Exposure assessment to azole resistant mycobiota

A demand in high load occupational environments

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BOHS The Chartered Society for Worker Health Protection

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Exposure to azole resistant mycobiota

- A large number of fungal species can cause severe infections, specially among immunocompromised individuals. (Springer et al. 2016)

- Most important fungi related with fungal exposure: *Cladosporium, Alternaria, Stachybotris, Penicillium,* ***Aspergillus.*** (Sabino, 2018)

- **Resistant isolates of *Aspergillus fumigatus*** strains have been found in **environmental and clinical samples** from several countries. (Bader, 2015)

- Azole resistance could become a **global public health threat** with fungal spores able to disperse great distances on air currents. (Verweij et al., 2015)

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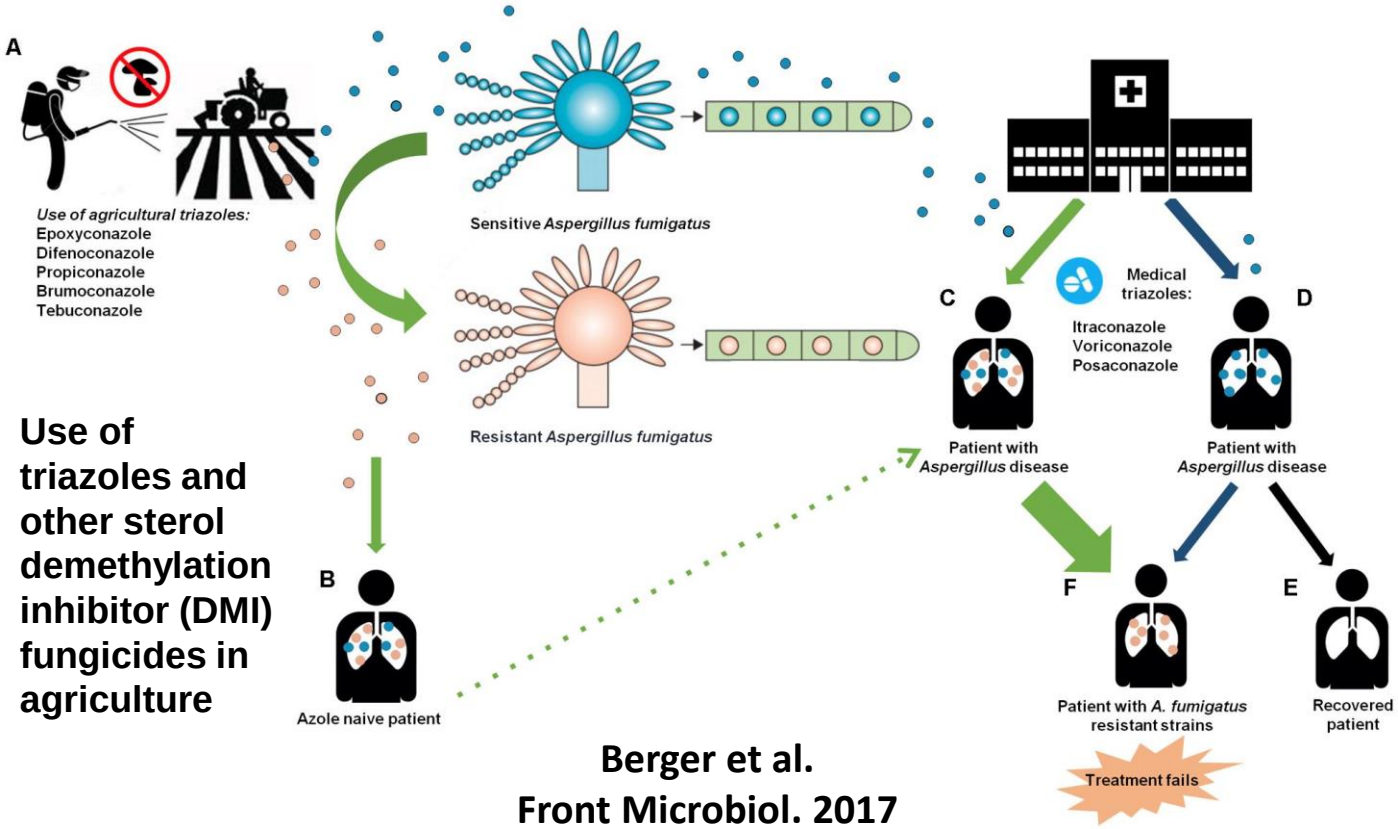
Emergence of antifungal drug resistance in the environment



