JOURNAL OF CLINICAL MICROBIOLOGY, July 2008, p. 2155–2159 Vol. 46, No. 7
0095-1137/08/$08.00+0 doi:10.1128/JCM.00493-08

Clinical Evaluation of the Sensititre YeastOne Colorimetric Antifungal Panel for Antifungal Susceptibility Testing of the Echinocandins Anidulafungin, Caspofungin, and Micafungin▽

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Received 12 March 2008/Returned for modification 25 April 2008/Accepted 29 April 2008

A commercially prepared, dried colorimetric microdilution panel (Sensititre YeastOne Trek Diagnostic Systems, Cleveland, OH) was compared in three different laboratories with the Clinical and Laboratory Standards Institute (CLSI) reference microdilution method by testing 2 quality control strains, 25 reproducibility strains, and 404 isolates of *Candida* spp. against anidulafungin, caspofungin, and micafungin. Reference MIC endpoints and YeastOne colorimetric endpoints were read after 24 h of incubation. YeastOne endpoints were determined to be the lowest concentration at which the color in the well changed from red (positive, indicating growth) to blue (negative, indicating no growth). Excellent essential agreement (within 2 dilutions) between the reference and colorimetric MICs was observed. Overall agreement was 100% for all three agents. Categorical agreement ranged from 99.3% (anidulafungin) to 100% (caspofungin, micafungin) and interlaboratory reproducibility was 99%. The YeastOne colorimetric method appears to be comparable to the CLSI reference method for testing the susceptibility of *Candida* spp. to the echinocandins anidulafungin, caspofungin, and micafungin.

All three available echinocandin antifungal agents—anidulafungin (Pfizer), caspofungin (Merck), and micafungin (Astellas)—provide excellent clinical efficacy coupled with low toxicity for the treatment of serious candidal infections (17, 22, 25, 26, 33). Standardized broth microdilution (BMD) susceptibility testing of *Candida* spp. against the echinocandins has been available since 2004 (24, 29), and the establishment of quality control strains and validated interpretive breakpoints (3, 4; M. A. Pfaller, D. J. Diekema, J. H. Rex, B. D. Alexander, D. Andes, S. D. Brown, V. Chaturvedi, M. A. Ghannoun, C. C. Knapp, L. Ostrosky-Zeichner, D. J. Sheehan, and T. J. Walsh, submitted for publication) now make it feasible for this method to be used more broadly for clinical testing (32). Notably, data from in vitro surveys document the presence of rare strains of otherwise susceptible species of *Candida* that exhibit unusually high MICs for one or more echinocandins (7, 32). These high-MIC strains are sufficiently rare that they have not been encountered with any frequency in clinical trials (15, 17, 22, 25, 26, 33), although several isolates with echinocandin MICs of >2 μg/ml have recently been associated with clinical resistance to echinocandin therapy in published case reports (2, 5, 6, 11, 13, 14, 16, 18, 20, 23, 27, 28, 34). These observations underscore the importance of antifungal susceptibility testing of echinocandins in detecting unusual resistance profiles, as these agents are used more broadly worldwide (32).

The Clinical and Laboratory Standards Institute (CLSI) BMD method for the testing of caspofungin has served as the reference standard for the development of both broth- and agar-based procedures designed to provide simple, flexible, and commercially available susceptibility testing methods for use in the clinical laboratory (1, 9, 10, 19). Among the commercially available BMD antifungal testing systems, only the Sensititre YeastOne system (Trek Diagnostic Systems, Cleveland, OH) offers an echinocandin (caspofungin) on a dried 96-well BMD panel (1, 9, 21). The YeastOne system is available in a dry-form 96-well panel with the colorimetric growth indicator Alamar Blue and has a shelf life of 24 months at ambient temperature. It has been used widely in the United States and elsewhere with excellent results in terms of accuracy and reproducibility (31). A study designed to establish the interlaboratory reproducibility of the YeastOne system for testing caspofungin has shown excellent agreement (96%) when a set of 100 isolates was tested in three different laboratories and MIC results were read after 24 h of incubation (9). Subsequent evaluation of the YeastOne panel in a single clinical microbiology laboratory found a 92% essential agreement (EA; MIC of ±2 dilutions) for MICs for caspofungin versus the CLSI BMD results (1). Recently, anidulafungin and micafungin have been added to a panel containing caspofungin, reflecting the increased use of these agents clinically.

