Real-World Experience with Echinocandin MICs against *Candida* Species in a Multicenter Study of Hospitals That Routinely Perform Susceptibility Testing of Bloodstream Isolates

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Reference broth microdilution methods of *Candida* echinocandin susceptibility testing are limited by interlaboratory variability in caspofungin MICs. Recently revised Clinical and Laboratory Standards Institute (CLSI) breakpoint MICs for echinocandin nonsusceptibility may not be valid for commercial tests employed in hospital laboratories. Indeed, there are limited echinocandin susceptibility testing data from hospital laboratories. We conducted a multicenter retrospective study of 9 U.S., Australian, and New Zealand hospitals that routinely tested *Candida* bloodstream isolates for echinocandin susceptibility from 2005 to 2013. Eight hospitals used Sensititre YeastOne assays. The *Candida* spp. were *C. albicans* (*n* = 1,067), *C. glabrata* (*n* = 911), *C. parapsilosis* (*n* = 476), *C. tropicalis* (*n* = 185), *C. krusei* (*n* = 104), and others (*n* = 154). Resistance and intermediate rates were ≤1.4% and ≤3%, respectively, for each echinocandin against *C. albicans*, *C. parapsilosis*, and *C. tropicalis*. Resistance rates among *C. glabrata* and *C. krusei* isolates were ≤7.5% and ≤5.6%, respectively. Caspofungin intermediate rates among *C. glabrata* and *C. krusei* isolates were 17.8% and 46.5%, respectively, compared to ≤4.3% and ≤4.4% for other echinocandins. Using CLSI breakpoints, 18% and 19% of *C. glabrata* isolates were anidulafungin susceptible/caspofungin nonsusceptible and micafungin susceptible/caspofungin nonsusceptible, respectively; similar discrepancies were observed for 38% and 39% of *C. krusei* isolates. If only YeastOne data were considered, interhospital modal MIC variability was low (within 2 doubling dilutions for each agent). In conclusion, YeastOne assays employed in hospitals may reduce the interlaboratory variability in caspofungin MICs against *Candida* species that are observed between reference laboratories using CLSI broth microdilution methods. The significance of classifying isolates as caspofungin intermediate and anidulafungin/micafungin susceptible will require clarification in future studies.

The echinocandin antifungals are frontline agents for the treatment of candidemia and other forms of invasive candidiasis, including infections caused by non-*albicans* species, such as *Candida glabrata* and *C. krusei*, that typically exhibit diminished susceptibility to fluconazole. Recent reports suggest that echinocandin resistance may be emerging with widespread use of these agents (1, 2). Resistance is mediated primarily through mutations in hot-spot regions of *FKS* genes, which encode the echinocandin target enzyme (1,3-β-D-glucan synthase) (2). The Clinical and Laboratory Standards Institute (CLSI) and European Committee on Antimicrobial Susceptibility Testing (EUCAST) have developed reference broth microdilution methods for evaluating echinocandin MICs (3–6). Using data generated by their reference method, CLSI proposed *Candida* species-specific echinocandin clinical breakpoint MICs, which were designed to identify *fks* mutant isolates that are less likely to respond to treatment (7). Both CLSI and EUCAST reference methods, however, are limited by significant interlaboratory variability in caspofungin MICs against *C. albicans*, *C. glabrata*, *C. tropicalis*, and *C. krusei* (8, 9). In contrast, the methods have performed more reliably between lab-

oratories when testing anidulafungin and micafungin (9). As a result, investigators and EUCAST have suggested that laboratories report anidulafungin or micafungin MICs as a surrogate for the class, rather than caspofungin MICs (5, 8, 10).

The utility of the CLSI breakpoints in predicting responses to echinocandin treatment among patients with candidiasis remains unproven. In studies at a large tertiary-care center using the CLSI broth microdilution method, the vast majority of *C. glabrata* bloodstream isolates were classified as nonsusceptible to caspo-

TABLE 1 Echinocandin MIC distributions

Species	Agent	n	% with MIC of[a]:							
			≤0.06	0.125	0.25	0.5	1	2	4	≥8
C. albicans	ANF	555	93.7	3.2	1.1	**0.5**	0.5	0.7	0.2	0
	CSF	906	77.9	15.6	4.3	**1.2**	0.3	0.1	0.0	0.6
	MCF	704	88.8	4.8	3.0	**2.0**	0.4	0.9	0.0	0.1
C. glabrata	ANF	480	72.7	19.2	**2.1**	1.5	0.6	1.7	2.3	0
	CSF	754	42.3	34.0	**17.8**	1.7	0.9	0.4	0.0	2.9
	MCF	634	88.3	**4.3**	2.1	1.3	0.3	0.3	1.1	2.4
C. parapsilosis	ANF	241	1.2	2.5	3.7	14.5	32.4	42.3	**2.9**	0.4
	CSF	396	3.0	6.6	30.8	42.2	15.9	1.3	**0.3**	0.0
	MCF	329	0.3	0.3	5.2	15.5	44.1	31.6	**3.0**	0.0
C. tropicalis	ANF	90	70.0	24.4	5.6	**0.0**	0.0	0.0	0.0	0.0
	CSF	135	77.8	14.1	7.4	**0.0**	0.7	0.0	0.0	0.0
	MCF	140	92.1	3.6	3.6	**0.7**	0.0	0.0	0.0	0.0
C. krusei	ANF	45	62.2	28.9	4.4	**4.4**	0.0	0.0	0.0	0.0
	CSF	71	0.0	4.2	43.7	**46.5**	2.8	0.0	2.8	0.0
	MCF	79	13.9	49.4	31.6	**2.5**	0.0	0.0	2.5	0.0
C. lusitaniae[b]	ANF	31	12.9	51.6	22.6	12.9	0.0	0.0	0.0	0.0
	CSF	44	15.9	15.9	34.1	27.3	4.5	0.0	0.0	2.3
	MCF	44	50.0	38.6	2.3	9.1	0.0	0.0	0.0	0.0
C. dubliniensis[b]	ANF	16	50.0	37.5	0.0	0.0	0.0	0.0	0.0	12.5
	CSF	26	61.5	30.8	0.0	0.0	0.0	0.0	0.0	7.7
	MCF	26	88.5	3.8	0.0	0.0	0.0	0.0	0.0	7.7
C. guilliermondii	ANF	4	0.0	0.0	0.0	25.0	50.0	25.0	**0.0**	0.0
	CSF	14	0.0	0.0	57.1	28.6	7.1	0.0	**0.0**	7.1
	MCF	26	0.0	11.5	15.4	30.8	26.9	11.5	**3.8**	0.0
C. kefyr[b]	ANF	2	0.0	100	0.0	0.0	0.0	0.0	0.0	0.0
	CSF	3	33.3	33.3	0.0	33.3	0.0	0.0	0.0	0.0
	MCF	17	5.9	23.5	17.6	5.9	17.6	0.0	0.0	29.4

