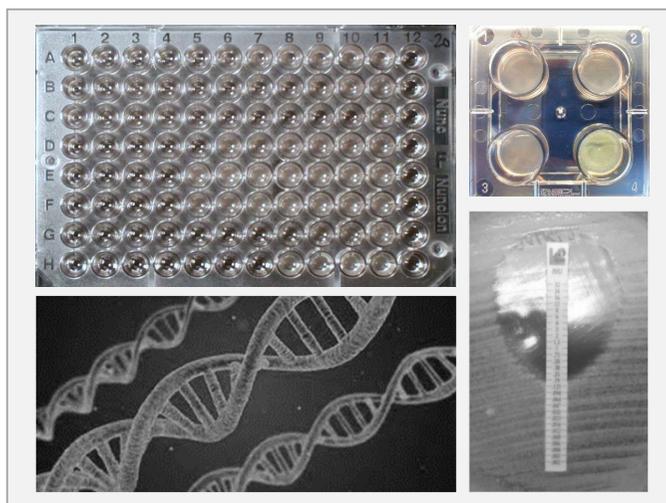


Principles and pitfalls of yeast AST



Maiken Cavling Arendrup,

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Rigshospitalet

University of Copenhagen

Copenhagen, Denmark

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Disclosures:

Research grants/contract work (Paid to SSI): Amplyx, Basilea, Cidara, F2G, Gilead, Novabiotics, Pfizer, Scynexis & T2Candida

Speaker honoraria: Astellas, Basilea, Gilead, MSD, Novartis, Pfizer & T2Candida

Chair(wo)man for EUCAST-AFST

Agenda



❖ Acquired Resistance

- size of the problem 😊

❖ EUCAST BMD method and alternatives

- Microdilution
- Molecular methods
- Commercial tests

❖ New I category

❖ Species without breakpoints



Acquired resistance, size of the problem, *Candida*

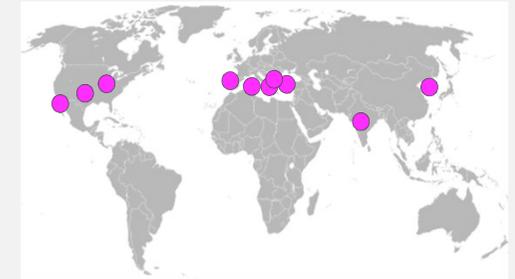


❖ *Candida*

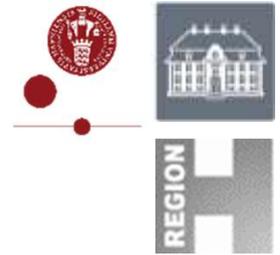
- Fluconazole R

Blood isolates	 ECMM	 surveillance
<i>C. albicans</i>	0%	0.5%
<i>C. glabrata</i>	14%	10%
<i>C. parapsilosis</i>	21%	~2.5%
<i>C. tropicalis</i>	5%	~2.5%

- *C. glabrata*: uniform
- *C. parapsilosis* (Y132F dominates)
 - Spain, Italy, Greece, Turkey, Israel
 - past 3 y also in
 - California, Indiana, Texas
 - India, S-Korea



Acquired resistance, size of the problem, *Candida*

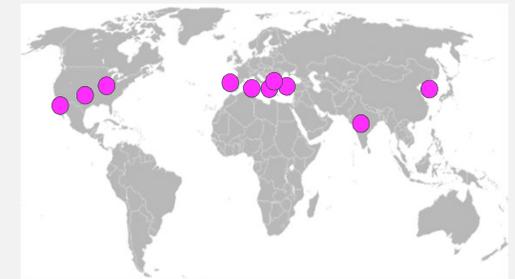


• *Candida*

- Fluconazole R

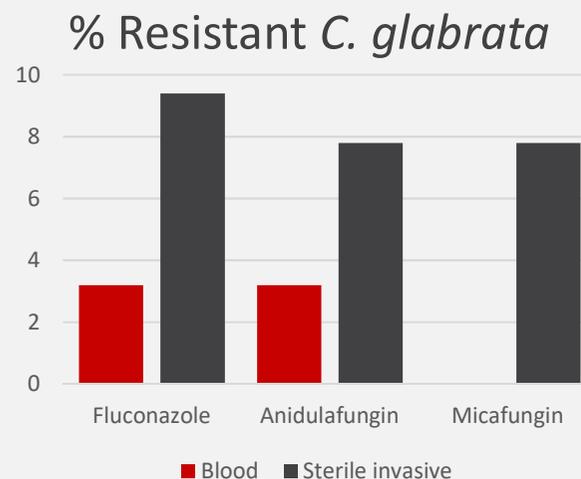
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 - Spain, Italy, Greece, Turkey, Israel
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 - California, Indiana, Texas
 - India, S-Korea



- Echinocandin R

- Low in blood & stationary
- Higher in
 - Foci (intra-abd...)
 - Mucosa
 - *C. glabrata*
 - *C. auris*
- Emerging in
 - *C. parapsilosis*



1st resistant *C. parapsilosis*

- **Greece**
F652S Fks1 alteration
- **Turkey**
L657W Fks1 alteration (& Fluco R)
- **China**
S256P Fks1 alteration (& Fluco R)

Is acquired resistance a problem in moulds?

❖ *Candida*

- Fluconazole R

Blood isolates	ECMM	surveillance
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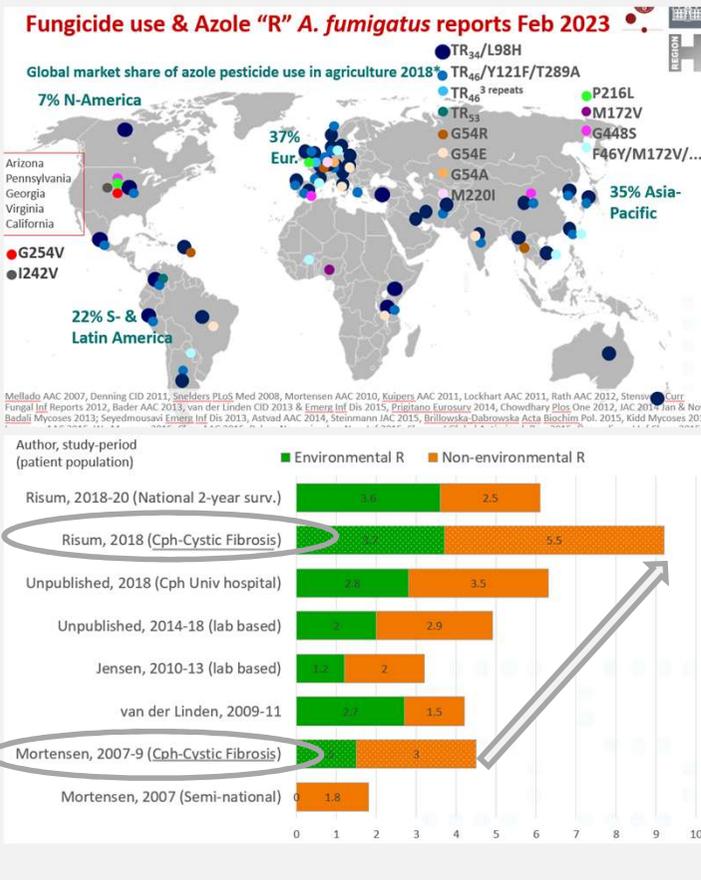
- Echinocandin R

- Low in blood & stationary
- Higher in
 - Foci (intra-abd...)
 - Mucosa
 - *C. glabrata*
 - *C. auris*
- Emerging in
 - *C. parapsilosis*

❖ *Aspergillus fumigatus*

- Azole R

- Environmental
- Medical route



Is acquired resistance a problem in dermatophytes?



❖ *Candida*

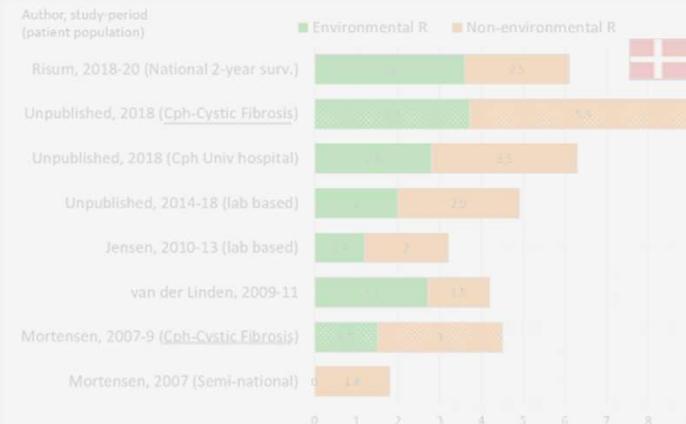
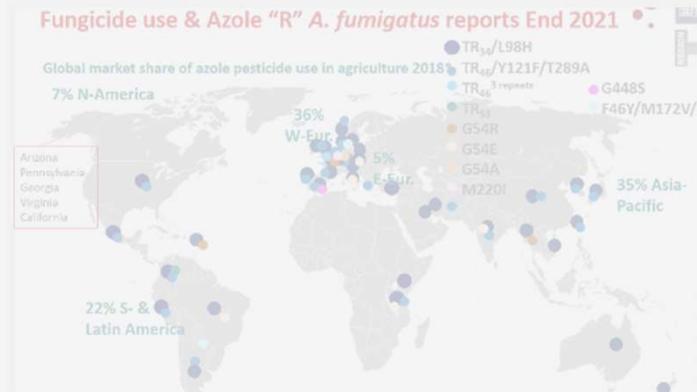
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Blood isolates	ECMM study	Danish surveillance
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 - *C. parapsilosis*

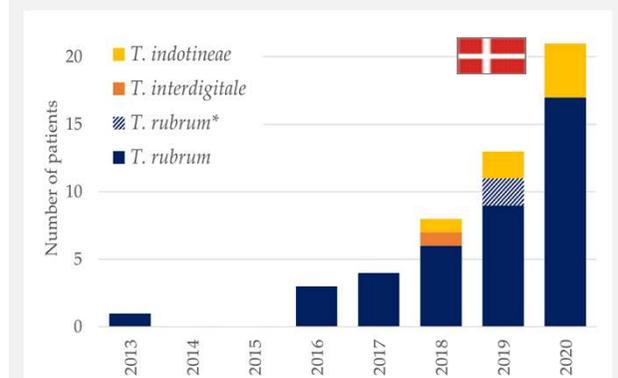
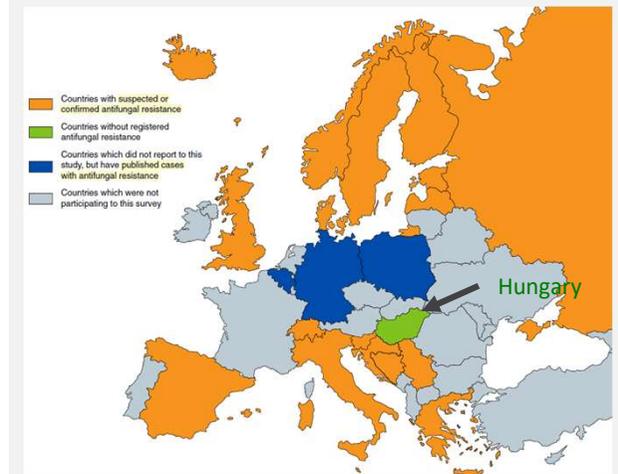
❖ *Aspergillus fumigatus*

- Azole R
 - Environmental
 - Medical route



❖ *Trichophyton*

- Terbinafine R
 - →30% in India



1.6 → 13.1 R-isolates/y
2001-20 vs 2021-22

Agenda



❖ Acquired Resistance

- size of the problem 😊

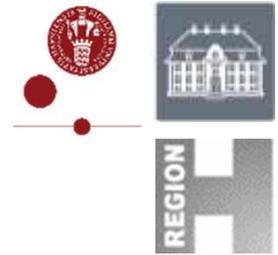
❖ EUCAST BMD method and alternatives

- Microdilution
 - Principle, strengths and pitfalls
- Molecular methods
- Commercial tests

❖ New I category

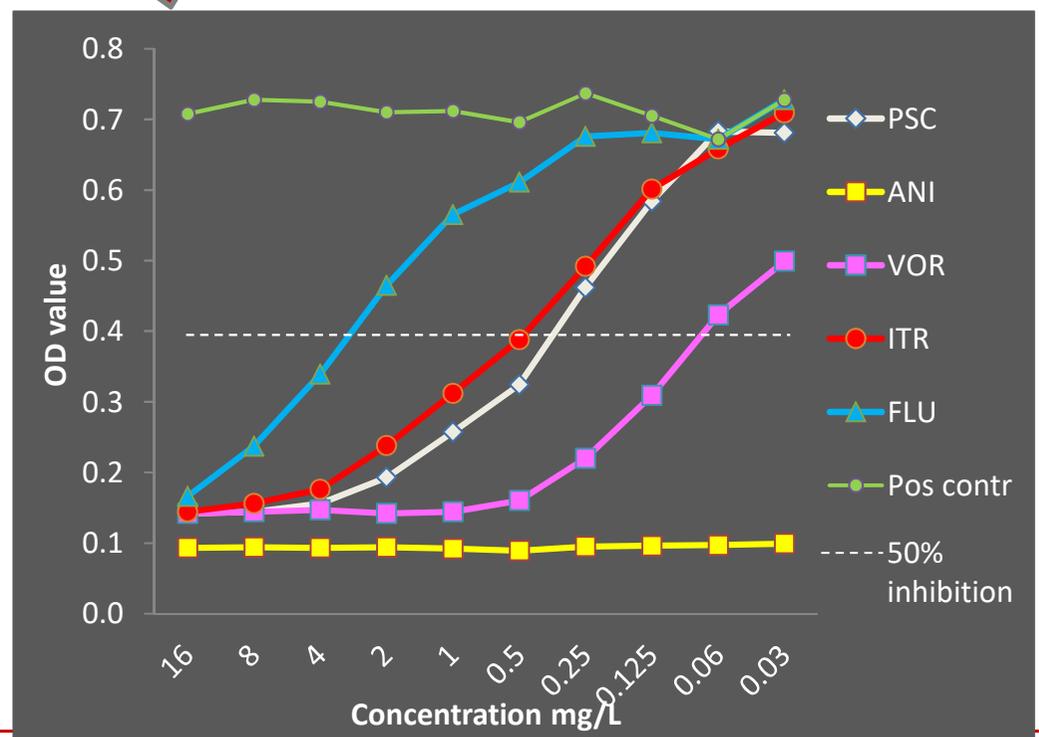
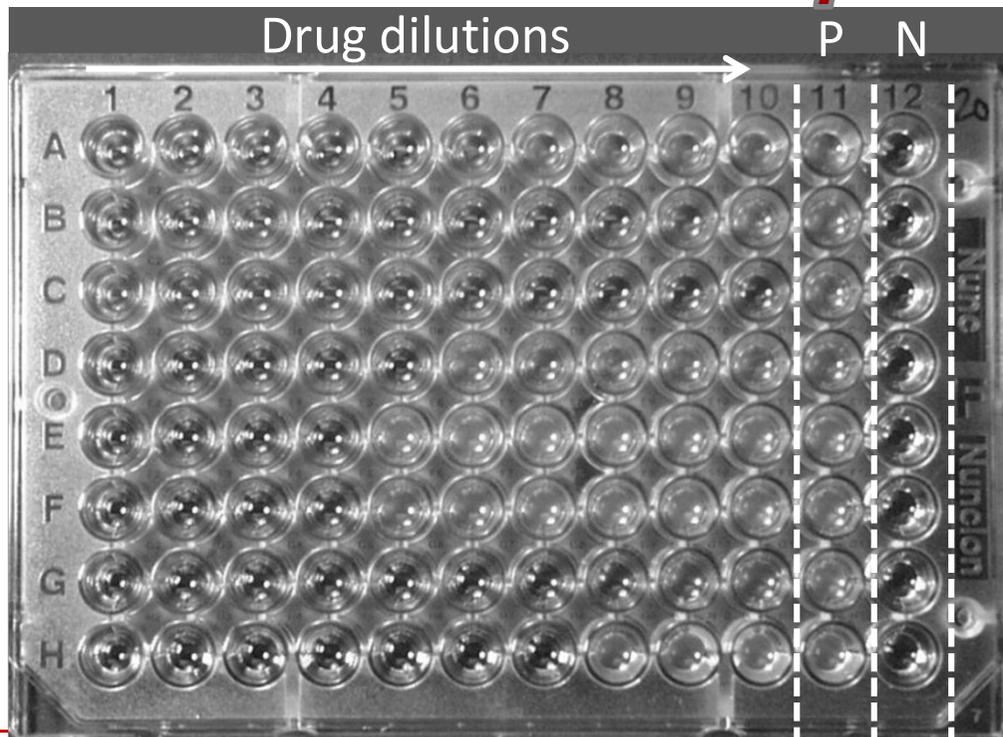
❖ Species without breakpoints