The purpose of the present study was to validate the performance of the YeastOne panel with anidulafungin, caspofungin, and micafungin against a broad range of clinical isolates of *Candida* spp. in three independent laboratories and to compare the results from these panels with those from a frozen reference BMD method performed according to CLSI guidelines.

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▽ Published ahead of print on 7 May 2008.

MATERIALS AND METHODS

Study design. The MIC results for anidulafungin, caspofungin, and micafungin obtained with the YeastOne system were compared to those obtained by the CLSI M27-A3 BMD method (3) in three laboratories. Each laboratory tested at

least 100 clinical isolates of *Candida* spp. (range, 100 to 103 isolates) with the YeastOne system and the CLSI frozen reference BMD panel (a total of 304 clinical isolates). In addition, a challenge set of 100 well-characterized stock isolates was tested by both methods in all three laboratories. The interlaboratory reproducibility of the echinocandin MICs determined by the YeastOne system was examined by testing a panel of 25 *Candida* spp. with on-scale MIC endpoints in each of the three participating laboratories. The MIC results obtained with the YeastOne system following 24 h of incubation were compared with those obtained with the reference BMD panel read after 24 h.

Test organisms. The test organisms included two American Type Culture Collection (ATCC) strains that have been established as quality control strains (*Candida parapsilosis* ATCC 22019 and *Candida krusei* ATCC 6258) by the CLSI (3, 4). A challenge set of 100 isolates of *Candida* spp. selected to provide on-scale MIC results and to represent clinically important species was tested in all three laboratories. The challenge set included 38 isolates of *C. albicans*, 24 of *C. glabrata*, 16 of *C. tropicalis*, 10 of *C. parapsilosis*, 7 of *C. krusei*, and 5 of *C. lusitaniae*. An additional 304 recent clinical isolates of *Candida* spp. were also tested (101, 100, and 103 isolates tested in laboratories 1, 2, and 3, respectively). The clinical isolates included 60 isolates of *C. albicans*, 15 of *C. glabrata*, 25 of *C. tropicalis*, 60 of *C. parapsilosis*, 60 of *C. krusei*, 60 of *C. lusitaniae*, and 24 of *Candida* spp. not identified to the species level. Reproducibility among laboratories was assessed by using a panel of 25 isolates: 5 isolates each of *C. albicans*, *C. parapsilosis*, *C. krusei*, and *C. lusitaniae*; 3 of *C. glabrata*; and 2 of *C. tropicalis*. All isolates were identified by standard methods (12). Before the tests were performed, each isolate was passed at least twice on Sabouraud dextrose agar (Hardy Diagnostics, Santa Maria, CA) to ensure its purity and viability.

Antifungal agents and microdilution panels. The YeastOne panels and the frozen reference BMD trays containing serial twofold dilutions of anidulafungin (0.008 to 16 μg/ml), caspofungin (0.008 to 16 μg/ml), and micafungin (0.008 to 16 μg/ml) were provided by Trek Diagnostic Systems. The YeastOne panels were shipped in sealed packages and stored at room temperature until testing was performed. The BMD trays, which were prepared by following the M27-A3 additive procedure (3), were shipped frozen to each participating laboratory and stored at −70°C until the day of the test.

Inoculum preparation. Stock inoculum suspensions of the *Candida* spp. were obtained from 24-h cultures on Sabouraud dextrose agar at 35°C. The turbidity of each yeast suspension was adjusted by Trek's nephelometer following the M27-A3 guidelines (3).

