucormycosis (IM) with isavuconazole (VITAL Study) or amphotericin formulations (FungiScopeTM): case matched analysis

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Objectives Invasive fungal diseases (IFD) are on the rise due to the increasing numbers of immunosuppressed patients including those undergoing high-dose chemotherapy and haematopoietic stem-cell transplantation. Isavuconazole is a novel, broad-spectrum antifungal triazole, available as a water-soluble prodrug in IV and oral formulations. IM is a life-threatening IFD with significant mortality and limited treatment options.
Methods The VITAL study was a Phase 3, multicentre, open-label, single-arm trial conducted to evaluate efficacy and safety of isavuconazole treatment in patients with rare IFD, including IM. Eligibility criteria and evaluated outcomes are outlined in clinicaltrials.gov, NCT00634049. Patients received IV or PO isavuconazole 200 mg TID for 2 days followed by 200 mg day^{-1}. FungiScope[TRADEMARK] - Global Emerging Fungal Infection Registry maintains a global web-based database on rare IFD, including IM. Entrance criteria are outlined in clinicaltrials.gov, NCT01731353. Twenty-one patients from the VITAL study who received isavuconazole for the primary treatment of proven/probable IM were matched (blinded to mortality status) to up-to three proven/probable patients with IM who received a formulation of amphotericin B entered in FungiScope [TRADEMARK] based on three dichotomous risk factors: severe disease (i.e. CNS/disseminated), surgical debridement, and haematologic malignancy. All-cause mortality through day 42 was summarised.
Results Thirty-three FungiScope[TRADEMARK] matched controls were identified; 14 VITAL cases were matched to a single control each ($n = 14$), two VITAL cases were matched to two controls each ($n = 4$), and five VITAL cases were matched to three controls each ($n = 15$). Demographics, treatments, matching criteria and mortality outcomes are shown in Table 1. The crude mortality rate (33.3%) through day 42 from the VITAL cases was similar to the mortality rate (39.4% crude; 41.3% weighted) from the matched controls of FungiScope[TRADEMARK].
Conclusions Isavuconazole was as effective as amphotericin B formulations in the primary treatment of invasive mucormycosis based on this matched case comparison of mortality rates from the VITAL study and FungiScope[TRADEMARK].

(Encore abstract: Previously presented at the American Society of Hematology [ASH] annual meeting; San Francisco, CA, USA; December 6–9, 2014; 1151).

Table 1

Parameter	Isavuconazole VITAL Cases (n=21)	FungiScope™ Amphotericin B Matched Controls (n=33)
Treatment, n (%)		
Isavuconazole	21 (100)	0
Deoxycholate amphotericin B	0	7 (21)
Liposomal amphotericin B	0	22 (67)
Amphotericin B Lipid Complex	0	4 (12)
Case year, range	2008–2013	2005–2013
Age, median (range)	51 (25–77)	57 (22–81)
Male, n (%)	17 (81)	22 (67)
Race, n (%)		
White	12 (57)	31 (94)
Black/African American	1 (5)	0
Asian	8 (38)	2 (6)
Severe Disease,[1] n (%)	12 (57)	13 (39)
Surgical Debridement,[2] n (%)	9 (43)	13 (39)
Haematologic Malignancy, n (%)	11 (52)	18 (55)
Crude Mortality, n (%)	7 (33.3)	13 (39.4)
Weighted Mortality[3] (%)	Not Applicable	41.3

[1]CNS Involvement and/or Disseminated Disease
[2]Surgery performed +/– 7 days of the initiation of isavuconazole or amphotericin
[3]Weights were applied according to the ratio of the number of controls matched to each VITAL case

P407

Successful Outcomes in Patients with Invasive Fungal Disease due to *C. gattii* and *C. neoformans* Treated with Isavuconazole: Experience from the VITAL Trial

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Objectives Cryptococcosis is associated with significant morbidity and mortality. Isavuconazole is a novel, broad-spectrum, triazole antifungal agent (IV and PO) developed for treatment of invasive fungal diseases (IFD). It displays potent activity *in vitro* against *Cryptococcus* spp. We report outcomes in a subset of patients enrolled in the VITAL trial with cryptococcosis.
Methods VITAL was a Phase 3, open-label, multi-center trial conducted to evaluate efficacy and safety of isavuconazole in patients with emerging IFD. Patients received isavuconazole 200 mg TID for 2 days followed by 200 mg QD (IV or PO). Proven/probable IFD (EORTC/MSG criteria) and overall response at end-of-treatment (EOT) were determined by an independent, data-review committee. Mortality and safety were also assessed.
Results Nine patients were treated with isavuconazole for cryptococcosis. The pathogens isolated were *Cryptococcus neoformans* ($n = 4$) and *C. gattii* ($n = 3$); one patient had histological evidence only, and one had positive antigen testing only. Three patients had isolated pulmonary disease and two had central nervous system (CNS) disease. The remaining patients had disseminated disease in the lung,

CNS, and/or blood or other organ. CLSI MICs were available for seven patients and ranged from 0.008 to 0.12 mg l^{-1}. Mean duration of therapy was 132 days (range 6–182 days). Six patients had primary therapy with isavuconazole; two were intolerant to amphotericin B (AmB; alone or with fluconazole), and one was refractory to AmB + fluconazole. Eight patients were alive through ≥84 days: six were treatment successes (two complete, four partial) and two were treatment failures (both stable) at EOT. All patients with *C. gattii* infection were treatment successes. One of the stable patients was switched to amphotericin B + 5-flucytosine at Day 25. One of the nine patients died on Day 7 and was considered a treatment failure (progression) at EOT. Adverse events (AEs) and drug-related AEs were experienced by eight and three patients, respectively.

Conclusions Cryptococcosis, caused by either *C. neoformans* or *C. gattii*, was successfully managed in this small group of patients, supporting further research into this disease.

ClinicalTrials.gov Identifier

NCT00634049 (Encore abstract: Previously presented at the Interscience Conference on Antimicrobial Agents and Chemotherapy [ICAAC] annual meeting, Washington, DC, USA; September 5–9, 2014; M-1773)

P408

HEOR analysis of patients in SECURE trial comparing isavuconazole to voriconazole for primary treatment of invasive fungal disease caused by *Aspergillus* and other filamentous fungi

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Objectives Invasive aspergillosis (IA) is an emerging clinical problem and remains an important complication especially among immunocompromised patients. IA is associated with significant increases in morbidity, mortality, length of hospitalization stay (LOS) and costs[1]. Case fatality is estimated to be at 60% in immunocompromised populations[2-4]. Despite improved treatment options and advances in diagnostic testing, patient outcomes remain suboptimal. The SECURE trial was a Phase III, double-blind, randomized, multi-center, non-inferiority study of isavuconazole versus voriconazole**.** Patients >18 years of age, who had proven, probable or possible invasive fungal disease caused by *Aspergillus* species or other filamentous fungi were randomized 1:1 to receive isavuconazole or voriconazole.

Methods Primary objective of this analysis was to compare initial LOS during hospitalization and 30-day all-cause hospital readmission rates in patients who received isavuconazole or voriconazole. Outcomes in subgroups of interest by age, Body Mass Index (BMI) and renally-impaired patients (eGFR-MDRD category <60 ml/min/1.73 m^2) were conducted. Ratio of total days on IV over total number of days of (IV + oral) therapy for study drugs and total number of additional days on potentially mould-active systemic antifungal therapy after end of study treatment were also analyzed.

Results A total of 516 patients were included in the Intent-to-Treat (ITT) group. Median LOS was calculated with descriptive statistics (13.0 vs 15.0 days) and using Kaplan–Meier estimates (15.0 vs 16.0 days, $P = 0.607$) for isavuconazole vs voriconazole, respectively. The 30-day readmission rate for isavuconazole compared to voriconazole patients was 18.3% vs. 24.4% ($P = 0.114$), respectively. Ratio of days on IV formulation to total days of (IV + oral) therapy were similar (isavuconazole 0.38 [SD 0.39]; voriconazole 0.38 SD [0.38]). Median additional days on potentially mould-active systemic antifungal therapy were comparable isavuconazole vs voriconazole (32.0 days vs. 33.0 days). Median LOS days were similar across various subgroups (Figure 1), except in renal impaired subgroup where the difference was statistically significant in favor of isavuconazole (isavuconazole 9.0 days; voriconazole 19.0 days, $P = 0.0032$).

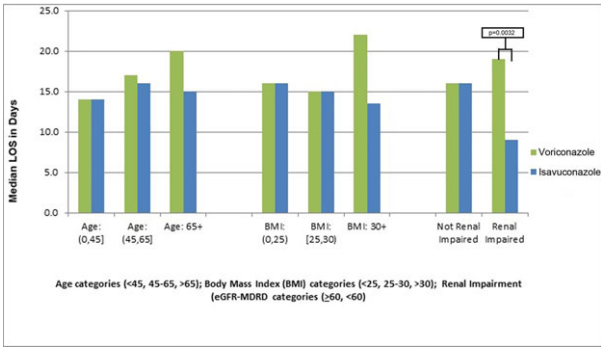

Figure 1. Comparison of Median LOS days for ITT patients receiving Isavuconazole vs. Voriconazole, by Age, BMI and Renal Impairment categories.

Conclusions Median LOS days was found to be lower for patients treated with isavuconazole vs voriconazole; this difference was statistically significant in patients with renal impairment.

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P409

An open-label phase 3 study of isavuconazole (VITAL): focus on mucormycosis

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Objective Isavuconazole is a novel, broad-spectrum triazole antifungal, available as a water-soluble prodrug in IV and oral formulations, for the treatment of invasive fungal disease (IFD). The objective of this analysis is to report the overall response, survival, and safety in a subset of patients with invasive mucormycosis (IM) who were treated with isavuconazole.

Methods VITAL was a Phase III, multicenter, open-label trial conducted to evaluate safety and efficacy of isavuconazole treatment in patients with rare IFD. Eligibility criteria and evaluated outcomes are outlined in clinicaltrials.gov, NCT00634049. Patients received IV or PO isavuconazole 200 mg TID for 2 days followed by 200 mg day^{-1} until day 180, end of treatment (EOT). An independent data review committee (DRC) categorized patients as having proven or probable IFD by EORTC/MSG criteria. DRC-assessed overall response at EOT, survival, and adverse events (AEs) using standard definitions are reported for patients with proven/probable IM.

Table 1. Baseline demographics, study outcomes, and survival.

Parameter	All patients (N=37)
Baseline characteristics	
Age (years), median (range)	50 (22–79)
Male, n (%)	30 (81)
Primary underlying condition, n (%)	
Hematological malignancy	22 (60)
Allogeneic bone marrow transplant	13 (35)
Diabetes mellitus	3 (8)
Immune status n, (%)	
Neutropenic	10 (27)
Corticosteroid use	10 (27)
T-Cell immunosuppressant use	18 (49)
Organ involvement	
Pulmonary	22 (59)
Sinus	16 (43)
Central nervous system	6 (16)
Skin	2 (5)
Bone	5 (14)
Disseminated	11 (30)
Efficacy	
Overall Response at EOT, n (%)	
Complete	5 (14)
Partial	6 (16)
Stable	10 (27)
Progression	14 (38)
Missing[a]	2 (5)
Survival rate[b], % (95% CI)	
Day 42	65 (47, 78)
Day 84	59 (42, 73)
Day 120	56 (39, 71)
Day 180	53 (35, 68)

[a]Two people continued treatment at EOT cutoff
[b]Kaplan–Meier method

Results Overall 37 patients with IM received isavuconazole for a median of 84 days (range 2–882). Baseline demographics, study outcomes, and survival are shown in Table 1. Survival at 180 days was 53%. Overall 95% of patients experienced an AE, 76% experienced a serious AE (SAE). Only 3 (8%) SAEs were attributed to isavuconazole.
Conclusion Isavuconazole appears to be a safe and promising agent for the treatment of mucormycosis in a particularly high-risk immunocompromised population.

(Encore abstract: Previously presented at IDWeek, Philadelphia, PA, USA; October 8–12, 2014; A824)

P410

Antifungal activity of a new econazole imprinted textile

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Objective Fungal skin infections are widespread, potentially contagious and have important economic consequences. Failure in treatment is most often due to low patient compliance, since antifungal cream should be applied daily for several weeks or months. In this work, the antifungal activity of a textile imprinted with solid lipid microparticles of econazole (ECN) was investigated.
Materials Solid lipid microparticles containing econazole were imprinted on textiles using a screen-printing method. Antifungal activity was investigated on 13 clinical isolates of *Candida spp. C. albicans* ($n = 5$) *C. kefyr* ($n = 2$), *C. krusei* ($n = 2$), *C. glabrata* ($n = 2$), *C. lusitaniae* ($n = 2$) and 4 *Trichophyton spp, T. rubrum* ($n = 2$) and *T. mentagrophytes* ($n = 2$) by a disk diffusion assay. *In vivo* efficacy of the econazole imprinted textile was evaluated on a murine cutaneous candidiasis model using econazole cream as reference and the textile alone as placebo.
Results *In vitro* antifungal disk diffusion tests showed a comparable activity to commercial formulation on all *Candida* species and slightly lower on dermatophytes species. Nine days post-infection, the retrocultures of biopsy samples demonstrated an effective infection in the control with high density of *Candida albicans*. In contrast, the treatment with the econazole imprinted textile lead to a high reduction of the yeast cutaneous burden. This reduction was less important in the group treated by econazole cream.
Conclusions Overall, the econazole imprinted textile exhibited a significant *in vitro* antifungal activity against *Candida* species and *Trichophyton*. These results showed that this technology is promising to develop pharmaceutical textiles for the treatment of fungal superficial infections. Furthermore this technology could improve patient compliance while maintaining drug activity.

P411

Propolis potential activity against *Candida tropicalis* adhered cells and its biofilms

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Objectives Invasive fungal infections, such as candidiasis, represent a public health problem of major importance and *Candida tropicalis* has been highlighted among the main agents of candidiasis. Adhesion, which is the attachment of microbial cells to biological or non-biological substrates, has a critical role in the initial stages of biofilm formation. Additionally, one of the major contributions to *C. tropicalis* virulence is its versatility in adapting to a variety of different habitats and the formation of microbial communities known as biofilms. Moreover, from the clinical perspective, the most important feature of *Candida* biofilms is its intrinsic tolerance to conventional antifungal therapy. This scenario encourages the search for alternative therapies. Natural matrixes, such as propolis, compromise a multitude of bioactive properties, in particular phenolic extracts have evidenced significant antimicrobial properties against a multiple of opportunist invaders, including *Candida* species. Thus, the main objective of the present work was to evaluate the potential antifungal effect of propolis against *Candida tropicalis* adhered and biofilm cells.
Methods This study was conducted with four clinical strains of *C. tropicalis* and one reference strain, from the American Type Culture Collection (ATCC 40042). Adhesion and biofilm formation were carried out on 96-well microplates containing a cellular suspension of 1×10^5 cells ml^{-1} and incubated for 2 h and 24 h, respectively at 37 °C. Adhered cells and pre-formed *C. tropicalis* biofilms were treated with propolis (ranging from 0.47 to 1.42 mg ml^{-1}), during 24 h at 37 °C and its effect assessed through quantification of the number of colony forming unit (CFU).
Results It was evident that all *C. tropicalis* strains tested were able to adhere and form biofilm and that propolis was able to reduce around 2.5 logs ($P < 0.05$) and 3 logs ($P < 0.05$) on adhered cells and pre-formed biofilm, respectively. Moreover, in general the propolis effect was similar among all the *C. tropicalis* clinical isolates.
Conclusions The results of this study are promising, considering that biofilms are difficult to control by conventional antibiotherapy, propolis can be considered an efficient and safe antifungal alternative strategy to combat yeast biofilm associated infections.

P412

Antifungal activity of compound selected by virtual screening against chorismate synthase from *Paracoccidioides brasiliensis*

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The aim of the present work was to evaluate the CP1 antifungal activity with focus in the development of effective and safe antifungal. Firstly, the minimal inhibition concentration (MIC) determination was performed to *P. brasiliensis* (five isolates) and *P. lutzii* (Pb01 isolate), according to M27-A3 guidelines (CLSI) with modifications. The CP1 was prepared in a range of concentrations from 64 to 0.5 μg ml^{-1}, diluted in DMSO and Amphotericin B ranging from 16 to 0.03 μg ml^{-1}. The yeast cells were collected aseptically in sterile saline (0.85%). The inoculum suspensions were diluted in RPMI 1640 medium (the final concentration was 0.5 to 1×10^4 cells ml^{-1}). The plates were incubated 15 days at 35 °C. The MIC endpoint was defined as the lowest concentration of amphotericin B and CP1, showing 90% and 100% growth inhibition, repectively. The *in vitro* fungicidal and fungistatic activities were determined for CP1 after 15 days by subculture in brain heart infusion (BHI) agar plates, at 37 °C. The CP1 cytotoxicity was performed in HeLa, J774 macrophages and HUVEC cells, using the CellTiter 96 assay (Promega, Madison, WI, USA), based on the reduction of MTS (3-[4,5-dimethylthiazol-2-yl]-5-[3- carboxymethoxyphenyl]-2-[4-sulfophenyl]-2H-tetrazolium). The Chorismate Synthase (CS) has been shown to be an excellent target for antifungal drug development, as it is involved in a metabolic pathway that is present only in plants, fungi and bacteria. By *in silico* methodologies, a new compound, which acts as CS inhibitor from *Paracoccidioides brasiliensis*, was selected by virtual screening. The dynamic molecular simulation showed a strong interaction with this enzyme from *P. brasiliensis*. This thermally dimorphic fungus causes Paracoccidioidomycosis (PCM) is a systemic granulomatous disease. It is a prevalent systemic mycosis in Latin America which requires prolonged antifungal treatment. These therapies have several limitations, such as drug interactions, infusion-related events and nephrotoxicity. Therefore, the development of drugs that act selectively in target pathogenic fungi without producing collateral damage to mammalian cells is a daunting pharmacological challenge. All isolates tested were sensible the amphotericin B, with MIC values ranging from 0.5 to 1 μg ml^{-1} and for CP1 32 to 64 μg ml^{-1}. Regarding, the fungicidal activities of the CP1 is illustrated in Figure 1. The CP1 demonstrated to have fungicidal activity against *P. brasiliensis*. The cytotoxicity assays in J774 macrophages, HUVEC and HeLa cells exposed to CP1 revealed absence of cytotoxic effect in the MIC concentration (64 μg ml^{-1}) for 24 h. Therefore, the virtual screening of chemicals libraries offered alternative for new antifungal agents development against human pathogens. Then, this study showed that CP1 is capable of killing *P. brasiliensis in vitro* and lower cytotoxicity to human cells. Thus, our results show great therapeutic potential for compound CP1 owing to its important antifungal activity *in vitro*.

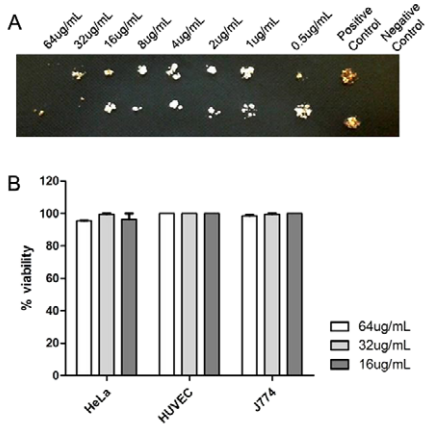

Figure 1 – The new compound with antifungal activity (A) and lower cytotoxicity (B)

P413

In vitro antifungal activity of two perylene bisimide derivatives and a dehydroabietylamine against *Fusarium* and *Scytalidium* species causing onychomycosis

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Objective To evaluate the antifungal activity of two perylene bisimide derivatives and a dehydroabietylamine against *Fusarium* and *Scytalidium* species causing onychomycosis.
Methods *In vitro* antifungal activity of twoperylene bisimide derivatives (72M and 73M) and a dehydroabietylamine (81M) was tested. Molecules were dissolved in DMSO, and five concentrations 25, 12.5, 6.25, 3.125 and 1.56 μg ml^{-1} were evaluated. Antifungal activity of the compounds was determined following the European Committee on Antibiotic Susceptibility Testing Subcommittee on Antifungal Susceptibility Testing protocols. Fifteen clinical isolates of *Fusarium* spp, an ATCC strain (*F. oxysporum* ATCC 48112) and six clinical isolates of of *Scytalidium* spp were evaluated. The minimum inhibitory concentration (MIC) was defined as the lowest compound concentration that resulted in total inhibition of growth. A molecule was considered active with a MIC value of ≤25 μg ml^{-1}. Plates with inocula and compounds were incubated at 28 °C for 48 h. The minimum inhibitory concentration tests were performed by duplicate in two different assays. As a positive control, itraconazole (Sigma) and terbinafine (Recalcine Laboratories, Santiago de Chile, Chile), in concentration ranges from 16 to 0.5 μg ml^{-1} and from 4 to 0.5 μg ml^{-1}, respectively, evaluated with *Fusarium oxysporum* ATCC 48112, were included. Also, all clinical isolates were evaluated with these two antifungals.
Results Overall*Scytalidium* spp isolates were more sensitive to the three molecules (MIC values range 6.25–12.5 μg ml^{-1}) than *Fusarium* spp (6.25 to ≥ 25 μg ml^{-1}). Also, MIC values with terbinafine were lower for *Scytalidium* spp (MIC value range 0.5 to ≥4 μg ml^{-1}), while for *Fusarium* spp, MIC value was ≥4 μg ml^{-1} in all cases. The susceptibility to itraconazole was low in all isolates evaluated (MIC values ≥16 μg ml^{-1}). Dehydroabietylamine 81M was active against 19/21 isolates (MIC value range 12.5 to 25 μg ml^{-1}) and with *F. oxysporum* ATCC 48112 (6.25 μg ml^{-1}). On the other hand, perylene bisimide derivative 73M was more active against *Scytalidum* spp (MIC value 6.25 μg ml^{-1}), but *Fusarium* spp susceptibility was strain dependent (MIC value range 6.25 to ≥25 μg ml^{-1}). Perylene bisimide derivative 72M was active against *Scytalidum* spp (MIC value 12.5 μg ml^{-1}), but it was not active against 11 *Fusarium* spp isolates (MIC value ≥25); the other isolates showed an MIC value of 25 μg ml^{-1}.
Conclusions Although onychomycosis is most frequently caused by dermatophytes and *Candida* spp., filamentous fungi such as *Fusarium* spp and *Scytalidium* spp are also increasing as a cause of this infection in tropical countries. Species of these genera are highly resistant to antifungals used in onychomycosis treatment. In addition, some *Fusarium* species are phytopathogenic. Therefore, identifying active molecules against these fungi could be important for both human health and treatment of plant infections.

