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Sensititre YeastOne Caspofungin Susceptibility Testing of *Candida* Clinical Isolates: Correlation with Results of NCCLS M27-A2 Multicenter Study

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The ability of Sensititre YeastOne to discriminate isolates with reduced caspofungin susceptibility was determined against 36 *Candida* spp. (6 with a known *FKS1* mutation). Results were compared with those of M27-A2. The MIC endpoint was 100% growth inhibition. Overall agreement (±2 $\log_2$) was 87.16%. Sensititre YeastOne detected strains with reduced caspofungin susceptibility.

The NCCLS proposed reference standard methodology (M27-A2) (13) to test susceptibility of *Candida* spp. is labor-intensive and requires 48 h of incubation. The marketed Sensititre YeastOne (SYO) microdilution colorimetric method has proved to be efficient for susceptibility testing of *Candida* and *Aspergillus* spp. (6, 11, 12, 18, 21).

Caspofungin is a semisynthetic echinocandin with a broad spectrum of in vitro activity (5, 15, 19, 22) that acts to inhibit (1,3)-β-D-glucan synthesis. Methods for susceptibility testing of caspofungin have not been standardized. To facilitate the susceptibility testing for clinical laboratories and avoid the drawbacks of the NCCLS method, we have assayed the capacity of SYO to detect isolates with reduced caspofungin susceptibility.

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Five Spanish laboratories collaborated in this study. Every participant tested 34 encoded strains, 25 blood culture isolates (Table 1), and 9 *Candida albicans* strains, 6 with reduced caspofungin susceptibility (2 heterozygous and 4 homozygous for *FKS1* gene mutation) provided by Merck Research Laboratories (Rahway, N.J.) (9). *Candida krusei* ATCC 6258 and *C. albicans* ATCC 90028 were included as a quality control (QC) strain (13, 14) and reference strain, respectively.

Stock solutions of fluconazole (Pfizer, Spain), flucytosine (Roche, Spain), itraconazole (Janssen Pharmaceutica, Belgium), amphotericin B (Sigma-Aldrich, Spain), and caspofungin (Merck Sharp & Dome, Spain; batch no. L-743,872-003M023) were frozen and sent to each participant the same day to prepare their own trays and perform the susceptibility tests (within 3 weeks) following the NCCLS microdilution methodology (13). The same batch of RPMI 1640 (RPMI) and RPMI with 2% dextrose (RPMI-G) (Oxoid, Spain) was used. The final concentrations were 64 to 0.12 μg/ml for fluconazole and flucytosine and 16 to 0.03 μg/ml for caspofungin, amphotericin B, and itraconazole. SYO panels with amphotericin B, itraconazole, and caspofungin at final concentrations of 16 to 0.016 μg/ml and fluconazole and flucytosine at final concentrations of 64 to 0.12 μg/ml were prepared by Trek Diagnostics Systems (United Kingdom).

MICs were determined at 24 and 48 h and after 24 h in RPMI-G for the antifungal agents other than caspofungin. For each strain, 30 caspofungin MICs were recorded (5 for each test condition). The MIC endpoints were 100% growth inhibition (MIC_0) for caspofungin and amphotericin B and ≥50% growth inhibition (MIC_2) for azoles and flucytosine. SYO MIC endpoints were interpreted as the lowest concentration at which the culture remained blue or slightly purple for azoles and blue (no growth) for the other agents. On-scale and off-scale results were included in the analysis and were left unchanged. For each strain or test condition, the minimum, maximum, and geometric mean (GM) of caspofungin MICs were determined. For each method evaluated, GM MICs for all strains were calculated per center. Agreement within 2 dilutions was estimated as the percentage of strains with equivalent results between centers and/or methods.

MICs for the reference and QC strains were within the acceptable range (1, 13, 14, 17, 18). Caspofungin MICs (24 h) by SYO for *C. krusei* ATCC 6258 were 0.25 and 1 μg/ml at 48 h. Although the endpoint for caspofungin MIC was 100% of growth inhibition, the values obtained were within the established NCCLS range (1, 13) and in accordance with those of Espinel-Ingroff et al. (7). For the reference strain, caspofungin MICs (24 h) were lower by the SYO method (GM MIC, 0.068 versus 0.284 μg/ml). MICs of agents tested (Table 1) for the blood isolates were similar to those published (3, 6, 7, 18, 19); the caspofungin mutant strains were categorized as susceptible to fluconazole, itraconazole, flucytosine, and amphotericin B, in agreement with the results of Douglas et al. (4). For each

