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Evaluation of a Protocol for Scalable Preparation of Broth Microdilution Panels for Antifungal Susceptibility Testing Using the EUCAST Reference Method

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Introduction

- Understanding the different susceptibility profiles of *Candida* provides crucial guidance to antifungal therapy.
- Antifungal susceptibility testing (AFST) is essential to monitor antifungal resistance; however, preparing reagents for antifungal broth microdilution (BMD) testing is cumbersome and may contribute to the limited use of reference susceptibility methods by clinical laboratories.
- In this study, we compared an automated method to produce EUCAST antifungal BMD panels to the EUCAST reference method against 5 *Candida* species.
  - This method employs similar dilution schemas for the preparation of reference panels but allows for the production of a large number of panels that can be stored until used.

Materials and Methods

- A total of 108 *Candida* spp. isolates from the SENTRY Antifungal Surveillance Program, including 30 *C. albicans*, 30 *C. glabrata*, 20 *C. parapsilosis,* 17 *C. tropicalis*, and 11 *C. krusei*, were tested.
- All fungal isolates were submitted to matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) using the MALDI Biotyper (Bruker Daltonics, Billerica, Massachusetts, USA) following the manufacturer's instructions.
- EUCAST BMD reference panels and panels produced using the Bravo Liquid Handling Platform (Agilent Technologies) were prepared using the same drug stock solutions and tested in parallel.
- Automated BMD panels were prepared by dispensing 10 µL of a 20X antifungal stock solution into panels containing 90 µL of RPMI broth and mixing the solution by aspirating and dispensing the solution 5 times.
- Both the EUCAST reference and automated BMD panels were produced using RPMI 1640 broth supplemented with 2% glucose and inoculated with 0.5 to 2.5 X 10^5 cells/ml suspensions.
- MIC reading conditions and interpretative criteria were applied as outlined in document EDef 7.3.2 and described by Arendrup *et al*.
- Quality Control (QC) was performed each day of testing, as recommended in the EUCAST guideline (2020), using *C. parapsilosis* ATCC 22019 and *C. krusei* ATCC 6258 strains.
- Essential agreement (+/– 1 $\log_2$ dilution), categorical agreement, and error rates, where breakpoints were available, were assessed between methods according to EUCAST guidelines (v.10.0, 2020).

Results

- Overall, the essential agreement and categorical agreement rates for *Candida* spp. were 97.5% and 99.3%, respectively (Table 1).
- The essential agreement rates to amphotericin B and echinocandins were 100%, except for caspofungin that had a 95.3% essential agreement rate.
  - Only 5 caspofungin MIC results (3 *C. tropicalis*, 1 *C. albicans*, and 1 *C. glabrata*) exhibited >1 dilution difference between methods.
- Fluconazole, posaconazole, voriconazole, and itraconazole displayed essential agreement rates of 99.1%, 98.1%, 95.0%, and 92.5%, respectively.
- No significant trends toward low or high MIC values were observed for any antifungal agent (Table 1).
- All antifungal agents with available, published interpretative criteria showed categorical agreement rates of >90.0% for the *Candida* isolates tested.
  - Anidulafungin, micafungin, amphotericin B, and fluconazole yielded the highest categorical agreement rates (100.0%), followed by posaconazole (98.5%), itraconazole (98.5%), and voriconazole (96.6%).
  - Only 2 major errors (1 for itraconazole and 1 for posaconazole, both against *C. parapsilosis*) and 2 minor errors (2 for voriconazole against *C. tropicalis*) were observed.