Yeast Testing: EUCAST E.DEF 7.3.2 (2020)



- ❖ Flat bottom plates
- ❖ RPMI with 2% glucose & MOPS
 - plates with 100 µl double conc medium/well
- ❖ 2-fold drug dilutions
 - plates with double conc drug /well)

- ❖ $0.5-2.5 \times 10^5$ cells/mL
 - Prepared double conc in water, 100 µl /well
- ❖ Incubation at $35 \pm 2^\circ\text{C}$
- ❖ 24h and \rightarrow OD \uparrow by ≥ 0.2
- ❖ 50% inhibition, Spec. reading

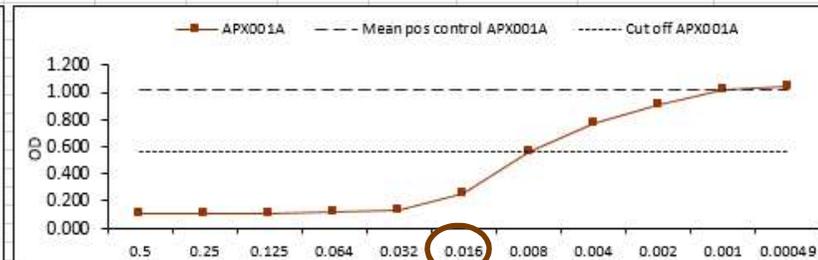
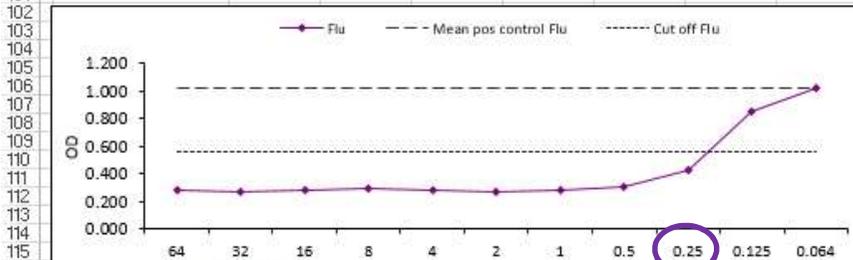
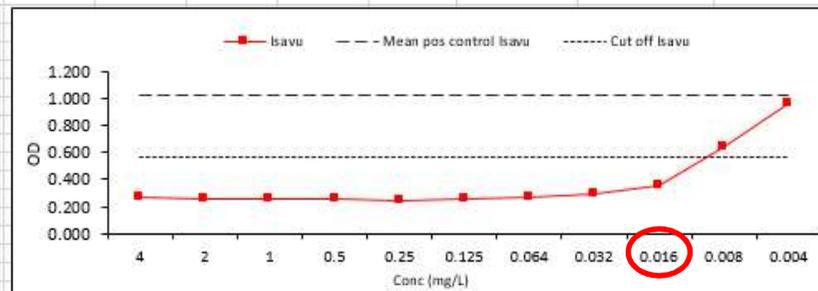
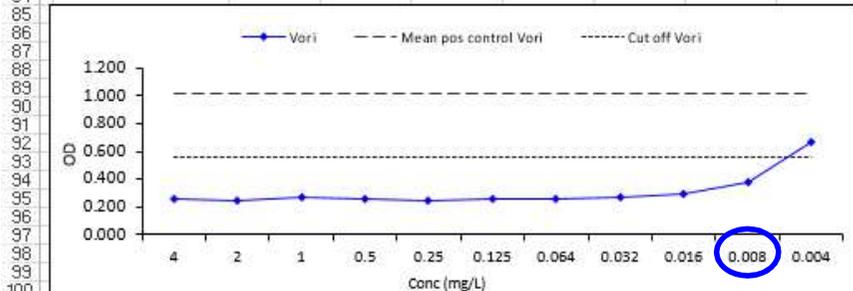
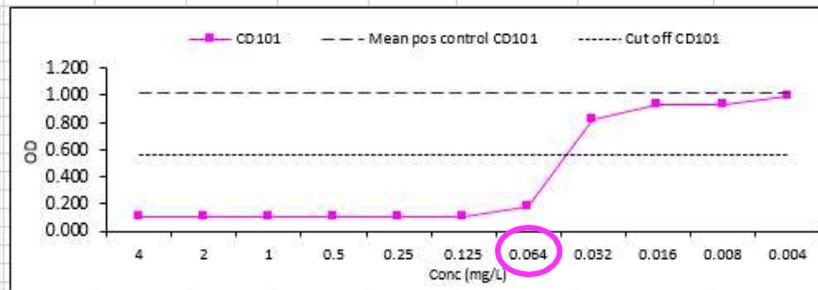
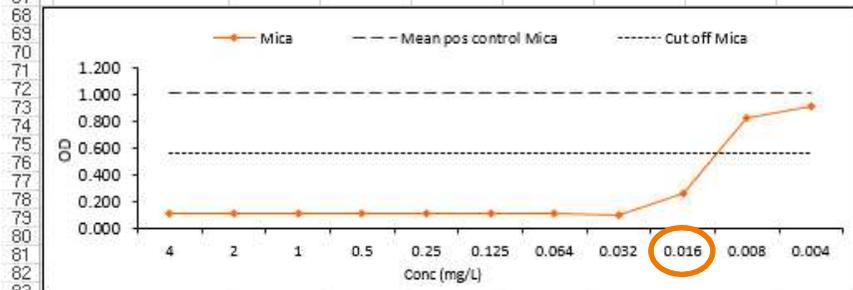
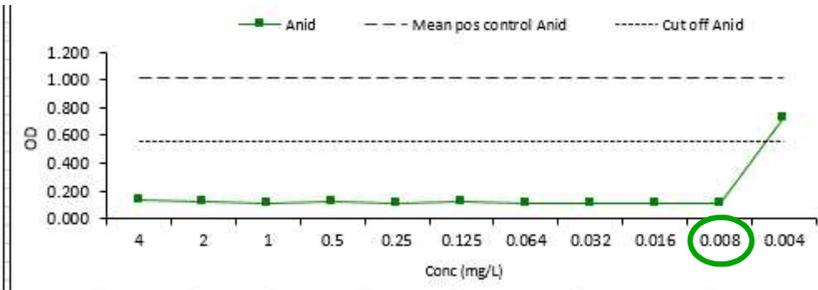
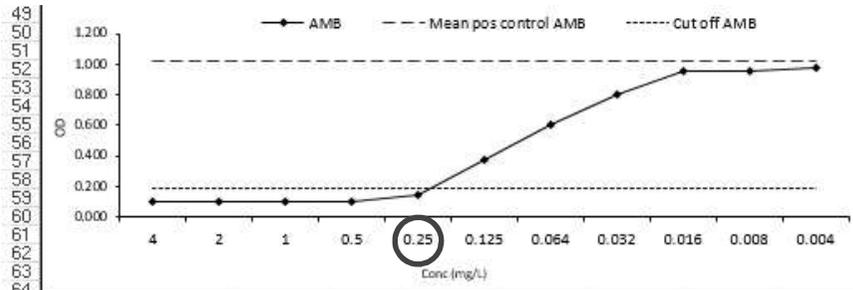


EUCAST Excel read out (upper part of the sheet)



KC4 report																
ProtocolFileName:	Ny hosp.gærplade vandret.prt											490				
Data:	NewPlate															
Opsat af dato:	211118 BBR															
Aflæst af:	221118 BBR															
Comment:	BATCH NR.061118															
Isolat nr.:	T32073															
Isolat navn:	C.ALBICANS															
	Beregn MIC					Zoom (ind/ud)										
	1	2	3	4	5	6	7	8	9	10	11	Pos Con	MIC OD	Mean pos con	90/50% cut off	
AMB	0.100	0.099	0.098	0.101	0.144	0.375	0.601	0.803	0.953	0.959	0.977	0.953	0.25	1.020	0.192	
Anid	0.140	0.118	0.115	0.119	0.117	0.118	0.114	0.112	0.108	0.110	0.727	0.983	0.008	1.020	0.560	
Mica	0.107	0.112	0.115	0.115	0.114	0.112	0.108	0.104	0.259	0.823	0.920	1.044	0.016	1.020	0.560	
CD101	0.109	0.112	0.109	0.109	0.106	0.110	0.175	0.822	0.935	0.935	0.990	1.018	0.064	1.020	0.560	
Vori	0.259	0.250	0.264	0.254	0.247	0.254	0.256	0.270	0.298	0.377	0.663	1.025	0.008	1.020	0.560	
Isavu	0.269	0.259	0.252	0.261	0.246	0.258	0.265	0.288	0.359	0.641	0.959	1.033	0.016	1.020	0.560	
Flu	0.277	0.270	0.282	0.286	0.275	0.265	0.280	0.305	0.422	0.850	1.018	1.094	0.25	1.020	0.560	
APX001A	0.108	0.108	0.110	0.118	0.132	0.257	0.562	0.777	0.907	1.023	1.042	1.010	0.016	1.020	0.560	
Mean pos control AMB	1.020	1.020	1.020	1.020	1.020	1.020	1.020	1.020	1.020	1.020	1.020	1.020				
Cut off AMB	0.192	0.192	0.192	0.192	0.192	0.192	0.192	0.192	0.192	0.192	0.192	0.192				
Mean pos control Anid	1.020	1.020	1.020	1.020	1.020	1.020	1.020	1.020	1.020	1.020	1.020	1.020				
Cut off Anid	0.560	0.560	0.560	0.560	0.560	0.560	0.560	0.560	0.560	0.560	0.560	0.560				
Mean pos control Mica	1.020	1.020	1.020	1.020	1.020	1.020	1.020	1.020	1.020	1.020	1.020	1.020	AMB	0,125-1	0,125-1	0,06-0,5
Cut off Mica	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56	Anid	0,016-0,06	0,25-1	
Mean pos control CD101	1.020	1.020	1.020	1.020	1.020	1.020	1.020	1.020	1.020	1.020	1.020	1.020	Mica	0,03-0,125	0,5-2	
Cut off CD101	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56	CD101			
Mean pos control Vori	1.020	1.020	1.020	1.020	1.020	1.020	1.020	1.020	1.020	1.020	1.020	1.020	Vori	0,03-0,25	0,016-0,06	0,5-2
Cut off Vori	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56	Isavu	0,016-0,06	0,008-0,03	
Mean pos control Isavu	1.020	1.020	1.020	1.020	1.020	1.020	1.020	1.020	1.020	1.020	1.020	1.020	Flu	16-64	0,5-2	32-128
Cut off Isavu	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56	APX001A			
Mean pos control Flu	1.020	1.020	1.020	1.020	1.020	1.020	1.020	1.020	1.020	1.020	1.020	1.020				
Cut off Flu	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56				
Mean pos control APX00	1.020	1.020	1.020	1.020	1.020	1.020	1.020	1.020	1.020	1.020	1.020	1.020				

EUCAST Excell read out (lower part of the sheet)



MIC OD	
0.25	
0.008	
0.016	
0.064	
0.008	
0.016	
0.25	
0.016	

Microdilution growth curves contain information

- Referred *C. parapsilosis* isolate for AFST
 - Info MALDI-TOF (Bruker, score >2)
- EUCAST E.Def 7.3 MIC results:

Compound	MIC OD
AMB	0.5
Anid	0.032
Mica	0.016
CD101	0.064
Vori	0.064
Isavu	0.032
Flu	4

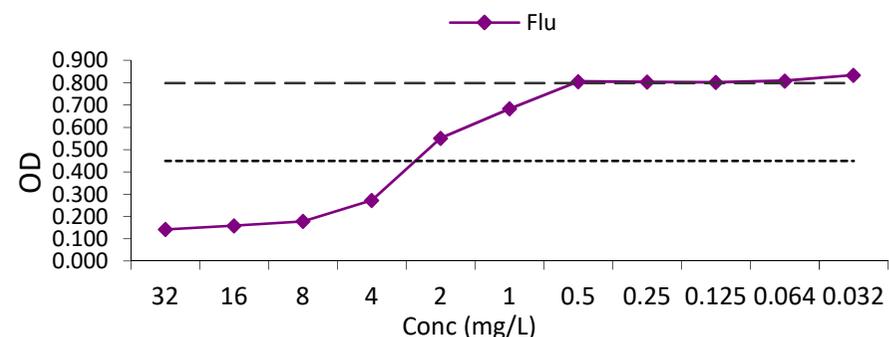
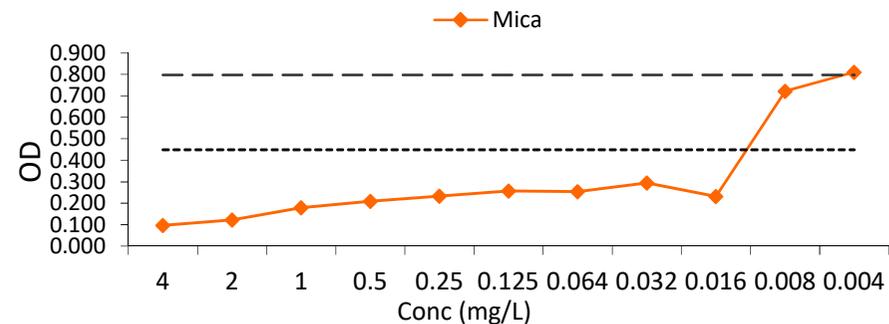
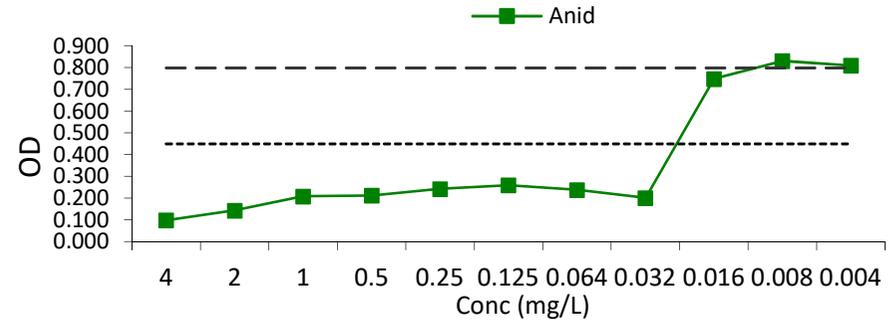
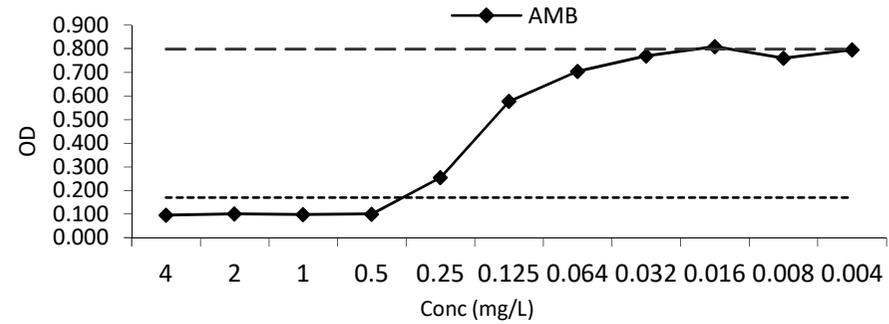
- Considerations?