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TABLE 1. GM MICs and MIC ranges for the 34 isolates by the indicated method[a]

Species (no. of strains)	Method	GM MIC in μg/ml (range)				
		Caspofungin	Fluconazole	Itraconazole	Flucytosine	Amphotericin B
C. albicans (17)	M27-A2 (24 h)	0.806 (0.06–>16)	0.298 (0.12–64)	0.059 (0.03–16)	0.144 (0.12–1)	0.800 (0.12–2)
	M27-A2 (48 h)	1.126 (0.06–>16)	0.372 (0.12–64)	0.065 (0.03–16)	0.206 (0.12–4)	1.54 (0.25–4)
	RPMI-G (24 h)	0.899 (0.03–>16)	0.243 (0.12–64)	0.042 (0.03–0.5)	0.164 (0.12–0.5)	0.53 (0.12–4)
	SYO (24 h)	0.327 (0.03–>16)	0.546 (0.12–256)	0.08 (0.03–16)	0.086 (0.03–4)	1.78 (1–2)
	SYO (48 h)	0.669 (0.03–>16)	ND[b]	ND	ND	ND
C. parapsilosis (5)	M27-A2 (24 h)	1.180 (0.25–4)	0.292 (0.12–1)	0.057 (0.03–0.25)	0.132 (0.12–0.25)	1 (0.25–4)
	M27-A2 (48 h)	1.84 (1–16)	0.46 (0.12–1)	0.07 (0.03–0.25)	0.16 (0.12–0.5)	1.95 (0.5–4)
	RPMI-G (24 h)	1.39 (0.5–16)	0.32 (1–8)	0.05 (0.06–0.25)	0.18 (0.12–2)	0.64 (0.25–2)
	SYO (24 h)	0.59 (0.25–4)	1.14 (0.12–2)	0.11 (0.008–0.25)	0.14 (0.03–0.25)	1.51 (0.5–2)
	SYO (48 h)	1.39 (0.25–16)	ND	ND	ND	ND
C. krusei (3)	M27-A2 (24 h)	0.658 (0.12–2)	12.670 (1–32)	0.299 (0.03–1)	8 (4–16)	2 (1–4)
	M27-A2 (48 h)	0.72 (0.12–2)	27.86 (4–64)	0.46 (0.25–1)	10.08 (4–16)	2.64 (2–4)
	RPMI-G (24 h)	0.95 (0.03–2)	19.25 (1–32)	0.26 (0.06–1)	9.19 (4–64)	0.72 (0.5–4)
	SYO (24 h)	0.42 (0.03–0.5)	46.3 (32–64)	0.42 (0.25–0.5)	9.19 (8–16)	3.03 (2–4)
	SYO (48 h)	0.83 (0.12–2)	ND	ND	ND	ND
C. glabrata (3)	M27-A2 (24 h)	0.376 (0.03–2)	3.482 (2–16)	0.272 (0.06–1)	0.12 (0.12)	1.587 (1–4)
	M27-A2 (48 h)	0.41 (0.03–2)	4.19 (1–32)	0.50 (0.06–4)	0.13 (0.12–0.25)	2.00 (1–4)
	RPMI-G (24 h)	0.47 (0.03–2)	3.32 (1–16)	0.19 (0.06–1)	0.14 (0.12–0.25)	0.57 (0.12–2)
	SYO (24 h)	0.14 (0.03–0.25)	13.3 (4–128)	1.15 (0.5–16)	0.03 (0.03–0.03)	2.00 (2–2)
	SYO (48 h)	0.31 (0.12–0.5)	ND	ND	ND	ND
C. tropicalis (2)	M27-A2 (24 h)	0.435 (0.25–1)	0.38 (0.25–4)	0.098 (0.03–0.5)	0.149 (0.12–0.25)	1.515 (1–2)