Results indicate that mainly perylene bisimide derivative 73M and dehydroabietylamine 81M have important antifungal activity *in vitro*,

suggesting that these molecules are candidates for further studies of toxicity on skin cell lines, determination of efficacy in animal models and mechanisms of action.

P414

Thioredoxin reductase from *Candida albicans* as a promising immunogen against candidiasis

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Objective The aim of this work was the heterologous expression of thioredoxin reductase from *Candida albicans* in the *Escherichia coli* system, evaluate its structural stability at different temperatures and pH, contributing to an potential antigen candidate to vaccine development against candidiasis. Since the vaccination could be an important complementary strategy to pharmacotherapy.
Methods The gene coding for the thioredoxin from *Candida albicans* was cloned into pET21a expression vector, achieving the best expression condition. The protein with high purity was subjected to enzymatic assays using 5,5′-dithiobis(2-nitrobenzoate) (DTNB) and thioredoxin from *Candida albicans* (Trx1-Ca) and NADPH as a substrate and cofactors, respectively. Then, the secondary structures and protein stability at different pHs and temperatures were evaluated by circular dichroism (CD) and the study the intrinsic fluorescence information was obtained for the structure of the native protein and its possible conformational changes by changes in pH, temperatures and binders. Trr1 recombinant was administrated to mice by subcutaneous injection before challenging them with a lethal dose of *C. albicans*. After vaccination of Trr1, antibody responses were observed. To evaluate the vaccination effect of Trr1, 5 days after the challenge they were sacrificed and the number of viable *C. albicans* cells in the kidney and spleen were determined by number of CFUs.
Results Thioredoxin reductase (Trr1) is a flavoprotein widely distributed that catalyzes the NADPH-dependent reduction of thioredoxin, which compose the thioredoxin system. This system plays a critical role in maintaining the cytoplasm redox state, participating in important functions to the cellular viability this fungus. Therefore, this system has proven not only to be a promising target for the development of new antifungal drugs using rational approaches, against problems as few therapeutic options and the emergence of resistant strains to antifungal agents, as well as candidate for a vaccine against candidiasis. The heterologous Trr1 was efficiently expressed in *Escherichia coli*, resulting a protein with molecular weight (Mr) of approximately 35 kDa, in the soluble fraction of the bacterial lysate. Trr1 was purified with high purity and presented specific enzymatic activity. Structural changes of the protein were observed at different pHs and temperatures, showing high thermal stability at pH's 7.0 and 8.0. The administration of Trr1 recombinant increased titers of the Trr1-specific antibody (Figure A) and appeared to induce protective immunity against *C. albicans*, since reduced number of CFUs in kidney as compared with the control (Figure B).
Conclusion Therefore, our results demonstrated the facility to obtaining with the heterologous expression, high stability and efficacy in the immunogenic response of thioredoxin reductase from *C. albicans*,narrowing our search for a probable and promising antifungal target, as well as candidate for a vaccine against candidiasis.

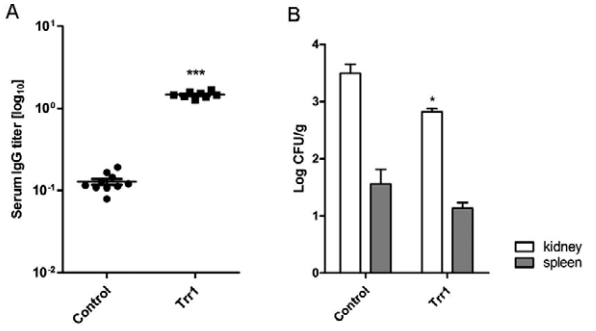

Figure: (A) Assessment of the titers of the Trr1-specific antibody by Elisa method. (B) Determination of CFU (colony forming units) per gram of tissue after immunization of mice with Trr1.

P415

Anti-*Candida albicans* activity of acetonic phenols-enriched fraction of Buchenavia tomentosa extract and gallic acid

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Objectives The aim of this study was to *in vitro* evaluate the anti-*Candida albicans* activity of acetonic phenols-enriched extract (FE) from *B. tomentosa* leaves and gallic acid (GA). Their influence on virulence factors was also assessed.
Methods Minimal inhibitory (MIC) and minimal fungicide concentrations (MFC) of FE and GA were determined for *C. albicans* reference strains (ATCC 18804, SC 5314) and 29 clinical isolates by EUCAST methodology. Pre-formed 24, 48 and 72 h biofilms of reference strains and 3 clinical isolates were exposed to concentrations of 2, 4 and 10 times MIC to evaluate the eradication potential. The effect of the presence of sub-inhibitory concentrations of FE and GA on the development of biofilms for 24, 48 and 72 h was assessed. The effects on the production of hydrolytic enzymes (proteinase and phospholipase) and adherence to buccal cells were also studied. All the experiments were performed in triplicate. The results of effect on biofilm viability (expressed in number of fungal cells) and adherence to buccal cells (expressed in number of yeast cells adhered to 25 cells) were compared by ANOVA and post hoc Tukey's test at level of significance of 5%. For production of hydrolytic enzymes, the results were expressed by Pz values ranges.
Results MIC values ranged from 10 to 1.25 mg ml^{-1} to FE and 10 to 2.5 mg ml^{-1} to GA. No fungicide activity was detected. Gallic acid and FE were able to promote significant reduction in the number of viable cells of pre-formed 24 h biofilm ($P = 0.021$ and $P = 0.034$, respectively). The presence of sub-inhibitory concentrations of gallic acid reduced significantly the development of biofilms of 24 ($P = 0.008$) and 48 h ($P = 0.002$). No effect on proteinase (Pz range: 0.22–0.58) and phospholipase (Pz range: 0.36–0.54) was found. Significant reduction in the adherence ability was detected after exposition to FE ($P < 0.001$) and GA ($P < 0.001$).
Conclusion Acetonic phenols-enriched fraction of *Buchenavia tomentosa* extract and gallic acid showed promising anti-*Candida albicans* activity, with inhibitory effect on biofilm and cell adherence.

P416

Evaluation of propolis and its subproduct as an inhibitor of growth and biofilm formation in vaginal yeast from pregnant women

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Objectives The treatment of vulvovaginal candidiasis (VVC) is still unsatisfactory, especially in pregnant women, being promising to the utilization of alternative therapies. Propolis extract solution (PES) has demonstrated antifungal efficacy and low toxicity. In addition, the subproduct of propolis extract solution (SPES) is produced during the process of preparing PES and is usually discarded, but can still submit substances responsible for biological effects, such as the polyphenols, responsible for the therapeutic activity of propolis. SPES have not been investigated or used as an antimicrobial agent. Thus, the objective of the present study was to investigate the effect of PES and SPES on *Candida* spp. isolated from the vaginal material of pregnant women.
Methods Vaginal samples from 291 pregnant women were collected and cultivated for yeasts, which were identified by the classical method and performing susceptibility tests against PES, SPES and conventional antifungal agents. The anti-biofilm effect and cytotoxicity tests of the PES and SPES were evaluated.
Results In 38.48% (112/291) of culture was positive for *Candida* species. There were patients with two different species, being a total of 115 yeasts (82.61% *C. albicans*; 6.08% *C. glabrata*; 5.22% *C. tropicalis*; 5.22% *C. parapsilosis* and 0.87% *C. krusei*). PES and SPES were effective, even against isolates resistant to conventional antifungal (Table 1) and reduced about 25% *C. tropicalis* biofilm, besides presenting its low toxicity in the concentrations of fungicides.
Conclusion Thus, in addition to the PES, SPES can also be a promising alternative treatment, especially in this population.

Table 1. Minimum inhibitory concentration (MIC) values (μg ml^{-1}) to amphotericin B, nystatin, fluconazole, PES and SPES.

Species (n) antifungal agente	MIC[d] (μg/mL) MIC Range	MIC 50[a]	MIC 90[b]	Mean
***C. albicans* (95)**				
Amphotericin B	0.25 - 1.0	0.5	1.0	0.5
Nystatin	0.125 - 0.5	0.125	0.25	0.125
Fluconazole	0.125 - 4.0	0.125	0.125	0.125
PES[c]	68.35 – 1093.75	136.71	273.43	206.50
SPES[c]	22.29 – 356.71	40.58	22.29	59.36
***C. glabrata* (7)**				
Amphotericin B	0.5 - 2.0	1.0	2.0	1.0
Nystatin	0.06 - 0.125	0.125	0.25	0.125
Fluconazole	0.125 - 4	1.0	4.0	1.0
PES[c]	68.35 – 536.87	68.35	546.87	146.47
SPES[c]	11.14 – 89.17	44.58	89.17	38.21
***C. tropicalis* (6)**				
Amphotericin B	0.5 - 1.0	0.5	1.0	0.5
Nystatin	0.125	0.125	0.125	0.125
Fluconazole	0.125 - 1.0	0.125	1.0	0.125
PES[c]	136.72-1093.75	546.87	1093.75	455.72
SPES[c]	22.29 – 178.35	89.17	178.35	89.17
***C. parapsilosis* (6)**				
Amphotericin B	0.5 - 1.0	0.5	1.0	0.5
Nystatin	0.125	0.125	0.125	0.125
Fluconazole	0.125	0.125	0.125	0.125
PES[c]	273.47–1093.75	273.43	1093.75	410.15
SPES[c]	89.17 – 178.35	89.17	178.35	104.03
***C.krusei* (1)**				
Amphotericin B	1.0	-	-	-
Nystatin	0.125	-	-	-
Fluconazole	16.0	-	-	-
PES[c]	136.71	-	-	-
SPES[c]	22.29	-	-	-

[a] the lowest concentration of drug that was able to inhibit 50% of isolates of each type.
[b] a lowest concentration of drug that was able to inhibit 90% of isolates of each species
[c] PES e SPES values in μg/mL of total phenol content in gallic acid
[d] MFC test presented the same value as the one found for the MIC

P417

Antifungal activity and time-kill assay of essential oil from Cymbopogon nardus against *Candida parapsilosis*

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Objectives *Candida parapsilosis* has emerged as one of the most important opportunistic pathogens, and the second most commonly isolated *Candida* species from blood cultures in Latin America, Europe, Canada and Asia. In addition, drug-resistant isolates are emerging quickly with the increasing clinical use of conventional drug, contributing to the therapeutic failure. Thus, the aim of this study was to test the antifungal activity of essential oil from *Cymbopogon nardus* (EO) against *C. parapsilosis* ATCC 22019 and clinical isolate.
Methods The minimal inhibitory concentration (MIC) of EO was determined according to the protocol described by Araújo et al., with modifications. The initial concentration of EO was 2000 μg ml^{-1}. 0.1 mL was placed in a 96-well microtiter plate containing RPMI 1640 medium. Each well was inoculated with 0.1 mL of a suspension containing 2.5x10^3 cfu ml^{-1} of yeast. Amphotericin-B and fluconazole were used as controls of the antifungal activity. The plates were incubated for 48 h at 37 °C. The MIC of sample was detected following the addition of 0.02 mL 2.0% triphenyltetrazolium chloride. The time-kill assay was performed according to Zore et al., with modifications. In brief, Sabouraud broth medium (10 mL) containing 2.5 × 10^3 cfu ml^{-1} of *C. parapsilosis* and 2 × MIC of EO were

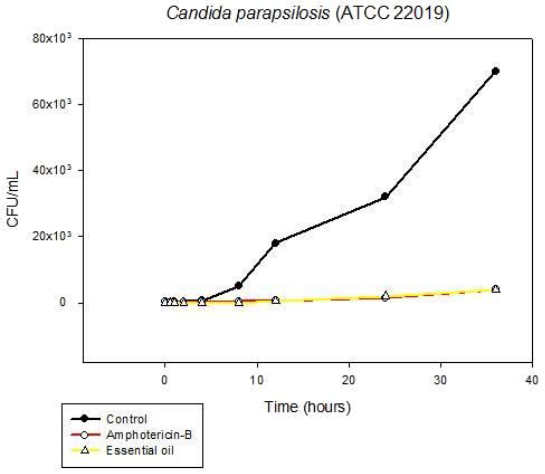

Figure 1: Effect of essential oil of *Cymbopogon nardus* on time-kill assay of *C. parapsilosis* (ATCC 22019)

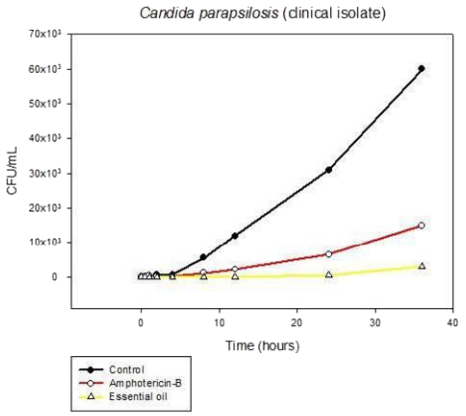

Figure 2: Effect of essential oil of *Cymbopogon nardus* on time-kill assay of *C. parapsilosis* (clinical isolate)

incubated and aliquots of 0.4 mL were removed at different time intervals (30 min, and 1, 2, 4, 8, 12, 24 and 36 h), and re-suspended in Sabouraud broth medium and 100 µL were inoculated on Sabouraud agar plates. All plates were incubated at 37 °C for 48 h. Amphotericin-B (32 µg ml^{-1}) was used as control. The number of colonies was counted and compared with controls.

Results The results showed EO had effective antifungal activity with MIC of 1000 µg ml^{-1} (ATCC and clinical isolates), while fluconazole and amphotericin-B had MIC >64 µg ml^{-1} (resistant) and 8.0 µg ml^{-1} (resistant), respectively. The time-kill assay curve showed that EO killed 100% of the ATCC strain and clinical isolate within 24 h of exposure (Fig. 1 e Fig. 2).

Conclusion In conclusion, the findings show that EO is a promising antifungal agent in the treatment of candidiasis caused by *C. parapsilosis*. Furthermore, on time-kill assay, EO exhibit similar or higher antifungal activity than amphotericin-B.

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P418

Modulating antifungal activity of amphotericin B through its conversion to a salt form

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Amphotericin B (AmB) is still the number one choice in invasive aspergillosis therapy. AmB interacts with ergosterol in the plasma membrane and its main molecular targets in *Aspergillus fumigatus* are ergosterol biosynthesis, oxidative stress response, cell wall maintenance and transport proteins.[1] Resistance to AmB has been rarely reported, except for some *A. terreus* strains.

AmB is insoluble in saline at normal pH and therefore it is usually administrated with sodium deoxycholate. The major drawback in AmB use is its high toxicity. Three commercialised lipid-AmB formulations have slower release and therefore reduced toxicity; however, increased cost is also associated.

Aiming at improved physical and antifungal properties, novel AmB derivatives are herein proposed, namely salts combining the anionic form of AmB with several cations, e.g. cholinium (ChAmB) or cetylpyridinium (C_{16}PyAmB). We focused on *A. fumigatus* biofilm, as it presents increased resistance to antifungal agents and is the prevailing growth mode *in vivo*. The mode of action of these AmB-based salts and their cytotoxicity were also subject of investigation.

Table 1. Inhibitory effects of amphotericin B (AmB) and its salts (ChAmB and C16PyAmB) and C16PyCl are reported as EC50 values (µM).

compound	*Aspergillus fumigatus*	*Aspergillus terreus*	mIMCD-3 cells
AmB	0.70	3.01	> 100
ChAmB	0.57	2.52	28.63
C_{16}PyAmB	0.22	0.83	2.02
C_{16}PyCl	5.95	n.d.	n.d.

Methods The synthetic route and characterisation of AmB salts will be reported elsewhere (collaborative work with Prof. Luís C. Branco). *Aspergillus fumigatus* and *A. terreus* biofilm susceptibility assays (EC50 values determination and checkerboard assay) and cytotoxicity assays (mIMCD-3) were performed. Expression analysis of AmB-responsive genes was performed by qRT-PCR.

Results ChAmB and C_{16}PyAmB displayed improved solubility in physiological solution and significantly enhanced antifungal activity against *A. fumigatus* biofilm when compared with AmB (Table 1). *Aspergillus terreus* showed higher resistance to ChAmB and C_{16}PyAmB, which could be seen as a preliminary evidence of the preserved mode of action. The individual contribution of the ions in C_{16}PyAmB was investigated by combining C_{16}PyCl and AmB in a checkerboard assay. Surprisingly, equimolar concentrations of the two compounds caused inhibitory effect similar to AmB alone. Therefore, a possible explanation for the stronger antifungal activity of C_{16}PyAmB may rely in the fact that AmB lost its zwitterionic nature. We are currently analysing further this hypothesis. In addition, cytotoxicity of ChAmB and C_{16}PyAmB towards mIMCD-3 cells increased tremendously when compared to AmB and this aspect needs to be carefully considered.

Conclusion Creating new opportunities in antifungal therapy is crucial for defeating induced resistance occurrence and emergence of novel fungal pathogens. The concept of converting biologically active molecules to a salt form as to improve their physical properties was explored before. We have herein demonstrated that such alteration potentiates the antifungal activity of AmB and possibly leads to dual biological action. A recent mechanistic study which proposed that large extramembranous AmB aggregates actually extract ergosterol from lipid bilayer is defying the well-established ion channel model.[2] We will attempt molecular dynamics simulation to further elucidate the possible interaction of AmB salts with ergosterol and we believe that our findings can contribute to the better understanding of AmB mode of action, guiding novel synthetical routes for efficient AmB derivatives.

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P419

Antifugal activity of Brazilian Cerrado *Pouteria ramiflora* plant extract against *Candida* spp

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Objectives Invasive candidiasis is recognized as a cause of morbidity and mortality in tertiary care hospitals worldwide. Although the majority of candidiasis infection cases are attributed to *Candida albicans*, rates of infection by non-*C. albicans* species are increasing

worldwide. Additionally, the limited number of available antifungal drugs and the increasing frequency of resistant cases stresses the urgent need for developing new drugs with antifungal activity. In this context compounds originating from medicinal plants become an important field of research. The genus *Pouteria* belongs to the family Sapotaceae and shows a widespread distribution around the World, being mainly found in tropical and subtropical regions of Asia and South America. Species of this genus have shown several biological activities such as antioxidant, anti-inflammatory, antibacterial and antifungal properties. However, the potential of this genus as source of new drugs or phytomedicines remains unknown. Therefore, this study aim to determine the antifungal activity of *Pouteria ramiflora* specie, originated from the Brazilian Cerrado.

Methods Three standard strains from the American Type Culture Collection (ATCC) of *Candida* species, *Candida albicans* (ATCC 40277), *Candida glabrata* (ATCC 40136) and *Candida parapsilosis* (ATCC 40038) were used in this study. The strains were grown on Sabouraud dextrose agar at 25 °C for 24 h. The evaluation of the antifungal activity of *Pouteria ramiflora* extract was performed using the microdilution method M27-A2 broth (CLSI). From pure ethanol extract serial dilutions were realized using a solution containing DMSO <1% and Ethanol <10%. The final concentration of the extract in each well ranged from 50 mg ml^{-1} to 0.1 mg ml^{-1}. For the assay, the plant extract was added to the 1×10^3 cells ml^{-1} yeast suspension in Sabouraud medium that had been previously seeded in each well. The cultures were observed at 6, 24 and 48 h to measure the activity profile of the plant extract on the yeast. After this incubation period, the contents of each well was seeded in Sabouraud dextrose agar medium at 25 °C for 24 h to allow the counting of the Colony Forming Units (CFU) and determining the Minimum Inhibitory Concentrations (MICs). All assays were performed in triplicate.

Results The *Pouteria ramiflora* extract was able to inhibit all three *Candida* species disclosing MIC values ranging from 6,25 mg ml^{-1} to 12.5 mg ml^{-1}. *Candida glabrata* showed MIC with a lower range when compared to *Candida albicans* and *Candida parapsilosis*.

Conclusion *In vitro* results obtained with ethanol extract of the *Pouteria ramiflora* showed potential antifungal effect against *Candida albicans, Candida glabrata* and *Candida parapsilosis*.

P420

In vitro effect photodynamic therapy with methylene blue, rose bengal, protoporphyrin IX and curcumin on *Candida albicans, C. parapsilosis* and *C. krusei*.

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Objectives *Candida albicans, C. parapsilosis* and *C. krusei* are yeasts that can be important pathogens commonly involved in infections. Antimicrobial photodynamic therapy (APDT), based on the application of a photosensitizer (Ps) activated by visible light to generate reactive oxygen species that are cytotoxic to microorganisms, could be an alternative treatment. Methylene blue (MB), rose bengal (RB), protoporphyrin IX (PpIX) and curcumin (CUR) are promising Pss for APDT.

The aim is to compare the efficacy of APDT on *C. albicans, C. parapsilosis* and *C. krusei* using MB, RB, PpIX and CUR.

Methods *C. albicans* ATCC10231, *C. parapsilosis* ATCC 22019 and *C. krusei ATCC 6258* suspensions containing $>10^7$cells ml^{-1} were prepared. Different concentrations two-fold serial dilution of PSs (from 0.15 μg ml^{-1} to 2560 μg ml^{-1}) were added. Irradiation at a fluence of 36 J/cm^2 was carried out using white light-emitting diode (LED) (0.024 w/cm^2) in all cases and different LEDs lamps depending on the most appropriate wavelength for each Ps: red LED light (625 nm, 0.0070 w/cm^2) for MB, green LED light (511 nm, 0.0058 w/cm^2) for RB and blue LED light (460 nm, 0.013 w/cm^2) for PpIX and CUR. The resultant suspensions were subcultured onto agar Sabouraud to determine the viable yeasts by colony-forming units counting (CFU ml^{-1}). Appropriate control experiments were carried out.

Results APDT with MB reached a reduction of 6 log10 in the number of CFU/mL of *C. albicans* using concentrations between 80 and 160 μg ml^{-1} with white light and 80 μg ml^{-1} with red light. In the case of *C.krusei*, the same log reduction was allowed with 320–640 μg ml^{-1} and 320 μg ml^{-1} of MB with white and red light respectively. In the case of *C.parapsilosis*, 160–320 μg ml^{-1} were needed either with white and red light.