The weird *C. parapsilosis*

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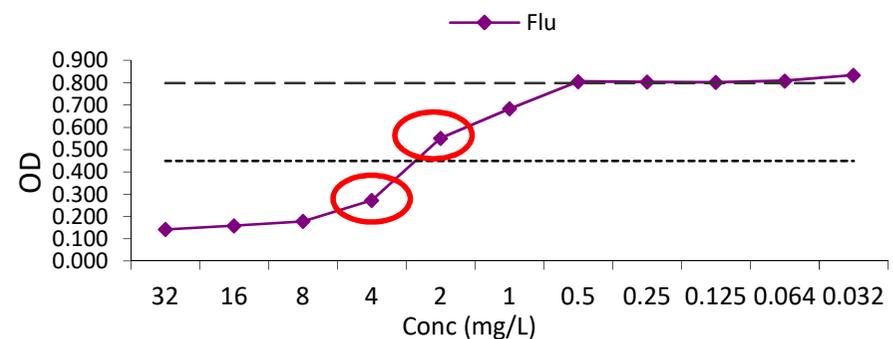
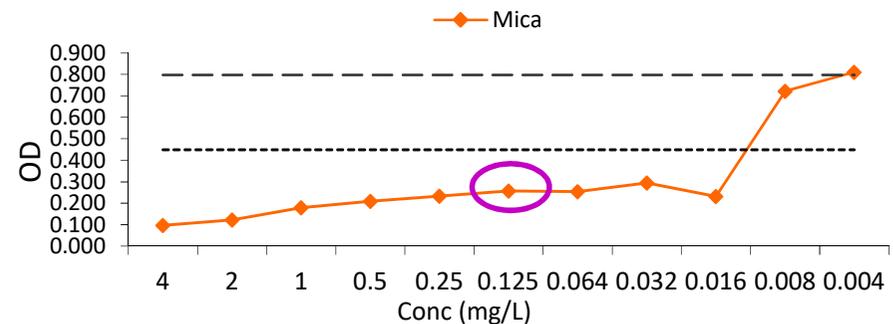
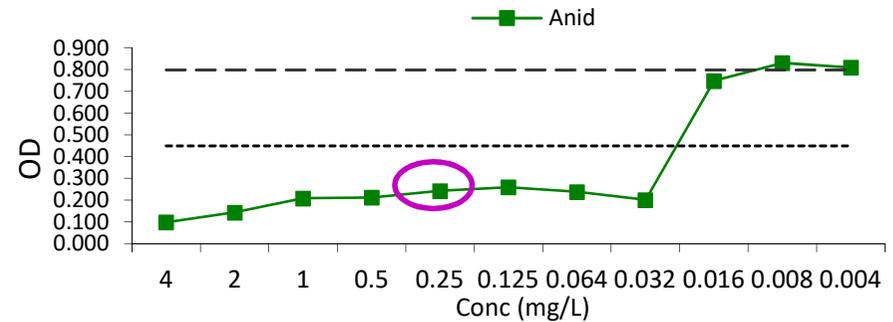
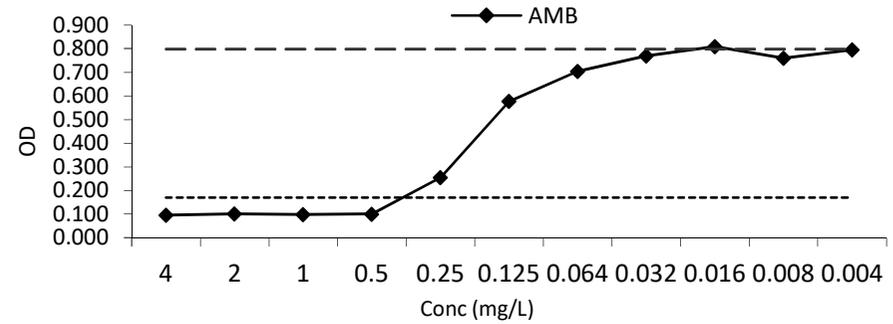


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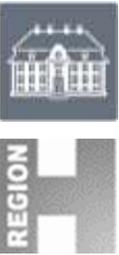
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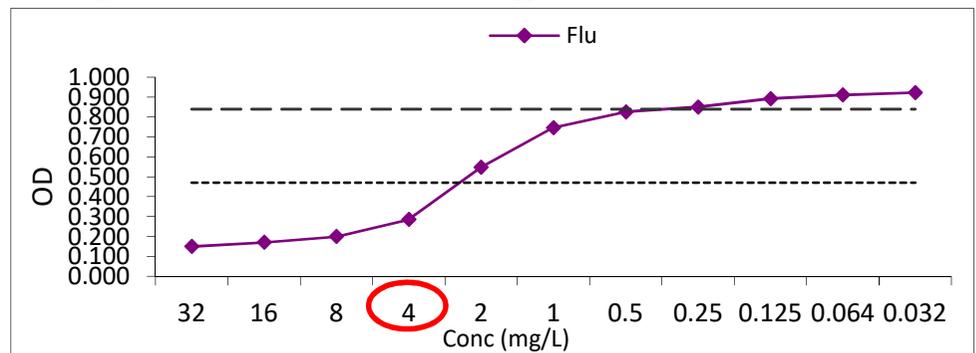
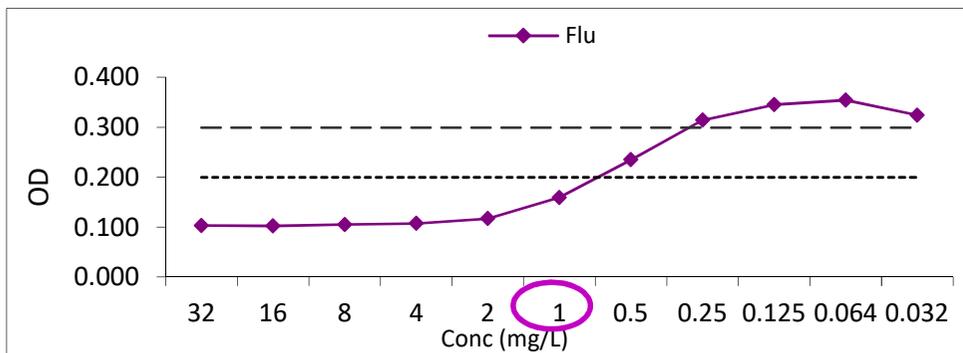
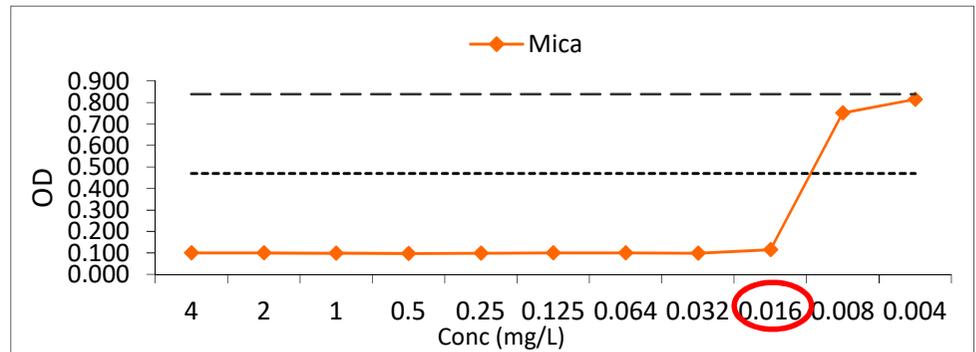
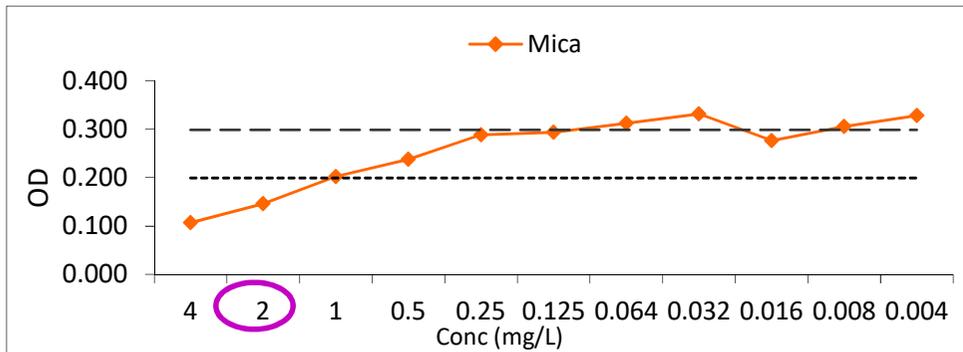
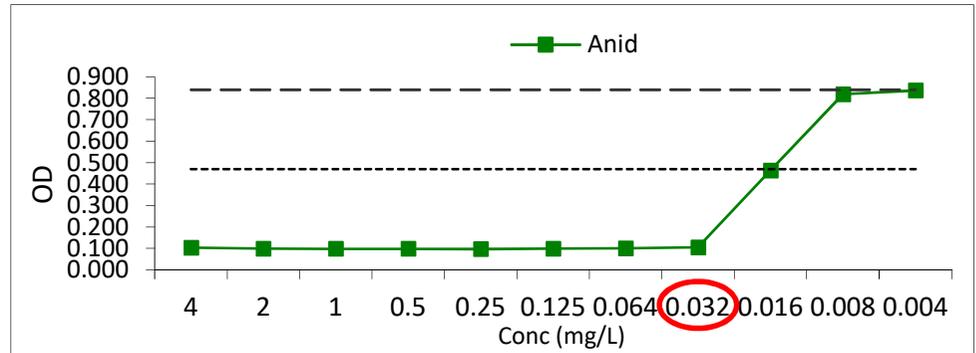
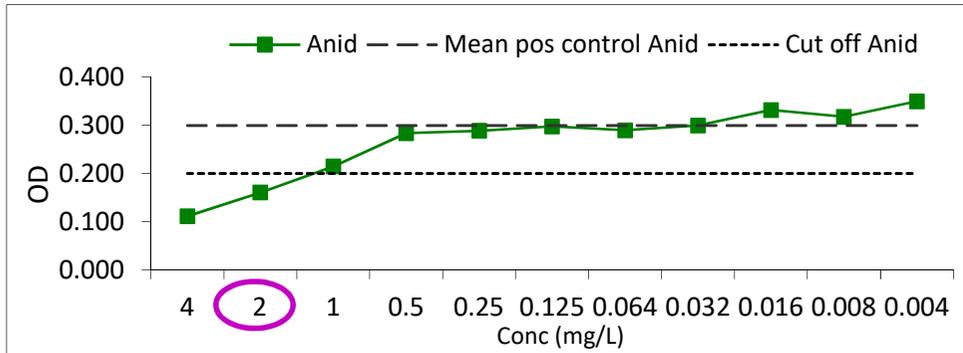
Cultured the wells indicated by ○

Weird *C. parapsilosis* → WT *C. parapsilosis* & *C. glabrata*



• *C. parapsilosis* (MICs encircled)

• *C. glabrata* (MICs encircled)

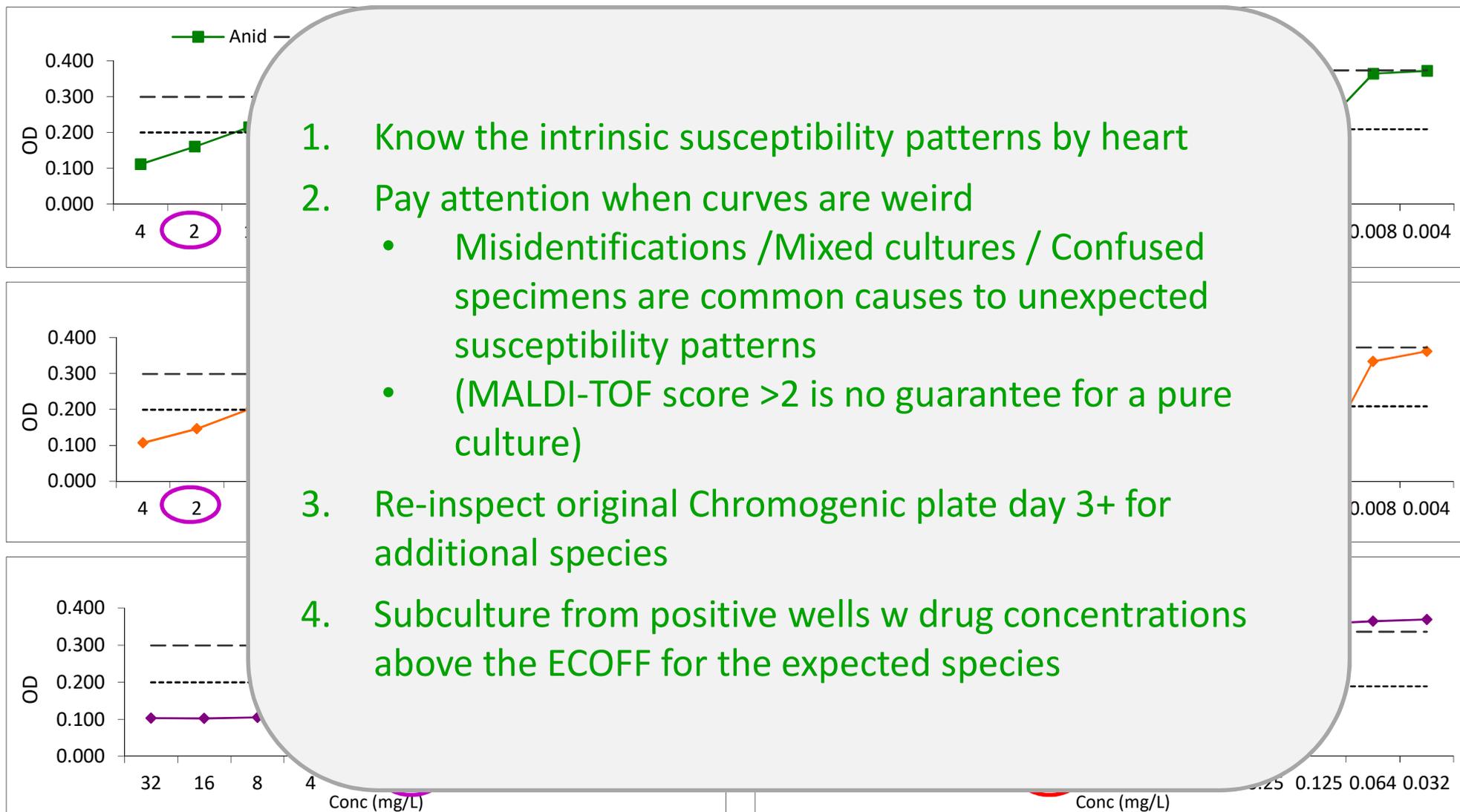


Weird *C. parapsilosis* → WT *C. parapsilosis* & *C. glabrata*



❖ *C. parapsilosis* (MICs encircled)

