

## AZOLE-RESISTANCE OF MUCORALES IN THE WASTE SORTING INDUSTRY

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## AZOLE-RESISTANCE OF MUCORALES IN THE WASTE SORTING INDUSTRY

- Resistance to antibiotics is a well-known threat to public health. **Fungal drug resistance** is more recently being described as an emergent threat.

Stop neglecting fungi. *Nat Microbiol.* 2017

- The emergence of resistance to azoles among fungal species **in the environment** challenges the management of severe fungal infections.

Verweij et al. *Clin Infect Dis.* 2016

- **Azole resistance** could become a global public health threat with fungal spores able to disperse great distances on air currents.

Jeanvoine et al. *Med Mal Infect.* 2019

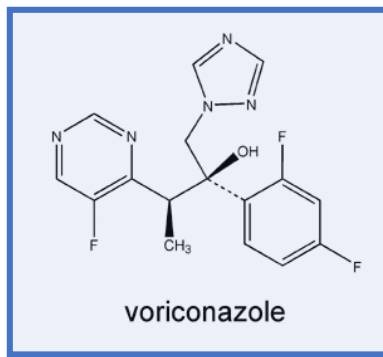
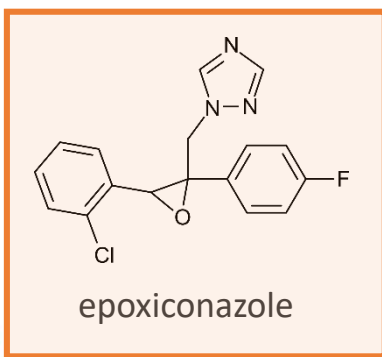
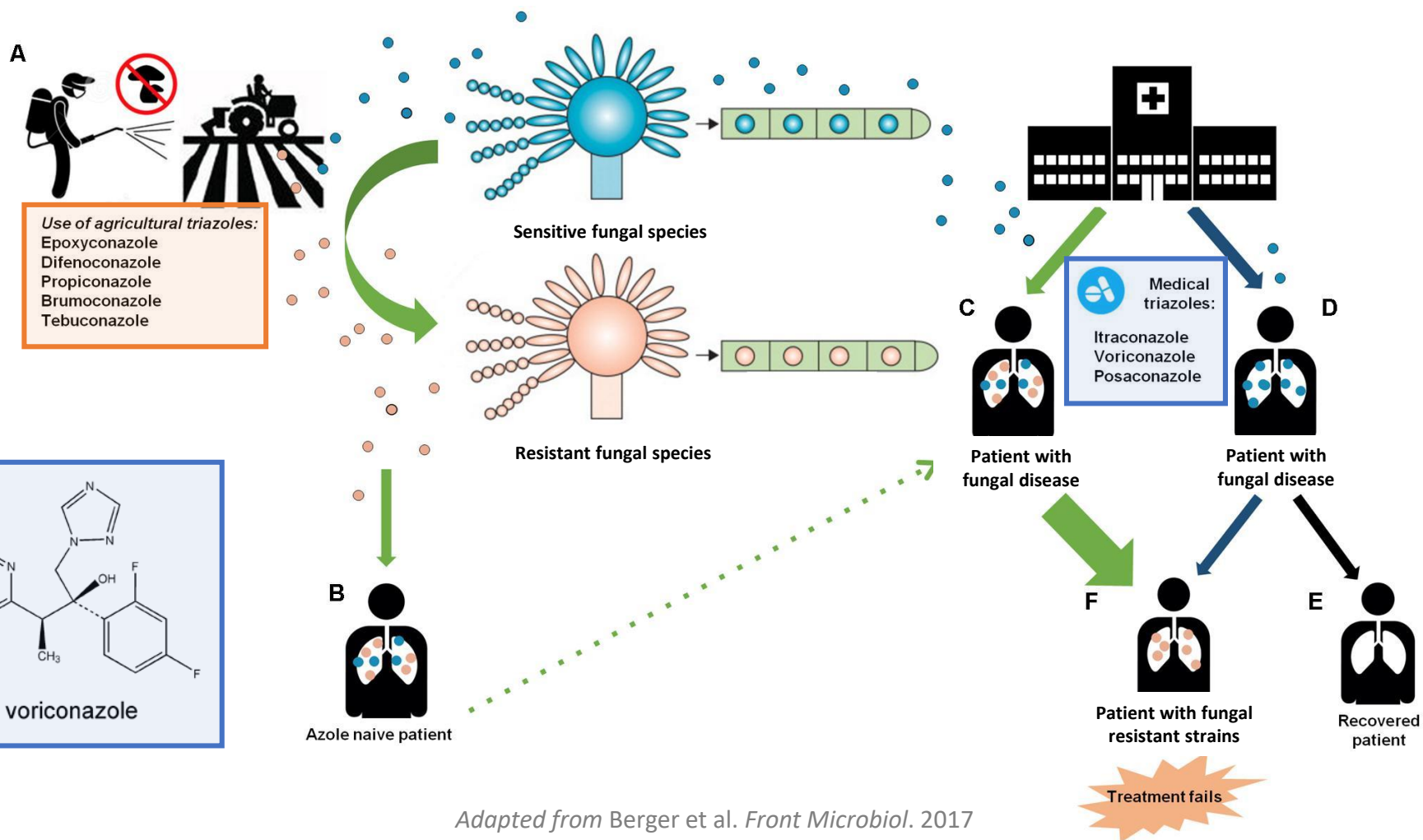


