Antifungal Susceptibility Testing of Molds: When, Why, and How

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- F2G
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- Scynexis
- Viamet/Mycovia

Member, CLSI Antifungal Susceptibility Subcommittee

Road Map

1. Why and when antifungal susceptibility testing should be considered against molds
   - What information should you expect from these laboratory tests
   - How is this information useful & how might it be limited

2. What breakpoints are available for molds and how are these established

3. Recommendations for susceptibility testing against molds

Key Take Away: Antifungal susceptibility results may help guide therapy for some infections, but this information should not be the sole basis for treatment decisions

What is Antifungal Susceptibility Testing?

In vitro assay that answers the following questions:

1. Does a drug or compound have activity against a fungal isolate?

2. If activity is observed, how potent is that activity?

Uses - Antifungal Susceptibility Testing

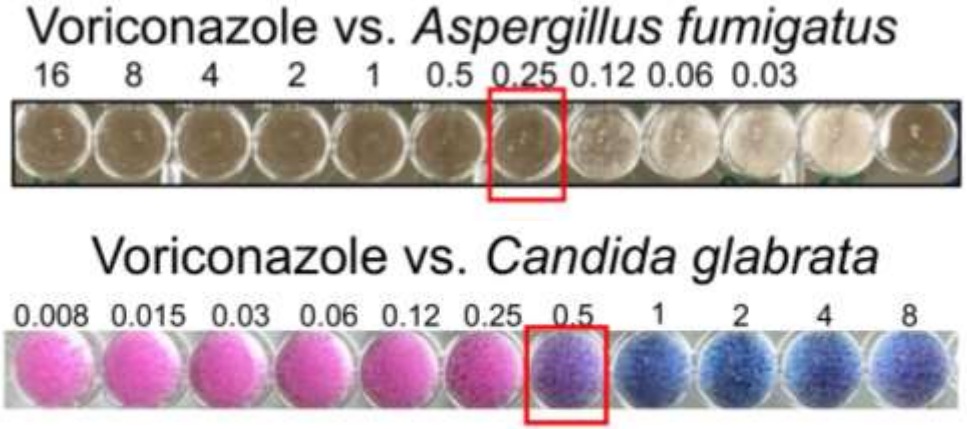
- Clinical diagnostic testing
  - Determine if a particular fungal isolate cultured from a patient is susceptible or resistant to antifungals
  - Information used to aid in making clinical decisions regarding treatment

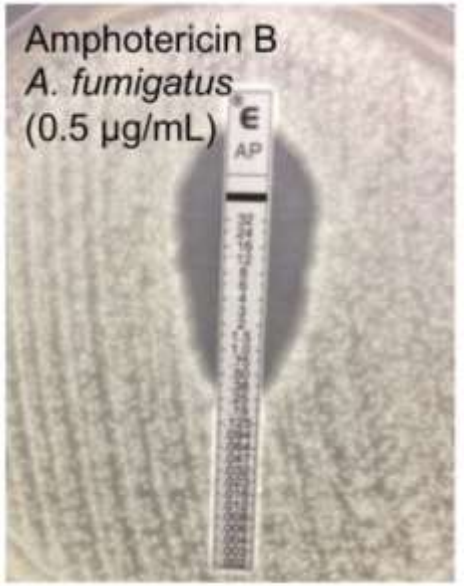
- Surveillance
  - Screen isolates for changes in susceptibility patterns (over time)
  - Is resistance (secondary/acquired) developing in a larger population?

- Pre-clinical drug development
  - Determine *in vitro* spectrum of activity and potency of investigational agents
  - Go/No Go decisions and what fungal infections to potentially target

Methods for Mold Susceptibility Testing

- Broth microdilution (CLSI & EUCAST)
- Colorimetric (Sensititre YeastOne)
- Gradient diffusion (Etest, MTS)
- Agar-based screen (VIPCheck)





Buill JB. et al. *Antimicrob Agents Chemother* 2017;61:e01250-17.
Guinea J. et al. *Clin Microbiol Infect* 2019;25:681-687.