Conversely, concentrations of RB, CUR or PpIX higher than 2560 μg ml^{-1} were not enough to show a significant reduction in any of the experimental conditions.

Conclusion *In vitro* PDT-MB has a significant fungicidal effect on *Candida* (*C. albicans*>*C. parapsilosis*>*C. krusei*)whereas RB, CUR and PpIX were not effective PSs.

As it was expected, red light is the most efficient, although white light can be also a good option.

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P421

Modulation of morphogenesis in *Candida albicans* by atmospheric pressure plasma jet

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Objectives The aim of this study was to evaluate the effect of atmospheric pressure plasma jet on *Candida albicans* viability, morphogenesis and hydrolytic enzymes (proteinase and phospholipase) production.

Methods Standardized suspensions of *C. albicans* SC 5314 and ATCC 18804 (10^6 cell ml^{-1}; λ = 550, OD: 0.380) were exposed to atmospheric pressure plasma jet working with helium (flow: 5.0 L min^{-1}) at 1.5 cm distance from the device tip to the liquid surface. The exposition times were 30, 60, 90 and 120 seconds. To determine the minimum exposition time necessary to reduce cell viability, cell suspensions were ten-fold diluted and cultivated on Sabouraud dextrose agar (37 °C, 24 h) to obtain CFU ml^{-1} values. The effect of plasma jet on *C. albicans* morphogenesis was evaluated by exposing standardized cell suspensions to plasma jet for 30 and 60 seconds (sub-inhibitory exposition times). After exposition to plasma jet, cell suspensions were supplemented with fetal bovine serum (20%) and after incubation (4 h, 37 °C) hyphae and yeasts were counted using a hemocytometer. To evaluate protease and phospholipase production, the suspensions were exposed to plasma jet also for 30 and 60 seconds. Then, they were inoculated on agar containing bovine albumin or egg yolk emulsion. After 5 days of incubation (37 °C), halos diameters were measured and Pz values were determined. All the experiments were performed in triplicate in three different situations. Non-exposed control groups were included in all experiments.

Results were compared statistically by ANOVA and post-hoc Tukey test. Results: Significant reduction in cell viability was observed from 90 seconds of exposition to plasma jet ($P < 0.001$). After exposition of 30 and 60 seconds, the percentage of filamentation was significantly reduced for both ATCC 18804 and SC 5314 ($P < 0.001$) cells. No difference in enzymes production was observed between exposed and non-exposed groups, $P > 0.05$).

Conclusion Atmospheric helium plasma jet showed promising effects on *Candida albicans*, reducing cellular viability and modulating morphogenesis.

P422

Molecular modeling of a heat shock protein (HSP90) as strategy for drug development against paracoccidiodomycosis

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Objectives In order to develop new antifungal treatments against *Paracoccidioides lutzii*, we proposed to evaluate the heat shock protein of 90 kDa (HSP90) as drug target. As there is no crystallographic structure presently available for HSP90 of *P. lutzii*, we performed the homology modeling of the N-terminus domain of *P. lutzii* HSP90 protein and the molecular dynamics simulations to obtain the HSP90 3D model with stability.

Methods The 3D model of HSP90 *P. lutzii* was constructed by homology modeling based on known structures with high percentage of identity in amino acid sequences. The known template structures were searched in the Protein Data Bank (PDB). The amino acid residue sequences of HSP90 were compared with the primary sequences of the structures deposited in the PDB using the BLAST program. The homologous sequences allowed the construction of a 3D model of HSP90 using the homology module of the Insight II software package (Biosym/MSI, San Diego, Accelrys Inc. 2001). This model was further submitted to molecular dynamics simulations in order to gain a better relaxation and a more correct arrangement. The NAMD program version 2.6 was employed in conjunction with the CHARMM22 force field.

Results The 3D model of N-terminus domain of *P. lutzii* HSP90 protein was obtained. To investigate the stability of 3D model, molecular dynamics simulations were performed and revealed that the evolution of the system is very stable. The folding stability of the model during the simulations was checked by monitoring secondary structure conservation over simulation time. With the model stable, virtual screening for select the main small molecules that interact with this target will be performed.

Conclusion This work describes the prediction of HSP90 model of *P. lutzii* and its stability by molecular dynamics simulations aiming to identify new antifungal compounds against paracoccidioidomycosis. In the absence of structures solved experimentally, the available homology modeling tools were extremely useful for the structural prediction of the HSP90 protein of *P. lutzii*. From these steps we obtained relevant information for future technological development. Moreover, these results are being used to virtually screen chemical libraries, which are under progress, generating new perspectives on technological development and innovation of antifungal agents to these human pathogens.

P423

Antifungal activity of the human uterine cervical stem cells conditioned medium against medically important species of *Candida*

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Candidiasis are major causes of human morbidity and mortality. Although *Candida albicans* is the prevalent aetiology of these fungal diseases, other species, such as *Candida parapsilosis*, *Candida glabrata* or *Candida krusei*, cause an increasing number of candidiasis. These emergent species frequently show a reduced susceptibility to common antifungal drugs. New compounds are needed for the treatment of invasive and recalcitrant superficial candidiasis. Human uterine cervical stem cells conditioned medium (HUCSCCM) has antitumour and antibacterial activities [1,2] and it could be a potent antifungal drug.

Objectives To evaluate and to compare the antifungal activities of human uterine cervical stem cells conditioned medium (HUCSCCM) and adipose tissue stem cells conditioned medium (ATSCCM).

Methods Antimicrobial *in vitro* activities of HUCSCCM and ATSCCM against one strain each of *Candida albicans*, *Candida dubliniensis*, *Candida glabrata*, *Candida krusei*, *Candida lusitaniae* and *Candida parapsilosis* were compared with a microdilution method on RPMI 1640 using the BioScreen C microbiological incubator.

Results Antifungal activity recorded as a reduction of cell growth in respect to the control at 24 h of culture at 37 °C is shown in table 1. The inhibitory activity of HUCSCCM was excellent against *Candida glabrata* (91.5%, Figure), *Candida dubliniensis* (73.4%), *Candida albicans* (57.5%), and *Candida lusitaniae* (46.8%). However, there was not antifungal activity against *Candida krusei* and this activity was low against *Candida parapsilosis*. ATSCCM showed also a very good activity against *Candida albicans* (96.8%), *Candida krusei* (83.6%), *Candida parapsilosis* (74.98%), and *Candida dubliniensis* (74.7%). Conversely, *Candida glabrata* was resistant to ATSCCM, with a paradoxical growth of this species in its presence, and the activity against *Candida lusitaniae* was low.

Conclusions HUCSCCM exerts an excellent antifungal activity against the most common species of *Candida* but *Candida krusei*. ATSCCM shows very good antifungal activity but it is ineffective against *Candida glabrata*.

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Table. In vitro activity of HUCSCCM and ATSCCM against *Candida* at 24 hours.

	OD	GR (%)	P
Candida albicans ATCC 90028			
HUCSCCM	0.075	57.5	<0.0001
ATSCCM	0.006	96.8	<0.0001
Candida dubliniensis ATCC MYA646			
HUCSCCM	0.069	73.4	<0.0001
ATSCCM	0.066	74.7	0.001
Candida glabrata ATCC 90030			
HUCSCCM	0.005	91.5	<0.0001
ATSCCM	0.120	-91.4	<0.0001
Candida krusei ATCC 6258			
HUCSCCM	0.159	-11.9	0.725
ATSCCM	0.023	83.6	<0.0001
Candida lusitaniae ATCC 200953			
HUCSCCM	0.057	46.8	<0.0001
ATSCCM	0.068	36.4	0.02
Candida parapsilosis ATCC 22019			
HUCSCCM	0.114	15.1	0.095
ATSCCM	0.033	74.98	<0.0001

OD: Optical density. GR: Growth reduction growth in respect to the control. HUCSCCM: Human uterine cervical stem cells conditioned medium.

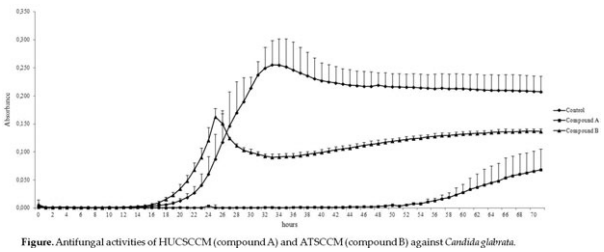

Figure. Antifungal activities of HUCSCCM (compound A) and ATSCCM (compound B) against *Candida glabrata*.

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P424

Photoinactivation with red LED lamp of *Candida albicans* using Methylene Blue, alone or in combination with fluconazole or sertaconazole

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Objectives *Candida albicans* is a yeast frequently found in skin and mucous that can be important pathogen commonly involved in infections.

Antifungals like fluconazole or sertaconazole are used to treat the infection by *C. albicans.* Antimicrobial photodynamic therapy (APDT), based on the application of a photosensitizer like Methylene Blue (MB) activated by visible light to generate reactive oxygen species that are cytotoxic to microorganisms, could be an alternative treatment.

The aim of this study was to compare the efficacy of APDT-MB on *C. albicans* at different times of preincubation in darkness alone and joined to antifungals.

Methods *C. albicans* ATCC 10231 suspensions containing $>10^7$cells ml^{-1} were prepared. Different concentrations two-fold serial dilution of MB (from 2.5 μg ml^{-1} to 640 μg ml^{-1}) combined with 0, 10, or 20 μg ml^{-1} of fluconazole and sertaconazole were tested using different preincubation times in darkness (0, 0.5, 1, 2 or 24 h). Irradiation was performed with a red light-emitting diode (LED) lamp (625 nm, 0.007 w/cm2) at a fluence of 18 J/cm2. The resultant suspensions were subcultured onto agar Sabouraud with added chloramphenicol to determine the viable yeasts by colony-forming units counting (CFU/mL). Dark and irradiated controls, with and without MB, fluconazole or sertaconazole were included.

Results A reduction of 6 log10 CFU ml^{-1} were reached without and with 0.5, 1 and 2 h of preincubation with 80–160 μg ml^{-1} of MB diminishing the concentration to 80 μg ml^{-1} at 24 h. Adding 10 or 20 μg ml^{-1} of fluconazole or sertaconazole, the MB concentrations required remain the same.

Conclusion *In vitro* PDT-MB has a significant fungicidal effect on *C.albicans* and 0 min of preincubation with the MB is enough to obtain the maximum effect. This is very convenient to translate this therapy into the clinic.

PDT-MB seems to have not synergic effect *in vitro* with fluconazole or sertaconazole against *C. albicans.*

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P425

Effects of Local Herbs on Common Dermatophytes and causative agent of *Pityriasis versicolor* in Rivers State, Nigeria

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Background The antifungal activities of seven (7) local herbs used by traditional medicine practitioners against the Dermatophytes (*Trichophyton, Microsporum, Epidermophyton*) and *Malassezia furfur* (the etiological agent of *Pityriasis versicolor*), were studied by well-in-agar diffusion technique using different concentrations of methanolic and ethanolic extracts.

Methods Isolates from the scalp, skin, toes and feet of forty individuals (mainly children) were obtained from four locations namely Aluu, Choba, Rumuosi and Emohua areas of Rivers State. The methanolic and ethanolic herb extracts that were tested are *Ficus exasperata* vahl, *Ocimum gratissimum*, *Jatropha curcas* leaves, *Commelina benghalensis*, *Mitracarpus villosus, Harungana madagascariensis* and *Chlorophora excelsa*. The phytochemical contents of the extracts were also determined.

Results The qualitative phytochemical analysis showed that the extracts contained alkaloids, cardiac glycosides, tannins, saponins and flavonoids. Anthraquinones and phlobatanins were absent. The study showed significant inhibitory effect by *O. gratissimum*, *F. exasperata vahl* and *J. curcas* on the fungal isolates, at five different concentrations (250 mg ml^{-1}, 200 mg ml^{-1}, 150 mg ml^{-1}, 100 mg ml^{-1} and 50 mg ml^{-1}) used. The other plant extracts of *C. excelsa*, *M. villosus, C. benghalensis,* and *H. madagascariensis* showed some levels of inhibition at higher concentrations (250 mg ml^{-1} and 200 mg ml^{-1}).

Conclusion Assessment of the various minimum inhibitory concentrations (MIC) showed that *Ocimum gratissimum* had the most potential for use as an antidermatophytic agent. The phytochemical constituents revealed may also be useful to the pharmaceutical industries and could be used as effective nutraceuticals. However, further studies are needed to isolate and purify the bioactive compounds of these useful traditional plants for industrial drug formulation.

P426

Nepenthes rafflesiana pitcher liquid has antifungal activity against Candida spp.

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Objectives To develop new effective antifungals, it is essential to search for antifungal compounds from plants such as *Nepenthes* spp., which have their greatest diversity in Indonesia. Since chitin-induced liquid (CIL) from *Nepenthes khasiana* pitchers has antifungal activity, due to their naphthoquinone content, this study aimed to evaluate antifungal activity of *Nepenthes rafflesiana* pitcher liquids on *Candida* spp.

Methods Collected pitcher liquids were of 2 types: non-induced liquid (NIL) and chitin-induced liquid (CIL). NIL was collected from fresh naturally opened pitchers and CIL from closed pitchers after 5 days of chitin solution injection. Chitin solution was prepared from dried prawn exoskeleton. The antifungal activity of the liquids against *C. albicans*, *C. glabrata*, *C. krusei*, and *C. tropicalis* were detected by disc diffusion. The medium was Mueller Hinton agar adding with glucosa 2% and metilen blue dye 0.5 mg l^{-1}. The 5 mm sterile discs were made from Whatman filter paper No. 42. The sterile disc soaked in aquabidest and placed on the seeded medium was used as a control. The test was performed in triplicate. The inhibition zone diameters were recorded.

Results The colour of NIL was white cloudy. On chitin induction, the colour of pitcher liquid changed to orange red (figure 1). Acidity of NIL was 3.1 and CIL was 2.6. All samples were slimy. Inhibition zone diameters of NIL and CIL against *C. albicans* were 36.44 ± 2.49 mm and 30.00 ± 2.00 mm, respectively, while for *C. glabrata* the zone diameters were 22.55 ± 3.53 mm and 28.89 ± 1.17 mm, respectively (figure 2). There were significant differences ($P < 0.05$) between NIL and CIL to both of fungi. No inhibition zones were found for NIL and CIL against *C. krusei* and *C. tropicalis*.

Conclusions NIL and CIL of *N. rafflesiana* have antifungal activity to *C. albicans* and *C. glabrata*, but no antifungal activity to *C. krusei* and *C. tropicalis*. The pitcher liquid of *N. rafflesiana* has antifungal

Figure 1. Pitcher liquid of *N. rafflesiana*: non-induced liquid (A) and chitin-induced liquid (B)

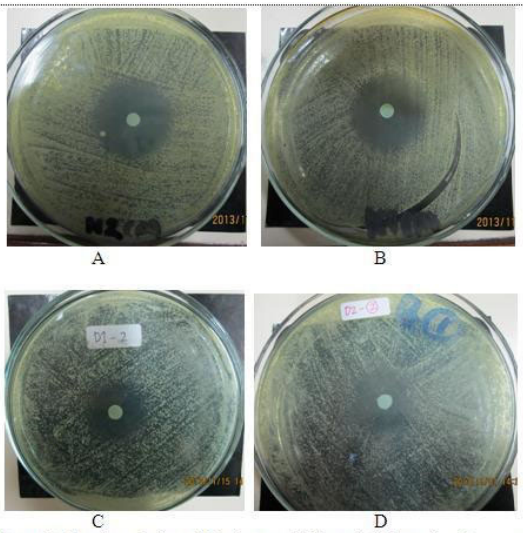

Figure 2. Results of disc diffusion test NIL and CIL to *C. albicans* and *C. glabrata*. Inhibition zone of NIL to *C. albicans* (A), CIL to *C. albicans* (B), NIL to *C. glabrata* (C), CIL to *C. glabrata* (D).

properties, presumably due to the presence of many potentially active substances, such as naphthoquinones, as has been proven in other studies.

Key words antifungal, chitin-induced, disc diffusion, *Nepenthes*, *Candida*

P427

The effectiveness of different denture disinfection methods on *Candida albicans* biofilm activity

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Objective Denture stomatitis is a widespread infection of complete denture users. The condition is related to multiple factors such as using old dentures, denture trauma and insufficient oral hygiene. Among all of these factors, the candidal biofilm formed on tissue surfaces of dentures are believed to be the primary etiological factor of denture stomatitis. Various types of candida species are contributed in this process, while *Candida albicans* is believed to be the most important one. Because of this, to prevent and to treat denture stomatitis, it is believed that elimination of *C. albicans* biofilm with various types of disinfection procedures is of paramount importance. Recently, in addition to conventional microwave and sodium hypochlroide disinfection regimes, strong and weak acidic electrolyzed waters are introduced. Regarding to this information, this study evaluated the effect of different disinfection regimes on *C. albicans* biofilm which is formed on acrylic denture base resin, *in vitro*.

Methods The samples were subjected to the previously mentioned disinfection process. At this stage, samples were transferred into 200 ml of sterile distilled water for the microwave disinfection at 650 W for 3 min. The chemical disinfection method was carried out transferring each sample into 1 ml disinfection solution. The disinfection process was carried out with weak and strong electrolyzed acid water and sodium hypochlorite for 1, 1 and 10 min, respectively. The disinfection was not adjusted on the control group.

The *C. albicans* ATCC (90128) suspensions were subcultured into fresh medium and incubated at 37 °C for 24 h. After 24 h of incubation, liquid cultures were washed three times. The optical density of the yeast stock solution was adjusted to a 0.5 MacFarland solution. 1 ml yeast suspension was incubated with the sample for 48 h at 37 °C in a horizontal shaker. To remove non-adhering fungi, all samples were carefully washed three times in PBS. Samples taken from the experimental group and control group were transferred into 1 ml of PBS and vortexed for 60 sec. to resuspend any microorganisms present. 0.1 ml of this resuspensions was plated on Sabouraud dextrose agar and incubated aerobically for 48 h at 37 °C. After incubation, the microbial colony count of each plated denture was quantified by the eye counting method and the logarithm of colony forming units (CFU) per ml was then calculated.

Results All four types of disinfection methods are completely eliminated *C. albicans* biofilm on acrylic resin specimens.

Conclusion This results revealed that electrolyzed acid waters are effective against *C. albicans* biofilm and they can be used to disinfect dentures for the patients with denture stomatitis.

P428

Antifungal Activity of Tesso Nilo Wild Honey Against *Candida albicans in Vitro*

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Objectives *Candida sp.* is a normal flora inhabiting the human skin and mucosa. *Candida sp.* may cause a disease called candidiasis, especially in immunocompromised patients. *Candida albicans* is the main pathogen in candidiasis cases. Candidiasis among immunocompromised patient often requires a long term usage of antifungal agents which leads to the emergence of the side effects. *Candida sp.* resistance against antifungal agents is also often reported. This condition has been an important issue in medical scope, so the various studies had been performed to find a better alternative antifungal agents. Honey is used as an alternative antifungal agent that had been proven by researchers. Tesso Nilo Wild Honey (TNWH) which originated from Tesso Nilo National Park - the largest rain forest in Sumatera, Indonesia - account as one of the Green and Fair Product by World Wildlife Fund Indonesia, so the quality of this honey can be guaranteed. This study is performed with the aim of investigating the antifungal activity of TNWH against *C. albicans in vitro*.

Methods This is an experimental *in vitro* study using the macrodilution method. *C. albicans* which has been incubated on 37°°C for 24 h is dissolved by normal saline with optical density equal to 0.5 McFarland in 530 nm wavelength. Furthermore, the inoculum is diluted by 1:2000 in Sabouraud Dextrose Broth (SDB). In this study there are 4 test groups with concentration of TNWH of 250 mg ml^{-1}, 500 mg ml^{-1}, 750 mg ml^{-1}, and 1000 mg ml^{-1}. The positive control used in this study is *C. albicans* in SDB and also 3 negative control consist of TNWH, SDB and mixture of both. All test groups are inoculated on Sabouraud Dextrose Agar and incubated on 37 °C for 24 h. The main idea of this study is to compare

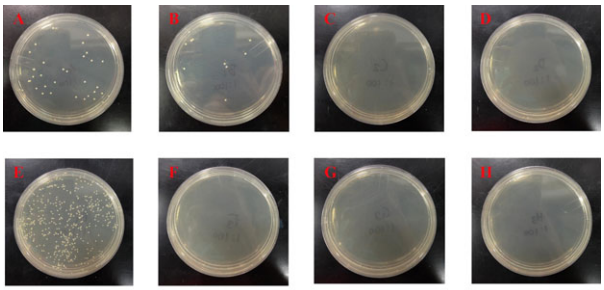

Figure 1. Colony Distribution
Note: TNWH 250 mg/mL (A); TNWH 500 mg/mL (B); TNWH 750 mg/mL (C); TNWH 1000 mg/mL (D); positive control (E); negative control TNWH (F); negative control SDB (G); negative control mixture of TNWH and SDB (H) All groups was diluted 1:100, except positive control which was diluted 1:1000

Figure 1. Colony Distribution.

the quantity of *C. albicans* numbers of colony in each test group to the positive control.
Results The numbers of colony for test group with concentration of TNWH of 250 mg ml^{-1}, 500 mg ml^{-1}, and 750 mg ml^{-1} are 34 (33–49), 10 (9–10), and 0 (0–1), consecutively, whereas the positive control is 2760 (2520–4400). The test group with concentration of TNWH of 1000 mg ml^{-1} and all negative control have no growth of *C. albicans*. Results are processed with SPSS using the Kruskal-Wallis statistic test and continued with the Post Hoc Mann-Whitney statistic analysis. The test group with the concentration of 500 mg ml^{-1} and 750 mg ml^{-1} had proven to had significant difference if compared to the positive control with p equals to 0.043 and 0.046 respectively.
Conclusion TNWH has an antifungal activity against *C. albicans in vitro* according to the macrodilution method. The test group with the concentration of 500 mg ml^{-1} shows an optimal result with the significant difference in numbers of colony.
Key words antifungal, *Candida albicans*, macrodilution, Tesso Nilo Wild Honey.

