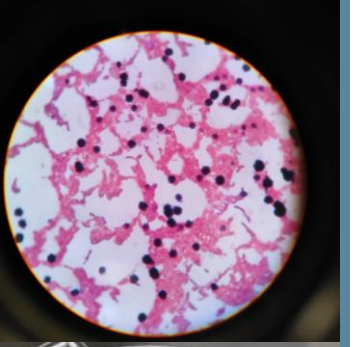
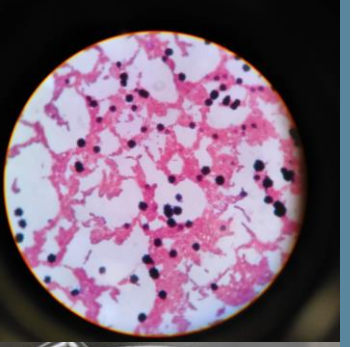



ExPOSE - Avaliação da exposição a micobiota resistente a antifúngicos

Liliana Aranha Caetano, Carla Viegas

HTRC Health and Technology Research Center
ESCOLA SUPERIOR DE TECNOLOGIA DA SAÚDE DE LISBOA
INSTITUTO POLITÉCNICO DE LISBOA
FCT Fundação para a Ciência e a Tecnologia
NORTE2020 PROGRAMA OPERACIONAL REGIONAL DO NORTE
Lisb@20[20]
PORTUGAL 2020
UNIÃO EUROPEIA Fundo Europeu de Desenvolvimento Regional



MYCOBIOTA IN CLINICAL ENVIRONMENTS

- Poor hospital indoor air quality (IAQ) may lead to **hospital-acquired infections**, sick hospital syndrome and various occupational hazards.

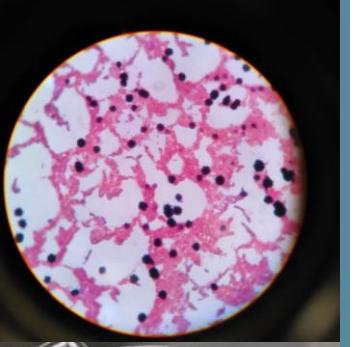
Cabo-Verde et al. Res. Microbiol. 2015

- **Microbiological IAQ monitoring and control in hospitals** is currently a necessary and integral part of prevention strategies against hospital-acquired infections.

Zahar et al. J Mycol Med. 2017

- Implementation of **sampling and analysis methods** should be adapted to hospital environment.

Baurès et al. Sci Total Environ. 2018



MYCOBIOTA IN CLINICAL ENVIRONMENTS

- Bacterial, viral and **fungal infections** are frequently acquired via inhalation, among them **pulmonary aspergillosis** and pneumocystosis still represent high disease burden.

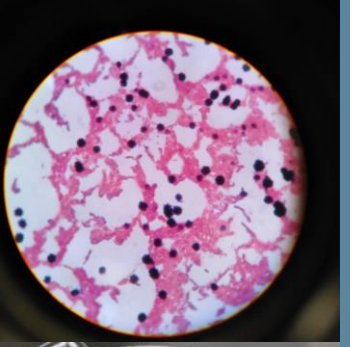
Gangneux et al. J Mycol Med. 2016

- A large number of fungal species can cause severe infections, specially among **immunocompromised** individuals.

