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Development and validation of a modified EUCAST yeast broth microdilution MIC method for rezafungin to mitigate nonspecific binding through incorporation of Tween 20

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INTRODUCTION

- The EUCAST broth microdilution (BMD) methodology generates potent echinocandin MIC values for *Candida* species, which can be problematic at lower drug concentrations if nonspecific binding occurs.
- In a prior multicentre EUCAST study, the novel echinocandin rezafungin (RZF) demonstrated nonspecific binding sufficient to cause significant variability for more susceptible species (e.g. *C. albicans* and *C. tropicalis*) between different MIC plate types.[1]
- Herein we investigated the use of surfactants as a means to mitigate nonspecific binding and generate a viable EUCAST BMD method for rezafungin.

METHODS

- Initial antifungal-surfactant range finding checkerboard assays utilized an abbreviated EUCAST BMD "stamping" method (4 µL surfactant and 4 µL drug at 50X were dispensed into MIC plates containing 196 µL of pre-inoculated RPMI-1640 media).
- All other MICs were performed according to EUCAST E.Def 7.3.2.
- Fluconazole was selected as a control for all experiments due to it being minimally impacted by nonspecific binding or surfactants.
- Depending on the experiment, upwards of 3 surfactants (Tween 20 [T20], 80, and Triton X-100), 4 tissue culture–treated MIC plates (Corning-CLS3596, NUNC-167008, Grenier-655180, and Falcon-353072), and a variety of WT and *fks* mutant strains representing 7 *Candida* species (*C. albicans*, *C. glabrata*, *C. tropicalis*, *C. krusei*, *C. parapsilosis*, *C. auris*, and *C. dubliniensis*) were evaluated.

RESULTS

- RZF-surfactant checkerboard assays conducted on a subset of WT and *fks* mutants demonstrated similar abilities of the 3 surfactants to mitigate nonspecific binding (data not shown).
- T20 was selected for further evaluation due to its existing use in EUCAST mould MIC/MEC testing (E.Def 9.3.2) for preparing conidia suspensions.
- 0.002% T20 was the optimal concentration to mitigate RZF nonspecific binding across strains (data not shown).

RESULTS (con't)

Fig. 1 Impact of T20 on WT *Candida* spp. RZF MICs between higher and lower binding plates

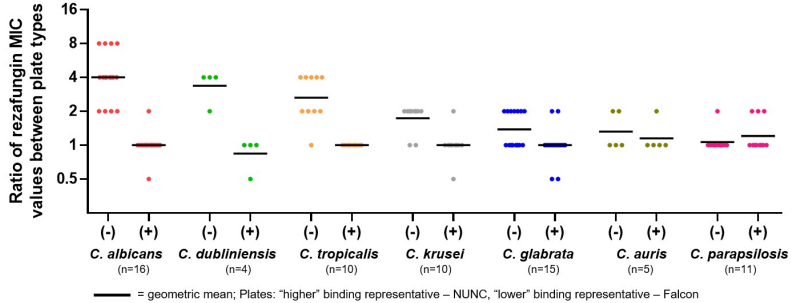
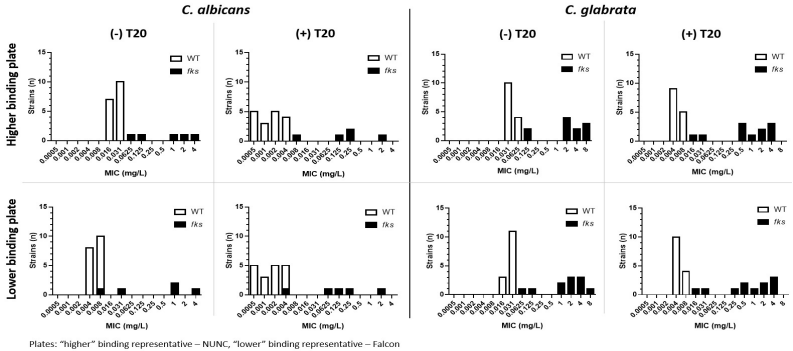


Fig. 2 WT and *fks* *C. albicans* and *C. glabrata* RZF MICs in higher and lower binding plates +/- T20



- Comparison of plates exhibiting higher (NUNC) and lower (Falcon) binding showed that 0.002% T20 successfully normalized MIC values for panels of WT strains of all 7 *Candida* species (Fig. 1) without compromising the ability to distinguish *fks* mutant and WT strains (Fig. 2).

RESULTS (con't)

Table 1. Pooled RZF MIC distributions across 4 plate types for EUCAST QC strains +/- T20

Strain	T20	MIC (mg/L) 0.0005	0.001	0.002	0.004	0.008	0.016	0.031	0.063	0.125	0.3	0.5	1	2	4
C. albicans ATCC 64548	(-)				1	7	**13**	**13**	6						
	(+)		2	**32**	6										
C. albicans ATCC 64550	(-)					2	12	**19**	7						
	(+)			1	**27**	12									
C. albicans CNM-CL-F8555	(-)					1	7	**16**	**16**						
	(+)			1	**28**	11									
C. krusei ATCC 6258	(-)								7	**23**	9	1			
	(+)					7	**33**								
C. krusei CNM-CL-3403	(-)								5	**24**	11				
	(+)						**38**	2							
C. parapsilosis ATCC 22019	(-)											2	**22**	16	
	(+)									**29**	11				

10 independent replicate MICs per strain per plate type; **mode**

- T20 normalization of rezafungin MIC values was further validated in a 10-replicate analysis of the 6 EUCAST *Candida* QC strains across all 4 plate types (Table 1).

CONCLUSIONS

- Incorporation of 0.002% T20 into EUCAST BMD MIC assays for rezafungin diminishes nonspecific binding, normalizes MIC values across plate types, and does not impact differentiation of WT vs. *fks* mutant strains.
- The replicate runs of all EUCAST *Candida* spp. QC strains demonstrated high reproducibility of this method across the same plate types used in the prior multicentre study in which bimodal MIC distributions were observed.[1]
- This promising methodology adaptation is currently undergoing further validation in a multicentre study and, if successful, could potentially benefit other antifungal agents for which interlaboratory variation, nonspecific binding, and/or plastics choice issues have been documented.[2,3,4]

REFERENCES

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