[a] Boldface denotes intermediate susceptibility; shading denotes resistance. ANF, anidulafungin; CSF, caspofungin; MCF, micafungin. When data from center 3 were removed, differences in categorical agreement were ≤4% for all species.
[b] CLSI breakpoints are not available for the species.

fungin if the recently revised CLSI breakpoints were applied ([1]). The clinical breakpoint MICs were not useful for identifying *fks* mutant isolates or predicting caspofungin responses among patients with *C. glabrata* candidemia. By receiver operating characteristic (ROC) analysis, investigators identified an institutional resistance breakpoint (>0.5 µg/ml) that both detected *fks* mutants and correlated with caspofungin treatment failures ([1]). In these studies, CLSI anidulafungin and micafungin breakpoints performed better than CLSI caspofungin breakpoints, but results were still superior using ROC-defined cutoffs (resistant, >0.06 µg/ml and >0.03 µg/ml for anidulafungin and micafungin, respectively) ([10]).

To date, echinocandin MICs have been reported largely from research or reference laboratories that used the CLSI or EUCAST broth microdilution methods. In contrast, echinocandin susceptibility data on *Candida* clinical isolates generated by hospital clinical microbiology laboratories and reported to clinicians are more limited. Furthermore, for ease of testing and time considerations, clinical microbiology laboratories often employ commercialized assays rather than reference broth microdilution methods. In gen-

eral, commercialized tests, like Sensititre YeastOne (Thermo Fisher Scientific, Waltham, MA) or Etest (AB Biodisk, Solna, Sweden), generate echinocandin MICs that show high levels of essential agreement (defined as MICs within 2 doubling dilutions) with the reference methods, but categorical agreement (i.e., agreement in the classification of an isolate as susceptible, intermediate, or resistant) may be lower ([11], [12]). Therefore, even if the uncertainties about broth microdilution methods and the echinocandin MICs they generate are resolved, it is not clear that these data will be applicable to clinical practice in most hospitals.

We conducted a multicenter study of hospitals in which clinical microbiology laboratories performed routine testing on *Candida* bloodstream isolates. Our objectives were to report on testing methods, echinocandin MICs, and susceptibility patterns using the CLSI breakpoints.

MATERIALS AND METHODS

We performed a retrospective, multicenter, descriptive analysis of echinocandin MICs against bloodstream *Candida* isolates recovered from adult patients between 1 January 2005 and 1 June 2013. The study was
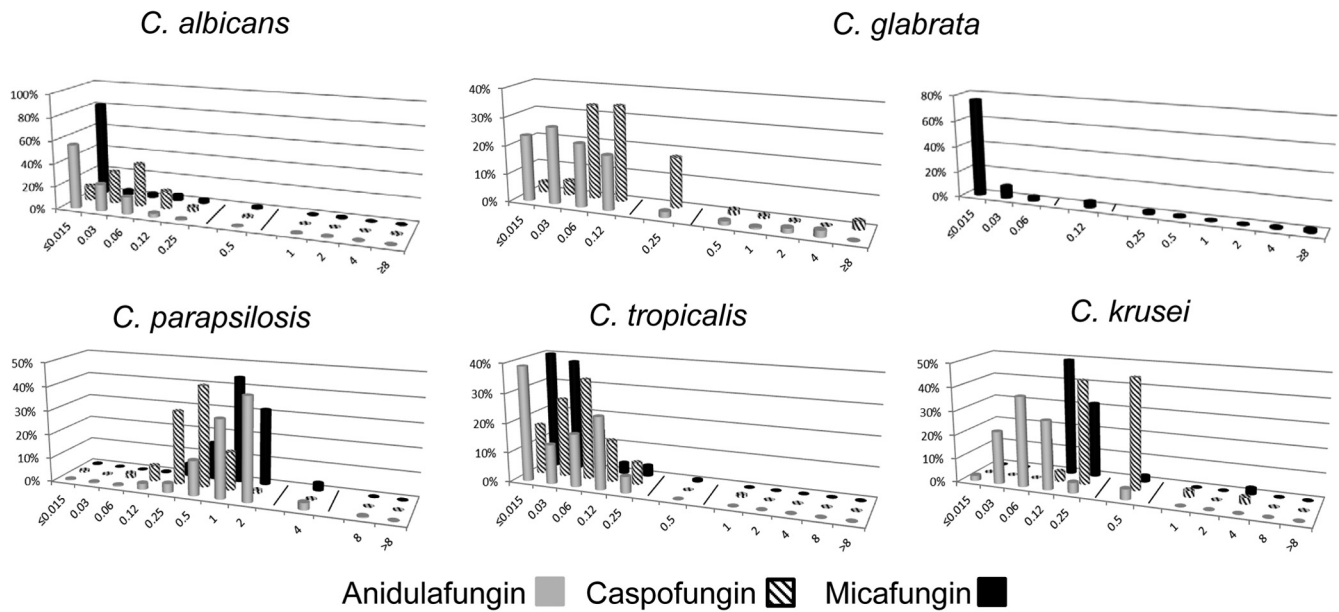
C. albicans

