Occupational exposure to environmental resistant fungi and possible implications in human health

Raquel Sabino
INSA, Portuguese NIH
ISAMB, Instituto de Saúde Ambiental

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raquel.sabino@insa.min-saude.pt

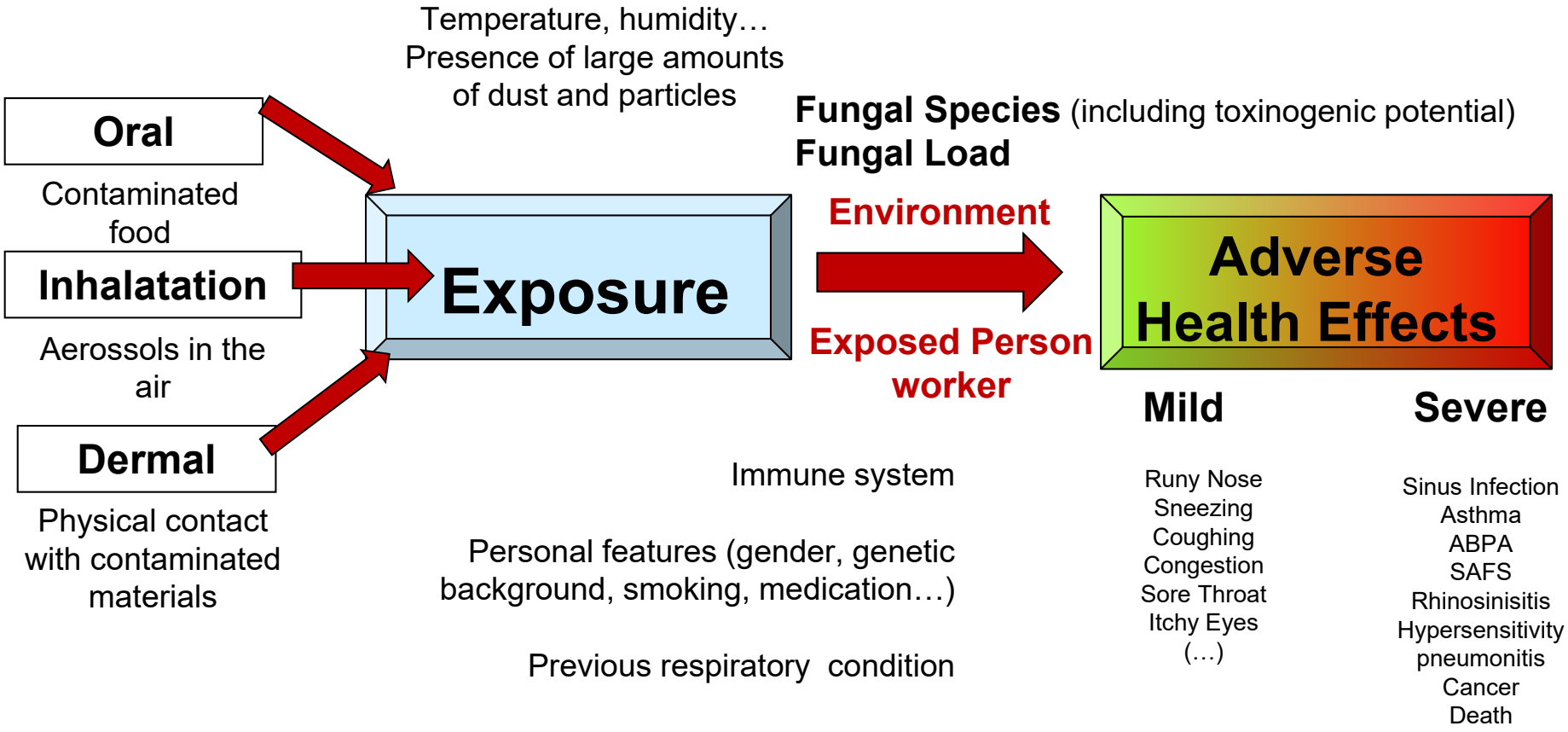
Ministério da Saúde

Instituto_Nacional de Saúde
Doutor Ricardo Jorge

Summary

- Occupational exposure and sources of fungal exposure
- *Aspergillus* exposure and infection
- Antifungal resistance in *Aspergillus* (how, when and why) and associated health implications
- Sources of environmental *Aspergillus* resistance

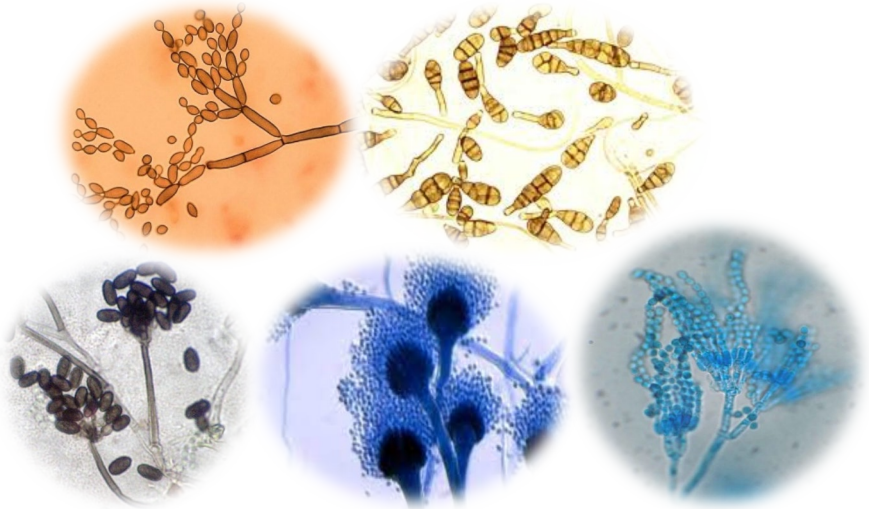
Occupational exposure vs. development of fungal disease

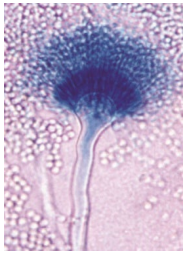
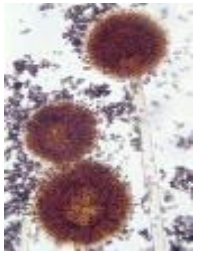
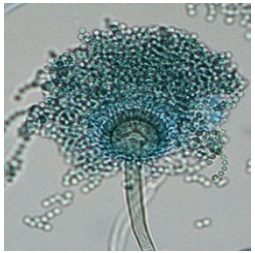
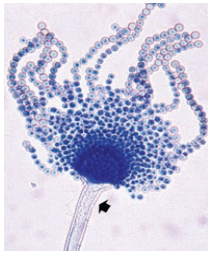


