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Aspergillus spp. and azole-resistance characterization on Mechanic Protection Gloves from waste sorting industry

1st CHRC Annual Summit

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Outline

1. Background
2. Materials and methods
3. Results
4. Main findings
5. Main conclusions

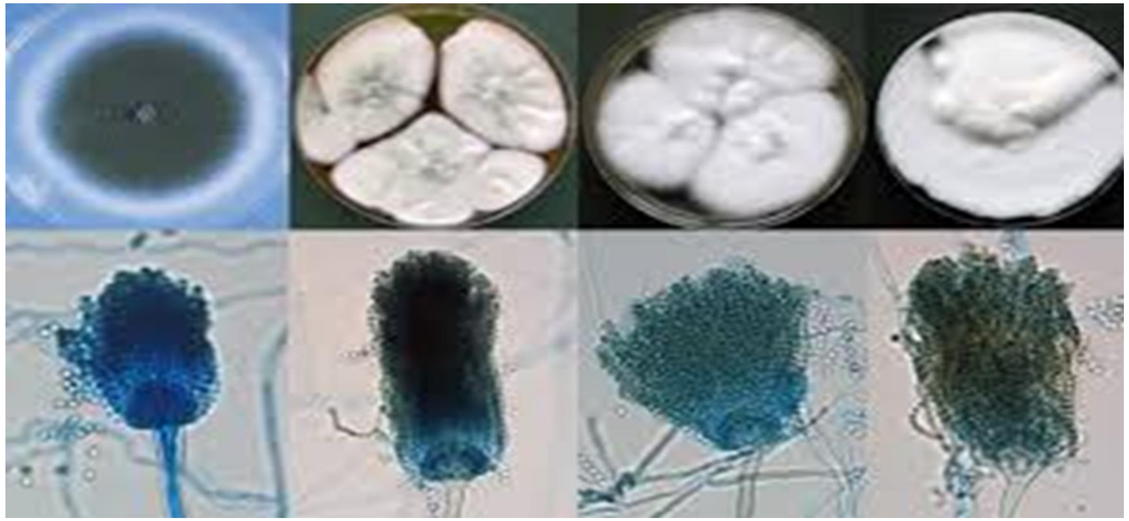
1. Background

- *Aspergillus* spp. species are filamentous fungi commonly found in different environmental compartments, where they thrive as saprophytes. Some species among the *Aspergillus* genera can be occasionally harmful to humans.

(Seyedmousavi et al. 2015)