Springer et al. 2016

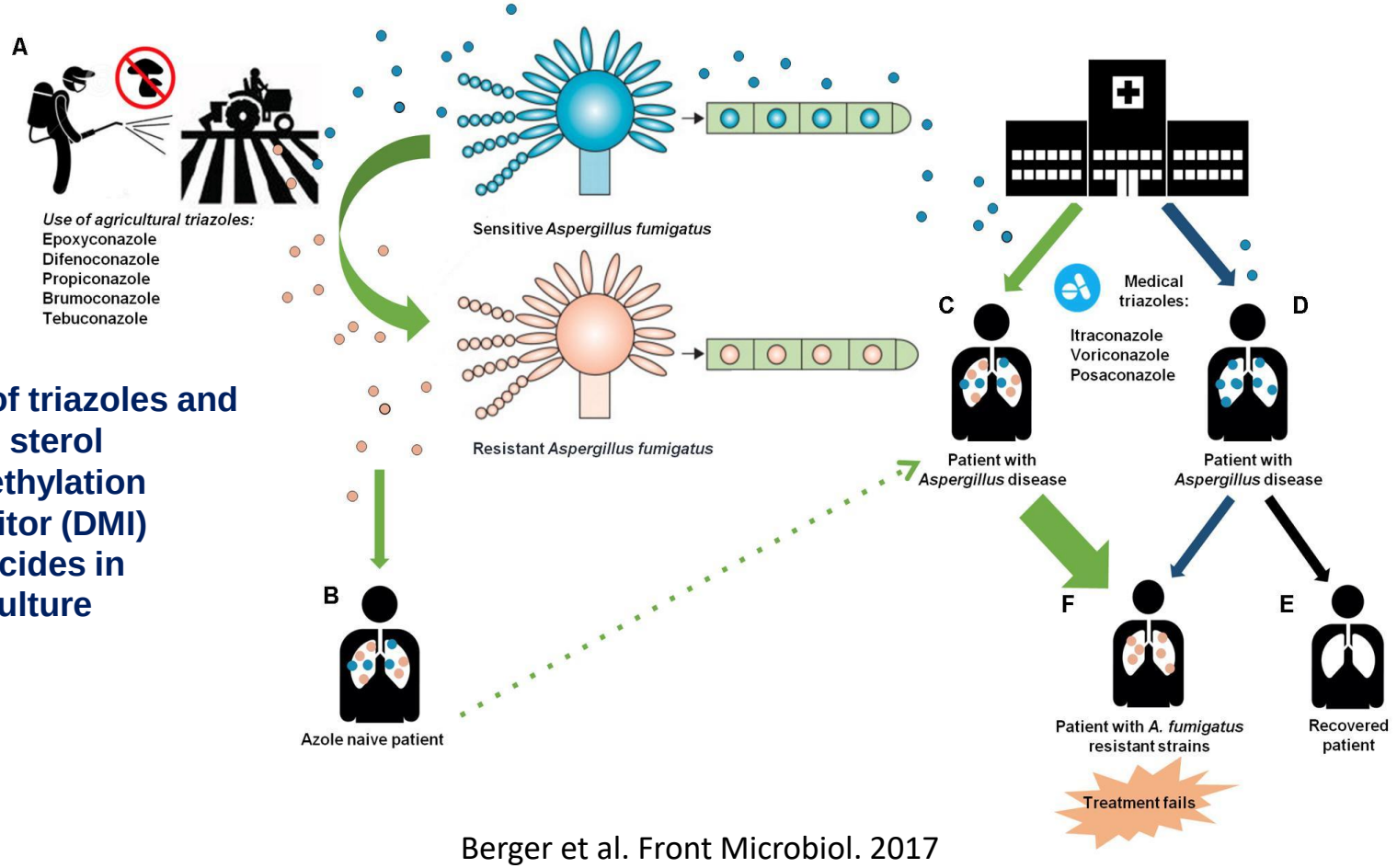
- Most important fungi related with fungal exposure: *Cladosporium, Alternaria, Stachybotris, Penicillium,* ***Aspergillus.***

Sabino et al. 2018

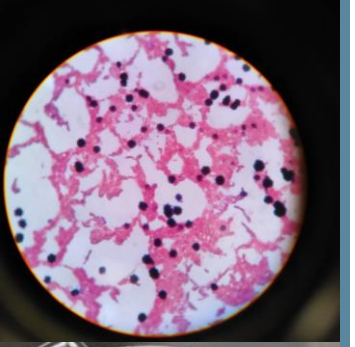


EMERGENCE OF AZOLE RESISTANCE

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



Berger et al. Front Microbiol. 2017

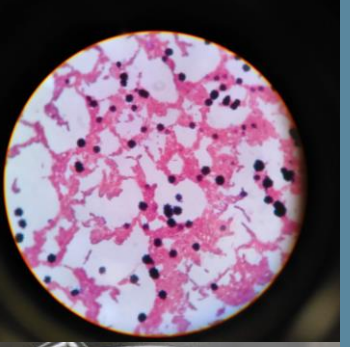


AZOLE RESISTANCE AS A PUBLIC HEALTH THREAT

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

- Exposure to fungal isolates with less susceptibility to antifungals. Intrinsic vs. Secondary (azole-induced) resistance. (Meis et al. 2016)