Use of triazoles and other sterol demethylation inhibitor (DMI) fungicides in agriculture

Berger et al. Front Microbiol. 2017

- Exposure to fungal isolates with less susceptibility to antifungals
- Intrinsic vs. Secondary (azole-induced) resistance

Meis et al. Phil Trans R Soc B. 2016

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Mycobiota in high load environments

- Flour and other raw materials contain several contaminants, namely, fungi, being the principal contamination sources in **bakery industry**.

(Milanowski et al. 2002; Karpinski 2003; Viegas et al. 2016, 2018)

- **Waste application facilities** are considered critical regarding occupational exposure to fungal burden since provide optimal conditions for fungal growth (moisture and decaying matter).

(Heinonen-Tanski et al. 2009;Thirumala et al. 2012; Abdel Hameed and El Gendy 2013; Viegas et al. 2014 – 2016)

- Large-scale and confined **swine production** promotes waste by-products and feed concentration, increasing workers' exposure to organic dust.

(Donham et al. 1989; Attwood et al. 1987; Viegas et al. 2013 - 2017)

Monitoring mycobiota in high load environments

Multi-approach sampling strategy

Active air sampling methods

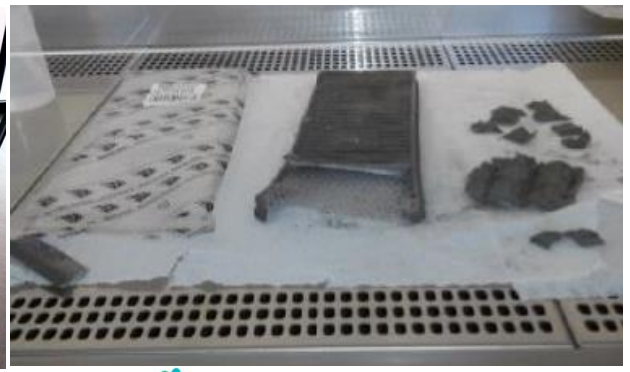
- Air samples of 100 liters (impaction)
- 600 liters (impinger)

Passive sampling methods

- Surface samples
- Electrostatic dust cloths (EDC)
- Raw materials
- Air filters (from HVAC or air-conditioning systems)







Occupational exposure to mycobiota in high load environments

toxics

MDPI

Article

A Novel Multi-Approach Protocol for the Characterization of Occupational Exposure to Organic Dust—Swine Production Case Study

Carla Viegas [1,2,*], Tiago Faria [1,3], Ana Monteiro [1], Liliana Aranha Caetano [1,4], Elisabete Carolino [1], Anita Quintal Gomes [1,5] and Susana Viegas [1,2]

AIMS Microbiology

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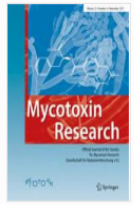
Research article

Assessment of occupational exposure to azole resistant fungi in 10 Portuguese bakeries

Liliana Aranha Caetano [1,2,*], Tiago Faria [1], Ana Crespo Batista [1], Susana Viegas [1,3], and Carla Viegas [1,3]

Aspergillus spp. prevalence in different Portuguese occupational environments: What is the real scenario in high load settings?

Carla Viegas, Tiago Faria, Liliana Aranha Caetano, Elisabete Carolino, Anita Quintal Gomes & Susana Viegas



Mycotoxin Research
November 2017, Volume 33, Issue 4, pp 285–295 | Cite as

A new approach to assess occupational exposure to airborne fungal contamination and mycotoxins of forklift drivers in waste sorting facilities

Authors | Authors and affiliations

Carla Viegas, Tiago Faria, Ana Cebola de Oliveira, Liliana Aranha Caetano, Elisabete Carolino, Anita Quintal-Gomes, Magdalena Twarużek, Robert Kosicki, Ewelina Soszczyńska, Susana Viegas

