Insights into Antifungal Resistance & Newer (Other) Moulds

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Disclosures

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- Astellas
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- Viamet

Speakers Bureau

- Gilead

Member, CLSI Antifungal Susceptibility Subcommittee

Road Map

- Antifungal activity/resistance in newer/ cryptic species (& other moulds)
  - *Aspergillus fumigatus* & azole resistance
  - Cryptic *Aspergillus* spp.
  - Mucorales
  - *Fusarium* spp.
  - *Scedosporium* spp.
  - Other moulds (intrinsic microbiologic resistance)
- Challenges with mould susceptibility testing & interpretation of results

Azole Resistant *A. fumigatus*

Continent/Country	Percent Resistance	Isolate Source
United States	0.6 – 11.8%	Clinical & Environmental
Colombia (TR_{53})	3.3%	Environmental
Africa (TR_{34}/L98H, TR_{46}/Y121F/T289A)		
Tanzania	13.9%	Environmental
Asia & Australia (G54, M222, G448S, TR_{34}/L98H, TR_{46}/Y121F/T289A)		
Australia	2.6%	Clinical
Taiwan, China, India, Iran, Japan, Kuwait, Pakistan	1.9 – 11.1%	Clinical & Environmental

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Isolation of azole-resistant *Aspergillus fumigatus* from the environment in the south-eastern USA

Steven F. Hurst[1], Elizabeth L. Berkow[1], Katherine L. Stevenson[2], Anastasia P. Litvintseva[1] and Shawn R. Lockhart[1]*

Rivero-Menendez *J Fungi* 2016;21; doi:10.3390/jof2030021. Wu et al. *Mycoses* 2015;58:544-549.

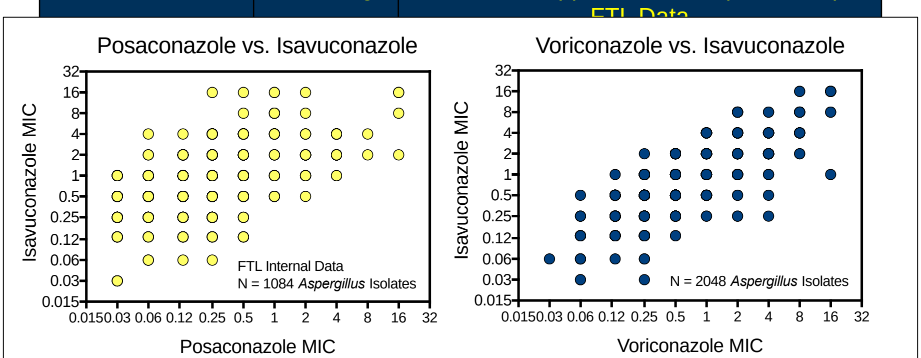
Cryptic *Aspergillus* Species

TRANSNET (US 2001-2006)	218 *Aspergillus* isolates 11% cryptic species	*A. lentulus* (1.8%) *A. udagawae* (1.4%) *A. tubingensis* (2.8%) *A. calidoustus* (2.8%)
FILPOP (Spain Oct 2010 & May 2011)	323 *Aspergillus* isolates 14.5% cryptic species	*A. lentulus* (1.1%) *A. alliaceus* (1.1%) *A. tubingensis* (7.9%) *A. calidoustus* (1.4%)

Section	Cryptic Species	Antifungal Susceptibility
Fumigati	*A. lentulus* *A. thermomutatus* *A. udagawae* *A. felis*	Reduced azole susceptibility
Flavi	*A. alliaceus*	Reduced susceptibility to amphotericin B and echinocandins
Nidulantes	*A. spinulosporus* *A. quadrilineatus*	Variable amphotericin B susceptibility azole susceptible
Nigri	*A. tubingensis*	Variable azole susceptibility
Usti	*A. calidoustus*	Reduced amphotericin B and azole susceptibility
Versicolores	*A. sydowii* *A. versicolor*	Variable amphotericin B susceptibility & reduced azole susceptibility