Occupational exposures to fungi

Most important fungi related with fungal exposure:

- *Cladosporium*
- *Alternaria*
- *Stachybotris*
- *Penicillium*
- *Aspergillus*




Aspergillus conidia

With dry wall spores, dessication-resistant

Very light

Produced in large amounts

Easy to dissociate

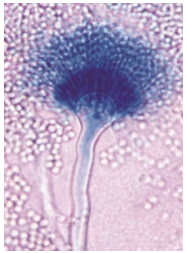
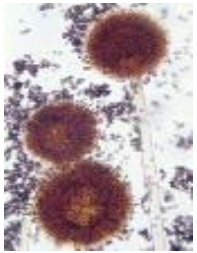
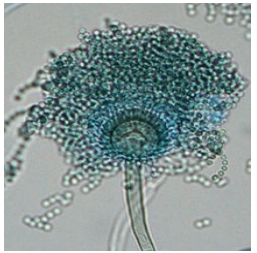
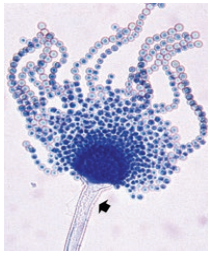
Very easy to disperse

Long time in the air, associated to other particles

Easily airborne and inhaled

Why are *Aspergillus* so commonly found in occupational environments?

- Ability to grow at a high range of temperatures
- High nutritional versatility
- Moisture environments
- Good growth on a high variety of construction materials (concrete, acrylic paints, wood based and cellulose based materials)
- Associated with decomposing organic mater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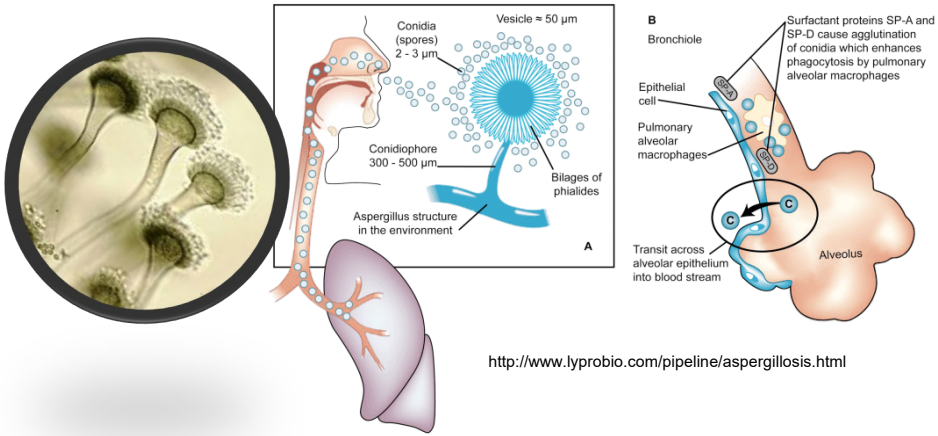


http://www.lyprobio.com/pipeline/aspergillosis.html

Diseases associated with occupational exposures to *Aspergillus*

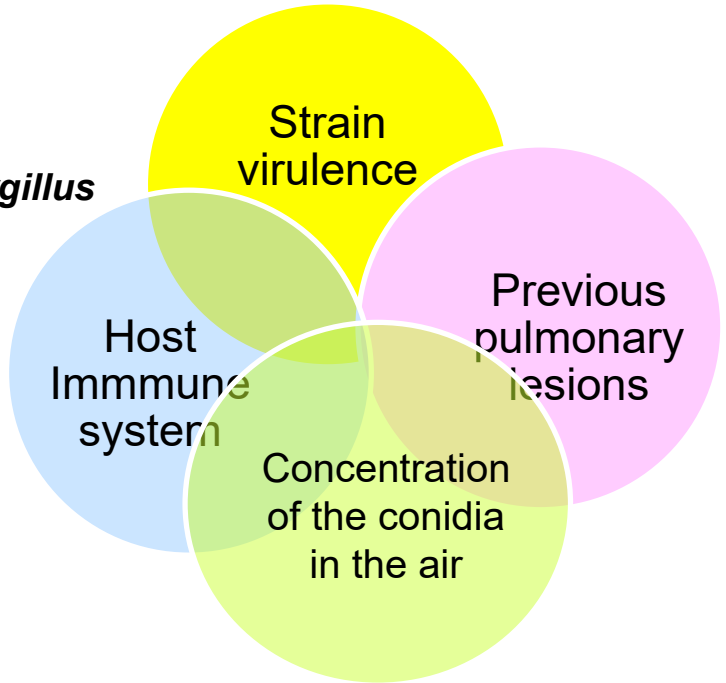
1) Allergic and other hypersensivity responses

2) Mycotoxicosis

3) Irritant effects caused by mold exposure

4) Opportunistic infection

Mycotoxins
MVOC's
Thermotolerance
Antifungal resistance
(…)



Invasive Pulmonary Aspergillosis

- More than **30 milions of patients** are at risk for invasive aspergillosis (corticosteroids, immunosuppressors).
- Incidence in Europe/ US between 1.1 and 4.6/ 100 000 population - trends show increase
- *Aspergillus* infection is associated with an increased length of stay of 5 to 10 days and excess costs of $15,000-$45,000, costs twice as high as in matched non- *Aspergillus* patients.
- About **300,000 patients** will develop the disease **annually**. More than 125,000 of these patients have COPD. Diagnosis is difficult; treatment often too late, only partially effective; about **50% mortality** if treated, **>99%** if not.
- Crude mortality rate between 14 and 44%
- Azole resistance in 1.5- 13% of the cases

http://www.life-worldwide.org/fungal-diseases/invasive-aspergillosis

Occupational Exposure to *Aspergillus*

- Home
- Workplace
- During hospitalization (especially during construction or renovation work)