Conclusions

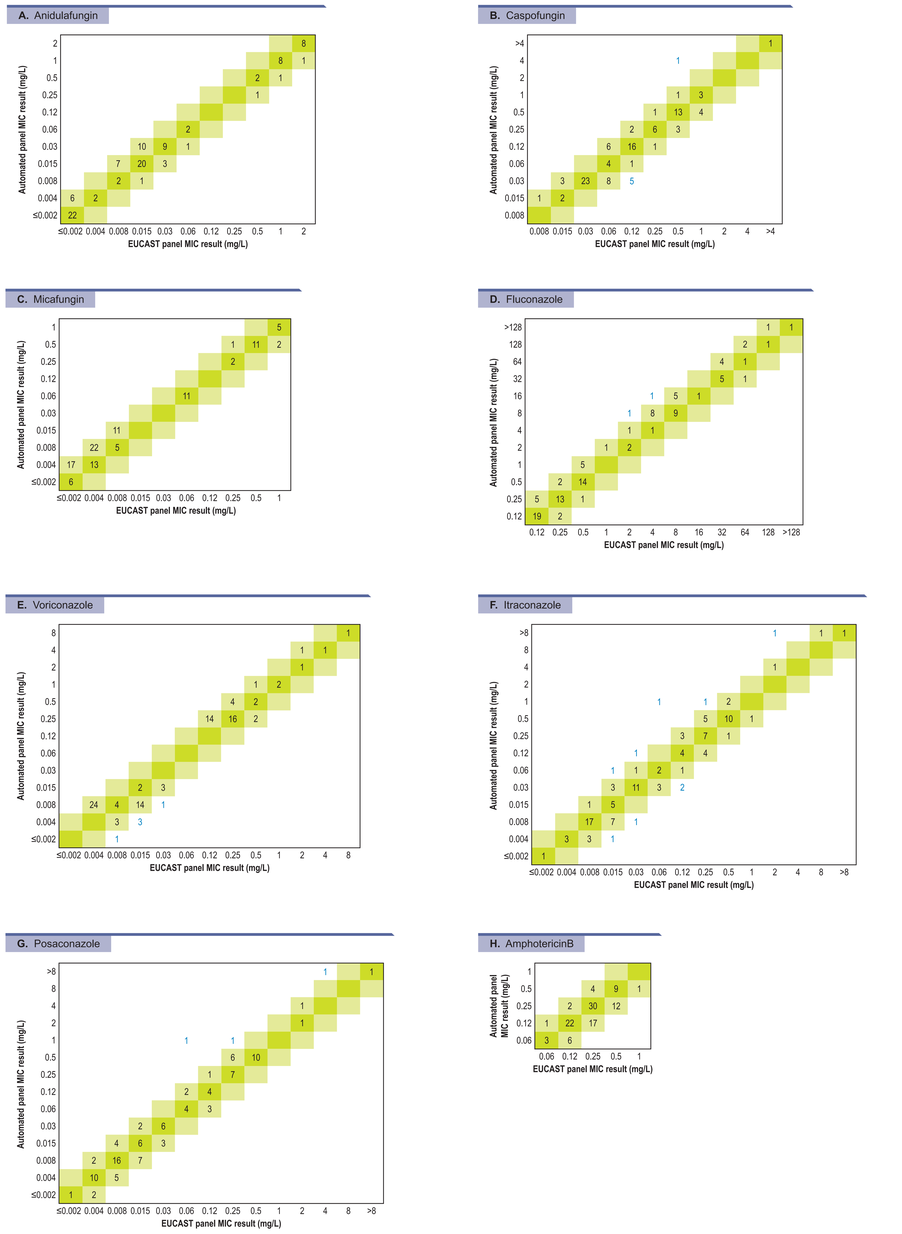
- Compared to the EUCAST reference method, the BMD panels produced using the automated protocol exhibited high overall essential and categorical agreement rates (>90%) when testing *Candida* spp.
- This method allowed for large-scale panel production and generated accurate results for the susceptibility testing of *Candida* species.

Table 1. Essential and categorical agreement rates between the EUCAST reference and automated protocol panels tested against *Candida* spp.

$\log_2$ dilution difference	ANF	CSF	MCF	AMB	FLC	ITR	PSC	VRC	Total
+2	0	0	0	0	1	4	2	0	7
+1	23	14	51	7	34	17	18	44	208
0	75	70	53	64	68	62	67	29	488
-1	8	17	2	36	4	20	20	22	129
-2	0	5	0	0	0	4	0	5	14
EA (±1 dil)	100.0%	95.3%	100.0%	100.0%	99.1%	92.5%	98.1%	95.0%	97.5%
CA	100.0%	NA	100.0%	100.0%	100.0%	98.5%	98.5%	96.6%	99.3%

ANF, anidulafungin; CSF, caspofungin; MCF, micafungin; AMB, amphotericin B; FLC, fluconazole; ITR, itraconazole; PSC, posaconazole; VRC, voriconazole; EA, essential agreement rate; CA, categorical agreement rate; NA, breakpoint not available.

Figure 1. Comparison of BMD panels produced by the automated protocol and EUCAST reference methods when susceptibility testing *Candida* spp.



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