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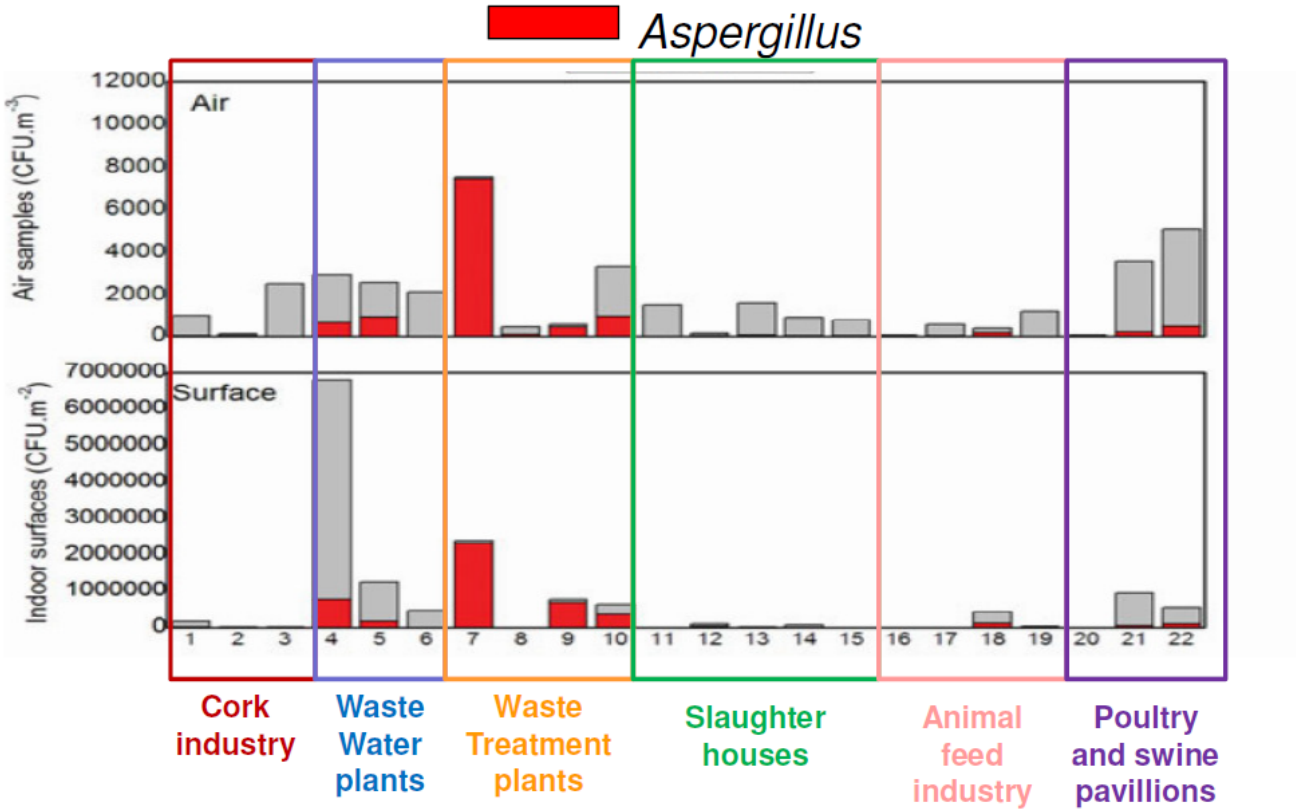
Occupational exposure to mycobiota in high load environments

Prevalence of *Aspergillus* spp. in highly contaminated occupational environments



125 air samples
125 surface samples

Viegas C, Faria T, Aranha Caetano L, Carolino E, Quintal Gomes A, Viegas S (2017) Aspergillus spp. prevalence in different Portuguese occupational environments: What is the real scenario in high load settings?, Journal of Occupational and Environmental Hygiene, 14:10, 771-785

