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A multicenter (three centers) study compared MICs obtained by the Sensititre YeastOne Colorimetric Antifungal plate to reference microdilution broth (NCCLS M27-A2 document) MICs of three new triazoles (posaconazole, ravuconazole, and voriconazole) and the echinocandin caspofungin acetate for 100 isolates of Candida spp. In addition, amphotericin B and fluconazole were tested as control drugs. Colorimetric MICs of caspofungin and amphotericin B corresponded to the first blue well (no growth), and MICs of the other agents corresponded to the first slightly purple or blue well. Two comparisons of MIC pairs by the two methods were evaluated: 24-h colorimetric MICs were compared to NCCLS MICs at 24 and at 48 h. The interlaboratory reproducibility of YeastOne and reference MICs was also examined. The best performance of the YeastOne plate was with 24-h MICs (overall, 95 to 99% agreement) for all the species and antifungal agents. These results suggest the potential value of the YeastOne plate for use in the clinical laboratory for the four new antifungal agents evaluated.

The National Committee for Clinical Laboratory Standards has developed a reference microdilution broth method (NCCLS document M27-A2) for antifungal susceptibility testing of yeasts (9). Alternatively, more simplified and efficient approaches also have been developed. Among them, the Sensititre YeastOne Colorimetric Antifungal plate (TREK Diagnostics Systems, Cleveland, Ohio) is a disposable plate that contains dried serial dilutions of three established antifungal agents and the colorimetric indicator Alamar Blue in individual wells. Based on earlier multicenter comparisons of NCCLS and YeastOne colorimetric MIC data (6, 12), the YeastOne plate was recently cleared by the Food and Drug Administration (FDA) for testing Candida spp. with fluconazole, itraconazole, and flucytosine in the clinical laboratory.

The purpose of this study was to extend the evaluation of the performance of the YeastOne plate for testing four new antifungal agents: caspofungin acetate, posaconazole, ravuconazole, and voriconazole. The evaluation was performed in three independent laboratories on a set of 100 isolates of Candida spp. Two established antifungal agents (amphotericin B and fluconazole) were included as controls. Colorimetric MICs (at 24 and 48 h) of the six agents were compared to reference NCCLS M27-A2 MICs for each isolate-drug combination in each of the three centers. The interlaboratory reproducibility of colorimetric and reference MICs was also examined.

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MATERIALS AND METHODS

Study design. The study was designed to compare MICs obtained by the YeastOne plate with those obtained by the NCCLS reference M27-A2 broth microdilution method (9) in the three independent laboratories (each laboratory tested 100 isolates by each method with six agents). Two MIC readings were performed by each method, e.g., at 24 and 48 h. Each first and second day, colorimetric MICs for each isolate-drug combination were compared to both corresponding first- and second-day M27-A2 MICs. In addition, the interlaboratory reproducibility of the colorimetric and reference MIC results was determined by performing repetitive testing (three times on three different days in each center) with the two quality control [QC] strains.

Clinical isolates. A total of 100 isolates from the culture collection of the University of Iowa College of Medicine included 38 Candida albicans, 24 C. glabrata, 7 C. krusei, 5 C. tropicalis, 10 C. parapsilosis, and 16 C. non-albicans isolates. The NCCLS QC isolates, C. krusei ATCC 6258 and C. parapsilosis ATCC 22019, were also tested each time a set of clinical isolates was evaluated by the two procedures. The isolates were recovered from oral cavities, blood, or other body fluids and included strains with different susceptibility patterns to fluconazole (resistant, susceptible dose-dependent, and susceptible isolates). Each isolate represented a unique strain from a single patient. The isolates were maintained in sterile water and subcultured onto antimicrobial-free medium to ensure viability and purity prior to testing.

