Antifungal Susceptibility Testing of Candida; Challenging the gold standard method

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Introduction

- After aspergillosis, candidiasis is the second most common cause of invasive fungal infection, with recent estimates of 750,000 cases worldwide (1).
- *Candida albicans* is the main species associated with human infection, however there has been a significant increase in infections caused by non-albicans species in the past two decades (2,3).
- Infections caused by Candida have often been treated with azole antifungals, however due to increasing rates of invasive fungal disease and the emergence of antifungal resistance, the requirement for the implementation of an easy-to-perform, in-house antifungal susceptibility testing (AFT) method has never been more apparent (3).
- The aim of this study was to test clinical isolates of Candida by disc diffusion and MIC gradient strip methods and compare susceptibility test interpretation and MIC against the gold standard broth microdilution method.

Methods

- 132 clinical isolates were tested in this study; *C. albicans* (n =80), *C. parapsilosis* (n=8), *C. tropicalis* (n = 4), *Nakaseomyces glabrata* (n=22), *Pichia kudriazveii* (n=2), *Kluyveromyces marxianus* (n=4), *Clavispora lusitaniae* (n=3), *C. dubliniensis* (n=5) *C. metapsilosis* (n=2), *Nakaseomyces nivariensisa* (n=2), isolated from a range of clinical samples: swabs, blood cultures, bronchial lavages, sterile tissues and fluids.
- Clinical isolates were tested by the gold standard broth microdilution method for AFT at UKHSA Mycology Reference Laboratory.
- In-house testing of fluconazole, voriconazole and caspofungin discs and MIC gradient strips (Liofilchem) was performed by inoculating 0.5 McFarland suspensions onto Mueller-Hinton agar with 2% glucose and methylene blue as per CLSI guideline M44 (4).
- Susceptibility agar plates were incubated aerobically at 35-37°C and zones of inhibition were read after 24-48 hours in accordance with CLSI guideline M60 (5).




Results

- Results of this study showed a 100% concordance in antifungal susceptibility interpretation between disc diffusion and the broth microdilution reference method.
- There was ~98% concordance in MIC values between gradient strips and broth microdilution methods, however a general trend of slightly higher MIC values was observed with the gradient strip method, and 2 major interpretation errors were noted.
- *C. albicans* was found at times difficult to interpret due to the presence of micro-colonies and some isolates required 48 hours incubation to adequately interpret the susceptibility result.
- Adherence to Liofilchem species-specific interpretation guidance, preparing appropriate McFarland suspensions and careful inoculation and incubation of agar plates is crucial to reduce erroneous interpretation of results (6).

Antifungals	Broth Microdilution %			Disc Diffusion %			Concordance %
	S	DD	R	S	DD	R	
Fluconazole	80	16	4	80	16	4	100
Voriconazole	88	6	6	88	6	6	100
Caspofungin	100	0	0	100	0	0	100

Table 1. Concordance of susceptibility interpretation between disc diffusion and broth microdilution

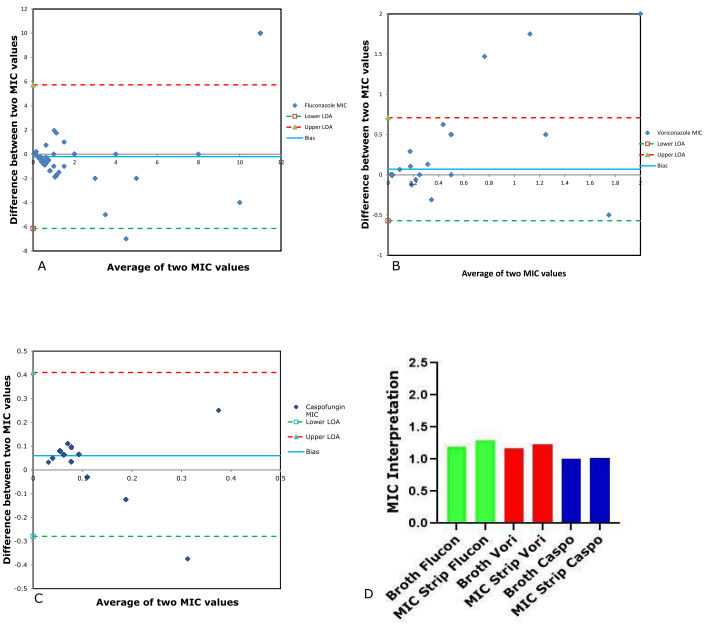

Figure 1. Bland Altman plots showing estimated agreement of MIC values for fluconazole (A), voriconazole (B) and caspofungin (C), for MIC gradient strip and broth microdilution methods.
Column bar graph for MIC interpretation comparison (D).

Conclusion

- This study has shown that in-house AFT susceptibility interpretation is concordant with the gold standard reference method.
- Implementation of in-house AFT testing is quick and easy to perform and can be used as an alternative to broth microdilution for first line antifungal agents.
- Implementation of in-house AFT reduces turnaround times from 7 days to 24 hours, improving patient management and reducing morbidity and mortality.
- Implementation of in-house AFT testing reduces costs on referring isolates to reference laboratories.
- CLSI zone diameter breakpoints are currently only available for fluconazole, voriconazole, caspofungin and micafungin.
- Susceptibility testing for other 2nd gen. azoles, anidulafungin and polyenes still require broth microdilution testing performed at the UKHSA Mycology reference laboratory.

References

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