P429

Antifungal activity of soursop leaf infusion against *Candida albicans*

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Objectives Candidiasis is a disease caused by *Candida* sp, mainly *Candida albicans*. Antifungals used to treat candidiasis have side effects such as disruption in liver and kidney functions, also produce resistency. *Annona muricata*, known as soursop, have been used as folk medicine as antimicrobe and anticancer. In Indonesia, traditional remedies are often processed by boiling, such as infusion and decoction. The aim of this study is to detect antifungal activity of soursopleaf infusion (SLI) against *C. albicans in vitro*.
Methods This was a laboratory experimental study. The SLI was prepared in various concentrations: 20% w/v, 40% w/v, 60% w/v, 80% w/v, and 100% w/v. Antifungal activity of SLI was assessed by macrodilution methods and continued with colony counting. Ketoconazole 0.5 mg ml^{-1} was used as positive control. Negative control was fungal suspension in Sabouroud dextrose broth. Statistical analysis was performed using Kruskal-Wallis test and post hoc Mann-Whitney from SPSS 17.
Results Total colony counting from SLI 20% w/v, 40% w/v, 60% w/v, 80% w/v, and 100% w/v were 206 (185–262), 162 (161–176), 14 (9–25), 8 (6–12), 3 (0–3), consecutively. Whereas, total colony counting from negative control was 451 (371–561), and no colonies was found in positive control. Total colonies from all concentrations of SLI were less than negative control. Antifungal activity of SLI 100% w/v has no significantly difference with positive control ($P = 0.114$).
Conclusions The SLI has antifungal activity against *C. albicans*.

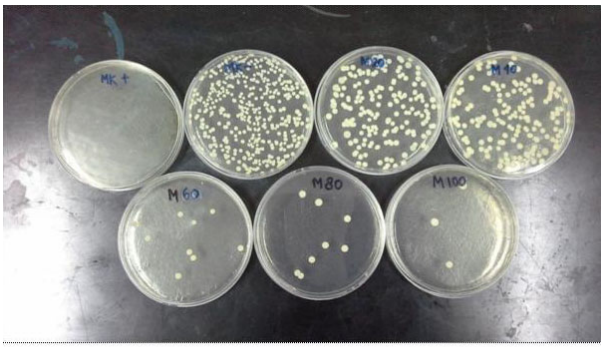

Figure 1. The colonies of *C. albicans* in SDA for counting.
Upper line, from left to right : positive control, negative control, SLI 20% w/v, 40% w/v
Lower line, from left to right : SLI 60% w/v, 80% w/v, 100% w/v

Figure 1. The colonies of C. albicans in SDA for counting

Table 1. Statistical analysis of total colonies among groups

Table 1. Statistical analysis of total colonies among groups

	n	Median (minimum-maximum)	p
Positive control (K+)	3	0	
Negative control (K-)	3	451 (371-561)	
SLI 20% w/v (A)	3	206 (185-262)	
SLI 40% w/v (B)	3	162 (161-176)	0.003
SLI 60% w/v (C)	3	14 (9-25)	
SLI 80% w/v (D)	3	8 (6-12)	
SLI 100% w/v (E)	3	3 (0-3)	

Post Hoc Mann Whitney Test: K+ vs K- p= 0.037, K+ vs A p= 0.037, K+ vs B p= 0.037, K+ vs C p= 0.037, K+ vs D p= 0.037, K+ vs E p= 0.114, K- vs A p= 0.050, K- vs B p= 0.050, K- vs C p= 0.050, K- vs D p= 0.050, K- vs E p= 0.046, A vs B p= 0.050, A vs C p= 0.050, A vs D p= 0.050, A vs E p= 0.046, B vs C p= 0.050, B vs D p= 0.050, B vs E p= 0.046, C vs D p= 0.127, C vs E p= 0.046, D vs E p= 0.046.

P430

Anti fungal activity of three Terpyridine derivates on pathogenic *Candida* species

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Objectives Recently, some Terpyridine based chemical compounds have been used as antibacterial agents. In this study, three Terpyridine based compounds including [Cd(Clphtpy)$(NO_3)_2H_2O$], [Cd (Clphtpy)$(N_3)_2$ and [Cd (Clphtpy)(NCS)$(NO_3)_n$ with similar structural and antibacterial activities were studied. This is a experimental study to develop using Terpyridine derivates on human pathogenic *Candida albicans*.
Methods A hundred Candida isolates of human infections and a standard Candida strain were profiled to the agar diffusion method by using a pour plate culture of Candida isolates which punched and loaded by 100 lμ of each three drugs. The cultures incubated at 30 °C for 48 h. Fungal activity was determined by measuring inhibited zone of the drug wells. The antifungal amphotericine B were used as positive control of inhibitory activity.

Results One hundred clinical isolates of pathogenic *Candida albicans* were used in this study. The clinical sources included sputum, broncho alveolar lavage and vaginal discharge of suspected patients to fungal infections. The average inhibitory zone of Terpyridine based compounds on studied Candida isolates included 18.2 mm, 29.6 mm and 20.8 mm for drugs 1,2 and 3 respectively.
Conclusion Comparing data of Terpyridine derivates with that of amphotericine B showed a low level of antifungal activity for mentioned drugs. Perhaps these compounds are useful to develop some forms of antifungal drugs.

P435

Insights into the synthesis of β-1,3-glucan in *Candida albicans*: relevance for echinocandins inhibition

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Fungal β-1,3-glucan synthase is an essential enzyme with no human homologue responsible for the biosynthesis of b-1,3-glucan. This polysaccharide is the main structural component of fungal cell wall providing structural stability and rigidity through covalent cross-linking with chitin and mannoproteins. The catalytic subunit of β-1,3-glucan synthase (known by FKS) is inhibited by the echinocandins, the newest antifungal class.
Objectives In order to get insights into the synthesis of β-1,3-glucan, a biochemical approach was established for heterologous expression and purification of FKS cytoplasmic domains. The *Candida albicans* FKS has two cytoplasmic domains: a N-terminal domain and the catalytic domain surrounded by two sets of transmembrane helices. The functional role of N-terminal and transmembrane regions is currently unknown.
Methods *C. albicans* Fksp cytosolic domains were cloned into different expression vectors. The approximate boundaries of each cytoplasmic-facing region were predicted using multiple protein sequence alignments and predictions of secondary structure or unfolded/disordered regions.
Results The N-terminal cytoplasmic domain was successfully expressed in bacteria and purified at large-scale using an affinity tag. Recombinant purified protein was subsequently analyzed by analytical gel filtration chromatography to assess protein stability and homogeneity. TheN-terminal FKS domain is stable and elutes as a monomer although this domain shows tendency to oligomerize when protein concentration increases. Domain oligomerization could be relevant for the assembly of β-1,3-glucan synthase in the plasma membrane since β(1,3)-glucan exists as a triple-helical structure in the cell wall.

Similarly to N-terminal domain, expression and purification of *C. albicans* catalytic domain was also attempted. However, the catalytic domain is unstable and its expression as soluble protein is very low. Different approaches were therefore employed to increase domain expression including co-expression of both catalytic and N-terminal domains. All these strategies failed suggesting that Fksp catalytic domain is unstable in the absence of the Fksp transmembrane region. In order to understand FKS, a theoretical three-dimensional model was generated by comparison with the crystal structure of bacterial cellulose synthase.
Conclusion *C. albicans* FKS catalytic domain is not stable alone in the absence of the transmembrane region, in contrast with the N-terminal domain.

P436

Predisposing factors to Vulvovaginal Candidiasis (VVC) and Recurrent Vulvovaginal Candidiasis (RVVC)

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Objectives Vulvovaginal candidiasis (VVC) and recurrent vulvovaginal candidiasis (RVVC) are diseases commonly diagnosed in women in reproductive age and is mainly caused by yeasts belonging to the genus *Candida*. It has been demonstrated that cellular immune response and defective neutrophil function may increase susceptibility to infections by *C. albicans*. The cell mediated immunity appears to be important in limiting the proliferation of *Candida* spp., suggesting that clinical conditions like exposure to chronic stress or deficient antioxidant micronutrients that alters the proper functioning of the immune system, could facilitate the primary VVC and RVVC occurrence. A low overall antioxidant capacity has been associated with immune deficiency, and some authors suggest that the antioxidant mechanism action is the modulation of signal transduction factors involving transcribing cells and immune-mediated cytokine production. On the other hand, attenuated levels of morning salivary cortisol and hyporesponsiveness of the hypothalamus-pituitary-adrenal axis have been shown previously to be indicative of chronic stress. In addition, authors suggest that stress may increase the risk for impaired function of the immune processes. Examine chronic stress and low levels of overall antioxidants capacity as a host intrinsic factors that could predispose women to developing VVC and RVVC.
Methods In a population of 254 women with or without signs and symptoms of VVC and RVVC, vaginal cultures were performed for yeasts isolation and identification by classical methods. Cervical-vaginal cytology and vaginal bacterioscopy were performed to analyze inflammatory process and microbiota. Fasting blood was collected for plasmatic cortisol and overall antioxidant capacity analyses. Cortisol levels were determined employing chemiluminescent microparticle immunoassay in the equipment Architect (Abbott, IL, USA), and the overall antioxidant capacity was determined by Trolox equivalent antioxidant activity (TEAC), which was performed as described by Miller et al. and Re et al. to evaluate plasma non-enzymatic antioxidants.Data distributions were expressed as mean ± standard deviation (SD). Significant differences among means were identified using GraphPad Prism® 5.0 software. Bonferroni test was used to calculate the multiple comparisions and Chi-square (χ^2) test using the STATA for Statistics and Data Analysis 9.1 software was used to investigate variables between control group and positive culture group. All variables were expressed as absolute and relative frequencies. $P < 0.05$ were considered significant.
Results Yeasts were isolated from 48 (19%) women: 22 (46%) in acute episode (VVC) and 26 (54%) with RVVC, and mainly *C albicans* was isolated in all clinical profiles. Control group (negative culture) comprised 206 (81%) women. VVC and RVVC groups showed lower means level of cortisol than control groups. RVVC group showed lower mean levels of antioxidant capacity than all other groups. RVVC showed a similar vaginal inflammatory process than control and lower than VVC groups. Only CVV group showed a reduction in vaginal lactobacilar microbiota.
Conclusion Our data suggest that both, chronic stress (decreased early morning cortisol levels) and low overall antioxidant capacity can be considered specific host predisposing factors to VVC and RVVC.

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Aspergillus fumigatus transcriptome during a disseminated murine infection reveals the metabolic adjustments once the infection has established

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Introduction *Aspergillus fumigatus* is a saprophytic mold that can act as an opportunistic pathogen. In order to succeed in the development of the infection, not only does this pathogen have to evade and confront the immune system of the host, but it also has to deal with numerous microenvironments. On the other hand, murine models have been used to study the pathogenesis of this fungus, which enables to understand what happens during a real infection and whose results might be extrapolated to human cases.
Objective The aim of this study is to analyze the transcriptome of *A. fumigatus* throughout an experimental disseminated infection in order to determine the metabolic changes that take place once the infection has established.
Methods Three independent murine infections were performed, being one infected kidney from each day used for RNA isolation. RNA samples were hybridized with a customized *A. fumigatus* expression microarray. Transcriptomic data were compared with a previous study of the germination of this pathogen at 37 °C (Sueiro-Olivares et al., 2015) and results were validated by RT-qPCR. Moreover, a clustering process was performed to determinate which genes had an increasing expression. Finally, these genes were classified according to their functional classification and the enrichment p-value of the functional groups was determined.
Results Statistical analysis of each day of infection using the *A. fumigatus* germination at 37 °C as the control condition showed that 4,080, 377, 3,604 and 1,645 genes were differentially expressed on day 1, 2, 3 and 4 of infection, respectively. Genes involved in iron, zinc and nitrogen metabolism appeared down-regulated during the infection. However, genes related to gliotoxin biosynthesis as well as others encoding proteases and phospholipases described as virulence factors were up-regulated along the infection. Focusing on the clustering process, 5 clusters showed an increasing expression pattern relative to the germination at 37 °C or throughout the infection. According to the enrichment p-values of the functional categories, secondary metabolism and polysaccharide metabolism were significantly represented. Nevertheless, among the 257 genes included within these clusters, 79 and 36 coded for hypothetical or unclassified proteins.
Conclusion It seems that once *A. fumigatus* infection has established in the host, a reduction of nitrogen, iron and zinc requirements takes place and/or this pathogen obtains these nutrients through different mechanisms. Secondary and polysaccharide metabolisms are the functions significantly expressed during the infection, standing out as indispensable metabolisms to spread the infection. However, the large number of hypothetical and unclassified proteins stresses the importance to focus researches on them, as they could shed light on novel virulence factors, and even on possible diagnostic targets.

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P438

Effect of antimycotic drugs on human platelets and their fungal killing capacity

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Objectives Platelets become more and more established as crucial players of the innate immunity and thus influence the course of invasive fungal infections. Recent own studies showed that some antimycotic drugs modulate the activity of platelets. This effect might influence the capacity of platelets to mediate antifungal killing and to discharge their role in the immune network, but also impact processes such as excessive inflammation and thrombosis. For that reason we performed a broad screening of antimycotic drug effects on platelets.
Methods Platelets were either mock-treated or incubated with increasing concentrations of various antimycotics. Putative activation of the platelets was tested by FACS. Fungal killing by platelets in presence or absence of the antimcyotics was investigated by calcofluor quantification of *Aspergillus fumigatus* growth and life-time microscopy. Interaction of platelets with fungal hyphae was studied by confocal microscopy.
Results The antimycotics amphotericin B and caspofungin were both capable to stimulate platelets in a time- and dose-dependent manner, as shown by increase of the activation marker CD62P on the platelet surface. In contrast, the azoles fluconazole and voriconazole as well as the echinocandins micafungin and anidulafungin did not affect platelet activity.

Furthermore, we studied a putative synergistic effect between antimycotics and platelets on *Aspergillus* killing. For that purpose, the fungus was incubated with increasing numbers of platelets in the presence of partially inhibitory concentrations of the respective drugs. It could be demonstrated that platelets alone induced already fungal killing in a ratio-dependent manner. Interestingly, the platelet effect on fungi in the presence of caspofungin, micafungin and posaconazole is ambivalent: whereas low (1:100) and high (1:4000) ratios of platelets have an additive effect on drug-induced fungal killing, the drugs even appeared to protect the fungus against intermediate platelet ratios (1:1000).

We also studied the capacity of the antimycotics to alter the platelet binding rate to the fungal surface. For that purpose, *Aspergillus fumigatus* was grown in the presence of the drugs before adding the platelets and studying the interaction microscopically. It could be demonstrated that pretreatment of the fungus with partially inhibitory posaconazole concentrations resulted in enhanced binding of the platelets.
Conclusions Various antimycotics can affect the interaction between platelets and *Aspergillus*, either by altering the fungal surface in a manner that platelets show enhanced binding, or by modulating the activity of platelets and their fungal killing. These processes might crucially influence the course of invasive aspergillosis as well as other of fungal diseases and will be further studied in the future.

P439

Profiling *Candida albicans* gene expression during human intra-abdominal candidiasis by RNA-Seq affords insights into pathogenesis and antifungal responses

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Background Profiling *C. albicans* gene expression during human infections may provide unique insights into pathogenesis and responses to antifungal treatment. Intra-abdominal candidiasis (IAC)

is suited to Candida transcriptional profiling because yeast concentrations in infected fluid are relatively high, host cells are less abundant than in tissue or bloodstream infections, and drainage is part of treatment.
Methods We used an Illumina platform to perform RNA-Seq on biliary fluid recovered from a 61 year old man with *C. albicans* cholangitis. Day 1 (D1) samples were collected from two distinct sites at time of drainage (RUQ-D1, LUQ-D1); a follow-up sample was collected from one site 24 h later, after fluconazole treatment was instituted (RUQ-D2). Differentially expressed genes were identified using EdgeR Bioconductor, and defined by ≥4-fold change and false discovery rate <0.001.
Results Fluid pH was 8.0. Cultures revealed *C. albicans* as sole pathogen; all cells were yeasts on Gram stain. 1.8 to 3.8 million reads mapped to *C. albicans* coding sequences; ≤380 000 reads mapped to human sequences. Reads mapped to ≥93% of *C. albicans* open reading frames. Only 2% of genes were differentially expressed between D1 samples. Adhesion and oxidation-reduction were processes over-represented among upregulated genes at RUQ (cholecystostomy tube) *vs.* LUQ (drain). 23% of genes were differentially expressed in D2 *vs.* D1 samples. Adhesion, filmentation, pathogenesis and ergosterol synthesis were over-represented, upregulated processes on D2. D1 (non-fluconazole) results were compared to RNA-Seq data from *in vitro*, *ex vivo*, and mouse kidney and tongue datasets. By principal component analysis, samples from given conditions clustered most tightly. Similarity to human expression was: mouse kidney, tongue>*ex vivo*>*in vitro*. Oxidation-reduction, glyoxylate pathway, fatty acid oxidation, host entry and carbohydrate transport were over-represented, upregulated processes in human IAC *vs. in vitro*. Oxidation-reduction, glyoxylate pathway, fatty acid oxidation, host entry and carbohydrate transport were over-represented, upregulated processes in human IAC *vs. ex vivo*. Adhesion, pathogenesis, filamentation and biofilm were over-represented, upregulated processes in *ex vivo vs.* human IAC. Fatty acid oxidation, osmotic stress and TCA cycle were over-represented, upregulated processes in human IAC *vs.* mouse kidney. Adhesion, pathogenesis, filamentation and biofilm were over-represented, upregulated processes in mouse kidney *vs.* human IAC.
Conclusions *C. albicans* gene expression *in vitro* or under *ex vivo* conditions is poorly representative of gene expression during human IAC. RNA-Seq is a powerful tool for transcriptional profiling of *C. albicans in vivo*, offering a snap shot of genes involved in different stages of pathogenesis and in responses to fluconazole treatment.

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Role of *Candida albicans* RLM1 in the interaction with macrophages: the impact of the carbon source

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Objectives Previously we showed that *Candida albicans RLM1* participates in the cell wall biogenesis, with the mutant rearranging its metabolic pathways to allow the use of alternative carbon sources. In this study we aimed to evaluate the effect of different carbon sources in response to cell wall damaging stress agents and in the interaction with macrophages.
Methods *Candida albicans* â^†*rlm1*/â^†*rlm1* mutant, WT and complemented strains were adapted to lactic acid or glucose, and the hypersensitivity of these cells to Congo Red, Calcofluor White and caspofungin was evaluated. Expression levels of *GPD1, AGP2, GCV2, SOU1, PUT2* and *CIT1* were also evaluated by RT-PCR in cells adapted to the different carbon sources. The cell viability and pro-inflammatory capacity was evaluated after incubation with the J774 murine macrophages cell line.
Results *Candida albicans* â^†*rlm1*/â^†*rlm1* mutant displayed phenotypes associated to cell wall deficiency, such as hypersensitivity to Congo Red and caspofungin, both in glucose- and in lactate- grown cells. However, the *Δrlm1*/*Δrlm1* mutant grown in lactic acid was slightly more resistant to Congo red, was not able to grow in the presence of SDS, and presented sensitivity to caffeine, in comparison with glucose-grown cells.

The increased transcription of genes involved in cell adhesion correlated well with adhesion and biofilm assays, in which *Δrlm1*/*Δrlm1* mutant presented greater biofilm formation than WT in cells grown in both carbon sources. However, in cells adapted to lactate biofilm formation was more pronounced. In general, lactate-grown cells were less efficiently killed in comparison to glucose-grown cells, while *Δrlm1*/*Δrlm1* mutant cells were more resistant when adapted to glucose than to lactate. The production of TNF-α and IL-10 by macrophages was lower in response to *Δrlm1*/*Δrlm1* mutant and the cellular toxicity, measured as extracellular lactate dehydrogenase activity, was significantly lower in comparison with the WT and complemented strains in glucose-grown cells. However, when *C. albicans* cells were grown on lactate the *Δrlm1*/*Δrlm1* mutant was slightly less resistant to macrophage killing but enhancing the expression of anti-inflammatory IL-10 cytokine.
Conclusion *Candida albicans* regularly colonizes niches that are poor in glucose, depending thus upon alternative carbon sources for growth. *C. albicans* cells adapted to different carbon sources behave differently, affecting important virulence parameters, such as stress resistance, adherence, biofilm formation, and infection outcome. This study reinforces evidence that assimilation of alternative carbon sources increases the yeast fitness, impacting *Candida*-host interactions.

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Immunological features in patients with asthma and fungal sensitization

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Objectives To study immunological parameters in patients with asthma and fungal allergy.
Materials and methods We observed 32 patients with asthma. Main group included 16 patients with asthma and fungal allergy (8 men and 8 women), aged 18–71 years (median–49 years). The comparing group consisted of 16 patients with asthma without fungal allergy (9 men and 7 women), aged 21–69 (median - 34 years). The control group included 12 healthy people (median - 29 years).

Allergological examination included skin prick tests with six fungal allergens, determination of total IgE level (by ELISA) and specific fungal, epidermal, household allergens (MAST-panel, Hitachi Chemicals Diagnostic). Positive skin prick-test and/or detection of specific IgE to fungal allergen in serum (level of IgE antibodies to mold allergens in serum - class ≥1) were considered as the criteria of fungal sensitization.

Production of interleukines (IL-2, IL-4, IL-10, IL-8, TNF-α, GM-CSF, IL-6) and interferon-γ (IFN-γ) was determined in peripheral blood cell supernatants after 24 h stimulation with phytohemagglutinin using Bio-Plex Pro Human Cytokine 8-Plex Panel (Bio Rad). The obtained results were statistically processed using the STATISTICA for Windows (version 6.0).
Results In the main group the highest frequency of fungal sensitization was connected with Aspergillus spp. (64%), *Penicillium* spp. - 52%, *Alternaria* spp. - 36%, *Mucor* spp. - 30%, *Cladosporium* spp. - 27%, *Rhizopus* spp. - 12%. The total IgE level ranged from 3 to 2100 U ml^{-1} (a median – 591) in this group. In the comparing group total IgE level was significantly lower – 1–693 U ml^{-1} (a median – 181), $P < 0.05$.

Production of GM-CSF was increased in all patients with asthma as compared with the control group. Strong positive correlation were found between the IL-4 levels and total IgE, GM-CSF and IFN-γ ($r = 0.59$, $r = 0.90$, $r = 0.79$ $P < 0.05$, respectively). However,

significantly higher production of IFN-γ (median 1275 (634÷2163) vs 474 (321÷527) pg ml^{-1}; $P = 0.001$) and IL-2 (median 1416 (926÷1893) vs 573 (235÷970) pg ml^{-1}; $P = 0.001$) was detected in patients with asthma and fungal allergy as against the comparing group. The levels of IL-10, TNF-α, IL-6 and IL-8 did not differ from the control values in patients of both groups.
Conclusion Immunological features in patients with asthma and fungal sensitization were increased levels of total IgE, high frequency of sensitization to *Aspergillus* spp. and increased concentrations of IL-2 and IFN-γ, which suggested the mixed type of inflammation due to fungal sensitization.