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



MONITORING MYCOBIOTA IN CLINICAL ENVIRONMENTS

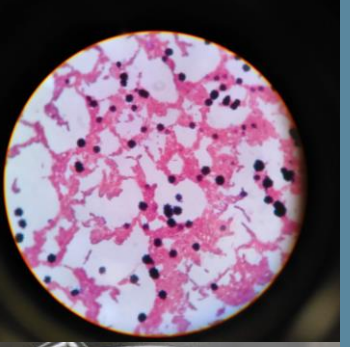
Multi-approach sampling strategy

Active air sampling methods

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

Passive sampling methods

- Surface and vaccumed dust samples
- Electrostatic dust cloths (EDC)
- Air-conditioning filters



Environmental Samples – Hospital and PHCC

Air sampling, Air conditioning filters , EDC, Vacumming and Surface swabs

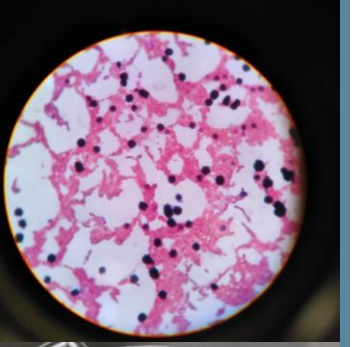
Culture methods for total mycobiota and molecular detection of *Aspergillus* section *Fumigati*

Identification/detection directly from the environmental matrix

Yes

No

Screening of azole resistance for total sample and for *A. fumigatus* isolates



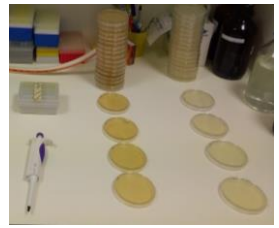

SCREENING FOR AZOLE RESISTANCE

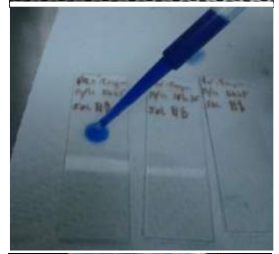
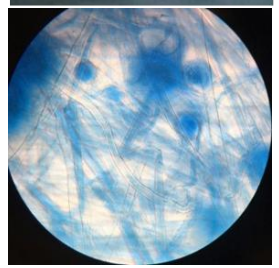
I. 150 µL of sample wash suspension on Saboraud agar supplemented with:
   - 4 mg/L itraconazole (ITC)
   - 1 mg/L voriconazole (VCZ)
   - 0.5 mg/L posaconazole (PSC) (EUCAST, 2017).

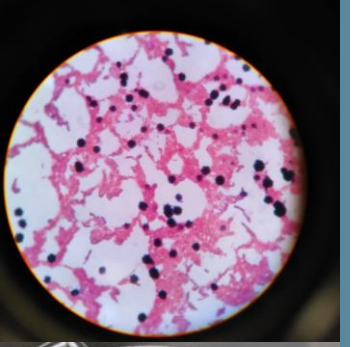
II. Incubation at 27 ºC for 3 to 5 days

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

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



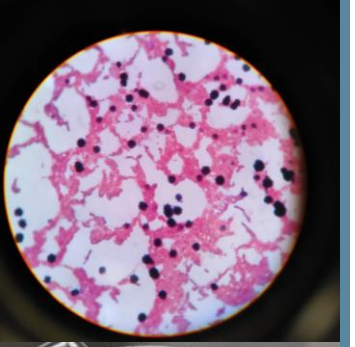



TOTAL FUNGAL BURDEN IN PRIMARY HEALTH CARE CENTERS (PHCC) – Passive sampling methods

