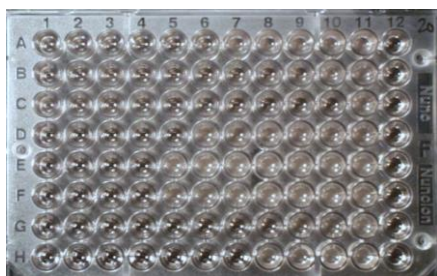


Multicentre determination of CD101 (rezafungin) susceptibility of *Candida* species by the EUCAST method

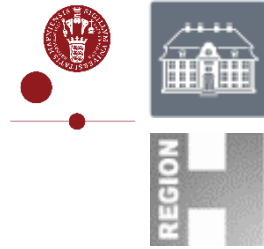
Maiken Cavling Arendrup*, Joseph Meletiadis, Oscar Zaragoza, Karin Meinike Jørgensen, Laura Judith Marcos-Zambrano, Lamprini Kanioura, Manuel Cuenca-Estrella, Johan Mouton, Jesus Guinea Ortega.



*Dept Clin Microbiol, Rigshospitalet, Unit of Mycology, Statens Serum Institut, Dept of Clin Medicine, University of Copenhagen, Copenhagen, Denmark, maca@ssi.dk

*Disclosures: Research grants/contract work (Paid to SSI): Amplyx, Basilea, Cidara, F2G, Gilead, Pfizer & T2Candida. Speaker honoraria: Astellas, Basilea, Gilead, MSD, Novartis, Pfizer & T2Candida, Chair(wo)man for EUCAST-AFST, Past advisory board (\leq 2014): MSD, Pcovery, Pfizer

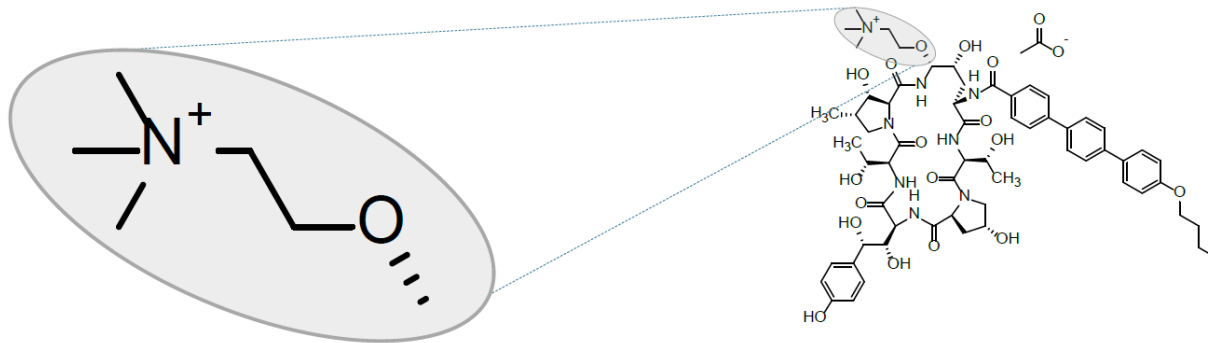
Background



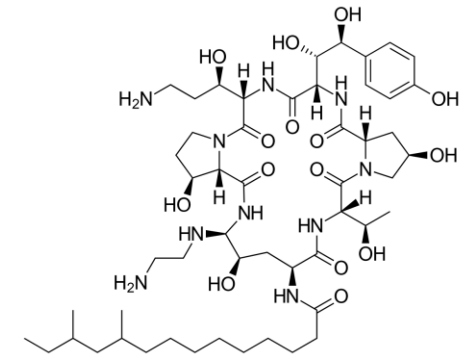
❖ Rezafungin (CD101)

- new echinocandin; long-acting → weekly dosing

Rezafungin



Caspofungin



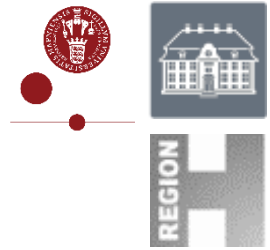
❖ Undergoing phase II clinical trials

- Candidaemia and Invasive Candidiasis.

