TIMM
Trends in Medical Mycology

ECMM/EFISG symposium: Multidrug resistance in fungi? A formidable foe

Resistance epidemiology

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Disclousure

- I have received grant, travel support or honoraria from
  - GILEAD
  - F2G
  - Scynexis
  - Amplix

Multidrug resistance

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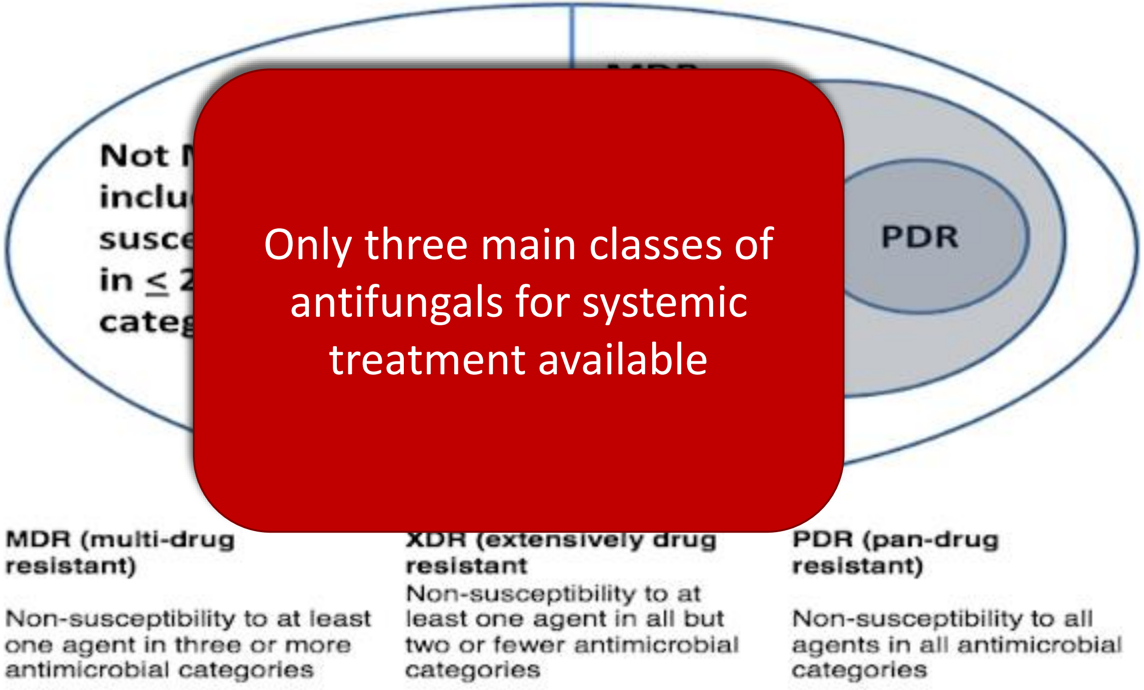
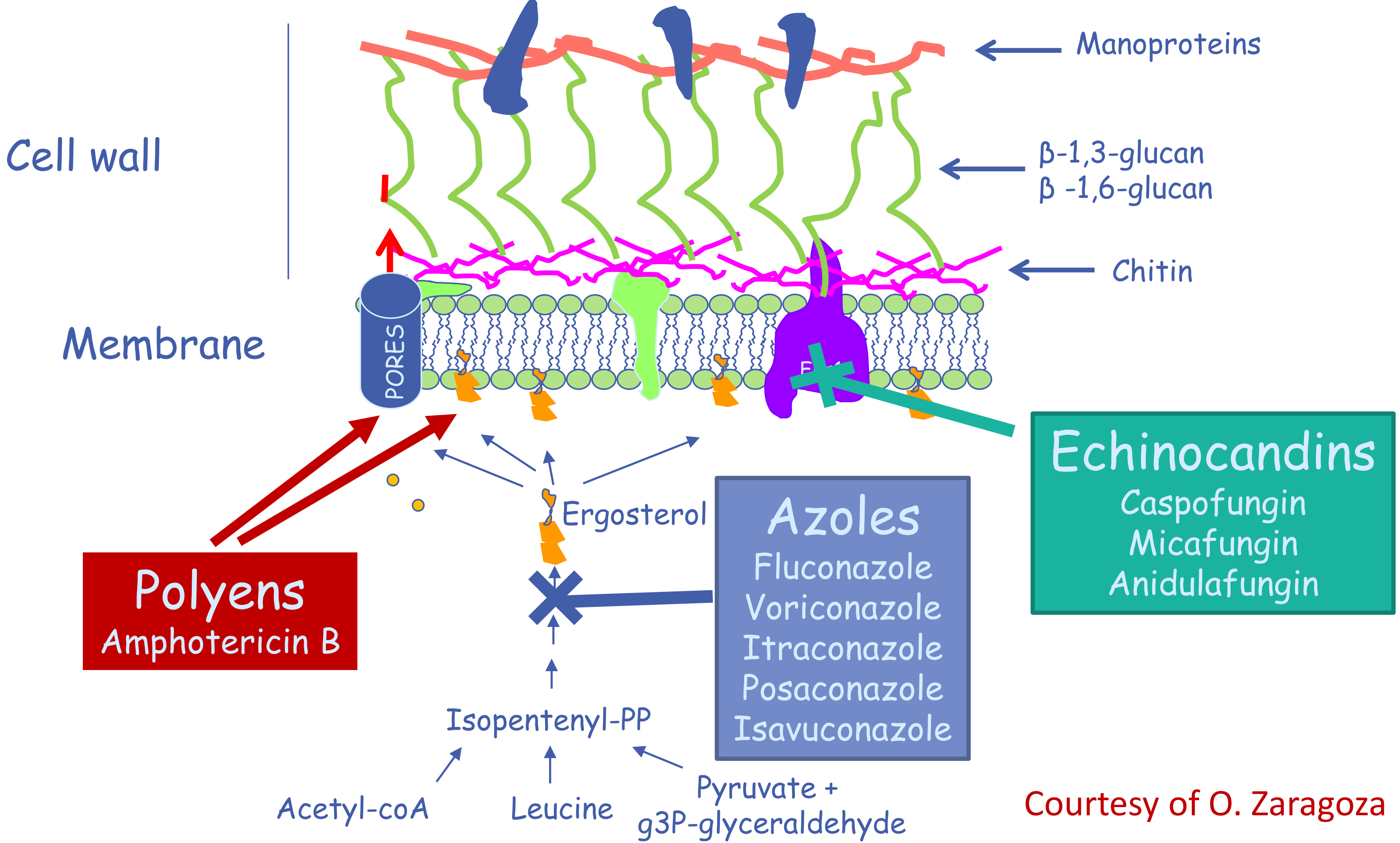


Magiorakos AP et al. Clin Microbiol Infect 2012; 18:268–81.


Manoproteins
Cell wall
β-1,3-glucan
β -1,6-glucan
Chitin
Membrane
PORES
Echinocandins
Caspofungin
Micafungin
Anidulafungin
Ergosterol
Azoles
Fluconazole
Voriconazole
Itraconazole
Posaconazole
Isavuconazole
Polyens
Amphotericin B
Isopentenyl-PP
Acetyl-coA
Leucine
Pyruvate +
g3P-glyceraldehyde
Courtesy of O. Zaragoza

Azole resistance

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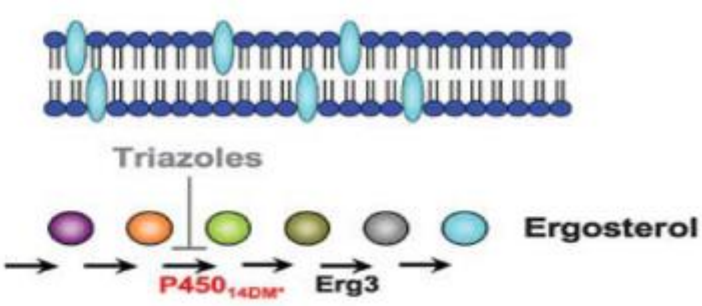
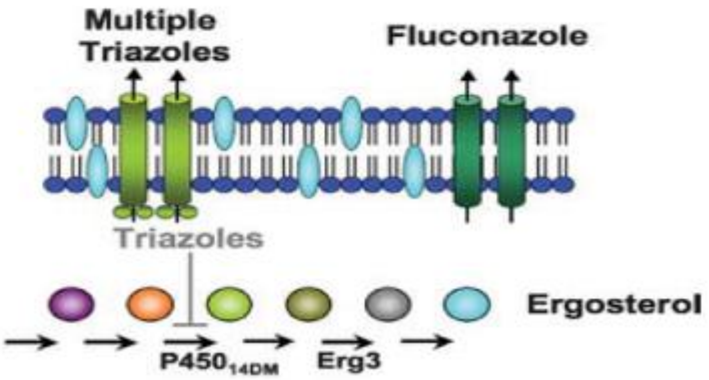
Mutations ERG11 /*Cyp*51



Overexpression of efflux pumps

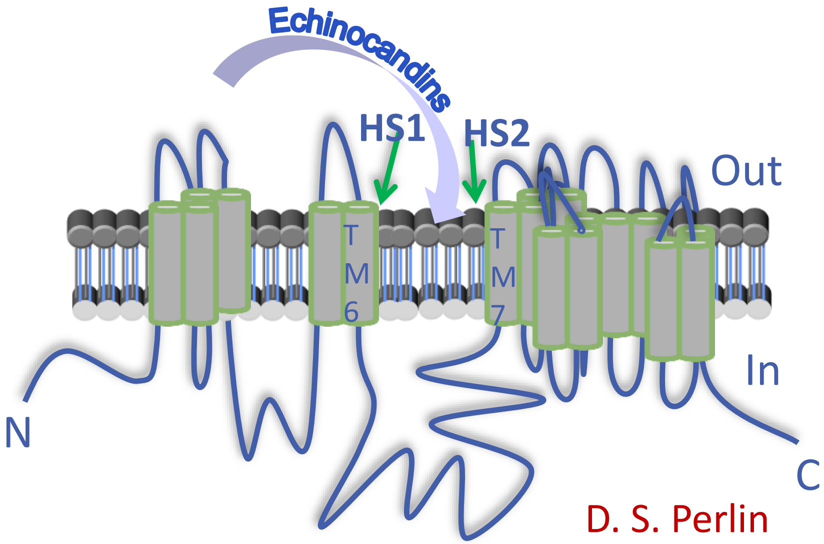


Cowen L E , and Steinbach W J Eukaryotic Cell 2008;7:747-764

Echinocandin resistance

Mutations FKS

Cowen L E , and Steinbach W J Eukaryotic Cell 2008;7:747-764



D. S. Perlin

Resistance in emerging

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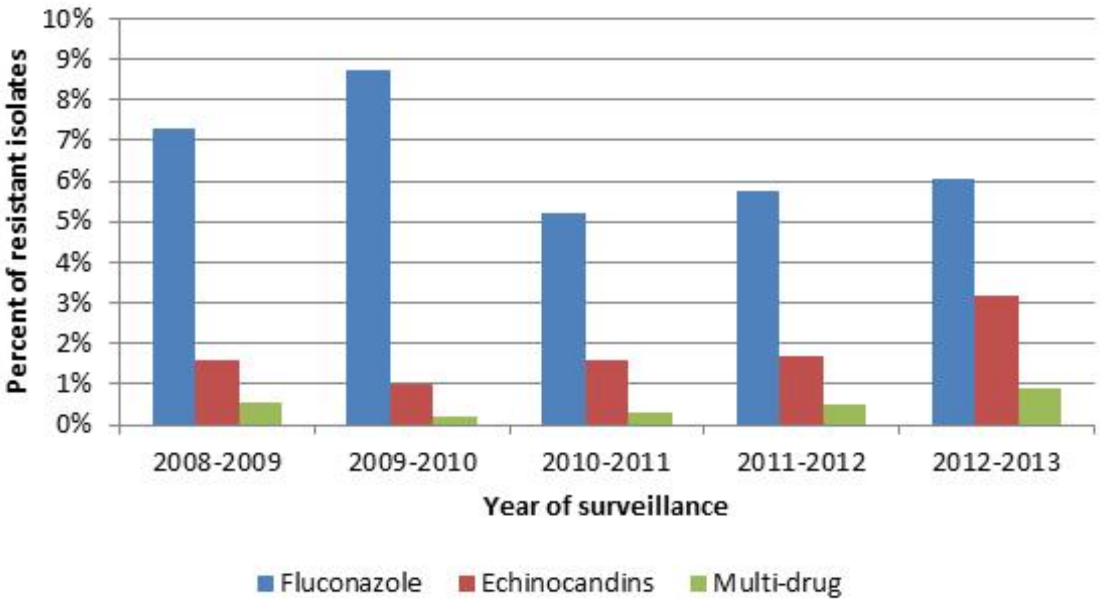