Antifungal agents. The YeastOne plates and reference microdilution plates, containing serial drug dilutions of caspofungin (Merck Research Laboratories, Rahway, N.J.), posaconazole (Schering-Plough Research Institute, Kenilworth, N.J.), ravuconazole (Bristol-Myers Squibb Pharmaceutical Research Institute, Wallingford, Conn.), voriconazole (Pfizer Pharmaceuticals, New York, N.Y.), and the echinocandin caspofungin (Bristol-Myers Squibb Pharmaceutical Research Institute) and fluconazole (Pfizer Pharmaceuticals), were provided by TREK. Drug dilutions of amphotericin B, caspofungin, and the new triazoles ranged from 16 to 0.008 μg/ml, and fluconazole concentrations ranged from 128 to 0.12 μg/ml in both the reference and YeastOne plates. YeastOne plates, which are individually packed in foil and silica gel desiccant, were stored at ambient temperature until testing was performed. The reference microdilution plates were prepared following the NCCLS M27-A2 procedure (9), shipped frozen to each laboratory, and stored at −70°C until the day of the test.
Inoculum preparation. Stock inoculum suspensions of the yeasts were obtained from 24-h cultures on Sabouraud dextrose agar at 35°C. The turbidity of each yeast suspension was adjusted by the spectrophotometric method (9).

SensitiSee YeastOne Colorimetric Antifungal plate procedure. On the day of the test, the dried YeastOne plates were rehydrated with the working yeast suspension (approximately 1.5 × 10⁵ CFU/ml) by dispensing 100 µl into each well. The YeastOne plates were sealed and incubated at 35°C in a non-CO₂ incubator and were read after 24 and 48 h of incubation by means of a view box. Colorimetric MICs were interpreted as the lowest concentration of antifungal solutions that remained blue or unchanged (indicating no growth) (amphotericin B and caspofungin) or the first dilution that either changed from blue to slightly purple (which is equivalent to the prominent growth inhibition [≥50%] seen by the NCCLS method) or remained unchanged (triazoles). QC isolates were tested in the same manner by each participating laboratory.

NCCLS broth microdilution method (M27-A2). Following inoculation of the reference microdilution plates, plates were incubated at 35°C in a non-CO₂ incubator, and MICs were determined after 24 and 48 h. Reference MICs corresponded to the lowest drug dilution that showed complete growth inhibition (caspofungin and amphotericin B) and the lowest drug dilution that showed prominent (50% or more) growth inhibition (the triazoles). QC isolates were tested in the same manner by each participating laboratory.

Data analysis. Both on-scale (e.g., 0.12 and 128 µg/ml) and off-scale (e.g., <0.12 and >128 µg/ml) MICs were included in the analysis. For the comparison between the two methods, MICs for each drug-organism combination by each method obtained in the three laboratories were compared as follows: (i) 24-h YeastOne MICs had the same range at 24 h as the reference MICs (e.g., 0.5 and 4 µg/ml) and other isolates (≤1 log₂ dilution was then used to obtain the percentages of agreement among the three sites.

RESULTS AND DISCUSSION

Table 1 lists the established MIC ranges for the two QC isolates (1, 9) and the percentages of agreement of colorimetric MICs with reference MIC ranges at both incubation times. Colorimetric MICs for the C. krusei ATCC 6258 isolate were within the established range at 24 h, but two caspofungin and posaconazole MICs were one dilution higher at 48 h than the upper limit established by the NCCLS. Although similar results were observed for the C. parapsilosis ATCC 22019 strain and MICs were clustered within a three- to two-dilution range with four of the six agents, one fluconazole and four ravuconazole colorimetric MICs were higher than the reference range at 24 h. Therefore, it appears that different fluconazole and ravuconazole MIC ranges should be established for the QC isolate C. parapsilosis ATCC 22019 for testing by the YeastOne method. In the evaluation of YeastOne performance with the two QC isolates by Pfaller et al. (12), fluconazole MICs for C. parapsilosis ATCC 22019 were within the reference range at both incubation times, but optimal agreement with the NCCLS range was achieved only at 48 h for amphotericin B. In the present study, the amphotericin B colorimetric results were not dependent on the time of incubation (Table 1). There is no reasonable explanation for these contrasting results; however, they underscore the influence of the incubation time on MIC data. Reproducibility results for the two QC isolates confirm the superior reliability of 24-h YeastOne MIC data for the established agents and extend previous reports for the four new antifungal agents tested, caspofungin, posaconazole, ravuconazole, and voriconazole.