❖ Limited EUCAST MIC data published

- no multicentre MIC studies yet

Aim

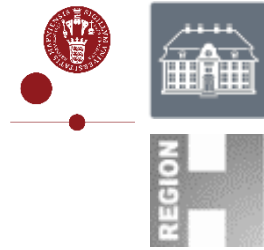


- ❖ To establish multicentre EUCAST MIC data against the 5 most common *Candida* species
 - *C. albicans*
 - *C. glabrata*
 - *C. krusei*
 - *C. parapsilosis*
 - *C. tropicalis*

- ❖ To generate multicentre EUCAST MIC data for 6 QC strains

- ❖ To set Wild-type upper limits (WT-ULs)
 - defined as the MIC value where the wild-type distribution ends

Materials and Methods



- ❖ 4 EU laboratories 2,018 European clinical *Candida* isolates
 - ~100 isolates / species / laboratory

- ❖ 6 QC strains tested; ~10 times in each laboratory
 - *C. parapsilosis* ATCC 22019
 - *C. krusei* ATCC 6258; CNM-CL-3403
 - *C. albicans* ATCC 64548; ATCC 64550; CL F8555

- ❖ EUCAST E.Def 7.3.1 MIC determination¹

- ❖ Wild-type upper limits (WT-ULs)
 - following the principles for EUCAST ECOFF-setting
 - visually
 - statistically using the
 - ECOFFinder programme (with 97.5% and 99% endpoints)²
 - The derivatization method³

Results – MICs for QC strains (non-albicans)



Modal MIC in bold and grey shading

QC strain and Centre	MIC (mg/L)													
	0.002	0.004	0.008	0.016	0.031	0.063	0.125	0.25	0.5	1	2	4	>4	
<i>C. parapsilosis</i> ATCC 22019														
Laboratory 1									4	6	1			
Laboratory 2										1	9			
Laboratory 3								2		9				
Laboratory 4								3		13	1			
Combined								9		29	11			
<i>C. krusei</i> ATCC 6258														
Laboratory 1					3	4	4							
Laboratory 2						10	1							
Laboratory 3					2	9								
Laboratory 4					53 (7)^a	34 (4)								
Combined					58 (12)	57 (27)	5							
<i>C. krusei</i> CNM-CL-3403														
Laboratory 1				2	8	1								
Laboratory 2					5	5								
Laboratory 3					6	5								
Laboratory 4					5	5								
Combined				2	24	16								

^aNumber in () is normalised to ~10 repeatitions/strain/lab

Results – MICs for QC strains (*C. albicans*)



Modal MIC in bold and grey shading

QC strain and Centre	MIC (mg/L)													
	0.002	0.004	0.008	0.016	0.031	0.063	0.125	0.25	0.5	1	2	4	>4	
<i>C. albicans</i> ATCC 64548														
Laboratory 1			5	6										
Laboratory 2				6	4									
Laboratory 3		6	5											
Laboratory 4		9	1											
Combined		15	11	12	4									
<i>C. albicans</i> ATCC 64550														
Laboratory 1			1	6	4									
Laboratory 2				9	1									
Laboratory 3			11											
Laboratory 4			8	2										
Combined			20	17	5									
<i>C. albicans</i> CL F8555^a														
Laboratory 2				6	4									
Laboratory 3			5	6										
Laboratory 4			1	9										
Combined			6	21	4									

^aNot tested in laboratory 1

Results – MICs for Clinical isolates



Modal MIC in bold and grey shading

Species and Centre	MIC (mg/L)												
	0.002	0.004	0.008	0.016	0.031	0.063	0.125	0.25	0.5	1	2	4	>4
<i>C. parapsilosis</i>													
Laboratory 1						1			1	52	35	8	1
Laboratory 2										1	87	12	
Laboratory 3										29	67	4	
Laboratory 4									5	44	51		
All						1			6	126	240	24	1
<i>C. glabrata</i>													
Laboratory 1					24	58	29	1					
Laboratory 2					4	69	27						
Laboratory 3			16		72	11	1						
Laboratory 4			32		69								
All			48		169	138	57	1					
<i>C. krusei</i>													
Laboratory 1					17	40	41	1					
Laboratory 2					29	59	12						
Laboratory 3			7		25	68							
Laboratory 4			4		51	46	2						
All			11		122	213	55	1					

Results – MICs for Clinical isolates



Modal MIC in bold and grey shading

Species and Centre	MIC (mg/L)												
	0.002	0.004	0.008	0.016	0.031	0.063	0.125	0.25	0.5	1	2	4	>4
<i>C. albicans</i>													
Laboratory 1			5	33	41	17	7						
Laboratory 2			1	44	48	7							
Laboratory 3	5	12	77	6									
Laboratory 4	1	47	47	5									
All	6	59	130	88	89	24	7						
<i>C. tropicalis</i>													
Laboratory 1		1	1		17	29	42	11	1				
Laboratory 2					7	51	41	1					
Laboratory 3		1	2	30	55	12							
Laboratory 4			2	41	44	13							
All		2	5	71	123	105	83	12	1				