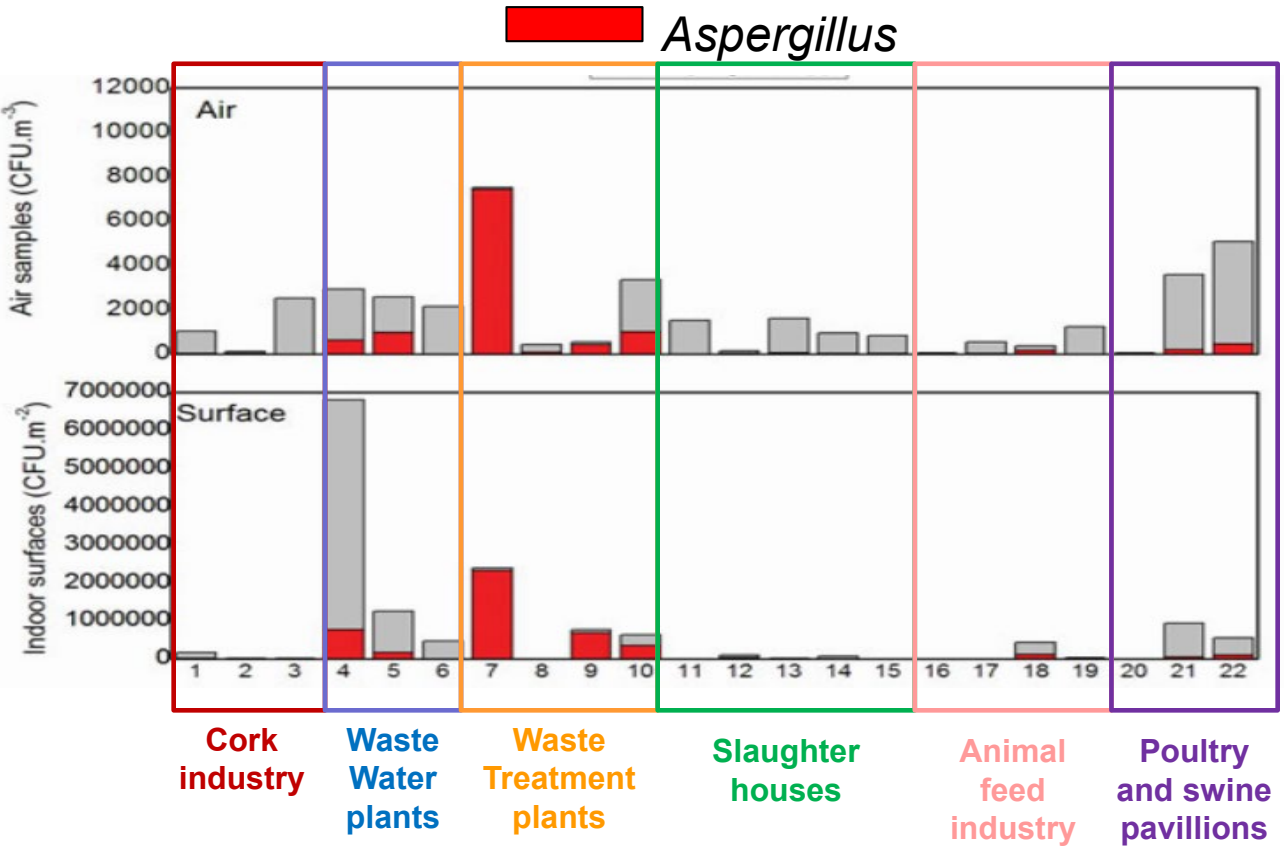









Prevalence of *Aspergillus* spp. in highly contaminated occupational environments



125 air samples
125 surface samples

12% of the air samples had an *Aspergillus* load that surpassed the limit of 150 CFU/m3 (WHO)

All air samples presented a higher fungal load indoor when compared to the outdoor samples

Viegas C et al Journal of Occupational and Environmental Hygiene 2017, 14:10, 771-785

Figure reproduction kindly authorized by Prof. Carla Viegas

Exposure to *Aspergillus* in agricultural settings

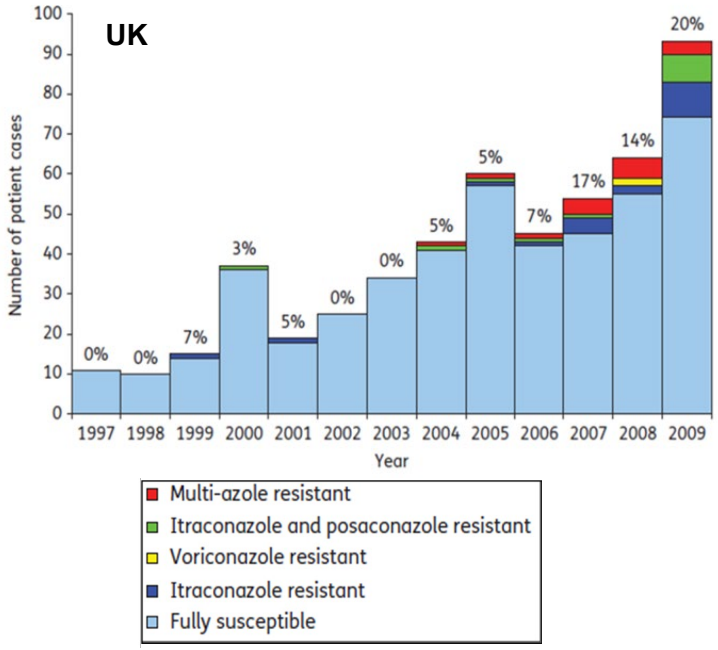




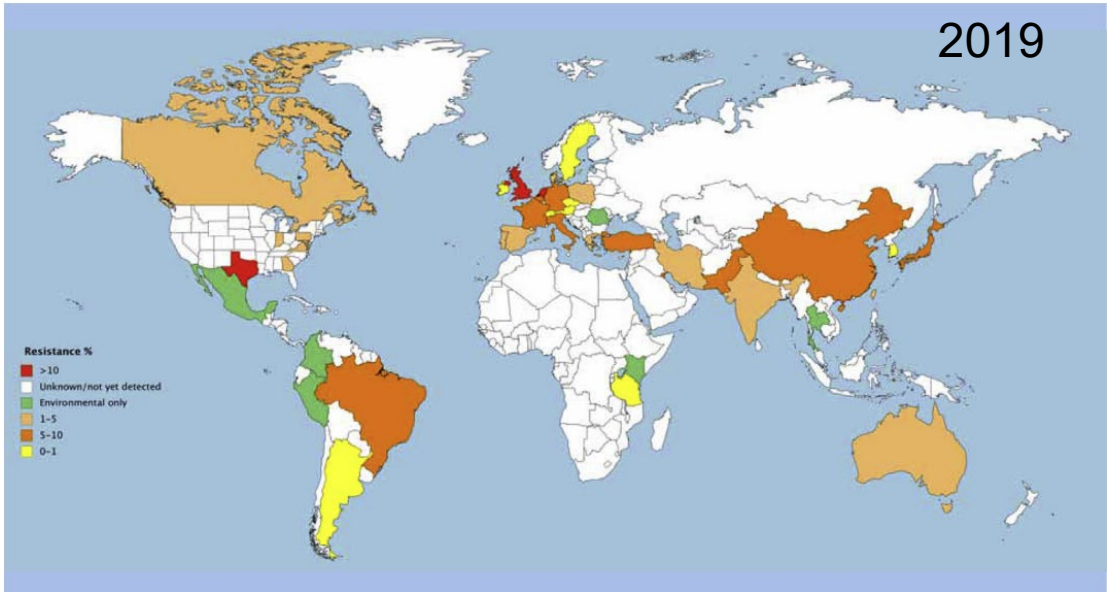

- High amounts of inhaled fungal spores
- Exposure to antifungal resistant spores