Public Health Threat of drug resistant fungal infections
Subimtted by HLeSueur on Fri, 04/22/2016
http://www.aspergillus.org.uk/content/public-health-threat-drug-resistant-fungal-infections

AF resistance

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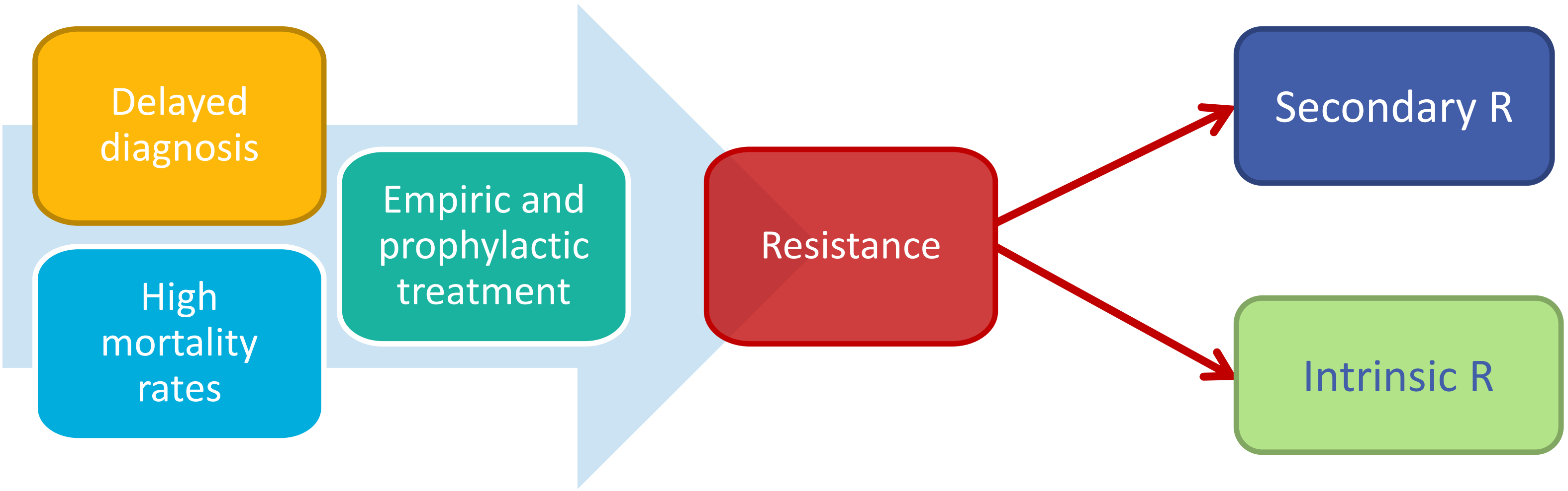


Candida

Intrinsic resistance

- Epidemiological change
- Species such as *C. krusei or C. guilliermondii*

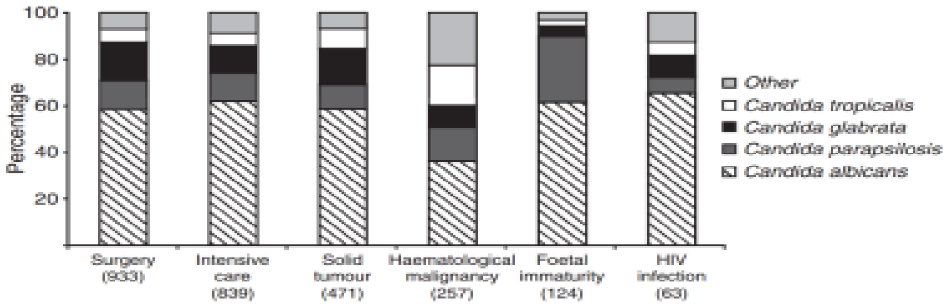
Secondary resistance:

- Acquire azole resistance→ mucosal candidiasis (AIDS)
- Echinocandin resistance mainly in *C. glabrata*

Epidemiology

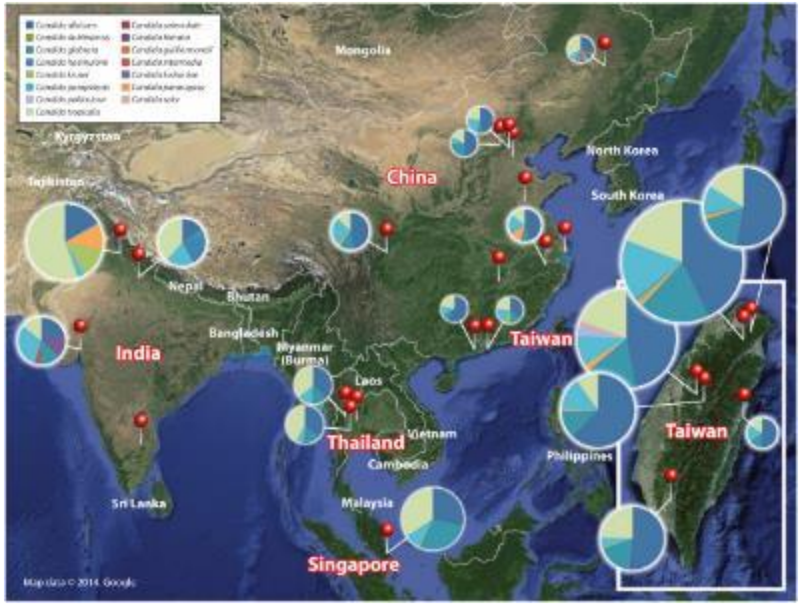
Species	AMB	FCZ	EC
C. albicans	S	S	S
C. glabrata	S	I	S
C. parapsilosis	S	S	I
C. tropicalis	S	S	S
C. krusei	S	R	S

Lass-Flörl, Mycoses 2009

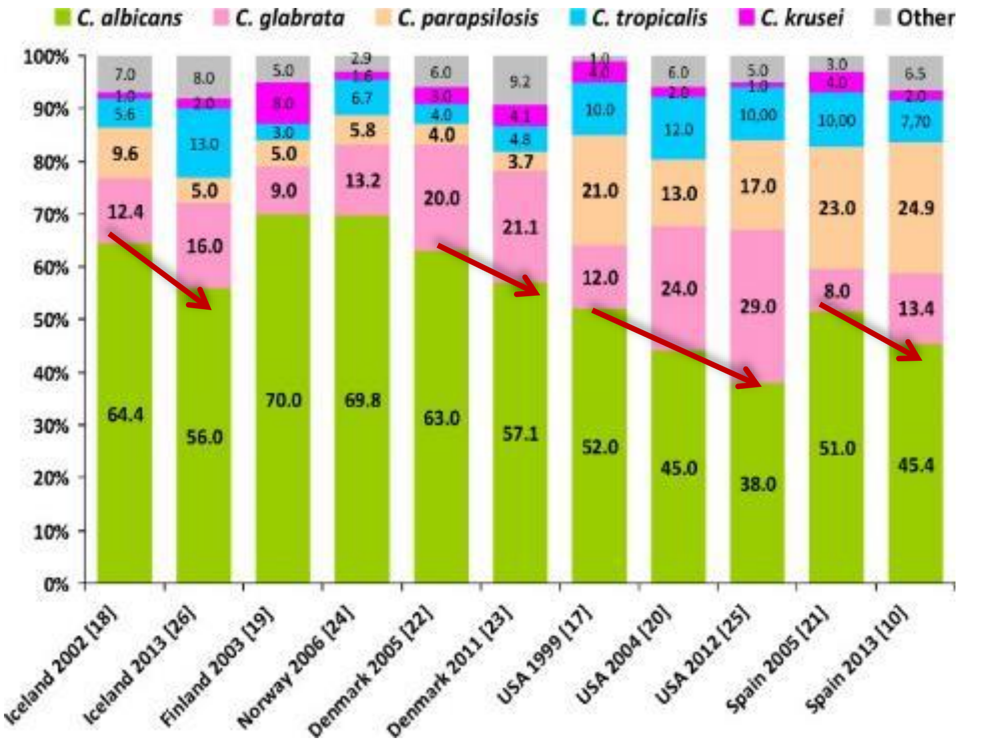


Quindos RIAM 2014

Epidemiology



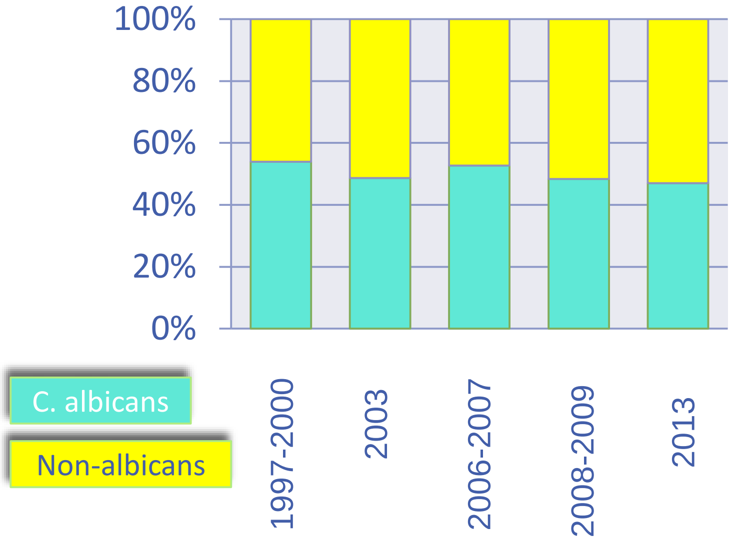
Tan et al. CID 2015



Guinea Clin. Microb. Infection 2014

Change in the epidemiology

SENTRY



Adapted from Fernández-Ruiz ECCMID 2017 *J Clin Microbiol* 2002;40:852-6

J Clin Microbiol 2006;44:1782-7

J Clin Microbiol 2009;47:1942-6

J Clin Microbiol 2011;49:396-9

Diagn Microbiol Infect Dis 2016;85:200-4

ARTEMIS

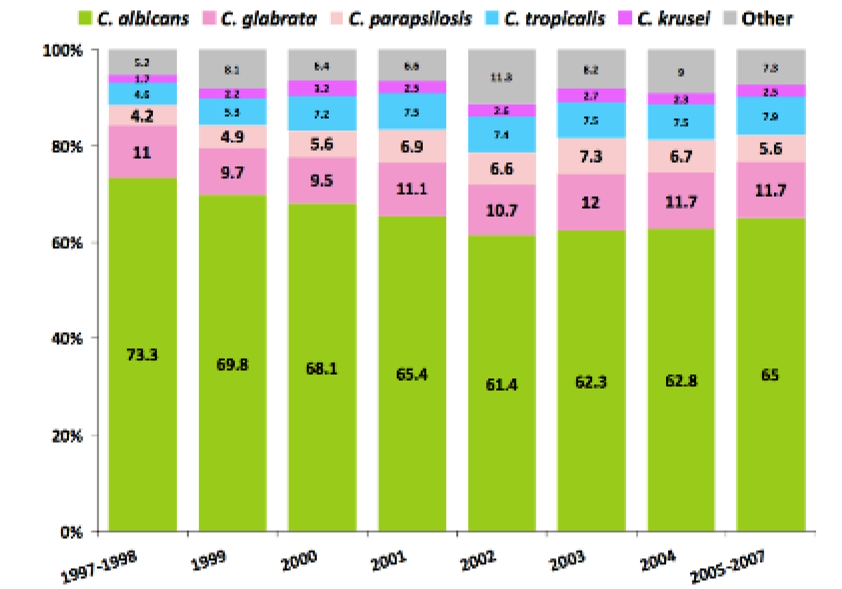


FIG. 2. Data compiled from the ARTEMIS DISK registry on the temporal change in species distribution among invasive Candida isolates from 1997 to 2007.

