

Interpretation of Antifungal Susceptibility Testing

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Pathology &
Laboratory Medicine

Disclosures

Funding to FTL

- Basilea
- Bruker
- Elion (prev. Sfunga)
- F2G
- Mycovia
- Scynexis

Collaborations through NIH

- Amplyx
- Elion
- F2G
- Fujifilm/Toyama/Appili
- Scynexis
- Viamet/Mycovia

Chairholder, CLSI Antifungal Susceptibility Subcommittee

Roadmap

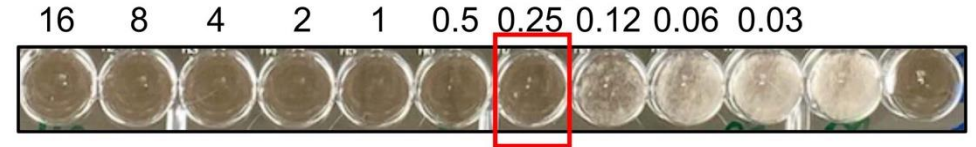
- Antifungal Susceptibility testing
 - How performed and used
 - Available breakpoints (BPs) and how they are set
 - Putting MICs and into context
 - How to make use of results when no breakpoints exist

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

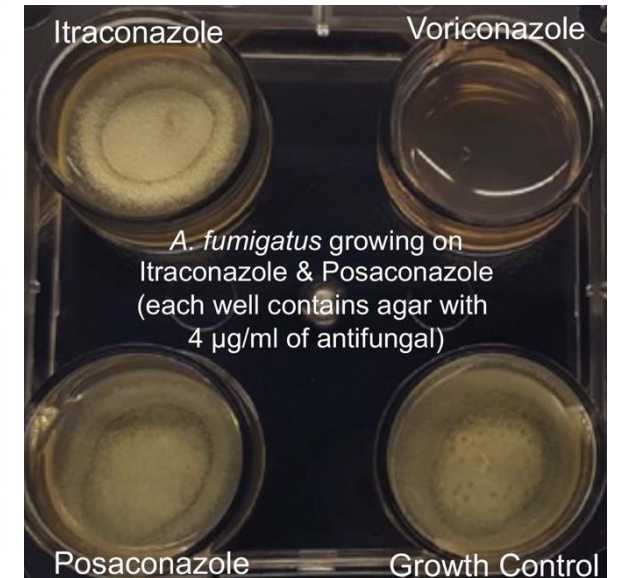
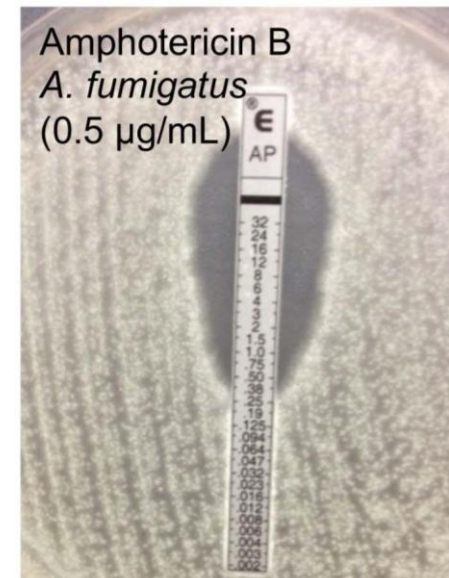
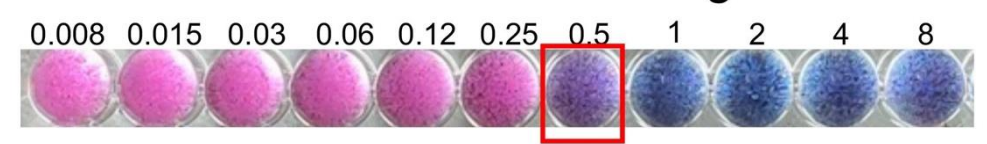
AFST – Clinical Use & Methods

- Clinical diagnostic testing
 - Determine if a particular fungal isolate cultured from a patient is susceptible or resistant to antifungals
 - Information used as an aid in making clinical decisions regarding treatment
- Methods
 - Broth microdilution ([CLSI & EUCAST – the standards](#))
 - Colorimetric ([Sensititre YeastOne](#))
 - Gradient diffusion ([Etest](#), [MTS](#))
 - Automated ([VITEK 2](#))
 - Agar-based screen ([VIPCheck](#))