Aspergillus Species

U.S. Isolates (2015-2018)



Section	Percentage	Prevalent spp. in Sections (% Overall) FTL Data
Circumdati	1.0%	*A. subramanianii* (0.3%)
Aspergillus & Cremei	0.6%	---

FTL Internal Data (1889 *Aspergillus* human isolates, excluding those from ears and nails)

Mucorales

Common causes of non-*Aspergillus* invasive mould infections

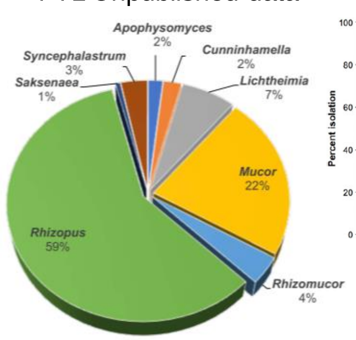
Genera	Representative Species (not comprehensive)
Rhizopus (most prevalent)	*R. arrhizus / delemar, R. microsporus, R. schipperae*
Mucor	*M. circinelloides* (f. *circinelloides* & f. *janssenii*), *M. irregularis*
Rhizomucor	*R. pusillus*
Lichtheimia (formerly *Absidia*)	*L. corymbifera, L. ornata, L. ramosa*
Cunninghamella	*C. bertholletiae, C. echinulata*
Apophysomyces	*A. elegans, A. trapeziformis, A. variabilis, A. mexicanus*
Saksenaea	*S. erythrospora, S. oblongispora, S. vasiformis*
Syncephalastrum	*S. racemosum*

- Risk factors
  - Hematologic malignancies (± HSCT)
  - Prolonged neutropenia or corticosteroid use
  - Poorly controlled DM
  - Trauma (traumatic inoculation)

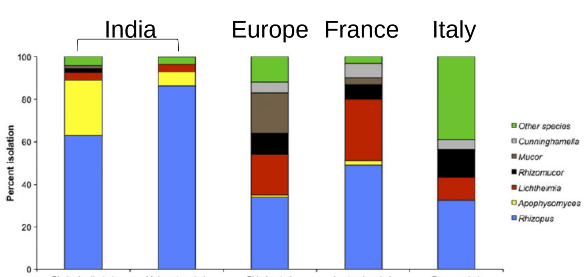
Order Mucorales

Species Distribution by Geographic Area

FTL Unpublished data



- 655 isolates (2.5 years)
- *Rhizopus* spp. most prevalent
  - *43.1% R. arrhizus*
  - *22.3% R. delemar*

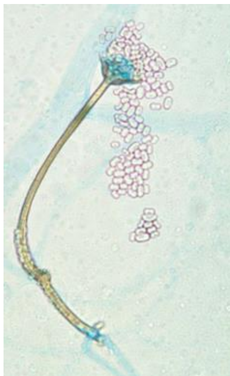


Species distribution may vary by region

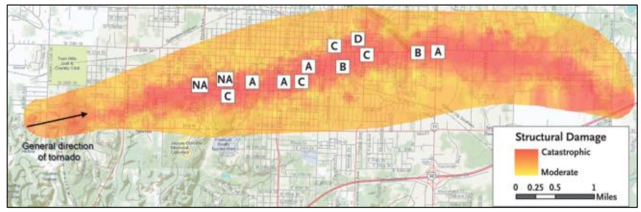
- *Rhizopus* most prevalent, followed by *Lichtheimia, Mucor,* & *Rhizomucor*
- *Apophysomyces* common in India

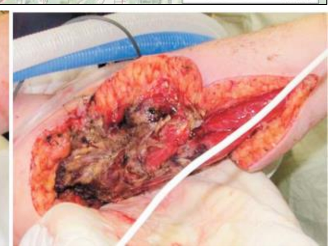
Alvarez et al. *J Clin Microbiol* 2009 47:1650-1660.
Gomes et al. *Clin Microbiol Rev* 2011;24:411-445.
Chakrabati & Singh *Mycoses* 2014;57(Suppl. 3):85-90.

