Antifungal susceptibility testing for *Candida* spp. Agreement between EUCAST reference method and commercial tests

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Introduction

Candida spp. are the most common cause of invasive fungal infection. Patients at risk of candidemia are those who are immunocompromised and those treated with antibiotics or invasive catheters. The last annual epidemiological report of the European Centre for Disease Prevention and Control (ECDC) stated that *Candida* spp. are the fifth most frequently isolated microorganism in intensive care unit (ICU)-acquired bloodstream infections in the European Union. Mortality rates associated with *Candida* bloodstream infections vary from 45% to 53% depending on the population investigated.

Candida isolates must be tested for susceptibility so that the most appropriate antifungal drug can be selected.
The microdilution method for the determination of antifungal MICs for fermentative species of yeast was developed by the Antifungal Susceptibility Testing Subcommittee of the European Committee on Antibiotic Susceptibility Testing (AFST-EUCAST). However, most clinical microbiology laboratories use commercial methods to perform antifungal susceptibility testing (AST).

Purpose

The aim of this study was to compare the concordance of MIC results of the AFST-EUCAST reference method to two commercial tests (Vitek 2, MICRONAUT-AM) for five common *Candida* spp.

Methods

- 23 isolates of 5 different *Candida* spp. (*C. albicans*, *C. glabrata*, *C. krusei*, *C. parapsilosis* and *C. tropicalis*) collected from patients with invasive candidiasis were evaluated.
- We prepared the microdilution plates following the EUCAST procedure and antifungal are listed in the Figure 1.
- The commercial MICRONAUT-AM (Merlin, Germany) and Vitek AST-YS07 (bioMérieux, France) methods were performed according the manufacturer instructions. The antifungal tested in the MICRONAUT-AM panel are represented in Figure 2.
- A schematic representation of the antifungal concentration in the Vitek AST-YS07 card (bioMérieux, France) is reported in Figure 3.
- MICs were compared and agreement was defined as a discrepancy in MIC of no more than two doubling dilutions and a p value of <0.01 was considered to be statistically significant.
- *C. parapsilosis* ATCC 22019 and *Issatchenkia orientalis* ATCC 6258 were used as quality control strains.

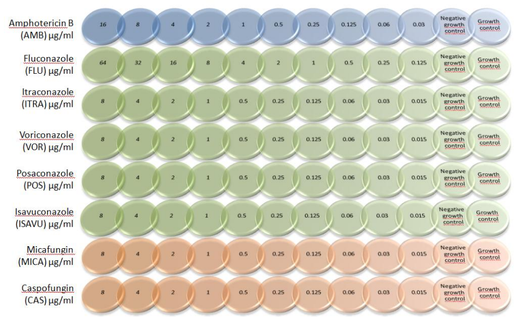

Fig. 1. Antifungal and concentration tested with EUCAST procedure

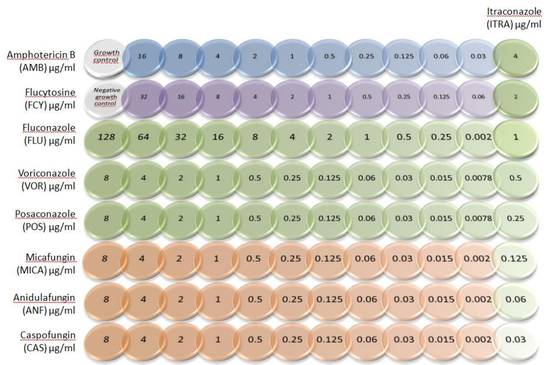

Fig. 2. Antifungal and concentration tested with MICRONAUT-AM

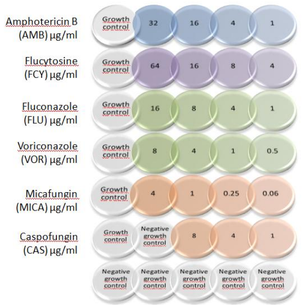

Fig. 3. Schematic representation of antifungal and concentration tested with Vitek AST-YS07