Meis et al. *Phil Trans R Soc B.* 2016

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## AZOLE-RESISTANCE OF MUCORALES IN THE WASTE SORTING INDUSTRY

### EMERGENCE OF RESISTANCE TO AZOLES IN THE ENVIRONMENT



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## AZOLE-RESISTANCE OF **MUCORALES** IN THE WASTE SORTING INDUSTRY

- Mucorales order includes a large number of ubiquitous saprophytes species that can cause severe infections with high morbidity. Prevalence of mucormycosis worldwide is poorly known, with infections by *Fusarium* and *Mucorales* on the rise.

Bitar et al. *Emerg Infect Dis.* 2009

Auberger et al. *J Antimicrob Chemother.* 2012

Lackner et al. *Future Microbiol.* 2014

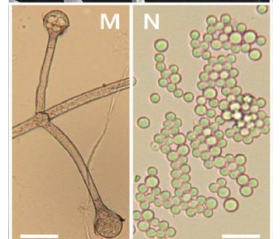
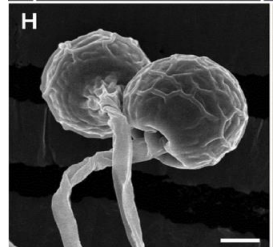
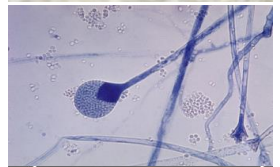
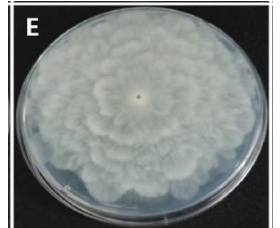
- Invasive mucormycosis affects up to 13% high-risk patients (e.g. immunocompromised patients with diabetes mellitus) and is lethal in up to 96% of cases.

Petrikkos et al. *Clin Infect Dis.* 2012

Espinel-Ingroff. *Antimicrob Agents Chemother.* 2015

- Worldwide, the most common genera found to cause mucormycosis are *Rhizopus*, followed by *Lichtheimia*, and *Mucor*.

Ribes et al. *Clin Microbiol Rev.* 2000





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## AZOLE-RESISTANCE OF MUCORALES IN THE WASTE SORTING INDUSTRY

- Mucormycetes have **innate resistance to short-tailed triazole** (Fluconazole, Voriconazole).

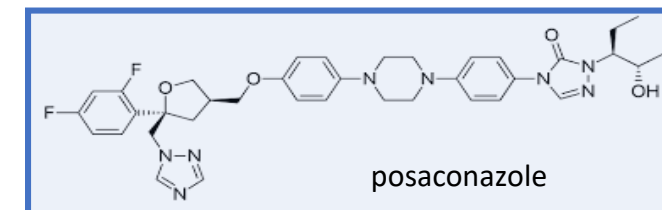
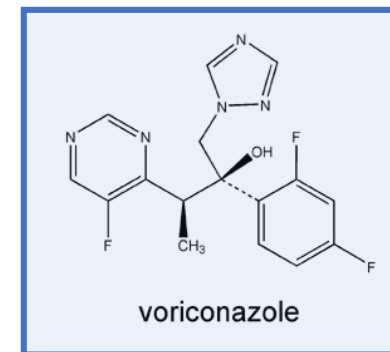
Almyroudis et al. *Antimicrob Agents Chemother.* 2007

- First-line treatment of mucormycoses with antifungal therapy with amphotericin B, and **long-tailed triazole** Posaconazole as salvage therapy.