Aspergillus fumigatus azole-resistance

Evolution along the years and geographic distribution of azole resistant isolates?

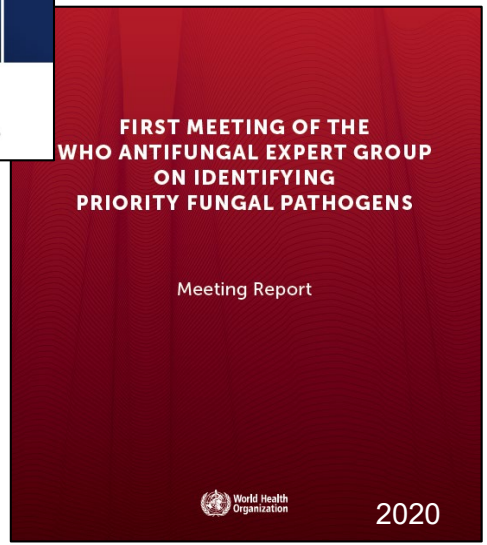


Bueid et al., J Antimicrob Chemother 2010; 65: 2116–2118
Figure reproduction kindly authorized by Prof David Denning



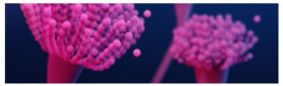
Lestrade et al., Clinical Microbiology and Infection 25 (2019) 799e806
Figure reproduction kindly authorized by Prof

CDC's Antibiotic Resistance Threats in the United States, 2019



Watch List

CDC's Watch List includes three threats that are uncommon, or the full burden of these germs is not yet understood in the United States. There is the potential for these resistant germs to spread across borders and cause significant morbidity and mortality. CDC and other public health experts are closely monitoring these germs, which have the potential to be included as listed threats in the future. Early detection of resistant germs within the United States, followed by implementation of prevention strategies, could reduce spread and public health impact.



AZOLE-RESISTANT
ASPERGILLUS FUMIGATUS

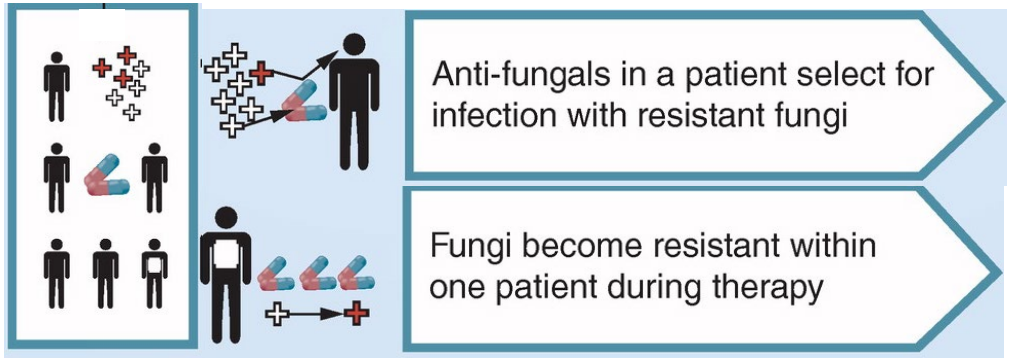
Summary of discussion points on fungal pathogen selection and prioritization

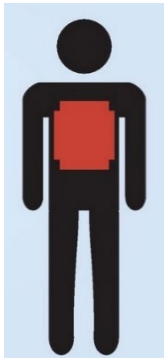
There was overall consensus that the fungal pathogens initially set out by the WHO secretariat (*Candida auris*; azole-resistant *Candida spp.*, azole-resistant *Aspergillus fumigatus*; *Cryptococcus neoformans & gattii; Pneumocystis jirovecii*; *Mucorales; and* potentially *Histoplasmosis*) were all of global public health importance and should be evaluated based on limitations of treatment options due to resistance and/or existing treatability issues (e.g. *Mucorales* has limited treatment options and poor outcomes).

Aspergillus azole-resistance

- **Primary resistance** (intrinsically resistant)
  - All individuals of the same species are resistant to a specific antifungal
- **Secondary resistance** (acquired resistance)
  - Only some isolates of a certain species are resistant to a specific antifungal

Clinical settings





Adapted from Halliday, Chen & Sorrell Future Microbiol. (2018) 13(10), 1175–1191
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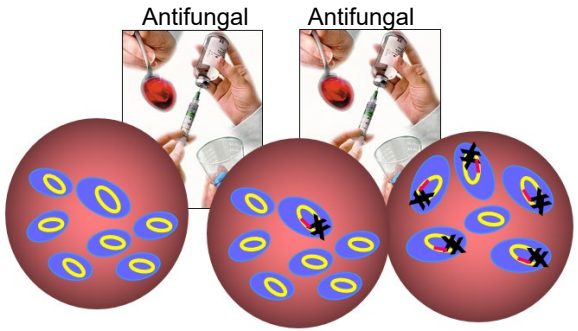
Medical Mycology April 2011, **49**(Suppl. 1), S82–S89