Guinea Clin. Microb. Infection 2014

SENTRY 2008-2009

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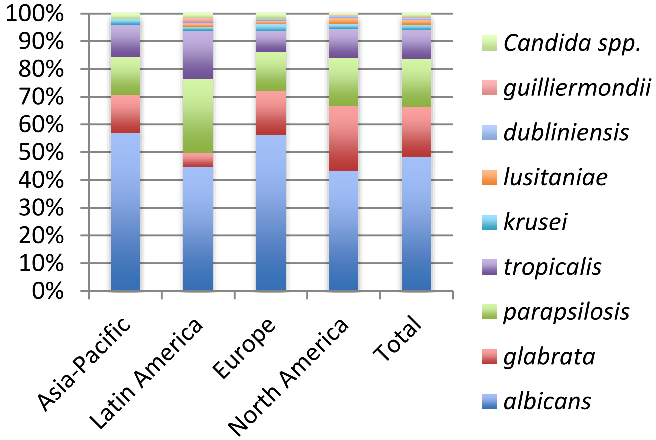

2,085 isolates
79 medical centers
Asian-Pacific (16 centers; 51 isolates),
European (25/750),
Latin American (10/348)

North American (28/936)

TABLE 2. Frequency of antifungal resistance among *Candida* bloodstream infection isolates by geographic region: SENTRY Surveillance Program, 2008 to 2009

Species	Antifungal agent	No. (%) of isolates resistant to each antifungal agent by region[a]				
		Asia-Pacific	L. America	Europe	N. America	Total
C. albicans	Anidulafungin	29 (0.0)	161 (0.0)	414 (0.2)	406 (0.0)	1,010 (0.1)
	Micafungin	29 (0.0)	161 (0.0)	414 (0.2)	406 (0.0)	1,010 (0.1)
	Fluconazole	29 (3.4)	161 (0.0)	414 (0.0)	406 (0.0)	1,010 (0.1)
	Posaconazole	29 (0.0)	161 (0.0)	414 (0.0)	406 (0.0)	1,010 (0.0)
	Voriconazole	29 (0.0)	161 (0.0)	414 (0.0)	406 (0.0)	1,010 (0.0)
C. glabrata	Anidulafungin	7 (0.0)	18 (0.0)	131 (1.5)	220 (3.2)	376 (2.4)
	Micafungin	7 (0.0)	18 (0.0)	131 (0.8)	220 (2.7)	376 (1.9)
	Fluconazole	7 (0.0)	18 (0.0)	131 (2.3)	220 (8.2)	376 (5.6)
	Posaconazole	7 (0.0)	18 (0.0)	131 (1.5)	220 (5.5)	376 (3.7)
	Voriconazole	7 (0.0)	18 (0.0)	131 (0.0)	220 (5.9)	376 (3.5)
C. parapsilosis	Anidulafungin	7 (0.0)	89 (0.0)	103 (0.0)	160 (0.0)	359 (0.0)
	Micafungin	7 (0.0)	89 (0.0)	103 (0.0)	160 (0.0)	359 (0.0)
	Fluconazole	7 (0.0)	89 (6.7)	103 (3.9)	160 (5.0)	359 (5.0)
	Posaconazole	7 (0.0)	89 (0.0)	103 (0.0)	160 (0.0)	359 (0.0)
	Voriconazole	7 (0.0)	89 (0.0)	103 (0.0)	160 (0.0)	359 (0.0)
C. tropicalis	Anidulafungin	6 (0.0)	59 (0.0)	55 (0.0)	98 (1.0)	218 (0.5)
	Micafungin	6 (0.0)	59 (0.0)	55 (0.0)	98 (0.0)	218 (0.0)
	Fluconazole	6 (0.0)	59 (1.7)	55 (3.6)	98 (4.1)	218 (3.2)
	Posaconazole	6 (0.0)	59 (0.0)	55 (0.0)	98 (2.0)	218 (0.9)
	Voriconazole	6 (0.0)	59 (1.7)	55 (3.6)	98 (2.0)	218 (2.9)
C. krusei[b]	Anidulafungin	1 (0.0)	5 (0.0)	19 (0.0)	15 (0.0)	40 (0.0)
	Micafungin	1 (0.0)	5 (0.0)	19 (0.0)	15 (0.0)	40 (0.0)
	Posaconazole	1 (0.0)	5 (0.0)	19 (0.0)	15 (0.0)	40 (0.0)
	Voriconazole	1 (100.0)[c]	5 (0.0)	19 (0.0)	15 (0.0)	40 (2.5)

[a] Resistance is defined as an MIC > 0.5 μg/ml for anidulafungin and micafungin versus *C. albicans*, *C. tropicalis* and *C. krusei*, an MIC > 4 μg/ml versus *C. parapsilosis*, an MIC > 0.5 μg/ml for anidulafungin and an MIC > 0.12 μg/ml for micafungin and *C. glabrata*, an MIC > 4 μg/ml for fluconazole versus *C. albicans*, *C. tropicalis*, and *C. parapsilosis* an MIC > 32 μg/ml versus *C. glabrata*, and an MIC > 2 μg/ml for posaconazole and voriconazole for all species. L., Latin; N., North.
[b] All isolates of *C. krusei* were defined as resistant to fluconazole as per CLSI criteria.
[c] Represents one isolate only.

Pfaller J. Clin. Micro 2011

SENTRY 2013

Table 1
Activity of antifungal agents tested against fungal organism groups with ≥10 isolates collected in a global surveillance study during 2013.

Organism (no. tested) antifungal agent	MIC/MEC$_{50}$ (μg/mL)	MIC/MEC$_{90}$ (μg/mL)	Range (μg/mL)	CLSI %S/%R	ECV %WT/%NWT
C. albicans (712)					
Anidulafungin	0.015	0.06	≤0.008 to 0.12	100.0/0.0	100.0/0.0
Caspofungin	0.03	0.03	≤0.008 to 0.06	100.0/0.0	100.0/0.0
Micafungin	0.015	0.03	≤0.008 to 0.03	100.0/0.0	100.0/0.0
Fluconazole	0.12	0.25	≤0.06 to >128	99.6/0.4	98.5/1.5
Voriconazole	≤0.008	0.015	≤0.008 to >8	99.7/0.3	99.2/0.8
Posaconazole	0.06	0.06	≤0.008 to >8	—/—	95.1/4.9
C. glabrata (251)					
Anidulafungin	0.06	0.12	0.015–2	96.0/2.4	96.0/4.0
Caspofungin	0.03	0.06	0.015–1	98.0/2.0	98.0/2.0
Micafungin	0.015	0.03	≤0.008 to 0.5	98.8/0.8	97.6/2.4
Fluconazole	8	64	0.12 to >128	—/12.0	79.3/20.7
Voriconazole	0.12	1	≤0.008 to 8	—/—	88.0/12.0
Posaconazole	1	2	0.06 to >8	—/—	95.6/4.4
C. parapsilosis (215)					
Anidulafungin	2	2	0.25–4	95.3/0.0	100.0/0.0
Caspofungin	0.25	0.5	0.06–1	100.0/0.0	100.0/0.0
Micafungin	1	2	0.12–2	100.0/0.0	100.0/0.0
Fluconazole	1	2	0.25–32	94.0/2.3	80.9/19.1
Voriconazole	0.015	0.06	≤0.008 to 0.5	99.1/0.0	99.1/0.9
Posaconazole	0.12	0.12	0.03–0.5	—/—	98.6/1.4
C. tropicalis (155)					
Anidulafungin	0.015	0.03	≤0.008 to 2	98.7/1.3	98.7/1.3
Caspofungin	0.03	0.03	0.015–2	98.7/0.6	98.7/1.3
Micafungin	0.03	0.06	≤0.008 to 1	98.7/0.6	97.4/2.6
Fluconazole	0.5	32	0.12 to >128	88.4/11.6	87.7/12.3
Voriconazole	0.03	1	≤0.008 to >8	88.4/10.3	87.7/12.3
Posaconazole	0.06	0.25	0.015 to >8	—/—	84.5/15.5

Castanheira Diagn. Micro Infec. Dis. 2013

Latin America: Azole resistance

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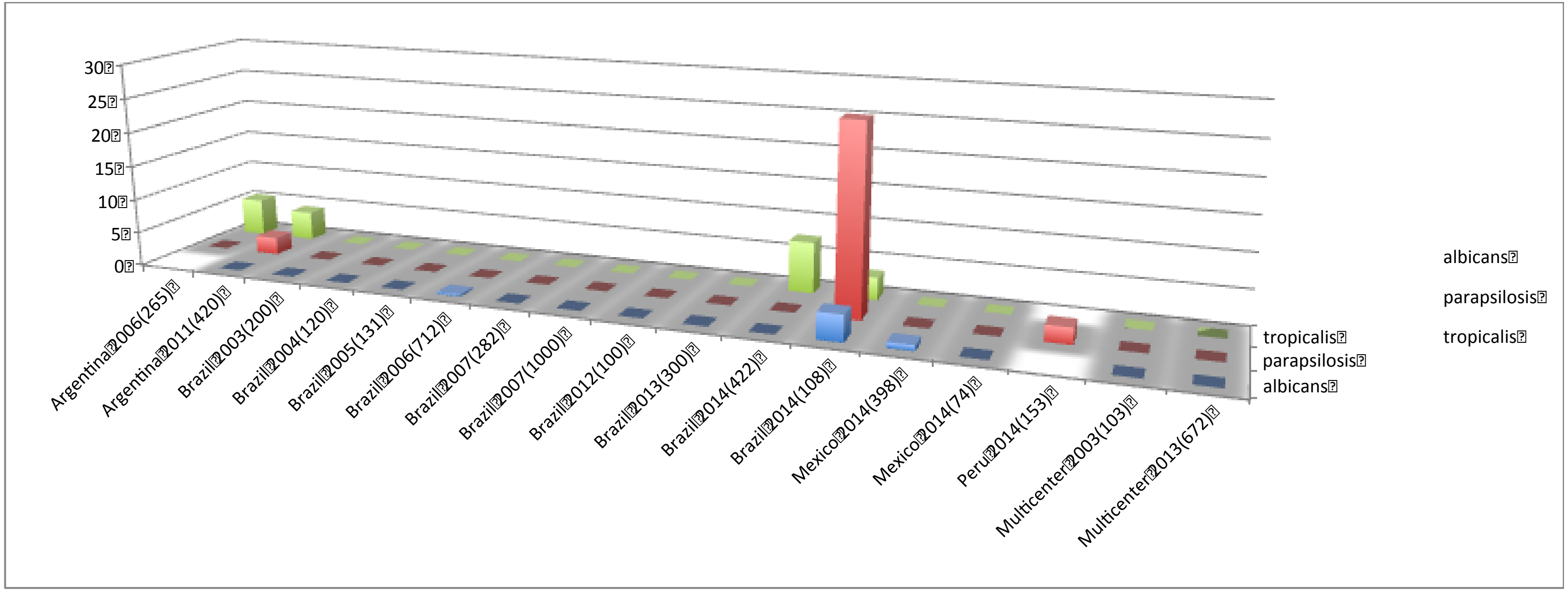
% Fluco

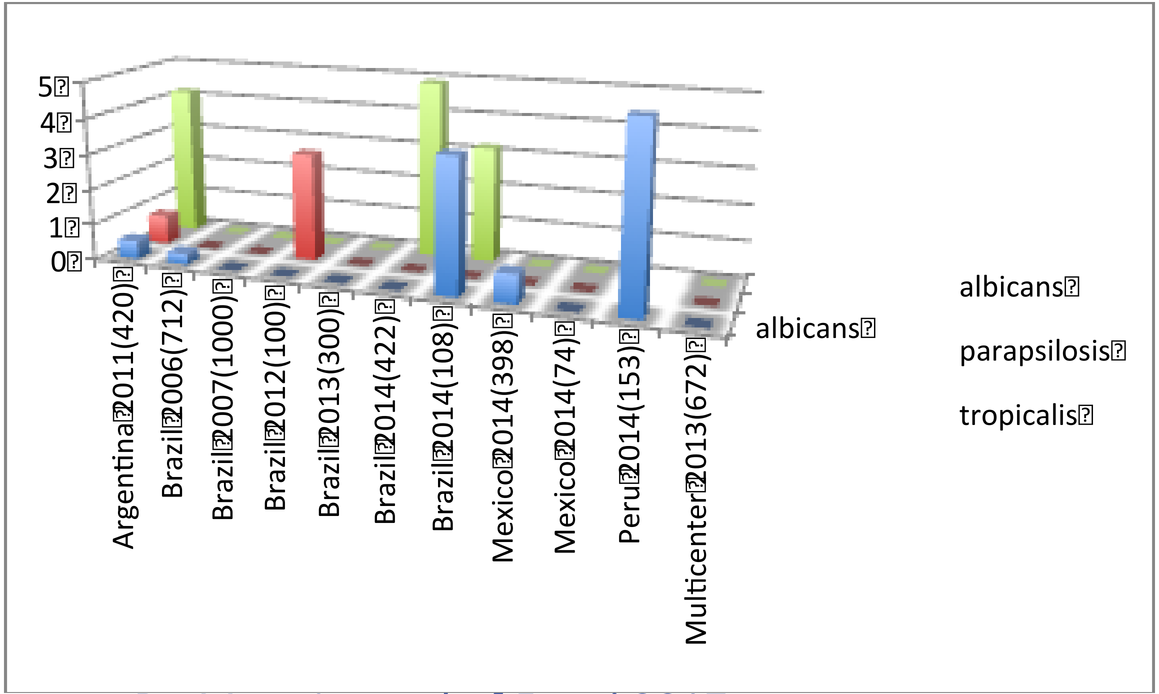


Da Mata Journal of Fungi 2017

Latin America: Azole resistance

Alastruey-Izquierdo TIMM 2017

% VCZ R



Da Mata Journal of Fungi 2017

Echinocandin resistance

	AFG EUCAST ECOFF (mg/L)	FKS1p Hot spot 1 1st AA no.	FKS1p Hot spot 1 AA sequence	FKS1p Hot spot 2 1st AA no.	FKS1p Hot spot 2 AA sequence	FKS2p Hot spot 1 1st AA no.	FKS2p Hot spot 1 AA sequence	FKS2p Hot spot 2 1st AA no.	FKS2p Hot spot 2 AA sequence
C. albicans	0.03	641	FLTLSLRDP	1357	DWIRRYTL				
C. dubliniensis	0.03	641	FLTLSLRDP	1357	DWIRRYTL				
C. glabrata	0.06	625	FLILSLRDP	1340	DWVRRYTL	659	FLILSLRDP	1374	DWI^{R}RYTL
C. kefyr	(0.03)	54*	FLTLSLRDP	769*	DWVRRYTL				
C. krusei	0.06#	655	FLILSIRDP	1364	DWIRRYTL				
C. lusitaniae	(0.06)	634*	FLTLSLRDP	**	DWIRRYTL				
C. tropicalis	0.06	76*	FLTLSLRDP	792*	DWIRRYTL				
C. parapsilosis	4	652	FLTLSLRDA	1369	DWIRRYTL				
C. metapsilosis	(4)	104*	FLTLSLRDA	821*	DWIRRYTL				
C. orthopsilosis	(4)	39*	FLTLSLRDA	756*	DWVRRYTL				
C. guilliermondii	(4)	632	FMALSLRDP	1347	DWIRRYTL				
C. lipolytica	NA	662	FLILSLRDP	1387	DWIRRCVL				
S. cerevisiae	(1)	639	FLVLSLRDP	1353	DWVRRYTL	658	FLILSLRDP	1372	DWVRRYTL