Modes deviated 2 dilutions from the most common modal MIC

→ Distributions not acceptable for aggregation

Results – Rezafungin WT-ULs for Clinical isolates



Species and Centre	GM	WT-UL visual	WT-UL ECOFFinder 97.5% endpoint	WT-UL ECOFFinder 99% endpoint	WT-UL Derivatization
<i>C. parapsilosis</i>					
Laboratory 1	1.414	4	2	2	4
Laboratory 2	2.158	8	NP ^a	NP	4
Laboratory 3	1.682	4	4	4	4
Laboratory 4	1.376	4	NP	NP	4
All	1.657	4	4	4	4
<i>C. glabrata</i>					
Laboratory 1	0.065	0.125	0.125	0.25	0.125
Laboratory 2	0.071	0.125	0.125	0.125	0.125
Laboratory 3	0.030	0.063	0.063	0.063	0.063
Laboratory 4	0.025	0.063	NP ^a	NP	0.063
All	0.048	0.125	0.125	0.125	0.125
<i>C. krusei</i>					
Laboratory 1	0.075	0.25	0.25	0.25	0.25
Laboratory 2	0.054	0.125	0.125	0.125	0.125
Laboratory 3	0.047	0.125	NP	NP	0.125
Laboratory 4	0.043	0.125	0.125	0.125	0.125
All	0.055	0.125	0.125	0.125	0.125

^aNP: Not Possible due to truncation

Results – MICs for Clinical isolates



Modal MIC in bold and grey shading

Species and Centre	MIC (mg/L)												
	0.002	0.004	0.008	0.016	0.031	0.063	0.125	0.25	0.5	1	2	4	>4
<i>C. glabrata</i>													
Laboratory 1					24	58	29	1					
Laboratory 2					4	69	27						
Laboratory 3					72	11	1						
Laboratory 4					1	1	1						
All					9	138	57	1					
<i>C. krusei</i>													
Laboratory 1						40	41	1					
Laboratory 2						59	12						
Laboratory 3						68							
Laboratory 4						46	2						
All					2	213	55	1					
<i>C. parapsilosis</i>													
Laboratory 1						1			1	52	35	8	1
Laboratory 2										1	87	12	
Laboratory 3										29	67	4	
Laboratory 4									5	44	51		
All						1			6	126	240	24	1

Adopting these WT-ULs
 1/413 *C. glabrata* (0.24%)
 1/402 *C. krusei* (0.25%)
 1/398 *C. parapsilosis* (0.25%)
 were categorised as non-WT
 all of which derived from
 laboratory 1

In conclusion

- Rezafungin displayed species-specific activity similar to other echinocandins.
 - *C. albicans* < *C. tropicalis* < *C. glabrata* & *C. krusei* << *C. parapsilosis*

- WT-ULs (awaiting formal EUCAST ECOFFs)

Species	WT-UL
<i>C. parapsilosis</i>	4
<i>C. glabrata</i>	0.125
<i>C. krusei</i>	0.125

- QC MIC ranges (awaiting formal EUCAST recommendations)

QC strain	Target	Range
<i>C. parapsilosis</i> ATCC 22019	1	0.5-2
<i>C. krusei</i> ATCC 6258	0.06	0.03-0.125
<i>C. krusei</i> CNM-CL-3403	0.03-0.06	0.016-0.125
<i>C. albicans</i> CL F8555	0.016	0.008-0.03

- Interlaboratory variation

- *C. albicans* clinical and QC strains, which warrants further investigation.

Acknowledgements:

Co-authors

Joseph Meletiadis, Oscar Zaragoza, Karin Meinike Jørgensen, Laura Judith Marcos-Zambrano, Lamprini Kanioura, Manuel Cuenca-Estrella, Johan Mouton, Jesus Guinea Ortega.