Results

When analysed by *Candida* species, Vitek 2 MICs where in agreement with the AFST-EUCAST reference method for 75.9 to 87.5% of strains tested (p<0.01). Similarly, agreement of the MICRONAUT-AM with the reference AFST-EUCAST method ranged from 75.5 to 89.3% (p<0.01) (Table 1). When analysed by antifungal tested, MICRONAUT-AM MIC results agreed with AFST-EUCAST method for fluconazole, itraconazole, posaconazole and micafungin, while Vitek 2 and AFST-EUCAST agreed for fluconazole, caspofungin and micafungin (p<0.01) (Table 2). The limited number of resistant strains among tested clinical isolates precluded comparisons of major error/minor errors. Agreement between Vitek 2 and AFST-EUCAST for voriconazole MICs is low but is affected by differences in the lower limit of quantitation between the two methods (0.016 vs. 0.12 mg/L) that is greater than the 2 dilution threshold.

Table 1. Agreement values and correlation coefficients between EUCAST and other methods grouped according to *Candida* species

	VITEK2			EUCAST			MICRONAUT-AM		
Species	**Agreement (%)**	**ICC**	**95% CI IC**	**Agreement (%)**	**ICC**	**95% CI IC**	**Agreement (%)**	**ICC**	**95% CI**
C. albicans	75.9	0.72[a]	0.49-0.86	91.7	0.90[a]	0.83-0.94	79.0	0.89[a]	0.81-0.94
C. parapsilosis	75.9	0.72[a]	0.49-0.86	91.7	0.90[a]	0.83-0.94	78.5	0.89[a]	0.80-0.94
C. tropicalis	80.0	0.85[a]	0.66-0.94	100	0.94[a]	0.89-0.97	89.3	0.85[a]	0.70-0.93
C. glabrata	80.0	0.93[a]	0.85-0.97	97.5	0.97[a]	0.94-0.98	80.0	0.91[a]	0.82-0.95
C. krusei	87.5	0.97[a]	0.90-0.99	88.9	0.96[a]	0.91-0.98	83.3	0.93[a]	0.84-0.97

ICC, intraclass correlation coefficient, ND, not done
[a] P<0.01

Table 2 Agreement values and correlation coefficients between EUCAST and other methods grouped according to antifungal

	VITEK2			EUCAST			MICRONAUT-AM		
Species	**Agreement (%)**	**ICC**	**95% CI IC**	**Agreement (%)**	**ICC**	**95% CI IC**	**Agreement (%)**	**ICC**	**95% CI**
Amphotericin B	100	0.40	0.02-0.70	91.3	0.84[a]	0.64-0.93	77.2	0.19	0-0.56
Fluconazole	86.9	0.83[a]	0.63-0.93	91.3	0.92[a]	0.82-0.97	86.9	0.81[a]	0.59-0.92
Itraconazole	ND	ND	ND	90.9	0.73[a]	0.45-0.88	90.9	0.83[a]	0.62-0.93
Voriconazole	22.7*	0.71[a]	0.41-0.87	100	0.93[a]	0.85-0.97	87.0	0.76[a]	0.49-0.89
Posaconazole	ND	ND	ND	100	0.83[a]	0.70-0.90	ND	ND	
Isavuconazole	ND	ND	ND	91.3	0.69[a]	0.38-0.86	ND	ND	
Caspofungin	100	0.92[a]	0.82-0.97	95.4	0.60[a]	0.24-0.82	68.2	0.44	0.03-0.73
Micafungin	90.5	0.88[a]	0.74-0.95	90.9	0.89[a]	0.75-0.95	91.3	0.93[a]	0.82-0.97

ICC, intraclass correlation coefficient, ND, not done
[a] P<0.01

Conclusions

This study suggests that there is a good agreement between the reference AFST-EUCAST method and the tested commercial assay, but a larger evaluation including more resistant strains is required.