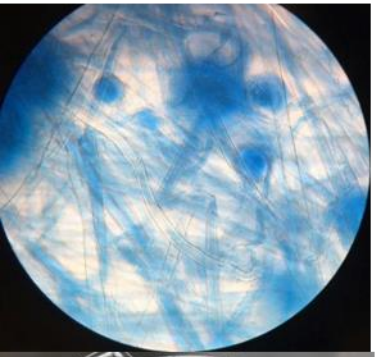

Is the quantitative cut-off a suitable surrogate to ensure a good indoor air quality regarding the mycobiota in health care facilities?

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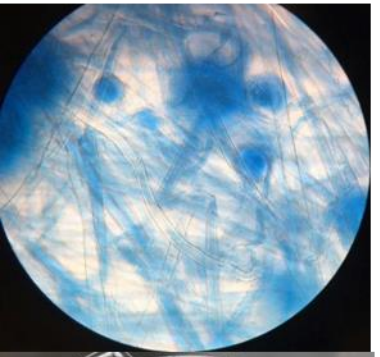


1. Background

Studies have reported that there is a significant relationship between hospital infections and bioaerosols.

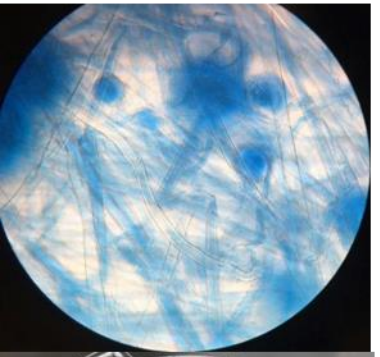
(Lim et al. 2010)

Fungi are present in clinical indoor environments and play important roles in human diseases in patients and staff.

(Lal et al. 2017)

Aspergillus is a common fungus in invasive infections, however, other fungi are enrolled as pathogens in immunosuppressed patients.

(Knibbs et al. 2011)

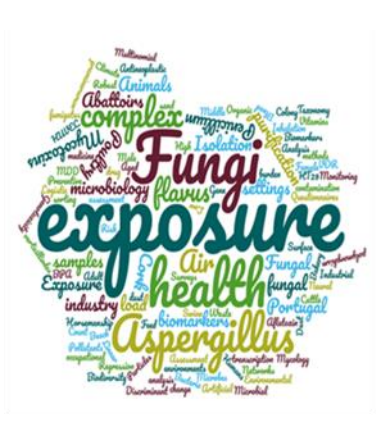


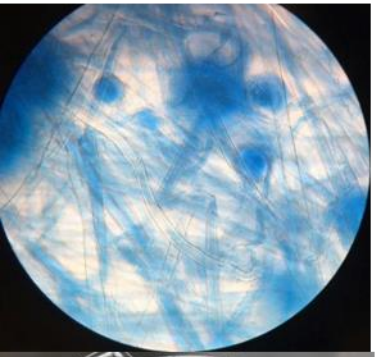
The azole-resistant *Aspergillus* sp. has up to 30% prevalence in some European hospitals, which report higher than 90% mortality rates.

(Van Paassen et al. 2016)

Control measures are crucial in clinical environments for reducing concentrations of airborne fungal contamination and to avoid invasive infections acquired from indoor air.

(Pfaller & Diekema 2010)





We cannot fully eliminate fungi from indoor clinical environments and enforcement is needed to ensure, at least, proper Indoor Air Quality (IAQ) assessments.

(Liu et al. 2017)

Portuguese legislation established limit values for air bioburden in indoor environments in general, without any specific requirement for health care facilities.

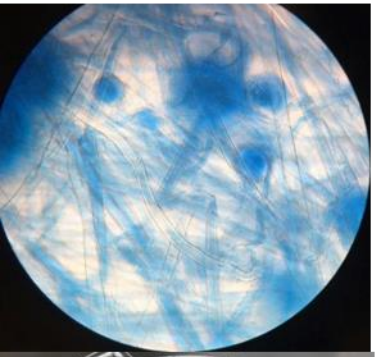
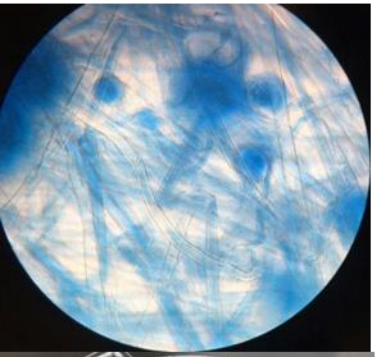


Table 1. Portuguese legal compliance for mycobiota according to Ordinance no. 359-A/2013.

	Fungi
1st requirement	• [indoor] < [outdoor]
2nd requirement (to be applied when the 1st requirement is not fulfilled)	• No visible fungal growth on surfaces; • Species should be evaluated according Table 2

Table 1. Fungal conformity based on the species according to Ordinance no. 359-A/2013.