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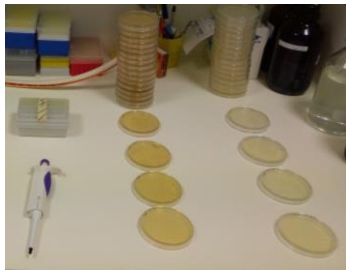
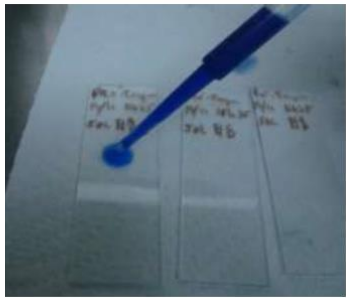
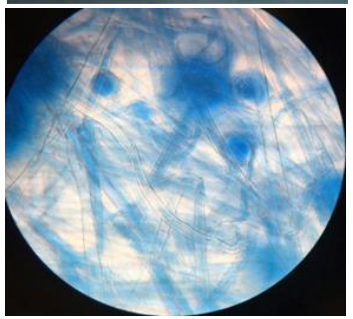
Monitoring antifungal drug resistance in occupational settings

Setting	Samples collected
Bakeries	Settled dust
	EDC
	Raw materials
Waste industry	Air-conditioning filters from waste
	forklifters
Swine farms	Bedding material
	Feed

Jan-Jul 2017







Screening for azole resistance

I. 150 µL of sample wash suspension on Saboraud agar supplemented with:
   - 4 mg/L itraconazole (ITC)
   - 1 mg/L voriconazole (VCZ)
   - 0.5 mg/L posaconazole (PSC) (EUCAST, 2017).

II. Incubation at 27 ºC for 3 to 5 days

III. Fungal densities (colony-forming units (CFU) per 1 m^2 of filter/EDC area, or per 1 gram of settled dust/raw material/bedding/feed)

IV. Fungal species identified microscopically using lactophenol cotton blue mount procedures (Caetano et al., 2017).

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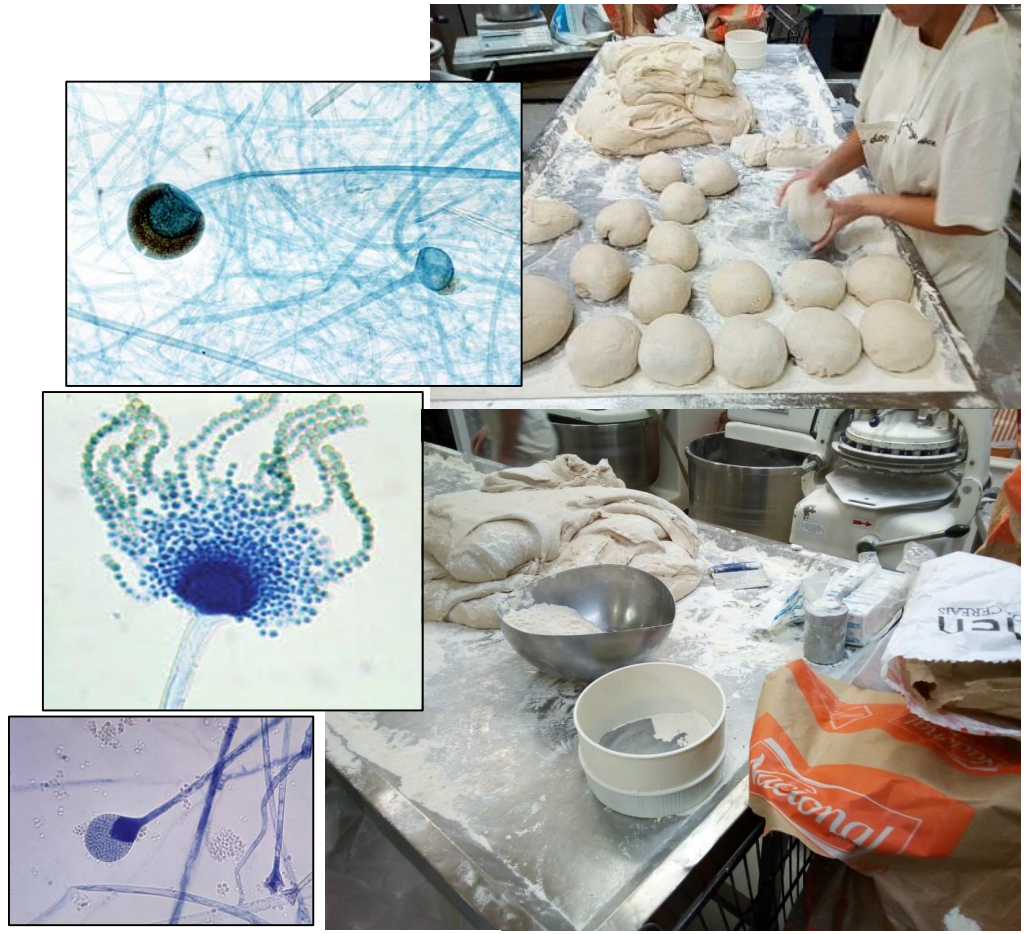
Results Bakeries