	SDA			ITRA			VORI			POSA		
PHCC	EDC	Filter	Dust	EDC	Filter	Dust	EDC	Filter	Dust	EDC	Filter	Dust
1	53715	0	10	0	0	1	318	0	1	0	0	1
2	267196	500	5	0	0	1	212	500	1	0	0	0
3	424628	1500	2	212	500	0	159554	1000	1	212	0	0
4	165496	56500	7	955	21500	0	107643	15500	0	0	19500	0
5	107219	0	3	53503	0	0	53185	0	0	0	0	0
6	1004	1000	514	954	0	0	212	0	3	0	0	0
7	1166	3000	10	53715	500	0	106	1500	1	106	0	0
8	2120	1000	30	318	1000	1	318	3500	2	0	4500	1
9	348	1500	17	107	0	502	2	0	5	1	0	0
10	3077	n.d.	13	531	n.d.	6	2547	n.d.	0	212	n.d.	0

EDC (CFU.m-2), HVAC filters (CFU.m-2), and settled dust (CFU.g-1). n.d. – not determined

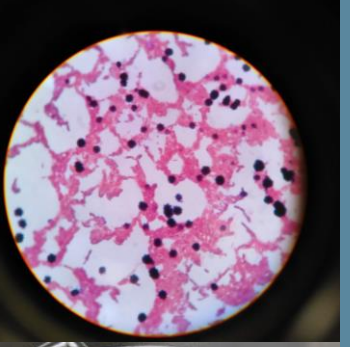
Caetano et al. 2019
Proceedings of the Healthcare Ergonomics and Patient Safety, HEPS, 3-5 July, Lisbon, Portugal




FUNGAL SPECIES/GENERA BY SAMPLING METHOD

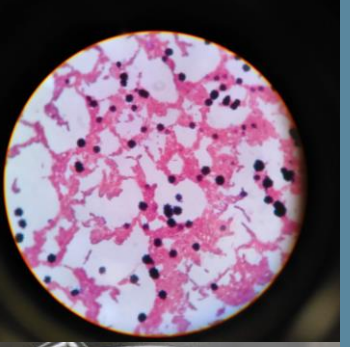
Sample	Fungi	SDA		ITRA		VORI		POSA	
		n	%	n	%	n	%	n	%
EDC (CFU/m2)	Alternaria sp.	156	0.02	212	0.19				
	Aspergillus section Candidi	318	0.04						
	Aspergillus section Circumdati	106	0.01						
	Aspergillus section Fumigati	106	0.01						
	Aspergillus section Nigri	106	0.01						
	Aspergillus section Versicolores	849	0.12						
	Aureobasidium sp.			106	0.10	106	0.03		
	Chrysonilia sitophila	636944	90.05	106476	96.45	318579	98.30		
	Chrysosporium sp.	1485	0.21			106	0.03	212	40.00
	Cladosporium sp.	7742	1.09	1167	1.06	1484	0.46	106	20.00
	Fusarium verticilloides	424	0.06						
	Fusarium solani			212	0.19				
	Paecilomyces sp.	53079	7.50						
	Penicillium sp.	5834	0.82	2015	1.83	3821	1.18	212	40.00
	Rhizopus sp.	212	0.03						
	Others			212	0.19				
HVAC filter (CFU/m2)	**Aspergillus section Cremei**	500	0.77						
	Aspergillus section Versicolores	500	0.77						
	Alternaria sp.	500	0.77						
	Chrysosporium sp.	1000	1.54			1000	4.55		
	Cladosporium sp.	9000	13.85	21500	91.49	11000	50.00	19500	81.25
	Mucor sp.	500	0.77	500	2.13				
	Penicillium sp.	53000	81.54	1500	6.38	10000	45.45	4500	18.75
Settled dust (CFU/g)	Alternaria sp.	4	0.65			1	7.14		
	Aspergillus section Fumigati	3	0.49						
	Chrysonilia sitophila	503	82.32	500	97.85				
	Chrysosporium sp.	13	2.13	1	0.20	1	7.14		
	Cladosporium sp.	10	1.64					1	100.00
	Penicillium sp.	76	12.44	10	1.96	6	42.86		
	Stemphilium sp.	2	0.33						
	Others					6	42.86		
Vacuum bag (CFU/m2)	Chrysosporium sp.			500	7.14				
	Penicillium sp.	1000	100.00	6500	92.86	1500	33.33		
	Rhizopus sp.					3000	66.67		