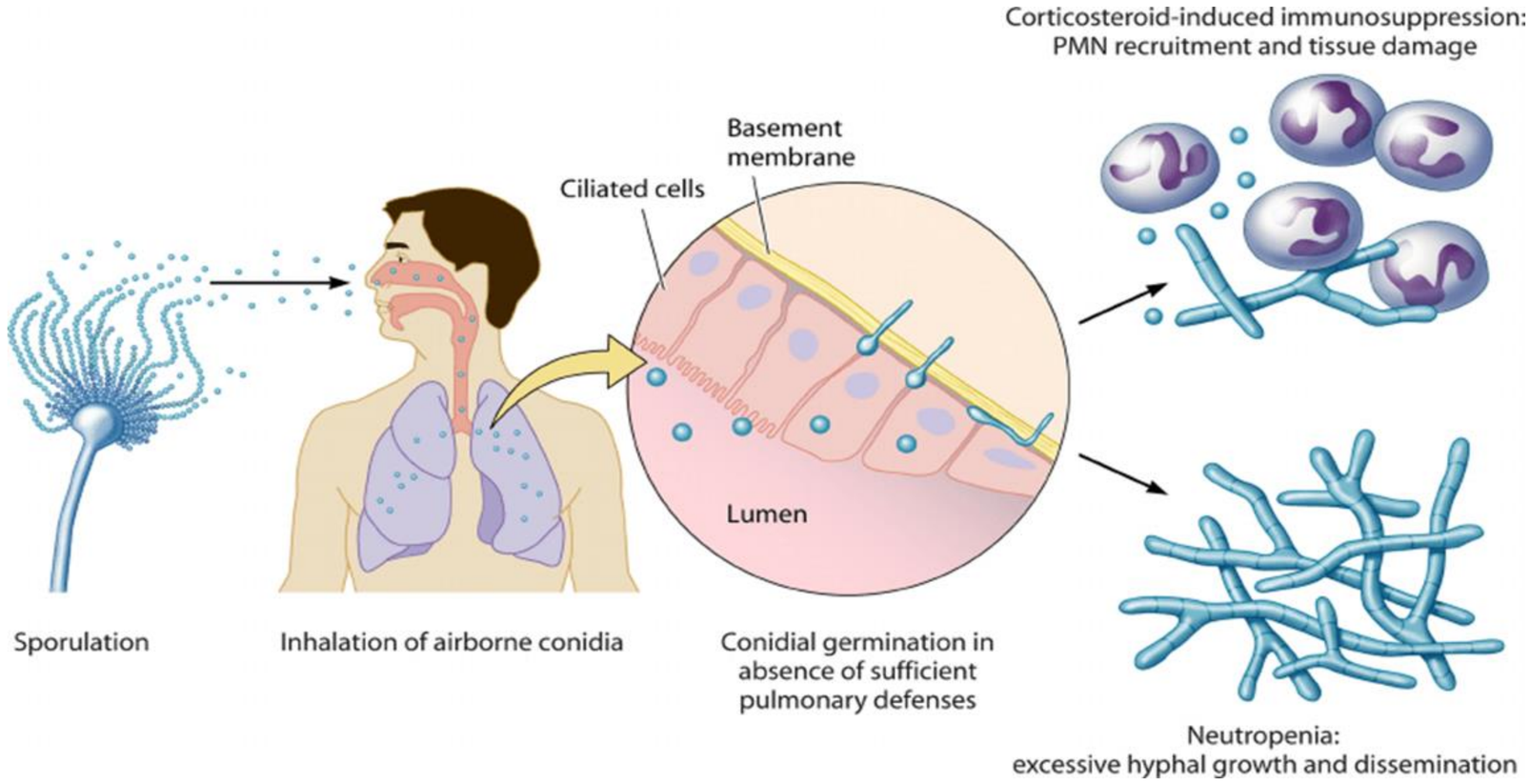
- Only a few species are considered as significant opportunistic pathogens in humans.

(Heitman 2011)



- *Aspergillus* section *Fumigati* is an opportunistic pathogen of immunocompromised hosts and one of several *Aspergillus* species that cause a wide range of respiratory disorders.

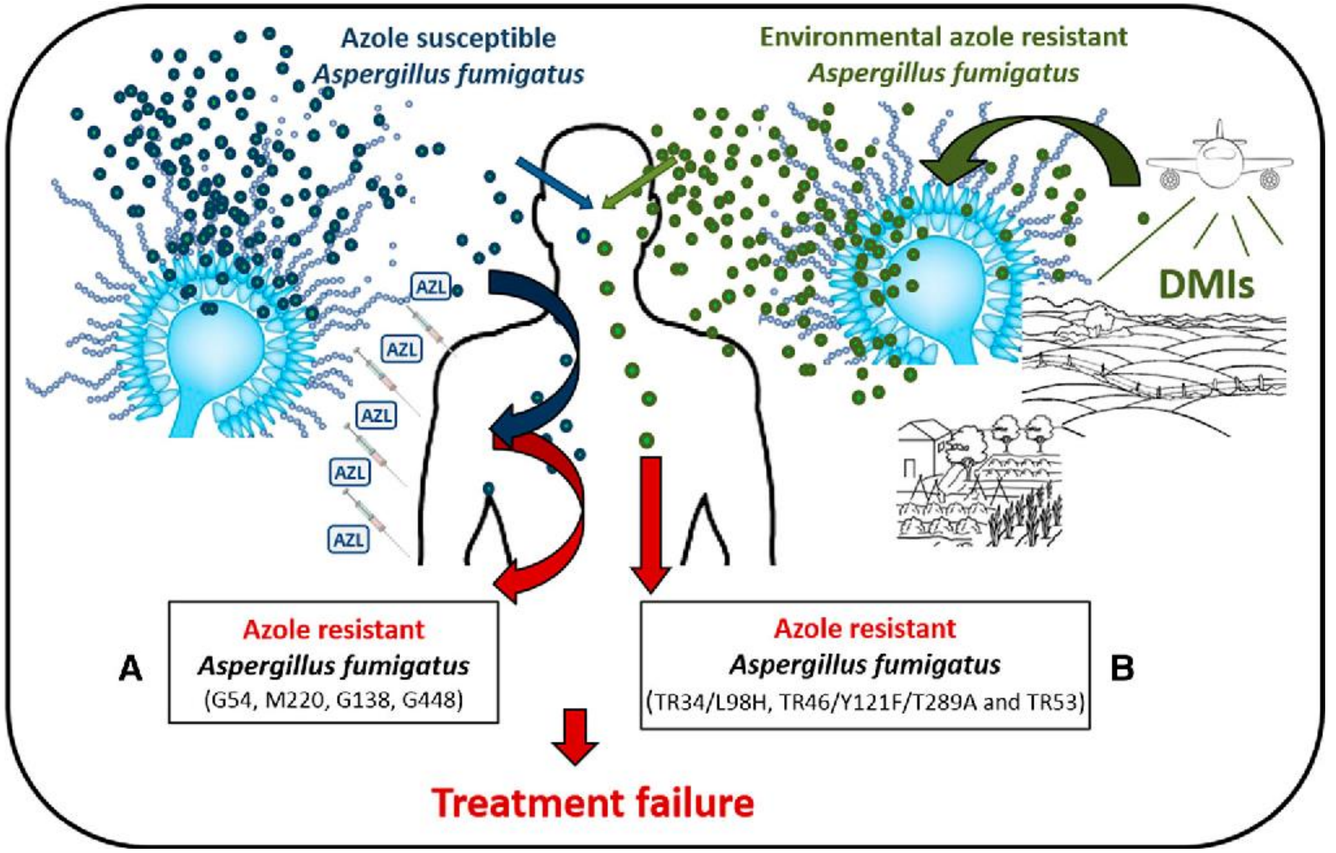
(Hope et al. 2008)