Review Article

Aspergillus species intrinsically resistant to antifungal agents

JAN W. M. VAN DER LINDEN*†‡, ADILIA WARRIS†‡ & PAUL E. VERWEIJ*†‡



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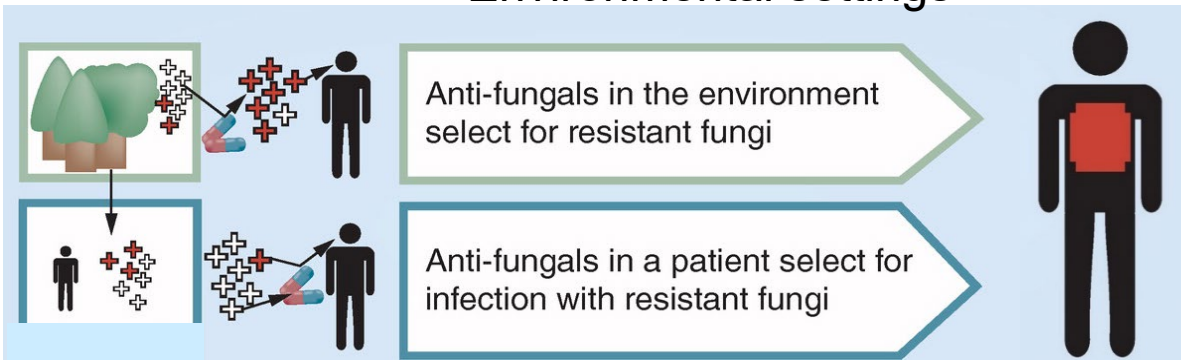
Environmental settings



Adapted from Halliday, Chen & Sorrell Future Microbiol. (2018) 13(10), 1175–1191
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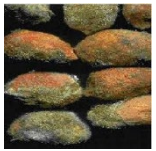
EXPOSURE to high levels of environmental isolates with intrinsic / less susceptibility to antifungals

EXPOSURE to environmental isolates with induced azole-resistance (secondary resistance): in agricultural / swammils settings– PESTICIDES/ OTHER FUNGICIDES????

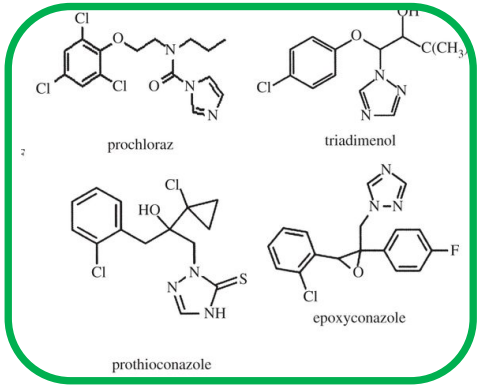
Development of *Aspergillus* resistance in environmental settings – How, Why?



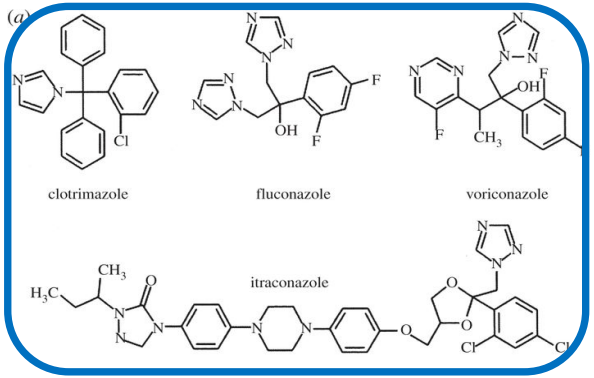





Use of triazoles and other sterol demethylation inhibitor (DMI) fungicides in agriculture

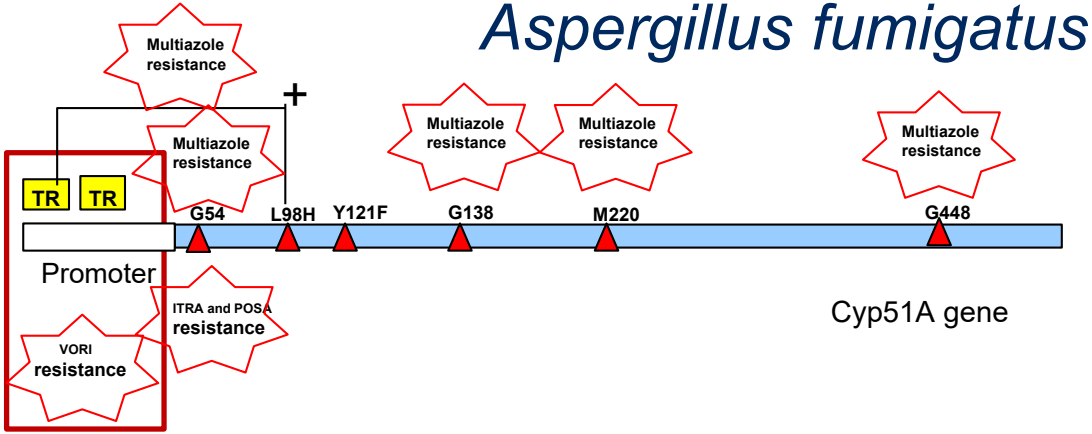


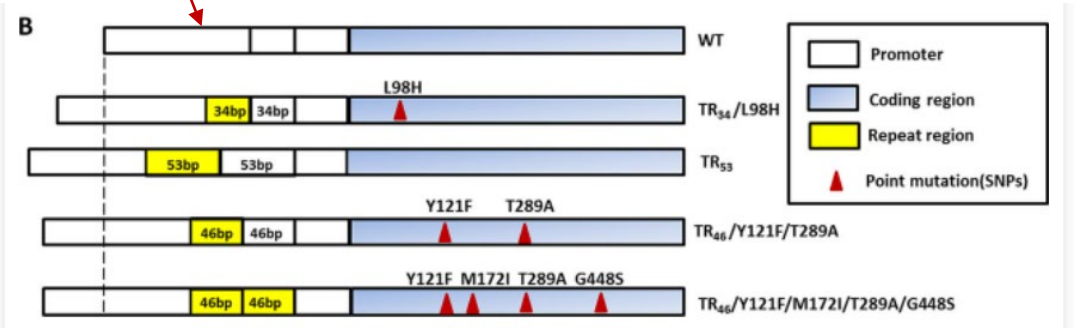
Prochoraz,
Difenoconazole
Brumoconazole
Triadimenol,
Epoxyconazole
Prothioconazole
Tebuconazole