Cornely et al. *Clin Microbiol Infect.* 2014

species	Whole cell susceptibility to VCZ <sup>a</sup>
	Median (range) MIC (mg/L) (n strains tested)
<i>R. arrhizus</i>	16.00 (4.00 -> 16.00) (n = 17)
<i>R. microsporus</i>	16.00 (4.00-16.00) (n = 13)
<i>M. circinelloides</i>	16.00 (16.00-> 16.00) (n = 18)

Caramalho et al. *Sci Rep.* 2017



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## AZOLE-RESISTANCE OF MUCORALES IN THE **WASTE SORTING INDUSTRY**

- Previous studies of exposure assessment in our group revealed the presence of Mucorales in occupational environments, including the **waste sorting industry**.

Caetano et al. *AIMS Microbiology*. 2017

Viegas et al. *8AAA*. 2018

- Waste manual sorting is associated with exposure to bioaerosols and has been related with diarrhea, skin irritation, organic dust toxic syndrome and inflammation of the airways.

Binion and Gutberlet. *Int. J. Occup. Environ. Health*. 2012

Poole and Basu. *Occup. Med.* 2017

- **Filtering Respiratory Protective Devices (FRPD)** and **Gloves** are used in waste-sorting industry in Portugal as preventive of workers' exposure to bioaerosols.

89/686/EEC. *Off. J. Eur. Communities*. 1989



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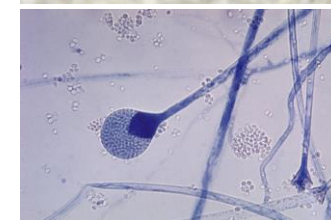
## AZOLE-RESISTANCE OF MUCORALES IN THE WASTE SORTING INDUSTRY

Considering the clinical relevance of Mucorales order, fungal exposure assessments should evaluate their prevalence and azole-resistance patterns.

*Viegas et al. Waste Manag. 2020*

### AIMS:

- To determine **Mucorales distribution in Personal Protective Equipment used by waste sorting workers** in one waste sorting industry located in Lisbon district
- To perform an **azole-resistance screening** using three medical azole drugs (itraconazole, voriconazole and posaconazole) for the samples collected in the waste sorting industry





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## AZOLE-RESISTANCE OF MUCORALES IN THE WASTE SORTING INDUSTRY

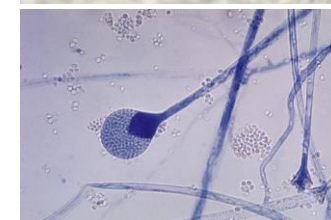
I. Samples collected between Jan and Feb 2019 after normal use (1 work shift) from waste sorting workers

- **120 Filtering Respiratory Protective Devices (FRPD) - interior layer and exhalation valves**
- **67 Gloves**

II. Samples extracted with 0.1% Tween™ 80 saline solution (NaCl 0.9%) and inoculated in:

- Malt extract agar (MEA) supplemented with chloramphenicol (0.05%)
- Dichloran-glycerol agar (DG18)
- Sabouraud dextrose agar (SDA) supplemented with 4 mg/L itraconazole (ITRA), 1 mg/L voriconazole (VORI), or 0.5 mg/L posaconazole (POSA)

III. After plates incubation at 27°C for 5 days, Mucorales densities (CFU/m<sup>2</sup>) and morphological identification were determined.