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Sph3: a glycosyl hydrolase required for biofilm formation by *Aspergillus fumigatus* and potential therapeutic agent.

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Objectives *Aspergillus fumigatus* is the second most common cause of hospital acquired fungal infections. In the infected lungs, *A. fumigatus* lives in multicellular communities encapsulated in self-produced matrices, termed biofilms. The galactosaminogalactan (GAG) polysaccharide is an important adhesin in *A. fumigatus* biofilm formation and has immunosuppressive effects *in vivo*. A cluster of genes in the *A. fumigatus* genome has been linked to GAG biosynthesis and biofilm formation. Bioinformatic analysis suggests that one of these genes, *sph3*, is a membrane bound protein that may adopt a fold similar to known glycosyl hydrolases (GHs). We hypothesized that Sph3 may play a role in the synthesis of GAG and in GAG-dependent biofilm formation.
Methods Recombinant Sph3 from *A. fumigatus* and *Aspergillus clavatus* were expressed and purified from E. coli. Structural and functional studies were carried out using the resulting proteins. A deletion mutant of Sph3 was constructed using the split marker technique. Biofilm assays were conducted in 96 well plates and the quantified with crystal violet staining. GAG quantification on hyphae was performed using fluorescent lectin staining and confocal microscopy.
Results Deletion of *sph3* resulted in a complete loss of GAG production and biofilm formation by *A. fumigatus*. Complementing this mutant with a wild-type allele of *sph3* restored GAG production and biofilm formation. Recombinant Sph3 from *A. fumigatus* (*Af*Sph3) was recalcitrant to crystallization, however, an Sph3 ortholog from *A. clavatus* (*Ac*Sph3) formed crystals that could be optimized using streak seeding. The structure was solved using selenium incorporation and the single- wavelength anomalous dispersion technique. The structure reveals a (β/α)8 barrel with similarity to GHs from families 18 and 27. Reducing sugar assays using crude GAG isolations show glycoyl hydrolase activity. Low concentrations of the recombinant hydrolase domain of Sph3 hydrolase decreased cell-associated GAG, disrupted preformed *A. fumigatus* biofilms. Introduction of a point mutation into the predicted catalytic domain of Sph3 reduced the anti-GAG and anti-biofilm activity of recombinant Sph3.
Conclusion We propose that Sph3 is a GAG membrane bound glycosyl hydrolase responsible for processing GAG during biosynthesis, and that recombinant Sph3 hydrolase domains could be developed as a novel therapeutic enzyme against biofilm- dependent *A. fumigatus* infections.

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The value of a multimodal imaging approach for the non-invasive assessment of invasive pulmonary aspergillosis in a mouse model

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Objectives Preclinical research plays an important role in gaining a better understanding of the pathogenesis of *Aspergillus* infections. Standard techniques to evaluate experimental infections in small animals are histology and fungal load quantification in tissue samples. Due to their invasive character, these techniques cannot provide us with any insight in dynamic disease processes as the available information is based on snapshot data of individual animals in a highly variable disease model. Thereby, disease severity may be underestimated due to a low sensitivity of culture techniques (1,2).

We aimed at developing a multimodal imaging approach to non-invasively investigate the pathogenesis of invasive pulmonary aspergillosis in a mouse model. The combination of non-invasive imaging techniques, such as computed tomography (CT), magnetic resonance imaging (MRI) and fibered confocal fluorescence microscopy (FCFM), were evaluated to obtain dynamic information on disease progression in individual animals.
Methods BALB/c mice were immune suppressed by intraperitoneal injections of cyclophosphamide 4 and 1 day prior to intranasal instillation of 20 μL GFP-expressing *A. fumigatus* FGSC A1258 (5.10^5 spores, $n = 5–8$) or saline ($n = 5$). The animals were scanned at baseline and daily after infection using CT (SkyScan 1076, Bruker microCT), MRI (9.4 T, Bruker Biospin) and FCFM (Cellvizio, Mauna Kea Technologies). On day 4 after infection, the lungs were isolated for validation of the imaging results by CFU counts and histology.
Results Within the lungs of infected mice, an increased attenuation was observed on the CT images and hyperintense signals were detected on the MR images (Fig 1A-B). The relative signal intensity, lesion volume and total lung volume calculated from the CT and MR images gradually increased over time, which corresponded to lung disease progression. In addition, hyphae and sporulating structures could successfully be visualized at a microscopic scale within the lungs of living animals by using *in vivo* FCFM (Fig 1C).
Conclusion We successfully visualized the development of fungal lesions within the lungs of mice infected with *A. fumigatus*. Lung CT and MR imaging techniques provided valuable insight in the global extent and 3D distribution of developing lesions, while FCFM allowed for the real-time visualization of fungal hyphae independent of surrounding inflammation and necrosis. In addition, disease onset and progression was successfully quantified in individual animals by calculating signal intensities and fungal lesion volume from the CT and MR images. By combining these complementary imaging techniques, an overall longitudinal picture can be provided about the dynamics of fungal lung infections on both a macroscopic and microscopic level in a completely non-invasive manner in individual animals.

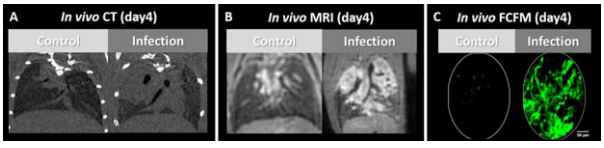

Figure 1. *In vivo* CT, MR and FCFM images of the lungs 4 days after intranasal instillation with A. fumigatus (right) or saline (left).

References

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Correction added on 19 October 2015, after online publication. Affiliation of author Liesbeth Vanherp was changed from University Hospital Leuven, Leuven, Belgium to KU Leuven, Leuven, Belgium.

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Adhesion of s and m strains of *Candida albicans* to hacat cell line

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Objectives In the frameofour study which focuses on a comparison of phenotypic and genotyping characteristics of *Candida albicans* isolates from patients with blood stream Candida infection (S strains) with isolates from patients with vaginal infection (M strains), we compared the adhesion to a human keratinocyte cell line - HACAT.

Methods *Cell Line* - The HACAT cell line was maintained in Dulbecco's Modified Eagle Medium (DMEM) at 37 °C under 5% CO_2. For the adhesion assay the cells were seeded in 96 well microtiter plates at a concentration of $2x10^5$ ml and grown for 48 hrs. to complete confluence.

Strains' maintenance, growth and FITC labelling - The *C.albicans* strains were maintained on Sabouraud's dextrose agar (SDA) at 4 °C. For the adhesion assay fresh 18 hrs. cultures grown in yeast extract broth under shaking were adjusted to a concentration of 1×10^8 ml and labelled with Fluorescein isothiocynate (FITC) at a concentration of 2.5 mM/0.5% DMSO in carbonate buffer.

Adhesion- The adhesion assay was carried out in the 96 well microtiter plates containing 2×10^4 HACATcells/well to which $1x10^7$ FITC labelled *C.albicans* yeasts were added for 2 h. at 37 °C. Assessment was done by TECAN Infinite 2000 microtiter plates reader measuring the fluorescence by an excitation and emission at a wave lengths of 480 and 530 nm, respectively.

Results We assessed 20 S and 24 M strains. As control we used the *C.albicans* strain CBS 562. Each strain was tested at least 3 times. The M strains were compared to the S strains as a group using the Student T test. The evaluation showed that M strains demonstrated higher adhesion values than the S strains, with a statistically significant difference. This observation is in contrast to our concurrent *in vivo* experiments in mice, where no such differences between virulence of the S and M strains, as expressed in survival rates and mean survival time, were noted.

Conclusions These data indicate that apparently the M strains representing mucosal infection exhibit as a major virulence attribute, higher tendency to adhere than strains from blood stream infection.

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Quantitative expression of ALS family genes of *Candida albicans* in the experimental cervical epithelial infections

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Objectives Investigate the potential interaction of vulvovaginal candidiasis isolates asymptomatic (ASS), with one episode (VVC) and recurrent VVC (RVVC), with a human vaginal epithelium and the relative expression of ALS genes family in same condition.

Methods Four strains of *Candida albicans* were used, one reference strain (ATCC 90028) and three clinical isolates (5V, 7V and 9V) obtained from female vaginal secretions. The clinical isolates were separated according to symptoms presented by the patients. To assess the adhesion ability of *C. albicans* to human epithelial cells by 2 and 6 h of incubation, was used as model *HeLa* cells. After adhesion assays, adherent *Candida albicans* cells were harvested by detaching the cervical cells monolayer to perform transcriptional analyses. The total RNA was extracted using Trizol® reagent (Invitrogen), adding to solution acid-washed glass beads (Sigma) and vortexing for 10 min with 30 second on ice. RNeasy® Mini kit (Qiagen, Germany) was used for purification. The concentration and purity of RNA was evaluated with NanoDrop (Thermo Scientific 200c). The cDNA was obtained using Superscript III RT® Supermix kit (Invitrogen), according to the kit manufacturer. For detection of *ALS* family transcripts, specific pairs primers were designed. The complete gene sequences of *ALS* genes for *C. albicans* were obtained from the GenBank database. Real-time PCR (CF × 48 Real-Time PCR System; Applied Biossystems) was used to determine the relative levels of *ALS* family mRNA transcripts, with *ACT1* (actin)and *CEF3* (translation elongation factor 3)as reference housekeeping genes for normalization. The relative quantification of *ALS* family expression was performed by the DDCt method. Each reaction was performed in triplicate and mean values of relative expression were determined for each gene.

Results The results of the adhesion assay are shown in Fig. 1. All *ALS* genes were expressed, however, the amount of *ALS* genes expressed were strain- dependent. The ASS isolate expressed the highest number of *ALS* genes and the expression was homogeneous among 2 and 6 h of adherence to cervical cells. For this strain, only *ALS2* and *ALS3* expression differed significantly between 2 and 6 h, with a decrease of 90 and 60%, respectively ($P < 0.05$). VVC isolate was also able to express a large number of adhesins, however, most of the genes were expressed in low amounts. Among these genes, *ALS3* was significantly expressed compared to *ALS6* (six-fold, $P < 0.05$). In general, RVVC isolate expressed a lowest number of adhesins. Although, the expression levels of *ALS2* and *ALS3* at 6 h were significantly higher to RVVC than ASS and VVC isolates, $P < 0.05$. Furthermore, it was possible to observe that *ALS3* increase expression over time (2 h to 6 h), significantly.

Conclusions Were observed that despite the ASS isolate, have greater adhesion capacity, showed down-expression of pathogenicity-related genes. And yet, that despite VVC and RVVC having less potential adhesion, which were able to markedly up- regulation genes involved in the pathogenicity as *ALS1* and *ALS3.*

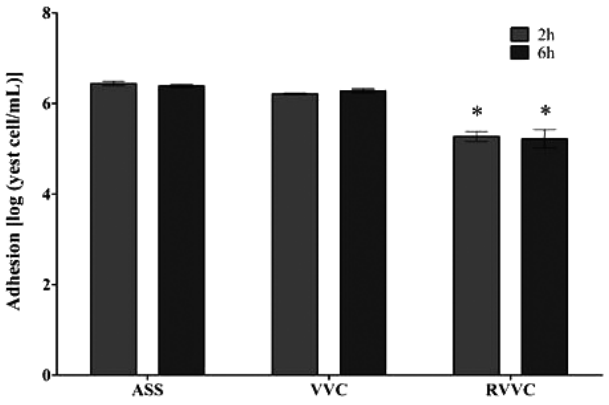

Fig. 1. Average log of CFUs of isolates of the *Candida albicans* (ASS, VVC and RVVC) obtained in adherence assays *in vitro* on cervical cells (HeLa). *p≤0.05, significant difference among RVVC isolate and the others samples tested in 2h and 6h of the adherence assy.

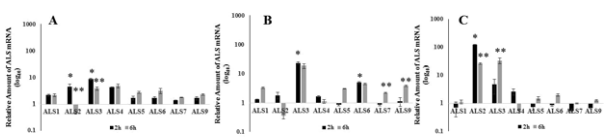

Fig 2. Gene expression fold-change values derived from qPCR data. The changes in expression of *ALS* genes of the (A) ASS isolate, (B) VVC isolate and (C) RVVC (isolate) in Hela-affected cells. The results were normalised to ACT1 and CEF3, and plotted with respect to equivalent yeast from control. The data are expressed as the mean ± SD of three separate experiments. *p≤0.05, significant difference among two and six hours, and **means higher expression among the samples analyzed

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Could the determination of *Aspergillus fumigatus* mating type have prognostic value in invasive aspergillosis?

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Objectives A clear link between mating type and virulence has been demonstrated for some fungal pathogens, but has not been established yet for *Aspergillus fumigatus*. However, some research in the field has pointed out genes associated with or linked to mating type idiomorphs could play a role in pathogenicity. In addition, a statistically significant association between mating type and invasiveness has recently been established. The main aim of this work was to search for an association between mating type and virulence in an alternative host model of infection, and also in the clinical setting.
Methods We determined mating type proportion (Mat1.1:Mat1.2) of a collection of 200 *A. fumigatus,* (150 azole susceptible and 50 azole resistant strains). In order to investigate the pathogenicity of Mat1.1 and Mat1.2 from both, azole susceptible and resistant strains, we tested 4–5 strains of each group in the *Galleria mellonella* model of infection. Moreover, we retrospectively analyzed the outcome of patients that were infected with *A. fumigatus* of each mating type.
Results The results obtained showed 48.5% of the analyzed strains were Mat1.1 and 51.5% were Mat1.2, which is in agreement with previous studies. However, we found that these percentages changed when the strain collection was divided in azole susceptible and resistant strains. The 150 azole susceptible strains kept these proportions with 44.2% Mat1.1 and 55.7% Mat1.2, but among the 50 azole resistant strains we found 60.8% Mat 1.1 and 39.2% Mat1.2.

In the infection model, strains with Mat1.1 genotype were consistently more pathogenic than strains Mat1.2, independently of their phenotype (azole susceptible or resistant). There was a significant difference ($P < 0.0009$) in median survival time between larvae infected withmating type Mat1.1 strains (3.1 days) and those ones infected with the Mat1.2 (5.1 days). In addition, when we looked at the clinical outcome associated to 25 patients infected with *A. fumigatus* strains, we found that Mat 1.1 was linked to a mortality rate of 64% while the ones associated to Mat1.2 genotype were 15%.
Conclusions (i) Our collection presents an equal strain distribution of Mat1.1/Mat1.2 as previously reported. (ii) Strains with Mat1.1 genotype were consistently more pathogenic than strains Mat1.2, indicating that differences in pathogenicity are dependent on the mating type genotype but are not related to the strain phenotypic drug susceptibility. (iii) In the clinical setting, Mat1.1 was linked to a higher mortality than Mat1.2 genotype. Altogether, it would suggest that the mating type determination at the time of the diagnosis might have a prognostic value of disease development. However, further studies would be necessary to a better understanding of the relationship between differences in pathogenicity observed in the *G. mellonella* model and the clinical outcome.

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Extra cellular enzymes activity of *Penicillium* spp

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Objective To screen for the production of important fungal enzymes (Protease, Lipase, Cellulase and ∞ - amylase) which could act as putative virulence factors among the *Penicillium* isolates and to elucidate if there is any difference in the spectrum of enzymes secreted by Penicillium marneffei and other non marneffei strains.
Methods For analysis of protease, cellulase, lipase and α-amylase, the isolates are inoculated onto skimmed milk agar, potato dextrose agar, tributyrin agar and starch agar respectively. For cellulase and lipase, 0.5% carboxymethyl cellulose and 1% tributyrin oil is added as an enzyme substrate. All the plates are incubated at 30 °C for 6 to 7 days and observe for zone of lysis around the fungal colony. Enzyme activity index was calculated for each isolate by taking ratio of lysis diameter to colony diameter as described by Blanco *et al.* (2002).
Results 51 *Penicillium* sps, comprising 23 clinical strains (including *P. marneffei*, $n = 13$) and 28 strains of environmental *Penicillium* spp. was evaluated for extra cellular enzymes activity. It was noted that no single strain of *P. marneffei* showed activity in protease and few strains of the isolates have mild activity in lipase (Table 1). However, cellulase ($n = 7$) and ∞ amylase ($n = 12$) activity were detected in significant number of *P. marneffei*. Although, the study detected non-*marneffei Penicillium* spp. with activity of cellulase and ∞ amylase, 43% of non *marneffei Penicillium* spp. exhibited to have hydrolytic activity in protease**.** Additionally, clinical isolates produced 8 different spectrum of enzymes hydrolysis of which ∞ amylase (30.4%) was commonly hydrolyzed followed by combined cellulase and ∞ amylase (21.7%). Similarly, environmental isolates could produce 12 different spectrums of extra cellular enzymes. Further, the enzyme activity index to determine the invasiveness and aggressive nature of the fungal strains showed higher index from clinical source with a value as much as 1.8 (cellulase) and 2.2 (∞ amylase) compared to environmental *Penicillium* spp. (1.3; cellulase and 2.1; ∞ amylase).
Conclusion Although *P. marneffei* showed no activity in protease, its potential in causing infection cannot be underestimated. Further, their ability to hydrolyze cellulase and ∞ amylase could be a suggestion of environmental origin.

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Lactobacillus spp. directed inhibition of *Candida albicans* in a *Galleria mellonella* infection model

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Although a naturally colonizing microbe, the fungus *Candida albicans* can be responsible for disease and health problems during weakened health or changes to dental structures. A potential means of controlling this infective microbe is through interactions with a probiotic bacteria.
Objectives Our study evaluates the inhibitory effect of *Lactobacillus* species against *C. albicans* using the greater wax moth *Galleria mellonella* as an *in vivo* infection model .
Methods We used nine clinical strains of *Lactobacillus* recovered from the oral cavity to investigate the influence to *C. albicans*. The

Table 1. Spectrum of extra cellular enzymes activity of Penicillium spp.

Table showing Spectrum of extra cellular enzymes activity of *Penicillium* spp.

Extra cellular enzymes	Clinical isolates		Environmental isolates	Total
	P. marneffei	Other *Penicillium* spp.		
α-Amylase	5	2	3	10
Cellulase	1	1	2	4
α-Amylase, lipase	1	-	2	3
Cellulase, α-amylase	4	1	1	6
Cellulase, α-amylase, lipase	2	-	2	4
Protease, cellulase, α-amylase	-	4	3	7
Protease, α-amylase	-	1	2	3
Protease, cellulase, α-amylase, lipase	-	1	7	8
Protease, α-amylase, lipase	-	-	3	3
Cellulase, lipase	-	-	1	1
Protease, lipase	-	-	1	1
No hydrolysis	-	-	1	1
Total	**13**	**10**	**27**	**51**

oral *Lactobacillus* strains were isolated from the saliva of 41 healthy patients including the following species: *L. paracasei* ($n = 5$), *L. rhamnosus* ($n = 2$) and *L. fermentum* ($n = 2$). *In vitro* analysis interrogated the effects of *Lactobacillus* on the biofilm formation. Since *C. albicans* uses biofilm formation during a lethal infection in *G. mellonella*, the changes to larvae survival were monitored during a dual infection process.

Results The *in vitro* results showed that *Lactobacillus* spp. were able to inhibit *C. albicans* biofilm formation. In the *in vivo* study, injection of *Lactobacillus* into *G. mellonella* larvae infected with *C. albicans* prolonged survival of these animals. The number of *C. albicans* CFU/mL recovered from the larval hemolymph was lower in the group inoculated with *Lactobacillus* compared to the control group. Furthermore, some groups with strains of *Lactobacillus* were able to increase the hemocyte count compared to the control group with *C. albicans*.

Conclusion *Lactobacillus* spp. inhibited *in vitro* biofilm formation by *C. albicans* and protected *G. mellonella* against experimental candidiasis *in vivo* suggesting prokaryotic-eukaryotic interactions.

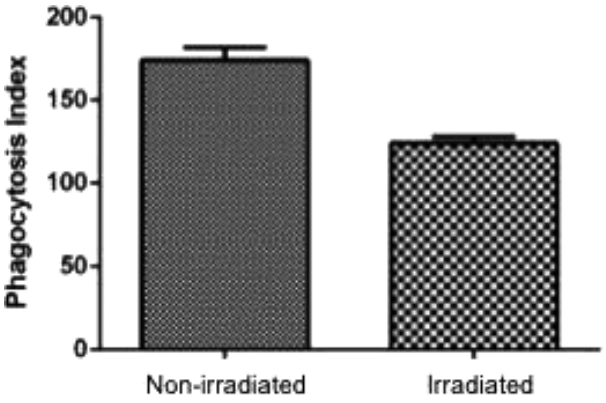

Figure 2. Phagocytosis of C. tropicalis *in vitro* by peritoneal macrophages.

P450

Candida tropicalis isolated from oral colonization of patients with head and neck cancer present changes when exposed to gamma radiation

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Candida tropicalis isolated from the oral cavity of a patient with head and neck cancer submitted to *in vitro* radiation therapy in order to simulate a procedure used in cancer patients. A culture containing $1x10^5$ yeast ml^{-1} on Sabouraud Dextrose Broth tubes were exposed to gamma radiation. The following conditions were calculated and employed: irradiated (area 9 × 26 cm) to the right and left sides with spine angle 270° and 90° respectively. The source skin distance equipment was 80 cm; target depth 6.5 cm 72.8% depth dose. The irradiation rate was 50.93 cGy/min lasting 2m25s in 40 applications of 180 cGy/day for each side, totaling 7,200 cGy. Thus, cultures were irradiate once a day, five days a week using a diary fraction dose of 180 cGy for 8 weeks. After irradiation of yeast, it was evaluated the colony morphology and phagocytosis *in vitro* using mouse peritoneal macrophages added at a ratio of 5:1 yeast to macrophages. Phagocytic indices were determined by counting with microscope analysis and the phagocytic index (PI). All experiments were done in duplicate. It was to observe that all colonies before irradiation exhibited macro morphology of annular spicules (Figure 1a and 1b) and after irradiation the colonies missed these characteristics completely, becoming smooth (Figure 1c and 1d). The PI of irradiated yeast was significantly smaller than non-irradiated yeast (Figure 2). These results suggest that gamma radiation may influence the virulence factors of yeast, making them more aggressive. Thus, it is interesting to evaluate whether the impact on increased yeast virulence *in vitro* is the same *in vivo*, since invasive fungal infections caused by C. tropicalis are emerging significantly, especially in tropical countries such as Brazil, affecting frequently in patients with neoplasia. The authors thank Sant'ana Radiation Oncology Center of Maringá for radiation procedure and Fundação Araucaria and CNPq for financial support of this study. The authors declare that they have no conflict of interest.