Clinical Breakpoints (CBP)

- MIC threshold used to classify organisms as susceptible or resistant to a particular antimicrobial

- Based on different components (considered by committees)
  1. *MIC distributions & Epidemiologic cut-off values (ECVs)*
  2. *Pharmacokinetics/pharmacodynamics*
  3. *Clinical outcomes correlated with MICs*

Pfaller MA, Rex JH, Rinaldi MG. *Clin Infect Dis* 1997;24:776-784.
Rex JH, Pfaller MA. *Clin Infect Dis* 2992;35:982-989.

Current Mold Breakpoints – *A. fumigatus*

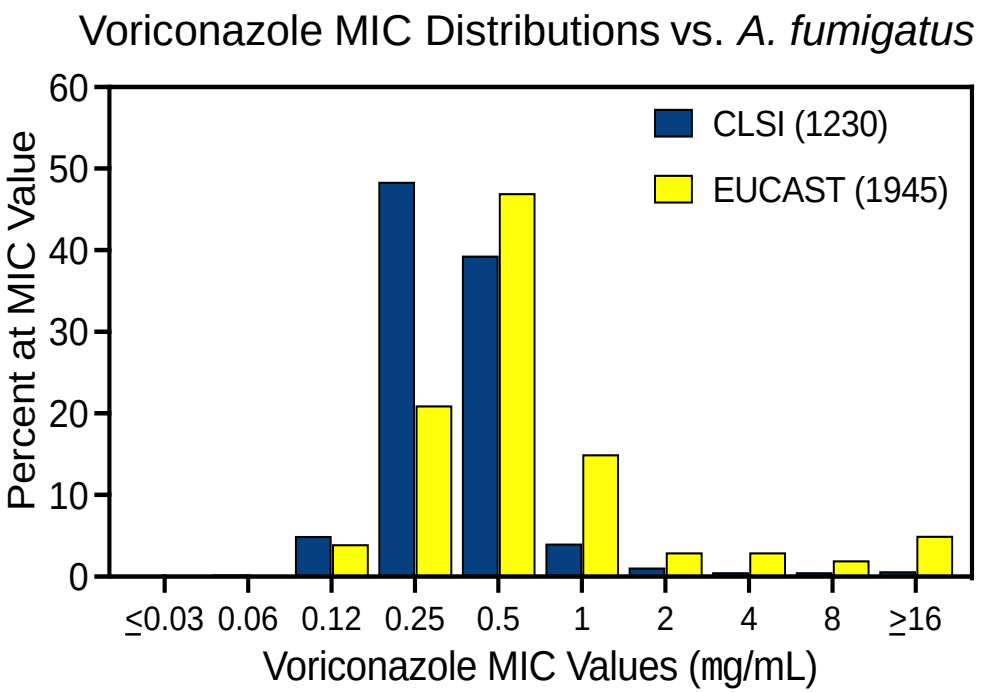
CLSI (M61)	Susceptible	Intermediate	Resistant
Voriconazole	≤0.5	1	≥2
EUCAST	**Susceptible**	**Resistant**	**ATU**
Amphotericin B	≤1	>1	---
Isavuconazole	≤1	>2	2
Itraconazole	≤1	>1	2
Posaconazole	≤0.125	>0.25	0.25
Voriconazole	≤1	>1	2

