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Research Paper

# Comparative assessment of Sensititre YeastOne and Micronaut-AM EUCAST for antifungal susceptibility testing in candidaemia isolates

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ABSTRACT

*Purpose:* Antifungal susceptibility testing (AFST) is essential to ensure appropriate antifungal therapy in candidaemia. This study compared two commercial colorimetric broth microdilution tests: Sensititre YeastOne (SYO; Thermo Scientific) and Micronaut-AM EUCAST AFST (M-AM; Bruker) for the AFST of *Candida* spp.
*Material and Methods:* A total of 74 yeast strains, including *C. albicans* ($n$ = 40) and non-*albicans Candida* species (NACS) ($n$ = 34), were obtained from blood cultures of patients admitted to a tertiary care hospital in Belgium from 2017 to 2022. AFST by SYO and by M-AM were performed according to the manufacturers' protocols and interpreted using CLSI and EUCAST guidelines, respectively. Essential and categorical agreements (EA and CA), very major, major and minor discrepancies were calculated for amphotericin B, echinocandins and azoles considering SYO as the reference method.
*Results:* In total, 441 and 392 isolate-antifungal results were evaluable for EA and CA, respectively. SYO and M-AM, showed a high level of concordance for *C. albicans* strains, with an EA and CA ≥90 % for all tested antifungals. However, we noted significant discordances for NACS, the lowest EA were observed with micafungin (50 %) and voriconazole (58.8 %). These discrepancies were likely due to differences in the raw MIC values obtained by the two methods and the different interpretation breakpoints used by CLSI and EUCAST.
*Conclusion:* Our study showed excellent agreement between SYO and M-AM for AFST of *C. albicans*, while the equivalency was lower for NACS. AFST method should be carefully selected, considering the results might impact the choice of antifungals for non-*albicans* candidaemia.
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## Introduction

Candidaemia, defined by the presence of *Candida* species in the bloodstream, is a severe and life-threatening fungal infection associated with high morbidity and mortality rates [1]. Several factors contribute to an increased susceptibility in patients to develop candidaemia, including hematological malignancies, prior abdominal surgeries, immunosuppression, exposure to broad-spectrum antibiotics and the presence of indwelling catheters [2].

*C. albicans* has traditionally been the leading pathogen causing candidaemia. However, there has been a noticeable rise of non-*albicans Candida* species (NACS), particularly *C. glabrata* in Europe [3]. Additionally, the emergence of antifungal resistance has become a significant concern in the management of candidaemia. A previous nationwide study in Belgium showed resistance rates to fluconazole of 11.3 % for *C. glabrata*, 5.6 % for *C. parapsilosis*, and 20 % for *C. tropicalis* [4]. These rates were comparable to those observed in the United States (US) for *C. glabrata* but differed for *C. tropicalis*, where lower resistance rates were reported [5]. The emergence of azoles-resistant and echinocandins-resistant strains is concerning, limiting available options for antifungal therapy. Notably, there has been a significant increase in resistance to echinocandins for *C. glabrata* in the US, a phenomenon not yet observed in Europe [5]. This highlights the importance of preserving the effectiveness of echinocandins and considering a step-down approach to fluconazole whenever feasible. Achieving this goal relies on implementing an accurate and reliable antifungal susceptibility testing (AFST) method.

The Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) have each developed a broth microdilution reference method to ensure standardised AFST for *Candida* species. However, these reference methods are labor-intensive and may yield different minimal inhibitory concentration (MIC) values due to inherent methodological differences. As a practical and time-saving alternative, the use of commercial tests offers a convenient approach.