Voriconazole vs. *Aspergillus fumigatus*



Voriconazole vs. *Candida glabrata*



MIC Results Most Useful with Breakpoints

- MIC threshold used to classify organisms as susceptible or resistant to a particular antimicrobial
- Based on different components (considered by committees – CLSI, EUCAST, FDA)
 1. MIC distributions & Epidemiologic cut-off values (ECVs)
 2. Pharmacokinetics/pharmacodynamics
 3. Clinical outcomes correlated with MICs

Available Yeast Breakpoints

Antifungals	Species	CLSI	EUCAST
Anidulafungin / Micafungin	<i>C. albicans</i> , <i>C. glabrata</i> , <i>C. guilliermondii</i> , <i>C. krusei</i> , <i>C. parapsilosis</i> , <i>C. tropicalis</i> [+ <i>C. dubliniensis</i> , - <i>C. guilliermondii</i> EUCAST]	✓*	✓
Caspofungin	Same 6 <i>Candida</i> species	✓*	✗
Fluconazole	<i>C. albicans</i> , <i>C. glabrata</i> , <i>C. parapsilosis</i> , <i>C. tropicalis</i>	✓*	✓
Voriconazole	<i>C. albicans</i> , <i>C. krusei</i> , <i>C. parapsilosis</i> , <i>C. tropicalis</i> [+ <i>C. dubliniensis</i> , - <i>C. krusei</i> EUCAST]	✓*	✓
Itraconazole	<i>C. albicans</i> , <i>C. dubliniensis</i> , <i>C. parapsilosis</i> , <i>C. tropicalis</i>	✗	✓
Posaconazole	<i>C. albicans</i> , <i>C. dubliniensis</i> , <i>C. parapsilosis</i> , <i>C. tropicalis</i>	✗	✓
Amphotericin	<i>C. albicans</i> , <i>C. dubliniensis</i> , <i>C. glabrata</i> , <i>C. krusei</i> , <i>C. parapsilosis</i> , <i>C. tropicalis</i> , <i>C. neoformans</i>	✗	✓

*FDA follows CLSI breakpoints (<https://www.fda.gov/drugs/development-resources/antifungal-susceptibility-test-interpretive-criteria>)
 No CLSI or EUCAST breakpoints against yeasts for isavuconazole or flucytosine

Available Mold Breakpoints

CLSI Breakpoints	Species
Isavuconazole*	<i>A. fumigatus</i>
Voriconazole*	<i>A. fumigatus</i>

EUCAST Breakpoints	Species
Isavuconazole	<i>A. fumigatus</i> as well as <i>A. flavus</i> , <i>A. nidulans</i> , <i>A. terreus</i>
Voriconazole	<i>A. fumigatus</i> & <i>A. nidulans</i>
Posaconazole	<i>A. fumigatus</i> & <i>A. terreus</i>
Itraconazole	<i>A. fumigatus</i> as well as <i>A. flavus</i> , <i>A. nidulans</i> , <i>A. terreus</i>
Amphotericin B	<i>A. fumigatus</i> & <i>A. niger</i>

*FDA follows CLSI breakpoints (<https://www.fda.gov/drugs/development-resources/antifungal-susceptibility-test-interpretive-criteria>)

Rezafungin Breakpoints

Species	CLSI Breakpoint (Susceptible)	FDA Breakpoint (Susceptible)	EUCAST	
			Susceptible	Resistant
<i>C. albicans</i>	≤ 0.25	≤ 0.12	≤ 0.008	> 0.008
<i>C. auris</i>	≤ 0.5	---	IE	IE
<i>C. dubliniensis</i>	≤ 0.12	---	≤ 0.016	> 0.016
<i>C. glabrata</i>	≤ 0.5	≤ 0.12	≤ 0.016	> 0.016
<i>C. krusei</i>	≤ 0.25	---	≤ 0.03	> 0.03
<i>C. parapsilosis</i>	≤ 2	≤ 2	≤ 4	> 4
<i>C. tropicalis</i>	≤ 0.25	≤ 0.12	≤ 0.03	> 0.03

CLSI rezafungin breakpoints based on nonclinical PK/PD targets & PTA, and clinical outcomes

- Importance of shape of concentration-response curve (AUC & Cmax important)

EUCAST rezafungin MICs and BPs lower than CLSI

- Use of 0.002% Tween 20 in RPMI due to interlaboratory variability observed

No BP for
resistance set by
CLSI or FDA yet

https://www.accessdata.fda.gov/drugsatfda_docs/nda/2023/217417Orig1s000IntegratedR.pdf

https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Rationale_documents/Rezafungin_RD_v1.0_final.pdf

Arendrup et al. *J Antimicrob Chemother* 2022. Zuill et al. *J Antimicrob Chemother* 2023.