Caetano et al.
HEPS 2019




AZOLE RESISTANCE FINDINGS IN 10 PHCC

- Mycobiota able to grow on azole-media observed in 10/10 PHCC
- Most common scenario: fungal growth in 1 azole only
  - Chrysosporium sp. in ITRA, VORI or POSA
  - C. sitophila in ITRA or VORI
- Multi-azole resistance (fungal growth in >1 azole) in 9/10 PHCC
  - Penicillium sp. (PHCC 2, 3, 4, 6, 7, 8 and 10)
  - C. sitophila (PHCC 5 and 9)
  - Cladosporium sp. (PHCC 4, 6, 7 and 8)
- No azole resistance observed for *Aspergillus* sp.

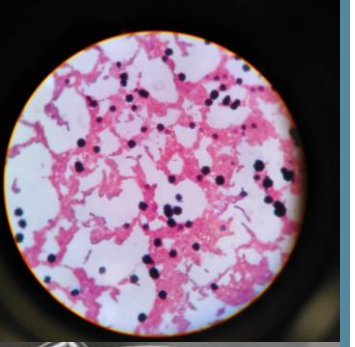



LIMITATIONS/OPPORTUNITIES

- 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**

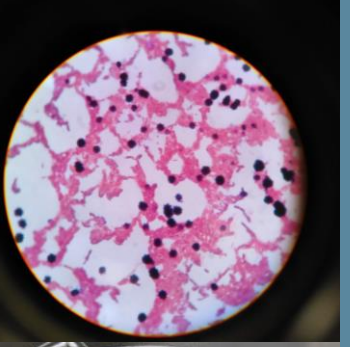



TAKE HOME MESSAGES

- The presence of azole-resistant fungal species in clinical settings may potentially place patients and healthcare staff at high health risk
  - **Exposure to resistant fungi may reach infectious levels within a confined space more readily**

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

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



Surveillance of azole-resistant *Aspergillus*

Collection of environmental samples
(Passive sampling)

Air sampling, Air conditioning filters , EDC, Settled dust, Filter protection devices (masks), Litter, Wood shavings, Sand, Food commodities

Sample treatment and fungal growth (MEA and DG18 media)

Avoid duplicates by selecting only 1 media to isolate *Aspergillus* section *Fumigati*

Identification of *Aspergillus* section *Fumigati*
(morphological and microscopy criteria)

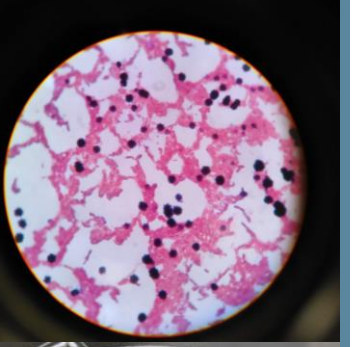
Detection of cryptic species
(sequencing)

Screening of fungal resistance
(azole-supplemented media with multi-resistant and susceptible strains as control)

Aspergillus fumigatus
sensu stricto

Future work!

Detection of mutations (Aspergenius™ kit)




ACKNOWLEDGEMENTS

The authors are grateful to FCT – Fundação para Ciência e Tecnologia for funding the project EXPOsE – Establishing protocols to assess occupational exposure to microbiota in clinical settings (02/SAICT/2016 – Project nº 23222).

THANK YOU FOR YOUR ATTENTION