(Dagenais and Keller 2009)

Increased occurrence of opportunistic *Aspergillus* infections in immunocompromised patients, and the emergence of antifungal resistance, either in the clinical and in the environment had been reported.

(*Fairlamb et al. 2016; Nature Microbiology 2017)*



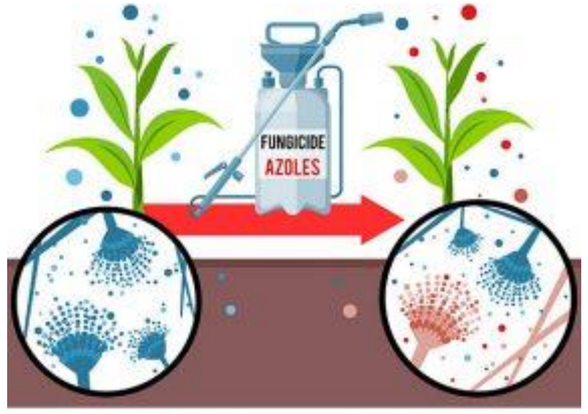
(Garcia-Rubio, Cuenca-Estrella and Mellado 2017)

Climate change

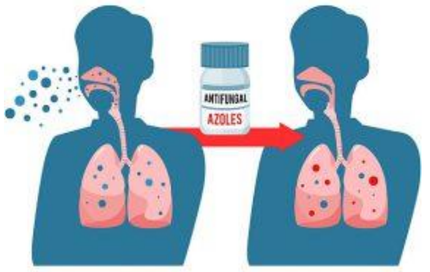
Increase of fungi and mycotoxins contamination

Intensive use of azoles in crops to avoid toxigenic species

Resistant species can multiply and survive (*A. fumigatus*)



Infections are hard to treat and life-threatening.



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Waste Management

journal homepage: www.elsevier.com/locate/wasman

Are workers from waste sorting industry really protected by wearing Filtering Respiratory Protective Devices? The gap between the myth and reality

Carla Viegas [a,b,*], Marta Dias [a], Beatriz Almeida [a], Liliana Aranha Caetano [a,c], Elisabete Carolino [a], Anita Quintal Gomes [a,d], Magdalena Twarużek [e], Robert Kosicki [e], Jan Grajewski [e], Geneviève Marchand [f], Susana Viegas [a,b]

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Environmental Research

journal homepage: www.elsevier.com/locate/envres

Cytotoxic effect of filtering respiratory protective devices from the waste sorting industry: is *in vitro* toxicology useful for risk characterization?