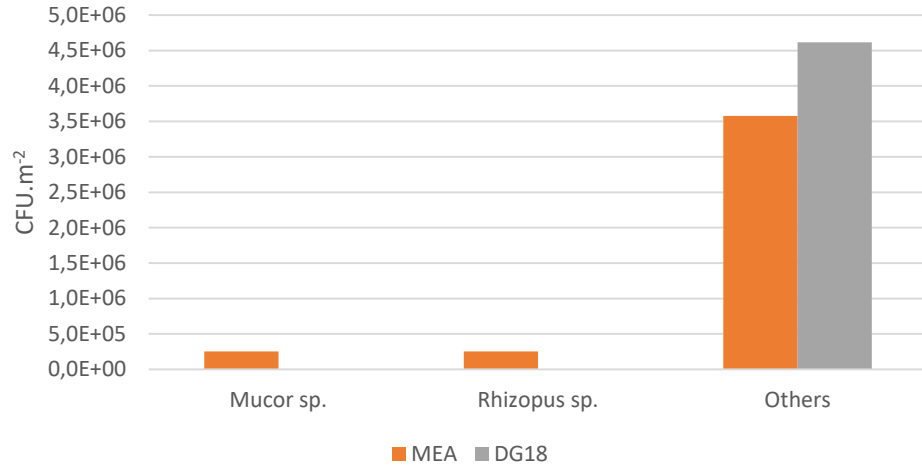




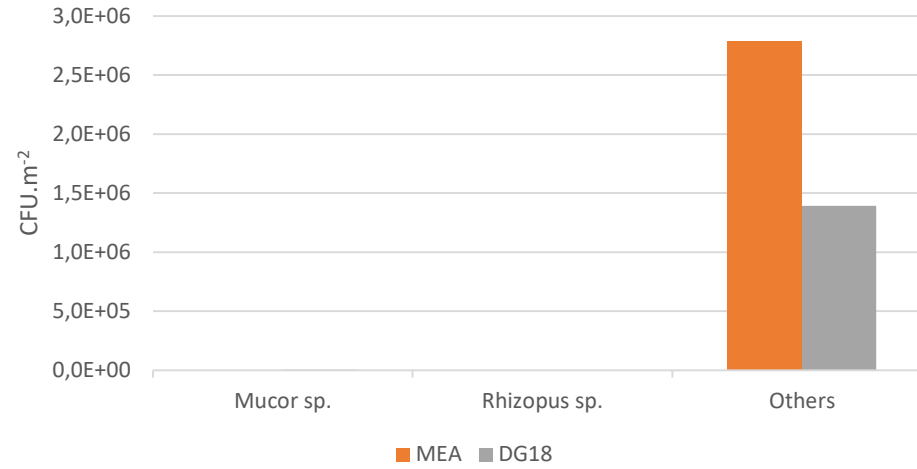


## Mucorales distribution in Personal Protective Equipment used by waste sorting workers

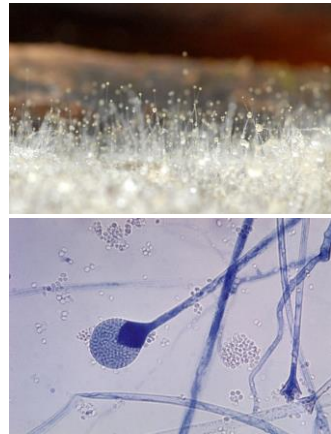
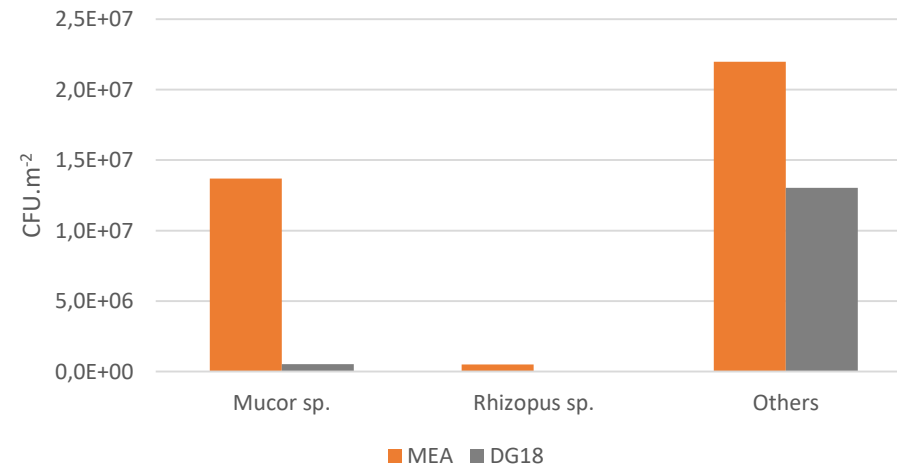
FRPD Interior layer