Necrotizing Cutaneous Mucormycosis after a Tornado in Joplin, Missouri, in 2011



Apophysomyces trapeziformis





13 patients with soft-tissue infections; 5 died; all in zone with most severe damage

Neblett et al. *N Engl J Med* 2012;367:2214-2225.

Mucorales Antifungal Susceptibility

What about Isavuconazole?

Study	Arendrup et al. *AAC* 2015 (CLSI methodology)						Pfaller et al. *AAC* 2018	
Drug	Amphotercin		Posaconazole		Isavuconazole		Isavuconazole	
Species	MIC Range	MIC50	MIC Range	MIC50	MIC Range	MIC50	MIC Range	MIC50
R. oryzae (arrhizus)	0.03-0.25	0.06	0.12-0.5	0.5	0.12-2	1	0.25-4	1
R. microsporus	0.03-0.25	0.12	0.06-0.5	0.25	0.12-1	0.5	0.25->16	1
M. circinelloides	0.03-0.12	0.06	0.125->16	2	1-8	8	2->16	8
Lichtheimia spp.	0.03-0.12	0.03	0.12-0.5	0.25	0.5-2	1	1->16	4
	Chowdhary et al. *Mycoses* 2014						FTL Unpub. Data	
Apophysomyces	0.06-0.12	---	0.25-1	---	0.25-8	---	1-4	---
Cunninghamella	---	---	---	---	---	---	0.5->16	8
Saksenaea	---	---	---	---	---	---	0.25-2	---
Syncephalastrum	0.03-0.12	0.06	0.06-1	0.5	0.125-8	1	0.25->16	>16

In clinical setting, is reduced isavuconazole susceptibility offset by higher exposure?

Chowdhary et al. Mycoses 2014;57(Suppl. 3):97-107. Arendrup et al. *Antimicrob Agents Chemother* 2015;59:7735-7742. Pfaller et al. *Antimicrob Agents Chemother* 2018 (Epub ahead of print).

Scedosporium & Scedosporiosis

- Invasive infections in immunocompromised patients (may mimic aspergillosis)
- Common colonizers in CF patients

Species	Taxonomic / Nomenclature Changes	FTL % Isolates	Lackner *AAC* 2012			
			AMB	VOR	ISA	PSC
Scedosporium apiospermum	Previously considered to be anamorph of *Pseudallescheria boydii*	57.2%	0.5->16	0.25-8	1->16	0.25->16
Scedosporium boydii	Previously *Pseudallescheria boydii*	22.5%	0.5->16	0.12-2	0.5->16	0.12->16
Scedosporium aurantiacum	Reduced amphotericin & azole susceptibility	1.4%	≥16	0.5-1	4-16	1->16
Scedosporium dehoogii	Rare Reduced voriconazole susceptibility	1.8%	2->16	0.5->16	2->16	0.5->16
Scedosporium minutispora	Previously *Pseudallescheria minutispora*	0.7%	1-4	0.25-2	2-16	0.5->16
P. ellipsoidea	Still *Pseudallescheria* species	1.8%	4->16	0.5-4	2->16	0.5->16
Lomentospora prolificans	Previously *Scedosporium prolificans* - Highly resistant	12.3%	8->16	4->16	8->16	>16

Lackner et al. *Antimicrob Agents Chemother* 2012;56:2635-2642. FTL internal data (N = 285; 9/12 through 8/18)

Fusarium Species & Fusariosis

Significant cause of morbidity and mortality in immunocompromised hosts

- Invasive & disseminated infections (high mortality in neutropenic hosts)
- Keratitis & onychomycosis in immunocompetent hosts
  - Fungal keratitis in contact lenses
  - Peritonitis in peritoneal dialysis patients

