

Are we neglecting the importance of azole resistance screening in Indoor Air Quality assessments?

Carla Viegas^{1,2,3}; Beatriz Almeida¹; Bianca Gomes¹; Marta Dias¹; Renata Cervantes¹; Liliana Aranha Caetano^{1,4}

* Carla Viegas
✉ carla.viegas@estesl.ipl.pt

1 H&TRC- Health & Technology Research Center, ESTeSL - Escola Superior de Tecnologia da Saúde, Instituto Politécnico de Lisboa; 2 NOVA National School of Public Health, Public Health Research Centre, Universidade NOVA de Lisboa.; 3 Comprehensive Health Research Center (CHRC), 1169-056 Lisbon, Portugal; 4 Research Institute for Medicines (iMed.Ulisboa), Faculty of Pharmacy, University of Lisbon, Lisbon, Portugal

The microbial exposure as well as their metabolic products has been frequently reported as a high risk of respiratory effects and general health hazards¹.

Regarding the Portuguese legislation for IAQ, the microbial assessment covers **only air sampling and culture-based methods** (malt extract agar as only culture media to be applied)²

Fungal legal criteria consist of fungal load ratio between indoor and outdoor, which should be lower than 1 (I/O<1)¹.

Purpose: Analyze the importance of including the characterization of the azole resistance profile in indoor air quality assessments.



Active sampling methods (air sampling)



Passive sampling methods (EDC, swabs, settled dust, mops and cleaning cloths, HVAC filters,)



• 0.1% Tween™ 80 saline solution (NaCl 0.9%)
• 30 min, 250 rpm

• Malt extract agar (MEA) with 0.05% chloramphenicol
• Dichloran-glycerol agar (DG18)
• 5-7 days, 27 °C

• Azole-supplemented Sabouraud (SAB) media
• 4 µg/mL itraconazole (ITR)
• 2 µg/mL voriconazole (VOR)
• 0.5 µg/mL posaconazole (POS)
• 3-4 days, 27 °C

• Macro and microscopic morphology
• Minimum inhibitory concentration (MICs for amphotericin B, POS, ITR and VOR with *Aspergillus* section *Fumigati* isolates by E-test gradient tips

RESULTS



- In six PHCC, four fungal species, collected from the HVAC filters, were observed in at least one azole-supplemented media: *Chrysosporium* sp., *Cladosporium* sp., *Mucor* sp. and *Penicillium* sp..
- The most prevalent species were *Cladosporium* sp. (91.5%, 50%, and 81.3%) and *Penicillium* sp. (6.4%, 45.5%, and 18.8%) on ITR-, VOR- and POS-media, respectively.
 - No *Aspergillus* sp. was observed in azole-supplemented media.



- The highest *Cladosporium* sp. prevalence among passive sampling (electrostatic dust cloths - EDC, cleaning cloths, mops, uniform name tags, filters, and settled dust) was observed on filters (91.1%, 72.1%, and 77.9%) on ITR-, VOR- and POS-media, respectively.
- *Aspergillus* sp. low frequencies (0.1% to 1.1%) were observed, mostly from EDC (3.4% and 3.2% on ITR- and VOR-media, respectively), and in filters (0.03% on ITR).
 - Six *Aspergillus* sections were observed on SAB (80.6% *Candidi*; 8.9% *Fumigati*; 3.3% *Nidulantes*; 3.3% *Circumdati*; 2.9% *Nigri*; 1.2% *Flavi*); 100% *Fumigati* on ITR; and 97.1% *Fumigati* and 2.9% *Nidulantes* on VOR.
- Considering the MIC values of >2 µg/ml for amphotericin B, ITR or VOR, or > 0.25 µg/ml for POS as cutoff values, 36% *Fumigati* isolates could be considered resistant.

DISCUSSION

- The **passive sampling methods allowed to obtain the azole resistance profile in both indoor environments**, enabling the characterization of contamination from a longer period of time than air sampling¹.
- Even when complying with IAQ legislation (quantitative cut off), fungal species should be identified¹.
- **Significant levels of azole resistance**, specifically for *Aspergillus* section *Fumigati*, **was found on FFH**, following the trend of other non-clinical environments³.

CONCLUSION

- The occurrence of fungi with reduced azole susceptibility in PHCC and FFH raises awareness and concern about **antifungal resistance in these settings**.
- **Passive sampling** should be considered when assessing IAQ.
- Other indoor environments besides health care facilities should be assessed for **azole resistance when performing IAQ assessments**.

REFERENCES: (1) Viegas, C., Almeida, B., Monteiro, A., Caetano, L.A., Carolino, E., Gomes, A.Q., Twaruzek, M., Kosicki, R., Marchand, G., Viegas, S., 2019. Bioburden in health care centers: Is the compliance with Portuguese legislation enough to prevent and control infection? *Build. Environ.* 160. (2) Agência Portuguesa do Ambiente, 2010. *Qualidade do Ar em Espaços Interiores Um Guia Técnico 2010*. (3) Viegas, C., Caetano, L.A., Viegas, S., 2021f Occupational exposure to *Aspergillus* section *Fumigati*: Tackling the knowledge gap in Portugal. *Environ. Res.* 194. <https://doi.org/10.1016/j.envres.2020.110674>

ACKNOWLEDGEMENTS: Instituto Politécnico de Lisboa, for funding the Projects "Waste Workers' Exposure to Bioburden in the Truck Cab during Waste Management - W2E Bioburden" (IPL/2016/W2E_ESTeSL), and "Occupational exposure of ambulance drivers to bioburden" (IPL/2020/BIO-AmbuDrivers_ESTeSL); National funds through the FCT - Fundação para a Ciência e Tecnologia, I.P., within the scope of the PhD Grant UI/BD/151431/2021; FCT/MCTES national support through the UIDB/05608/2020 and UIDP/05608/2020.