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Comparison of European Committee on Antimicrobial Susceptibility Testing (EUCAST) and Etest Methods with the CLSI Broth Microdilution Method for Echinocandin Susceptibility Testing of *Candida* Species[▽]

M. A. Pfaller,[1,2]* M. Castanheira,[2] D. J. Diekema,[1,3] S. A. Messer,[1,2] G. J. Moet,[2] and R. N. Jones[2,4]

Department of Pathology, University of Iowa Carver College of Medicine, Iowa City, Iowa[1]; JMI Laboratories, North Liberty, Iowa[2]; Department of Medicine, University of Iowa Carver College of Medicine, Iowa City, Iowa[3]; and Tufts University School of Medicine, Boston, Massachusetts[4]

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The antifungal broth microdilution (BMD) method of the European Committee on Antibiotic Susceptibility Testing (EUCAST) and the Etest agar diffusion method were compared with the Clinical and Laboratory Standards Institute (CLSI) BMD method M27-A3 for anidulafungin, caspofungin, and micafungin susceptibility testing of 133 clinical isolates of *Candida* species. The isolates were characterized for the presence or absence of *fks1* and/or *fks2* gene mutations and included 34 isolates of *C. glabrata* (4 mutant strains), 32 of *C. albicans* (1 mutant strain), 25 of *C. parapsilosis*, 19 of *C. guilliermondii*, 12 of *C. tropicalis* (2 mutant strains), and 11 of *C. krusei*. Excellent essential agreement (EA; within 2 dilutions) between the CLSI and EUCAST and CLSI and Etest MIC results was observed. The overall EA between the EUCAST and CLSI results ranged from 89.5% (caspofungin) to 99.2% (micafungin), whereas the EA between the Etest and CLSI results ranged from 90.2% (caspofungin) to 93.2% (anidulafungin). The categorical agreement (CA) between methods for each antifungal agent was assessed using previously determined epidemiological cutoff values (ECVs). Excellent CA (>90%) was observed for all comparisons between the EUCAST and CLSI results with the exceptions of *C. glabrata* and caspofungin (85.3%) and *C. krusei* and caspofungin (54.5%). The CA between the Etest and CLSI results was also excellent for all comparisons, with the exception of *C. krusei* and caspofungin (81.8%). All three methods were able to differentiate wild-type (WT) strains from those with *fks* mutations. With anidulafungin as the test reagent, the CLSI method identified 5 of 7 mutant strains, whereas the EUCAST method and the Etest identified 6 of 7 mutant strains. With either caspofungin or micafungin as the test reagent, the CLSI method identified all 7 mutant strains and the EUCAST method identified 6 of 7 mutant strains. The Etest identified all 7 mutant strains using caspofungin as the reagent. All three test methods showed a high level of agreement and of ability to distinguish *fks* mutant strains of *Candida* species from WT strains using each of the echinocandins.

The echinocandin class of antifungal agents is currently represented by three drugs, anidulafungin, caspofungin, and micafungin. All three of the echinocandins are acknowledged as first-line agents for the treatment of invasive candidiasis, including candidemia (29). Despite the broad utilization of these agents (5, 19, 38, 42), longitudinal surveillance studies have documented the excellent and sustained potency of all three echinocandins since the introduction of caspofungin in 2001 (6, 10, 11, 14, 15, 30–32). Although resistance to echinocandins remains uncommon among cases of invasive candidiasis, sporadic examples of clinical failure associated with elevated MICs to one or more of these agents have been reported (1, 3, 16, 26, 39). In the majority of these cases, it has been demonstrated that the clinically resistant isolates of the *Candida* species *C. albicans*, *C. glabrata*, *C. tropicalis*, and *C. krusei* have acquired resistance mutations in the *fks1* and/or *fks2* gene (encoding the glucan synthase [GS] target enzyme) associated with altered GS enzyme kinetics for all three echinocandins (3, 12, 13, 16–18, 23–25, 30–32, 39, 41).