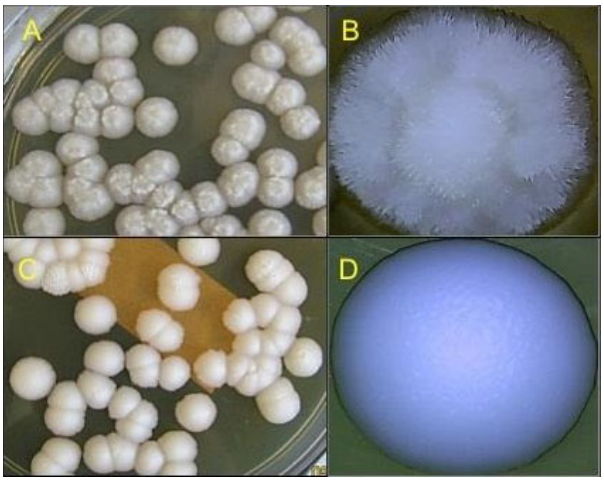

Figure 1. A e B) C. tropicalis without radiation exposure; C e D) C. tropicalis after 7,200 cGy radiation exposure.

P451

Incubation period and early events of the natural history of the acute form paracocciodioidomycosis: lessons from patients with a single *Paracoccidioides* spp. exposure

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Introduction At difference from the most prevalent, chronic form of Paracoccidioidomycosis, the acute form represents a continuum from the initial respiratory infection to full-blown disease. However, as this form of the disease generally develops in individuals living in endemic areas and thus repeatedly exposed to the fungus, it has been difficult to determine when the initial infection with *Paracoccidioides* spp. has occurred. This missing information has hindered the understanding of the first steps of natural history of this disease and the determination of its period of incubation.

Objectives and Methods Herein we describe for the first time two cases of AF PCM in patients with a single time point exposure to *Paracoccidioides* spp. in whom we were able to determine precisely the time lapsed from exposure to the fungus to the onset of signs and symptoms.

Results The two patients always lived downtown São Paulo city. The first patient went to a one-day ecological trekking and the other traveled for a weekend in a farm in São José dos Campos, both cases at the Paraiba do Sul river valley. In case 1 the initial pulmonary

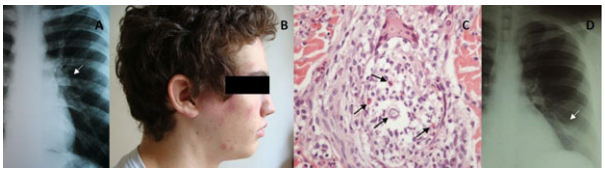

Figure 1

infection was unapparent, and after forty-one days laboratory data of an ongoing but subclinical infectious process such as marked eosinophilia and radiological chest X-ray showing normal pulmonary parenchyma but an enlargement of left peri-hilar node, compatible with deep lymphatic involvement (Fig. 1A) wasdemonstrated. The first manifestations of the AF disease appeared 4 months after *Paracoccidioides* spp. exposure, such as fever, weight loss, papular erythematous cutaneous lesion (Fig. 1B), lymph node enlargement (cervical, *supra*-clavicular, pre-auricular, axilar) and tender hepatosplenomegaly. A new chest X-ray showed normal pulmonary parenchyma and no peri-hilar enlargements. After 1 month a biopsy of a dorsal cutaneous lesion provided the diagnosis of PCM (Fig. 1C). Serum anti-*P. brasiliensis* antibodies titer was 1:16. Treatment with itraconazole was started and improvement was seen after 1 week. He was discharged and continued his treatment with sulfadiazine. The patient has been cured of PCM. In case 2, the patient presented fever, non-productive cough and night sweats 45 days after infection. Fifteen days later, she referred diffuse abdominal pain, erythematous patches on the face, trunk, and upper limbs and palpable bilateral cervical and axillar lymph nodes. The persistent cough was investigated initially with a chest X-ray, which showed a mild left lung basal infiltrate (Fig. 1D). The cough subsided but the other signs and symptoms progressed. Laboratorial results revealed eosinophilia and anemia. A cervical lymph node biopsy diagnosed PCM and serum anti-*P. brasiliensis* antibodies titer was 1:256. The patient was treated with Amphotericin B followed by itraconazole and has been cured of PCM.

Conclusion Our patients confirm that, few weeks after infection, *Paracoccidioides* spp. affect the pulmonary lymphatic system,causing no or mild to moderate self-limited symptoms, and eventually abnormalities on chest X-ray, all of which spontaneously subside. These cases provide some insights on the natural history of this mycosis, the extent of the host exposure to the fungus, and the determination of its incubation period.

P452

Intestinal mucositis in hematological malignancy patients is not a cause of elevated serum 1,3-beta-d-glucan levels

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Background In the absence of an active invasive fungal infection (IFI) serum 1,3-beta-d-glucan (BDG) may be a reasonable indicator of gut mucosal barrier impairment and microbial translocation. The aim of this study was to investigate whether serum BDG levels are elevated in hematological malignancy patients without IFI but with intestinal mucositis.

Methods We determined same day serum BDG and citrulline levels in 40 samples obtained from 23 patients with underlying hematological malignancies and without IFI/signs of pneumonia. BDG evaluation was performed at the Medical University of Graz, Austria, using the Fungitell® assay (Associates of Cape Cod, Falmouth, MA). BDG levels were interpreted as follows: <60 pg ml^{-1} negative, 60–80 pg ml^{-1} intermediate, >80 pg ml^{-1} positive.

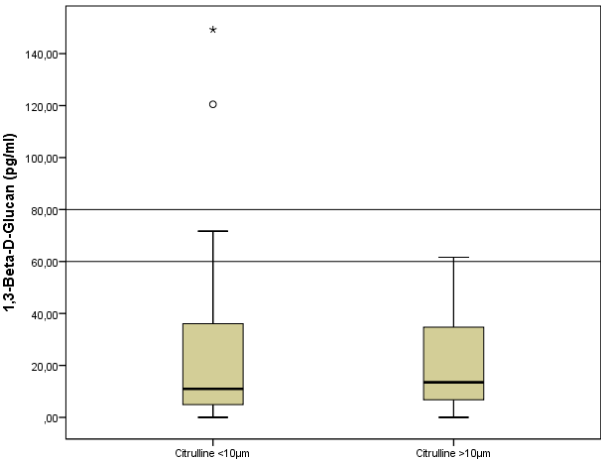

Figure 1. Serum beta-d-glucan levels according to citrulline levels

Citrulline serum levels were used as a surrogate marker for mucositis. Citrulline levels were determined at the University Hospitals Leuven, Belgium, using liquid chromatograph-tandem mass spectrometry, and interpreted as follows: <10 µm diffuse destructive mucositis, >10 µm mild mucositis or no evidence for intestinal mucositis.

Results Primary underlying disease was acute myeloid leukemia in 17 patients (73.9%), acute lymphoid leukemia in 4 patients (17.4%) and myelodysplastic syndrome in 2 patients (8.7%).

17 cases (42.5%) had citrulline levels <10 µm and 23 cases (57.5%) >10 µm.

Median BDG levels according to citrulline levels are displayed in Figure 1.

Spearman-Rho analysis did not show a correlation between serum BDG and citrulline levels ($r = -0.034$, $P = 0.833$).

Conclusion Our results suggest that intestinal mucositis is not a cause of elevated serum BDG levels in hematological malignancy patients.

P453

A morphological study on evolution of cutaneous chromoblastomicotic infection before and after therapies

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Objectives This study compared patients with elevated lesions of chromoblastomycosis and patients with flat lesions evaluating the morphological components of biopsies in cases with regular follow-up before and after treatments.

Methods: Historical cohort study with before and after type of analysis without control group.

Results The study included 67 patients who were divided into two groups, according to the clinical course of disease and to the type of dermatological lesion: 24 of them had flat and 43 had elevated lesions, biopsied before and after therapies. "Bad responders"were found in 95% of cases with elevated lesions. "Good responders"were found in 92% of cases with flat lesions. Patients with elevated lesions had persistence of high intensity of the histopathological findings associated with a unsatisfactory response to therapy and those with flat lesions showed more decreasing of the intensity of this findings (with significant statistical analisys to all elements through odds ratio

and $P = 0.01$) with a satisfactory response to therapies. After treatments the relative risk of high intensity of these components in the elevated lesions were higher than those of flat lesions ($P < 0.001$).
Conclusion The high and low intensity of the histopathological elements in association with the type of lesion denoted the presence of polarity of the granulomas in this disease either before or after therapies. The pathogenic events may be better understood and its histopathology findings better associated with clinical aspects. Little is known regarding the pattern of histopathological aspects in flat and elevated lesions before and after therapies. The cutaneous lesion presenting as a verrucous plaque had a granulomatous reaction with a suppurative granuloma (with high intensity of the elements) with several fungi cells. It was suggested that patients with this type of lesion have a type Th2 immunological response ("bad response"to therapy), while in erythematous atrophic plaque had a granulomatous reaction less supurative resembling a tuberculoid granuloma (low intensity of the elements) with few fungi cells and a type Th1 response ("good response"to therapy). These findings confirm the hypotheses that is a polar disease relating either to polar granuloma or polar lesions respectively.

P460

Pharmacokinetic/pharmacodynamic simulation of anidulafungin in an *in vitro* model against *Candida parapsilosis* clade

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Objectives To simulate the expected time-kill curves of anidulafungin for *Candida parapsilosis*, *Candida metapsilosis* and *Candida orthopsilosis* using a previously developed mathematical E_{max} sigmoid model and human pharmacokinetic data for a typical dosing regimen.
Methods Three reference strains were included in this study, *C. parapsilosis* ATCC 22019, *C. metapsilosis* ATCC 96143 and *C. orthopsilosis* ATCC 96139. Time-kill curve analysis and mathematical modelling of the time-kill curve data were performed using a nonlinear mixed-effect approach as appropriate with NONMEM 7 (ICON Development solutions, USA). A previously described adapted E_{max} model [1] was tried to fit to the log-transformed data of the static time-kill curve experiments of anidulafungin. Simulations of the expected time-kill curves for each isolate were made using the estimated pharmacodynamic parameters, from the E_{max} model, and human pharmacokinetic data obtained from *in vivo* pharmacokinetic parameters of anidulafungin ($t_{1/2}$ = 25.6 h; V*d* = 33.4 L) [2]. Phoenix WinNonlin (Certara, USA) software program was used to simulate plasma concentration-time profiles for recommended multiple intravenous dosing regimen of anidulafungin (loading dose/maintenance dose, 200/100 mg with a dosing interval of 24 h up to 6 days) and then applied to the adapted E_{max} model to simulate *in vivo* time-kill curves.
Results The results of the simulations showed that anidulafungin at the selected dosing schedule would be effective in a species-dependent way at easily achievable serum levels. A rapid fungicidal activity would be exerted against *C. metapsilosis* ATCC 96143. Similarly, against *C. orthopsilosis* ATCC 96139, anidulafungin would be fungicidal. A prolonged fungistatic activity against *C. parapsilosis* ATCC 22019 was observed, with reductions from starting inocula that were predicted to exceed 1 log.
Conclusion The mathematical E_{max} sigmoid model can be used to simulate expected time-kill data for typical anidulafungin dosing regimen. Our approach of combining *in vitro* time-kill data with existing *in vivo* pharmacokinetic data, might serve in the future to define optimal antifungal regimens against candidiasis.
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P461

Posaconazole Pharmacokinetics among Lung Transplant Recipients

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Background Posaconazole (POS) is a promising agent for prevention and treatment of invasive fungal infections among lung transplant recipients (LTR), but there are very limited pharmacokinetic (PK) data in this population. We previously showed that POS trough levels >0.5 are associated with successful outcomes following LT. We hypothesized that LTR, in particular those with cystic fibrosis (CF), would have difficulty achieving PK targets.
Methods We conducted a prospective study of POS oral suspension PK among LTR with or without CF. Steady-state (SS; after a minimum of 5 days of therapy) blood samples were obtained at 0, 2, 4, 6, 8, and 12 h after oral administration. POS levels were determined by a validated HPLC with fluorescence detection assay; calibration curves were linear over 0.02–3 μg ml^{-1}. Maximum, minimum, and average plasma concentrations were calculated (Cmax, Cmin, Cavg). Area under the curve (AUC) was determined from time 0 to 12 h.
Results 20 LTR were enrolled (7 with CF). 60% and 85% were men and white, respectively. POS doses ranged from 600 - 1200 mg day^{-1}. There was no correlation between dose and level. Median age and weight were lower for CF pts (36 yrs and 57 kg) compared to non-CF pts (61 yrs and 78 kg). Mean time to Cmax was 4.7 h. Only 20% of pts had Cavg levels >0.5 μg mL^{-1}. Mean (standard deviation) Cmax (0.4 [±0.3]), Cmin (0.3[±0.2]), Cavg (0.3 [±0.3], and AUC (3.7 [±3.2]) were lower among CF pts than non-CF pts (1 [±0.8], 0.6 [±0.5], 0.7 [±0.6], and 8.6 [±7.4], respectively) (P = 0.04, 0.05, 0.03, and 0.03, respectively). Mean oral clearance (L h kg^{-1}) was 18× higher among CF pts (10.6 ± 18) versus non-CF pts (0.6 ± 0.4; P = 0.03). After dose normalization, mean POS AUC for CF pts was decreased by 57% compared to non-CF pts (P = 0.06). Trough levels were well correlated with AUC (R^2=0.95, $P < 0.0001$); however, significant inter-patient variability was observed for both CF and non-CF pts.
Conclusions POS levels are suboptimal among LTR, particularly those with CF. Trough levels are representative of SS AUC and ideal for therapeutic drug monitoring. Given the challenging PK of POS oral suspension in this population, there is a pressing need to study PK of new formulations.

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Voriconazole blood levels and its major metabolite, voriconazole-N-oxide: Utility on Therapeutic Drug Monitoring

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Background Voriconazole is considered the first-line agent in the treatment of invasive aspergillosis and one of the best positioned alternatives to handle emerging fungal infection with high resistance profiles (*Scedosporium*, *Fusarium* ...). However, certain peculiarities

such as nonlinear pharmacokinetic profile are reflected in large variability in exposure and therefore in difficulties in adjusting the effective dose. One of the mechanisms responsible for such variability is related to their hepatic metabolism conditioned by the presence of concomitant therapy and individual genetic factors. Primary route of metabolism involves oxidation of N-fluoropyrimidine ring leading to the formation of major metabolite, N-oxide VRC (VRC-NO) which has lower activity. The evaluation of the relationship [metabolite/active drug] while monitoring blood concentration is proposed as a useful strategy to predict metabolic profile and individually estimate the drug exposure. This strategy might help to maximize the effectiveness of this antifungal treatment.

Objective The objective of this study is firstly, to develop and validate a chromatographic method to detect and quantify VRC and its major metabolite in serum samples, valid for estimating individual metabolic rate. Secondly, to describe the metabolic rate from serum samples received in our department to monitor voriconazole concentrations.

Method A new high-pressure liquid chromatography assay was developed using a stepwise gradient elution profile. The proposed method enables the simultaneous quantification of VRC and its metabolite in 150μL serum aliquots, after a first step of protein precipitation and direct injection of resulting supernatant. The analytical run lasting <16 min per sample. For the validation procedure linearity, accuracy and precision parameters were determined.

Results The validation procedure establishes that the method was linear between concentrations ranging from 0.125 and 8 mg l^{-1} for both components (analytical range). The correlation coefficient was higher than 0.9. The retention time for VRC-NO and VRC was 7.3 ± 0.06 and 9.3 ± 0.14 min, respectively. In 42% of the samples evaluated voriconazole concentration remained at therapeutic levels (1–5.5 mg l^{-1}, average 2.6 mg l^{-1}). The metabolic rate in these samples remained at values close to 1 (average 1.29). 50% of the samples showed subtherapeutic levels (<1 mg l^{-1}, mean 0.31 mg l^{-1}). For these samples the average metabolic rate exceeded the value of 1 (mean 6.65). The remaining samples showed voriconazole levels above the therapeutic value (> 5.5 mg l^{-1}), and significantly lower metabolic rate (mean 0.43).

Conclusions 1) The method proposed allows the simultaneous quantification of VRC and its major metabolite VRC-NO in serum samples. 2) The estimation of the metabolite/active drug relationship could quickly detect patients with impaired voriconazole metabolism.

P463

Evaluation of the methodological approach for therapeutic drug monitoring of prophylactic posaconazole in haematology patients

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Objectives Although tablet and IV formulations of posaconazole are now available, the oral suspension is still widely used and the systemic exposure of this formulation is significantly affected by acid, fat and food. It has a long half-life and levels reach steady state towards the end of the first week of use. A recommended target for prophylaxis is a trough level of 0.7 mg l^{-1}. It is metabolized mainly in the liver, interacting with several drugs that use similar pathways.

The aims of the study were: to analyse intra and inter-day stability and assess whether a unique daily specimen could be used for TDM thus facilitating and lowering the cost of the procedure; to determine if a bioassay and an HPLC procedure produce similar results to allow use of whichever method is available; to reveal if there is an influence on the hepatic and renal function due to the use of the drug and if there are differences in patients undergoing bone marrow transplantation or in patients taking cyclosporine.

Methods Thirty haematology patients under prophylactic treatment with posaconazole as mono-therapy (600 mg d^{-1} in three divided doses) were enrolled after written consent and record of all their relevant medical details. Serum specimens were collected at the end of the first and the second week of treatment, just before the morning dose, 2 h and 6 to 8 h afterwards. Levels were measured with a validated bioassay in 157 specimens and with HPLC in 51 of them. ALT, AST, ALP, bilirubin, γ-GT, urea and creatinine were measured on each occasion. Analysis was done with non-parametric methodology.

Results All inter and intra daily posaconazole levels were correlated ($r > 0.8$, $P < 0.05$), and there was not a statistical difference between them irrespective of the timing ($P > 0.05$). Interestingly, there was a slight decrease at the 2 h post dose specimens, although not statistically significant. The same results were found with HPLC measurements. There was not a statistical difference ($P = 0.056$) between bioassay (1.59 ± 1.03 mg l^{-1}) and HPLC levels (1.24 ± 0.80 mg l^{-1}), while they were significantly correlated ($r = 0.86$, $P = 0.000$). There was no inter-day variation of the hepatic or renal function markers which were within the reference ranges, nor a correlation with posaconazole levels, while the levels of the drug were similar, independently of the cyclosporine or other immunosuppressive regimen and previous bone marrow transplantation or not.

Conclusions Thus, it appears that in clinically stabilized patients a random specimen on any day after steady state serum concentrations have been achieved is adequate in order to monitor posaconazole levels. Moreover, when the drug is used as monotherapy a bioassay is an acceptable, alternative to the HPLC. The drug was well and safely tolerated in all thirty patients in this study, there was no breakthrough fungal infection during treatment and the levels were not affected by the use of cyclosporine or other immunosuppressive drugs or previous bone marrow transplantation.

P464

Drug interaction profiles of isavuconazole, voriconazole and posaconazole with immunosuppressants metabolized by CYP4503A4 (CYP3A4)

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Objectives Isavuconazole is the active moiety of isavuconazonium sulfate, a water-soluble prodrug for oral and intravenous administration. Isavuconazole is a broad-spectrum triazole with *in vitro* activity against clinically relevant pathogens including *Aspergillus* spp., *Candida* spp., *Cryptococcus* spp., and Mucorales.

This makes isavuconazole potentially useful to treat patients with invasive mould disease (IMD) including those receiving immunosuppressants (bone marrow/solid organ transplant recipients), at risk of renal/hepatic impairment and with an increased potential for drug-drug interactions (DDIs) due to multiple co-medications.

Isavuconazole shows predictable pharmacokinetics[i], high oral bioavailability[i] and no food effect[ii]. Isavuconazole is a moderate CYP3A4 inhibitor. Unlike voriconazole, isavuconazole does not inhibit CYP2C9 or CYP2C19. A review of clinical pharmacology information was therefore performed to compare the potential for DDIs between immunosuppressants metabolized by CYP3A4 and the triazoles isavuconazole, voriconazole, or posaconazole.

Methods A review of US and EU prescribing information was conducted to identify clinical pharmacology information related to multiple doses posaconazole or voriconazole on co-administration with midazolam (typical CYP3A4 probe substrate for clinical DDI studies) and immunosuppressants metabolized by CYP3A4. A literature

Table 1

CYP3A4 substrate (oral administration)	n-fold increase in the AUC of midazolam or immunosuppressants (CYP3A4 substrates) by the respective triazole		
	Isavuconazole (capsule)	Posaconazole (oral suspension)	Voriconazole (tablet)
Midazolam[1]	↑ 2[iii]	↑ 5*	↑ 10[v]
Ciclosporin	↑ 1.3[x1,a]	↑[2]	↑ 1.7*[3,b]
Sirolimus[1]	↑ 2[vi]	↑ 9*	↑ 11*
Tacrolimus[1]	↑ 2.3[vii]	↑4.6*,[viii]	↑3*

[1]US prescribing information and/or EU Summary of Product Characteristics for POS (Jun/Apr 2014) and VRC (Jan/Aug 2014)
[1]healthy subjects; [2] increased trough concentrations (not quantified) in heart-transplant patients ; [3]renal transplant patients
[a]single dose ciclosporin in subjects after 4d ISA; [b]chronic ciclosporin treatment in patients receiving 8d VRC;

search was also performed to obtain missing information. Clinical pharmacology information for isavuconazole was derived from published abstracts. The effects of isavuconazole, voriconazole or posaconazole on the exposure of the respective CYP3A4 substrates - expressed as area under the curve (AUC) - were compared.

Results The AUC of oral midazolam (probe substrate for CYP3A4) was increased 2-fold with isavuconazole, 5-fold with posaconazole and 10-fold with voriconazole. Results with immunosuppressants metabolized by CYP3A4 are presented in the table below.