FRPD Exhalation valves

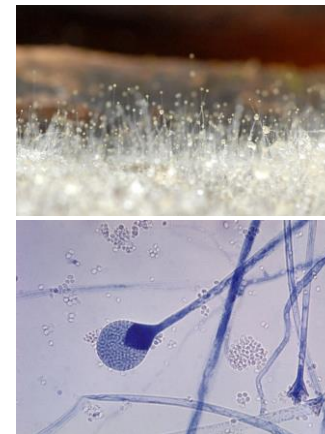
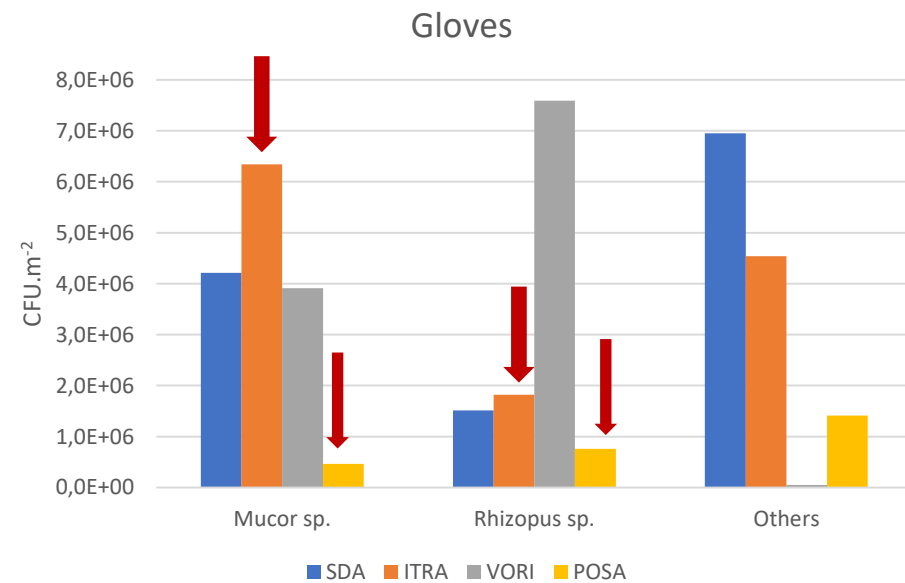
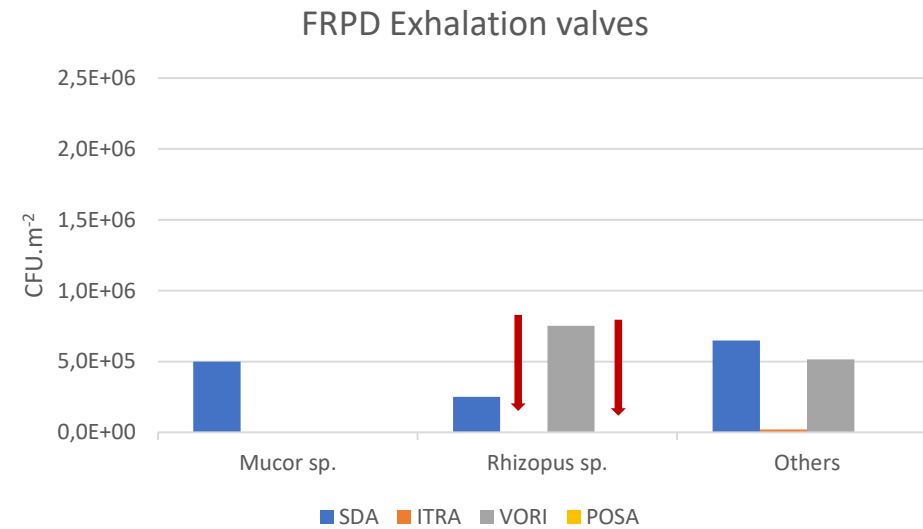
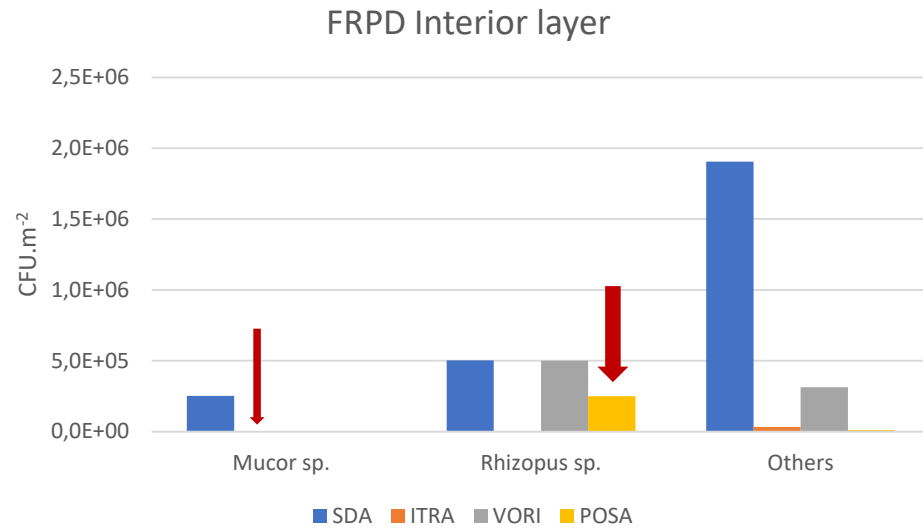