❖ *C. glabrata* (MICs encircled)



Pitfalls - Two important sources for variation

❖ Choice of plastic microtitre plates

- Treated or non-treated plates
 - EUCAST recommends treated polystyrene plates

❖ Method of drug-dilution may impact the MIC

- serial dilution vs ISO method
- number of plastic tube contacts
 - (tips, tubes, reservoirs)

In general

- Affects the
- Low MIC organisms the most
- Not always picked up with QC strains

C. parapsilosis ATCC 22019

C. krusei ATCC 6258

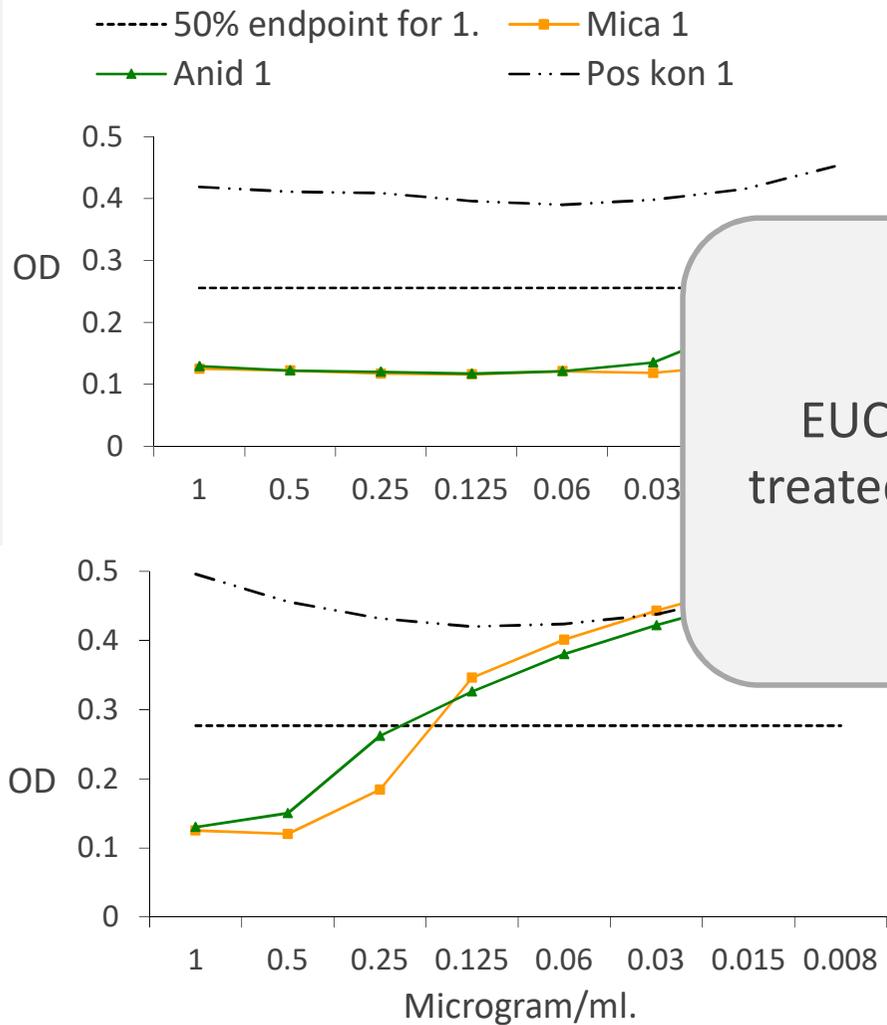
Choice of plastic plates may greatly impact MIC



Nunc 167008

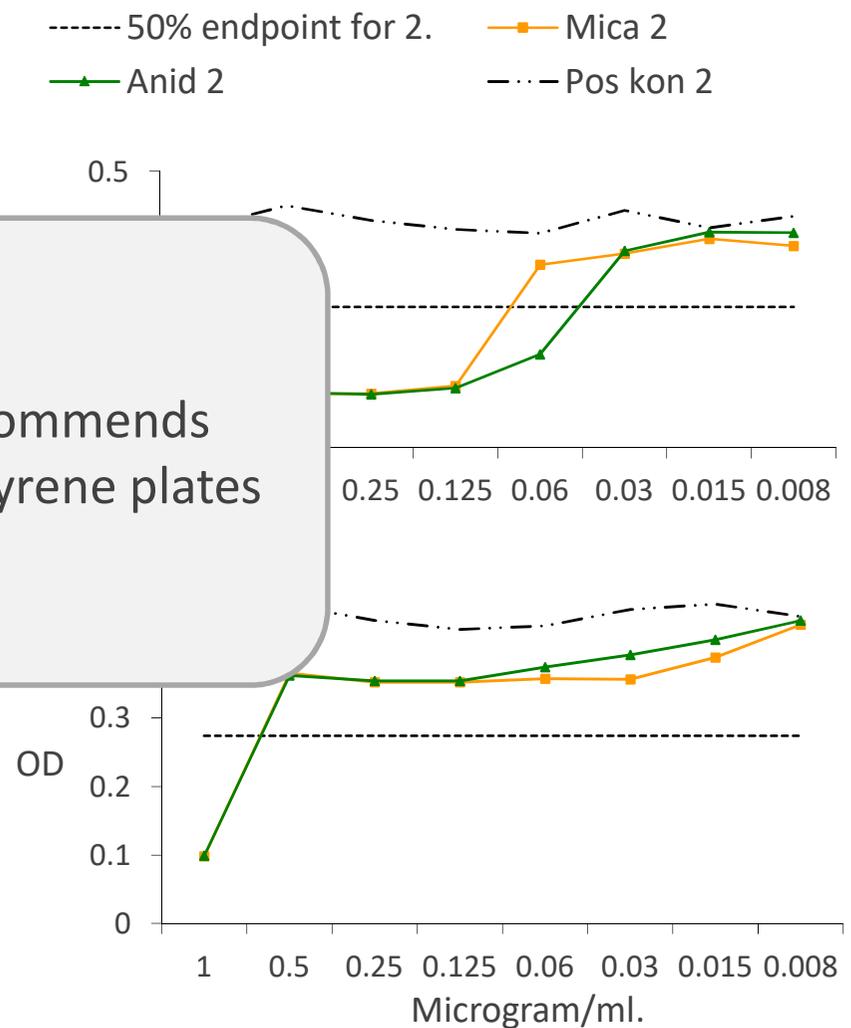
Sterile Nunc 174927 96F

C. albicans ATCC64550



Anidulafungin MIC: 0.015 → 0.25 mg/L (4 dil.)
 Micafungin MIC: ≤ 0.008 → 0.25 mg/L (5 dil.)

C. krusei ATCC6258



Anidulafungin MIC: 0.06 → 1 mg/L (4 dil.)
 Micafungin MIC: 0.125 → 1 mg/L (3 dil.)

EUCAST recommends treated polystyrene plates

Method of drug-dilution may impact the MIC

• E.Def 7.3.3

Preparation of working solutions

The range of concentrations tested will depend on the organism and the antifungal drug being tested. The
.....
...

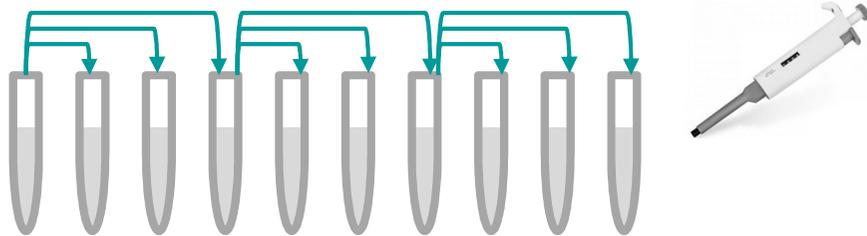
Dilutions should be prepared according to ISO recommendations [12]. For example, an alternative which uses smaller volumes to prepare a dilution series with final concentrations 0.125-64 mg/L is shown in Table 4 (also see Table 3 to check the solvents required for each antifungal). A summary of the steps required to prepare
.....
...

Alternative dilution schemes may be used if they are shown to perform as well as the reference method [13].

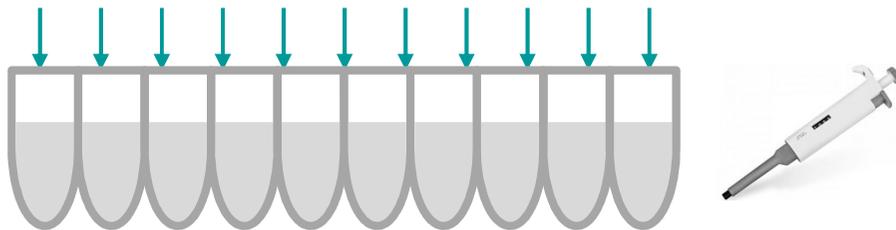
ISO Method for 2-fold drug dilutions

drug stock
in solvent 

- Drug dilution(s) series in solvent in tubes



- 1:100 dilution into medium (1% solvent)



- Transfer to a 12-channel reservoir (1% solvent)

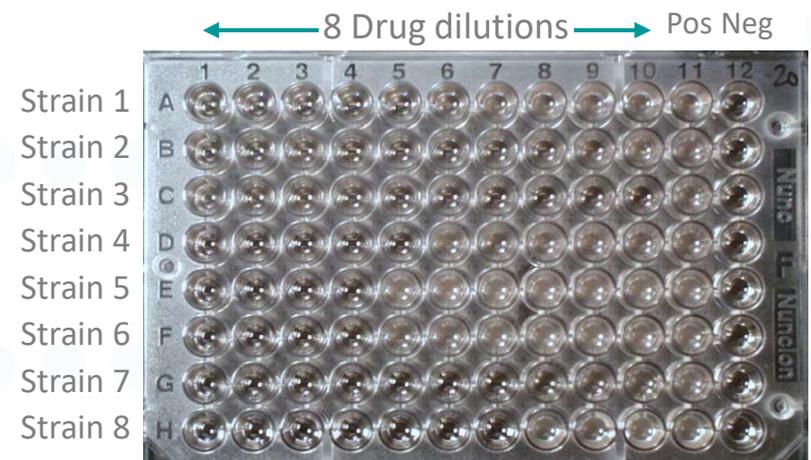


- Transfer to a microtitre plate (1% solvent)



- Best suitable for:

- 1 drug, 8 isolates
 - Adjust to specific clinical situation
- Flexible drug choice
 - Determination of 50% endpoint based on
 - Single pos control well OR
 - Mean for positive wells performed across on several plates
- But! - Only 1 growth control /plate

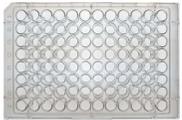


Serial dilution Methods for 2-fold drug dilutions

drug stock
in solvent



- Fill a microtitre plate w medium w 1% solvent



- Initial drug dilution(s) in medium w 1% solvent



- Transfer 8 initial drug dilution(s) to 8-channel reservoir

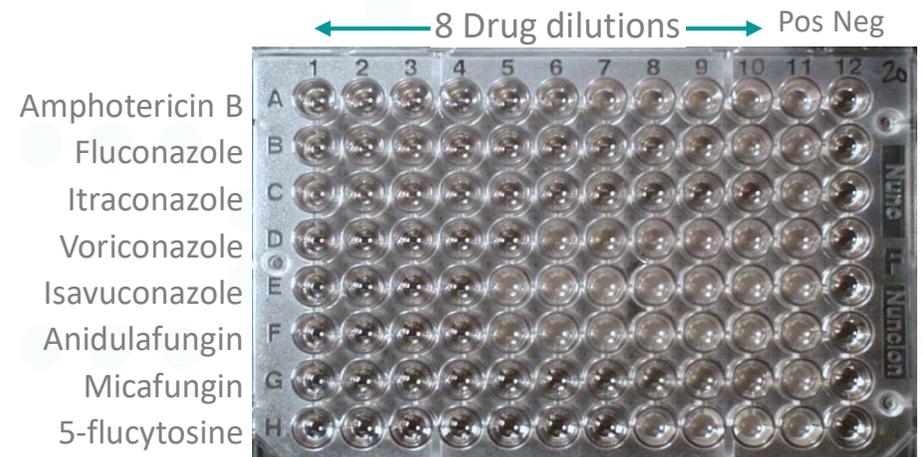


- Transfer to a microtitre plate & serial dilute



• Allows

- 8 drugs tested on a single plate
- All MICs on one plate
 - ↓ risk of errors reading AF-biogram.
- 8 growth controls on the plate
 - Allow automated calculation of mean controls and 50% endpoint



Dilution method and impact on MICs

❖ Background

- Serial dilution much more feasible than ISO dilution
- Documented as appropriate for fluconazole as ISO*

❖ Systematic investigation for most common compounds**

- Nine antifungals
- Serial (0–10 tip changes) and ISO dilution.
- 4-5 QC strains
- 5 clinical isolates each of 4-6 species
 - *C. albicans*, *C. dubliniensis*, *C. glabrata*, *C. krusei*, *A. flavus* and *A. terreus*

> [J Antimicrob Chemother.](#) 2021 Mar 18;dkab088. doi: 10.1093/jac/dkab088. Online ahead of print.

ISO standard 20776-1 or serial 2-fold dilution for antifungal susceptibility plate preparation: that is the question!