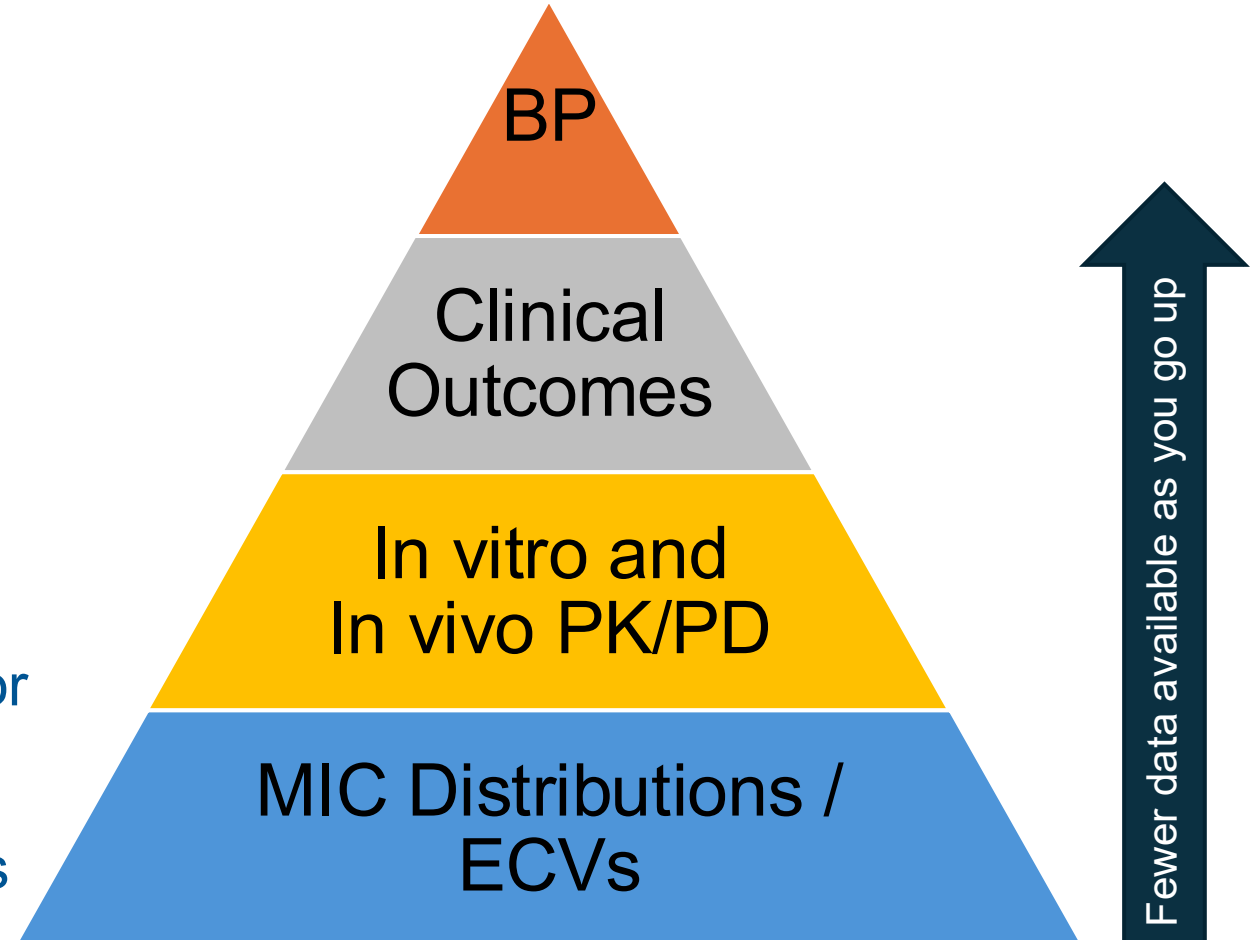
AFST is **NOT** the Be-All and End-All

- Low MIC does not predict successful outcomes in each case
- High MIC does not predict failure
 - *In vitro* resistance may predict those less likely to respond
- Other factors are most likely more 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
 - Adverse effects/ toxicities

“...it is but a single laboratory test that may provide useful information and which should be used judiciously and in context with other factors.”