Kelly S L , and Kelly D E Phil. Trans. R. Soc. B 2013;368:20120476

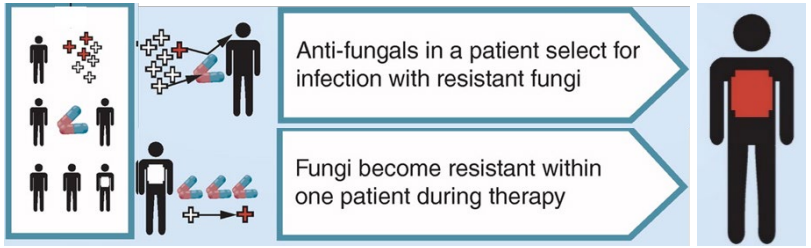
Aspergillus fumigatus azole-resistance





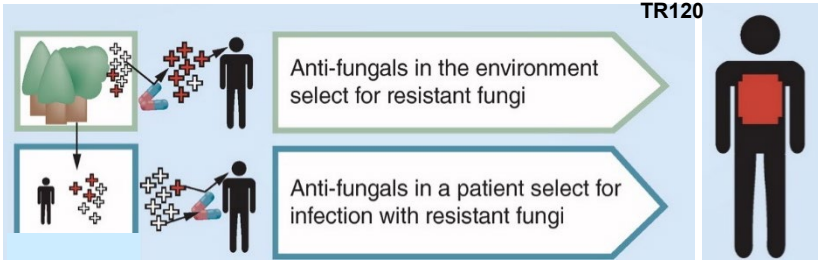
Point Mutations
G54
G138
F219
M220
Y431
G448

Clinical settings



TR34
TR46
TR53
TR120
+ Point Mutations

Environmental settings



Zhang, J et al., mBio 8 (3), e00791–17. doi: 10.1128/mBio.00791-17

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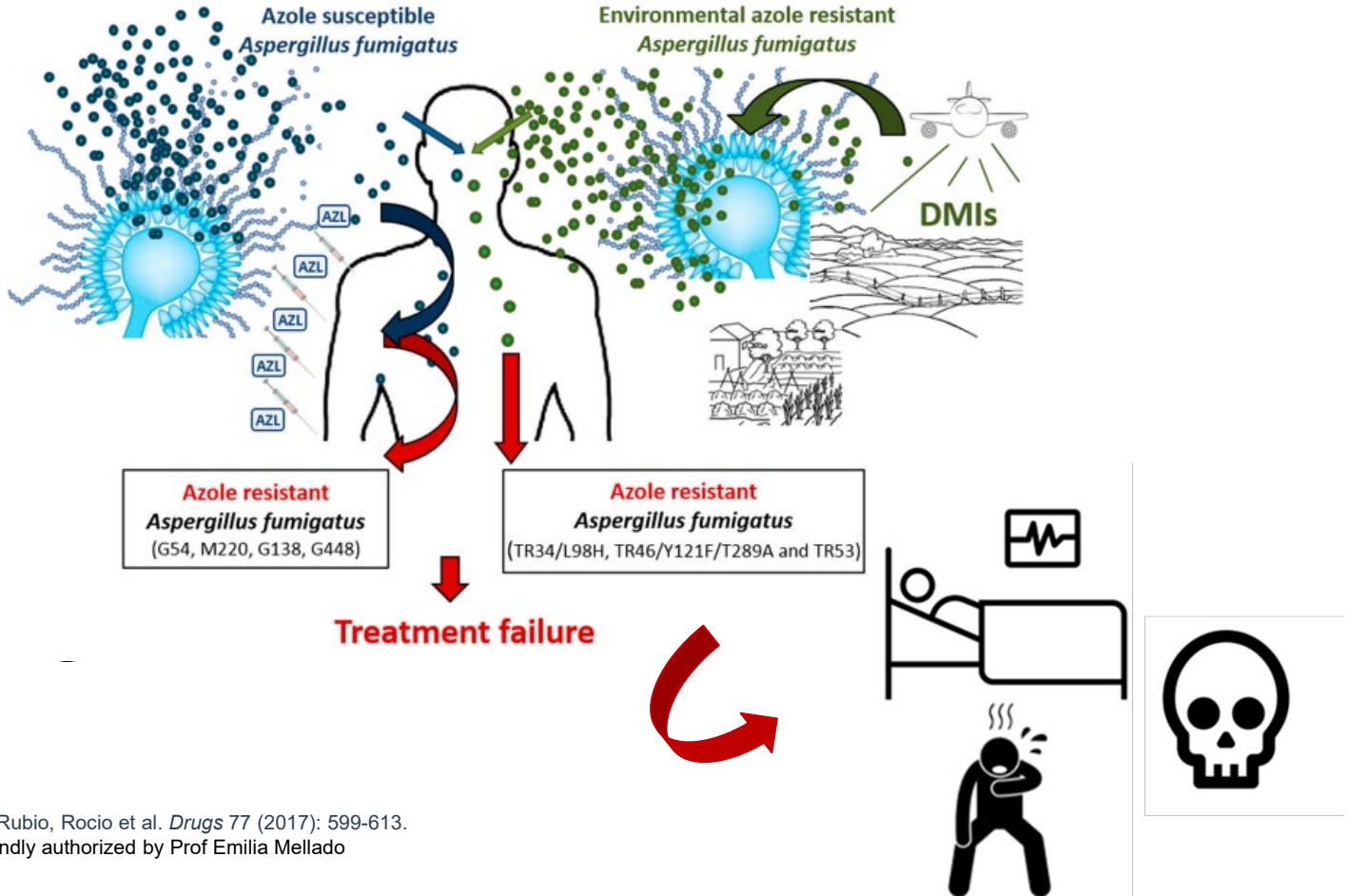

Adapted from Garcia-Rubio, Rocio et al. *Drugs* 77 (2017): 599-613.
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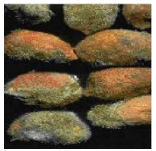
Development of *Aspergillus* resistance in environmental settings – How, Why?