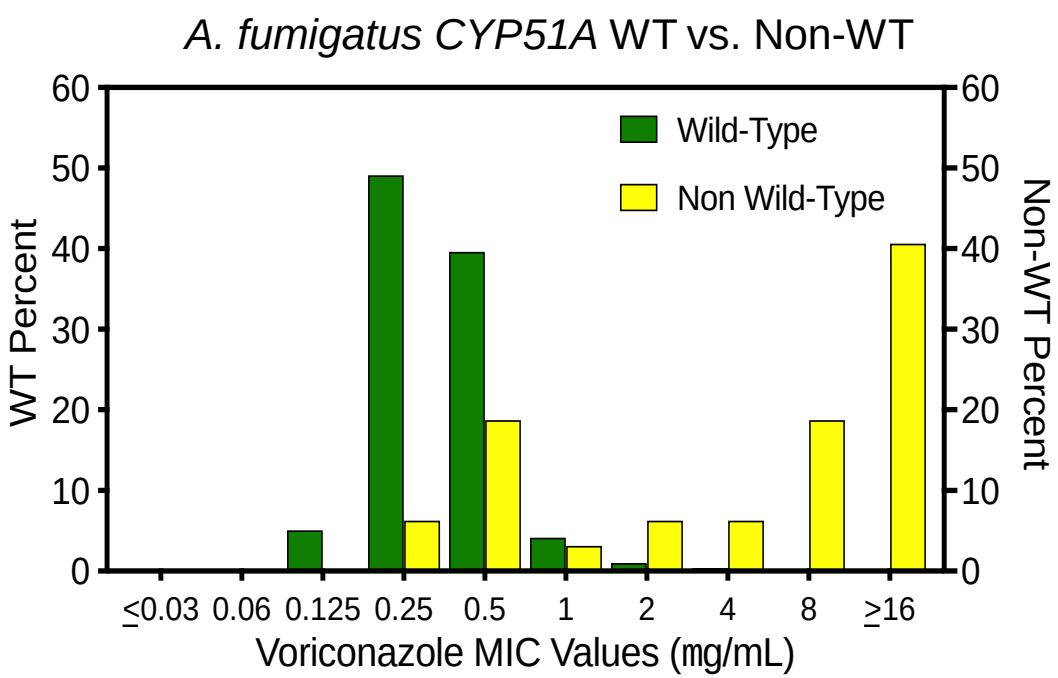
EUCAST has also set breakpoints for some antifungals against *A. flavus, A. nidulans, A. niger,* and *A. terreus*

ATU = Area of Technical Uncertainty

CLSI. Performance Standards for Antifungal Susceptibility Testing of Filamentous Fungi, 2nd Ed., supplement M61. 2020.
https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/AFST/Clinical_breakpoints/AFST_BP_v10.0_200204_updatd_links_200924.pdf

A. fumigatus Voriconazole MIC





Epidemiologic Cut-Off Values (ECV or ECOFFs) for voriconazole against *A. fumigatus*:

CLSI = 0.5 µg/ml

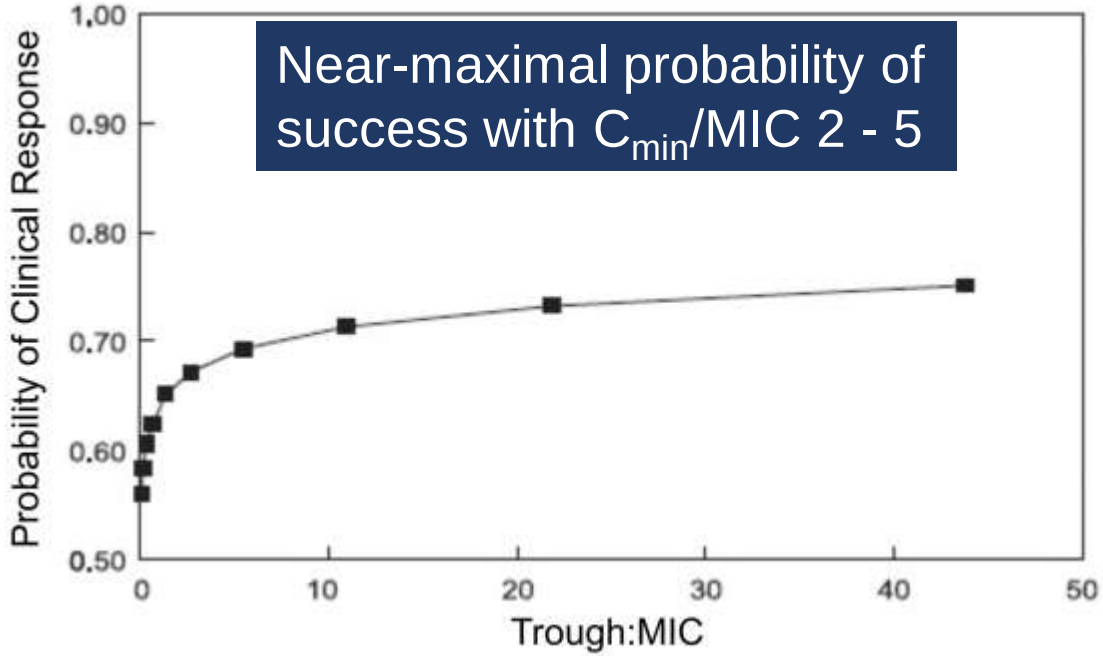
EUCAST = 1 µg/ml

NOTE: ECVs should not be used when CBP available

Presented to CLSI Subcommittee on Antifungal Susceptibility Testing, January 2019

Voriconazole PK/PD (human data)

Target attainment *A. fumigatus* ($fAUC_{24}$/MIC = 15)			
MIC	200 mg x 2	300 mg x 2	4 mg/kg x 2
0.25	99	100	100
0.5	92	100	99
1	62	88	74
2	21	34	14
4	3	2	0



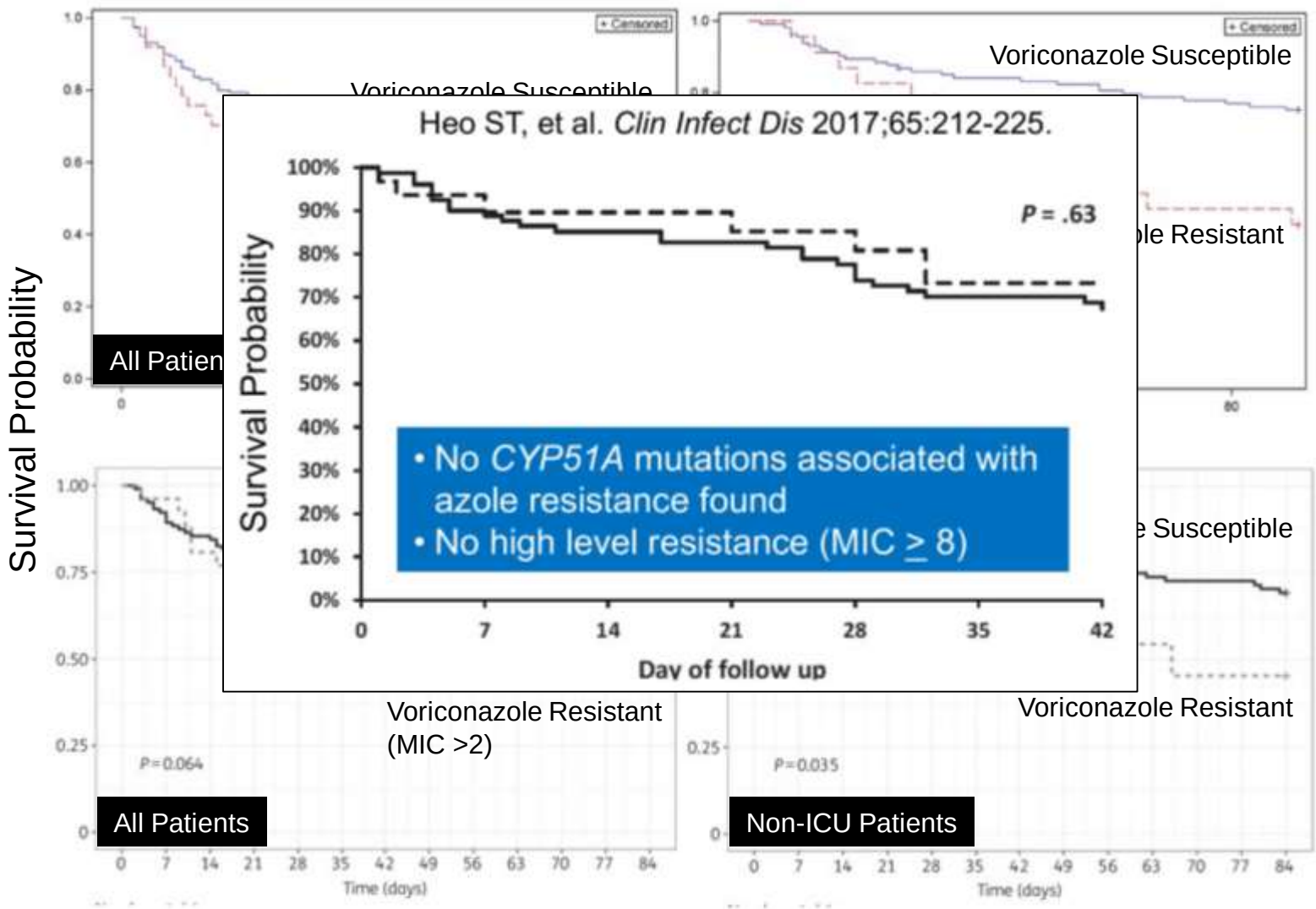
Scenario 1	C_{min}	MIC	C_{min}/MIC
	1	0.25	4
	2	0.25	8
	4	0.25	16