Reality Check for Antifungal Breakpoints

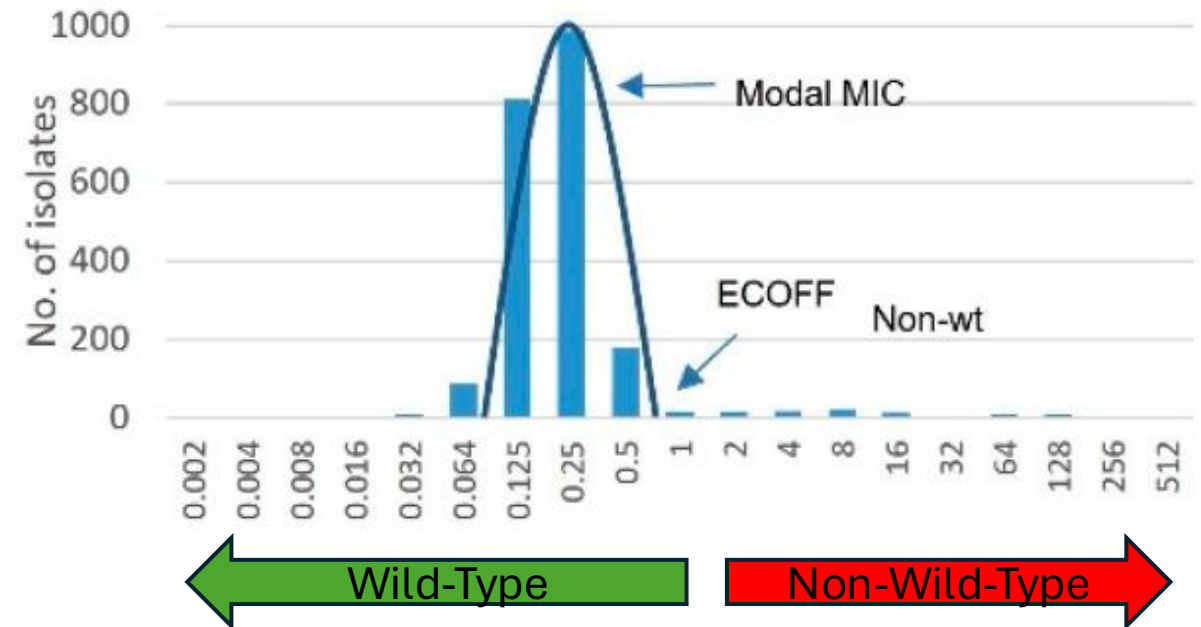
- ~700 fungal species associated with disease in humans and animals
 - ~100 true pathogens capable of causing serious disease
- Vast majority of bug/drug combinations will never have breakpoints
 - Often no clinical outcome studies or even PK/PD for rare yeasts/molds
 - Cultures often negative; patients enrolled with probable IFI; no MICs



Epidemiological Cut-Off Values

- Within a normal Gaussian distribution MIC_{50} = Modal MIC
- Upper limit of wild-type population = *epidemiologic cut-off value* (ECV or ECOFF)
 - Calculated statistically
 - *In general*, encompasses ~97.5% of wild type isolates

C. albicans & Fluconazole



Threshold value that separates fungal populations into those with and without (presumed) acquired resistance based on their phenotypes (MIC)

Breakpoints vs. ECVs

Breakpoints

- MIC distributions & ECVs
- PK/PD results
- Clinical outcomes correlated with MICs

Try to help answer the question
“Is my patient likely to respond to this particular therapy?”