The objective of this study was to compare two commercial colorimetric broth microdilution tests available for AFST: Sensititre

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YeastOne (SYO; Thermo Scientific), and the more recent Micronaut-AM EUCAST AFST (M-AM; Bruker). These commercial tests are standardised against CLSI and EUCAST broth microdilution reference methods, respectively. As an European clinical laboratory, we strongly desire to adopt EUCAST guidelines for AFST. However, MIC values obtained with SYO cannot be interpreted using EUCAST criteria. By testing *Candida* strains isolated from candidaemia cases over the past five years in our tertiary care hospital in Belgium, we aimed to investigate the concordance and discordance between SYO and M-AM. Furthermore, we assessed the impact of changing the AFST method on the susceptibility profiles of antifungal agents.

## Material and methods

### *Yeast strains*

Between 2017 and 2022, CHU UCL Mont-Godinne, a tertiary-care 386-bed hospital in Belgium, recorded 74 cases of candidaemia. All yeast strains from this five-year period comprising *C. albicans* ($n$ = 40) and NACS ($n$ = 34) were retrieved and included in the study. The NACS included *C. parapsilosis* ($n$ = 11), *C. krusei* ($n$ = 8), *C. glabrata* ($n$ = 6), *C. tropicalis* ($n$ = 5), *C. guilliermondii* ($n$ = 2), *C. kefyr* ($n$ = 1) and *C. lusitaniae* ($n$ = 1). Reference strains *C. krusei* ATCC 6258, *C. parapsilosis* ATCC 22019 and *C. albicans* ATCC 24433 were tested for the reproducibility evaluation.

The candidaemia isolates stored at −80 °C were thawed and subcultured twice on Sabouraud agar plates (BD, Germany) to ensure purity and viability. Species identification was carried out using matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS, Bruker, Germany).

### *Sensititre YeastOne (SYO)*

SYO is a colorimetric broth microdilution test for AFST. SYO allows the testing of one isolate per plate and includes 9 antifungal agents with the following concentrations ranges: 5-flucytosine (0.06 −64 mg/L), amphotericin B (0.12−8 mg/L), anidulafungin (0.015 −8 mg/L), caspofungin (0.008−8 mg/L), fluconazole (0.12−256 mg/L), itraconazole (0.015−16 mg/L), micafungin (0.008−8 mg/L), posaconazole (0.008−8 mg/L) and voriconazole (0.008−8 mg/L).

AFST was performed according to the manufacturer's protocol [6]. We added 20 $\mu$L of the 0.5 McFarland suspension using pure yeast culture into 11 mL of a culture medium (Yeast Broth, Thermo Scientific). We filled each well of the SYO microdilution 96-well plate (YeastOne AST Plate, Thermo Scientific) with 100 $\mu$L of the inoculum broth using an automated delivery system (Sensititre AIM, Thermo Scientific). The plate was sealed and incubated at 35 °C for a minimum of 24 h.

### *Micronaut-AM EUCAST AFST (M-AM)*

M-AM is also a colorimetric broth microdilution test designed for AFST. M-AM allows the testing of 2 isolates per plate and includes 6 antifungal agents with the following concentrations ranges: amphotericin B (0.25−2 mg/L), anidulafungin (0.002 mg/L; 0.016−4 mg/L), micafungin (0.002 mg/L; 0.008−2 mg/L), fluconazole (0.002 mg/L; 0.5−32 mg/L), posaconazole (0.03−4 mg/L) and voriconazole (0.03 −4 mg/L).

AFST was performed according to the manufacturer's protocol [7]. We added 200 $\mu$L of the 0.5 McFarland suspension using pure yeast culture to 4 mL of 0.85 % saline. Next, we added 200 $\mu$L of the 1:20 suspension to 11.5 mL of growth media (Micronaut-RPMI-1640 medium, Bruker). To the growth media, we added 100 $\mu$L of AST-Indicator and 50 $\mu$L of methylene blue solution (AST-Reagent Kit, Bruker). Using a multichannel pipette, we inoculated the microtitration plate (Micronaut-AM EUCAST AFST, Bruker) with 100 $\mu$L per well. The plate was sealed and incubated at 35 °C for a minimum of 24 h.