Setting	Samples collected		N. of samples with azole-resistant isolates		
	Sample type	N	Total	*Aspergillus* sp.	Mucorales
Bakeries (10 units)	Settled dust	7	0	0	0
	EDC	27	15	1	2
	Raw materials	26	2	0	1
		60	17	1	3

	EDC (CFU/m^2 EDC) (*n*; %)			
Fungal species	4 mg/L ITC	1 mg/L VRC	0.05 mg/L PSC	Total
Chrysonilia sitophila	0; 0	49,761; 65.8	0; 0	49,761; 64.8
Rhizopus sp.	0; 0	24,930; 33.0	0; 0	24,930; 32.5
Cladosporium sp.	498; 71.4	249; 0.3	249; 55.6	995; 1.3
Penicillium sp.	100; 14.3	398; 0.5	149; 33.3	647; 0.8
Chrysosporium sp.	0; 0	100; 0.1	50; 11.1	149; 0.2
Aureobasidium sp.	50; 7.1	50; 0.1	0; 0	100; 0.1
Aspergillus section *Circumdati*	0; 0	50; 0.1	0; 0	50; 0.1
Paecilomyces sp.	50; 7.1	0; 0	0; 0	50; 0.1
Chrysonilia sp.	0; 0	50; 0.1	0; 0	50; 0.1
Alternaria sp.	0; 0	50; 0.1	0; 0	50; 0.1

	Raw material (CFU/g) (*n*; %)			
Fungal species	4 mg/L ITC	1 mg/L VRC	0.05 mg/L PSC	Total
Chrysosporium sp.	1; 100			1; 50
Mucor sp.		1; 100		1; 50

ITC, itraconazole; VRC, voriconazole; PSC, posaconazole.



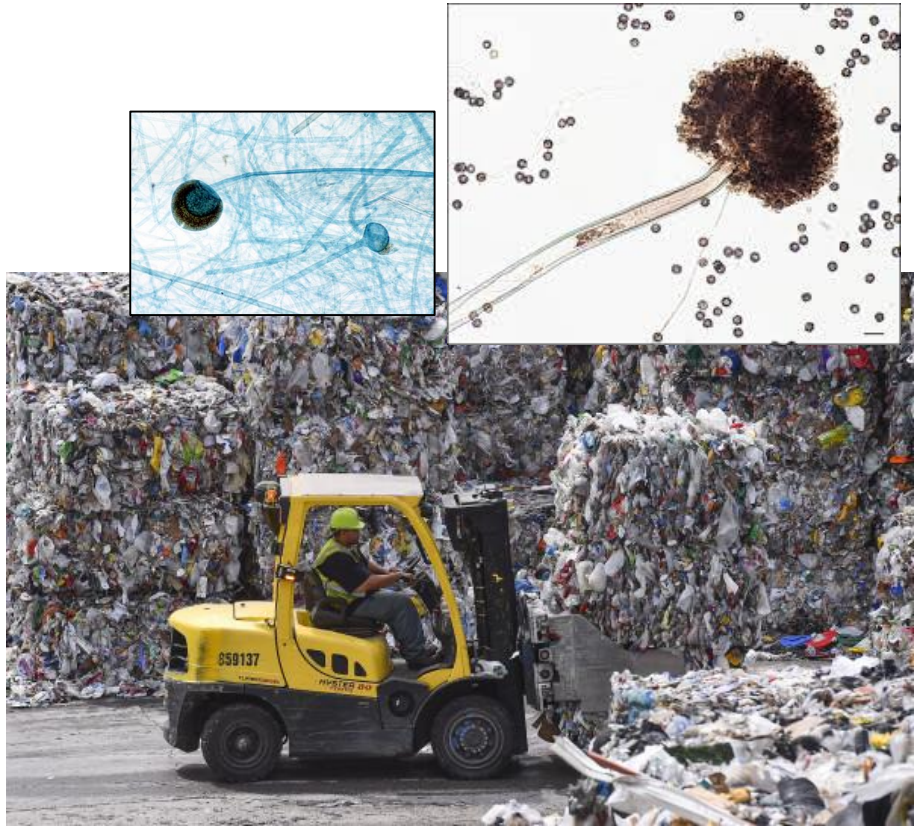
80% Bakeries (56% EDC and 8% raw materials)

