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EUCAST reference testing of rezafungin susceptibility: impact of choice of plastic plates

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Background

Rezafungin is a new long-acting echinocandin currently undergoing Phase 3 clinical trials. Epidemiological cut-off values are necessary for clinical breakpoint setting but have not been established, in part due to an unexplained interlaboratory variation observed particularly for *C. albicans*. Here we investigated if the choice of microtitre susceptibility testing (AFST) trays contributed to interlaboratory variability of rezafungin. Anidulafungin was included as comparator.

Materials/methods

Laboratory methods:

1. EUCAST E.Def 7.3.1 AFST: rezafungin & anidulafungin
2. 4 tissue/cell-culture treated (TC-plates) and 4 untreated polystyrene plates (UT-plates) (Cat. no. Sigma-Aldrich: 243656, 167008, 655161, 655180, 3370, CLS3596 and Cultek: 351172, 353072).
3. QC: *C. albicans* CNM-CL-F8555, ATCC 64548 & ATCC 64550; *C. krusei* CNM-CL-3403 & ATCC 6258; *C. parapsilosis* ATCC 22019) (→520 MICs).
4. Clinical isolates: 5-6 wild-type and 4-5 FKS mutant of *C. albicans, C. glabrata, C. krusei,* and *C. tropicalis* and 5 wild-type *C. parapsilosis* (→580 MICs).

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Results

QC strain testing

Repetitive MICs for QC strains fell within 2/3 dilutions for rezafungin in 82%/100% of the cases and for anidulafungin in 90%/98% of the cases. The modal MIC for rezafungin and collated *C. albicans* control strain distributions were 0.016 mg/L across TC-plates but 0.03 mg/L across UT-plates. The modal anidulafungin MICs were 0.004 mg/L and 0.016 mg/L for TC-plates versus UT-plates. The difference was most pronounced with Falcon plates (TC-plates: rezafungin MICs 0.008-0.016 mg/L versus UT-plates: 0.016-0.125 mg/L) but not observed for *C. krusei* and *C. parapsilosis* (data for *C. parapsilosis* is not shown).

Table. Rezafungin and anidulafungin MIC results for repetitive testing of five QC strains. Most common MIC is highlighted in bold and underlined font.

QC strain and Plate type	Rezafungin MICs (mg/L)						Anidulafungin MICs (mg/L)						Total
	0.008	0.016	0.03	0.06	0.125	0.25	0.004	0.008	0.016	0.03	0.06	0.125	
***C. albicans* QC strains combined**													
TC plates (all)	5	**22**	18	3			**25**	19	4				48
UT plates (all)		12	**29**	12	4		1	20	**26**	10			57
Falcon TC (specifically)	**4**	2					2	**4**					6
Falcon UT (specifically)		1	**2**	**1**	2			3	3				6
***C. krusei* QC strains combined**													
TC plates (all)			6	**42**	16	1		1	6	**43**	15		65
UT plates (all)		4	24	**28**	4					**29**	24	7	60
Falcon TC (specifically)			1	**14**					3	**12**	1		15
Falcon UT (specifically)			**12**	3						**12**	3		15

Wild-type and *fks* mutant clinical *Candida* isolate testing

For rezafungin, MICs for 11 mutants overlapped with the MIC range for wild-type isolates (TC-plates on 4 occasions; UT-plates on 7 occasions). For anidulafungin, overlaps were observed on 5 occasions (all UT-plates). Most overlaps (n=5 for rezafungin; n=3 for anidulafungin) were caused by a *C. tropicalis* harbouring a F650F/L alteration and a C. *glabrata* harbouring a D666Y alteration (n=2 for rezafungin; n=1 for anidulafungin). On 12 occasions the MICs of mutant isolates were at the highest MIC of the wild-type range.

Table. Rezafungin and anidulafungin MIC ranges (mg/L) for *Candida fks* mutant isolates and the number of overlaps (specific AA alteration) between MICs for *Candida fks* mutant and wild-type isolates are shown.

AF & plate	Lab #	*C. albicans* MIC range	*C. albicans* n. overlaps	*C. glabrata* MIC range	*C. glabrata* n. overlaps	*C. krusei* MIC range	*C. krusei* n. overlaps	*C. tropicalis* MIC range	*C. tropicalis* n. overlaps
Rezafungin									
NUNC TC	Lab 1	0.125-1	0	0.125-2	1 (D666Y)	0.25-8	0	0.125-1	1 (F650F/L)
Greiner TC	Lab 1	0.06-0.5	0	0.25-2	0	0.25-8	0	0.125-1	1 (F650F/L)
	Lab 2	0.25-1	0	0.5-4	0	0.25-2	1 (P663Q)	0.25-1	0
Costar TC	Lab 2	0.25-1	0	0.25-4	0	0.25-2	0	0.25-1	0
Falcon TC	Lab 2	0.06-1	0	0.25-4	0	0.25-2	0	0.25-1	0
NUNC UT	Lab 1	0.125-1	0	0.125-2	0	0.25-8	0	0.125-1	1 (F650F/L)
Greiner UT	Lab 1	0.125-1	0	0.125-2	0	0.25-8	0	0.125-1	1 (F650F/L)
Costar UT	Lab 1	0.06-1	1 (R647G)	0.125-4	1 (D666Y)	0.25-8	0	0.06-1	1 (F650F/L)
	Lab 2	0.125-1	1 (R1361H)	0.25-4	0	0.25-2	0	0.5-1	0
Falcon UT	Lab 2	0.125-1	1 (R1361H)	0.25-4	0	0.125-2	0	0.5-1	0
Anidulafungin									
NUNC TC	Lab 1	0.016-0.5	0	0.06-2	0	0.125-4	0	0.03-0.5	0
Greiner TC	Lab 1	0.016-0.5	0	0.125-2	0	0.25-8	0	0.06-0.5	0
	Lab 2	0.03-1	0	0.125-2	0	0.25-4	0	0.125-0.5	0
Costar TC	lab 2	0.03-0.25	0	0.125-2	0	0.125-2	0	0.125-0.25	0
Falcon TC	Lab 2	0.03-2	0	0.25-2	0	0.25-4	0	0.125-0.5	0
NUNC UT	Lab 1	0.03-1	0	0.125-2	0	0.25-8	0	0.06-0.5	1 (F650F/L)
Greiner UT	Lab 1	0.06-1	0	0.125-2	0	0.25-8	0	0.06-1	1 (F650F/L)
Costar UT	Lab 1	0.06-2	0	0.125-4	2 (F659S; D666Y)	0.25-8	0	0.125-1	1 (F650F/L)
	Lab 2	0.125-2	0	0.25-4	0	0.25-4	0	0.125-0.5	0
Falcon UT	Lab 2	0.125-2	0	0.25-4	0	0.25-4	0	0.25-0.5	0

Conclusions

- Intra-laboratory variation was low for both compounds and all plates.
- Treated plates resulted in lower MICs, most profoundly for *C. albicans*, for Falcon plates, and more for anidulafungin than rezafungin.
- Standardisation of plate choice for EUCAST AFST would help minimise inter-laboratory variation.