Serial dilution: *N* of tip changes – AMB & candins

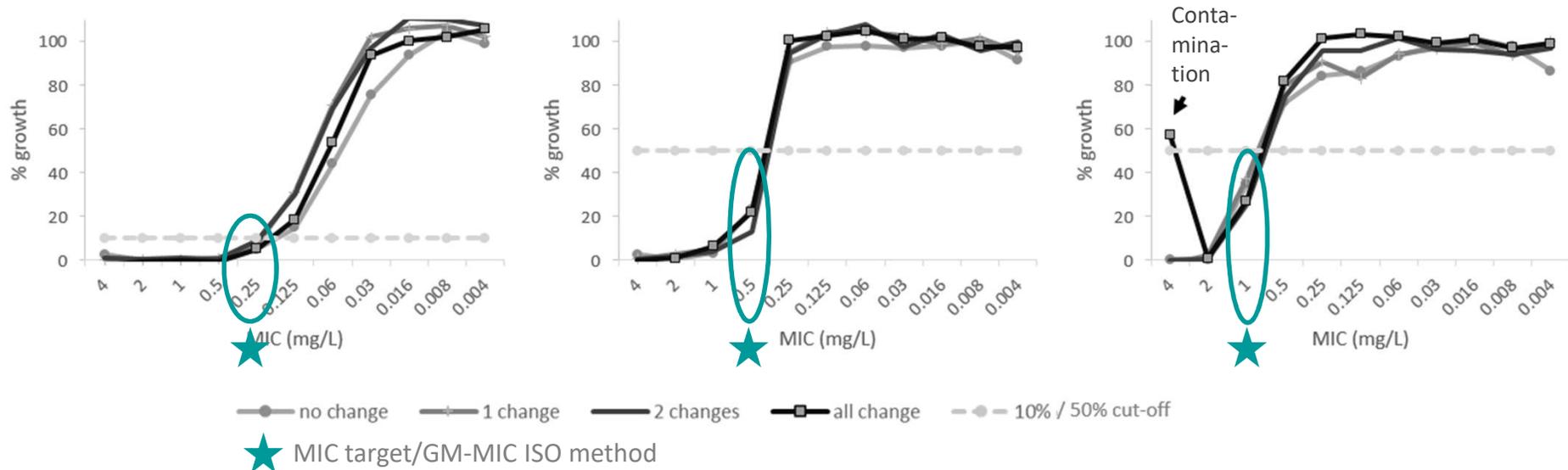


Amphotericin B

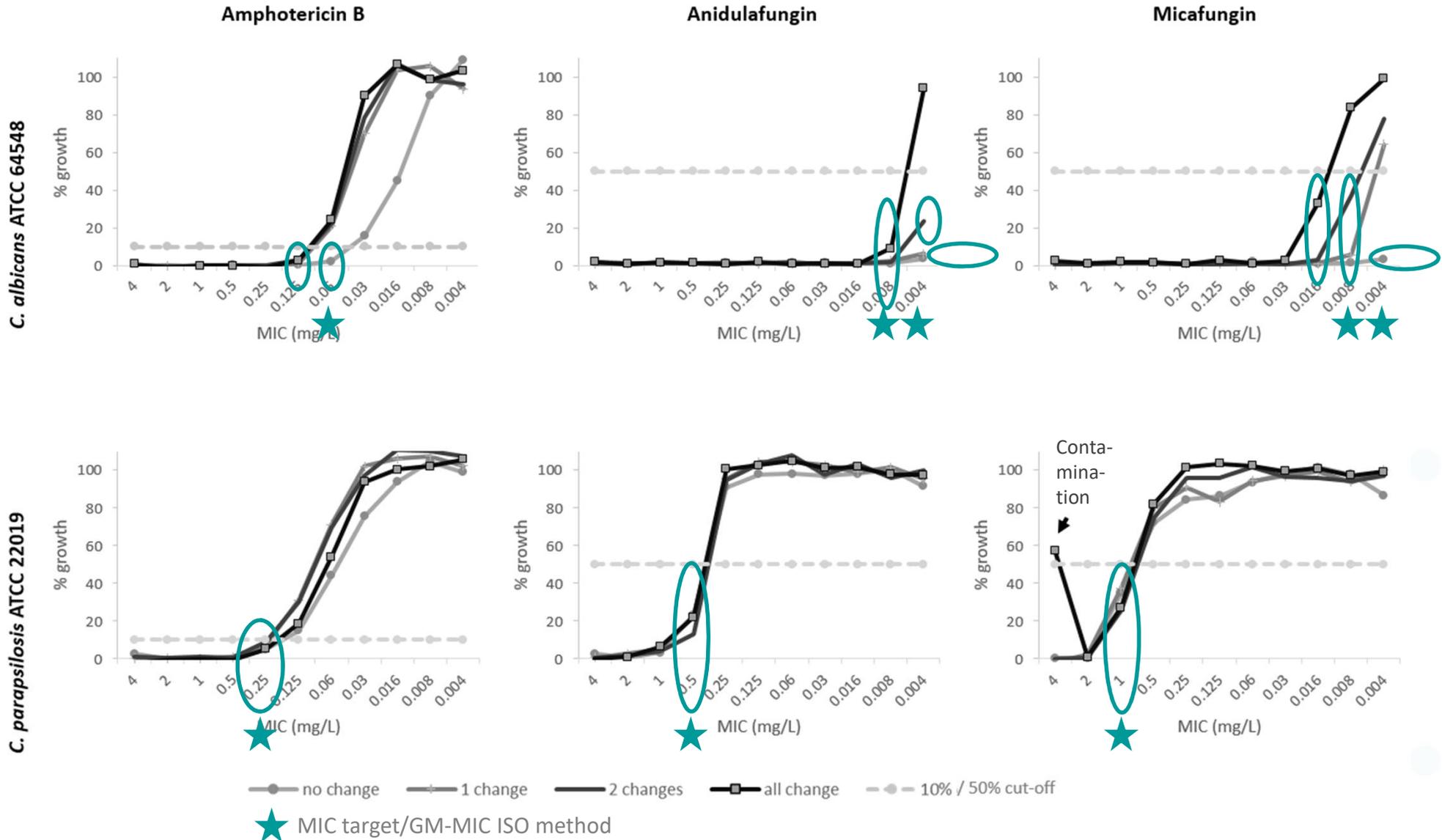
Anidulafungin

Micafungin

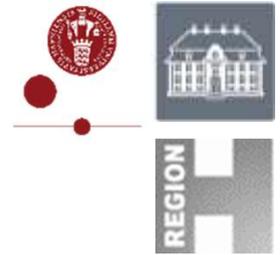
C. parapsilosis ATCC 22019



Serial dilution: *N* of tip changes – AMB & candins



Serial dilution w. 2 tip changes >< ISO-method



- ❖ For 4 *Candida* QC-strains & 8 compounds (352 MICs)
 - 85% (35/41) GM-MIC deviated <1 dilution from the QC target
 - Best performance compared to QC values
 - ISO method in 30.8%
 - Serial dilution in 34.6%
 - Equal performance in 34.6% of the cases.
 - 14.6% (6/41) deviated with 1–1.8 dilution from the target
- ❖ For 4 clinical species & 8 compounds (5 isolates of each 4 species, 4 repetitions (920 MICs))
 - 78.3% (18/23) GM-MIC pairs deviated ≤ 0.6 dilution
 - 21.7% (5/23) GM-MIC pairs deviated 1.0–1.1 dilutions.
- ❖ Take home message
 - Some drugs bind to plastic ware (tips, tubes, reservoirs, trays...)
 - Potential consequences: drug loss \rightarrow MIC \uparrow or drug release \rightarrow MIC \downarrow
 - MICs affected by number of tip changes when serial dilution is performed
 - The ISO and serial dilution (two tip changes) method were in acceptable agreement and thus equally applicable for EUCAST testing

Agenda



❖ Acquired Resistance

- size of the problem 😊

❖ EUCAST BMD method and alternatives

- Microdilution
- Molecular methods
- Commercial tests

❖ New I category

❖ Species without breakpoints

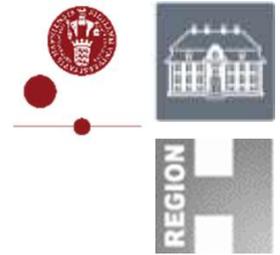
J Antimicrob Chemother
<https://doi.org/10.1093/jac/dkac161>

Journal of
Antimicrobial
Chemotherapy

Molecular mechanisms of acquired antifungal drug resistance in principal fungal pathogens and EUCAST guidance for their laboratory detection and clinical implications

Thomas R. Rogers ^{1*}, Paul E. Verweij ^{2,3†}, Mariana Castanheira ^{4†}, Eric Dannaoui ^{5,6†}, P. Lewis White ^{7†} and Maiken Cavling Arendrup ^{8,9,10} on behalf of the Subcommittee on Antifungal Susceptibility Testing (AFST) of the ESCMID European Committee for Antimicrobial Susceptibility Testing (EUCAST)‡

Molecular detection of AF-resistance



Fungal pathogen (Specimen types)	Antifungal drug	In-house detection of drug resistance mechanism(s)	Commercially available assays for detection of drug resistance mechanism(s)
<i>Aspergillus</i> spp. (Sputum, bronchoalveolar lavage, serum, or plasma)	Triazoles	Real-time PCR studies ²⁴⁻³⁰ Pyrosequencing ³¹	Aspergenius [®] multiplex real-time PCR detects <i>Aspergillus</i> TR ₃₄ /L98H; TR ₄₆ /T289A; TR ₄₆ /Y121F gene mutations in <i>Aspergillus</i> spp. ^{32-33,36-38} Aspergenius [®] detects G54 and M220 mutations in <i>A. fumigatus</i> Mycoguard MycoG detects gene mutations Fungiplex PCR
<i>Candida</i> spp.	Triazoles	No assays for azole resistance due to multiple mechanisms playing in concert	Exception "endemic" Fluconazole R <i>erg11</i> Y132F <i>C. parapsilosis</i>
	Echinocandins	PCR assay to detect Glucan synthase (<i>FKS</i>) gene(s) for subsequent sequencing to identify mutations in the hotspots of <i>fk1</i> and <i>fk2</i> (<i>C. glabrata</i> only) ^{39,40}	
<i>Cryptococcus</i> spp.	Fluconazole	No assays due to variable mechanisms of resistance	
Dermatophytes	Terbinafine	PCR assay to detect squalene epoxidase (<i>SQLE</i>) gene for subsequent sequencing to identify mutations in <i>Trichophyton mentagrophytes/interdigitale</i> and <i>T. rubrum</i> ^{17,42}	DermaGenius [®] Resistance Multiplex real-time PCR kit. ⁴³ Detects: <i>T. rubrum/Trichophyton soudanense</i> , <i>T. interdigitale/mentagrophytes</i> , <i>T. mentagrophytes</i> (ITS type IV), <i>T. tonsurans</i> , <i>T. violaceum</i> , <i>Trichophyton quinckeanum/Trichophyton schoenleinii</i> and <i>SQLE</i> alterations: L393F, F397L, L393S, F397I, F397V.

Target gene mutations → R: *Candida* Echinocandin FKS



AFG EUCAST ECOFF (mg/L)	FKS1p				FKS2p				
	Hot spot 1		Hot spot 2		Hot spot 1		Hot spot 2		
	1st AA no.	AA sequence	1st AA no.	AA sequence	1st AA no.	AA sequence	1st AA no.	AA sequence	
<i>C. albicans</i>	0.03	641	<u>F</u> LT <u>L</u> <u>S</u> LR <u>D</u> P	1357	D <u>W</u> IR <u>R</u> YTL				
<i>C. dubliniensis</i>	0.03	641	<u>F</u> LT <u>L</u> <u>S</u> LRDP	1357	DWIRRYTL				
<i>C. glabrata</i>	0.06	625	<u>F</u> L <u>I</u> <u>L</u> <u>S</u> LR <u>D</u> P	1340	D <u>W</u> <u>V</u> RRYTL	659	<u>F</u> L <u>I</u> <u>L</u> <u>S</u> LR <u>D</u> P	1374	D <u>W</u> <u>I</u> <u>R</u> YTL
<i>C. kefyr</i>	(0.03)	651	<u>F</u> LT <u>L</u> <u>S</u> LR <u>D</u> P	769*	DW <u>V</u> RRYTL				
<i>C. krusei</i>	0.06#	655	<u>F</u> L <u>I</u> <u>L</u> <u>S</u> IR <u>D</u> P	1364	DWIR <u>R</u> YTL				
<i>C. lusitaniae</i>	(0.06)	634	FLTL <u>S</u> LRDP	1348	DWIRRYTL				
<i>C. tropicalis</i>	0.06	650	<u>F</u> LT <u>L</u> <u>S</u> LRDP	1366	DWIRRYTL				
<i>C. auris</i>		635	<u>F</u> LIL <u>S</u> LRDP	1350	DWIR <u>R</u> YTL				
<i>C. parapsilosis</i>	4	652	<u>F</u> LT <u>L</u> <u>S</u> LR <u>D</u> <u>A</u>	1369	DWIRRYTL				
<i>C. metapsilosis</i>	(4)	104*	FLTL <u>S</u> LR <u>D</u> <u>A</u>	821*	DWIRRYTL				
<i>C. orthopsilosis</i>	(4)	645	FLTL <u>S</u> LR <u>D</u> <u>A</u>	1362	DW <u>V</u> RRYTL				
<i>C. guilliermondii</i>	(4)	632	F <u>M</u> ALSLRDP	1347	DWIRRYTL				
<i>C. lipolytica</i>	NA	662	FL <u>I</u> LSLRDP	1387	DWIRR <u>C</u> VL				
<i>S. cerevisiae</i>	(1)	639	FL <u>V</u> LSLRDP	1353	DW <u>V</u> RRYTL	658	FLILSLRDP	1372	DW <u>V</u> RRYTL

X "strong R" mutation, low letters indicate the codon involves a mutation or deletion; high letters indicate the codon involves a mutation or stop codon;

X "weak R" mutation;

X "silent" mutation, acquired or naturally occurring;

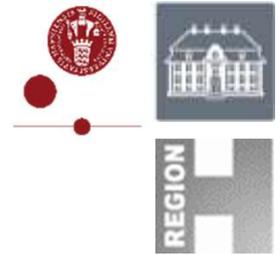
X naturally occurring mutation proven or possibly related to the intrinsic lower susceptibility;

X naturally occurring mutation of unknown impact; * Inaccurate annotation, sequencing of entire gene-sequence required;

Micafungin ECOFF elevated for *C. krusei* compared to *C. albicans* and *C. glabrata*, but not the anidulafungin ECOFF.

Target gene mutations: echinocandins & *Candida*

Overview of MIC ↑ by spp, codon & alteration



Species	≥3 ANF MIC ↑	≥3 ANF MIC ↑	1-2 ANF MIC ↑	1-2 ANF MIC ↑	1-2 ANF MIC ↑	0 ANF MIC ↑
	≥3 MCF MIC ↑	1-2 MCF MIC ↑	≥3 MCF MIC ↑	1-2 MCF MIC ↑	0 MCF MIC ↑	1-2 MCF MIC ↑
<i>C. albicans</i>	F641S (2) R1361G (2) S645P (6) R1361R/S (1) S645S/P (1) R1361S (3) P1354S (1)	D648V (2) D1337Y (1) ^{A,B} P1354P/S (2) ^{A,B} R1361H (5) ^B	R647G (2) ^B	R647G (2) ^B P1354S (2) ^{A,B} R1361C (1) ^B WT (18)	WT (1)	WT (4)
<i>C. dubliniensis</i>	F641S (1) R1361S (1) S645S/P (1) S645P (3)			WT (4)	WT (1)	P1354Q (1) ^A WT (14)
<i>C. glabrata</i>	S629P/L640STOP (1) ^A F659DEL (4) S663F (2) S663F/L630Q (1) S663P (9)	F659S (1) F659V (1)	R665G (1) ^B	F625S (1) ^B L662F (1) ^B F625Y/WT (1) ^B L662W (2) ^{B(1)} S629P (2) ^B S663C (1) ^B A651V (1) ^{A,B} D666E (2) ^{B(1)} F659I (1) D666Y (1) ^B F659L (1) ^B P667I (1) F659S (3) ^{B(1)} WT (9) F659Y (2)	L628I (1) ^B WT (6)	K1323E (1) ^{A,B} WT (4)
<i>C. krusei</i>	S659F (1) E651E/Q + D662D/Y (1) ^A		S659P (2)	WT (1)		
<i>C. parapsilosis</i>		F652S (1) ^C L657W (1) ^C			WT (4)	
<i>C. tropicalis</i>	F650S (2) S654S/P (1) S654P (1)	F650F/L (1)	R656R/G (1) ^B	WT (2) ^D		V1404I (1) ^{A,B} WT (2)
<i>C. auris</i>	F635Y (1) S639F (8) ^E					

^AOutside hot spot, ^BCategorised anidulafungin S, ^CMIC for MCF is higher than the tested range (>4 mg/L); therefore >1 dilution step elevated, ^DOne isolate is FKS WT but has an ERG11 V48G alteration, ^EBy comparison to modal MICs in doi: 10.1128/AAC.02136-19.