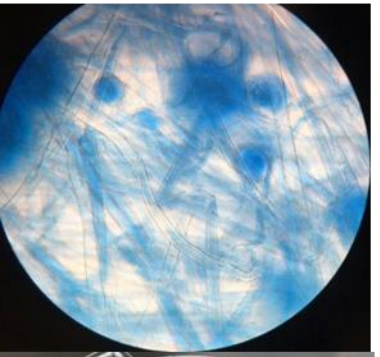
Genera/Species		Specific Condition of Conformity
Common species	*Cladosporium* sp. *Penicillium* sp. *Aspergillus sp.* *Alternaria* sp. *Eurotium spp* *Paecilomyces* sp. *Wallemia* sp.	Mixture of species: ≤ 500 CFU.m^{-3}
Non-common species	*Acremonium* sp. *Chrysonilia* sp. *Tricothecium* sp. *Curvularia* sp. *Nigrospora* sp.	One species: < 50 CFU.m^{-3} Mixture of species: < 150 CFU.m^{-3}
Pathogenic species	*Chryptococcus neoformans* *Histoplasma capsulatum* *Blastomyces dermatitidis* *Coccidioides immitis*	Absence of any species
Toxigenic species	*Stachybotrys chartarum* *Aspergillus versicolor* *Aspergillus flavus* *Aspergillus ochraceus* *Aspergillus terreus* *Aspergillus fumigatus* *Fusarium moniliforme* *Fusarium culmorum* *Trichoderma viride*	One species: < 12 CFU.m^{-3} *(Several colonies per plate)*



- This study focuses on the mycobiota assessment in 10 Primary Health Care Centers (PHCC) applying the IAQ Portuguese legislation as guidance.

- After the quantitative cut-off analyses (I/O) the fungal species identification was performed to verify if the fungal conformity was achieved even in those that comply with the I/O ratio.

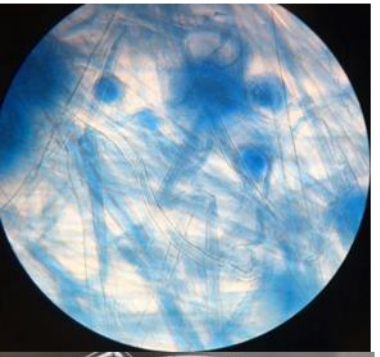
2. Materials and methods



10 PHCC in the Lisbon area

- Active sampling
  - Air sampling by impaction
- Passive sampling
  - Surface swabs
  - Electrostatic Dust Collectors (EDC)
  - Settled dust
  - HVAC Filters

Waiting room, treatments room, vaccination room, back and front office, medical office, cleaning supplies room, oral hygiene office, sterilization, and canteen

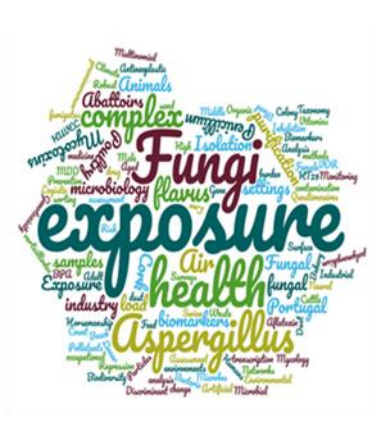


Bioburden exposure assessment in 10 PHCC

- **Bacteria**
  - Inoculation in tryptic soy agar (TSA), incubation at 30ºC for 7 days
  - Inoculation in violet red bile agar (VRBA), incubation at 37ºC for 7 days
- **Fungi**
  - Inoculation in 2% malt extract agar (MEA) with 0.05 g/L chloramphenicol media, incubation at 30ºC for 5 to 7 days
  - Inoculation in dichloran glycerol (DG18) agar-based media, incubation at 30ºC for 5 to 7 days

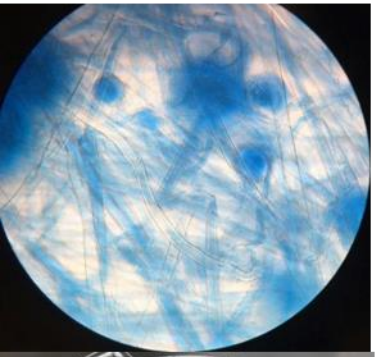
Other performed assessments in the same matrices:

- Fungal biomass
- Toxigenic fungal strains detection
- MRSA prevalence (all passive methods)
- Azole resistance prevalence among the recovered mycobiota
- Mycotoxins
- Endotoxins



3. Results

The median values on MEA ranged from 164 CFU.m^{-3} (in the clean area of the sterilization room) to 508 CFU.m^{-3} (in the treatments room). Among the 10 PHCC 60% (6 out of 10) presented I/O >1.