X "strong R" mutation, letters indicate the codon involves a mutation or deletion; letters ind

X "weak R" mutation;

X "silent" mutation, acquired or naturally occurring;

X naturally occurring mutation proven or possibly related to the intrinsic lower susceptibility;

X naturally occurring mutation of unknown impact;

Arendrup & Perlin Curr. Opin. Infect. Dis 2015

Candida glabrata

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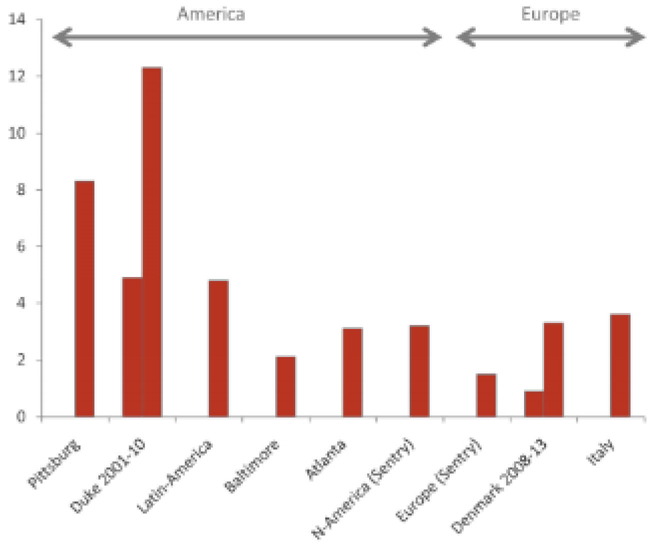


Fig. 1.
Echinocandin resistance in *C. glabrata* in Europe and America. Proportion of invasive isolate with resistance at tertiary centres (Pittsburgh, Duke and 7 Latin American hospitals), Shields AAC 2013; Alexander CID 2013; Nucci Plos One 2013; In population based surveys (Baltimore, Atlanta, Denmark) and Italy and from the global sentry study. Zimbeck AAC 2010; Pfaller JCM 2011; Arendrup Unpubl data; Tortorano Infection 2013.

Arendrup & Perlin Curr. Opin. Infect. Dis 2015

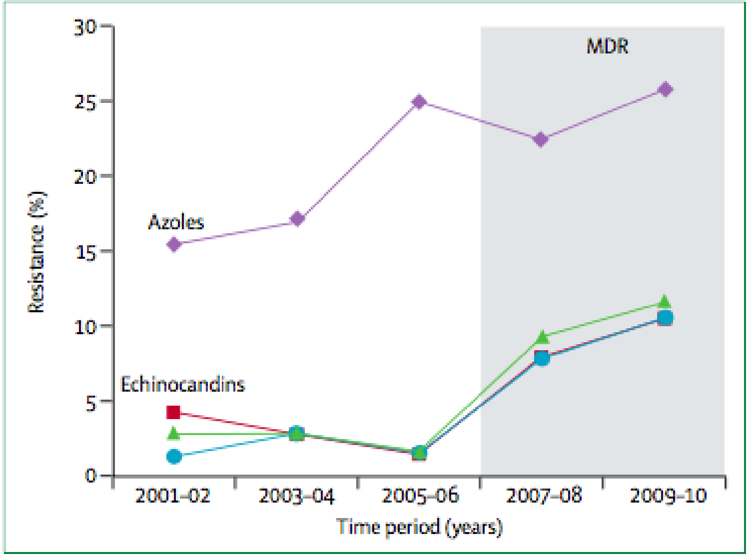


Figure 3: **Parallel rise in azole and echinocandin resistance in *Candida glabrata* bloodstream isolates over a 10-year period, showing emergence of multidrug-resistant strains**
The grey-shaded box shows the time of emergence of substantial multidrug resistance. The three echinocandin-class drugs are shown: red, anidulafungin; green, caspofungin; blue, micafungin (adapted from Alexander et al[73]).
MDR=multidrug resistance.

Perlin et al. Lancet Inf. Dis 2017
Alexander et al. Clin Inf. Dis 2013

Candida auris

2009	2010	2011	2012	2013	2014	2015	2016	2017
Japan		Korea, Venezuela		India	S. Africa Kenya Kuwait	Pakistan Israel	Colombia Panama USA Spain	Oman

Some hospitals have reported 40% candidemia by *C. auris*

Slide kindly provided by Diego Caceres CDC

Candida auris

Pan American Health Organization
World Health Organization
Americas

Epidemiological Alert

Candida auris outbreaks in health care services

3 October 2016

Instituto Nacional de Salud

TODOS POR UN NUEVO PAÍS
PAZ EQUIDAD EDUCACIÓN

Alerta por emergencia global de infecciones invasivas causadas por la levadura multirresistente, *Candida auris*

ecdc

RAPID RISK ASSESSMENT

***Candida auris* in healthcare settings – Europe**

19 December 2016

Public Health England

See more information about this Research and analysis

Research and analysis

Candida auris identified in England

Published 1 July 2016

- Clinical Alert to U.S. Healthcare Facilities – June 2016

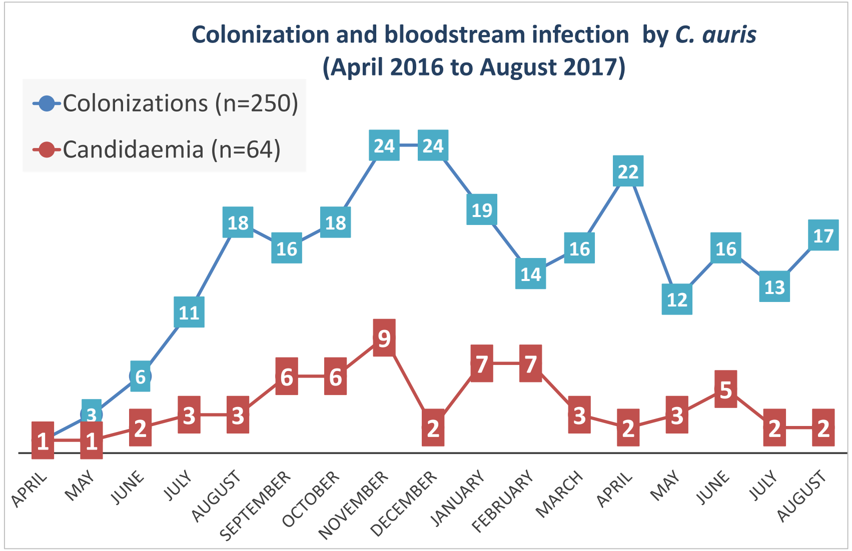
Clinical Alert to U.S. Healthcare Facilities - June 2016

This clinical alert has been updated. Please read the September 2017 *C. auris* Clinical Update with important information from investigations of U.S. cases of *C. auris* for clinicians, laboratorians, and public health officials.

Global Emergence of Invasive Infections Caused by the Multidrug-Resistant Yeast *Candida auris*

Candida auris

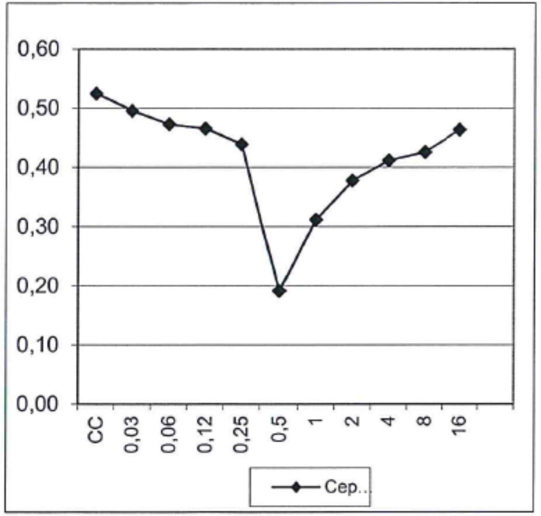
- Not correctly identified with classical methods
- Difficult to control
  - Colonization
  - Environment
- Antifungal resistance



* S18 08/10/17 17:30 Alba Ruiz
Room: Mediterranean/Adriatic

Candida auris

- 93% resistant to Fluconazole
- 54% resistant to voriconazole
- 35% resistant to amphotericin B
- 7% resistant to echinocandins
- 41% multidrug resistance
- 4% resistant to all antifungals



Lockhart et al. Clin Infect Dis 2017
Chowdary et al. Hosp Infect 2016

What about *Aspergillus*??

AF resistance

Delayed diagnosis

High mortality rates

Empiric and prophylactic treatment

Resistance

Agricultural use of azoles

Intrinsic R

secondary R

Secondary resistance

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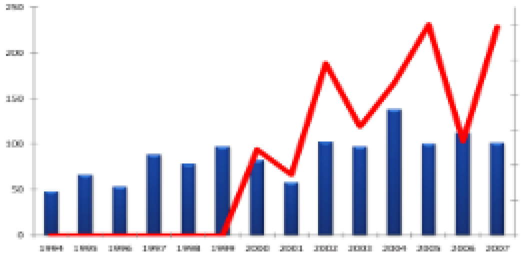
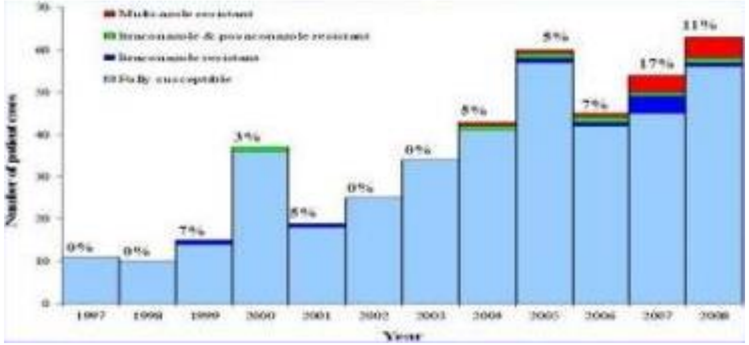
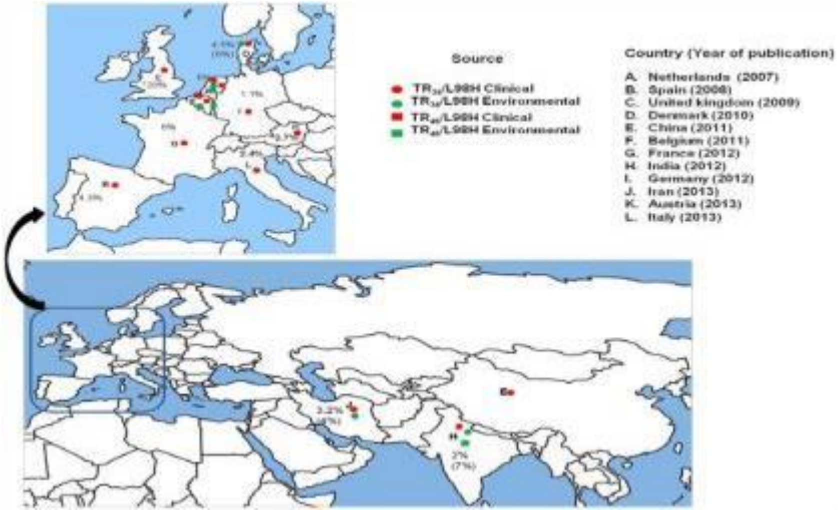