Agenda



- Acquired Resistance
 - size of the problem 😊
- EUCAST BMD method and alternatives
 - Microdilution
 - Molecular methods
 - Commercial tests
- New I category
- Species without breakpoints



Can I adopt EUCAST BP for a commercial AFST MIC test?



• Well,... that depends

- Essential agreement (± 1 dilution) between Etest and EUCAST for *Candida*

	AMB		FLU		Mica		Vori	
	N. isolates	EA%	N. isolates	EA%	N. isolates	EA%	N. isolates	EA%
<i>C. albicans</i>	345	81%	354	67%	21	93%	308	88%
	54	15%	54	91%	159	100%	54	87%
<i>C. glabrata</i>	104	39%	110	73%	40	89%	98	82%
	11	44%	11	82%	152	99%	11	60%
<i>C. krusei</i>					10	80%		
					127	98%		
<i>C. parapsilosis</i>	68	65%	69	78%	27	75%	59	86%
	38	2.7%	38	82%	152	97%	37	95%
<i>C. tropicalis</i>	54	61%	54	85%	28	93%	48	81%
	33	22%	33	58%	152	99%	33	73%

EUCAST AFST Advise regarding commercial MIC tests for AFST



• Before implementing a reference test - check alignment with the reference method

1. run the EUCAST QC strains, 10 times each,

– Check:

- mode on the target?
- MICs within the range?

Random variation is permissible (max 1/10 MIC value outside range)
Systematic deviation (mode systematically to one side of the target) is not OK.

QC for EUCAST antifungal testing

Candida krusei ATCC 6258^a
Version 5.0, valid from 2020-09-24

See EUCAST E.Def 7.3.2 for MIC methodology

Antimicrobial agent	MIC ^b (mg/L)	
	Target	Range
Amphotericin B	0.25-0.5	0.125-1
Anidulafungin	0.03	0.016-0.06
Caspofungin	NA ^c	NA ^c
Fluconazole	32	16-64
Flucytosine	2	1-4
Isavuconazole	0.03	0.016-0.06
Itraconazole	0.06	0.03-0.125
Micafungin	0.06	0.03-0.125
Voriconazole	0.06-0.125	0.03-0.25
Posaconazole	0.03	0.016-0.06

EUCAST AFST Advise regarding commercial MIC tests for AFST



❖ Before implementing a reference test - check alignment with the reference method

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Caspofungin	NA ^c	NA ^c
Fluconazole	32	16-64
Flucytosine	2	1-4
Isavuconazole	0.03	0.016-0.06
Itraconazole	0.06	0.03-0.125
Micafungin	0.06	0.03-0.125
Voriconazole	0.06-0.125	0.03-0.25
Posaconazole	0.03	0.016-0.06

1. if the above is OK – then run:

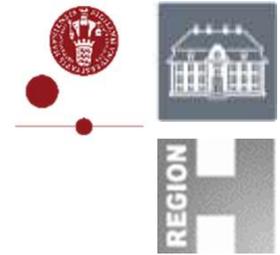
- 10 clin *C. albicans*
- 10 clin *C. glabrata*
- 10 clin *C. tropicalis*
- 10 clin *C. krusei*

3b. MIC distributions (%) and epidemiological cut-off (ECOFF) values (mg/L) Amphotericin B

	No.	0.002	0.004	0.008	0.016	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	ECOFF/ [T-ECOFF]
<i>Aspergillus flavus</i>	207	0	0	0	0	0	0	0	3	20	40	29	7	0	0	0	0	0	0	0	4
<i>Aspergillus fumigatus</i>	2627	0	0	0	0	0	0	15	23	46	8	0	0	0	0	0	0	0	0	0	1
<i>Aspergillus nidulans</i>	121	0	0	0	0	0	2	3	7	31	31	17	3	2	4	0	0	0	0	0	[4] ^a

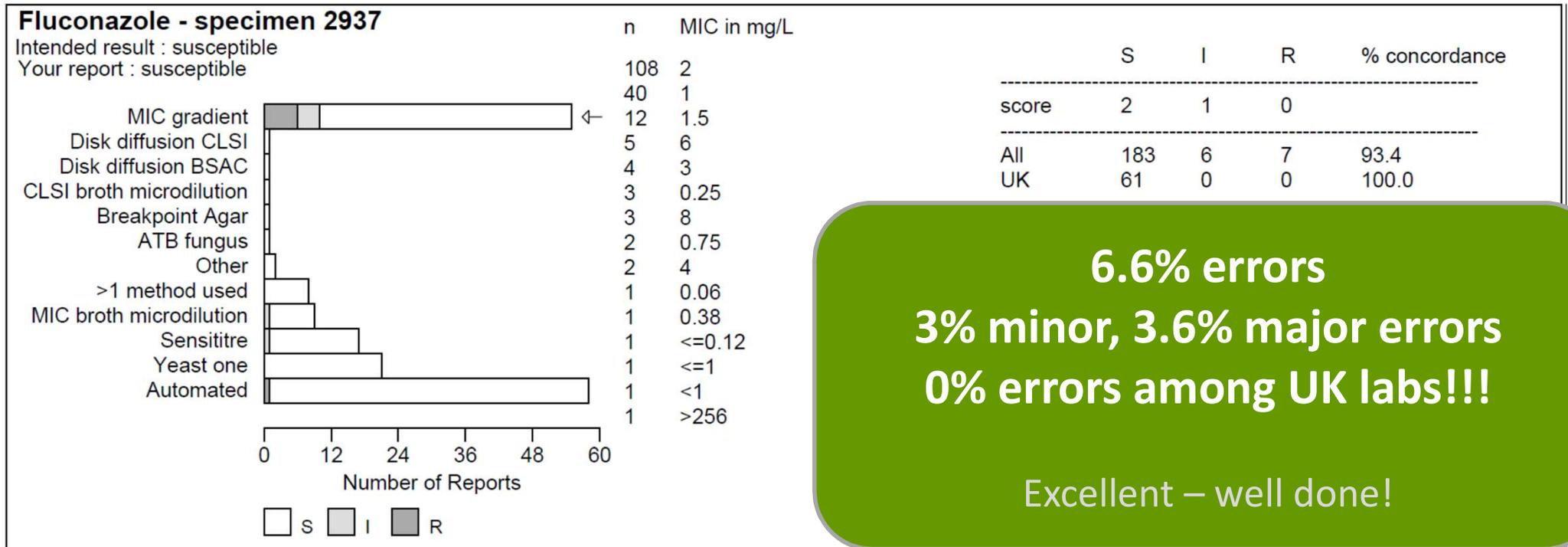
- Check the modal MICs are within ± 1 dilution of the modal MICs for each of the drug bug combinations in the EUCAST rationale documents
 - If they are, your method perform fine for wildtype isolates when adopting EUCAST breakpoints.
 - If not, your method will misclassify isolates.

UK NEQAS AFST performance for a susceptible wt strain



UK NEQAS AFST performance for a susceptible *C. parapsilosis* isolate

	Antifungal susceptibility	Laboratory :
	Distribution : 3789	Page 3 of 7
	Dispatch Date : 04-Jan-2016	

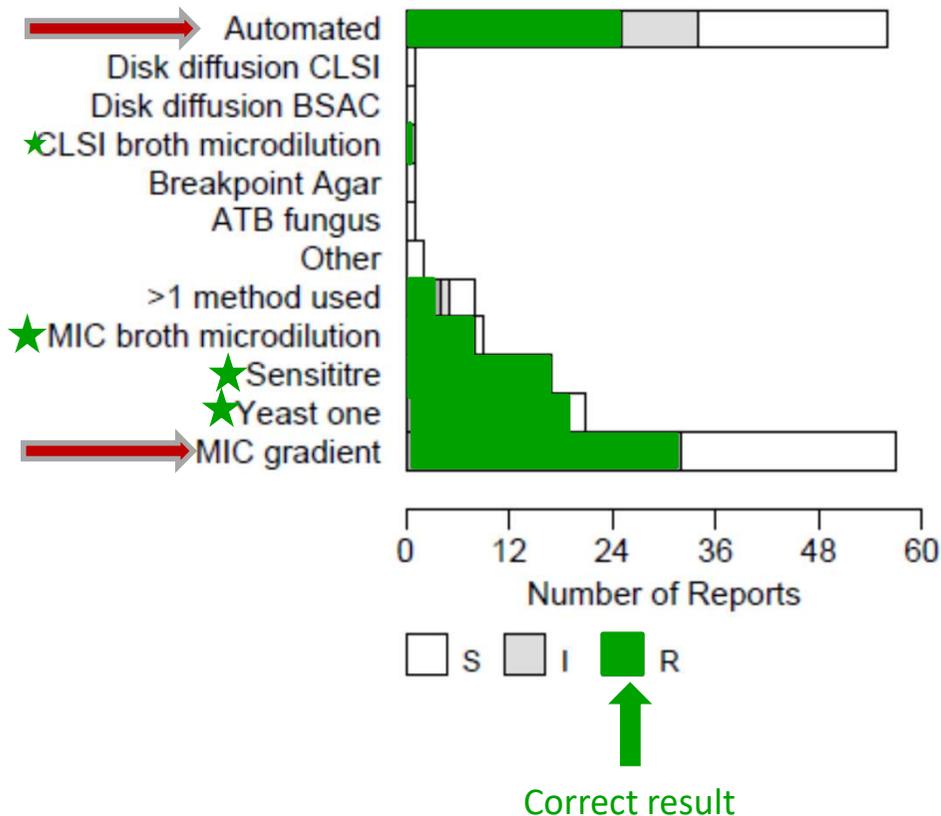


Routine testing not always sensitive in detecting R

UK NEQUAS AFST performance for a resistant *C. albicans* isolate

Fluconazole - specimen 2938

Intended result : resistant



	S	I	R	% concordance
score				
All	62	10	124	63.3
DK	1	0	2	66.7

37% errors
32% very major errors!!

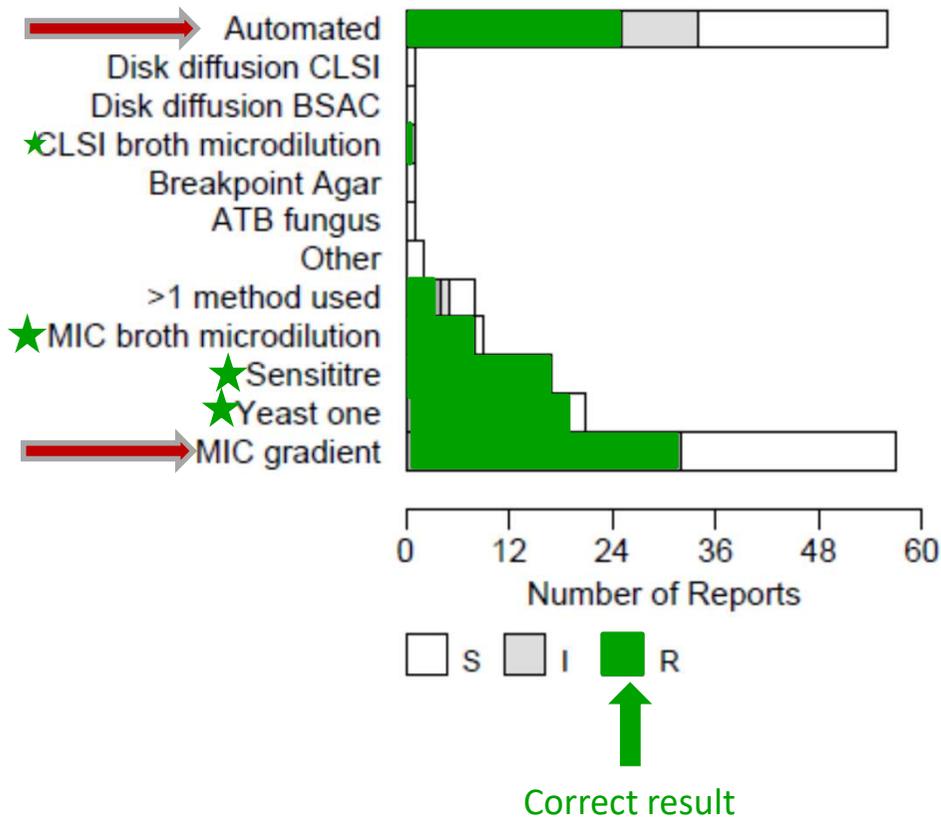
Despite the fact that:
 EQA typically overestimate performance
 Many laboratories don't participate

Routine testing not always sensitive in detecting R

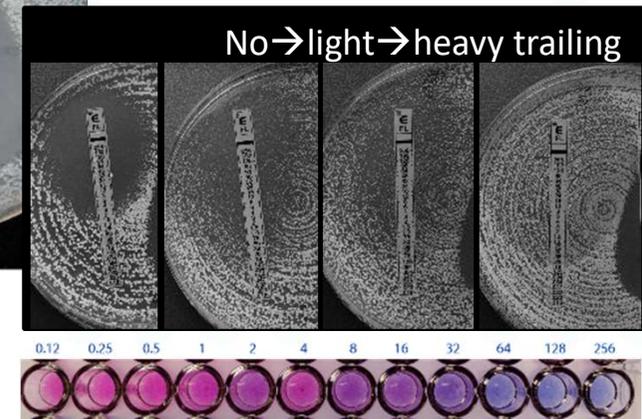
• UK NEQUAS AFST performance for a resistant *C. albicans* isolate

Fluconazole - specimen 2938

Intended result : resistant



	S	I	R	% concordance
score				
All	62	10	124	63.3
DK	1	0	2	66.7



Agenda



❖ Acquired Resistance

- size of the problem 😊

❖ EUCAST BMD method and alternatives

- Microdilution
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❖ New I category

❖ Species without breakpoints

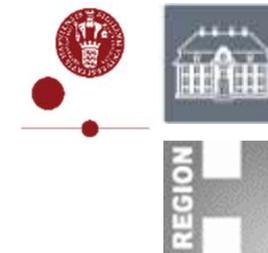


Narrative review

How to interpret MICs of antifungal compounds according to the revised clinical breakpoints v. 10.0 European committee on antimicrobial susceptibility testing (EUCAST)

M.C. Arendrup^{1,2,3,*}, N. Friberg⁴, M. Mares⁵, G. Kahlmeter⁶, J. Meletiadis^{7,8,†}, J. Guinea^{9,10,11,†}, on behalf of the Subcommittee on Antifungal Susceptibility Testing (AFST) of the ESCMID European Committee for Antimicrobial Susceptibility Testing (EUCAST)[‡]