C. glabrata

C. parapsilosis

C. tropicalis

C. krusei

Anidulafungin ▦ Caspofungin ▧ Micafungin ▪

FIG 1 Echinocandin MIC distributions against the five most common *Candida* spp. The lines represent breakpoints. Note that if data from center 3 are removed, the modes remain the same as shown except for the following: *C. parapsilosis*, anidulafungin mode MIC of 1 µg/ml; *C. tropicalis*, anidulafungin, caspofungin, and micafungin modes of 0.12, 0.03, and 0.03 µg/ml, respectively; and *C. krusei*, anidulafungin and caspofungin modes of 0.03 and 0.25/0.5 µg/ml, respectively. Since the micafungin CLSI breakpoint for *C. glabrata* differs from those of anidulafungin and caspofungin, the distribution of micafungin MICs is presented in a separate graph.

approved by the Institutional Review Board at each center. The participating centers were Johns Hopkins Hospital, Baltimore, MD; Henry Ford Hospital, Detroit, MI; New York Presbyterian Hospital, New York, NY; Shands Teaching Hospital at the University of Florida, Gainesville, FL; University of Michigan Health System, Ann Arbor, MI; University of Utah, Salt Lake City, UT; University of Pittsburgh Medical Center, Pittsburgh, PA; Auckland City Hospital, Auckland, New Zealand; and Westmead Hospital, New South Wales, Australia. In general, *Candida* isolates were identified to the species level using morphology on cornmeal agar, yeast assimilation tests (API 20C Aux Yeast Identification System or Vitek Yeast Biochemical Card), or mass spectrometry; if the species level identification was in doubt, internal transcribed spacer (ITS) sequencing was performed. MICs were determined by the clinical microbiology laboratory at each center as part of routine patient care. Duplicate isolates from a given patient (defined as the same species within any 7-day period) were excluded. The methods used for antifungal susceptibility testing were recorded. Isolates were classified as susceptible (S), intermediate (I), or resistant (R). The following CLSI breakpoints were used for anidulafungin and caspofungin: *C. albicans*, *C. krusei*, and *C. tropicalis*, S ≤ 0.25, I = 0.5, R > 0.5 µg/ml; *C. glabrata*, S ≤ 0.125, I = 0.25, R > 0.25 µg/ml; and *C. parapsilosis*, S ≤ 2, I = 4, R > 4 µg/ml. For micafungin, the CLSI breakpoints were concordant with those for anidulafungin/caspofungin for *C. albicans*, *C. krusei*, *C. tropicalis*, and *C. parapsilosis*, but for *C. glabrata*, the breakpoints were as follows: S ≤ 0.06, I = 0.12, R > 0.125 µg/ml.

Data analysis. For statistical analysis, MICs listed as "less than or equal to" were analyzed at the given concentration; MICs noted as "less than" were analyzed at a concentration 1 doubling dilution (here referred to as "1 dilution") lower than the given concentration. Statistical analyses were performed using the PASW Statistics 18 software program (SPSS Inc., Chicago, IL). A chi-square test was used to determine differences in rates of susceptibility between caspofungin and anidulafungin or micafungin for individual *Candida* spp. Significance was defined as a *P* value of ≤0.05 (two tailed).

RESULTS

Echinocandin susceptibility testing methods and *Candida* isolates. We surveyed 15 large tertiary-care teaching hospitals throughout the United States about echinocandin susceptibility testing of *Candida* bloodstream isolates. Fifty-three percent (8/15) of the centers routinely performed such testing in their clinical microbiology laboratories. Seven of the 8 U.S. centers and one hospital each from Australia and New Zealand participated in this study. Together, the centers provided data for 2,897 isolates. MICs were not always determined for all three echinocandins. Thus, anidulafungin, caspofungin, and micafungin were tested against 1,467, 2,355, and 2,004 isolates, respectively. Eight centers utilized Sensititre YeastOne for susceptibility testing during the study period, and one center (center 3) used the CLSI broth microdilution method. Overall, the five most common *Candida* spp. tested were *C. albicans* (*n* = 1,067), *C. glabrata* (911), *C. parapsilosis* (476), *C. tropicalis* (185), and *C. krusei* (104). Other, less common *Candida* spp. were *C. lusitaniae* (57), *C. dubliniensis* (36), *C. guilliermondii* (36), *C. kefyr* (17), *C. famata* (2), *C. lipolytica* (2), and one isolate each of *C. haemulonii*, *C. nivariensis*, *C. norvegensis*, and *C. pelliculosa*. This study focused on only the 5 most common *Candida* spp.