CLSI BMD method. Reference BMD testing was performed exactly as outlined in CLSI document M27-A3 (3) with a final inoculum concentration of $1.5 \times 10^3 \pm 1.0 \times 10^3$ cells/ml in RPMI 1640 medium buffered to pH 7.0 with 0.165 M morpholinepropanesulfonic acid (MOPS) buffer. The panels were incubated in air at 35°C and observed for the presence or absence of growth at 24 h. The MICs of all three agents were read as the lowest concentration that produced a prominent decrease in turbidity (approximately 50% reduction in growth) relative to the growth control (3).

Sensititre YeastOne colorimetric MIC procedure. On the day of the test, a working yeast suspension of approximately 1.5×10^3 cells/ml was prepared in YeastOne inoculum broth (Trek). The dried YeastOne panels were rehydrated with the working yeast suspension by use of an appropriate multichannel pipetting device by dispensing 100 μl into each well. The YeastOne panels were covered with adhesive seals and incubated at 35°C for 24 h in a non-CO_2 incubator. The colorimetric MIC endpoints were read using a reading mirror which displays the underside of the wells. Yeast growth was evident as a color change from blue (negative, no growth) to red (positive, growth). Colorimetric MIC results for all of the test agents were defined as the lowest concentration of antifungal agent that prevented the development of a red color (first blue well) (8, 9).

Quality control. Quality control was ensured by testing the CLSI-recommended quality control strains *C. parapsilosis* ATCC 22019 and *C. krusei* ATCC 6258 (3, 4). These isolates were tested at least 20 times in each of the three laboratories and all (100%) MICs (YeastOne and reference) were within the respective MIC ranges.

Analysis of results. The MIC results obtained with the YeastOne panels read at 24 h were compared with those of the reference panels read at 24 h. As with previous studies (9, 30), high off-scale MIC results were converted to the next highest concentration and low off-scale MIC results were left unchanged. Discrepancies among MIC endpoints of more than 2 dilutions (two wells) were used to calculate the EA. Interlaboratory agreement, assessed with the 25-isolate reproducibility panel, was defined when the MIC results were within a 3-dilution range (mode ± 1 $\log_2$ dilution). The recently established CLSI interpretive breakpoints for the echinocandins (susceptible, ≤2 μg/ml; nonsusceptible, ≥4 μg/ml) were used to obtain categorical agreement percentages between the MICs determined with the YeastOne system and by the reference BMD (3, 32; Pfaller et al., submitted). Very major errors were identified when the reference MIC indicated a nonsusceptible result and the YeastOne system MIC was susceptible. Major errors were identified when the isolate was classified as nonsusceptible by the YeastOne system and susceptible by the reference method.

RESULTS AND DISCUSSION

Table 1 summarizes the in vitro susceptibilities of 404 isolates of *Candida* spp. (100 challenge isolates and 304 clinical isolates) to anidulafungin, caspofungin, and micafungin, as determined with the YeastOne system and by the reference BMD read at 24 h. Due to the similarity in the results obtained with the YeastOne system compared with the 24-h BMD results for both the challenge isolates and the clinical isolates, the results for the two organism sets were combined in Table 1. In general, the MIC results for all three agents were typical of those for each species of *Candida* (32).

The overall and species-specific EA between the YeastOne system and the BMD MICs for each echinocandin was 100% in each of the three study sites (Table 1). This level of EA exceeds that previously reported for caspofungin in both multicenter (9) and single-center (1) studies, confirming the previous observations that the YeastOne system MIC results were highly predictive of the reference BMD results for all organism-drug combinations (9).

The YeastOne system MIC results for all three antifungal agents were highly reproducible, as determined by replicate testing of a panel of 25 *Candida* sp. isolates in the three laboratories (data not shown). Overall, 223 of 225 (99%) MIC results fell in a 3-dilution range (mode ± 1 dilution) for the three agents as follows: for anidulafungin, 98.7% (74/75 results); for caspofungin, 98.7% (74/75 results); and for micafungin, 100% (75/75 results). This high level of reproducibility underscored the excellent level of test standardization achieved with this colorimetric MIC system.