### *Mic readings*

Using a manual mirror viewer, two distinct operators determined MIC values through visual reading under standard laboratory lighting. A blue color indicated the absence of growth, while a pink color indicated growth in the well. For fungicides like amphotericin B and echinocandins, MIC values were determined based on the first well showing a blue color, representing total inhibition of noticeable growth. For fungistatic azoles, MIC values were defined as the lowest concentration resulting in a prominent reduction (50 % inhibition) of visual colorimetric growth compared to the positive growth control. MIC determination required a positive growth control. If a negative growth control was observed after 24 h, the plate was re-incubated and read at 36 or 48 h [6,7]. With M-AM, in cases of strong microbial growth leading to a significant reduction of resorufin to dihydroresorufin, the pink color may fade and turn white [7].

### *Essential agreement and categorical agreement*

Recorded raw MIC values obtained from SYO and M-AM were interpreted according to CLSI and EUCAST guidelines, respectively. We interpreted MIC values based on the availability of a clinical breakpoint (CBP), using the CLSI M27M44S 3rd Edition and the EUCAST version 10.0 [8,11]. In cases where a CBP was unavailable, we applied the epidemiological cutoff value (ECV), referring to the CLSI M57S 4th Edition and the EUCAST version 4.0 [9,10]. If neither CBP nor ECV was available, we did not interpret susceptibility nor calculate categorical agreement. CPB and ECV used to interpret MIC values are detailed in Supplementary data (Table S1). Essential agreement (EA) and categorical agreement (CA) were calculated for each antifungal agent, considering SYO as the reference method. We did not evaluate CA if an isolate-antifungal combination only had ECV and no CBP in EUCAST and CLSI guidelines.

For EA, agreement was achieved when the MIC value obtained with M-AM fell within $\pm$ 2 $\log_2$ dilution compared to the SYO value. We applied the following definition for CA: very major discrepancies (VMD) when the result was susceptible (S) with M-AM but resistant (R) with SYO; major discrepancies (MD) when the result was (R) with M-AM but (S) with SYO; minor discrepancies (mD) when the result was intermediate (I) or susceptible-dose dependent (SDD) with one method and either (S) or (R) with the other one. Categorical matches were met when the result ((S), (I/SDD) or (R)) was identical for both methods [12].

Acceptable performance rates were defined if $\geq$ 90 % agreement for CA and EA [12,13].

### *Reproducibility*

We used 3 reference strains to evaluate reproducibility: C. krusei ATCC 6258, C. parapsilosis ATCC 22019 and C. albicans ATCC 24433. These strains were tested 4 times for both methods. We calculated modal MIC values for each antifungal agent for both methods. As described previously, we determined the proportion of MIC values falling within the range of modal MIC $\pm$ 1 dilution [14−16].

## Results

In total, 441 and 392 isolate-antifungal results were evaluated for EA and CA, respectively. The CA for anidulafungin, micafungin, fluconazole and voriconazole was not evaluated due to the lack of CBP and ECV in EUCAST or CLSI guidelines for *C. guilliermondii, C. lusitaniae* and *C. kefyr*. CA was not calculated for voriconazole and *C. glabrata* (no CBP), and for fluconazole and *C. krusei* (no CBP). For

posaconazole, the CA was not evaluated for *C. lusitaniae, C. kefyr, C. guilliermondii, C. glabrata and C. krusei* (no CBP). For amphotericin B, CA was not assessed for *C. guilliermondii, C. lusitaniae* and *C. kefyr* (no CBP). Raw MIC values are provided in Supplementary data (Table S2).

*Essential and categorical agreement*

We observed no discrepancies for amphotericin B: EA and CA were 100 % for all tested Candida isolates.

For anidulafungin, EA and CA were 92.5 % ($n$ = 37/40) and 100 % ($n$ = 40/40) for *C. albicans*. NACS showed an EA of 85.3 % ($n$ = 29/34) and a CA of 86.7 % ($n$ = 26/30). We observed 4 MD (13.3 %) with *C. glabrata* and *C. krusei*.