Presently, there are two independent standards for broth microdilution (BMD) antifungal susceptibility testing of echinocandins against *Candida* species: the Clinical and Laboratory Standards Institute (CLSI) method (8) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) method (2, 34). These two methods are similar in that both use BMD, with RPMI 1640 broth as the base medium, a 24-h duration of incubation, and a prominent inhibition (50% relative to the growth control) MIC endpoint criteria (2). They differ in inoculum density (0.5×10^3 to 2.5×10^3 CFU/ml [CLSI] versus 0.5×10^5 to 2.5×10^5 CFU/ml [EUCAST]), glucose content of the medium (0.2% [CLSI] and 2.0% [EUCAST]), round-bottom (CLSI) versus flat-bottom (EUCAST) microdilution wells, and visual (CLSI) versus spectrophotometric (EUCAST) endpoint reading. In limited head-to-head comparisons of the two methods involving caspofungin, it has been shown that both produce very similar MIC values with an essential agreement (EA; ±2 dilutions) of 93 to 98% and an intraclass correlation coefficient of 0.81 (7, 36).

Although EUCAST has not proposed clinical breakpoints (CBPs) for the echinocandins and *Candida* spp., the CLSI has

* Corresponding author. Mailing address: Medical Microbiology Division, C606 GH, Department of Pathology, University of Iowa College of Medicine, Iowa City, IA 52242-1009. Phone: (319) 356-8615. Fax: (319) 356-4916. E-mail: michael-pfaller@uiowa.edu.

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established CBPs for susceptibility of ≤2 μg/ml for all three agents and all species of *Candida* (31, 32). Recently, however, it has become apparent that *Candida* spp. infections involving strains with mutations in *fks1* and/or *fks2* do not necessarily have MIC values above this CBP (1, 3, 12, 13, 16–18, 25, 31, 39). Furthermore, kinetic studies of the GS enzyme complex suggest that a lower MIC cutoff of 0.25 to 0.5 μg/ml may be more sensitive in detecting those strains with *fks1* and/or *fks2* mutations (17, 18, 41).

In contrast to both the CLSI and EUCAST BMD methods, the agar-based Etest method has been proposed as a more sensitive means of discriminating strains of *Candida* species with *fks* mutations from wild-type (WT) strains by virtue of much higher MIC results observed with the mutant strains (1, 3, 12, 13). As a result of these observations, we have used a large global collection of *Candida* species bloodstream infection (BSI) isolates to define the WT MIC distributions and to establish epidemiological cutoff values (ECVs) for each echinocandin and species of *Candida* using the CLSI BMD method (31). Notably, these ECVs are 8- to 64-fold lower than the CBP value for most *Candida* species.

A recent study by Arendrup et al. (2) examined the ability of the CLSI and EUCAST BMD methods and the agar-based Etest to discriminate *fks* mutant strains of *Candida* species from WT strains for each of the echinocandins. In this study, the authors observed that the majority of strains with *fks* mutations fell below the CBP for all three echinocandins. While Arendrup et al. (2) were able to demonstrate that all three methods were capable of differentiating *fks* mutants from WT strains, they did not provide an analysis of the EA and the categorical agreement (CA) between methods due to the fact that each method was performed in a different laboratory.

In the present study, we have utilized a collection of WT and non-WT strains of *Candida* species characterized with respect to the presence or absence of *fks* mutations to directly compare the performance of the CLSI, EUCAST, and Etest methods. In this way, we have not only determined the ability of the three methods to differentiate WT strains from those with acquired resistance mutations but also have assessed the levels of EA and CA between the methods using the previously determined ECVs from each species and echinocandin.

MATERIALS AND METHODS

Organisms. A total of 133 clinical isolates of *Candida* spp. were selected from global surveillance collections (28, 31) to represent both WT and non-WT MIC results for each of the three echinocandins. The study collection encompassed six species of *Candida*, including 34 isolates of *C. glabrata*, 32 of *C. albicans*, 25 of *C. parapsilosis*, 19 of *C. guilliermondii*, 12 of *C. tropicalis*, and 11 of *C. krusei*. Species identification was established using Vitek (bioMerieux, Hazelwood, MO), conventional reference methods (20), and 28S and internal transcribed spacer (ITS) sequencing as described elsewhere (27). All isolates were further characterized regarding the presence or absence of mutations in the hot spot (HS) regions of *fks1* and *fks2* (*C. glabrata* only) as described previously (4).

Antifungal susceptibility testing. All isolates were tested for *in vitro* susceptibility to anidulafungin, caspofungin, and micafungin using the CLSI and EUCAST BMD and Etest agar diffusion methods. Reference powders of each agent were obtained from their respective manufacturers. Personnel performing the *in vitro* susceptibility studies were blinded to the results of the *fks* resistance mutation studies.