Conclusion Considering midazolam probe substrate, isavuconazole exhibits moderate inhibition of CYP3A4 (≥2 and <5 -fold), whereas posaconazole and voriconazole are strong CYP3A4 inhibitors (≥5-fold). Clinical pharmacology studies demonstrate that the effect of isavuconazole on CYP3A4 substrates is substantially less pronounced than posaconazole or voriconazole. The clinical pharmacology profile indicates potential advantages of isavuconazole in treating patients receiving immunosuppressants metabolized by CYP3A4.

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ASCPT: American Society for Clinical Pharmacology & Therapeutics; ICAAC: Interscience Conference on Antimicrobial Agents & Chemotherapy.

(Encore abstract: Previously presented at the European Congress of Clinical Microbiology and Infectious Disease [ECCMID], Copenhagen, Denmark; April 25–28, 2015; P0216)

P465

Effect of NO-aspirin on susceptibility to fluconazole in *Candida albicans* clinical isolates obtained from denture stomatitis patients

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Candida albicans form biofilms on diverse biomaterials and is frequently present in denture prosthesis participating on developing of denture stomatitis, one of the most common oral pathology in elderly people. In a large percentage of denture stomatitis patients, *C. albicans* biofilms function as an infection reservoir that in susceptible individuals could lead to invasive candidiasis. Biofilms presence is crucial for their persistence in the host, acting as a multidrug resistance mechanism to antifungals. Because of the high resistance of *C. albicans* biofilms to antifungals, new pharmacological strategies to treat these infections are needed. Non-steroidal anti-inflammatory drugs (NSAIDs) have antibiofilm activity on *C. albicans*. On the other hand delivery of exogenous NO through a NO donor molecule, has shown to inhibit *C. albicans* biofilms formation and to potentiate the effect of conventional antifungals. NO releasing aspirin (NO-ASA), NCX-4040 has been proposed for treatment of different cardiovascular affections and inflammatory conditions, since maintain the anti-inflammatory effects of aspirin, besides the beneficial effects of NO, such as endothelial protection. According to this information, in this work we evaluated the antifungal/antibiofilm effect of NO-ASA on *Candida albicans* clinical isolates obtained from denture stomatitis patients.

Objectives 1) characterization clinical isolates of *Candida spp.* obtained from oral mucosa in denture stomatitis patients; 2) evaluate susceptibility of clinical *Candida spp.* strains to fluconazole, NO-ASA and their combination.

Methods *Candida spp.* obtained from oral mucosa of denture stomatitis patients ($N = 60$), were identified through CHROMagar *Candida* Medium BD™ and by sequencing the ITS1-5.8S rDNA-ITS2 region using the primers ITS1 and ITS4. To evaluate antifungal susceptibility, isolated strains were standardized to 0.5 McFarland and then grown on sabouraud agar plates and disk diffusion tests were performed in presence or absence of fluconazol, NO-ASA and their combination.

Results from the 60 strains, 55% ($N = 33$) were identified as *C. albicans* while 27% corresponded to *C. tropicalis* ($n = 16$) and remaining 18% was identified as *C. glabrata* ($n = 18$). According to susceptibility assays 6 strains of *C. albicans* were classified as resistant to fluconazole (10%) and the rest as susceptible. NO-ASA had no antifungal effect on any of the strains assayed. Addition of 25 ug of NO-ASA to fluconazole standard disks (25 ug) resulted in marginal increase in susceptibility, ranging from 6 to 10% increase. In resistant strains however, NO-ASA was able to increase the effect of fluconazole in 20–30% ($P < 0.05$, ANOVA), reversing the resistant behavior of those strains.

Conclusions NO-ASA was able to potentiate antifungal effect of Fluconazole in resistant strains obtained from denture stomatitis patients. Since NO-ASA has no antifungal effect, we hypothesize that prostaglandins synthesis inhibition or NO releasing effect alters drug resistance mechanisms. We are currently working to elucidate that postulate.

P470

Immunoreactive proteins of *Lichtheimia corymbifera* for farmer's lung serodiagnosis.

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Objective *Lichthteimia corymbifera* has been described as one of the main causative agents of farmer's lung disease (FLD). Serodiagnosis is currently routinely performed by immunoassays with crude antigens. Serodiagnosis is based on immunoprecipitation techniques with homemade crude antigens and is not standardized. The objectives of this study were to identify immunogenic proteins from a strain of *L. corymbifera* isolated from hay, and to synthesize the corresponding recombinant antigens for the development of a standardized ELISA test.

Methods An immunoproteomic approach based on comparative western blot analysis with serum samples from FLD patients and healthy exposed controls (HEC) was used to identify FLD specific proteins.

We recruited 41 FLD patients and 43 HEC from five university hospital pneumology departments in France and Switzerland. *L. corymbifera* proteins were extracted from the reference strain BBCM/IHEM 3809 isolated from FLD-linked hay. Proteins were separated by two-dimensional electrophoresis and subjected to western blotting, with sera from FLD patients ($n = 7$) or controls ($n = 9$). FLD-specific proteins were identified by mass spectrometry (LC-MS/MS) and were produced as recombinant antigens as previously described[1]. The diagnostic performance of ELISA tests using the recombinant antigens was assessed with all the sera from FLD patients and controls.

Result When compared western blot membranes revealed by FLD and HEC serum, 25 spots were considered as FLD specific. The 25 FLD specific spots were cut from the gel and analyzed by LC-MS/MS. Sixty-nine different proteins were identified from the 25 spots. Six proteins were selected to be produced as recombinant antigens: acylCoAdehydrogenase, proteasome alpha, pyruvate kinase, malate dehydrogenase, ATP synthase alpha, dihydrolipoyl dehydrogenase.

ELISA tests were performed using each recombinant antigen, with all the sera from FLD patients ($n = 41$) and controls ($n = 43$). Dihydrolipoyl dehydrogenase was the most effective recombinant antigens for discriminating FLD patients from controls, with AUC = 0.82 and with sensitivity and specificity of 81% and 77%, respectively. ELISA using proteasome a also showed AUC above 0.80, with sensitivity of 88%, but sensitivity was only 65%.

Conclusion Involvement of *L. corymbifera* in FLD has been described mainly in East of France and Finland. Combining recombinant antigens from *L corymbifera* with recombinant antigens from other micro-organisms (*Saccharopolyspora rectivirgula*, *Aspergillus*) involved in FLD would be probably helpful to produce a standardized ELISA kit effective for diagnosing FLD whatever the geographic location of the patient. A prospective study, using such a test combining most effective recombinant antigens from *S rectivirgula*[1]and from *Aspergillus2* with dihydrolipoyl dehydrogenase and proteasome alpha from *L. corymbifera,* is ongoing in our lab to assess diagnosis performance with patients from different geographic origins.

1. Barrera C, Millon L, et al. Immunoreactive proteins of *Saccharopolyspora rectivirgula* for farmer's lung serodiagnosis Proteomics Clin Appl. 2014: 8(11–12):971–81

2. Millon, Roussel et al. *Aspergillus* recombinant antigens for serodiagnosis of farmer's lung disease J Allergy Clin Immunol, 2012, 130(3):803–805.

P471

Variation in the polysaccharide capsule size interferes with identification of *Cryptococcus neoformans* and *C. gattii* by MALDI-TOF mass spectrometry

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Objectives to investigate the interference of *Cryptococcus*'s capsule size in MALDI-TOF MS species identification.

Methods four *C. neoformans* reference strains, WM 148 (VNI), WM 626 (VNII), WM 628 (VNIII), WM 629 (VNIV), and four *C. gattii*, WM 179 (VGI), WM 178 (VGII), WM 161 (VGIII), and WM 779 (VGIV) were used for capsule growth experiments. Initially the yeast cells of each strain were incubated overnight in acid Sabouraud broth at 30 °C with shaking. After centrifugation, the pellets were incubated in the capsule growth inducing medium (Sabouraud broth diluted 10 times, pH 7.3) at 37 °C with shaking. The capsule induction was carried out replacing medium every 48 h during a period of 20 days. To reduce the size of the capsules, the yeast cells were then seeded in acid Sabouraud agar plus 2.9% NaCl, and incubated at 30 °C for 48 h. For each strain, the capsule size was measured in light microscope using the software Axiovision. At least ten different fields of the slides were randomly chosen and 40 to 50 capsule cells were measured. Subsequently, the strains were analyzed by MALDI-TOF mass spectrometry after standard extraction protocol with ethanol 70% and formic acid 70%. Then, the supernatants were placed in MALDI target plate wells, dried at room temperature and overlaid with the matrix containing a saturated solution of HCCA. Mass spectra (MS) were generated by the Autoflex MALDI-TOF mass spectrometer and compared to main spectra of both *C. neoformans* and *C. gattii* from the database Biotyper 3.1. To ensure the reproducibility of spectra each strain was tested in quadruplicate in eight different experiments. Results were expressed as log score of 1.700–1.999 indicating a probable genus identification and log score above 2.000 indicating secure genus and species identification.

Results the strains of *C. gattii* developed larger capsule sizes in comparison with *C. neoformans* strains (mean 16.8 μm × 5.6 μm, $P = 0.002$) when incubated in the induction medium. Capsule size was inversely related to MS quality. All replicates with capsule size above 10 μm had unreliable species identification. The mean capsule size of *Cryptococcus* strains with correct species identification was 2.18 μm, whereas for those assigned with log score below 2.000 was 5.77 μm ($P = 0.03$).

Conclusion The capsule of *Cryptococcus* negatively interfered with the performance of MALDI-TOF MS species identification. Capsule size reduction is recommended to achieve a reliable laboratory identification of *Cryptococcus* isolates by this technology.

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Dermatophyte infections in the Lisbon and Tagus valley

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Objective A retrospective study was done in the Portuguese National Institute of Health, in order to establish the prevalence and characterize dermatophytic infections within the NUTSII region of Lisbon and Tagus Valley.

Material and methods This retrospective study included 4193 biological samples from patients with medical suspicion of fungal infection, collected from 2004 to 2013. Samples were obtained by extracting hairs and scraping skin and nails using a sterile curette over the affected areas. Samples were microscopically examined after 30% W/V for 20 min potassium hydroxide preparation and cultured on Sabouraud dextrose agar with cicloheximide. Species were identified by the observation of morphological features that included colony pigmentation, texture, growth rate and distinctive microscopic structures. Physiological tests (urea, vitamin and amino acid test agars) were used whenever necessary.

Results The average frequency of dermatophyte infections was 21%, ranging from 18% to 26%; 841 individuals (425 female and 410 male) had positive cultures. *Tinea capitis* was confirmed in 236 (28%) patients and was more prevalent in children from the group of 1–9 years old. In scalp dermatomycosis, *Microsporum audouinii* was the most frequently isolated species ($N = 120$, 51%), followed by *T. soudanense* ($N = 44$, 19%). Males were more affected (58%) than females (42%).

Onychomycosis caused by dermatophytes were confirmed in 385 cases (46%). Other fungi recognized as cause of onychomycosis were not considered for this report. Skin samples were positive in 220 cases (26%).

The most prevalent dermatophytes isolated in nail and skin samples were *T. rubrum* (206 and 93 isolates, respectively) and *T. mentagrophytes* with (49 and 39 isolates respectively).

E. floccosum was the species less frequently found in skin samples (1%).

Conclusion The frequency of infections by dermatophytes has revealed itself steady for the last 10 years. The spectrum of dermatophytes is similar to those reported in other studies for *tinea corporis* and onychomycosis, being *T. rubrum* the main aetiological agent.

However, our results differed from several studies that describe a raise, during the recent years, in the number of cases of *tinea capitis* caused by *M. canis*, especially in other Mediterranean countries. Our results showed a high prevalence of anthropophilic species (*M. audouinii* and *T. soudanense)* in the region of Lisbon and Tagus valley, where the number of foreign citizens from African countries is higher.

Like in other studies the number of infections by *E. floccosum* was very low suggesting that there was a replacement of this aetiological agent by other dermatophytes like *T. rubrum*.

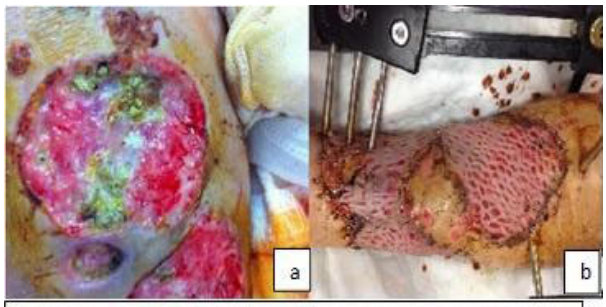

Figure 1a; Before treatment, 1b; After treatment

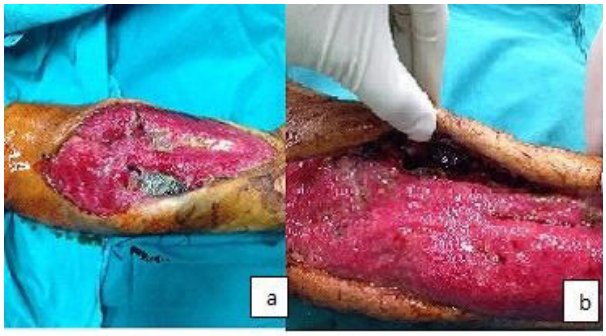

Figure 2a; Before treatment, 1b; After treatment

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In treatment of primary cutaneous Aspergillosis, systemic antifungal therapy and surgical management; Presentation of two cases

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Objectives Aspergillus skin involvement occurs by hematogenous spread or local inoculation. Primary cutaneous aspergillosis that appears with local inoculation is rarely determined on immunocompetent patient. However, it is an important cause of morbidity and mortality in surgical patients. Treatment is controversial and different medical and/or surgical approaches can be applied. Here, two cases of primary cutaneous aspergillosis diagnosed with the growth of *Aspergillus flavus* on tissue samples are presented. In the treatment of patients, voriconazole and surgical treatment was applied together. Different methods were used for the wound care.

Methods Patient 1: 53 years old female, who had a motorcycle accident resulted with left leg injury. She had a wound with a large tissue defect on her left cruris with tibia and fibula fracture. Together with fracture treatment repeated surgical debridement were applied on her wounds that had wide tissue defects. But in 3 weeks of surgical treatment due to the appearance of infected necrotic tissue, the wounds were debrided again for the tissue cultures. *A. flavus* has grown in culture. For the distinction of colonisation and infection, tissue cultures were repeated for twice. *A. flavus* has also grown in both. Fungal spores and hyphae structure branching with narrow angle were demonstrated. Because of having necrotic areas with the continuing infected appearance and growing of *A. flavus* in 3 consequent tissue cultures she was treated with recurrent surgical debridement and iv voriconazole. Antibacterial absorbent polymer dressing - Sorbact absorption dressing; SB was also used for the wound care. After third week of the treatment there was no fungus growth in cultures. Reconstruction was made with the skin graft taken from lateral side of right thigh. Voriconazole therapy was completed in 12 weeks (Figure 1).

Patient 2: 39-years old male patient applied to emergency service with burn and carbon monoxide intoxication. Because of the development of right forearm, right thigh and cruris compartment syndrome, fasciotomies were performed for both extremities. He stayed in ICU for 16 days. Infected necrotic wounds were debrided for twice. *A. flavus* has grown in the tissue culture. He was treated with voriconazole and tigecycline. Vacuum-assisted closure (VAC) therapy was used for wound care. There was not any growth in the tissue cultures taken in the second week of the antifungal treatment. Following negativity in the tissue cultures, wound was primarily sutured. He was treated successfully with voriconazole for 8 weeks (Figure 2).

Discussion Primary cutaneous aspergillosis may develop at intravenous catheter insertion sites and under adhesive tape dressings and bandages or in cases whose skin integrity is impared. Both of the reported cases are primary cutaneous aspergillosis. It has been thought that slow progressive massive necrosis might be guiding for the opportunistic fungus infection in both cases. In treatment of cases, repeated debridement/ different wound care and systemic voriconazole treatment was administered together. In slow but progressively destructive post-traumatic wound infections, primary cutaneous aspergillosis should be considered in differential diagnosis.

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Fusarium solani isolated from patients with onychomycosis: nail infection potential and biofilm formation ability

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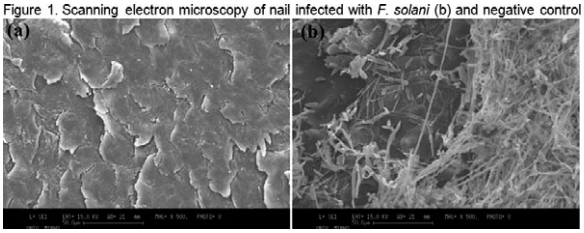
Objective The aim of the present study was to perform an epidemiological study, evaluate the ability of *Fusarium solani* to use the human nail as a single source of nutrients and its potential for biofilm formation.

Methods We first performed an epidemiological study to determine the frequency of *F. solani* in patients with onychomycosis. The study included data from all patients who attended the Teaching and Research Laboratory of Clinical Analysis (LEPAC), Division of Mycology, Universidade Estadual de Maringá (UEM), between January and December 2013 with suspected onychomycosis. This descriptive, retrospective, cross-sectional, observational study was

Figure 1. Scanning electron microscopy of nail infected with *F. solani* (b) and negative control (a)



performed in Maringá, Paraná, Brazil, and approved by the Research Ethics Committee of UEM (approval no. 615.643/2014). To *in vitro* study, it was randomly chosen one clinical isolate of *F. solani* that was subcultured on Potato Dextrose Agar for 7 days at 25 °C. *In vitro* nail infection was performed with 5×10^4 conidia ml^{-1} in 0.85% sterile saline on sterilized nail fragments (collected from healthy female volunteer) and incubated for 30 days at 25 °C. After incubation, nail fragments were clarified with 40% KOH and observed under a light microscope. Biofilm formation was carried out on nails containing a cellular suspension of $1x10^6$ conidia ml^{-1} in Sabouraud Dextrose Broth and incubated for 3 days at 37 °C. The ability of *F. solani* to form biofilm on nail was evaluated by scanning electron microscopy.

Results It was possible to observe that in the period of January to December 2013, 695 patients were attended in the LEPAC-UEM with clinical suspicion of dermatomycoses. Of these, onychomycosis was confirmed by culture in 131 clinical samples. *Fusarium* spp. were isolated in 26 (19.8%) cases of onychomycosis, and it was the most isolated among nondermatophyte molds. *F. solani* was the most frequently (48%) isolated species. Patients with onychomycosis caused by this species had an average age of 47 ± 9 years, moreover women were more frequently infected than men. Concerning *in vitro* nail infection, the growth was observed in 3 days and microscopic analysis revealed evidence of hyaline, thick and septate hyphae in the entire nail, chlamydospores, and microconidia. With regard to the ability to form biofilm on nail, *F. solani* showed great potential of biofilm formation (Figure 1).

Conclusion The frequency of onychomycosis caused by *Fusarium* spp. in our region is high and *F. solani* is able to use nail as a single source of nutrient and it has the potential to invade the nail by itself. Moreover, *F. solani* was able to form biofilm on nail. Thus, the model of nail infection proposed herein may be useful for future studies on the pathogenesis of onychomycosis.

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Clinico-mycological and antifungal susceptibility profile of tinea corporis and tinea cruris cases in India

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Objectives The study was carried out to know the clinico-mycological profile and antifungal susceptibility profile of tinea corporis and tinea cruris cases.

Methods Skin scrapings were collected from the margins of the lesions and a 10% KOH mount was prepared. Various dermatophyte species were tested for antifungal susceptibility to antifungal agents viz. fluconazole, voriconazole, itraconazole and ketoconazole, as per protocols of the Clinical and Laboratory Standards Institute (CLSI M38-A) for molds by broth-microdilution and E-strip methods.

Results A total of 122 patients consented to participate in the study. Samples of 68 patients revealed fungal hyphae in the KOH mount and revealed growth on culture. Among these, 64 were dermatophytes. Of these patients, 45 were males and 19 were females. Seven were clinically diagnosed as tinea cruris, as 39 were affected by tinea corporis and 18 had both. Amongst the dermatophytes, 14 were *T. rubrum*, 18 were *T.* mentagrophytes, 3 were *T. tonsurans*, 2 were *T. verrucosum*, while 16 isolates belonged to other Trichophyton species. Five isolates were identified as *Microsporum gypseum*, 1 was *M. distortum*, 3 was *M. canis* and 2 were other *Microsporum* species. The minimum inhibitory concentration (MIC) ranged from 0.313–32.0 mcg ml^{-1} for fluconazole, 0.313–0.5 mcg ml^{-1} for voriconazole, 0.313–1.0 mcg ml^{-1} for ketoconazole, 0.313–4.0 mcg ml^{-1} for itraconazole.

Conclusions Amongst dermatophytes, lowest MICs were obtained with voriconazole. Around 80% of the isolates revealed high MIC with fluconazole. *In vitro* analysis of antifungal activity of these agents would allow for comparison between different systemic antifungal agents which may clarify reasons for lack of clinical response or serve as an effective therapy in patients with chronic infection. Since the MIC breakpoints of antifungal agents have not been established for dermatophytes, more number of large scale studies are warranted to predict their clinical outcome to assist clinicians in treatment strategies.

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Clinical signs associated with dermatophyte onychomycosis

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Objectives The diagnostics of tinea unguis (dermatophyte onychomycosis) is complex because the clinical signs are non-specific and the mycological analyses have a relatively high false-negative rate. This prospective study aimed to evaluate which clinical signs are significantly associated with tinea unguis.

Methods From September 2011 to June 2013, outpatients at the Podiatric School of Marseille in whom a tinea pedis and/or tinea unguis was suspected were prospectively included in the study. Demographic data, personal history, topical and systemic treatment and clinical signs were collected on a standardized form. Dermatophyte culture (Sabouraud medium with antibacterials and cycloheximide) and dermatophyte-specific real-time PCR was performed on each nail sample at the Parasitology-Mycology of the University Hospital of Marseille. The tinea unguis diagnostic criteria were a positive dermatophyte culture and/or a positive dermatophyte PCR. The association between each clinical sign and tinea unguis diagnosis was tested via univariate and multivariate logistic regression analysis.