Carla Viegas [a,b,c,*,1], Magdalena Twarużek [d,1], Marta Dias [a], Beatriz Almeida [a], Elisabete Carolino [a], Ewelina Soszczyńska [d], Iwona Ałtyn [d], Susana Viegas [a,b,c], Liliana Aranha Caetano [a,e]

- **Filtering respiratory protection devices (**FRPD) were assessed
- High bioburden contamination
- High counts of *Aspergillus* spp. and *Aspergillus* section *Fumigati*
- No mycotoxins detected
- **TR34/L98H mutation detected in *A. fumigatus* isolates** *(to be published)*

- Mechanical Protection Gloves (MPG) are mandatory in waste-sorting industry in Portugal.

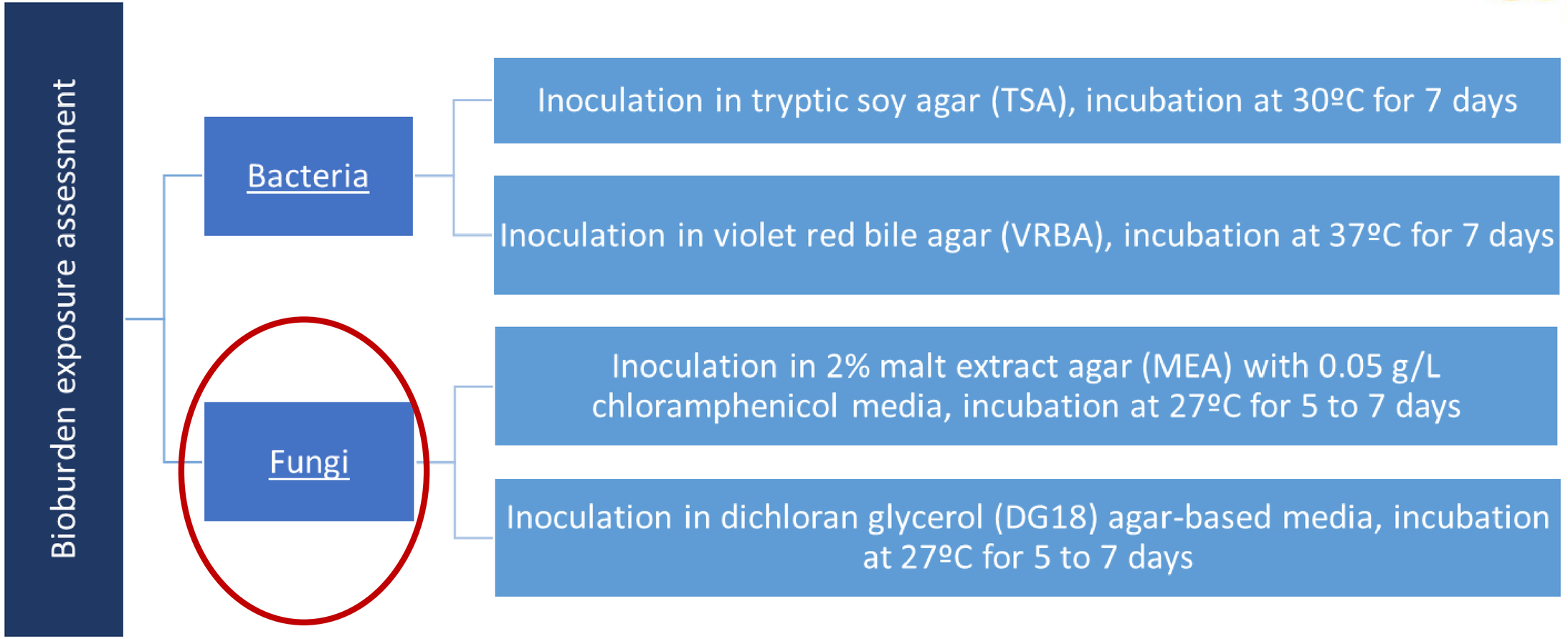
- The use of Mechanic Protection Gloves (MPG) by workers from waste sorting promotes humidity and temperature conditions providing a favorable environment for the growth of retained *Aspergillus.*

(Jankowska et al. 2000; Majchrzycka et al. 2016)

The aim of this study was to characterize *Aspergillus* spp. presence in MPG as well as to detect possible azole-resistant isolates.