Human infections can be caused by 8 species complexes

- *Fusarium solani* spp. complex (FSSC)
- *Fusarium oxysporum* spp. complex (FOSC)
- *Fusarium fujikuroi* spp. complex (FFSC)
- *Fusarium chlamydosporum* spp. complex (FCSC)
- *Fusarium dimerum* spp. complex (FDSC)
- *Fusarium incarnatum-equiseti* spp. complex (FIESC)
- *Fusarium sambucinum* spp. complex (FSAMSC)
- *Fusarium tricinctum* spp. complex (FTSC)

Campo et al. *J Infect* 2010;60:331-337. Kerr et al. Ann Intern Med *Ann Intern Med* 1983;99:334-336.
O'Donnell et al. *J Clin Microbiol* 2007;45:2235-2248.

Fusarium solani Species Complex

Species	Haplotype	Proposed Name
F. petroliphilum	haplotype 1	*Neocosmospora petroliphila*
F. keratoplasticum	haplotype 2	*Neocosmospora keratoplastica*
F. falciforme	haplotype 3+4	*Neocosmospora falciforme*
F. solani	haplotype 5	*Neocosmospora solani*
F. metavorans	haplotype 6	*Neocosmospora metavorans*
FSSC 7	haplotype 7	*Neocosmospora gamsii*
F. lichenicola	haplotype 16	*Neocosmospora lichenicola*
FSSC 20	haplotype 20	*Neocosmospora suttoniana*
FSSC 43	haplotype 43	*Neocosmospora catenata*

Genus *Neocosmospora* (FSSC) contains significant plant pathogen species, important agents of veterinary infections, and clinically relevant species that cause infections in immuncompromised hosts.

Lombard et al. *Stud Mycol* 2015;80:189-245. Sandoval-Denis & Crous. *Persoonia* 2018;41:109-129.

Fusarium & Antifungal Susceptibility

	Espinel-Ingroff *AAC* 2015 MIC Ranges (Mode)				Pfaller et al. *AAC* 2018
Species	Amphotericin	Itraconazole	Posaconazole	Voriconazole	Isavuconazole
F. solani SC	≤ 0.25 - 16 (2)	0.5 - >16 (16)	1 - >16 (8)	0.5 - >16 (8)	>4
F. oxysporum SC	≤ 0.25 - 16 (2)	1 - >16 (16)	0.5 - >16 (2)	0.5 - >16 (4)	---
F. fujikuroi SC	0.5 - 2 (1)	≥16 (16)	0.5 - 4 (2)	2 - 16 (4)	---
Fusarium spp.	---	---	---	---	>4 (8->16; >16)*

Limited in vitro activity of available antifungals against *Fusarium*
Clinical relevance in vitro susceptibility testing?

Espinel-Ingroff et al. *Antimicrob Agents Chemother* 2015;1079-1084.
Pfaller et al. *Antimicrob Agents Chemother* 2018 (Epub ahead of print).*FTL internal data

Things to Remember…

- Low MIC _does not_ predict successful outcomes
- High MIC _does not_ predict failure
  - *In vitro* resistance may predict a population less likely to respond
- Other factors are important predictors of outcomes
  - *Host immune status & other comorbidities*
  - *Site of infection*
  - *Time to diagnosis/start of treatment*
  - *Drug concentrations at site of infection*
  - *Drug-drug & drug-disease interactions*

Both antibacterial AND antifungal susceptibility testing share these limitations

Antifungal Susceptibility Moulds
Challenges

- No automation for mould susceptibility testing
  - No FDA-cleared panels
  - Some labs use YeastOne Sensititre plates (*Aspergillus*)
    - Utility against other moulds?
- Few clinical laboratories in U.S. perform susceptibility testing against moulds
  - Sendout lab (delays in results)
- Clinical breakpoints not established against most moulds
  - Limited breakpoints against *Candida* species
  - EUCAST breakpoints against some *Aspergillus* spp.