The most important role of antifungal susceptibility testing is to identify resistant isolates among susceptible strains, which requires wide MIC ranges. Table 2 summarizes NCCLS MIC ranges for the actual number of isolates per species tested by both methods, as well as the percent agreement between YeastOne MICs and those reference values at the listed incubation times. YeastOne MICs had the same range at 24 h as the reference MICs, which indicated that the colorimetric method could identify fluconazole resistance (≥64 µg/ml) and other isolates for which high reference MICs were obtained with the other agents. Although two major errors were observed with 24-h fluconazole colorimetric results (0.5 and 4 µg/ml versus 64 µg/ml by the reference method), they represented the low reproducibility among the laboratories for two isolates (only one of the three centers reported the low values). However, minor errors were observed among C. albicans (7 errors, 91% categorical agreement) and C. glabrata (29 errors, 54% categorical agreement). For the other species, the categorical agreement was 100%. Interpretive breakpoints have not been established for amphotericin B, caspofungin, or the new triazoles against any fungal species. However, substantial disagreement between the methods was low for those agents (0.3 to 1%) when colorimetric MICs were read at 24 h (e.g., reference MICs of ≤1 µg/ml versus colorimetric MICs of ≥8 µg/ml). In general, results for established and new agents were similar to
especially, length of incubation for panel results was dependent on species, antifungal agent, and, these were the species where the major discrepancies were those previously reported (2, 3, 5, 8, 10, 11), but the higher MICs (≥2 μg/ml) of amphotericin B recently reported for C. krusei and C. glabrata (11) were obtained mostly at 48 h. In the earlier evaluation with >1,000 yeast isolates, the 24-h amphotericin B MICs by the YeastOne plate provided the lowest percentages of agreement between the methods (40 to 76% versus 90 to 99% at 48 h) (6). However, that evaluation included uncommon Candida spp., Cryptococcus spp., and other yeasts and yeast-like organisms, and these were the species where the major discrepancies were noted when this agent was tested. In the present study, we tested only the common and less fastidious Candida species for which the YeastOne plate has been cleared for clinical testing by the FDA. For the other agents, the overall agreement between the two methods for colorimetric MICs (all the species tested) was better at 24 (95 to 99%) than at 48 h (80 to 97%). The main reason for this discrepancy was that colorimetric 48-h MICs were consistently more than two dilutions higher than reference MICs, especially for isolates of C. krusei and C. glabrata.

The interlaboratory agreement among the three centers for
The colorimetric YeastOne MICs was excellent (96 to 99%) at 24 h; lower percentages of interlaboratory agreement were observed for ravuconazole (83%) and voriconazole (88%) at 48 h (Table 3). The reproducibility of YeastOne MICs at 24 h was comparable to that obtained with reference MIC endpoints (95 to 99%). Reproducibility was lower at 48 h for both methods, but the effect of the longer incubation was more evident for colorimetric MICs (83 to 99%) than for the reference MICs (90 to 99%). It is noteworthy that the shorter incubation time had yielded superior interlaboratory agreement for fluconazole NCCLS MICs in an earlier study (4).

Data from the present study corroborated our earlier results regarding the YeastOne performance for clinical and QC isolates (6, 12).

There are two commercially available antifungal susceptibility testing systems for fungal pathogens in the United States, the Etest (7) and the YeastOne plate (6, 12), but only the YeastOne plate has been cleared by the FDA for testing three established antifungal agents. Our evaluation of the performance of the Sensititre YeastOne Colorimetric Antifungal plate suggests its potential value also for use in the clinical laboratory for the antifungal susceptibility testing of most Candida spp. after 24 h of incubation with the new antifungal agents caspofungin, posaconazole, ravuconazole, and voriconazole. The clinical value of these in vitro results needs to be established.

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REFERENCES