Scenario 2	C_{min}	MIC	C_{min}/MIC
	1	0.5	2
	2	0.5	4
	4	0.5	8

Scenario 3	C_{min}	MIC	C_{min}/MIC
	1	2	0.5
	2	2	1
	4	2	2

Troke PF, Hockey HP, Hope WW. *Antimicrob Agents Chemother* 2011;55:4782-4788.
Rationale document for EUCAST clinical breakpoints (https://www.eucast.org/astoffungi/rationale_documents_for_antifungals/)

Clinical Data



Lestrade P, et al.
Clin Infect Dis 2019;68:1463-1471

- Retrospective analysis in patients with IA The Netherlands (3 centers, 2011-2015)
- 196 *A. fumigatus* culture positive cases (53% with hematologic malignancies)
- Voriconazole resistance 19%

Group	All-Cause Mortality (%)	P-value
Overall (day 42)	VRC Suscept. - 28% VRC Resistant - 49%	0.017
Non-ICU (day 42)	VRC Suscept. – 16% VRC Resistant – 35%	0.045

Resendiz-Sharpe A, et al.
J Antimicrob Chemother 2019;74:2759-2766

- Retrospective analysis in hematologic malignancy patients with IA (*A. fumigatus*)
- Belgium & The Netherlands (4 centers, 2012-2017)
- 129 *A. fumigatus* culture-positive cases
- Voriconazole resistance 20.2%

Group	All-Cause Mortality (%)	P-value
Overall (12 wks)	VRC Suscept. - 36.9% VRC Resistant - 57.7%	0.064
Non-ICU (12 wks)	VRC Suscept. – 30.7% VRC Resistant – 54.5%	0.035

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
  - Drug concentrations at site of infection
  - Time to diagnosis/start of treatment
  - Drug-drug & drug-disease interactions

Both antibacterial AND antifungal susceptibility testing share these limitations

Rex JH, Pfaller MA. *Clin Infect Dis* 2992;35:982-989.

Antifungal Susceptibility - Molds

Challenges

- Few clinical laboratories in U.S. perform susceptibility testing against molds
  - Send out lab (delays in results)
- No automation for mold susceptibility testing
- In U.S., no FDA-cleared microtiter panels for molds
  - Diffusion gradient strips available (Etest, MTS)
  - Some labs use YeastOne Sensititre plates
    - Off-label

Useful Information When CBPs Not Available

- Epidemiologic Cut-Off Values (ECVs or ECOFFs)
  - Distinguish wild-type from non-wild-type
    - Non-wild-type = acquired resistance
  - More ECVs than CBPs for molds, but still relatively few

- MIC Distributions for Species/Group of Organisms
  - Is antifungal concentration achievable above potential MIC?
  - Determination of intrinsic resistance

Intrinsic Resistance

- Intrinsic resistance –
  - Inherent or innate (*not acquired*) resistance
  - Reflected by high MICs for *all* OR *most all* representatives of a species
    - So common that susceptibility testing is ***unnecessary***
      (97% or more of isolates have *in vitro* resistance to a particular antifungal)

Antifungal Class or Agent	Examples of *In vitro* Intrinsically Resistant Fungi
Echinocandins	*Cryptococcus, Rhodotorula, Trichosporon*, Mucorales
Fluconazole	*Candida krusei*, *Aspergillus*, *Lomentospora prolificans*, Mucorales
Voriconazole	Mucorales, *Rasamsonia argillacea* species complex
Amphotericin B	*Purpureocillium lilacinum, Lomentospora prolificans*

CLSI. Performance Standards for Antimicrobial Susceptibility Testing, 30th ed., CLSI Document M100 (Appendix B). 2020.