The categorical agreement between the results obtained with the YeastOne system and those obtained by BMD with all three agents was assessed by combining the data obtained with the clinical and challenge organism collections in all three laboratories (data not shown). Excellent categorical agreement was observed for all comparisons. The overall categorical agreements for the comparison of the YeastOne system results with the 24-h BMD results were 99.3% (600/604 results) for anidulafungin and 100% (604/604 results) for both caspofungin and micafungin. The four discrepancies observed with anidulafungin all originated from one of the study sites and were confined to three isolates of *C. parapsilosis* and one isolate of *C. glabrata* for which anidulafungin MICs were 2 μg/ml as determined by the YeastOne system and 4 μg/ml as determined by the reference BMD method.

These findings demonstrate that in addition to susceptibility tests for the licensed antifungal agents amphotericin B, flucytosine, fluconazole, itraconazole, voriconazole, and posaconazole (9, 30), the YeastOne system also provides a means of determining the MICs for anidulafungin, caspofungin, and micafungin when they are tested against *Candida* spp. This system is the first commercially available BMD system to offer antifungal susceptibility testing of all three echinocandins and, as shown previously (31), provides excellent test standardization and reproducibility. In addition to providing highly repro-

TABLE 1. In vitro susceptibilities of *Candida* spp. isolates to anidulafungin, caspofungin, and micafungin as determined by the Sensititre YeastOne antifungal plate and CLSI BMD methods[a]