Echinocandin MIC distributions and susceptibility patterns. Echinocandin MIC distributions against the five most common *Candida* spp. are presented in Table 1. There were no significant trends in the emergence of resistance to each of the echinocandins over time (data not shown). For *C. albicans* and *C. tropicalis*, the modal MIC of each of the agents was at least 3 dilutions below the MIC designated intermediate by the CLSI (Fig. 1). For *C. parapsilosis*, the modal caspofungin and micafungin MICs were 3 and 2 dilutions below the intermediate MIC breakpoint, respectively. The modal anidulafungin MIC was a single dilution below the

TABLE 2 MIC distributions by center

Drug	Organism	MIC	No. of isolates at center[a]:								
			1	2	4	5	6	7	8	9	3
Anidulafungin	C. albicans	≤0.008	1	8	0	0	1	NT	6	0	0
		0.015	2	44	0	42	17	NT	10	28	150
		0.03	5	22	1	29	29	NT	7	18	18
		0.06	0	13	0	23	12	NT	4	15	15
		0.125	0	3	0	3	2	NT	2	6	2
		0.25	0	0	0	2	1	NT	1	0	2
		0.5	0	0	0	0	2	NT	0	0	1
		1	0	0	0	0	0	NT	0	1	2
		2	0	0	0	0	1	NT	0	0	3
		≥4	0	0	0	0	0	NT	0	0	1
	C. glabrata	≤0.008	2	1	0	0	0	NT	1	0	0
		0.015	2	29	0	14	7	NT	4	24	28
		0.03	5	50	0	33	22	NT	6	14	0
		0.06	0	9	0	14	11	NT	2	4	67
		0.125	0	1	0	4	8	NT	0	0	79
		0.25	0	3	0	1	1	NT	0	0	5
		0.5	0	1	0	0	1	NT	0	0	5
		1	0	2	0	1	0	NT	0	0	0
		2	0	1	0	5	1	NT	0	0	1
		≥4	0	0	0	5	0	NT	0	0	6
	C. parapsilosis	≤0.008	0	0	0	0	0	NT	0	0	0
		0.015	0	0	0	0	0	NT	0	0	1
		0.03	0	0	0	0	1	NT	0	0	0
		0.06	0	0	0	0	0	NT	0	0	1
		0.125	0	0	0	4	0	NT	1	0	1
		0.25	0	2	0	5	1	NT	0	0	1
		0.5	0	6	0	11	8	NT	3	2	5
		1	1	17	0	18	11	NT	8	4	19
		2	1	9	0	21	11	NT	4	3	53
		≥4	0	0	0	0	0	NT	0	0	8
	C. tropicalis	≤0.008	0	0	0	0	0	NT	0	0	0
		0.015	0	0	0	3	3	NT	0	2	27
		0.03	0	4	0	2	4	NT	0	2	0
		0.06	0	2	0	0	7	NT	0	2	5
		0.125	0	7	0	4	6	NT	1	4	0
		0.25	0	5	0	0	0	NT	0	0	0
		0.5	0	0	0	0	0	NT	0	0	0
		1	0	0	0	0	0	NT	0	0	0
		2	0	0	0	0	0	NT	0	0	0
		≥4	0	0	0	0	0	NT	0	0	0
	C. krusei	≤0.008	0	0	0	0	0	NT	0	0	0
		0.015	0	0	0	0	0	NT	0	1	0
		0.03	1	3	0	3	0	NT	0	3	0
		0.06	0	4	1	1	1	NT	1	1	8
		0.125	0	4	0	2	0	NT	2	1	4
		0.25	0	0	0	0	0	NT	1	0	1
		0.5	0	0	0	0	1	NT	0	1	0
		1	0	0	0	0	0	NT	0	0	0
		2	0	0	0	0	0	NT	0	0	0
		≥4	0	0	0	0	0	NT	0	0	0
Caspofungin	C. albicans	≤0.015	9	5	3	1	5	0	3	4	85
		0.03	56	48	2	41	29	21	19	28	10
		0.06	75	39	1	34	37	51	18	26	56
		0.125	32	4	0	20	22	20	9	8	26
		0.25	10	1	4	2	5	5	0	1	11
		0.5	1	0	0	0	6	1	0	0	3
		1	0	0	0	0	1	0	0	0	2
		2	0	0	0	0	0	0	0	0	1
		≥4	0	0	0	1	1	2	0	1	0

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TABLE 2 (Continued)