ECMM/ISHAM Rare Molds Recommendations

Summary of Susceptibility Testing Rare Molds

Fungal Group/Infection	Individual Clinical Decisions	Epidemiology/ Resistance Surv.	Development/ New Agents
Fusarium – fusariosis	Questionable	YES	YES
Scedosporium - scedosporiosis	YES (AMB?)	YES	YES
Lomentospora – lomentosporiosis (previously *Scedosporium prolificans*)	NO	NO (with current AFs)	YES
Rasamsonia	YES (not VRC - IR)	YES	YES
Purpureocillium lilacinum (previously *Paecilomyces lilacinus*)	YES (not AMB - IR)	YES	YES

IR = Intrinsic resistance; AMB = Amphotericin B; VRC = Voriconazole; AFs = Antifungals

Hoenigl et al. *Lancet Infect Dis* 2021;21:e246-257.

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 different 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 & 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	0.5 - >16	1 - >16	0.5 - >16	>4

Limited-to-variable *in vitro* activity of available antifungals against *Fusarium.* Clinical relevance *in vitro* susceptibility testing?

(Nucci et al. *J Antimicrob Chemother* 2021;76:1063-1069)

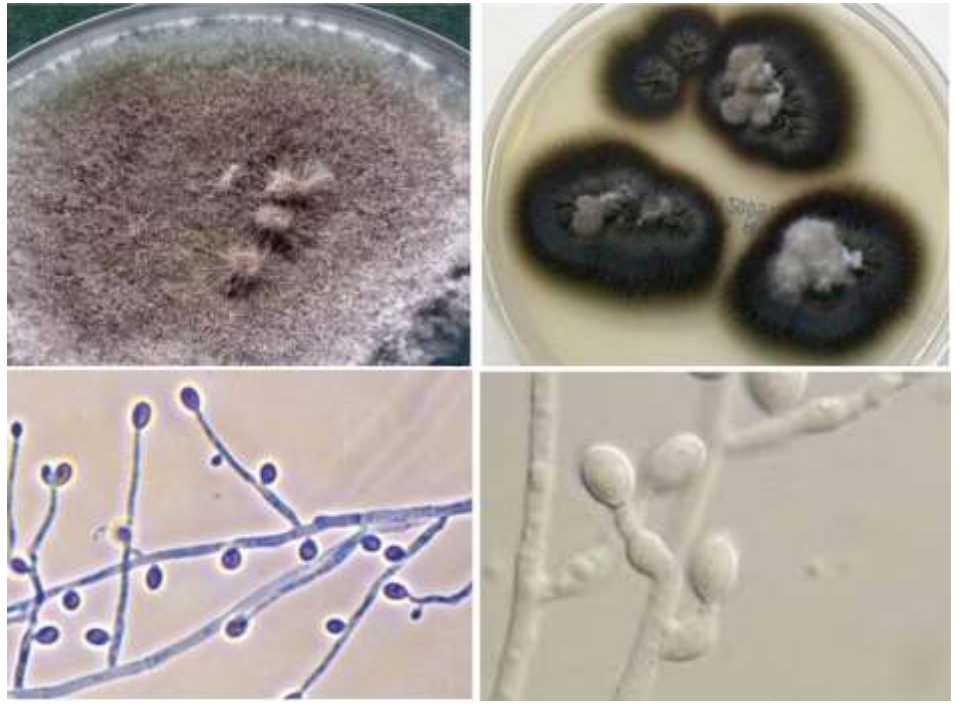
ECMM / ISHAM Recommendations

- Susceptibility testing used for epidemiologic purposes in defining range of MIC distributions
- Studies demonstrating utility of these assays to inform antifungal choice for individual patients are lacking