Organism	Antifungal agent	Study site (no. of isolates)	Test method	MIC (μg/ml)[b]			EA (%)
				Range	50%	90%	
C. albicans	Anidulafungin	1 (58)	YstOne	≤0.008–0.12	0.06	0.12	100
			Ref.	≤0.008–0.06	0.03	0.06	
		2 (58)	YstOne	≤0.008–0.25	0.016	0.06	100
			Ref.	≤0.008–0.25	0.016	0.03	
		3 (58)	YstOne	≤0.008–0.12	0.06	0.12	100
			Ref.	≤0.008–0.06	0.03	0.06	
	Caspofungin	1 (58)	YstOne	0.016–0.12	0.03	0.06	100
			Ref.	≤0.008–0.25	0.06	0.06	
		2 (58)	YstOne	0.016–0.25	0.03	0.06	100
			Ref.	0.016–0.5	0.03	0.06	
		3 (58)	YstOne	0.03–0.25	0.06	0.12	100
			Ref.	0.03–0.5	0.06	0.06	
	Micafungin	1 (58)	YstOne	≤0.008–0.03	≤0.008	0.016	100
			Ref.	≤0.008–0.06	0.016	0.03	
		2 (58)	YstOne	≤0.008–0.06	0.016	0.03	100
			Ref.	0.016–0.12	0.016	0.03	
		3 (58)	YstOne	≤0.008–0.016	≤0.008	0.016	100
			Ref.	≤0.008–0.06	0.016	0.03	
C. glabrata	Anidulafungin	1 (29)	YstOne	0.03–2	0.06	0.12	100
			Ref.	0.03–4	0.06	0.25	
		2 (29)	YstOne	0.03–0.25	0.12	0.12	100
			Ref.	0.03–0.25	0.12	0.12	
		3 (29)	YstOne	0.03–.025	0.12	0.12	100
			Ref.	0.03–0.12	0.06	0.06	
	Caspofungin	1 (29)	YstOne	0.03–>8	0.06	0.12	100
			Ref.	0.03–>8	0.06	0.12	
		2 (29)	YstOne	0.06–1	0.12	0.25	100
			Ref.	0.06–1	0.12	0.25	
		3 (29)	YstOne	0.06–0.5	0.12	0.25	100
			Ref.	0.03–0.5	0.06	0.12	
	Micafungin	1 (29)	YstOne	≤0.008–8	≤0.008	0.016	100
			Ref.	≤0.008–8	0.016	0.016	
		2 (29)	YstOne	≤0.008–0.12	0.016	0.03	100
			Ref.	≤0.008–0.25	0.016	0.03	
		3 (29)	YstOne	≤0.008–0.016	≤0.008	0.016	100
			Ref.	≤0.008–0.03	0.016	0.016	
C. tropicalis	Anidulafungin	1 (26)	YstOne	0.016–0.5	0.12	0.25	100
			Ref.	0.016–0.06	0.03	0.06	
		2 (21)	YstOne	≤0.008–0.5	0.016	0.06	100
			Ref.	≤0.008–0.5	0.016	0.12	
		3 (26)	YstOne	0.06–0.25	0.12	0.12	100
			Ref.	0.016–0.12	0.03	0.06	
	Caspofungin	1 (26)	YstOne	0.016–0.12	0.03	0.12	100
			Ref.	0.016–0.06	0.03	0.06	
		2 (21)	YstOne	0.016–4	0.03	0.06	100
			Ref.	0.03–4	0.03	0.12	
		3 (26)	YstOne	0.03–0.25	0.06	0.25	100
			Ref.	0.03–0.25	0.06	0.06	
	Micafungin	1 (26)	YstOne	0.016–0.03	0.016	0.03	100
			Ref.	≤0.008–0.06	0.03	0.06	
		2 (21)	YstOne	≤0.008–2	0.016	0.03	100
			Ref.	≤0.008–2	0.016	0.06	
		3 (26)	YstOne	≤0.008–0.06	0.016	0.016	100
			Ref.	0.016–0.06	0.03	0.06	
C. parapsilosis	Anidulafungin	1 (30)	YstOne	0.5–2	1	2	100
			Ref.	1–4	2	2	
		2 (30)	YstOne	0.03–1	0.25	1	100
			Ref.	0.016–2	0.12	0.5	
		3 (30)	YstOne	0.5–4	1	2	100
			Ref.	0.5–4	1	2	
	Caspofungin	1 (30)	YstOne	0.25–2	0.5	1	100
			Ref.	0.25–1	0.5	1	
		2 (30)	YstOne	0.016–0.5	0.12	0.25	100
			Ref.	0.03–1	0.25	0.5	
		3 (30)	YstOne	0.25–2	0.5	1	100
			Ref.	0.12–1	0.5	0.5	
	Micafungin	1 (30)	YstOne	0.25–2	0.5	1	100
			Ref.	0.5–2	1	2	
		2 (30)	YstOne	0.03–0.5	0.25	0.25	100
			Ref.	0.016–1	0.25	0.5	
		3 (30)	YstOne	0.25–2	0.5	1	100
			Ref.	0.25–2	1	2	