Snelders et al., PLOS 2008 Vol. 5:11



Howard et al., EID 2009, Vol. 15, No. 7

Secondary resistance



Chowdhary et al. PLoS Pathog. Oct 2013; 9(10)

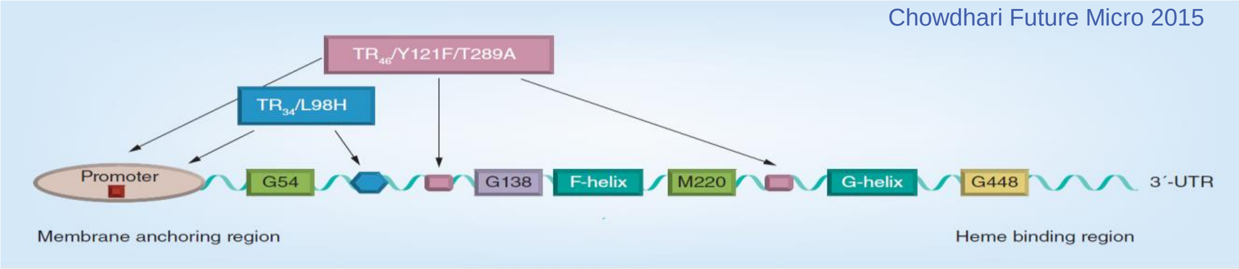
Secondary resistance

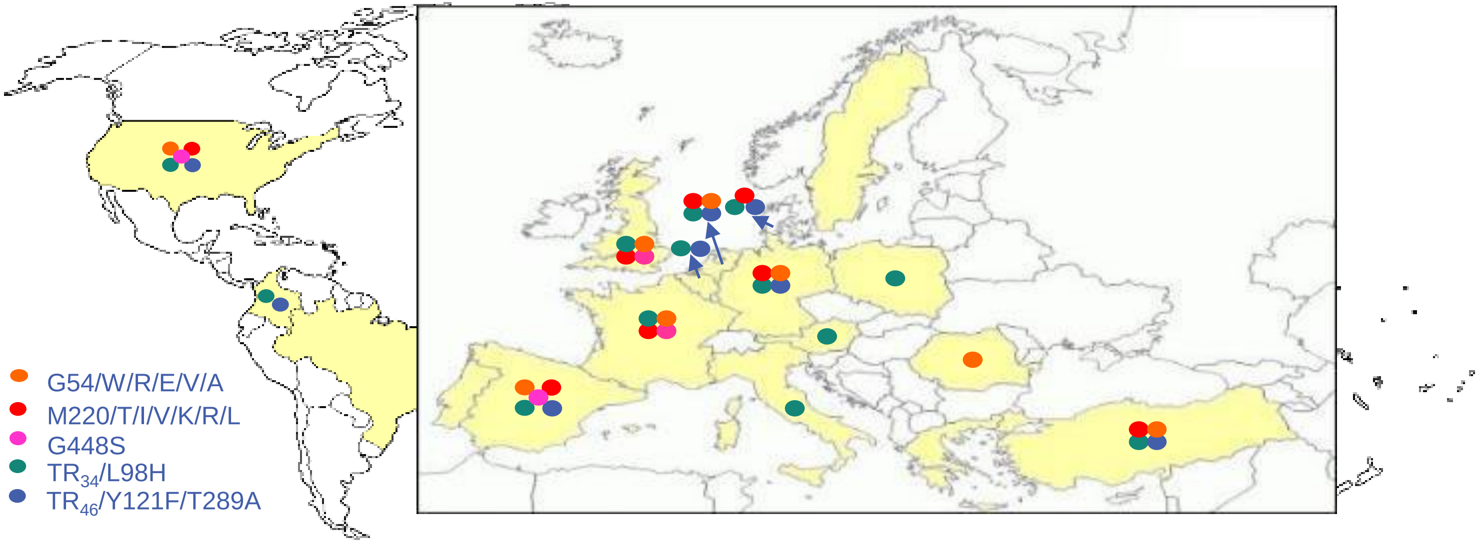
Figure 1. Shaded areas show countries that have reported the TR_{34}/L98H and TR_{46}/Y121F/T289A resistance mechanism in clinical or environmental *Aspergillus fumigatus* isolates.

Turn de world RED

Verweij Clin. Inf. Dis. 2016

Secondary resistance





Rivero-Menéndez Journal of Fungi 2016

Secondary resistance

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Table 2. Azole resistance prevalence in *A. fumigatus* by continent and/or country. Only significant publications with more than 50 isolates tested are reported.

Continent/Country	% Resistance	Source of the Isolates	References
	Europe		
Belgium	5.7	C	[76]
France	0.85–10.6	C	[30,48,50]
Germany	1.1–12	C and E	[32,47,60]
Netherlands	2.1–20	C and E	[20,53,67,74]
Poland	2.25	C	[69]
Spain	1.8	C	[63]
Turkey	10.2	C	[71]
United Kingdom	6.6–28	C	[17,33]
	Other continents		
Asia *	1.9–11.1	C and E	[55,77,78,80–86,121]
Africa (Tanzania)	13.9	E	[90]
America (USA)	0.6–11.8	C	[58,122]
Oceania (Australia)	2.6	C	[59]
	International surveillance studies		
America-Asia-Australia-Europe	1.4–5.8	C and E	[52,70,123,124]

C = clinical strains, E = environmental strains; * including China, India, Iran, Japan, Kuwait and Pakistan.

Rivero-Menéndez Journal of Fungi 2016

Clinical significance

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Table 4. Characteristics of patients with azole-resistant invasive aspergillosis, the Netherlands, 2007–2009*

Patient age, y/sex	Underlying disease	Disease	No. positive cultures†	Resistance mechanism	VCZ MIC, mg/L	Prior azole treatment (duration)‡	Treatment§	Outcome at 12 wk
66/M	Lung carcinoma	Proven pulmonary aspergillosis	1	TR/L98H	4	None	VCZ	Died
59/M	Hematologic malignancy, allo-SCT, GvHD	Proven pulmonary aspergillosis	4	TR/L98H	8	VCZ (>1 mo)	VCZ	Died
54/M	Acute myeloid leukemia relapse, all[illegible]	Proven pulmonary [illegible]	1	TR/L98H	8	ITZ (2–4 wk)	VCZ	Died
50/M	Non-Hodgkin [illegible] allo-SCT, Gv[illegible] caviti[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	VCZ	Died
36/F	Breast carcir[illegible] metast[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	VCZ	Died
13/F	Non-Hodgkin [illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]Z, CAS, AMB	Died
58/M	Liver transpla[illegible] hepatic failure after methotrexate treatment for arteritis	[illegible] CNS aspergillosis	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]B, VCZ	Died
60/M	Acute myeloid leukemia, allo-SCT, GvHD	Proven pulmonary and CNS aspergillosis	3	TR/L98H	4	FCZ (1–2 wk)	VCZ, CAS, AMB, POS	Survived

*VCZ, voriconazole; allo-SCT, allogeneic hematopoietic stem cell transplantation; GvHD, graft-versus-host disease; HSCT, hematopoietic stem cell transplantation; ITZ, itraconazole; CNS, central nervous system; CAS, caspofungin; AMB, amphotericin B; FCZ, fluconazole; POS, posaconazole.
† All cultures were *Aspergillus fumigatus*.
‡Azole treatment <12 wk before the first culturing of an azole-resistant isolate.
§Azole treatment after first culturing of resistant isolate.

	Aspergillosis	
	Susceptible strains	Resistant strains
Mortality	30-50%	88%

van der Linden et al. Emerg Infect Dis 2011; 17:1846–1854.

AF resistance

Delayed diagnosis

High mortality rates

Empiric and prophylactic treatment

Resistance

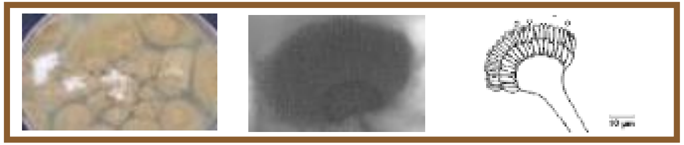
Agricultural use of azoles

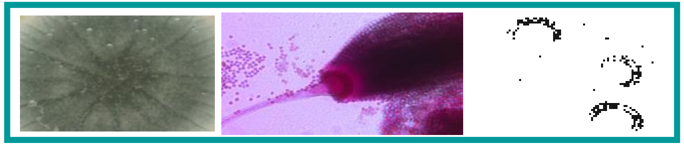
Intrinsic R

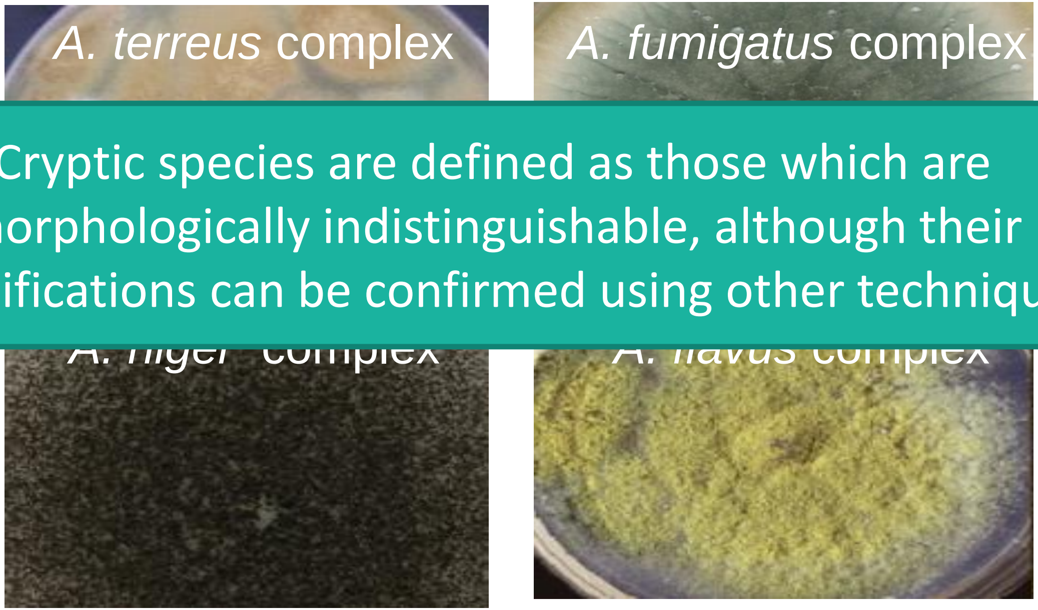
secondary R

Cryptic species

Alastruey-Izquierdo TIMM 2017







Cryptic species are defined as those which are morphologically indistinguishable, although their identifications can be confirmed using other techniques

Cryptic species

Journal of Clinical Microbiology, Feb. 2004, p. 925–928 Vol. 42, No.
0095-1137/04/$08.00+0 DOI: 10.1128/JCM.42.2.925–928.2004

Isolation of *Neosartorya pseudofischeri* from Blood: First Hint of Pulmonary Aspergillosis

H. Järv,[1*] J. Lehtmaa,[2] R. C. Summerbell,[3] E. S. Hoekstra,[3] R. A. Samson,[3] and P. Naaber[1]

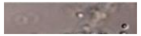
Bathoorn et al. BMC Infectious Diseases 2013, 13:59
http://www.biomedcentral.com/1471-2334/13/59

BMC Infectious Diseases

CASE REPORT Open Access

Involvement of the opportunistic pathogen *Aspergillus tubingensis* in osteomyelitis of the maxillary bone: a case report

Erik Bathoorn[1,2*], Natalia Escobar Salazar[3], Shahrzad Sepehrkhouy[4], Martin Meijer[3], Hans de Cock[3] and Pieter-Jan Haas[1]



Journal of Clinical Microbiology, June 2011, p. 2357–2360 Vol. 49, No. 6
0095-1137/11/$12.00 doi:10.1128/JCM.00134-11

Uncommon *Neosartorya udagawae* Fungus as a Causative Agent of Severe Corneal Infection

Brunella Posteraro,[1] Romano Mattei,[2] Fausto Trivella,[3] Andrea Maffei,[3] Antonio Torre,[3] Elena De Carolis,[1] Patrizia Posteraro,[4] Giovanni Fadda,[1] and Maurizio Sanguinetti[1*]

...nce-Based Methods

Mark D. Lindsley,[1] Naureen Iqbal,[1] James Ito,[2] ... Pappas,[3] and Mary E. Brandt[1]

Medical Mycology November 2010, 48, 995–999

informa healthcare

Case Report

Aspergillus alliaceus and *Aspergillus flavus* co-infection in an acute myeloid leukemia patient

BETIL OZHAK-BAYSAN*, ANA ALASTRUEY-IZQUIERDO†, RABIN SABAL, DILARA OGUNC*, GOZDE ONGUT*, AYSEN TIMURAGAOGLU§, GOKHAN ARSLAN#, MANUEL CUENCA-ESTRELLA† & JUAN LUIS RODRIGUEZ-TUDELA†