Drug	Organism	MIC	No. of isolates at center[a]:								
			1	2	4	5	6	7	8	9	3
	C. glabrata	≤0.015	0	0	0	0	0	1	1	0	26
		0.03	3	11	2	2	5	2	2	11	1
		0.06	27	46	11	26	24	25	14	20	59
		0.125	28	39	2	29	36	65	4	9	44
		0.25	3	8	13	8	41	10	1	2	48
		0.5	0	1	0	2	3	3	0	0	4
		1	0	0	0	2	4	0	0	0	1
		2	0	2	0	0	0	0	0	0	1
		≥4	0	0	1	8	2	4	0	0	7
	C. parapsilosis	≤0.015	0	0	0	0	0	0	0	0	3
		0.03	0	0	0	0	0	0	0	0	1
		0.06	2	1	0	0	1	0	1	0	3
		0.125	4	0	0	8	1	5	2	0	6
		0.25	19	12	1	29	10	19	5	5	22
		0.5	38	21	2	15	28	13	12	4	34
		1	9	5	0	5	14	1	5	0	24
		2	0	0	0	1	0	0	0	0	4
		≥4	0	0	0	0	0	0	0	0	1
	C. tropicalis	≤0.015	0	0	1	0	8	0	0	2	12
		0.03	3	9	4	4	6	5	0	5	0
		0.06	6	6	2	4	5	5	3	1	14
		0.125	3	3	0	1	4	1	0	1	6
		0.25	0	3	0	0	5	1	0	1	0
		0.5	0	0	0	0	0	0	0	0	0
		1	1	0	0	0	0	0	0	0	0
		2	0	0	0	0	0	0	0	0	0
		≥4	0	0	0	0	0	0	0	0	0
	C. krusei	≤0.015	0	0	0	0	0	0	0	0	0
		0.03	0	0	0	0	0	0	0	0	0
		0.06	0	0	0	0	0	0	0	0	0
		0.125	0	0	0	0	0	0	0	2	1
		0.25	3	5	6	5	0	1	3	3	5
		0.5	3	6	6	1	3	5	1	1	7
		1	0	0	0	0	1	1	0	0	0
		2	0	0	0	0	0	0	0	0	0
		≥4	0	0	0	0	0	0	1	1	0
Micafungin	*C. albicans*	≤0.008	8	42	73	70	48	NT	23	36	0
		0.015	0	40	65	24	9	NT	5	23	106
		0.03	0	1	17	3	3	NT	0	4	1
		0.06	0	0	3	1	1	NT	2	1	16
		0.125	0	1	1	0	1	NT	0	0	31
		0.25	0	0	0	1	0	NT	0	0	20
		0.5	0	0	0	0	1	NT	0	0	13
		1	0	0	0	0	0	NT	0	0	3
		2	0	0	0	0	2	NT	0	1	3
		≥4	0	0	0	0	0	NT	0	0	1
	C. glabrata	≤0.008	1	8	31	13	19	NT	7	19	0
		0.015	7	64	79	49	27	NT	6	18	133
		0.03	0	15	32	7	2	NT	0	2	1
		0.06	0	4	1	0	1	NT	0	1	13
		0.125	1	1	2	0	0	NT	1	0	22
		0.25	0	1	1	1	0	NT	0	0	10
		0.5	0	0	1	1	1	NT	0	0	5
		1	0	0	0	2	0	NT	0	0	0
		2	0	1	0	0	0	NT	0	0	1
		≥4	0	1	5	8	2	NT	0	0	6
	C. parapsilosis	≤0.008	0	0	0	0	0	NT	0	0	0
		0.015	0	0	0	0	0	NT	0	0	1
		0.03	0	0	0	0	0	NT	0	0	0

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TABLE 2 (Continued)

Drug	Organism	MIC	No. of isolates at center[a]:								
			1	2	4	5	6	7	8	9	3
		0.06	0	0	0	0	0	NT	0	0	0
		0.125	0	0	0	0	0	NT	0	0	1
		0.25	0	3	3	3	2	NT	2	0	4
		0.5	0	5	9	14	5	NT	3	2	13
		1	1	16	35	25	18	NT	8	7	35
		2	1	12	23	16	6	NT	5	0	41
		≥4	0	0	6	1	1	NT	0	0	2
	C. tropicalis	≤0.008	0	0	0	0	4	NT	0	0	0
		0.015	0	2	12	2	9	NT	0	4	23
		0.03	0	12	25	5	5	NT	1	5	0
		0.06	0	5	10	2	1	NT	0	1	1
		0.125	0	0	2	0	0	NT	0	0	3
		0.25	0	0	0	0	0	NT	0	0	5
		0.5	0	0	0	1	0	NT	0	0	0
		1	0	0	0	0	0	NT	0	0	0
		2	0	0	0	0	0	NT	0	0	0
		≥4	0	0	0	0	0	NT	0	0	0
	C. krusei	≤0.008	0	0	0	0	0	NT	0	0	0
		0.015	0	0	0	0	0	NT	0	0	0
		0.03	0	0	0	0	0	NT	0	0	0
		0.06	0	3	5	1	0	NT	1	1	0
		0.125	1	2	21	4	1	NT	2	4	4
		0.25	0	5	9	1	0	NT	0	1	9
		0.5	0	1	0	0	1	NT	0	0	0
		1	0	0	0	0	0	NT	0	0	0
		2	0	0	0	0	0	NT	0	0	0
		≥4	0	0	0	0	0	NT	1	1	0

[a] Modal MICs are shaded; modes are not indicated for institutions with <5 isolates. All centers utilized Sensititre YeastOne except center 3 (CLSI broth microdilution). NT, not tested.

intermediate breakpoint (Fig. 1). Despite the differences in modal MICs, resistance rates among *C. albicans*, *C. parapsilosis*, and *C. tropicalis* isolates using CLSI breakpoints were ≤1.4% for each of the echinocandins. Intermediate rates were ≤2%, ≤3%, and ≤0.7% among *C. albicans*, *C. parapsilosis*, and *C. tropicalis* isolates, respectively, for each of the agents.

For *C. glabrata*, the modal anidulafungin and micafungin MICs were 3 dilutions below the intermediate breakpoint MICs, while the modal caspofungin MIC was only a single dilution below the intermediate breakpoint (Fig. 1). The resistance rate for caspofungin was 5.9% compared to 6.1% for anidulafungin ($P = 1.0$) and 7.5% for micafungin ($P = 0.28$). The intermediate rate for caspofungin was 17.8%, compared to 2.1% for anidulafungin and 4.3% for micafungin ($P < 0.0001$ for both). For *C. krusei*, the modal anidulafungin and micafungin MICs were 3 and 2 dilutions below the intermediate breakpoint MICs, respectively. In contrast, the modal caspofungin MIC was the same as the intermediate breakpoint. The resistance rate was 5.6% for caspofungin, compared to 0% for anidulafungin ($P = 0.16$) and 2.5% for micafungin ($P = 0.42$). The intermediate rate for caspofungin was 46.5%, compared to 4.4% for anidulafungin and 2.5% for micafungin ($P < 0.0001$ for both).

Modal MICs and susceptibility patterns remained essentially the same if data were taken only from centers that used the YeastOne assay (Table 1 and Fig. 1). For example, the resistance and intermediate rates for caspofungin among *C. glabrata* at these centers were 5.7% and 15.3%, respectively. The resistance and inter-mediate rates for caspofungin among *C. krusei* isolates were 6.9% and 44.8%, respectively.