For micafungin, EA and CA were 100 % ($n$ = 40/40) for *C. albicans*, indicating no discrepancies. Among NACS, EA was 50 % ($n$ = 17/34) and CA was 93.3 % ($n$ = 28/30). We observed 2 mD (6.7 %) regarding *C. parapsilosis,* where MIC values were higher with SYO compared to M-AM.

Regarding fluconazole, EA was 92.5 % ($n$ = 37/40) and CA was 100 % ($n$ = 40/40) for *C. albicans*. Among NACS, EA was 96.8 % ($n$ = 30/31) and CA was 68.2 % ($n$ = 15/22). We noticed 7 mD (31.8 %) with *C. parapsilosis, C. tropicalis* and *C. glabrata*, where MIC values were higher with M-AM compared to SYO.

For posaconazole, *C. albicans* showed an EA and CA of 100 % ($n$ = 40/40), indicating no discrepancies. Among NACS, EA was 70.6 % ($n$ = 24/34) and CA was 81.3 % ($n$ = 13/16). We noticed 3 VMD (18.7 %) regarding *C. tropicalis,* where MIC values were higher with SYO compared to M-AM.

Concerning voriconazole, EA and CA were 100 % ($n$ = 40/40) for *C. albicans*, indicating no discrepancies. Among NACS, EA was 58.8 % ($n$ = 20/34) and CA was 87.5 % ($n$ = 21/24). We found 3 mD (12.5 %) with *C. tropicalis,* where MIC values were higher with SYO compared to M-AM.

Table A.1 shows the susceptibility ratios of *C. albicans* and NACS with SYO and M-AM for the 6 tested antifungals. All *C. albicans* isolates were reported (S) to amphotericin B, anidulafungin and micafungin with both AFST methods. For azoles (fluconazole, posaconazole, voriconazole), 1 strain was found to be (R) with both methods.

Regarding NACS, all isolates were (S) to amphotericin B with both methods. However, discordances were observed for other antifungals. For anidulafungin, 2 *C. glabrata* and 2 *C. krusei* strains were classified as (R) with M-AM while being (S) with SYO. For micafungin, 2 *C. parapsilosis* strains were categorized as (I) using SYO, whereas they were both (S) with M-AM. Discordances were observed for fluconazole between the two methods: with M-AM, 4 *C. parapsilosis* and 2 *C. tropicalis* strains were (I) while classified as (S) with SYO. Considering *C. glabrata*, with SYO, 5 strains were (I) and 1 was (R), but with M-AM, 4 of these strains were (I) and 2 were (R). Differences were also noted for posaconazole where 3 *C. tropicalis* strains were classified as (R) using SYO but (S) with M-AM. Finally, for voriconazole, the same 3 *C. tropicalis* strains were considered (I) with SYO, while being reported as (S) with M-AM.

It was obviously not possible to evaluate EA and CA for the 3 antifungal agents not included in the M-AM plate (5-flucytosine, caspofungin and itraconazole). It should be noted that for 5-flucytosine, neither the EUCAST nor the CLSI guidelines provide specific interpretive criteria for this antifungal. Concerning caspofungin, a strain is considered (S) if tested (S) to both micafungin and anidulafungin according to EUCAST guidelines [11]. For *C. albicans*, all strains were (S) to caspofungin using SYO and were also (S) to anidulafungin and micafungin with M-AM. On the other hand, among NACS, we observed 4 strains that were (R) to anidulafungin but (S) to micafungin with M-AM. In this scenario, EUCAST does not provide specific information regarding the inferred susceptibility to caspofungin. When tested with SYO, 3 of these strains were found to be (S) to caspofungin, while 1 (*C. krusei*) was considered (I).