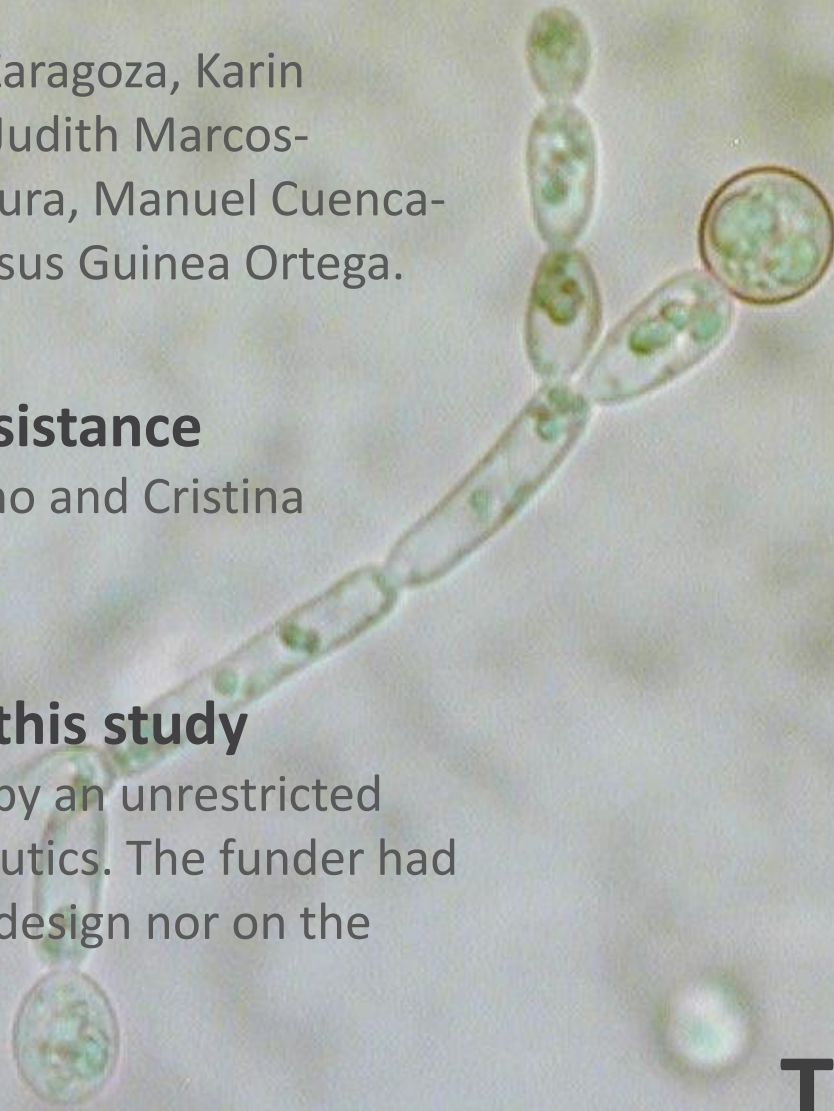
Excellent technical assistance

Birgit Brandt, Teresa Merino and Cristina Armentia.

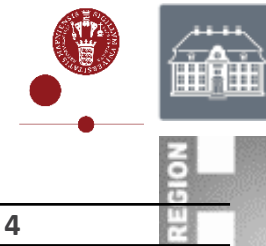
Financial support for this study

This study was supported by an unrestricted grant from Cidara Therapeutics. The funder had no influence on the study design nor on the analysis of the results.

**Thank you for
your attention**



Potential sources of variation



	Laboratory 1	Laboratory 2	Laboratory 3	Laboratory 4
Microtitre plate Brand	Corning*	NUNC	Grenier	Falcon
Microtitre Polystyrene plate type	Tissue treated.	Cell culture treated.	Cell Culture Treated.	Tissue culture-Treated.
Catalogue number	Costar cat no. CLS3596*	167008	655180	Falcon cat no. 353072*
Plate prep method	The ISO method for hydrophobic compounds as in Table 4 of E.Def 7.3.1	The ISO method for hydrophobic compounds as in Table 4 of E.Def 7.3.1	ISO method for hydrophobic compounds as in Table 4 of E.Def 7.3.1	ISO method for hydrophobic compounds as in Table 4 of E.Def 7.3.1
Plastics used during preparation				
DMSO dilutions	15 ml Falcon (Fisher 352095), tips (Mettler Toledo)	SafeSeal micro tube, 2 mL Sarstedt ref. 72.695.500	12 ml PS Tubes Greiner Bio-one Thermo Scientific Art Tips	2 mL glass tubes with screw cap. Ref.VB462001, Agrado
RPMI dilutions	Glass bottles (VWR215-1592), 3-5 ml plastic pipettes (Greiner 606180), Pasteurs pipette (Greiner 740365) 12-channell reservoir (Z370843 SIGMA)	Nunc EZFlip centrifuge tubes, 50 mL, Nunc cat.no. 362696. 12-channel reservoir (approx. 20 years old unknown origin)	100 ml sterile container Soria GenLab 25 ml serological plastic pipettes SPL 50ml reservoir Costar	50 mL Centrifuge tube Grade Polypropylene Ref 430829 Corning CentriStar 50 mL reservoir Ref 87097802, Costar 3 mL plastic Pasteur Pipettes, Nirco, ref 200037
CD101 lot number	C15071064-CF16001	C15071064-CF16001.	C15071064-CF16001	WuXi P05787-048-C-P2
Plates used for culturing strains	Sabouraud	CHROMagar	CHROMagar	Sabouraud
Manufacturer of RPMI1640	Sigma-Aldrich	SSI Diagnostica, 60984	Sigma-Aldrich, R6504-10X1L	Sigma-Aldrich
MOPS	Sigma-Aldrich M3183-1KG	(Included in the ready to use medium above)	Sigma-Aldrich M3183-500G	Sigma-Aldrich M1254-1Kg
Wavelength for the spectrophotometric reading	530 nm	490 nm	540 nm	530 nm
Shipment conditions for CD101 pure substance	Room temperature	Room temperature	Room temperature	Room temperature