Echinocandin MIC distributions and susceptibility patterns by center. MIC distributions by center are presented in Table 2. Modal micafungin MICs against the five most common *Candida* species were within a single dilution at each of the centers. Modal caspofungin MICs against *C. albicans*, *C. glabrata*, and *C. tropicalis* were distributed within 2 dilutions; modal MICs against other species were within a single dilution. Modal anidulafungin MICs against both *C. glabrata* and *C. tropicalis* were within 3 dilutions; modal MICs against other species were within a single dilution. Intercenter variability was lessened if only YeastOne data were considered. Modal caspofungin MICs were within 2 dilutions against *C. glabrata* and within a single dilution against other species. Modal anidulafungin and micafungin MICs were within a single dilution against each of the five most common *Candida* species.

The most notable differences in susceptibility between centers were with caspofungin against *C. glabrata* (Fig. 2). Resistance rates for the three agents were comparable across centers (range, 0 to 16%); however, intermediate rates varied more for caspofungin (5 to 45%) than anidulafungin (0 to 3.1%) or micafungin (0 to 11.5%). The interpretation of data for *C. krusei* is complicated by the small numbers of isolates at each center.

Categorical agreement among *C. glabrata* and *C. krusei* isolates. Rates of categorical agreement for caspofungin-anidula-fungin, caspofungin-micafungin, and micafungin-anidulafungin
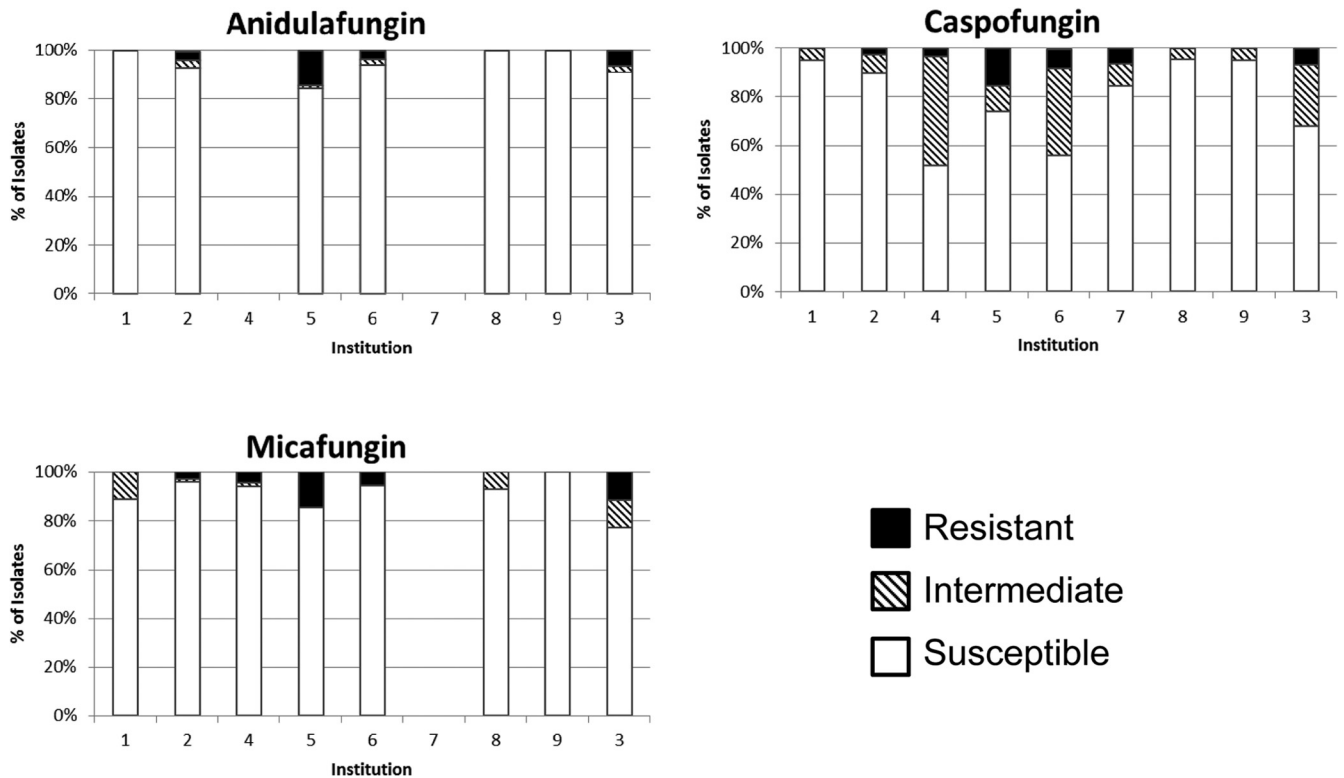
FIG 2 *C. glabrata* susceptibility pattern by specific center. Center 7 tested only caspofungin. Center 4 tested only caspofungin and micafungin.

among *C. glabrata* isolates that were tested against both agents were 80%, 72%, and 89%, respectively (Fig. 3). The most common categorical discrepancies included 18% of the *C. glabrata* isolates that were anidulafungin susceptible and caspofungin nonsusceptible (caspofungin intermediate, 16%; caspofungin resistant, 2%) and 19% of isolates that were micafungin susceptible and caspofungin nonsusceptible (caspofungin intermediate, 17%; caspofungin resistant, 2%). Eight percent of *C. glabrata* isolates were anidulafungin susceptible and micafungin nonsusceptible (micafungin intermediate, 4%; micafungin resistant, 4%). Other categorical discrepancies were noted in ≤6% of *C. glabrata* isolates.