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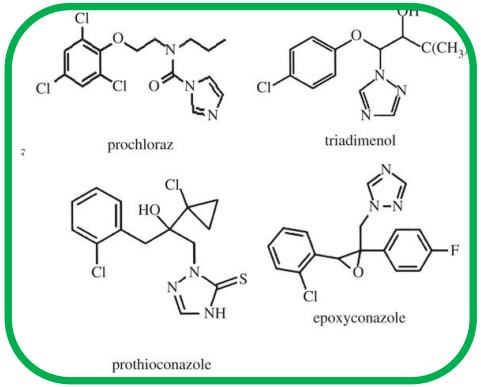

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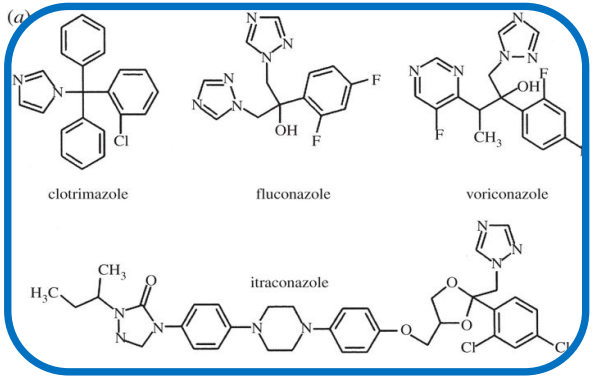
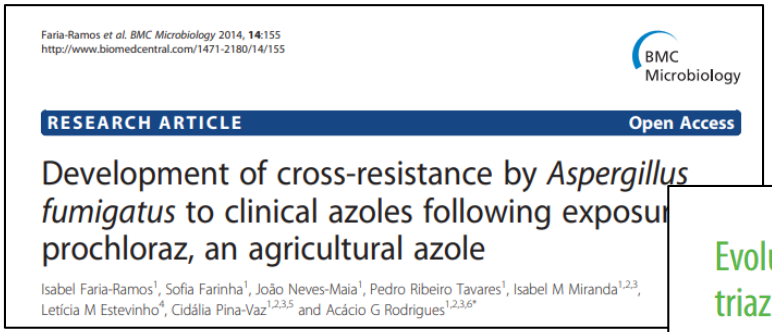


Kelly S L , and Kelly D E Phil. Trans. R. Soc. B 2013;368:20120476

Can the agricultural fungicides induce resistance to clinical azoles?

Development of *Aspergillus* resistance in environmental settings – How, Why?



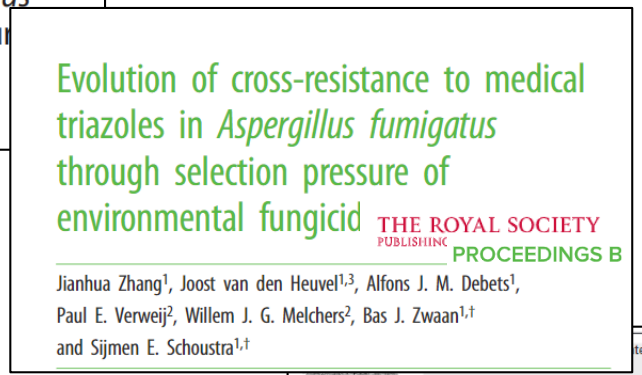
Faria-Ramos *et al. BMC Microbiology* 2014, **14**:155
http://www.biomedcentral.com/1471-2180/14/155

BMC Microbiology

RESEARCH ARTICLE Open Access

Development of cross-resistance by *Aspergillus fumigatus* to clinical azoles following exposu
prochloraz, an agricultural azole

Isabel Faria-Ramos[1], Sofia Farinha[1], João Neves-Maia[1], Pedro Ribeiro Tavares[1], Isabel M Miranda[1,2,3], Letícia M Estevinho[4], Cidália Pina-Vaz[1,2,3,5] and Acácio G Rodrigues[1,2,3,6*]



Evolution of cross-resistance to medical triazoles in *Aspergillus fumigatus* through selection pressure of environmental fungicid

THE ROYAL SOCIETY PUBLISHING PROCEEDINGS B

Jianhua Zhang[1], Joost van den Heuvel[1,3], Alfons J. M. Debets[1], Paul E. Verweij[2], Willem J. G. Melchers[2], Bas J. Zwaan[1,†] and Sijmen E. Schoustra[1,†]

tents lists available at ScienceDirect

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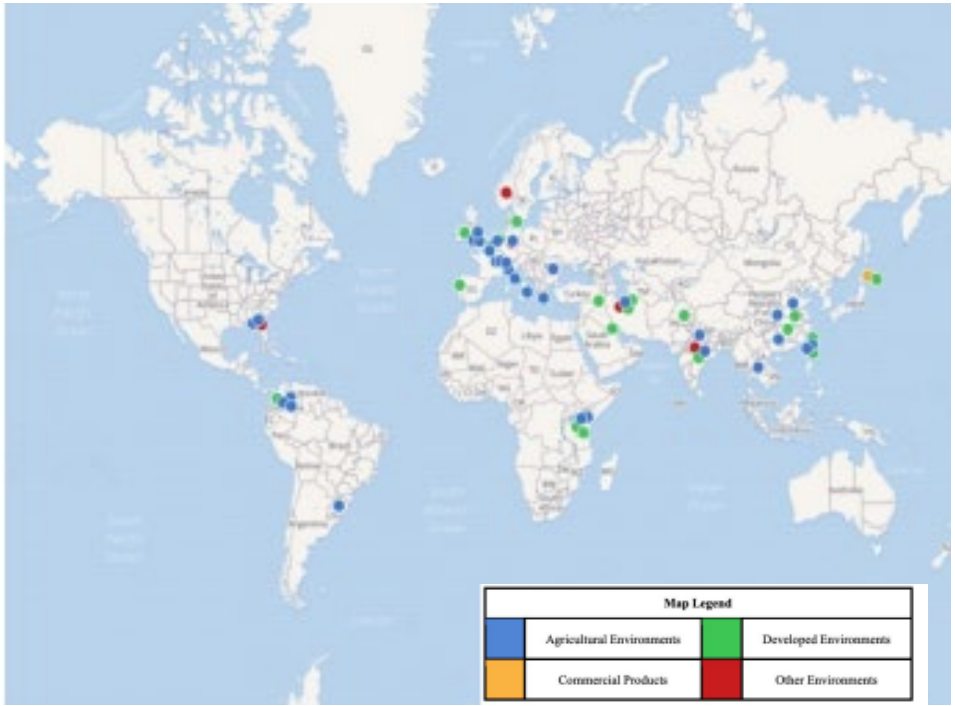
Journal of Hazardous Materials

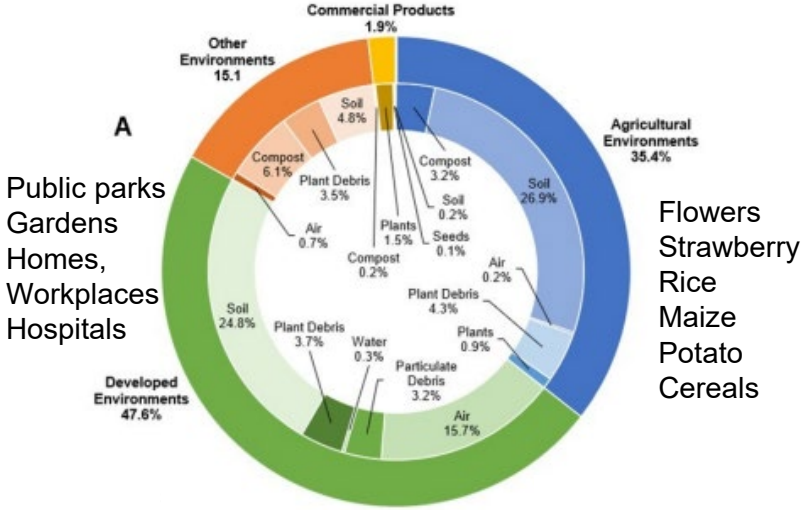
journal homepage: www.elsevier.com/locate/jhazmat