Caetano et al. AIMS Micro. 2017

Results Waste industry

Setting	Samples collected		N. of samples with azole-resistant isolates		
	Sample type	N	Total	*Aspergillus* sp.	Mucorales
Waste industry (2 units)	Filters from forklifters	16	15	10	2

WASTE	Number of CFUs			
Filter	**4 mg/L ITC**	**1 mg/L VCZ**	**0.5 mg/L PSC**	**Total**
Aspergillus section *Nigri*	3000	2009	0	5009
Penicillium sp.	0	1606	1500	3106
Chrysosporium sp.	0	514	514	1028
Syncephalastrum sp.	1000	0	0	1000
Rhizopus sp.	0	505	0	505
Phoma sp.	500	0	0	500



100% Waste sorting plants

(94% air filters from forklifters)

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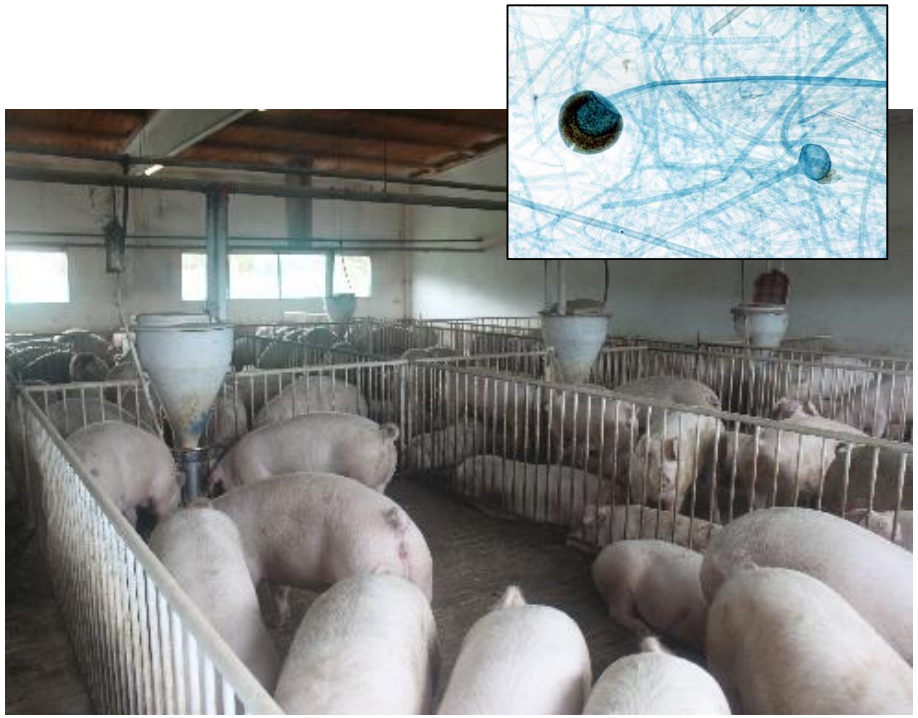
Results Swine farms

Setting	Samples collected		N. of samples with azole-resistant isolates		
	Sample type	N	Total	*Aspergillus* sp.	Mucorales
Swine farms (5 units)	Feed	10	5	0	0
	Bedding material	5	3	0	1
		15	8	0	1