PHCC	Mean (CFU.m^{-3})	Outdoor (CFU.m^{-3})	I/O	Toxigenic species*
1	28.57	144	0.20	
2	65	20	**3.25**	*Aspergillus* sections *Circumdati* and *Versicolores*
3	479	612	0.78	*Aspergillus* section *Versicolores*
4	402	260	**1.55**	
5	291	96	**3.03**	*Aspergillus* section *Fumigati*
6	155.5	228	0.68	*Aspergillus* section *Versicolores*
7	474.91	140	**3.39**	
8	526	384	**1.37**	
9	505.71	508	1.00	
10	520	208	**2.50**	

* Not complying with specific condition of conformity

- *Aspergillus* sections *Versicolores*, *Circumdati* and *Fumigati* were identified on air

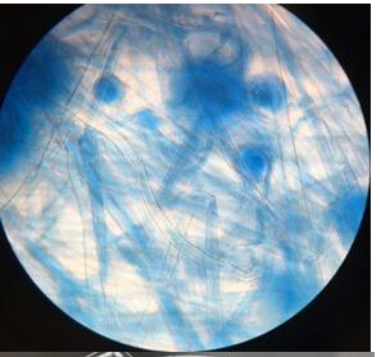
- **> 12 CFU.m^{-3}**

- Section ***Versicolores*** surpassed in PHCC 2, 3 and 6.

- Section ***Circumdati*** the load was surpassed in PHCC 2

- Section ***Fumigati*** in PHCC 5.

- Sections *Nigri*, *Aspergilli* and *Nidulantes* were also observed.

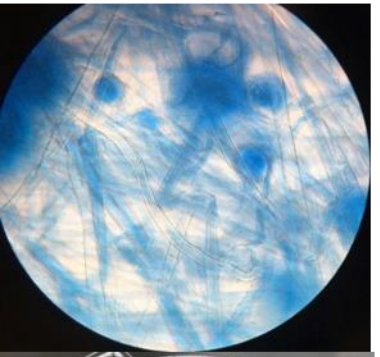


4. Main findings discussion

Mycobiota assessment should be always performed:

- to verify compliance with Portuguese legislation;
- fungal infections predominantly occur in immunocompromised patients and many are acquired in hospitals;
- *Aspergillus* genus should always be target in clinical environment and should be considered as a main IAQ concern.

(ACGIH 1999; Patterson 2016)

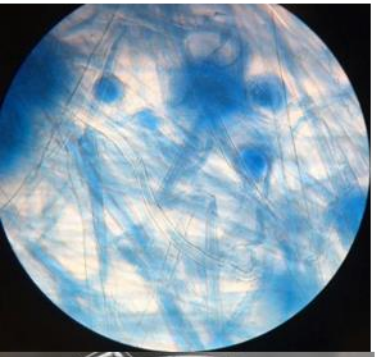


Even if the I/O ratio cut-off is in accordance with legislation

Qualitative assessment from the fungal burden (fungal species/sections identification) should be performed

Since toxigenic species can be present

Other critical points from Portuguese legislation:

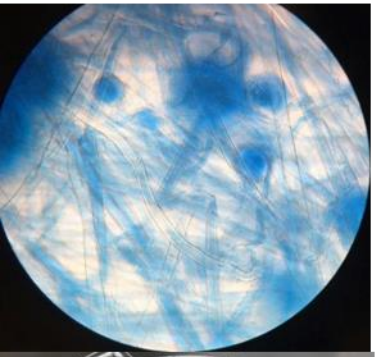


- Assessment should not be restrictive to the listed fungal species, since not all fungal/species with toxigenic potential, and common in indoor environments from Portugal, are listed (*Aspergillus* sections *Nigri*, *Aspergilli* and *Nidulantes* were also isolated).

- Portuguese legislation only relies on active methods (air sampling) and this can narrow the exposure assessment.

(Gralton et al. 2011)

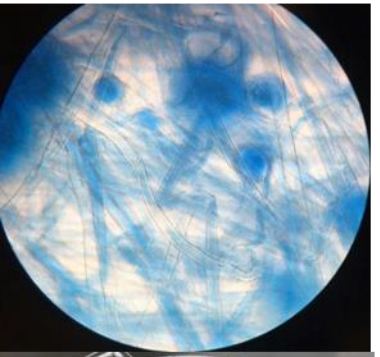
Take home messages (with further results):



- A **multi-approach in the sampling methods (active and passive) and analyses** applied should be the trend.

More refined risk characterization

Suitable risk control measures to reduce patients and workers health outcomes.



The quantitative cut-off applied to assess IAQ ***is not a suitable surrogate*** *to ensure an IAQ regarding the mycobiota in health care facilities.*

18 Junho at ESTeSL – EXPOsE Seminar

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