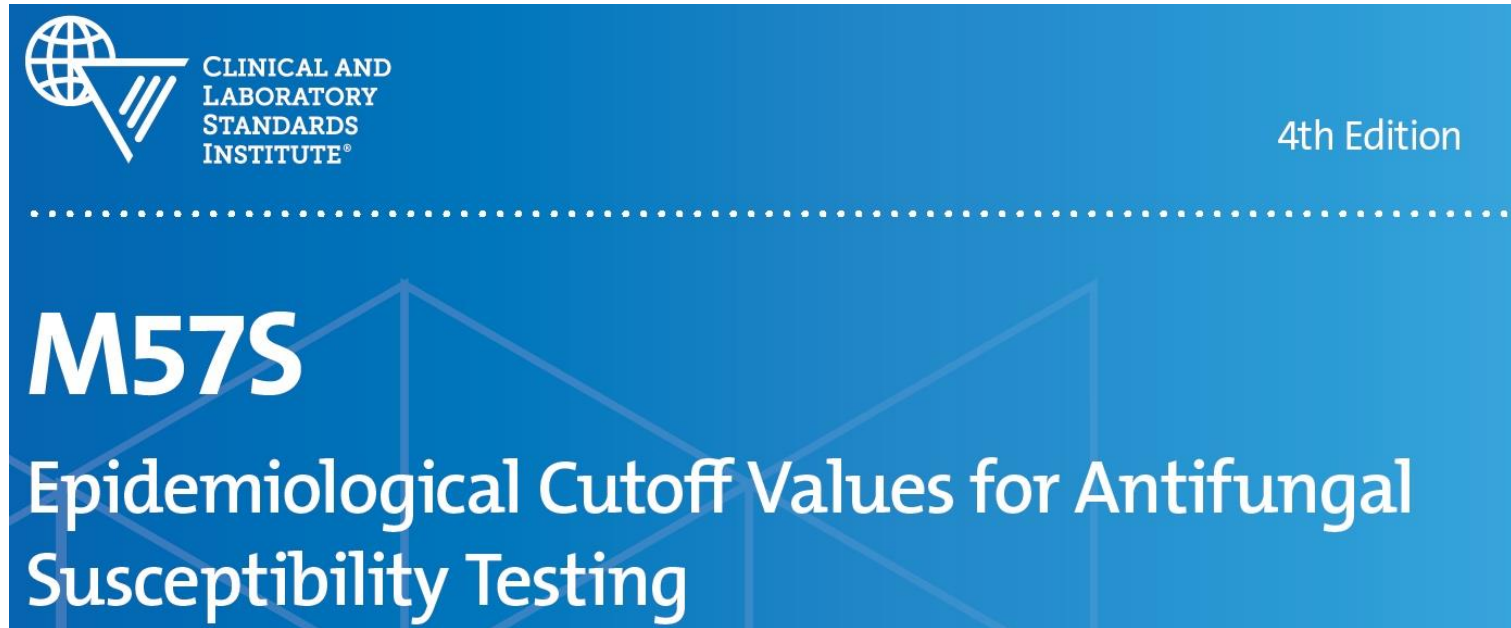
ECVs

- MIC distributions

Try to help answer the question
“Will the bug respond in the way we are used to it responding?”

Do not use/report ECV in interpreting MIC result if breakpoint is available
No association between ECVs and clinical outcomes (or PK/PD)

Where Can I Find ECVs?



European Committee on Antimicrobial Susceptibility Testing

Overview of antifungal ECOFFs and clinical breakpoints for yeasts, moulds and dermatophytes using the EUCAST E.Def 7.4, E.Def 9.4 and E.Def 11.0 procedures

What do I do when there is no BP or ECV?

- MIC distributions published in the literature (“ECV lite”)
 - How does this MIC compare to published values for this bug/drug combination?
- Achievable antifungal concentrations
 - Can we achieve concentrations above MIC values?
- Available data for closely related species

Review

A Pragmatic Approach to Susceptibility Classification of Yeasts without EUCAST Clinical Breakpoints

Karen Marie Thyssen Astvad ¹, Sevtap Arikan-Akdagli ^{2,†} and Maiken Cavling Arendrup ^{1,3,4,*}



EUCAST

EU
ON
SU

Anti-Fungal
Susceptibility
Testing
Subcommittee

EUCAST guidance on
**Interpretation of MICs for rare yeast without breakpoints in
breakpoint tables**

Two assumptions:

1. Pathogenicity and intrinsic susceptibility patterns are assumed to be similar between genetically related isolates
2. Even if species are not phylogenetically related, rare yeasts will likely respond to therapy provided the MIC is comparable to values observed in WT isolates of more prevalent susceptible species