*Reproducibility*

There was a high reproducibility for nearly all antifungals with 100 % of MIC values within the modal MIC ± 1 dilution (Table A.2). For *C. albicans* ATCC 24433 and anidulafungin a reproducibility of 75 % was obtained.

## Discussion

In this study, we compared two commercially available methods, SYO and M-AM, for AFST of *Candida* species. To our knowledge, this is the first study evaluating the Micronaut-AM EUCAST AFST (6 antifungals for 2 isolates per plate) following previous evaluations on the Micronaut Anti-Fungal Agents (9 antifungals for 1 isolate per plate) [14,16].

Our first objective was to assess the concordance between these two methods. However, due to differences in breakpoint criteria between EUCAST and CLSI, it was challenging to compare CA results accurately. Therefore, we evaluated the potential impact of a method change on the reported antifungal susceptibility results reported to clinicians.

SYO and M-AM, showed a high level of concordance for *C. albicans* strains, with an EA and CA ≥ 90 % for all tested antifungals. The results obtained with both methods were consistent, indicating that the choice of AFST method had minimal impact on susceptibility categorization and subsequent reporting to clinicians. On the other hand, significant discordances emerged when comparing the results for NACS. The lowest EA were observed with micafungin (50 %) and voriconazole (58.8 %). These discrepancies were likely attributed to differences in the raw MIC values obtained by the two methods and the different interpretation criteria used by CLSI and EUCAST. For example, considering *C. krusei* and anidulafungin, a MIC value of 0.12 mg/L would be interpreted as (S) according to CLSI ($S \leq 0.25$ mg/L, $I = 0.5$ mg/L, $R \geq 1$ mg/L) and (R) according to EUCAST ($S \leq 0.06$ mg/L, $R \geq 0.06$ mg/L). When examining susceptibility ratio, the change in testing methods had a noticeable impact on the reported results for NACS. Considering echinocandins, discordances in AFST results were observed for *C. krusei, C. glabrata* and *C. parapsilosis*. With azoles, discordances were noted for *C. glabrata, C. parapsilosis* and *C. tropicalis*.

The results obtained in our study align with those reported by Philips et al. in 2021 [16]. CA and EA were similar between the two studies, with the exception of a higher agreement observed for posaconazole and voriconazole in our study. This difference could be attributed to the higher proportion of *C. albicans* strains in our study (54 % vs. 13 % in their study), as the agreement between the two methods was generally better for *C. albicans* compared to NACS. The reasons behind the observed discrepancies were also similar. SYO method generally exhibited higher MIC values compared to M-AM method, except for anidulafungin, where errors were associated with higher values with M-AM [16].

The implementation of a new AFST method could impact laboratory workload and result turnaround time, ultimately affecting clinical outcomes. SYO offers the potential advantage of nearly fully automated testing of nine antifungals with one isolate per plate. On the other hand, M-AM requires additional manual preparation steps and allows testing of six antifungals for two isolates per plate. It is worth noting that for M-AM, a spectrophotometer is available for automated reading of MIC values. Pellaton et al. compared the automated system to visual reading in 2022, reporting good overall agreement but a tendency to underestimate susceptibility to echinocandins with automated reading compared to visual reading [14]. Another drawback is the extended incubation time required for 13.5 % of M-AM plates, as the growth control was negative after 24 h, while this phenomenon was not observed with SYO. This longer incubation time inevitably delays the diffusion of AFST results and could directly impact patient management.