Espinel-Ingroff et al. *Antimicrob Agents Chemother* 2015;60:1079-1084.
Pfaller et al. *Antimicrob Agents Chemother* 2018;97:115007.*FTL internal data

Scedosporium & Scedosporiosis

- Invasive infections primarily in immunocompromised hosts
  - Breakthrough infections in persistently neutropenic and/or lymphopenic hosts
  - Enters through sinopulmonary route & causes pulmonary infection; difficult to distinguish from invasive aspergillosis
- Common colonizers of cystic fibrosis patients
- Infections can occur in survivors of near-drowning events
  - Pulmonary infections with dissemination to the CNS



https://mycology.adelaide.edu.au/descriptions/

Scedosporium & Scedosporiosis

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

Species	Taxonomic / Nomenclature Changes	FTL % Isolates (US)	Lackner *AAC* 2012			
			AMB	VRC	ISC	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
S. ellipsoidea	Previously *Pseudallescheria ellipsoidea*	1.8%	4->16	0.5-4	2->16	0.5->16

Lackner et al. *Antimicrob Agents Chemother* 2012;56:2635-2642. FTL internal data (N = 285)

Scedosporium & Scedosporiosis

- Amphotericin B vs. *Scedosporium* similar vs. *Aspergillus terreus*
  - Marked variability with MICs
  - *In vitro* susceptibility NOT predictive of clinical outcomes
  - Does not meet definition of intrinsic resistance
    - Isolates with MIC values <4 μg/mL often observed
    - But failures can occur even with low MIC values

Don't trust AMB MICs vs. *Scedosporium*

- Variable susceptibility with azoles
  - Species dependent
  - Voriconazole typically has best *in vitro* activity
    - Surprisingly, little activity observed with isavuconazole
      - In contrast to agreement observed between these 2 azoles against *Aspergillus* spp.

In vitro Potency:
VRC > PSC >>> ITC & ISC

ECMM / ISHAM recommend for surveillance AND for clinical decision making (moderate)

Lomentospora prolificans

- Previously *Scedosporium prolificans* (*S. inflatum*)
- Antifungal resistance with high MIC values for all clinically available agents
  - "**>**" often used to report MICs (no activity at highest concentrations tested)
- *Occasionally* lower voriconazole MICs
  - Very rare (still elevated - ≥4 μg/mL)
- Treatment recommendation – combination therapy (ECMM/ISHAM)
  - Voriconazole + terbinafine + other (amphotericin B or echinocandin)

Any role for routine *in vitro* susceptibility testing with currently available antifungals?
- Probably not – does not change treatment recommendations (*combination tx*)

Other Antifungals in Development

Proven Mold Activity

- Olorofim (previously F901318; F2G)
  - Inhibition of fungal pyrimidine biosynthesis
  - Mold-active antifungal (including *L. prolificans*)
  - No activity against yeast or Mucorales
- Fosmanogepix (previously APX001; Amplyx & now Pfizer)
  - Prodrug (active moiety – manogepix)
  - Inhibition of GPI-anchored protein maturation
  - Broad-spectrum activity
  - Not *C. krusei*, questionable vs. Mucorales

Others

- ATI-2307 (previously T-2307; Fujifilm/Toyama/Appili)
  - Collapse of fungal mitochondrial membrane potential
- Ibrexafungerp (previously SCY-078; Scynexis)
  - Triterpenoid
  - Inhibition of glucan synthase (same target as echinocandins)
  - Now FDA approved for VVC (Brexafemme)
- Rezafungin (Cidara)
  - Long half-life echinocandin
- VMT-1161 (VT-1161; Mycovia)
  - Tetrazole
  - Inhibition of ergosterol biosynthesis

When in Doubt…

- Contact the microbiology laboratory or the reference laboratory that is testing or has performed testing
- Ask questions
- Ask for literature
- Ask for guidance

Key Take Away: Antifungal susceptibility results may help guide therapy for some infections, but this information should not be the sole basis for treatment decisions