Josep Guarro,* Esper G. Kallas,†‡ Patricio Godoy,† Anna Karenina,†‡ Josepa Gené,* Alberto Stchigel,* and Arnaldo Lopes Colombo†

We report the first case of infection by *Neosartorya hiratsukae*, an ascomycete in which the conidial state resembles *Aspergillus fumigatus*. The fungus caused a brain infection in a Brazilian woman, who died despite itraconazole treatment. Diagnosis was established by direct microscopic examination, computed tomographic scan, and magnetic resonance imaging of the brain, and repeated cultures from the lesions. The in vitro antifungal susceptibility of the isolate is provided.

and repetitively, with a loss of strength. Tendon reflexes were normal. The patient underwent surgical exploration, with drainage of the frontal and occipital lesions. Four samples of a yellowish, dense liquid were collected. Laboratory examination did not show neoplastic cells or neutrophils in the liquid. Direct microscopic examination showed septate hyphae in all the samples. Cultures were negative for aerobic and anaerobic bacteria and mycobacteria, and two samples were positive for a fungus, tentatively identified as *Aspergillus* sp. The patient was initially treated for 21 days with ceftriaxone, oxacillin, and metronidazole without major improvement. When the fungus was isolated, she was treated with amphotericin B and underwent postdrainage magnetic resonance imaging (MRI) of

Clinical... $08.00+0 ... 2005, Am... stake... 43, No. 12 ...h

Chronic Invasive Aspergillosis caused by *Aspergillus viridinutans*

Donald C. Vinh,[1] Yvonne R. Shea,[1] Pamela A. Jones, Alexandra F. Freeman, Adrian Zelazny, and Steven M. Holland

Aspergillus viridinutans, a mold phenotypically resembling *A. fumigatus*, was identified by gene sequence analyses from 2 patients. Disease was distinct from typical aspergillosis, being chronic and spreading in a contiguous manner across anatomical planes. We emphasize the recognition of fumigati-mimetic molds as agents of chronic or refractory aspergillosis.

infiltrate was noted. Antifungal therapy was changed to posaconazole. Over the next 2 months, there was expansion of the right lung infiltrates and lymphadenopathy. Treatment was modified to posaconazole and caspofungin. Serial imaging over the next 3 weeks showed regression of the lung consolidations and mediastinal mass. Four months later, with ongoing resolution of the thoracic disease, the patient began receiving a maintenance dosage of posaconazole. As of 5 years later, he had experienced no recurrence.

Patient 2 was an 8-year-old boy with hyperimmunoglobulin-E (Job) syndrome due to mutation in signal transducer and activator of transcription-3 in whom a right-sided pulmonary abscess developed and failed to improve after 1 month of antibacterial therapy. New left lung nodules were biopsied and specimens yielded a mold morphologically identified as *A. fumigatus*. During 3 months of treatment with voriconazole, the bilateral pulmonary lesions cavitated. Two months later, left lower lobe wedge resection yielded the same mold. Diaphragmatic injury required primary closure with sutures. One month later, enlargement of the residual left lung lesions prompted

Annette Fothergill,[4] and Kieren A. Marr[1,5*]

Cryptic species

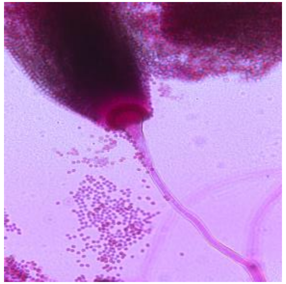


Table 1. *Aspergillus* species distribution according to epidemiological surveys from Spain and the U. S.[22]

Species	Section	Transnet		FILPOP	
		N isolates	%	N isolates	%
A. fumigatus	Fumigati	139	63.8	156	56.1
A. lentulus	Fumigati	4	1.8	3	1.1
A. udagawae	Fumigati	3	1.4	0	0.0
N. peudofischeri	Fumigati	1	0.5	1	0.4
A. viridinutans	Fumigati	0	0.0	1	0.4
A. fumigatiafinis	Fumigati	0	0.0	1	0.4
A. flavus	Flavi	29	13.3	27	9.7
A. alliaceus	Flavi	0	0.0	3	1.1
A. terreus	Terrei	11	5.0	26	9.4
A. carneus	Terrei	0	0.0	1	0.4
A. tubingensis	Nigri	6	2.8	22	7.9
A. niger	Nigri	13	6.0	21	7.6
A. calidoustus	Usti	6	2.8	4	1.4
A. insuetus	Usti	0	0.0	1	0.4
A. keveii	Usti	0	0.0	1	0.4
A. sydowii	Versicolores	2	0.9	1	0.4
A. versicolor	Versicolores	3	1.4	0	0.0
E. quadrilineata	Nidulantes	1	0.5	0	0.0
A. nidulans	Nidulantes	0	0.0	8	2.9
A. westerdijkiae	Circumdati	0	0.0	1	0.4
Total		218	100	278	100

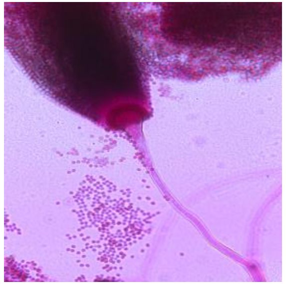
Balajee et al, JCM 2009 Alastruey-Izquierdo et al. AAC 2013

Alastruey-Izquierdo et al. ANYAS 2012

Cryptic species

11% cryptic species

14% cryptic species



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A. udagawae	Fumigati	3	1.4	0	0.0
N. peudofischeri	Fumigati	1	0.5	1	0.4
A. viridinutans	Fumigati	0	0.0	1	0.4
A. fumigatiafinis	Fumigati	0	0.0	1	0.4
A. flavus	Flavi	29	13.3	27	9.7
A. alliaceus	Flavi	0	[illegible]	[illegible]	[illegible]
A. terreus	Terrei	11	[illegible]	[illegible]	[illegible]
A. carneus	Terrei	0	[illegible]	[illegible]	[illegible]
A. tubingensis	Nigri	6	[illegible]	[illegible]	[illegible]
A. niger	Nigri	13	[illegible]	[illegible]	[illegible]
A. calidoustus	Usti	6	[illegible]	[illegible]	[illegible]
A. insuetus	Usti	0	0.0	1	0.4
A. keveii	Usti	0	0.0	1	0.4
A. sydowii	Versicolores	2	0.9	1	0.4
A. versicolor	Versicolores	3	1.4	0	0.0
E. quadrilineata	Nidulantes	1	0.5	0	0.0
A. nidulans	Nidulantes	0	0.0	8	2.9
A. westerdijkiae	Circumdati	0	0.0	1	0.4
Total		218	100	278	100

Chowdhary et al. (2016)
16.6% cryptic species

Balajee et al, JCM 2009

Alastruey-Izquierdo et al. AAC 2013

Alustruey- Izquierdo A et al. 2012, Ann N Y Acad Sci. 1273:18-24

Cryptic species

	n	AMB	ICZ	VCZ	PCZ	CPF	MCF	ANF
A. lentulus	26	**3**	**2.3**	**3.4**	**0.23**	1.6	0.1	0.1
N. hiratsukae	9	1.7	0.9	1.1	0.16	0.11	0.03	0.03
N. pseudofischerii	6	0.25	**4**	**2.51**	**0.22**	0.86	0.03	0.03
A. fumigatiaffiinis	6	**4.8**	**5**	**3.1**	**0.4**	0.22	0,03	0,03
N. udagawae	5	**2**	0.6	**2.3**	**0.25**	0.3	0.03	0.03
A. viridinutans	3	0,7	**16**	**4**	**0,25**	5,66	0,06	0,09
A. tubingensis	22	0.11	0.42	0.76	0.09	0.3	0.05	0.03
A. calidoustus	19	0.9	**8.6**	**6.2**	**6.8**	0.5	0.04	0.04
A. insuetus	2	0.7	**11.3**	**8**	**2.8**	5.6	1.4	0.9
A. keveii	1	0,25	**16**	**16**	**16**	16	16	16
A. alliaceus	30	**19.25**	0.2	0.5	0.11	12.15	3.8	1.9

A. lentulus

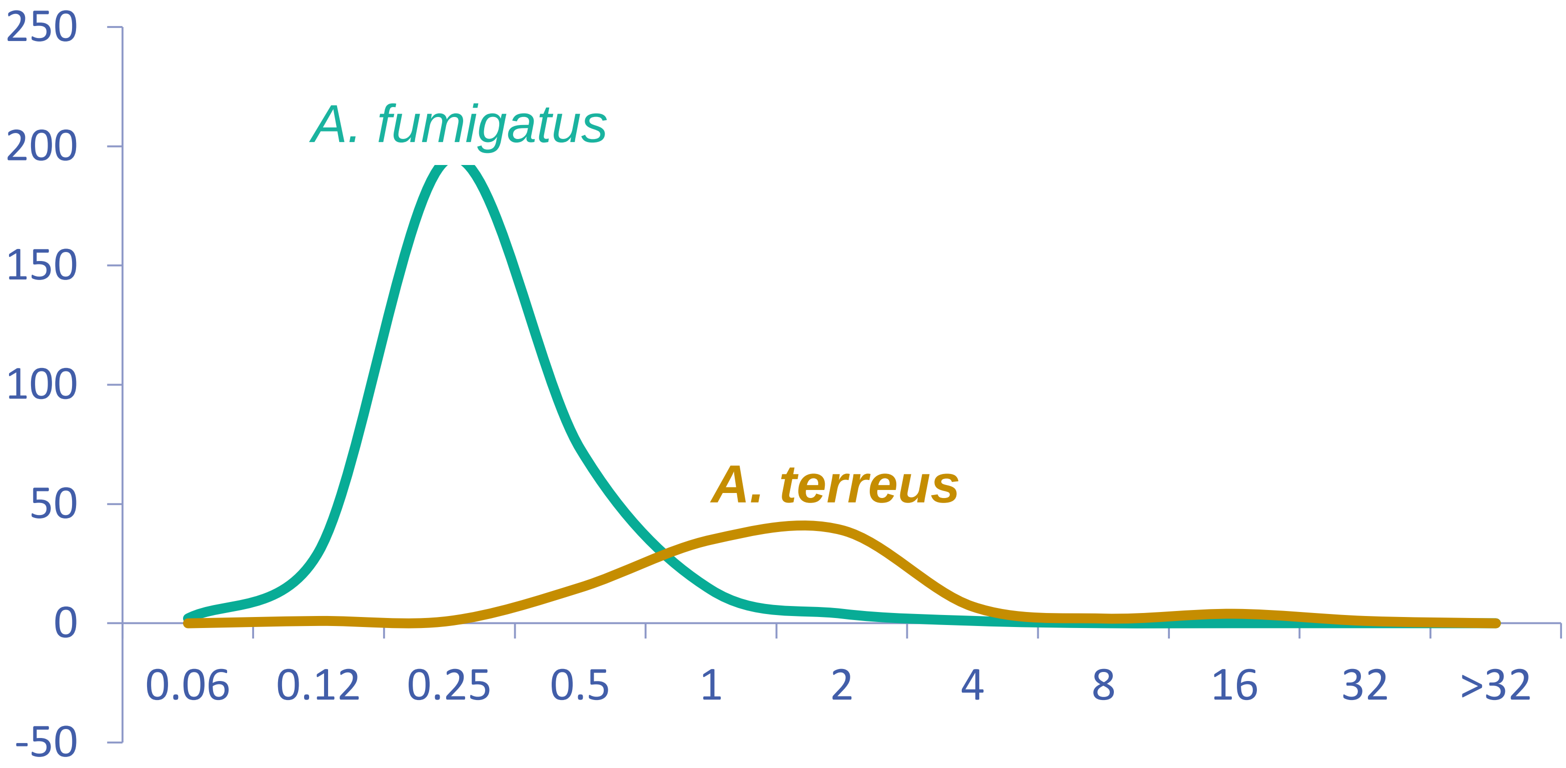
Summary on published clinical cases caused by *Aspergillus lentulus*

Year (Reference)	Number of patients	Underlying disease/ procedure	Source of isolate	Disease classification	Species identification	Susceptibility pattern	Treatment	Outcome
2004 (10)	4	Stem cell transplantation	Lung, mouth, kidney	Proven or probable IA	Genotypic	MIC Itra 0.5–2 mg/L, Vor 2–4 mg/L, AmB 1–4 mg/L; MEC Cas 4–>32 mg/L	NR	Died
2008 (11)	1	Chronic obstructive lung disease	Lung	Probable IA, Co-infection with *A. fumigatus*	Genotypic	MIC AmB 4 mg/L, Itra 3 mg/L, Vor 1.5 mg/L; MEC Cas >32 mg/L	Vor	Died
2009 (12)	1	Kidney transplantation	Lung	Probable IA, Co-infection with *A. fumigatus*	Genotypic	MIC Vor 2 mg/L, AmB 2 mg/L; MEC Cas >16 mg/L	L-AmB	Died
2009 (4)	4	Stem cell transplantation	Lung	Proven or probable IA	Genotypic	MIC AmB 0.5–2 mg/L, Itra 0.25–0.5 mg/L, Vor 1–4 mg/L, Pos 0.25 mg/L	NR	NR
2010 (13)	1	Cystic fibrosis	Lung	Colonization	Genotypic	MIC AmB 4 mg/L, Itra 2 mg/L, Vor 8 mg/L	NR	NR
2011 (14)	1	Cystic fibrosis	Lung	Colonization	Genotypic	MIC AmB 2 mg/L, Itra 2 mg/l, Vor 2 mg/L, Pos 0.5 mg/L	NR	NR
2012 (current report)	1	Heart transplantation	Lung	Proven IA	Genotypic	MIC AmB 2.0 mg/L, Itra 0.25 mg/L, Vor 1.0 mg/L, Pos 0.125 mg/L; MEC Cas 16 mg/L	Vor, Cas, AmB	Died

Zbinden et al, TID 2012

MICs distribution for AMB

Alastruey-Izquierdo TIMM 2017



Data obtained from Mycology Reference Laboratory. Spain

Aspergillus terreus

bjh research paper

Epidemiology and outcome of infections due to *Aspergillus terreus*: 10-year single centre experience

Table II. Response to antifungal therapy in patients with IA caused by *Aspergillus terreus* and non-*A. terreus* between 1994 and 2004.