Our study has some limitations. First, the high proportion of *C. albicans* isolates in our panel ($n$ = 40/74) may contribute to the improved agreement between methods. Moreover, the inclusion of some NACS lacking CBP or ECV values has led to varying of AFST results for different drugs. However, it is important to note that this distribution accurately reflects the epidemiological and clinical picture of candidaemia cases observed in our setting. *C. albicans* remains the most prevalent *Candida* species causing bloodstream infections, and its inclusion in our study allowed to estimate the practical implications of the method comparison for a significant portion of candidaemia cases. Despite the absence of CBP or ECV values for certain NACS, such as *C. guilliermondii, C. lusitaniae* and *C. kefyr*, we could still evaluate the EA. Another limitation is the absence of molecular confirmation of antifungal-resistant strains through the detection of *FSK1* mutations for echinocandin resistance or *ERG11, TAC1* and *PDR1* mutations for azole resistance. These mutations are known to play a significant role in mediating resistance to their respective antifungal classes [17]. In this study, we encountered 2 *C. parapsilosis* strains classified as (I) for micafungin with SYO but (S) with M-AM. It is noteworthy that these 2 strains were categorized as (S) for anidulafungin with both methods. We also found 2 *C. glabrata* and 2 *C. krusei* strains that were classified as (R) for anidulafungin with M-AM but (S) with SYO. These 4 strains were categorized as (S) for micafungin with both methods. Incorporating gene sequencing to seek for these specific mutations would provide further insights into the resistance mechanisms exhibited by the analyzed strains. Pellaton et al. observed that strains carrying mutations in antifungal resistance genes showed a more significant increase in MIC values when tested with SYO using CLSI criteria compared to M-AM using EUCAST criteria [14].

In conclusion, our study demonstrated excellent agreement between SYO and M-AM methods for AFST of *C. albicans*, indicating that the choice of method has limited implications for patient management in these cases. However, we observed discordances for non-*albicans Candida* species, which can have significant implications for the choice of appropriate antifungal therapy in the treatment of non-*albicans* candidaemia. Our findings confirmed the trend of an expected substantial impact resulting from a methodological shift.

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## Declaration of competing interest

None.

## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.mycmed.2024.101465.

## Appendix

**Table A.1**
Susceptibility ratios of C. albicans and non-albicans Candida species with Sensititre YeastOne (SYO) and Micronaut-AM EUCAST AFST (M-AM).

| | *C. albicans* | | | | | |
|---|---|---|---|---|---|---|
| | SYO | | | M-AM | | |
| | S | I/SDD | R | S | I | R |
| Amphotericin B | 100 % ($n$ = 40) | N/A | 0 % ($n$ = 0) | 100 % ($n$ = 40) | N/A | 0 % ($n$ = 0) |
| Anidulafungin | 100 % ($n$ = 40) | 0 % ($n$ = 0) | 0 % ($n$ = 0) | 100 % ($n$ = 40) | N/A | 0 % ($n$ = 0) |
| Micafungin | 100 % ($n$ = 40) | 0 % ($n$ = 0) | 0 % ($n$ = 0) | 100 % ($n$ = 40) | N/A | 0 % ($n$ = 0) |
| Fluconazole | 97.5 % ($n$ = 39) | 0 % ($n$ = 0) | 2.5 % ($n$ = 1) | 97.5 % ($n$ = 39) | 0 % ($n$ = 0) | 2.5 % ($n$ = 1) |
| Posaconazole | 97.5 % ($n$ = 39) | N/A | 2.5 % ($n$ = 1) | 97.5 % ($n$ = 39) | N/A | 2.5 % ($n$ = 1) |
| Voriconazole | 97.5 % ($n$ = 39) | 0 % ($n$ = 0) | 2.5 % ($n$ = 1) | 97.5 % ($n$ = 39) | 0 % ($n$ = 0) | 2.5 % ($n$ = 1) |