Gloves





## Azole-resistance screening in Personal Protective Equipment used by waste sorting workers

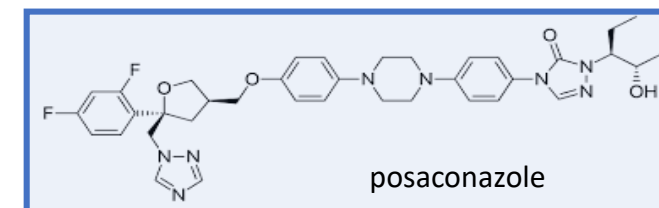
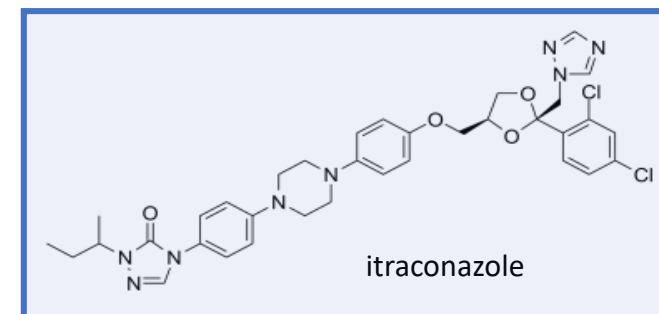


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## AZOLE-RESISTANCE OF MUCORALES IN THE WASTE SORTING INDUSTRY

### MAIN FINDINGS

- Higher fungal contamination on Gloves (total fungi, Mucorales) (MEA, DG18)
- Mucorales with reduced susceptibility to long-tailed azoles (ITRA, POSA)
  - **Environmental acquired resistance?**
  - **Menace to medical use of azoles as first line therapy against mucormycosis?**

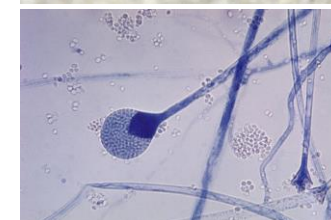


CFU.m <sup>-2</sup>	ITRACONAZOLE	VORICONAZOLE	POSACONAZOLE
Gloves	7.90x10 <sup>6</sup>	1.15x10 <sup>7</sup>	1.46x10 <sup>6</sup>
FRPD interior layer	1.00x10 <sup>3</sup>	5.01x10 <sup>5</sup>	2.50x10 <sup>5</sup>
FRPD exhalation valves	2.00x10 <sup>3</sup>	7.52x10 <sup>5</sup>	1.00x10 <sup>3</sup>



### LIMITATIONS & FUTURE PERSPECTIVES

- Protective efficacy of FRPD and gloves and azole-resistance profile of Mucorales should be determined to assess the risk of exposure in the waste sorting industry
- 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 (competition)
  - 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



### TEAM

Beatriz Almeida | Carla Viegas | Liliana Aranha Caetano | Marta Dias

### ACKNOWLEDGEMENTS

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