	A. terreus		Non-*A. terreus*	
Treatment	Patients (*n*)	R	Patients (*n*)	
Amphotericin B	13	1 (8)	14	5 (36)
LAMB	11	4 (36)	9	6 (67)
Itraconazole	4	2 (50)	2	1 (50)
Voriconazole	2	2 (100)	5	3 (60)
Caspofungin + voriconazole	1	1 (100)	3	3 (100)
Amphotericin B or LAMB + voriconazole	1	0 (0)	2	1 (50)

IA, invasive aspergillosis; LAMB, liposomal amphotericin B.

20%

47%

Lass-Florl *BJH* 2005

A. niger complex

Alastruey-Izquierdo TIMM 2017

TABLE 1. Source, molecular identification, MICs, and MECs for species of *Aspergillus* section *Nigri*[a]

Isolate	Source	Molecular identification (β-tubulin gene)	MIC (mg/liter)[b]						MEC (mg/liter)[c]	
			AMB	ITC	VCZ	RVC	POS	TRB	CAS	MICA
Isolates of *Aspergillus* section *Nigri* showing low ITC MICs										
CM-3236	Respiratory	*A. niger*	0.19	0.5	0.5	1.0	0.12	1.0	0.25	0.03
CM-3257	Respiratory	*A. niger*	0.25	1.0	1.0	1.0	0.25	1.0	0.25	0.03
CM-3506	Respiratory	*A. niger*	0.19	0.5	0.75	1.0	0.12	0.31	0.15	0.03
CM-3507	Respiratory	*A. tubingensis*	0.19	0.5	1.0	1.5	0.15	0.62	0.06	0.03
CM-3585	Environmental	*A. tubingensis*	0.19	0.5	1.0	1.67	0.12	0.42	0.37	0.03
CM-3586	Catheter	*A. niger*	0.25	0.5	1.0	2.0	0.12	0.12	1.0	0.03
CM-3636	Respiratory	*A. niger*	0.25	0.5	0.5	1.0	0.19	1.0	0.25	0.03
CM-3641	Respiratory	*A. niger*	0.25	0.5	1.0	1.0	0.125	0.25	0.5	0.03
CM-3672	Cutaneous	*A. niger*	0.12	0.5	1.0	1.5	0.19	0.07	0.15	0.03
CM-4004	Unknown	*A. niger*	0.25	1.0	1.0	1.67	0.25	0.13	0.10	0.03
CM-4213	Respiratory	*A. niger*	0.33	0.14	0.33	0.58	0.03	0.22	0.39	0.03
CM-4264	Blood culture	*A. tubingensis*	0.12	0.5	1.0	1.5	0.12	0.50	0.03	0.03
CM-4296	Respiratory	*A. tubingensis*	0.12	0.75	1.0	1.5	0.19	0.62	0.25	0.03
CM-4316	Respiratory	*A. niger*	0.19	0.5	0.5	1.0	0.125	0.5	0.25	0.03
CM-5094	Respiratory	*A. tubingensis*	0.12	0.5	0.75	2.0	0.06	0.62	0.19	0.03
CM-5095	Respiratory	*A. niger*	0.19	0.5	0.75	1.5	0.12	0.62	0.25	0.03
GM for group			0.20	0.56	0.82	1.31	0.14	0.50	0.28	0.03
Isolates of *Aspergillus* section *Nigri* showing much higher ITC MICs										
CM-3123	Respiratory	*A. tubingensis*	0.25	11	1.67	2.67	0.25	1.17	0.25	0.03
CM-3810	Respiratory	*A. tubingensis*	0.25	4.0	2.0	2.0	0.12	1.0	0.5	0.03
CM-4003	Unknown	*A. tubingensis*	0.12	16	2.0	4.0	0.25	1.0	0.25	0.03
CM-4005	Unknown	*A. tubingensis*	0.12	16	2.0	4.0	0.5	0.25	0.5	0.03
CM-4688	Respiratory	*A. tubingensis*	0.21	3.67	2.0	3.33	0.25	1.50	0.18	0.03
CM-5264	Respiratory	*A. foetidus*	0.12	16	2.0	8.0	0.5	0.5	0.06	0.03
GM for group			0.18	11.11	1.95	4.0	0.31	0.90	0.29	0.03
Isolates of *Aspergillus* section *Nigri* showing paradoxical effect against ITC										
CM-3125	Respiratory	*A. tubingensis*	0.12	0.5	1	1.67	0.12	0.5	0.05	0.03
CM-3177	Respiratory	*A. tubingensis*	0.16	1	2	3.33	0.25	0.67	0.05	0.03
CM-3551	Respiratory	*A. niger*	0.5	4.75	1	2	0.12	0.25	0.03	0.03
CM-3654	Blood culture	*A. tubingensis*	0.19	1	2	2.50	0.25	0.63	0.14	0.03
CM-4000	Unknown	*A. tubingensis*	0.16	1	2	2	0.25	0.33	0.10	0.03
CM-4001	Unknown	*A. tubingensis*	0.19	1	1.75	2.50	0.25	0.56	0.11	0.03
CM-4002	Unknown	*A. foetidus*	0.25	1	2	2.67	0.12	0.33	0.14	0.03
CM-4262	Ophthalmic	*A. niger*	0.25	1	2	2	0.25	0.29	0.13	0.03
CM-4352	Respiratory	*A. tubingensis*	0.28	1	0.88	2	0.25	0.31	0.15	0.03
CM-4897	Blood culture	*A. tubingensis*	0.16	1	2	2	0.25	0.42	0.10	0.03
CM-4899	Respiratory	*A. tubingensis*	0.16	1	2	2.67	0.25	0.33	0.10	0.03
CM-4995	Prosthesis	*A. foetidus*	0.21	1	2	2	0.16	0.33	0.14	0.03
GM for group			0.2	1.3	1.72	2.28	0.21	0.41	0.10	0.03

[a] GM, geometric means of MICs and MECs for the strains within each group.
[b] MIC geometric mean of amphotericin B (AMB), itraconazole (ITC), voriconazole (VCZ), ravuconazole (RVC), posaconazole (POS), and terbinafine (TRB).
[c] MEC geometric mean of caspofungin (CAS) and micafungin (MICA).

Alcazar-Fuoli et al AAC 2009

36% *A. awamori*
90% *A. tubingensis*
33% *A. niger*
100% *A. acidus*
67% unknown

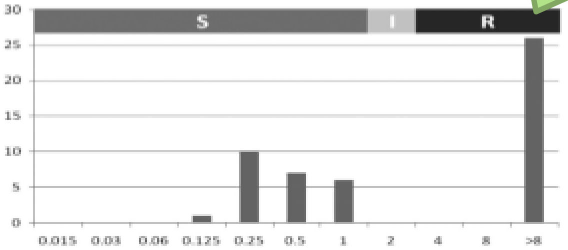
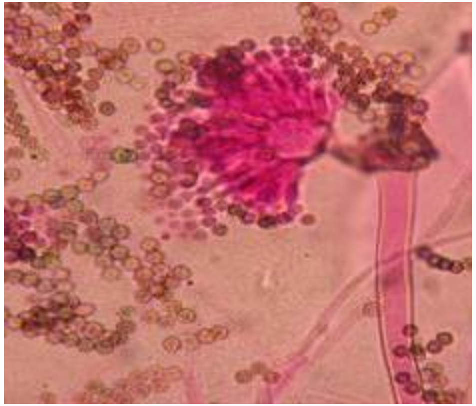


FIG. 1. Itraconazole MICs of *Aspergillus* section *Nigri* from the Mycology Reference Centre Manchester culture collection and proposed ECVs (S, susceptible; I, intermediate; R, resistant). The MIC is shown on the x axis, and the number of isolates is shown on the y axis.

Howard et al, AAC 2011

Aspergillus ustus complex

	n	AMB	ICZ	VCZ	PCZ
A. calidoustus	19	0.9	**8.6**	**6.2**	**6.8**
A. insuetus	2	0.7	**11.3**	**8**	**2.8**
A. keveii	1	0.25	**16**	**16**	**16**

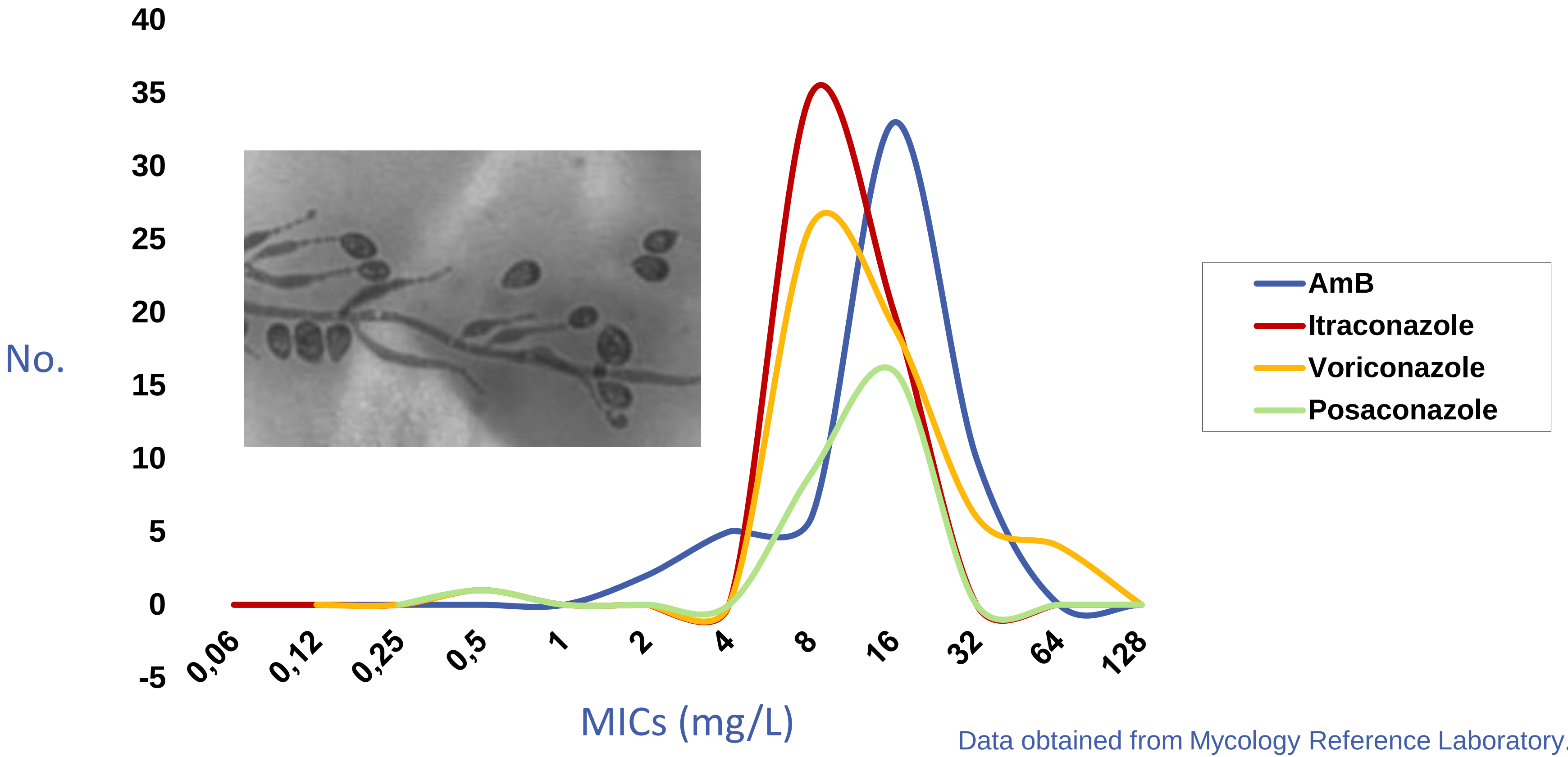


Alastruey-Izquierdo et al, Medical Mycology 2010

And Other species?