Astvad et al. *J Fungi* 2022.

https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/AFST/Files/EUCAST_guidance_for_Rare_yeast_with_no_breakpoints_final_clean_19-06-2024.pdf

Amphotericin B	Anidulafungin	Fluconazole	Voriconazole
REPORT: "Formal categorising of the susceptibility of the organism is not possible. A cautious interpretation suggests that the agent may be considered for therapy" when...			
<u>Confirmed MIC ≤1:</u> <i>Candida</i> spp. except <i>C. lusitaniae</i> Rare yeasts <i>Trichosporon</i> spp. (2 nd line)	<u>Confirmed MIC ≤ 0.06:</u> <i>C. inconspicua</i> <i>C. nivariensis</i> <i>C. norvegensis</i> <i>C. pelliculosa</i> <i>C. utilis</i> <i>L. elongisporus</i> <i>P. kluyveri</i>	<u>Confirmed MIC ≤2:</u> <i>C. intermedia</i> <i>C. kefyr</i> [MIC ≤1] <i>C. lusitaniae</i> <i>C. metapsilosis</i> <i>C. orthopsilosis</i> <i>C. utilis</i> <i>L. elongisporus</i>	<u>Confirmed MIC ≤0.03:</u> <i>C. intermedia</i> <i>C. kefyr</i> <i>C. lusitaniae</i> <i>C. metapsilosis</i> <i>C. orthopsilosis</i> <i>L. elongisporus</i>

Repeat MIC ≤ 0.125 or
absence of *fks* mutations
C. intermedia
C. lusitaniae
C. palmioleophila
C. kefyr

Amphotericin B	Anidulafungin	Fluconazole	Voriconazole
REPORT: "R" when indicated below. For the others, report: "Formal categorising of the susceptibility of the organism is not possible. An MIC in the indicated range suggests the organism is wild-type, but MICs are higher than for common <i>Candida</i> spp. and there is insufficient data to allow interpretation."			
<u>Confirmed MIC >1:</u> Any isolate → regard resistant	<u>Repeat MIC 0.5-1</u> No evidence that allows recommendation <i>C. fermentati</i> <i>C. guilliermondii</i> <u>Repeat MIC ≥1:</u> → Report as R <i>Cryptococcus</i> <i>Trichosporon</i> , <i>Magnusiomyces</i> , <i>Geotrichum</i> and <i>Rhodotorula</i> (Against due to intrinsic resistance)	<u>Confirmed MIC >16</u> → Report as R <i>C. inconspicua</i> <i>C. lipolytica</i> <i>C. magnoliae</i> <i>C. palmioleophila</i> <i>C. norvegensis</i> <i>P. kluyveri</i> <i>G. candidum</i> <i>R. mucilaginosa</i> <i>M. capitatus</i> <i>M. clavatus</i> <i>A. adenivorans</i>	<u>Confirmed MIC 0.25-1:</u> No evidence that allows recommendations <i>C. inconspicua</i> <i>C. norvegensis</i> <i>P. kluyveri</i> <i>M. capitatus</i> (1 st line Alt) <i>G. candidum</i> (1 st line Alt) <u>Confirmed MIC testing ≥ 2</u> → Report as R <i>A. adenivorans</i> <i>R. mucilaginosa</i> (Against)

Similar examples not published
for filamentous fungi

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 – will soon change to $\geq 90\%$)

Antifungal Class or Agent	Examples of <i>In vitro</i> Intrinsically Resistant Fungi
Echinocandins	<i>Cryptococcus</i> , <i>Rhodotorula</i> , <i>Trichosporon</i> (and similar genera)
Fluconazole	<i>Candida krusei</i> , <i>Aspergillus</i> , <i>Lomentospora prolificans</i> , Mucorales
Voriconazole	Mucorales, <i>Rasamsonia argillacea</i> species complex
Amphotericin B	<i>Purpureocillium lilacinum</i> , <i>Lomentospora prolificans</i>

Summary

- Antifungal susceptibility testing can be a useful tool in helping to guide therapy
 - Most useful when breakpoints are available
 - These can be few and far between
 - Other information can be used to help interpret MIC results
 - ECVs; closely related species
 - Other factors may be more important in determining clinical outcomes

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