SWINE	Number of CFUs			
Feed	**4 mg/L ITC**	**1 mg/L VCZ**	**0.5 mg/L PSC**	**Total**
F. culmorum	1	1	0	2
Ulocladium sp.	0	5	0	5
Cladosporium sp.	0	2	0	2
Penicillium sp.	0	1	0	1
Bedding				
Penicillium sp.	0	2	1	3
F. culmorum	1	0	0	1
Rhizopus sp.	0	1	0	1



100% swine farms

(50% feed and 60% bedding)

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Dairies and Aquaculture

Preliminary results

AQUACULTURE	Number of CFUs			
Feed	**4 mg/L ITC**	**1 mg/L VCZ**	**0.5 mg/L PSC**	**Total**
F. verticilloides	1	0	0	1

1/1 aquaculture
(14% feed, 0% water, 0% filters)

DAIRIES	Number of CFUs			
Feed + EDC	**4 mg/L ITC**	**1 mg/L VCZ**	**0.5 mg/L PSC**	**Total**
Cladosporium sp.	0	10	63	73
Mucor sp.	1	7	0	8

(42% samples)

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Main findings

- *Aspergillus* sp. isolates with reduced susceptibility to ITC and VCZ were observed in Bakeries and in Waste industry
  - ***Aspergillus* load might be underestimated**
- Mucor order growth was observed in VCZ in Bakeries, Waste industry and Swine farms (intrinsic resistance) and in ITC in Waste industry and in Dairies
  - **Acquired resistance?**
- The presence of azole-resistant fungal species in the 3 workplaces, in particular, azole-resistant *Aspergillus* sp. in the waste industry and in bakeries, may potentially place these workers at high health risk
  - **Exposure to resistant fungi may reach infectious levels within a confined space more readily**

Limitations

- Lack of standardized protocols for the screening of azole-resistance in environmental samples (heterogeneous environments and matrices)
  - **Further research in this field is necessary**

- Mycobiota able to grow in azole screening media might be underestimated as there is competition for nutrientes among fungal species in culture
  - **Target specific fungal species or genera by molecular identification**

- Lack of breakpoint values for azoles for species other than *Aspergillus*
  - **Susceptibility testing guidelines should evolve to outreach microbial resistance characterization in the environment**

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Take home messages

- Passive sampling methods are suitable to characterize the mycobiota in high load occupational settings (Viegas et al. 2015b; Viegas et al. 2017).
  - **Allow to collect contamination from a longer period compared with the active methods**

- Raw materials prone to fungal contamination should be considered for systematic bioburden characterization and azole-resistance screening
  - **Global warming is increasing the prevalence of crop fungal pathogens, and may increase the prevalence of fungal disease in humans as fungi adapt to survive in warmer temperatures** (Nature Microbiology, 2017).

- A multi-approach in sampling methods and fungal identification are recommended for a proper screening of azole-resistance in high load occupational settings
  - **To enable a better risk characterization and more suitable risk control measures to reduce workers health outcomes.**

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Surveillance of azole-resistant *Aspergillus*

Occupational environments (waste, hospitals, dairies, aquaculture, offices…)
Dwellings

Different sampling methods

Active air sampling (impaction and impinger)
Passive air sampling (EDC)
Passive methods (Air conditioning filters, surface swabs, raw materials)

Screening in azole-supplemented media

Samples are seeded (150 ul) in Sabouraud agar plates supplemented with 4 mg/L itraconazole, 1 mg/L voriconazole, and 0.5 mg/L posaconazole, and incubated at 27 °C for 3-5 days. MEA and DG18 innoculation is also performed.

Morphological identification of the collected *Aspergillus* isolates growing on all media

Molecular characterization from Aspergillus section Fumigati growing on all media

Mutation detection from Aspergillus section Fumigati growing on suplemmented media

Acknowledgements

- **Instituto Politécnico de Lisboa, Lisbon, Portugal** for funding the Projects: "Waste Workers' Exposure to Bioburden in the Truck Cab during Waste Management"(IPL/2016/W2E_ESTeSL); "Bacterial Bioburden assessment in the context of occupational exposure and animal health of swine productions" (IPL/2016/BBIOR_ESTeSL); "Bioburden and mycotoxigenic burden from feed applied in aquaculture – Risk assessment and control" (IPL/2018/AquaInvest_ESTeSL)

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Thank you for your attention