2. Materials and methods

Workstations	Tasks	Number of Samples
FMW	Feeding machines with waste	9
SW	Sorting waste	40
MI	Machines inspection	10
MSVO	Machines and special vehicles operator	8
Total		**67**



Assays performed

Azole resistance (Sabouraud dextrose agar media supplemented with 4 mg/L itraconazole (ITR), 1 mg/L voriconazole (VOR), and 0.5 mg/L posaconazole (POS)

Toxigenic fungal strains (*Aspergillus* sections *Flavi, Fumigati, Circumdati* and *Nidulantes)*

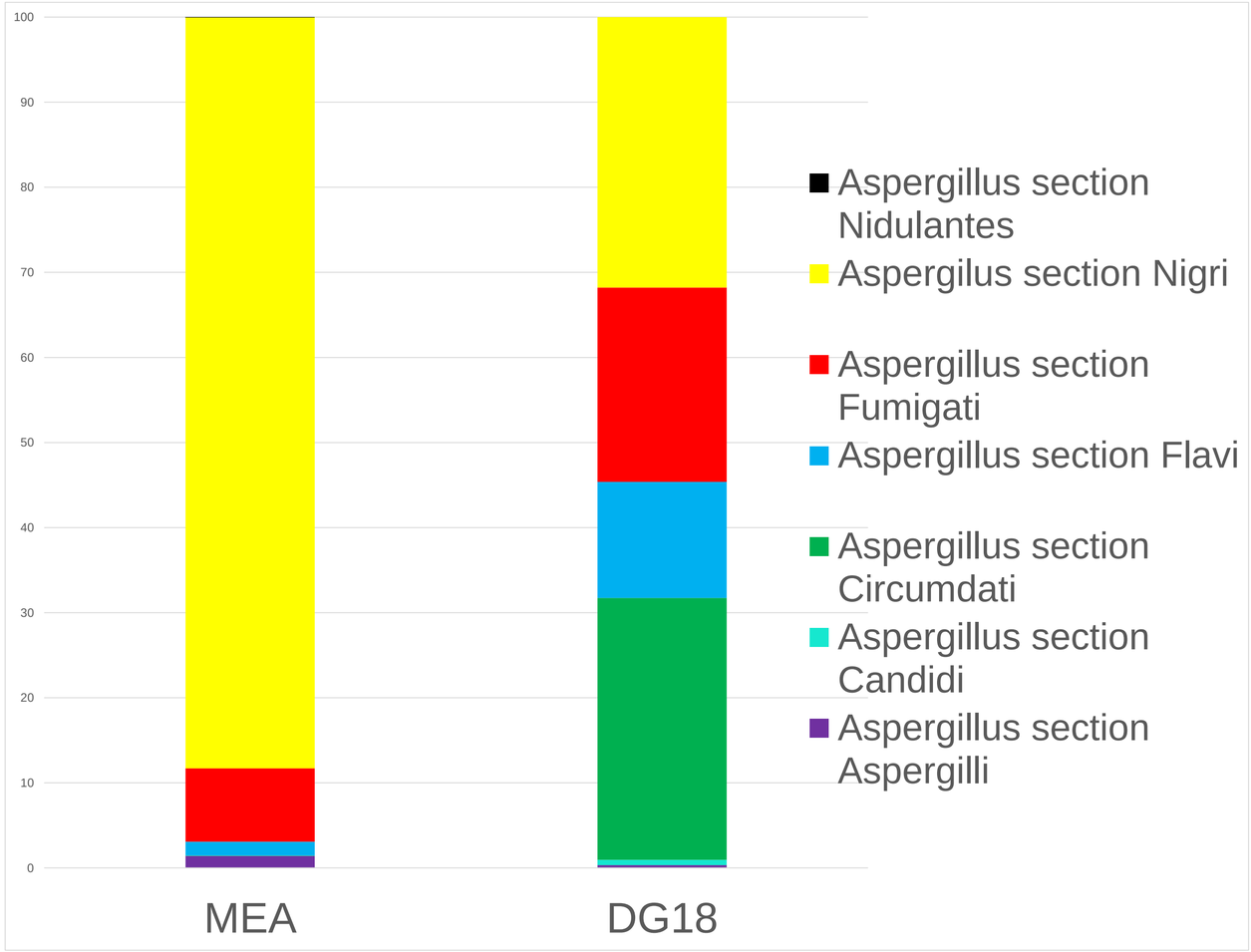
Mycotoxins

3. Results

Mycobiota

Species	MEA n	MEA CFU.m^{-2}	MEA %	DG18 n	DG18 CFU.m^{-2}	DG18 %
Aspergillus spp.	3.6504×10^{4}	1.8252×10^{7}	50.46	4.929×10^{3}	2.4645×10^{6}	18.18
Aureobasidium spp.	30	1.5×10^{4}	0.04			
Chrysosporium spp.				1.01×10^{2}	5.05×10^{4}	0.37
Cladosporium spp.	5.5×10^{2}	2.75×10^{5}	0.76	5.12×10^{2}	2.56×10^{5}	1.89
Fusarium spp.				10	5×10^{3}	0.04
Mucor spp.	2.7402×10^{4}	1.3701×10^{7}	37.88	1.053×10^{3}	5.265×10^{5}	3.88
Paecilomyces spp.				8	4×10^{3}	0.03
Penicillium spp.	6.82×10^{3}	3.41×10^{6}	9.43	2.0491×10^{4}	1.02455×10^{7}	75.59
Phoma spp.	29	1.45×10^{4}	0.04			
Rhizopus spp.	1.001×10^{3}	5.005×10^{5}	1.38			
Syncephalastrum spp.				5	2.5×10^{3}	0.02
TOTAL		3.6168×10^{7}	100		1.35545×10^{7}	100

Aspergillus sections



Azole screening

Aspergillus sections	SDA			ITR			POS		
	n	CFU.m^{-2}	%	n	CFU.m^{-2}	%	n	CFU.m^{-2}	%
Candidi	2	1.00×10^{3}	0.01	1	5.00×10^{2}	0.004			
Circumdati	41	2.05×10^{4}	0.16	5×10^{2}	2.50×10^{5}	1.97			
Clavati							1	5.00×10^{2}	0.02
Flavi	42	2.10×10^{4}	0.17	6	3.00×10^{3}	0.027			
Fumigati	3.77×10^{3}	1.88×10^{6}	14.86	2	1.00×10^{3}	0.01			
Nigri	2.11×10^{3}	1.06×10^{6}	8.33	5×10^{2}	2.50×10^{5}	1.97			
Versicolores				10	5.00×10^{3}	0.04			
Total		2.98×10^{6}	23.53		5.10×10^{5}	4.01		5.00×10^{2}	0.02

- Azole resistant *Aspergillus* sections were found in both ITR and POS supplemented media.
- In POS supplemented media only *Aspergillus* section *Clavati* was found.