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TABLE 1—*Continued*

Organism	Antifungal agent	Study site (no. of isolates)	Test method	MIC (μg/ml)[b]			EA (%)
				Range	50%	90%	
C. krusei	Anidulafungin	1 (27)	YstOne	0.06–0.25	0.12	0.12	100
			Ref.	0.03–0.5	0.25	0.25	
		2 (27)	YstOne	0.03–0.12	0.12	0.12	100
			Ref.	0.016–0.12	0.06	0.12	
		3 (27)	YstOne	0.06–0.5	0.12	0.25	100
			Ref.	0.03–0.25	0.06	0.12	
	Caspofungin	1 (27)	YstOne	0.12–0.5	0.25	0.5	100
			Ref.	0.12–1	0.5	0.5	
		2 (27)	YstOne	0.06–0.5	0.25	0.5	100
			Ref.	0.06–0.25	0.25	0.25	
		3 (27)	YstOne	0.12–0.5	0.25	0.5	100
			Ref.	0.06–1	0.12	0.5	
	Micafungin	1 (27)	YstOne	0.016–0.12	0.12	0.12	100
			Ref.	0.03–0.12	0.06	0.12	
		2 (27)	YstOne	0.016–0.25	0.12	0.12	100
			Ref.	0.016–0.25	0.12	0.25	
		3 (27)	YstOne	0.06–0.25	0.12	0.12	100
			Ref.	0.03–0.25	0.12	0.12	
C. lusitaniae	Anidulafungin	1 (25)	YstOne	0.06–0.25	0.12	0.25	100
			Ref.	0.06–1	0.5	0.5	
		2 (25)	YstOne	≤0.008–0.12	0.12	0.12	100
			Ref.	≤0.008–0.25	0.12	0.12	
		3 (25)	YstOne	0.12–2	0.25	0.5	100
			Ref.	0.06–2	0.25	1	
	Caspofungin	1 (25)	YstOne	0.016–1	0.12	0.25	100
			Ref.	0.06–1	0.25	0.5	
		2 (25)	YstOne	≤0.008–0.25	0.06	0.12	100
			Ref.	≤0.008–0.25	0.06	0.25	
		3 (25)	YstOne	0.03–0.25	0.25	0.25	100
			Ref.	0.06–0.5	0.5	0.5	
	Micafungin	1 (25)	YstOne	0.016–0.25	0.06	0.25	100
			Ref.	0.06–0.5	0.12	0.5	
		2 (25)	YstOne	≤0.008–0.06	0.03	0.06	100
			Ref.	≤0.008–0.12	0.06	0.12	
		3 (25)	YstOne	0.016–1	0.06	0.5	100
			Ref.	0.03–2	0.12	0.25	
Candida spp.	Anidulafungin	1 (6)	YstOne	≤0.008–2	1		100
			Ref.	0.03–2	1		
		2 (10)	YstOne	0.016–1	0.25	0.5	100
			Ref.	0.03–0.5	0.25	0.5	
		3 (8)	YstOne	0.06–0.12	0.06		100
			Ref.	0.016–0.12	0.06		
	Caspofungin	1 (6)	YstOne	≤0.008–0.5	0.25		100
			Ref.	0.03–0.5	0.5		
		2 (10)	YstOne	0.016–0.25	0.12	0.25	100
			Ref.	0.016–0.25	0.12	0.25	
		3 (8)	YstOne	0.016–0.03	0.03		100
			Ref.	0.016–0.12	0.06		
	Micafungin	1 (6)	YstOne	0.016–1	0.25		100
			Ref.	0.03–2	0.5		
		2 (10)	YstOne	0.016–0.5	0.12	0.5	100
			Ref.	0.03–0.5	0.12	0.25	
		3 (8)	YstOne	≤0.008–0.06	0.016		100
			Ref.	0.016–0.12	0.03		

[a] Abbreviations: YstOne, YeastOne; Ref., reference method.
[b] 50%, MIC encompassing 50% of all isolates tested; 90%, MIC encompassing 90% of all isolates tested.

ducible MIC results that reliably predict the MICs determined by the reference BMD, the YeastOne system provides results for all antifungal agents (polyenes, flucytosine, triazoles, and echinocandins) within 24 h.

The overall level of agreement (100%) in this study is consistent with or superior to that reported from other studies of the YeastOne system (1, 8, 9, 21, 30). By testing several clinically relevant species in multiple laboratories, we have validated the YeastOne colorimetric MIC system for testing of three additional antifungal agents that possess potent activity against *Candida* spp. Thus, the YeastOne system may be used for further investigation of the antifungal activity of anidulafungin, caspofungin, and micafungin with results comparable to those obtained with the reference BMD method.

ACKNOWLEDGMENTS

This study was supported in part by a grant from Trek Diagnostic Systems.

We thank Linda Elliot and Tara Schroder for secretarial assistance in the preparation of the manuscript. We acknowledge the excellent assistance of the technical personnel in the Iowa, Ohio, and New York laboratories.

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