Rates of categorical agreement for caspofungin-anidulafungin, caspofungin-micafungin, and micafungin-anidulafungin among *C. krusei* isolates that were tested against both agents were 59%, 58%, and 93%, respectively (Fig. 3). The most common categorical discrepancies included 38% of *C. krusei* isolates that were anidulafungin susceptible and caspofungin nonsusceptible (caspofungin intermediate, 36%; caspofungin resistant, 2%) and 39% of the isolates that were micafungin susceptible and caspofungin nonsusceptible (all caspofungin intermediate). Other categorical discrepancies were noted in ≤4% of *C. krusei* isolates.

DISCUSSION

To our knowledge, this is the first multicenter study to report, evaluate, and compare the echinocandin MICs against clinical *Candida* bloodstream isolates, using data that were generated as part of routine patient care by clinical microbiology laboratories and reported to clinicians. With one exception, the clinical laboratories used the commercial YeastOne assay to measure MICs, rather than the CLSI/EUCAST broth microdilution methods typ-

ically used by reference and research laboratories. An important outcome of this study was that echinocandin MICs measured by YeastOne were distributed comparably against the various *Candida* species at the different centers and that the modal MICs of each agent fell within narrow ranges (≤2 dilutions). The results for caspofungin, in particular, were remarkable, differing dramatically from a recent international study of 24 laboratories that measured caspofungin MICs using CLSI- or EUCAST-recommended broth microdilution methods (8). In the latter study, there was extraordinary modal variability in caspofungin MICs between laboratories against *C. albicans*, *C. glabrata*, *C. krusei*, and *C. tropicalis* (ranges of 4 or 5 dilutions) (8). These data corroborated smaller reports of interlaboratory variability for caspofungin MICs by CLSI broth microdilution methods, most notably for *C. glabrata* (9, 13). A prospective, multicenter study is warranted to test whether YeastOne or other commercial tests may overcome the variability in caspofungin MICs that has been reported to date, particularly since a significant percentage of clinical laboratories are employing such methodologies.

A second important finding in this study was that clinical breakpoint MICs, as proposed by CLSI based upon data obtained using CLSI broth microdilution methodology, identified large percentages of *C. glabrata* (24%) and *C. krusei* (52%) isolates as caspofungin nonsusceptible. In contrast, anidulafungin and micafungin nonsusceptibility rates were only 8% and 12% among *C. glabrata* isolates, respectively, and 4% and 5% among *C. krusei* isolates. The rates of caspofungin, anidulafungin, and micafungin resistance did not differ significantly among *C. glabrata* (≤7.5%) and *C. krusei* (≤6%) isolates. Rather, more *C. glabrata* and *C. krusei* isolates were found to be intermediate to caspofungin (18%