Fungicides induced triazole-resistance in *Aspergillus fumigatus* associated with mutations of TR46/Y121F/T289A and its appearance in agricultural fields

Jingbei Ren, Xiangxiang Jin, Qian Zhang, Yuan Zheng, Dunli Lin, Yunlong Yu*

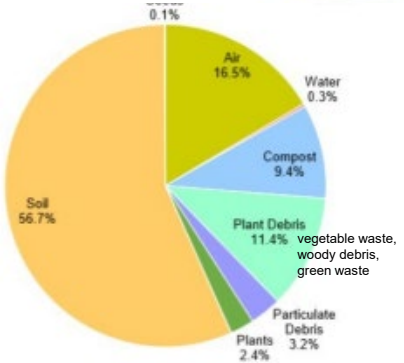
52 published studies
1,292 azole-resistant isolates

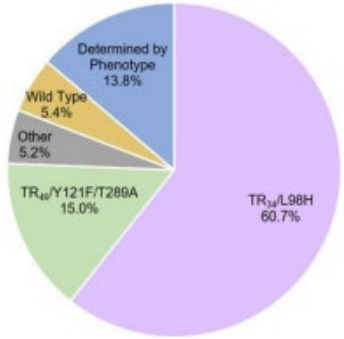




Public parks
Gardens
Homes,
Workplaces
Hospitals

Flowers
Strawberry
Rice
Maize
Potato
Cereals





Burks C, et al, PLoS Pathog 17(7): e1009711.

Resistance selection environments: the concept of the hotspot

(1) the physical, biotic and abiotic conditions facilitate the growth of the fungus and from which the fungus can spread;
(2) this growth can take place for prolonged periods and the fungus can complete all the stages of its growth cycle;
(3) azoles are present, in different concentrations sufficient to select in populations, and combinations;





Burks C, et al, PLoS Pathog 17(7): e1009711.

Aspergillus Resistance in occupational environments

microorganisms MDPI

Article

Azole-Resistant *Aspergillus fumigatus* Harboring the TR_{34}/L98H Mutation: First Report in Portugal in Environmental Samples

Paulo Gonçalves [1,2], Aryse Melo [1,3], Marta Dias [4], Beatriz Almeida [4], Liliana Aranha Caetano [4,5], Cristina Verissimo [1], Carla Viegas [4,6,7] and Raquel Sabino [1,8,*]

Table 3. Growth of resistant *A. fumigatus* isolates in different screening media, minimal inhibitory concentrations for ICZ, VCZ and PCZ, and mutations found on the *cyp51A* gene and its promoter.

Isolate Number	Source	Azole Screening Media			Minimal Inhibitory Concentration (mg/L)			*cyp51A* Mutations
		ICZ	VCZ	PCZ	ICZ	VCZ	PCZ	
VA299CP	Dairy air	+	+	+	4	4	2	TR_{34}/L98H
VA610CP	Hospital air	±	–	–	2	0.5	0.5	No mutation detected
VA873CP	Waste sorting plant FRPD	+	+	+	4	2	1	TR_{34}/L98H
VA978CP	Waste sorting plant FRPD	–	+	–	1	0.25	0.25	No mutation detected
V1207CP	Waste sorting plant FRPD	–	–	+	1	0.5	0.5	No mutation detected
VA1209CP	Waste sorting plant FRPD	+	–	+	8	4	1	TR_{34}/L98H
VA1215CP	Waste sorting plant FRPD	–	+	+	1	0.25	0.125	N248K
VA1216CP	Waste sorting plant FRPD	–	+	–	1	0.25	0.25	N248K

ICZ: itraconazole; VCZ: voriconazole; PCZ: posaconazole; –: negative (no growth); ±: residual growth (growth of only one or few small colonies; +: relevant growth (growth similar to positive control).

99 *A. fumigatus* sensu stricto isolates
3 mutant TR34/L98H

High Azole Resistance in *Aspergillus fumigatus* Isolates from Strawberry Fields, China, 2018

Yong Chen,[1] Fengshou Dong,[1] Jingya Zhao, Hong Fan, Chunping Qin, Runan Li, Paul E. Verweij, Yongquan Zheng,[2] Li Han[2]

Table 1. Prevalence of ARAF isolates in soil samples from different crops, China, 2018*

Crop	Soil depth, cm	No. ARAF-positive soil samples/no. samples (%)	No. ARAF isolates/no. isolates (%)
Watermelon	0	0/10	0/33
	20	0/10	0/13
Rice	0	1/16 (6.3)	1/20 (5.0)
	20	0/16	0/11
Vegetable	0	1/11 (9.1)	2/33 (6.1)
	20	0/11	0/18
Strawberry	0	6/10 (60.0)	16/44 (36.4)
	20	2/10 (20.0)	2/23 (8.7)
Tea leaf	0	0/5	0/6

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ARTICLE

Algorithm to assess the presence of *Aspergillus fumigatus* resistant strains: The case of Norwegian sawmills

Carla Viegas [a,b,c], Beatriz Almeida [a], Liliana Aranha Caetano [a,d], Anani Afanou[e], Anne Straumfors [e], Cristina Veríssimo [f], Paulo Gonçalves[f,g] and Raquel Sabino[f,h]

6 *A. fumigatus* sensu stricto isolates
1 mutant TR34/L98H

Take-home messages

- Fungicide treatments are, and will remain, essential for maintaining healthy crops and reliable, high-quality yields.

- Monitoring is vital, to determine whether resistance is the cause in cases of lack of disease control, and to check whether resistance management strategies are working.

- Recognizing possible exposure to antifungal resistance in occupational settings is fundamental because it will allow appropriate health interventions.

We are all linked…antifungal resistances too!!!

Thank you!!!

Hope to receive you in Lisbon!!!

raquel.sabino@insa.min-saude.pt