	M27-A2 (48 h)	0.54 (0.25–1)	0.54 (0.25–4)	0.19 (0.03–1)	0.25 (0.12–0.5)	2.14 (1–4)
	RPMI-G (24 h)	0.50 (0.06–1)	0.29 (0.12–0.25)	0.06 (0.03–0.25)	0.15 (0.12–0.5)	0.66 (0.25–2)
	SYO (24 h)	0.14 (0.03–0.5)	0.66 (0.25–2)	0.16 (0.06–0.25)	0.03 (0.03–0.06)	2.00 (2–2)
	SYO (48 h)	0.34 (0.06–8)	ND	ND	ND	ND
C. guilliermondii (2)	M27-A2 (24 h)	0.754 (0.12–4)	0.87 (0.25–2)	0.174 (0.03–0.5)	0.12 (0.12)	1.741 (1–4)
	M27-A2 (48 h)	0.87 (0.12–2)	1.23 (0.25–4)	0.31 (0.03–1)	0.13 (0.12–0.25)	1.87 (1–4)
	RPMI-G (24 h)	0.81 (0.25–2)	0.76 (0.25–2)	0.13 (0.03–0.5)	0.14 (0.12–0.25)	0.75 (0.12–4)
	SYO (24 h)	0.16 (0.03–0.5)	2.3 (1–4)	0.25 (0.06–0.5)	0.03 (0.03–0.06)	1.83 (1–2)
	SYO (48 h)	0.32 (0.06–2)	ND	ND	ND	ND
C. famata (1)	M27-A2 (24 h)	0.435 (0.25–1)	0.329 (0.25–0.5)	0.091 (0.03–0.5)	0.12 (0.12)	1.515 (1–2)
	M27-A2 (48 h)	0.5 (0.25–1)	0.43 (0.12–1)	0.16 (0.03–0.5)	0.12 (0.12)	2.30 (2–4)
	RPMI-G (24 h)	0.5 (0.25–1)	0.22 (0.06–0.5)	0.05 (0.03–0.12)	0.14 (0.12–0.25)	0.66 (0.25–4)
	SYO (24 h)	0.12 (0.03–0.25)	0.87 (0.5–2)	0.16 (0.12–0.25)	0.03 (0.03–0.06)	2.00 (2–2)
	SYO (48 h)	0.25 (0.12–0.5)	ND	ND	ND	ND
C. lusitaniae (1)	M27-A2 (24 h)	0.5 (0.5)	0.287 (0.25–0.5)	0.103 (0.03–0.5)	0.12 (0.12)	0.569 (0.12–2)
	M27-A2 (48 h)	0.5 (0.5)	0.43 (0.25–1)	0.04 (0.03–0.12)	0.19 (0.12–0.25)	1.14 (0.25–4)
	RPMI-G (24 h)	0.5 (0.5)	0.28 (0.12–1)	0.04 (0.03–0.25)	0.12 (0.12–0.12)	0.66 (0.5–1)
	SYO (24 h)	0.08 (0.03–0.12)	0.76 (0.5–4)	0.18 (0.06–16)	0.05 (0.03–0.06)	1.32 (1–2)
	SYO (48 h)	0.10 (0.06–0.12)	ND	ND	ND	ND
All strains (25)	M27-A2 (24 h)	0.728 (0.03–>16)	0.560 (0.12–64)	0.090 (0.03–16)	0.203 (0.12–16)	1.044 (0.12–4)
	M27-A2 (48 h)	0.957 (0.03–>16)	0.772 (0.12–64)	0.15 (0.03–16)	0.260 (0.12–16)	1.769 (0.25–4)
	RPMI-G (24 h)	0.847 (0.03–>16)	0.507 (0.06–64)	0.07 (0.03–1)	0.230 (0.12–64)	0.573 (0.12–4)
	SYO (24 h)	0.228 (0.03–>16)	1.345 (0.12–256)	0.18 (0.008–16)	0.109 (0.03–16)	1.88 (0.5–4)
	SYO (48 h)	0.603 (0.03–>16)	ND	ND	ND	ND