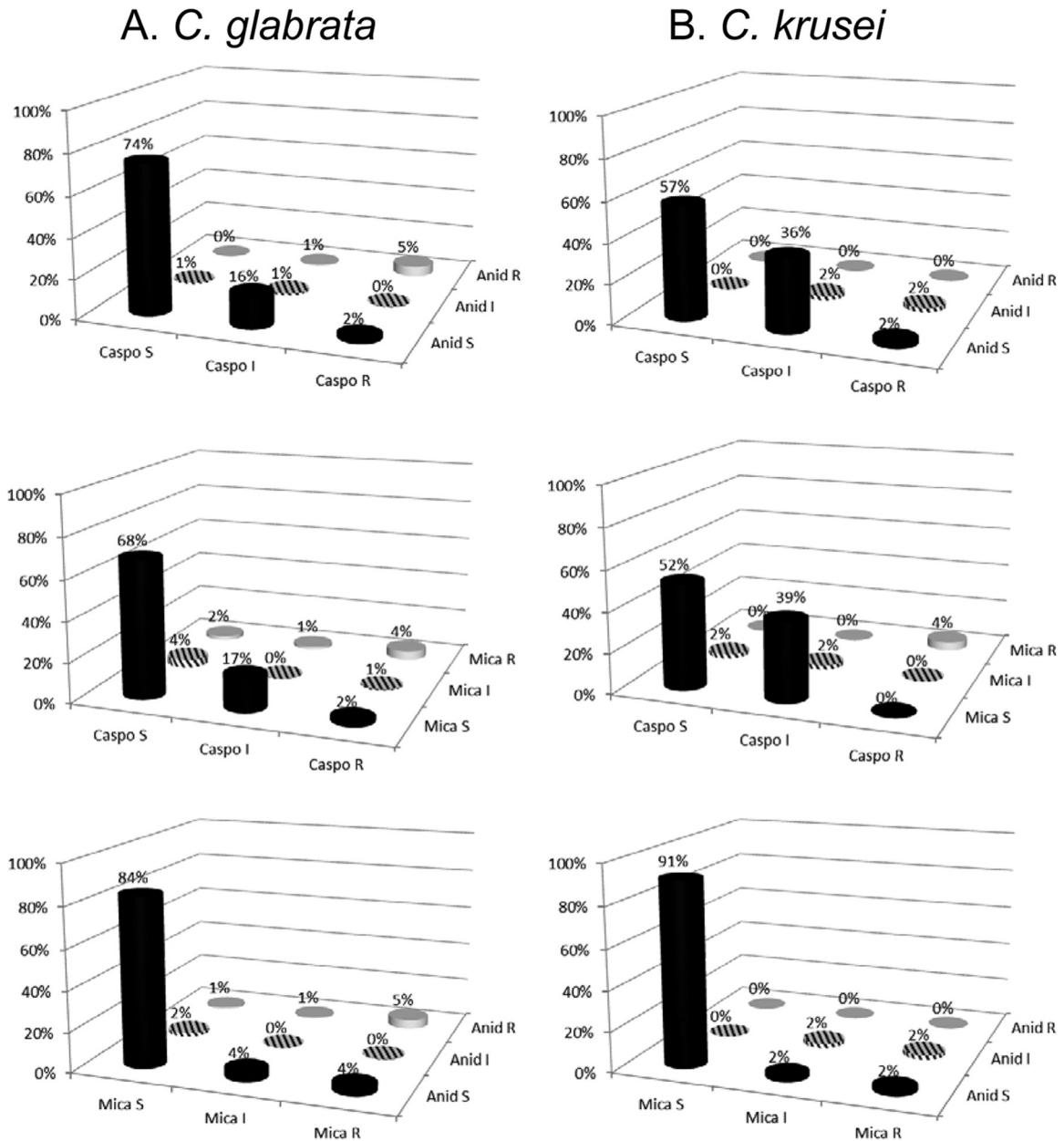## A. *C. glabrata*

B. *C. krusei*

FIG 3 Categorical agreement among *C. glabrata* and *C. krusei*. Percentages were essentially unchanged with data from center 3 removed from the analysis.

and 47%, respectively) than anidulafungin (2% and 4%, respectively) or micafungin (4% and 3%, respectively). Along these lines, the most common categorical discrepancies were described in caspofungin-intermediate *C. glabrata* and *C. krusei* isolates that were identified as anidulafungin and micafungin susceptible. For each of the agents, rates of nonsusceptibility against the other common *Candida* species were extremely low (<4%).

Overall, our data were similar to those in previous studies of commercial assays that used CLSI echinocandin breakpoints (11, 12). In one study, categorical agreement between YeastOne and broth microdilution MICs using CLSI breakpoints was excellent for all echinocandin-*Candida* species combinations, except for caspofungin-*C. glabrata* and caspofungin-*C. krusei*. Caspofungin nonsusceptibility rates by YeastOne were 16.1% and 23.5%

against *C. glabrata* and *C. krusei*, respectively, compared to <5% for other echinocandins (11). In a study evaluating Etest methodology, the use of CLSI breakpoints misclassified 33% and 73% of putatively wild-type *C. glabrata* and *C. krusei* isolates, respectively, as caspofungin nonsusceptible (12). In both studies, the overwhelming majority of the nonsusceptible isolates were identified as caspofungin intermediate (11, 12).

The significance of classifying isolates as caspofungin intermediate and anidulafungin/micafungin susceptible is not clear. It is biologically unlikely that such large percentages of *C. glabrata* and *C. krusei* isolates are nonsusceptible to caspofungin but remain susceptible to anidulafungin and micafungin. Although there is emerging evidence that certain *fks* mutations may confer differential relative resistance to echinocandins that correlates with treat-

ment responses in mice, the extent of this phenomenon and its clinical significance are unknown (14). Indeed, differences in clinical efficacy among the three echinocandins have never been demonstrated in clinical trials, and all of the members of the class are considered therapeutically equivalent (15). When setting the echinocandin breakpoints, CLSI acknowledged that as additional data are compiled, isolates currently classified as intermediate may need to be reclassified as susceptible or resistant (3).

A top priority in the future will be to validate proposed echinocandin breakpoints by systematically characterizing *FKS* genotypes of *Candida* isolates and correlating genetic variants with pharmacokinetic-pharmacodynamic data from mice and treatment responses among patients with candidiasis. Initial studies should be on *C. glabrata* and *C. krusei* isolates that are defined as caspofungin nonsusceptible but anidulafungin and micafungin susceptible by CLSI breakpoints. Afterwards, other *Candida* species that demonstrate discrepant susceptibility patterns should be evaluated. In pursuing future studies, researchers must be mindful of the fact that many, if not most, clinical microbiology laboratories will be employing commercial susceptibility assays, such as YeastOne, rather than reference broth microdilution methods. As such, comparative studies that include commercial assays will be particularly important.

It is imperative to resolve the ongoing uncertainties surrounding echinocandin susceptibility testing in *Candida* spp. and the clinical interpretation of breakpoint MICs. The performance of the YeastOne assay in this study argues that the shortcomings of CLSI/EUCAST broth microdilution methods are due to methodological considerations rather than lot-to-lot variations in caspofungin potency, as proposed previously (16). The clinical implications of failing to improve the performance of caspofungin susceptibility testing are clear from our data. Currently, MICs provided to clinicians by clinical laboratories may overstate the rates of caspofungin nonsusceptibility among *C. glabrata* and *C. krusei* isolates. Although echinocandin therapy for candidemia and other forms of invasive candidiasis caused by *C. glabrata* has not been shown to reduce mortality relative to other treatment options, it has been shown to result in higher rates of clinical response (17, 18). Therefore, if clinicians are discouraged from using caspofungin or other echinocandins due to the overstated reports of caspofungin nonsusceptibility, patient outcomes may be adversely impacted. In addition, uncertainties about the reproducibility and relevance of echinocandin susceptibility data may contribute to reluctance on the part of many hospitals to offer such testing. It was striking that only 53% of U.S. tertiary-care hospitals surveyed performed echinocandin susceptibility tests in house despite the importance of candidemia and candidiasis at such centers. The scientific implications of improving echinocandin testing are also significant, since accurate data are essential for epidemiologic studies and important in defining or determining differences in clinical activity and efficacy among these agents. In this regard, adopting either anidulafungin or micafungin susceptibility testing as a valid surrogate determinant for the echinocandin class is a less satisfactory course of action than developing and validating caspofungin testing methods that are more reliable than the current CLSI/EUCAST broth microdilution methods.

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