CLSI BMD testing was performed exactly as outlined in document M27-A3 (8) by using RPMI 1640 medium with 0.2% glucose, inocula of 0.5×10^3 to 2.5×10^3 cells/ml, and incubation at 35°C. MIC values were determined visually, after 24 h of incubation, as the lowest concentration of drug that caused a significant diminution (≥50% inhibition) of growth below control levels (8, 30–32).

EUCAST BMD testing was performed exactly as outlined in document EDef 7.1 (34) and as described by Arendrup et al. (2) by using RPMI 1640 medium with 2.0% glucose, inocula of 0.5×10^5 to 2.5×10^5 cells/ml, and incubation at 35°C. MIC values were determined spectrophotometrically (at 530 nm), after 24 h of incubation, as the lowest concentration of drug that resulted in ≥50% inhibition of growth relative to that of the growth control.

Etest agar diffusion testing was performed as recommended by the manufacturer (AB Biodisk, Solna, Sweden) using RPMI 1640–2% glucose agar. The agar surface was inoculated by using a nontoxic swab dipped in a cell suspension adjusted spectrophotometrically at 530 nm to the turbidity of a 0.5 McFarland standard. After excess moisture was absorbed into the agar and the surface was completely dry (15 min at room temperature), the Etest strips were applied to each inoculated plate. The plates were incubated at 35°C and read at 24 h. The MIC was taken as the lowest concentration of antifungal agent at which the zone of inhibition intersected the strips.

Quality control. Quality control was performed as recommended in CLSI document M27-A3 (8) using *C. krusei* ATCC 6258 and *C. parapsilosis* ATCC 22019.

Analysis of results. The MIC results for each echinocandin obtained with the EUCAST and Etest methods were compared to those of the CLSI BMD method. High off-scale BMD MIC results were converted to the next highest concentration, and low off-scale MIC results were left unchanged. The Etest MIC results were rounded up to the next even $\log_2$ concentration for comparison. Discrepancies of more than 2 dilutions among MIC results were used to calculate the EA. The recently described ECVs for each agent and species were used to obtain CA percentages between the MIC values determined with the EUCAST and Etest methods and by the CLSI method. The ECV for each echinocandin and each species of *Candida* was obtained as described by EUCAST (21, 22), by considering the WT MIC distribution (population of strains with no acquired resistance mechanisms), the modal MIC for each distribution, and the inherent variability of the test (usually ±1 $\log_2$ dilution). In general, the ECV encompasses at least 95% of isolates in the WT distribution (40). Statistical determination of the ECVs was performed as described by Turnidge and colleagues (40). Whereas clinical breakpoints are used to indicate those isolates that are likely to respond to treatment with a given antimicrobial agent administered at the approved dosing regimen for that agent, the ECV can be used as the most sensitive measure of the emergence of strains with reduced susceptibility to a given agent (21, 22, 37). Very major (VM) discrepancies were identified when the CLSI BMD MIC was greater than the ECV for each agent and species and when the EUCAST BMD or Etest MIC was less than or equal to the ECV. Major discrepancies (M) were identified when the isolate's echinocandin MIC was greater than the ECV by EUCAST or Etest and less than or equal to the ECV by the CLSI method. The ability of each method to discriminate those strains with *fks* HS mutations from WT strains (MIC less than or equal to the ECV) was assessed for each of the three echinocandins.

RESULTS AND DISCUSSION

Table 1 summarizes the *in vitro* susceptibilities of 133 isolates of *Candida* spp. to anidulafungin, caspofungin, and micafungin as determined by the CLSI, EUCAST, and Etest methods. The MIC results for each agent were typical of those for each species of *Candida* (2, 28, 31). The EUCAST and Etest MIC results tended to be ≤1 2-fold dilution lower than those determined by the CLSI method for most agents and species.

The overall EA between the EUCAST and CLSI methods ranged from 89.5% (caspofungin) to 99.2% (micafungin), whereas the EA between the Etest and CLSI methods ranged from 90.2% (caspofungin) to 95.5% (micafungin) (data not shown). Of the discrepancies noted between the EUCAST and CLSI BMD results, the MIC values generated by the CLSI method were higher than those obtained by the EUCAST method in 16 of 17 (94.1%) instances (1 of 2 with anidulafungin, 14 of 14 with caspofungin, and 1 of 1 with micafungin). Likewise, of the discrepancies noted between the Etest and

TABLE 1. *In vitro* susceptibilities of *Candida* spp. isolates to anidulafungin, caspofungin, and micafungin as determined by the CLSI and EUCAST broth microdilution methods and the Etest method