Lomentospora prolificans

Alastruey-Izquierdo TIMM 2017



Data obtained from Mycology Reference Laboratory. Spain

Mucorales

Species
Rhizopus arhizus
Rhizopus microspor
Mucor spp.
Rhizomucor spp.
Lichtheimia corymb
Cunninghamella be

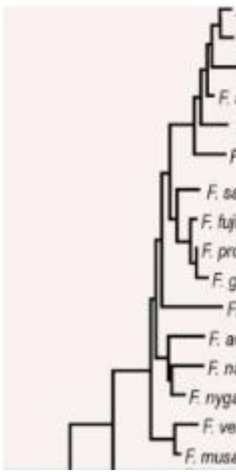
Data obtained fro
Alastruey

TABLE 1 *In vitro* susceptibilities of *Mucorales* ($n = 124$) to amphotericin B, posaconazole, isavuconazole, and voriconazole as determined by the CLSI and EUCAST broth microdilution methods

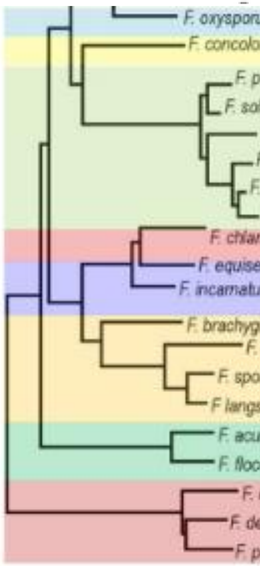
Genus (no. of isolates tested)	Species (no. of isolates tested)	Parameter	Test result (µg/ml) for: AMB CLSI	AMB EUCAST	POS CLSI	POS EUCAST	ISAV CLSI	ISAV EUCAST	VRC CLSI	VRC EUCAST
Rhizopus (74)	*arrhizus* var. *delemar* (25)	GM	0.05	0.24	1.43	3.78	5.42	6.96	10.55	15.56
		MIC_{50}	0.03	0.25	1	4	8	8	16	16
		MIC_{90}	0.25	0.5	8	8	8	8	16	16
		Range	0.03–0.5	0.03–0.5	0.125–8	0.5–8	1–8	2–8	4–16	8–16
	arrhizus var. *arrhizus* (22)	GM	0.07	0.40	0.66	1.45	1.76	3.20	8.25	15.02
		MIC_{50}	0.06	0.5	0.5	1	1	4	8	16
		MIC_{90}	0.25	2	8	8	8	8	16	16
		Range	0.03–0.5	0.06–2	0.125–8	0.5–8	0.5–8	1–8	4–16	8–16
	microsporus (23)	GM	0.11	0.53	0.78	2.70	2.32	4.65	9.02	15.06
		MIC_{50}	0.125	0.5	1	4	2	8	16	16
		MIC_{90}	1	1	2	8	4	8	16	16
		Range	0.03–1	0.125–1	0.125–8	0.5–8	0.5–8	1–8	1–16	8–16
	stolonifer (4)	Range	0.03–0.06	0.125–0.5	0.125–0.5	0.125–1	0.25–1	0.5–2	4–8	8–16
Mucor (7)	*circinelloides* (5)	Range	0.03–0.125	0.125–0.5	0.125–1	0.5–8	1–8	2–8	8–16	16
	ramosus (1)	MIC	0.25	0.25	1	1	8	8	16	16
	velutinosus (1)	MIC	0.06	0.125	2	8	2	8	16	16
Lichtheimia (13)	*ramosa* (7)	Range	0.06–1	0.125–1	0.5–8	0.5–8	0.5–8	1–8	8–16	8–16
	corymbifera (6)	Range	0.06–0.5	0.125–1	0.125–8	0.5–8	2–8	2–8	8–16	16
Rhizomucor (8)	*pusillus* (7)	Range	0.06–1	0.06–1	0.25–8	1–8	1–8	4–8	8–16	16
	miehei (1)	MIC	1	2	0.125	2	8	8	16	16
Cunninghamella (4)	*bertholletiae* (3)	Range	0.25–1	0.5–1	8	8	8	8	16	16
	elegans (1)	MIC	2	8	8	8	8	8	16	16
Apophysomyces (3)	*elegans* (2)	Range	0.06–0.125	0.125–0.5	0.25–0.5	1–2	0.25–4	8	4–16	16
	variabilis (1)	MIC	0.06	0.5	0.5	1	4	8	8	16
Syncephalastrum (15)	*racemosum* (15)	GM	0.06	0.15	0.62	1.14	2.40	4	11.05	16
		MIC_{50}	0.06	0.125	0.5	1	4	4	16	16
		MIC_{90}	0.125	0.5	4	8	8	8	16	16
		Range	0.03–0.5	0.03–1	0.125–8	0.25–8	0.125–8	1–8	4–16	16

Chowdhary et al. AAC 2015

Fusarium spp



species	AMB	5FC	FLC	ITC	VOR	POS	AND	CAS	Complex
	MIC/MEC (µg/ml)								
F. ananatum	1	>64	>64	>16	4	0.5	>16	>16	***fujikuroi***
F. guttiforme	0.5	>64	>64	>16	2	>16	>16	>16	
F. temperatum	0.25	>64	>64	>16	0.5	>0.25	>16	>16	
F. anthophilum	0.25	>64	>64	>16	2	0.5	>16	>16	
F. subglutinans	1	>64	>64	>16	1	0.25	>16	>16	
F. acutatum	1	>64	>64	>16	4	2	>16	>16	
F. sacchari	2	>64	>64	>16	4	>16	>16	>16	
F. fujikuroi	0.5	>64	>64	>16	2	1	>16	>16	
F. proliferatum	0.5	>64	>64	>16	4	2	>16	>16	
F. globosum	-	-	-	-	-	-	-	-	
F. thapsinum	2	>64	>64	>16	8	>16	>16	>16	
F. andiyazi	2	32	>64	>16	1	0.25	>16	>16	
F. napiforme	4	>64	>64	>16	1	0.25	>16	>16	
F. nygamai	4	>64	>64	>16	8	>16	>16	>16	
F. verticillioides	2	>64	>64	>16	1	0.25	>16	>16	
F. musae	4	>64	>64	>16	8	1	>16	>16	



species	AMB	5FC	FLC	ITC	VOR	POS	AND	CAS	Complex
	MIC/MEC (µg/ml)								
F. oxysporum	0.5	>64	>64	>16	2	1	>16	>16	***oxysporum***
F. concolor	2	>64	>64	>16	4	2	>16	>16	***concolor***
F. petroliphilum	0.5	>64	>64	>16	8	>16	>16	>16	***solani***
F. solani-6	2	>64	>64	>16	2	4	>16	>16	
F. lichenicola	1	>64	>64	>16	4	8	>16	>16	
F. keratoplasticum	2	>64	>64	>16	8	>16	>16	>16	
F. solani-5	1	>64	>64	>16	8	4	>16	>16	
F. falciforme	1	>64	>64	>16	4	8	>16	>16	
F. chlamydosporum	4	>64	>64	>16	2	>16	>16	>16	***chlamydosporum***
F. equiseti	1	>64	>64	>16	4	2	>16	>16	***equiseti***
F. incarnatum	0.5	>64	>64	>16	4	4	>16	>16	
F. brachygibbosum	-	-	-	-	-	-	-	-	***sambucinum***
F. armeniacum	-	-	-	-	-	-	-	-	
F. sporotrichioides	4	-	-	-	>16	-	-	-	
F. langsethiae	>32	>64	>64	1	0.5	-	-	-	
F. acuminatum	-	-	-	-	-	-	-	-	***tricictum***
F. flocciferum	-	-	-	-	-	-	-	-	
F. dimerum	0.5	>64	>64	>64	2	>16	>16	>16	***dimerum***
F. delphinoides	1	>64	>64	>16	4	>16	>16	>16	
F. penzigii	-	-	-	-	-	-	-	-	

Al Hatami et al. Plos. Path. 2016

Emerging species

Table 2. Epidemiological features of rare mould species

Species	Diseases	Specific characteristics	References
Mucorales	IFD in patients with risk factors	• Increasing prevalence in haematological patients • Higher mortality than aspergillosis • Resistance to voriconazole	42
Fusarium spp.	Local and disseminated mycoses in patients with risk factors	• Leading cause of IFD in haematological patients in some areas (Brazil) • Mortality >75% in IFD cases • Unpredictable resistance to some antifungal agents	43, 44
Scedosporium apiospermum complex	Colonization, local infections and IFDs	• More common in temperate areas • High mortality in IFD cases • Voriconazole is the most potent antifungal agent against them	43, 45
Scedosporium prolificans	Colonization, local infections and IFDs	• More common in southern Europe, Australia and California • Mortality >90% in IFD cases • Multiresistant organism	43, 46
Other rare mould species	Colonization, local infections and IFDs	• Unreliable data on prevalence and mortality • Identification at species level and AST are compulsory for correct management	43, 47

IFD, invasive fungal disease; AST, antifungal susceptibility testing.

Lass-Florl & Cuenca-Estrella JCM 2017

Conclusions

- Antifungal resistance is increasing
  - Both in yeast and moulds
    - Development of secondary resistance (*C. glabrata A. fumigatus*…)
    - Intrinsically resistant species (*C. auris*, *A. lentulus*, Mucorales…)
- Important to know local epidemiology
- Perform AFST to any isolate coming from invasive infection

Conclusions

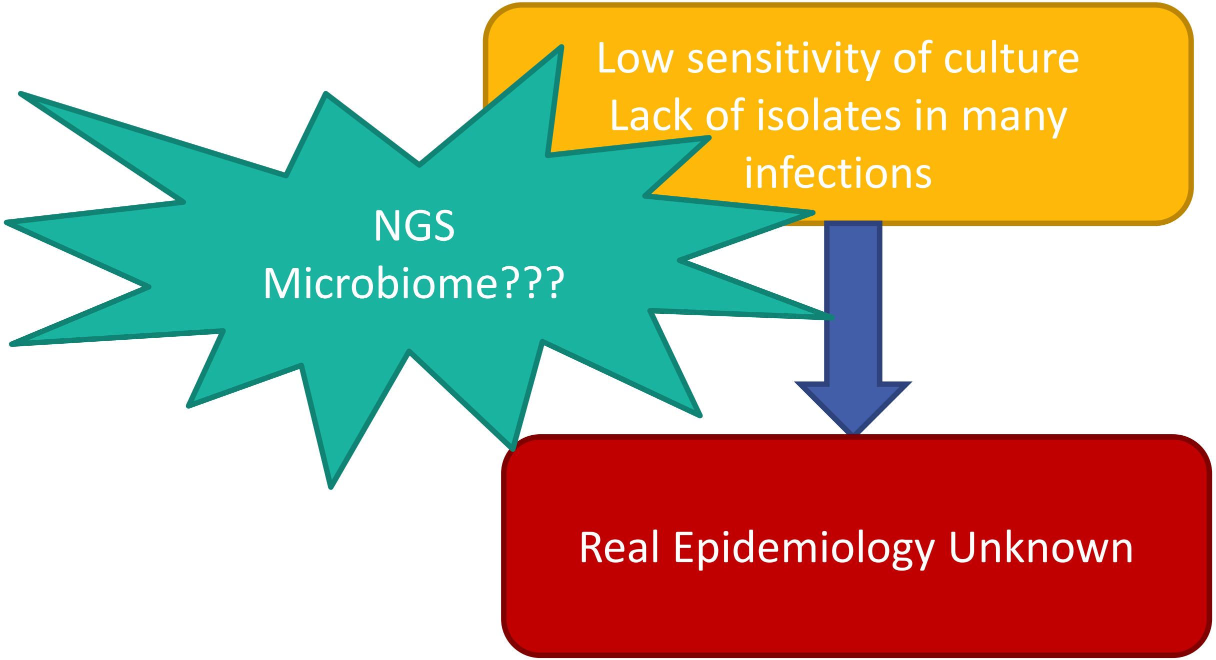
- Antifungal resistance is increasing
  - Both in yeast and moulds
    - Development of secondary resistance (*C. glabrata A. fumigatus…*)
    - Intrinsecally resistant species (*C. auris, A. lentulus,* Mucorales…)
- … know local epidemi…
  … any isolate com…

AFST

Monitor their prevalence

Epidemiological studies

But...


Low sensitivity of culture
Lack of isolates in many infections
NGS
Microbiome???
Real Epidemiology Unknown

Thanks for your attention

TIMM
Trends in Medical Mycology

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