Results A total of 468 nail samples were collected from 234 patients. Tinea unguis diagnosis was positive in 86 (18.4%). In the multivariate analysis, lesions of the distal free margin (OR = 7.71, 95%IC[3.37 -17.61], $P<10^{-4}$) and proximal matrix (OR = 2.34, 95% IC [1.38 -3.96], $P = 0.0016$) of the nail, and skin maceration of toe web spaces (OR = 3.71, 95% IC [1.37–10.03], $P = 0.0100$) were independently associated with tinea unguis.

Conclusion In the multivariate analysis, conventional clinical signs of tinea unguis, such as nail color, nail thickening, debris and distal lateral subungual localization did not significantly discriminate between onychomycosis and other nail diseases. Unexpectedly, among all dependent clinical signs of nail disease, the most strongly associated with tinea unguis was lesion of the distal free margin of the nail.

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Maintenance antifungal therapy of recurrent esophageal candidiasis in HIV-negative patients

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Objective The efficiency of maintenance antifungal therapy of recurrent esophageal candidiasis (REC) in HIV-negative patients is not well established.
Methods In prospective single-center study (2004–2014) we include 124 HIV-negative patients with REC. Median age was 53 y (46–65), women 91. Endoscopic examination with a biopsy, microscopy and culture was made in all patients. Identification of the pathogen was made with MALDI-TOF Mass Spectrometry. Determination of susceptibility of patogens to fluconazole and voriconazole was made with the disco-diffusion CLSI M44-A method. Criteria of RCE was $\geq$ 1 relapse in a year.
Results Risk factors were: thyroid disease (43%) with hypothyroidism (17%), bronchial asthma (12%), diabetes mellitus (9%), smoking (100%), inhaled (90%) and per os (52%) corticosteroids, and very hot food use (67%). The clinical signs were dysphagia (90%), retrosternal discomfort (50%), and odinophagia (18%). Endoscopic features were hyperemia (100%) and contact sensitivity (100%) of esophagus mucosa, and white fibrin plaques (75%). Main etiology agent was *C. albicans* (99%), sensitive *in vitro* to fluconazole and voriconazole (100%). Treatment of relapse fluconazole 150 mg day^{-1} for 3–4 weeks was effective in 100% patients. The patients were divided into two groups: with or without maintenance antifungal therapy – 50 mg fluconazole once a week for 6 months. If patients did not receive maintenance therapy rate of relapse in 6 months was 57%. All patients with maintenance therapy were in remission of REC in 6 months ($P < 0.005$). No side effects or drug-drug interactions in patients with maintenance antifungal therapy were detected.
Conclusions Maintenance antifungal therapy with 150 mg fluconazole once a week for 6 months is effective and safe in HIV-negative patients with recurrent esophageal candidiasis.

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Immunological and immunohistochemical features of recurrent esophageal candidiasis in HIV-negative patients.

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Objectives The immunological and immunohistochemical features of a reNAAAAA urrent esophageal candidiasis (REC) in HIV-negative patients are not well understood.
Methods We examined immunological and immunohistochemical parameters in 78 patients with REC. The median age of patients was 53 years. Criteria of REC was ≥1 relapse in a year. Subpopulations of lymphocytes were determined by immunocytochemical method using a monoclonal antibody «DAKO». Blood cell supernatants were tested for IFN-g and IL-10 by using an ELISA test 'Cytokine'. The antibodies used for immunohistochemical staining were monoclonal mouse anti-human CD3 + , CD4 + , CD8 + , CD68 + («DAKO»). Statistical analysis was done using Mann-Whitney U-test ($P < 0.05$).
Results Our study showed that in patients with REC was a reduction of the absolute numbers of CD3 + (1.216*109/L), CD4 + (0.735*109/L), CD8 + (0.483*109/L), CD16 + (0.157*109/L) lymphocytes, and the increase the spontaneously secreting IFN-γ (63.0 pg ml^{-1}) and IL-10 (144.0 pg ml^{-1}) ($P < 0.05$). Immunohistochemical parameters were characterized by a decreased of CD3 + and CD68 + cells and increased CD4 + and CD8 + cells in the epithelium of the esophagus and the underlying tissues.
Conclusions Immunological parameters, identified in this trial may be of interest in the pathogenesis of recurrent esophageal candidiasis in HIV-negative patients.

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Validation of the DermaGenius Nail plus multiplex assay, a new commercial PCR assay developed for the detection and identification of dermatophyte and *Candida* in nails

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Objectives Superficial dermatophytosis is the most common fungal infection in humans. Diagnosis of dermatophytosis is currently based on microscopy or histology associated with culture on specific agar media. However, direct microscopy lacks specificity and culturing has a long turn-around-time of 2–4 weeks. These limitations can be prevented by the use of molecular diagnostics. The DermaGenius (DG) multiplex kit (PathoNostics, the Netherlands) is a new commercial realtime-PCR kit, which can differentiate various dermatophytes species including the nail pathogens *T. rubrum, T. mentagrophytes, T. interdigitale* and 2 *Candida species (C. albicans and C. parapsilosis)*. This study aimed at the validation of the kit on nails clippings. Results were compared with histology and culture data.
Methods A set of 76 nail clippings was collected from 76 patients attending the dermatology consultation at the University Hospital of Liege on suspicion of onychomycosis. All nails were divided in three pieces for histology, culture and the PCR multiplex assay. Histologic preparations were stained with PAS staining. Cultures were performed on 2 different Sabouraud agar medium slants (bioMérieux). The DNA extraction protocol used a proteinase K pre-treatment followed by an automated DNA-extraction (EasyMag, bioMérieux). An Internal Control (IC) was included to monitor for PCR inhibition or manual errors. The realtime-PCR amplification was performed with the DG kit on a Rotor-Gene Q instrument (Qiagen) by using quantitative amplification and melting curve analysis.
Results In total, 35 of 76 cases (46%) were classified as confirmed onychomycosis based on positive microscopy (M+) with or without positive culture (C+) or just by positive culture of a confirmed pathogen. Based on negative microscopy (M-) and negative culture of a confirmed pathogen, 41 cases (54%) were reported as non-fungal onychodystrophy. Agreement between DermaGenius (DG) and culture was found in 52% of the cases while 86% agreement was reported when comparing positive DG with confirmed onychomycosis. Three positive cultures (microscopy negative) were not detected by DG (2 *T. rubrum*, 1 *C. albicans*). However, DG could detect 7 additional infections (9%). Eleven discrepancies DG+/C+ were determined which could be positively confirmed in favour of DG result by ITS sequence analysis. Most discrepancies could be explained by fungal/yeast species overgrowing the agar slant, including species of *Candida, Fusarium, Trichosporon*, which were not considered as the source of infection.
Conclusion The DermaGenius Nail *plus* multiplex was able to detect the most prevalent pathogenic dermatophytes species in clinical nail specimens and proved to be more sensitive and specific than culture and direct microscopy. The DNA extraction procedure has been shown to work efficiently in diagnostics which enables the physician in charge of the patient to start a dedicated treatment rapidly.

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Onychomycosis caused by uncommon agents in immunocompetent patients: two case reports

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Objective Onychomycosis is a fungal infection of nails and accounts for up to 50% of nail disorders. Dermatophytes are the most frequently causative agents in onychomycosis (nearly 70% of the cases) whereas non-dermatophytic molds cause 1.5–6% of the cases. We report two cases of onychomycosis of the halux in immunocompetent patients due to uncommon agents in this setting: *Aspergillus niger and Paecilomyces lilacinus.*
Methods In the first case a 45-year-old caucasian male was referred to the Dermatology department due to changes in the coloration and form of the left halux nail. Clinical examination revealed onycholysis involving one of the lateral nail margins and black pigmentation of the nail plate, with paranoquia. Other nails were normal. The second case is related to a 25-year-old caucasian woman with progressive right great toenail dystrophy since 1 year. Clinical examination revealed onycholysis which involved both lateral nail margins, subungual hyperkeratosis and yellowish discoloration of the nail plate. Other nails were all normal. In both cases nail scrapings were collected and sent to the Microbiology laboratory.
Results Direct microscopy of nail scrapings of the male patient showed thick septated hyaline hyphae and *Aspergillus niger* was isolated in all cultures of three different samples collected. The patient was treated with oral itraconazol in combination with topical sertaconazol for 4 months with clinical resolution of the infection. Direct microscopy of nail scrapings of the female patient showed septated hyaline hyphae. *Paecilomyces lilacinus* was isolated on several cultures of samples collected with one month interval. Susceptibility tests to antifungal agents revealed high MIC′s to itraconazole and fluconazole, but low MIC′s to ketoconazole and voriconazole. She initiated a treatment with oral ketoconazole combined with applications of 40% urea cream and amorolfine lacquer on the nail bed. This treatment was performed during 9 months without marked benefits. Clinical and microbiological resolution was reached with surgical removal of the nail plate and amorolfine cream application in the nail bed.
Conclusions Factors such as ageing, immunodeficiency, trauma, hyperhidrosis, socio-economic status, climatic conditions and paronychia predispose to onychomycosis. This mycosis can significantly impair the quality of life of a patient and may create a number of clinical and occupational complications. These successfully treated cases of onychomycosis caused by two uncommon agents document the fact that the identification of the causative fungi is therefore essential in all suspected cases of onychomycosis due to epidemiologic purposes and because the type and duration of treatment varies when the pathogen is a non-dermatophyte agent.

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Molecular Identification of Dermatophyte species causing cutaneus infections in West Azarbayjan of Iran

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Objectives The most common cutaneus fungal infections are caused by dermatophyte fungi including *Microsporum*, *Trichophyton*, and *Epidermophyton* species. In this study, the epidemiologic trends and the predominant organisms causing dermatophytosis in Urmia, North West of Iran were identified.
Methods The study samples were collected from clinically suspected cutaneus lesions. All the specimens were transported to Medical Mycology Center, UMS University of Medical Sciences. First of all, a conventional diagnosis was carried out which included microscopic examination and culture on sabouraud dextrose agar medium with antibiotics: chloramphenicol and cyclohexamide. All the dermatophyte isolates were then identified at the level of species by the molecular method of PCR-RFLP.
Results From the tested 357 clinical specimens, 30 dermatophytic isolates were identified. The dermatophyte species *Trichophyton mentagrophytis* (36%), *Microsporum canis* (32%), *Microsporum gypseum* (16%), *Trichophyton rubrum* (4%),and *Epidermophyton floccosum* (12%) were included, respectively.
Conclusion The results showed that some dermatophyte species including *T. rubrum* and *T. mentagrophytes* were mainly isolated from foot, whereas *E. fluccosum* was predominantly found in the scrapping of groin and hand.

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Co-existence of *Aspergillus niger* and *Trichophyton interdigitale* toenail onychomycosis in an immunocompetent 70-years-old patient

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Objectives Onychomycosis is the most frequently encountered nail disease. To our knowledge, this study represents an unusual case of toenail onychomycosis caused by the co-existence of *Aspergillus niger* and *Trichophyton interdigitale* in an immunocompetent patient.
Methods Samples obtained from two totally dystrophic toenails of the patient were processed separately. The patient was sampled two times, 3 weeks apart. Microscopic examination by KOH method and culture-based identification of the causative agents from scrapings of the two toenails were performed. Phenotypic identification of the strains was confirmed by sequencing of the beta tubuline gene and the ITS region. The patient was prescribed terbinafine 250 mg p.o. daily for three months.
Results The macroscopic appearances of the two toenails were totally different. The black-dotted nail was more dystrophic than the yellowish one. The microscopic features of the infecting strains from the two toenails were also different on KOH examination: while the sample from the black-dotted toenail had hyphae presumptive for an *Aspergillus* species, the yellowish one was for a dermatophyte species. Also, the growth characteristics of the strains were different from each other, and compatible with the presumptive microscopic identification. The two strains were phenotypically identified as *Aspergillus niger* and *Trichohpyton interdigitale* and the final identifications were confirmed by sequencing.
Conclusion An exact diagnosis of onychomycosis relies on clinical examination, microscopic investigation, culture identification and molecular methods when necessary. At least half of the causative agents of onychomycosis are dermatophytes, as *Trichophyton interdigitale* is a common worldwide dermatophytic fungus. Additionally, in cases of co-isolation of molds, aspergilli infections should not be easily considered saprophytic.

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The first Turkish case of onychomycosis caused by *Chaetomium globosum* in an immunocompetent patient

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Objectives Onycomycosis is a chronic fungal infections of toenail and fingernail. The most causative agents are observed as dermatophyte, yeast and nondermatophyte species. Several types of nondermatophyte mould such as *Aspergillus* spp.,*Fusarium* spp., *Chaetomium* spp may cause these infections. The genus *Chaetomium*, which belongs to (family *Chaetomiaceae*, class *Sordariomycetes*, phylum *Ascomycota*), is dematiaceous nondermatophyte fungus that are commonly found in deteriorating wood products, soil and cellulosic substrates.
Methods We report a case of distal subungual onychomycosis of the thumb of the right foot of a 25-year-old female. In direct microbiological examination septate hyphae was observed by using % 20 KOH. The same brown colonies were produced on repeated cultures by using Sabouraud's dextrose agar without cycloheximide. Antifungal susceptibiltiy test was performed and microplates were prepared as described in document M38-A2.
Results After growth of colony, slide cultures were prepared and brown-colored septated hyphae, perithecia, lemon- shaped ascospores were observed by light microscopy. The causative agent was identified as *Chaetomium globosum* based on DNA sequencing andmycological examination. Using CLSI M38-A2 microdilution method, minimum inhibitory concentration values of amfotericin B,fluconazole, itracanozole, miconazole, ketoconazole, flucytosine voriconazole were determined as 4, >64, 1, 0.125, 0.125, >64, 0.5 μg ml^{-1} respectively. Fluorocytosine and fluconazole were determined as resistant for *Chaetomium globosum* and the best effective antifungal was determined as miconazole and ketoconazole. The patient was treated by using oral itraconazole (250 mg/a day) and local application of amorolfine 5% nail lacquer for 12 weeks.
Conclusion we report the onychomycosis caused by *C. globosum* in an immunocompetent patient which was confirmed by mycological examination and molecular analysis. Antifungal susceptibility was performed and the most effective agent was determined as ketoconazole and miconazole, but clinical*recovery was provided by using* itraconazole.

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Dermatophytes isolating from foxes from private farm

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The aim of this study was to determine the prevalence of dermatophytes colonization in breeding foxes (Polish line of *Vulpes vulpes* with white neck).
Methods In this study, the routine method of isolation and identification of dermatophyte was supported by a method patented by Brillowska-Dabrowska and co-workers [2007]. This method involves extraction of fungal DNA and PCR amplification with pan-dermatophyte primers. To confirm the presence of dermatophytes in samples took from 5 foxes with typical ringworm lesions and 70 healthy farm animals (hair), after confirmation by culture, another two PCR assays were performed; with primers specific for *Trichophyton* genus and *T. mentagrophytes* complex. To investigate the strains differentiation GACA primers were used.
Results the frequency of isolation of dermatophytes in breeding of foxes was 20% among all investigated animals. Only *Trichophyton mentagrophytes* was detected after routine diagnostics. The PCR methods confirm this identification but RAPD results shows strains differentiation among the *T. mentagrophytes* isolates.
Conclusion The results obtain by molecular methods confirm the dermatophyte infection even in symptomless animals and suggests that infections may be due different strains of *T. mentagrophytes.*

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Protein profile of the main species of the *Sporothrix* complex associated with cases of sporotrichosis.

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Sporotrichosis, a subcutaneous mycosis caused by *Sporothrix* spp., is cosmopolitan and more frequent in Latin America and East Asia. In last years the number of sporotrichosis cases has significantly increased in Brazil, especially in the metropolitan area of Rio de Janeiro. At the last decade, it has been proposed that *S. schenckii* should not be considered a single taxon but a complex including the species *S. brasiliensis*, *S. globosa*, *S. mexicana*, *S. pallida* and *S. luriei*. The phylogenetic characterization of strains around of the world from patients treated with sporotrichosis has been reported that the the species often identified using phenotypic and genotypic tests were *S. brasiliensis*, *S. schenckii*, *S. globosa*, *S. mexicana* and *S. pallida*.
Objectives The objective of this study is to characterize the protein profile of these species of the *Sporothrix* complex.
Methods Five *Sporothrix* isolates of control strains of *S. brasiliensis* (IPEC16490), *S. schenckii* (IPEC27722), *S. globosa* (IPEC27135), *S. mexicana* (MUM11.02) and *S. pallida* (SPA8) were used in this study for characterization of the protein profile of these species. For analysis of these exoantigens from the mycelial phase the SDS-PAGE technique was applied. Briefly, fungi were growth in Sabouraud Dextrose medium with constant stirring for 14 days at 28 °C.
Results The exoantigens of *S. brasiliensis*, *S. schenckii*, *S. globosa*, *S. mexicana* and *S. pallida* showed protein complex profiles, composed of molecules with molecular weight ranging from 10-160 kDa.
Conclusion The characteristic profiles observed for each species of *Sporothrix* spp. allowed the clear distinction of the control strains suggesting that this tool can be used in the implementation of the polyphasic taxonomy of this complex and in the development of new molecular diagnostic methods with higher accuracy.

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Insight into the intricate taxonomy of *Aspergillus viridinutans* complex (section *Fumigati*) comprising opportunistic human and animal pathogens

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Objectives The aim of this study was to determine the species boundaries within the *Aspergillus viridinutans* complex. These species are increasingly isolated as opportunistic human and animal

pathogens. Their differentiation from each other and from *A. fumigatus* is clinically relevant due species-specific susceptibility patterns.
Methods A total number of ~100 isolates from various substrates and countries was subjected to morphological, physiological and molecular analysis (DNA sequences of RPB2, calmodulin, beta-tubulin and actin genes). Mating experiments were provided between isolates representing opposite mating types within and between all major clades of combined phylogenetic tree. The viability of ascospores was tested by flow-cytometry.
Results Phylogenetic analysis based on combined dataset supported recognition of at least eight species within *A. viridinutans* complex including one undescribed species sister to *A. aureolus*. *Aspergillus aureolus* and *A. siamensis* represent homothallic species whereas other species are heterothallic and isolates of both mating types were detected. Fertile ascomata were observed in three heterothallic species, *A. udagawae*, *A. felis* and *A. wyomingensis*. The morphology of ascospores and their viability was stable within major clades. Probable interspecies hybrides detected between isolates from four clades (representing phylogenetic species) can be distinguished by unstable morphology of ascospores and their decreased viability.
Conclusion *Aspergillus viridinutans* complex comprises at least eight species, four of them are clinically relevant - *A. udagawae*, *A. felis*, *A. wyomingensis* and *A. aureolus*. The taxonomy of this complex is complicated by relativelly simple induction of interspecies hybrides during in-vitro mating experiments. The stability of ascospore morphology and the viability of ascospores seem to be crucial features in definition of species boundaries. Flow-cytometry is a promissing tool to assess the percentage of viable ascopores in samples.

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Sporothrix chilensis sp. nov. (Ascomycota: Ophiostomatales), a soil-borne agent of human sporotrichosis with mild-pathogenic potential to mammals.

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Sporotrichosis, a cutaneous and subcutaneous mycosis affecting humans and animals worldwide, is caused by several members of the genus *Sporothrix*. Diagnostic characteristics of the genus comprise single-celled, hyaline conidia which are sympodially arranged on clusters of denticles in a bouquet-like fashion at the ends of conidiophores. This morphology is expressed in numerous environmental members of the order Ophiostomatales. A combination of phylogeny, evolution, morphologies and ecologies has enabled major advances in understanding the taxonomy of *Sporothrix* species, including members exhibiting distinct lifestyles such as saprobes, human/animal pathogens, and insect symbionts. Phylogenetic analyses of ITS1/2 + 5.8s sequences split *Sporothrix* genus in two well-defined groups with dissimilar ecologies. Species embedded in the *Sporothrix schenckii* complex are frequently agents of human and animal sporotrichosis, and some of these are responsible for large sapronoses and zoonoses around the warmer temperate regions of world. At the other extreme, basal saprophytic species evolved in association with decaying wood and soil, and are rarely found to cause human disease.
Objectives We propose to create new taxa, *Sporothrix chilensis* sp. nov., to accommodate strains collected from a clinical case of onychomycosis as well as from environmental origins in Chile.
Methods Recently we received some atypical cultures of *Sporothrix* recovered from a clinical case as well as from environmental origins in Viña del Mar, Chile. Morphological and molecular research suggested that an undescribed taxon was concerned. The aim of the present study was to characterize these atypical *Sporothrix* isolates using a polyphasic approach incorporating morphology, virulence and multigene data to recognize species boundaries among phylogenetically related species, and to develop diagnostic barcodes for the species concerned.
Results Multigene analyses based on ITS1/2 + 5.8s region, beta-tubulin, calmodulin and translation elongation factor 1α revealed that *S. chilensis* is a member of the *Sporothrix pallida* complex, and the nearest taxon is *Sporothrix mexicana*, a rare soil-borne species, non-pathogenic to humans. The ITS region serves as a primary barcode marker, while each one of the protein-coding *loci* easily recognized species boundaries providing sufficient information for species identification. A disseminated model of murine sporotrichosis revealed a mild-pathogenic potential, with lung invasion.
Conclusion We provided evidences based on a polyphasic approach, including examination of cultures, morphology and virulence in murine model combined with phylogenetic analyses of protein coding *loci* that *S. chilensis* is distinct from *S. pallida* and allied species. Therefore, a new taxa is proposed to accommodate *Sporothrix chilensis* sp. nov., and *CAL* is recommended as barcoding gene. Although *S. chilensis* is not a primary pathogen, accidental infection may have an impact in the immunosuppressed population. With the introduction of distinct species with similar routes of transmission but different virulence, identification of *Sporothrix* agents at the species level is mandatory.
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The Jabuti method for *Candida* species identification

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Several species of the genus *Candida* are common saprophytes which reside on the skin and mucous membranes in healthy persons. Opportunistic pathogen of *Candida* species, such as *C. albicans, C. glabrata, C. krusei, C. parapsilosis and C. tropicalis* are ordinarily weak pathogens which produce mild superficial infection, but occasionally may cause systemic diseases with involvement of the lung, kidney, endocardium, brain, or reticuloendothelial system. In this study we propose a new methodology for identification of *Candida* species and compare this approach with the system Vitec for identification, that has been validated, and the molecular biology profiles. The new methodology proposed is faster and presents an affordable cost. The results of the three methods were above 95% concordant, demonstrating that the new methodology for identification of *Candida* can be applied; favoring the laboratories that have limited financial resources, to oppose such hospitals network publishes health. This methodology is already Patented at the Brazilian government.