- in ITR supplemented media, 6 different *Aspergillus* sections were found.

Molecular tools - qPCR

Species Detected	Culture-based		Molecular	
	Number of samples	%	Number of samples	%
Aspergillus section *Circumdati*	9	13.43	22	32.84
Aspergillus section *Flavi*	22	32.84	6	8.96
Aspergillus section *Fumigati*	19	28.36	59	88.06
Aspergillus section *Nidulantes*	1	1.49	61	91.05

- In all sections but one (*Flav*i), the number of samples in which each specific section was detected was higher in the molecular detection compared with the culture-based methods.

Mycotoxins were detected in 89.6% (60 out of 67) MPG samples

4. Main findings

- ***Aspergillus*** genus was **the most prevalent**
- ***Aspergillus* sections** presented **different distribution in MEA and DG18**
- **Toxigenic strains** and mycotoxins **were detected**
- **Azole resistant *Aspergillus* sections were found** in both ITR and POS

MPG can be used to assess occupational exposure to *Aspergillus* burden (and mycotoxins) and to screen azole resistance in waste sorting industries

Accurate risk characterization

5. Main conclusions (FRPD and MPG)

- High exposure to bioburden (fungi and mycotoxins)
- Exposure can happen directly by inhalation and by hand-to-mouth contact
- Found fungal resistant species probably driven by the high use of fungicides in different contexts (e.g. crops, consumer products...)
- Both protection devices need to be replace more frequently to avoid exposure and guarantee the protection needed.

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Assessment of the microbial contamination of mechanical protection gloves used on waste sorting industry: A contribution for the risk characterization

Carla Viegas [a,b,c,*], Magdalena Twarużek [d,**], Marta Dias [a], Beatriz Almeida [a], Elisabete Carolino [a], Robert Kosicki [d], Ewelina Soszczyńska [d], Jan Grajewski [d], Liliana Aranha Caetano [a,e], Susana Viegas [a,b,c]

Thank you for your attention

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