Implications for amphotericin B BPs for *Candida*



• Amphotericin B Updates:

- No support for an “I” category – No change after BP definition revision
- Breakpoints have been established for *C. dubliniensis* S: ≤ 1; R: >1

Antifungal agent	MIC breakpoint (mg/L)																		Comments on the I category	
	<i>Candida albicans</i>			<i>Candida dubliniensis</i>		<i>Candida glabrata</i>		<i>Candida krusei</i>		<i>Candida parapsilosis</i>		<i>Candida tropicalis</i>		<i>Candida guilliermondii</i>		<i>Cryptococcus neoformans</i>		Non-species related breakpoints for <i>Candida</i> ¹		
	S ≤	R >	ATU	S ≤	R >	S ≤	R >	S ≤	R >	S ≤	R >	S ≤	R >	S ≤	R >	S ≤	R >	S ≤		R >
Amphotericin B	1	1		1	1	1	1	1	1	1	1	1	1	IE	IE	1	1	IE	IE	No data to support an I category according to the new definitions

Implications for Fluconazole BPs for *Candida*

• Fluconazole

- Former “I”s
 - *C. albicans*, *C. dubliniensis*, *C. tropicalis* (S: ≤ 2 , I: 4, R > 4 mg/L)
 - *C. glabrata* (I: ≤ 32 , R: > 32 mg/L)

- Action
 - Fluconazole ECOFF for *C. glabrata* revised from 32 to 16 mg/L based upon more MIC data

- Decision
 - Retain “I” with increased exposure: 800 mg/day
 - *C. albicans*, *C. dubliniensis*, *C. tropicalis* (S: ≤ 2 , I: 4, R > 4 mg/L)
 - *C. glabrata* (I: ≤ 16 , R: > 16 mg/L)

Antifungal agent	MIC breakpoint (mg/L)																		Comments on the I category	
	<i>Candida albicans</i>			<i>Candida dubliniensis</i>		<i>Candida glabrata</i>		<i>Candida krusei</i>		<i>Candida parapsilosis</i>		<i>Candida tropicalis</i>		<i>Candida guilliermondii</i>		<i>Cryptococcus neoformans</i>		Non-species related breakpoints for <i>Candida</i> ¹		
	S \leq	R $>$	ATU	S \leq	R $>$	S \leq	R $>$	S \leq	R $>$	S \leq	R $>$	S \leq	R $>$	S \leq	R $>$	S \leq	R $>$	S \leq		R $>$
Fluconazole	2	4		2	4	0.001 ⁴	16	-	-	2	4	2	4	IE ²	IE ²	IE	IE	2	4	See dosages table for appropriate dose

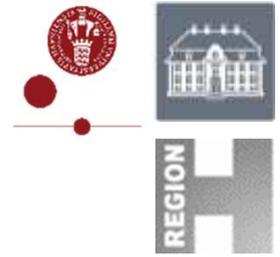
Implications for Echinocandin BPs for *Candida*

• Echinocandins Updates

- No support for an “I” category → Former “I”s need to be revised
- *C. parapsilosis*
 - Anidulafungin: $I \leq 4$ mg/L → ?
 - Micafungin: $I \leq 2$ mg/L → ?



Motivation behind reclassifying *C. parapsilosis* as S



- ❖ Echinocandins used for ~two decades in countries following CLSI (S)
- ❖ Mortality was equal (flu 9.5% vs candin 9.9%)
 - in a retrospective observational cohort study, including 307 unique patients *C. parapsilosis* BSI
- ❖ Flu resistance is ↑ in *C. parapsilosis*
 - in which case echinocandins are a valid alternative considering the study above and the amphotericin B related toxicity
- ❖ Treatment guidelines still emphasize that
 - fluconazole is the preferred agent for *C. parapsilosis* when the isolate is susceptible thus limiting the risk of increased persistent candidaemia

Implications for Echinocandin BPs for *Candida*

• Echinocandins Updates

- No support for an “I” category → Former “I”s need to be revised
- *C. parapsilosis*
 - Anidulafungin: I ≤4 mg/L → S ≤4 mg/L
 - Micafungin: I ≤2 mg/L → S ≤2 mg/L
- *C. albicans* and Micafungin
 - Former S breakpoint was stringent (S ≤0.016 mg/L, modal MIC 0.008 mg/L)
 - → frequent discrepant classifications of wildtype isolates as anidulafungin S and micafungin R
 - Action
 - Literature and MIC mutant database review
 - » No examples of *fks* mutant isolates misclassified as the combination of micafungin MIC 0.03 mg/L and anidulafungin MIC 0.03 mg/L.

Antifungal agent	MIC breakpoint (mg/L)																		Comments on the I category	Comments on the ATU	
	<i>Candida albicans</i>			<i>Candida dubliniensis</i>		<i>Candida glabrata</i>		<i>Candida krusei</i>		<i>Candida parapsilosis</i>		<i>Candida tropicalis</i>		<i>Candida guilliermondii</i>		<i>Cryptococcus neoformans</i>		Non-species related breakpoints for <i>Candida</i> ¹			
	S ≤	R >	ATU	S ≤	R >	S ≤	R >	S ≤	R >	S ≤	R >	S ≤	R >	S ≤	R >	S ≤	R >	S ≤			R >
Anidulafungin	0.03	0.03				0.06	0.06	0.06	0.06	4	4	0.06	0.06	IE ²	IE ²	-	-	IE	IE		
Micafungin	0.016	0.016	0.03			0.03	0.03	IE ⁵	IE ⁵	2	2	IE ⁵	IE ⁵	IE ⁵	IE ⁵	-	-	IE	IE		If S to anidulafungin, report as S and add the following comment: "Isolates susceptible to anidulafungin with micafungin MIC of 0.03 mg/L do not harbour an <i>fks</i> mutation conferring resistance to the echinocandins". If not S to anidulafungin, report as R and refer to reference laboratory for <i>fks</i> sequencing and confirmation of MICs.

Agenda



- Acquired Resistance
- EUCAST BMD method and alternatives
- New I category
- Species without breakpoints
 - Astvad et al; JoF 2022; doi 10.3390/jof8020141

Not an official EUCAST document, but a pragmatic approach.

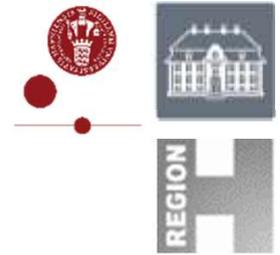


Review

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,*}

Pragmatic approach... Underlying Assumptions



❖ Rare yeasts are less virulent

- Otherwise they would not be rare

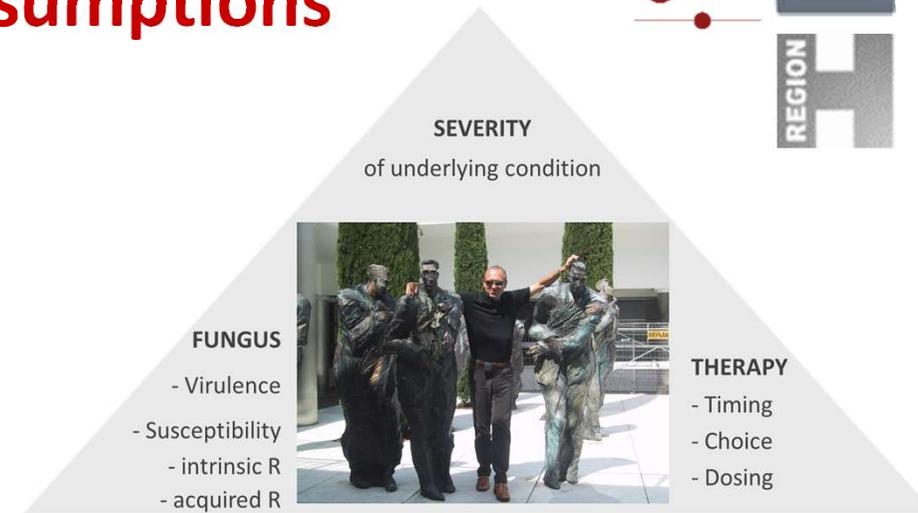
❖ Consequently

- Comparison to a “related” common organism

- will be safe
 - Because, if efficacious against a more virulent organism → it probably also is against a less virulent one with the same susceptibility
- but may be too strict
 - as “less may be enough “ is the organism is truly low virulent
- If apparently non-wild-type
 - Select alternative therapy

❖ Approach

- Reviewed SSI MIC-data 2017-2021
- Compared/verified against published EUCAST data
 - Set single centre wild type upper limits for species ≥ 15 isolates
- Reviewed clinical guideline recommendations
- Traffic light classification according to
 - MIC comparison to related common species
 - guideline recommendations



Amphotericin B EUCAST MICs 2015-21



• Full table layout in the publication

Species	n	Amphotericin MIC (mg/L)								ECOFF/ WT susc. ¹	ECMM/ISHAM/ASM Recommendation (SoR/QoE) [14] ²	Number; MIC ₅₀ /MIC ₉₀ (Range), [Reference] ³
		≤0.016	0.03	0.06	0.125	0.25	0.5	1	2			
<i>C. dubliniensis</i>	235	26	57	102	41	9					0.25/S	n = 146; 0.06/0.25 [34]
<i>C. albicans</i>	1260		2	81	337	707	132	1			1/S	n = 1342; 0.25 /0.5 [34]
<i>C. glabrata</i>	947		6	28	149	433	323	8			1/S	n = 907; 0.25/0.5 [34]
<i>C. tropicalis</i>	147				5	75	64	3			1/S	n = 257; 0.25/0.5 [34]
<i>C. krusei</i>	150				1	3	73	73			1/S	n = 262; 0.5/1 [34]
<i>C. parapsilosis</i>	128				3	35	81	9			1/S	n = 314; 0.5/0.5 [34]
<i>P. kluyveri</i>	2		2									
<i>C. intermedia</i>	1			1								n = 13; 0.25/1 [45], n = 1, (0.03) [46]
<i>P. manshurica</i>	1				1							n = 1; (0.25) [47]
<i>L. elongisporus</i>	2				2							n = 1, 0.03 [49]; n = 2 (0.03–0.12) [46]
<i>C. pararugosa</i>	2				1		1					n = 60; 1/1 [48], n = 6; 1/1 [45]
<i>C. utilis</i>	3				1	2						
<i>C. fermentati</i>	11			1	4	6						n = 29; 0.5/2 [45]
<i>C. pelliculosa</i>	12			3	3	4	2					n = 30; 0.5/1 [45]
<i>R. mucilaginosa</i>	7			1	1	3	2					n = 1; (0.25) [49]; n = 5; (0.5–1) [50]
<i>C. guilliermondii</i>	32			2	14	15		1			[0.5]	n = 88; 0.125/0.25 [34], n = 30, 0.125/0.25 [51], n = 27; 1/1 [45]
<i>C. lusitanae</i>	61			6	24	26	4	1			[0.5]	n = 59; 0.125/0.25 [34], n = 30; 0.06/0.25 [51], n = 14; 0.25/1 [45]
<i>C. orthopsilosis</i>	15				2	6	7					n = 5; 0.06/NA (0.03–0.12) [25]; n = 8; (0.03–0.12) [46]
<i>Cr. neoformans</i> SC	17		1	1	2	9	3	1			[1]	n = 1022; 0.25/0.5 [34], n = 106, 0.125/0.25 [52]
<i>S. cerevisiae</i>	58		1	4	11	26	15	1			[0.5]	n = 81; 0.25/0.5 [34]
<i>C. palmiophila</i>	1					1						n = 3; (0.125–0.5) [45]
<i>C. fabianii</i>	1					1						n = 2, (0.06–0.25) [46]
<i>C. inconspicua</i>	6				1		3	2				n = 168; 0.5/1 [48]; n = 5 (0.25–0.5) [46]
<i>C. kefyi</i>	47					9	32	6			[1]	n = 64; 0.5/1 [34], n = 17; 1/2 [45]
<i>C. nivariensis</i>	4					1	3					n = 4; 0.125/0.25 [45]
<i>K. ohmeri</i>	1						1					n = 1, 0.03 [49], n = 1; (0.125) [50]; n = 4 (0.03–0.12) [46]
<i>C. catenulata</i>	1						1					n = 1; (0.06) [47]
<i>C. norvegensis</i>	10					1	2	2				n = 18; 0.25/1 [45], n = 15; 1/2 [48]
<i>C. metapsilosis</i>	5				4	1						n = 6; 0.09/NA (0.06–0.12) [25]
<i>C. lipolytica</i>	2						1		1			n = 27; 0.5/1 [45]
<i>C. ciferrii</i>	1							1				n = 8; (1–2) [48]; n = 1, 0.25 [46]
<i>A. adeniniivorans</i>	3						2	1				n = 1; (2) [50]
<i>G. candidum</i>	4						2	2				n = 3; (0.25–1) [35]
<i>M. capitatus</i>	11						1	9	1			n = 27; 0.25/0.5 [35], n = 3 (0.125–0.5) [49]
<i>T. asahii</i>	1							1				n = 37; 2/16 [36], n = 29 (0.25–4) [37], n = 2 (2) [49], n = 1; (>8) [50]
<i>T. dermatis</i>	2							1				n = 1; (2) [36], n = 1 (0.13), [37], n = 1; (1) [50]
<i>T. inkin</i>	1											n = 3; (0.25–1) [37], n = 2; (>8) [50]

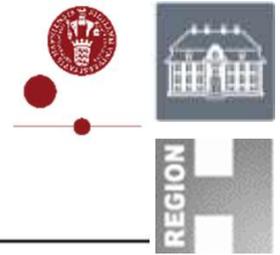
Amphotericin B EUCAST MICs 2015-21



Species	n	Amphotericin MIC (mg/L)									ECOFF/ WT susc. ¹	ECMM/ISHAM/ASM Recommendation (SoR/QoE) [14] ²
		≤0.016	0.03	0.06	0.125	0.25	0.5	1	2	4		
<i>C. dubliniensis</i>	235	26	57	102	41	9					0.25/S	
<i>C. albicans</i>	1260		2	81	337	707	132	1			1/S	
<i>C. glabrata</i>	947		6	28	149	433	323	8			1/S	
<i>C. tropicalis</i>	147				5	75	64	3			1/S	
<i>C. krusei</i>	150				1	3	73	73			1/S	
<i>C. parapsilosis</i>	128				3	35	81	9			1/S	
<i>P. kluyveri</i>	2		2									
<i>C. intermedia</i>	1			1								
<i>P. manshurica</i>	1				1							
<i>L. elongisporus</i>	2				2							
<i>C. pararugosa</i>	2				1		1					
<i>C. utilis</i>	3				1	2						
<i>C. fermentati</i>	11			1	4	6						
<i>C. pelliculosa</i>	12			3	3	4	2					
<i>R. mucilaginosa</i>	7			1	1	3	2					
<i>C. guilliermondii</i>	32			2	14	15		1			[0.5]	1st line (+/- 5FC) (BIIu/BIII)
<i>C. lusitaniae</i>	61			6	24	26	4	1			[0.5]	
<i>C. orthopsilosis</i>	15				2	6	7					
<i>Cr. neoformans</i> SC	17		1	1	2	9	3	1			[1]	1st line (+/- 5FC) (IDSA)
<i>S. cerevisiae</i>	58		1	4	11	26	15	1			[0.5]	1st line (BIII)
<i>C. palmiophila</i>	1					1						
<i>C. fabianii</i>	1					1						
<i>C. inconspicua</i>	6				1		3	2				
<i>C. kefir</i>	47					9	32	6			[1]	
<i>C. nivariensis</i>	4					1	3					
<i>K. ohmeri</i>	1						1					1st line (BIII)
<i>C. catenulata</i>	1						1					
<i>C. norvegensis</i>	10					1	7	2				
<i>C. metapsilosis</i>	5					4	1					
<i>C. lipolytica</i>	2						1		1			
<i>C. ciferrii</i>	1							1				
<i>A. adeninivorans</i>	3						2	1				
<i>G. candidum</i>	4						2	2				1st line (+/- 5FC) (BIII)
<i>M. capitatus</i>	11						1	9	1			1st line (+/- 5FC) (BIIu)
<i>T. asahii</i>	1							1				2nd line (CIIu)
<i>T. dermatis</i>	2							1		1		2nd line (CIIu)
<i>T. inkin</i>	1									1		2nd line (CIIu)