| | *C. glabrata* | | | | | |
|---|---|---|---|---|---|---|
| | SYO | | | M-AM | | |
| | S | I/SDD | R | S | I | R |
| Amphotericin B | 100 % ($n$ = 6) | 0 % ($n$ = 0) | 0 % ($n$ = 0) | 100 % ($n$ = 6) | 0 % ($n$ = 0) | 0 % ($n$ = 0) |
| Anidulafungin | 100 % ($n$ = 6) | 0 % ($n$ = 0) | 0 % ($n$ = 0) | 66.7 % ($n$ = 4) | 0 % ($n$ = 0) | 33.3 % ($n$ = 2) |
| Micafungin | 100 % ($n$ = 6) | 0 % ($n$ = 0) | 0 % ($n$ = 0) | 100 % ($n$ = 6) | 0 % ($n$ = 0) | 0 % ($n$ = 0) |
| Fluconazole | 0 % ($n$ = 0) | 83.3 % ($n$ = 5) | 16.7 % ($n$ = 1) | 0 % ($n$ = 0) | 66.7 % ($n$ = 4) | 33.3 % ($n$ = 2) |
| Posaconazole | 66.7 % ($n$ = 4) | 0 % ($n$ = 0) | 33.3 % ($n$ = 2) | 100 % ($n$ = 6) | 0 % ($n$ = 0) | 0 % ($n$ = 0) |
| Voriconazole | 16.7 % ($n$ = 1) | 0 % ($n$ = 0) | 83.3 % ($n$ = 5) | 100 % ($n$ = 6) | 0 % ($n$ = 0) | 0 % ($n$ = 0) |

| | *C. krusei* | | | | | |
|---|---|---|---|---|---|---|
| | SYO | | | M-AM | | |
| | S | I | R | S | I | R |
| Amphotericin B | 100 % ($n$ = 8) | 0 % ($n$ = 0) | 0 % ($n$ = 0) | 100 % ($n$ = 8) | 0 % ($n$ = 0) | 0 % ($n$ = 0) |
| Anidulafungin | 100 % ($n$ = 8) | 0 % ($n$ = 0) | 0 % ($n$ = 0) | 75 % ($n$ = 6) | 0 % ($n$ = 0) | 25 % ($n$ = 2) |
| Micafungin | 100 % ($n$ = 8) | 0 % ($n$ = 0) | 0 % ($n$ = 0) | 100 % ($n$ = 8) | 0 % ($n$ = 0) | 0 % ($n$ = 0) |
| Fluconazole | N/A | N/A | N/A | 87.5 % ($n$ = 7) | 0 % ($n$ = 0) | 12.5 % ($n$ = 1) |
| Posaconazole | 100 % ($n$ = 8) | 0 % ($n$ = 0) | 0 % ($n$ = 0) | 100 % ($n$ = 8) | 0 % ($n$ = 0) | 0 % ($n$ = 0) |
| Voriconazole | 100 % ($n$ = 8) | 0 % ($n$ = 0) | 0 % ($n$ = 0) | 100 % ($n$ = 8) | 0 % ($n$ = 0) | 0 % ($n$ = 0) |

| | *C. parapsilosis* | | | | | |
|---|---|---|---|---|---|---|
| | SYO | | | M-AM | | |
| | S | I/SDD | R | S | I | R |
| Amphotericin B | 100 % (*n* = 11) | 0 % (*n* = 0) | 0 % (*n* = 0) | 100 % (*n* = 11) | 0 % (*n* = 0) | 0 % (*n* = 0) |
| Anidulafungin | 100 % (*n* = 11) | 0 % (*n* = 0) | 0 % (*n* = 0) | 100 % (*n* = 11) | 0 % (*n* = 0) | 0 % (*n* = 0) |
| Micafungin | 81.8 % (*n* = 9) | 18.2 % (*n* = 2) | 0 % (*n* = 0) | 100 % (*n* = 11) | 0 % (*n* = 0) | 0 % (*n* = 0) |
| Fluconazole | 100 % (*n* = 11) | 0 % (*n* = 0) | 0 % (*n* = 0) | 63.3 % (*n* = 7) | 36.7 % (*n* = 4) | 0 % (*n* = 0) |
| Posaconazole | 100 % (*n* = 11) | 0 % (*n* = 0) | 0 % (*n* = 0) | 100 % (*n* = 11) | 0 % (*n* = 0) | 0 % (*n* = 0) |
| Voriconazole | 100 % (*n* = 11) | 0 % (*n* = 0) | 0 % (*n* = 0) | 100 % (*n* = 11) | 0 % (*n* = 0) | 0 % (*n* = 0) |