Epidemiologic Cut-Off Values
ECV or ECoff

- <u>ECVs</u> separate wild-type isolates from non-wild type isolates
  - *Encompass ~95 - 97.5% of isolates in wild type MIC distribution*
  - *Sensitive measure of emergence of resistance*
- <u>Clinical breakpoints</u> indicate isolates likely to respond to treatment with a given antimicrobial agent
  - *Established using PK/PD, ECV & MIC distributions, clinical data*

Genus	Antifungals	CLSI, EUCAST, or Other
Candida (not all species)	Amphotericin, Azoles, Echinocandins (AFG & MFG)	CLSI M59, EUCAST
C. neoformans & *C. gatti* (certain genotypes)	Amphotericin, Azoles, Flucytosine	CLSI M59, EUCAST
Aspergillus (not all species)	Amphotericin, Azoles, Caspofugnin	CLSI M59, EUCAST
Fusarium (by SC)	Amphotericin, Azoles (not Isavuconazole)	Espinel-Ingroff *AAC* 2015

Limited number ECVs established against moulds

Turnidge and Johnson. *Clin Microbiol Rev* 2007;20:391-408. Pfaller et al. *Drug Resist Updat* 2011;14:164-76.

AMERICAN SOCIETY FOR MICROBIOLOGY Antimicrobial Agents and Chemotherapy® 2018:62:e02599-17.
MECHANISMS OF RESISTANCE

2 of 3 *S. apiospermum*-specific amino acid changes (Y136F & G464S) corresponded to mutations in *A. fumigatus* CYP51A (Y121F & G448S) linked to azole resistance

Identification of 14-α-Lanosterol Demethylase (CYP51) in *Scedosporium* Species

Anne Bernhardt,[a] Wieland Meyer,[b,c] Volker Rickerts,[a] Toni Aebischer,[a] Kathrin Tintelnot[a]

MICROBIAL DRUG RESISTANCE
Volume 24, Number 6, 2018
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DOI: 10.1089/mdr.2017.0311

Increased expression of CYP51A observed following exposure to voriconazole, posaconazole, & tebuconazole, but not amphotericin B.

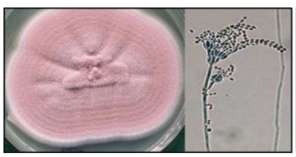
Overinduction of the *CYP51A* Gene After Exposure to Azole Antifungals Provides a First Clue to the Resistance Mechanism in the *Fusarium solani* Species Complex

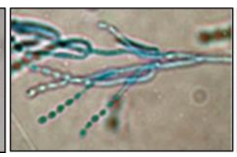
Maurine D'Agostino,[1] Thomas Lemmet,[1] Claire Dufay,[1] Amandine Luc,[2] Jean Pol Frippiat,[1] Marie Machouart,[1,3] and Anne Debourgogne[1,3]

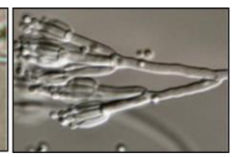
Mellado et a
Chamilos & I ... 92-2097.
Slaven et al. ... . *PLoS Pathog* 2008;4:e1000200. Camps et al. *PLoS One* 2012;7:E50034. Ammar et al. PLoS One 2013;8:e79042. Chowdhary et al. *J Infect Dis* 2017;216:S436-444.

Other Moulds & Resistance

Species	Reduced Susceptibility/Resistance
Purpureocillium lilacinum (formerly *Paecilomyces lilacinus*)	Amphotericin B (MIC >16) Low voriconazole MICs
Paecilomyces variotii	Voriconazole & Isavuconazole (MIC 4->16) Low Amphotercin B MICs
Various *Penicillium* spp. • *P. citrinum* • *P. steckii* • *P. sumatraense* *(plus others)*	Voriconazole (MIC >16) & Sometimes Itraconazole/Posaconazole (variable; MICs can range 4 - >16)







Rasamsonia
Intrinsic Voriconazole Resistance

- Thermotolerant and thermophilic *Penicillium*-like fungi
- *Rasamsonia argillacea* species complex
  - *R. argillacea*
  - *R. eburnean*
  - *R. piperina*
  - *R. aegroticola*