*Resistance mutations arise spontaneously in *C. lusitaniae*

Anidulafungin EUCAST MICs 2015-21



Species	n	Anidulafungin MIC (mg/L)									EUCAST ECOFF/WT susc. ¹	ECMM/ISHAM/ASM recommendation (SoR/QoE) [14] ²
		≤0.008	0.016	0.03	0.06	0.125	0.25	0.5	1	>1		
<i>C. albicans</i>	1928	1626	255	44	2			1			0.03/S	
<i>C. tropicalis</i>	200	41	89	56	13	1					0.06/S	
<i>C. glabrata</i>	1351	52	352	591	327	11	7	3	5	3	0.06/S	
<i>C. krusei</i>	204	4	27	102	63	6		1		1	0.06/S	
<i>C. parapsilosis</i>	164						5	43	77	39	4/S	
<i>C. dubliniensis</i>	276	107	130	36	3 ⁴	1 ⁵	2 ⁵					
<i>P. kluyveri</i>	2	2										
<i>C. inconspicua</i>	10	4	4	2								
<i>C. norvegensis</i>	10	1	6	3								
<i>C. pelliculosa</i>	14	4	10									
<i>C. utilis</i>	4	3			1							
<i>C. nivariensis</i>	6		3	1	2							
<i>L. elongisporus</i>	2		2									
<i>C. intermedia</i>	3		1	2								
<i>C. ciferrii</i>	1			1								
<i>C. fabianii</i>	1				1							
<i>C. palmiophila</i>	10		2	6	1	1						
<i>C. kefyri</i>	56	1	13	31	8	2 ⁴						1 ⁵
<i>C. lusitanae</i>	76		3	31	29	11	2					
<i>S. cerevisiae</i>	63		1	6	25	25	5	1				
<i>C. metapsilosis</i>	6				1	3	1	1				
<i>C. magnoliae</i>	2					1	1					
<i>C. pararugosa</i>	2					1						1
<i>A. adeninivorans</i>	3		1			1		1				
<i>C. orthopsilosis</i>	16					1	10	2	3			
<i>C. lipolytica</i>	2						2					
<i>C. fermentati</i>	24						4	10	7	3		
<i>K. ohmeri</i>	1								1			
<i>C. guilliermondii</i>	58					2	4	15	23	14		
<i>Cr. neoformans</i> SC	21									21		
<i>M. capitatus</i>	11								1	10		
<i>M. clavatus</i>	2									2		
<i>G. candidum</i>	4								1	3		
<i>T. asahii</i>	2									2		
<i>T. dermatis</i>	2									2		
<i>T. inkin</i>	1									1		
<i>R. mucilaginosa</i>	8									8		

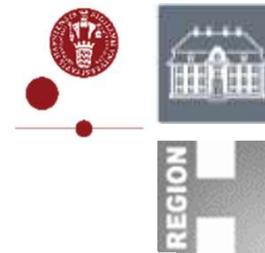
Alt: alternative therapy

1st line Alt (BIII) ⁶

1st line Alt (BIIu/BIII) ⁶

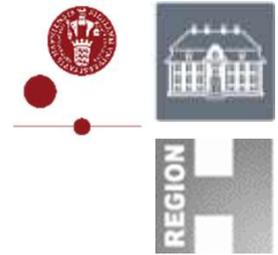
- Against (IDSA)
- Against (DIIu-DIII)
- Against (DIIu-DIII)
- Against
- Against
- Against
- Against

Fluconazole EUCAST MICs SSI 2015-2021



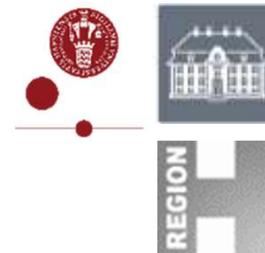
Species	n	Fluconazole MICs (mg/L)									EUCAST ECOFF/ WT susc. ¹	ECMM/ISHAM/ASM Recommendation (SoR/QoE) [14] ²
		≤0.125	0.25	0.5	1	2	4	8	16	≥32		
<i>C. albicans</i>	1972	927	895	120	14	4	4	1		7	0.5/S	
<i>C. dubliniensis</i>	280	116	85	49	15	2	1	1	3	8	[0.5]/S	
<i>C. tropicalis</i>	203	15	57	71	44	2	5	2	3	4	1/S	
<i>C. parapsilosis</i>	171		9	87	54	12	3	1	1	4	2/S	
<i>C. glabrata</i>	1385		1	3	38	377	671	117	27	151	16/I	
<i>C. krusei</i>	206							5	39	162	128/R	
<i>L. elongisporus</i>	2		2									
<i>C. kefyi</i>	57	3	20	23	8	2		1			[1]	
<i>C. lusitanae</i>	77	5	24	36	5	1			2	4		
<i>C. intermedia</i>	3			1	1	1						
<i>C. fabianii</i>	1			1								
<i>T. inkin</i>	1				1							1st line Alt (BIu)
<i>C. metapsilosis</i>	6				3	3						
<i>C. orthopsilosis</i>	16		2	4	3	2			1	4		
<i>C. utilis</i>	4				1	2			1			
<i>C. catenulata</i>	1					1						
<i>T. dermatis</i>	2					1	1					1st line Alt (BIu)
<i>C. fermentati</i>	24				4	11	4		2	3		
<i>C. nivariensis</i>	6					2	4					
<i>C. pararugosa</i>	2						1		1			
<i>C. pelliculosa</i>	14					6	7	1				
<i>Cr. neoformans</i> SC	21				1	3	7	6	4			2nd line (IDSA)
<i>C. guilliermondii</i>	59					6	18	21	2	12	[16]	
<i>S. cerevisiae</i>	64					4	20	24	10	6		1st line Alt (BIu)
<i>K. ohmeri</i>	1							1				1st line Alt (BIu-BIII)
<i>C. bovina</i>	1								1			
<i>T. asahii</i>	2								2			1st line Alt (BIu)
<i>M. capitatus</i>	11						2	3	3	3		(BIu-DIII)
<i>C. palmiophila</i>	10						1	1	3	5		
<i>R. mucilaginosa</i>	8									8		Against (BIu-DIII)
<i>C. norvegensis</i>	11								5	6		
<i>C. inconspicua</i>	10								3	7		
<i>C. lipolytica</i>	2						1			1		
<i>M. clavatus</i>	2								1	1		(BIu-DIII)
<i>C. magnoliae</i>	2									2		
<i>C. ciferrii</i>	1									1		
<i>A. adenivorans</i>	3									3		
<i>G. candidum</i>	4									4		
<i>P. manshurica</i>	1									1		
<i>P. kluyveri</i>	2									2		

Voriconazole EUCAST MICs 2017-21



Species	n	Voriconazole MIC (mg/L)											EUCAST ECOFF/WT susc. ¹	ECMM/ISHAM/ASM Recommendation (SoR/QoE) [14] ²	
		≤0.004	0.008	0.016	0.03	0.06	0.125	0.25	0.5	1	2	4			>4
<i>C. albicans</i>	865	597	237	15	7	4				1	1		3	0.03/S	
<i>C. dubliniensis</i>	184	40	106	26	3	1	2	2					4	0.03/S	
<i>C. parapsilosis</i>	94	2	30	47	10	3			1		1			0.06/S	
<i>C. tropicalis</i>	95		8	34	40	6	3			2			2	0.125/S	
<i>C. glabrata</i>	637			1	71	340	131	25	7	17	27	15	3	1/IE	
<i>C. krusei</i>	109						21	49	24	11	3		1	1/IE	
<i>C. kefyr</i>	43	2	25	13	2	1									
<i>C. lusitaniae</i>	49	5	33	8				2	1						
<i>L. elongisporus</i>	2	1	1												
<i>C. intermedia</i>	3		1		2	6									1st line (BIIIu-CIII)
<i>T. inkin</i>	1				1										
<i>C. metapsilosis</i>	5			2	3										
<i>C. catenulata</i>	1			1											
<i>C. orthopsilosis</i>	12		2	3	1	2		1		2	1				
<i>C. nivariensis</i>	4				2	2									
<i>K. ohmeri</i>	1					1									2nd line (BIII)
<i>C. lipolytica</i>	2					1						1			
<i>T. dermatis</i>	2					2									1st line (BIIIu-CIII)
<i>C. fermentati</i>	15				3	6	7	3		1		1			
<i>C. pelliculosa</i>	6					1		4	1						
<i>C. guilliermondii</i>	50				5	6	16	15	2	4	2	2	2	1	
<i>S. cerevisiae</i>	48						9	28	8	2	1				(BIII)
<i>Cr. neoformans SC</i>	10			2	1	3	3	1		2				0.5	
<i>C. palmiophila</i>	9							4	2	1	2				
<i>C. utilis</i>	3					1		2							
<i>C. bovina</i>	1								1						
<i>T. asahii</i>	1								1						1st line (BIIIu-CIII)
<i>C. inconspicua</i>	4						2	1	1						
<i>G. candidum</i>	3							1		2					1st line Alt (BIII)
<i>C. norvegensis</i>	9						1	4	3	1					
<i>M. capitatus</i>	10					2	2		2	3	1				1st line (BIIIu)
<i>C. ciferrii</i>	1								1						
<i>C. pararugosa</i>	1								1						
<i>P. manshurica</i>	1								1						
<i>P. kluyveri</i>	2								1		1				
<i>A. adenivorans</i>	2									1					
<i>R. mucilaginosa</i>	5							1		2	1	1	1		Against

Pragmatic recommendation for Species without BPs



Recommendation Regarding Treatment	Amphotericin B	Anidulafungin	Fluconazole	Voriconazole
Treat if wild-type	Confirmed MIC \leq 1: <i>Candida</i> species Rare yeasts (except those below)	Confirmed MIC \leq 0.06: →regard susceptible <i>C. dubliniensis</i> <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> Repeat MIC \leq 0.125 mg/L →regard susceptible (consider FKS sequencing if MIC > 0.06 mg/L) <i>C. intermedia</i> <i>C. lusitaniae</i> <i>C. palmiophila</i> <i>C. kefyr</i>	Confirmed MIC \leq 2: →regard susceptible <i>C. intermedia</i> <i>C. kefyr</i> [1] <i>C. lusitaniae</i> <i>C. metapsilosis</i> <i>C. orthopsilosis</i> <i>C. utilis</i> <i>L. elongisporus</i>	Confirmed MIC \leq 0.03: →regard susceptible <i>C. intermedia</i> <i>C. kefyr</i> <i>C. lusitaniae</i> <i>C. metapsilosis</i> <i>C. orthopsilosis</i> <i>L. elongisporus</i>
Consider use if wild-type and: Not severe/ Elevated dose/ Oral consolidation/ No better options		Confirmed MIC 0.125–0.5: →consider use in some situations (for ex. less severe infections, when no better option is available) <i>C. lipolytica</i> <i>C. magnoliae</i> <i>C. metapsilosis</i> <i>C. orthopsilosis</i> <i>C. pararugosa</i> <i>S. cerevisiae</i> <i>A. adenivorans</i>	Confirmed MIC 2–16: →consider use in some situations (increased dosage and less severe infections) <i>C. fermentati</i> <i>C. nivariensis</i> <i>C. pararugosa</i> <i>C. pelliculosa</i> <i>C. guilliermondii</i> [16] <i>C. bovina</i> <i>T. dermatis</i> (1st line Alt) <i>Cr. Neoformans</i> (2nd line) <i>S. cerevisiae</i> <i>T. asahii</i> (1st line Alt)	Confirmed MIC 0.06–0.125: →consider use in some situations (TDM confirmed sufficient exposure, less severe infections or when no better option is available) <i>C. fermentati</i> <i>C. guilliermondii</i> <i>C. lipolytica</i> <i>C. nivariensis</i> <i>C. palmiophila</i> <i>C. pelliculosa</i> <i>C. utilis</i> <i>S. cerevisiae</i> <i>Cr. neoformans</i> [0.5] <i>T. dermatis</i> (1st line)
Consider alternative therapy	Confirmed MIC >1: Any isolate →regard resistant <i>C. lusitaniae</i> [0.5] <i>Trichosporon</i> spp. (2nd line) <i>C. lusitaniae</i> [0.5] <i>Trichosporon</i> spp. (2nd line)	Repeat MIC 0.5–1 No evidence that allows recommendation <i>C. fermentati</i> <i>C. guilliermondii</i> Repeat MIC \geq 1: →regard resistant <i>Cryptococcus</i> <i>Trichosporon</i> , <i>Magnusiomyces</i> , <i>Geotrichum</i> and <i>Rhodotorula</i> (Against due to intrinsic resistance)	Confirmed MIC > 16 →regard resistant <i>C. inconspicua</i> <i>C. lipolytica</i> <i>C. magnoliae</i> <i>C. norvegensis</i> <i>C. palmiophila</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>	Confirmed MIC 0.25–1: No evidence that allows recommendations <i>C. inconspicua</i> <i>C. norvegensis</i> <i>P. kluyveri</i> <i>M. capitatus</i> (1st line Alt) <i>G. candidum</i> (1st line Alt) Confirmed MIC testing \geq 2 →Regard as resistant <i>A. adenivorans</i> <i>R. mucilaginosa</i> (Against)

Treat if wild-type

- Consider use if wildtype &
- Not severe infect.
 - Elevated dose
 - Oral consolidation
 - No better option

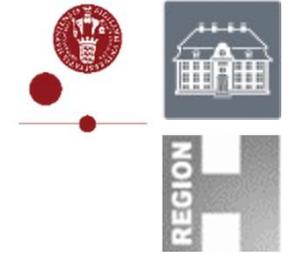
Consider alternative therapy

Species without breakpoints - Concluding remarks



- ❖ Much is yet to be learned concerning susceptibility and treatment of rare yeast
 - Patient population vary
 - no systematic clinical outcome studies
- ❖ MIC interpretation guidance suggested here
 - Is restricted to EUCAST testing
 - Can only be adopted for other AFST tests if agreement between test results are confirmed
 - Is based upon pragmatism and few isolates
 - Is not supported by clinical data
- ❖ Not all rare yeasts are covered in this presentation
 - But the approach used here may be applied on other species
- ❖ Use common sense
 - Change therapy (agent or use combination therapy) in case of poor response

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