| | *C. tropicalis* | | | | | |
|---|---|---|---|---|---|---|
| | SYO | | | M-AM | | |
| | S | I/SDD | R | S | I | R |
| Amphotericin B | 100 % (n = 5) | 0 % (*n* = 0) | 0 % (*n* = 0) | 100 % (n = 5) | 0 % (n = 0) | 0 % (n = 0) |
| Anidulafungin | 100 % (n = 5) | 0 % (n = 0) | 0 % (n = 0) | 100 % (n = 5) | 0 % (n = 0) | 0 % (n = 0) |
| Micafungin | 100 % (n = 5) | 0 % (n = 0) | 0 % (n = 0) | 100 % (n = 5) | 0 % (n = 0) | 0 % (n = 0) |
| Fluconazole | 100 % (n = 5) | 0 % (n = 0) | 0 % (n = 0) | 60 % (n = 3) | 40 % (n = 2) | 0 % (n = 0) |
| Posaconazole | 40 % (n = 2) | 0 % (n = 0) | 60 % (n = 3) | 100 % (n = 5) | 0 % (n = 0) | 0 % (n = 0) |
| Voriconazole | 40 % (n = 2) | 60 % (n = 3) | 0 % (n = 0) | 100 % (n = 5) | 0 % (n = 0) | 0 % (n = 0) |

Caption: SYO: Sensititre YeastOne; M-AM: Micronaut-AM EUCAST AFST; S: Susceptible; I: Intermediate; SDD: Susceptible-dose dependent; R: Resistant.

**Table A.2**
Reproducibility of Sensititre YeastOne (YO10) and Micronaut-AM EUCAST AFST (M-AM).

| Strains | Number of tests performed | Antifungals | YO10 Modal MIC (mg/L) | YO10 Percentage of MIC within modal MIC ± 1 dilution | M-AM Modal MIC (mg/L) | M-AM Percentage of MIC within modal MIC ± 1 dilution |
|---|---|---|---|---|---|---|
| ATCC 22019 *C. parapsilosis* | 4 | Amphotericin B | 0.5 | 100 | 0.5 | 100 |
| | | Anidulafungin | 0.5 | 100 | 0.25 | 100 |
| | | Fluconazole | 2 | 100 | 4 | 100 |
| | | Micafungin | 1 | 100 | 0.25 | 100 |
| | | Posaconazole | 0.03 | 100 | 0.03 | 100 |
| | | Voriconazole | 0.03 | 100 | 0.03 | 100 |
| ATCC 6258 *C. krusei* | 4 | Amphotericin B | 0.5 | 100 | 0.5 | 100 |
| | | Anidulafungin | 0.015 | 100 | 0.03 | 100 |
| | | Fluconazole | 32 | 100 | 16 | 100 |
| | | Micafungin | 0.25 | 100 | 0.03 | 100 |
| | | Posaconazole | 0.12 | 100 | 0.03 | 100 |
| | | Voriconazole | 0.25 | 100 | 0.03 | 100 |
| ATCC 24433 *C. albicans* | 4 | Amphotericin B | 1 | 100 | 0.25 | 100 |
| | | Anidulafungin | 0.015 | 75 | 0.016 | 100 |
| | | Fluconazole | 0.5 | 100 | 2 | 100 |
| | | Micafungin | 0.015 | 100 | 0.016 | 100 |
| | | Posaconazole | 0.03 | 100 | 0.03 | 100 |
| | | Voriconazole | 0.015 | 100 | 0.03 | 100 |

Caption: MIC: Minimal Inhibitory Concentration; YO10: Sensititre YeastOne; M-AM: Micronaut-AM EUCAST AFST; ATCC: American Type Culture Collection.

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