[a] The reference and QC strains are not included.
[b] ND, not determined.

single strain, 52.7, 66.65, and 87.44% of caspofungin MIC ranges were clustered within 3 twofold dilutions (e.g., 0.25 to 1 μg/ml) by the reference, RPMI-G, and SYO methods, respectively. In general, caspofungin MICs by SYO had a tendency to increase 1 or 2 dilutions at 48 h, except for a *Candida parapsilosis* strain (shifted from 1 to 16 μg/ml in four laboratories); one MIC for *C. tropicalis* (0.25 to 8 μg/ml); and MICs for one isolate each of *C. krusei*, *Candida glabrata*, and *Candida famata* (3 to 5 dilution increments in one laboratory, yet all MICs were <2 μg/ml). In agreement with other authors (10, 16), we found that the addition of 2% dextrose to the RPMI had little effect on caspofungin MICs. Table 2 shows the caspofungin results for the strains provided by Merck Research Laboratories. All participating centers reported a MIC_0 of ≥2 μg/ml for the caspofungin-resistant mutant strains, in agreement with those reported previously (2, 4). Table 3 lists the interlaboratory

TABLE 2. Influence of the methods tested on activity of caspofungin against Merck reference strains

Strain	GM MIC (range) in μg/ml					
	M27-A2		RPMI-G		SYO	
	24 h	48 h	24 h	48 h	24 h	48 h
CAI4	0.38 (0.25–0.5)	0.38 (0.25–0.5)	0.22 (0.03–0.5)	0.53 (0.25–0.5)	0.21 (0.12–0.25)	0.38 (0.25–0.5)
CAI4R1[a]	3.48 (1–16)	9.19 (4–>16)	5.27 (1–>16)	8.00 (4–>16)	4 (2–>16)	12.12 (4–>16)
T25[a]	5.28 (4–16)	9.19 (4–>16)	6.96 (4–16)	11.31 (4–>16)	6.96 (4–8)	12.12 (4–>16)
T26[a]	6.96 (4–16)	12.12 (4–>16)	6.96 (4–16)	13.45 (4–>16)	6.06 (4–8)	16 (16)
T28	0.33 (0.12–0.25)	0.33 (0.12–0.25)	0.21 (0.06–0.5)	0.50 (0.5)	0.08 (0.06–0.12)	0.16 (0.06–0.25)
T32	0.28 (0.12–0.5)	1.74 (0.25–16)	0.16 (0.03–0.5)	0.35 (0.25–0.5)	0.07 (0.03–0.12)	0.49 (0.12–16)
NR2[a]	4 (2–16)	6.96 (4–16)	4.59 (2–16)	5.65 (4–>16)	1.13 (1–2)	6.97 (4–16)
NR3[a]	6.96 (4–16)	9.19 (4–>16)	8 (4–16)	8.00 (4–16)	4 (4)	12.12 (4–>16)
NR4[a]	2 (2)	2.29 (2–4)	4 (2–16)	5.28 (3–>16)	2 (2)	6.06 (4–16)

[a] Caspofungin mutant with low caspofungin susceptibility.

agreement among centers. The agreement of the RPMI-G method was excellent (>90%) for all antifungals except for amphotericin B (71.1%), probably because MICs were determined at 24 h. Conversely, this drug showed the highest agreement by the SYO method (97.2%). The caspofungin agreement between the reference and the SYO method was 87.16%, less than previously reported (96%) (7). This discrepancy could be due to the caspofungin-resistant strains that showed greater variability. For the other agents tested, the results were in accordance with those published (6, 7, 18).

A previous multicenter report assessed the potential value of the colorimetric method for determining caspofungin MIC in *Candida* spp.; the present study confirms these results and extends them to documented caspofungin-resistant strains (7–9). Another multicenter survey established the test conditions for the best interlaboratory MIC reproducibility and separation of *FKS1* mutants (RPMI medium, with ≥50% growth inhibition as the MIC endpoint at 24 h) (16), which was validated by Pfaller et al. (20). Although we have used 100% inhibition of growth as the caspofungin MIC endpoint, two laboratories also provided the results of 50% inhibition of growth. In general, the MICs obtained by both MIC endpoints were within 2 twofold dilutions. The greatest differences (>2 dilutions) between MICs were found with the *FKS1* mutants. Since five MICs were obtained for each strain or test condition, the GM MIC was calculated instead of the mode MIC. The same as the previous multicenter studies (7, 16), we found variability among MIC ranges in some strains, generally in *C. albicans*, with the M27-A2 method in contrast with those observed with SYO (80.2% within 2 dilutions). Our multicenter study provides further validation of the SYO method for determining caspofungin susceptibility. In addition, we have shown that the SYO method identifies strains with reduced caspofungin susceptibility, which is the main purpose of susceptibility studies. However, a clinical evaluation will be required to establish the usefulness of these results.

TABLE 3. Interlaboratory agreement with the M27-A2 method (48 h)

Antifungal agent	% Agreement ± SD	
	RPMI-G (24 h)	SYO (24 h)
Caspofungin	91.16 ± 12.22	87.16 ± 8
Fluconazole	97.2 ± 3.38	86.7 ± 3.7
Itraconazole	93.3 ± 4.64	90.5 ± 5.77
Amphotericin B	71.1 ± 33.1	97.2 ± 4.85
Flucytosine	96.6 ± 2.34	92.8 ± 6.34

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