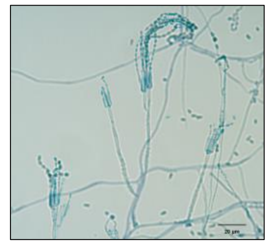
Previously known as *Geosmithia*
Often misidentified by morphology as *Penicillium* or *Paecilomyces*

- Emerging pathogens reported to cause invasive disease in CGD & patients with hematologic malignancies
- Chronic colonization in CF patients
  - No clear correlation between colonization & lung deterioration

Houbraken et al. *J Clin Microbiol* 2013;51:22-30.

Fatal Disseminated *R. aegroticola* Infection & Antifungal Susceptibility

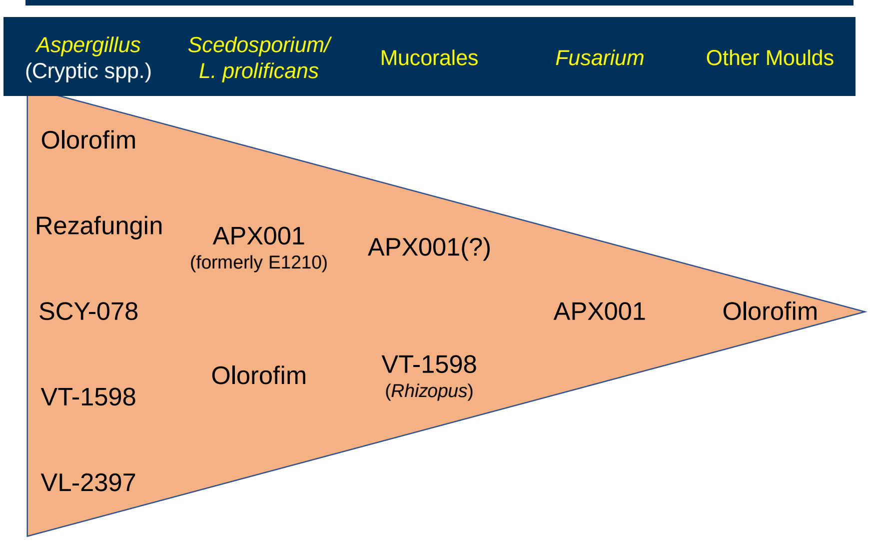
- 21 year old CF patient who developed disseminated invasive fungal infection post-lung transplantation
- Cultures pre-transplant: MRSA, *A. fumigatus*, *P. variotii*
- Voriconazole part of antifungal prophylaxis/treatment
  - Days 2 – 100; trough levels 2-5 µg/mL
- *R. aegroticola* cultured from BAL collected pre-mortem & tissue collected at autopsy
  - DNA sequence analysis (previous sputum cultures mis-identified by morphology as *P. variotii*)



Species	Isavuconazole	Itraconazole	Voriconazole	Posaconazole	Amphotericin	Micafungin
R. argillacea	>32	1->16	>32	1->16	0.5-32	0.125-0.25
R. piperina	>32	0.5-2	>32	0.5-1	1	0.03-0.25
R. aegroticola	>32	2->16	>32	1-4	1-8	0.125-0.25
R. aegroticola (pt. isolate)	NT	2	>16	1	2	≤ 0.015

Hong et al. *J Cyst Fibros* 2017;16:e3-7. Steinmann et al. *Antimicrob Agents Chemother* 2016;60:6890-6891.

Antifungals in Development



Summary

- Increased recognition of antifungal resistance in moulds
  - *Aspergillus fumigatus* & other *Aspergillus* species
  - *Fusarium, Scedosporium* & Mucorales (and others)
- Increased recognition of cryptic species
  - Reduced susceptibility to clinically available agents
- Understanding of clinical significance lags behind microbiologic data
  - Other factors of importance
    - Host response (or lack thereof)
    - Concentrations/exposures achieved at sites of infection