Species (no. of isolates tested)	Antifungal agent	Test	MIC (μg/ml)		EA (%)
			Range	Mode	
C. albicans (32)	Anidulafungin	CLSI	≤0.008–0.12	0.03	
		EUCAST	0.015–0.25	0.015	100.0
		Etest	0.003–0.25	0.004	78.1
	Caspofungin	CLSI	≤0.008–1	0.06	
		EUCAST	0.015–1	0.015	100.0
		Etest	0.015–2	0.047	93.8
	Micafungin	CLSI	≤0.008–0.25	0.015	
		EUCAST	0.015–0.25	0.015	100.0
		Etest	0.003–0.25	0.015	100.0
C. glabrata (34)	Anidulafungin	CLSI	0.03–4	0.06	
		EUCAST	0.015–4	0.03	97.1
		Etest	0.008–8	0.012	91.2
	Caspofungin	CLSI	0.03 to >8	0.06	
		EUCAST	0.015 to >8	0.015	91.2
		Etest	0.032 to >32	0.12	94.1
	Micafungin	CLSI	≤0.008–4	0.015	
		EUCAST	0.015–4	0.015	97.1
		Etest	0.003–8	0.015	94.1
C. parapsilosis (25)	Anidulafungin	CLSI	0.25–4	2	
		EUCAST	0.12–4	2	100.0
		Etest	0.12–4	2	100.0
	Caspofungin	CLSI	0.06–2	1	
		EUCAST	0.06–1	0.5	100.0
		Etest	0.12–1	0.5	100.0
	Micafungin	CLSI	0.25–4	2	
		EUCAST	0.12–4	1	100.0
		Etest	0.12–2	0.5	100.0
C. tropicalis (12)	Anidulafungin	CLSI	≤0.008–1	0.015	
		EUCAST	0.015–1	0.015	91.7
		Etest	0.004–1	0.008	100.0
	Caspofungin	CLSI	0.015–4	0.03	
		EUCAST	0.015–2	0.03	100.0
		Etest	0.03–6	0.06	91.7
	Micafungin	CLSI	≤0.008–0.5	0.03	
		EUCAST	0.015–0.5	0.03	100.0
		Etest	0.015–1	0.03	100.0
C. krusei (11)	Anidulafungin	CLSI	0.015–0.06	0.06	
		EUCAST	0.015–0.06	0.03	100.0
		Etest	0.012–0.25	0.03	100.0
	Caspofungin	CLSI	0.03–2	0.06	
		EUCAST	0.015–0.12	0.06	63.6
		Etest	0.09–0.5	0.38	90.9
	Micafungin	CLSI	0.03–0.06	0.06	
		EUCAST	0.015–0.06	0.06	100.0
		Etest	0.05–0.12	0.06	100.0
C. guilliermondii (19)	Anidulafungin	CLSI	2–4	2	
		EUCAST	0.5–4	2	100.0
		Etest	0.25–4	1	94.7
	Caspofungin	CLSI	0.25–8	1	
		EUCAST	0.06 to >8	0.12	63.2
		Etest	0.12–0.75	0.25–0.5	84.2
	Micafungin	CLSI	0.5–4	2	
		EUCAST	0.25–4	0.5	100.0
		Etest	0.05–1	0.5	79.0

CLSI results, the MIC values generated by the CLSI method were higher than those obtained by Etest in 24 of 28 (85.7%) instances (8 of 9 with anidulafungin, 9 of 13 with caspofungin, and 6 of 6 with micafungin). The largest number of discrepancies observed with the EUCAST and CLSI comparison occurred with *C. krusei* tested against caspofungin (4 discrepant results) and with *C. guilliermondii* tested against caspofungin (7 discrepant results), whereas the largest number of discrepan-

TABLE 2. Epidemiological cutoff values for anidulafungin, caspofungin, and micafungin and six species of *Candida*[a]

Species	No. of isolates tested	ECV[b] (% of isolates that were at or below the ECV)		
		Anidulafungin	Caspofungin	Micafungin
C. albicans	4,283	0.12 (99.7)	0.12 (99.8)	0.03 (97.7)
C. glabrata	1,236	0.25 (99.4)	0.12 (98.5)	0.03 (98.2)
C. tropicalis	996	0.12 (98.9)	0.12 (99.4)	0.12 (99.1)
C. krusei	270	0.12 (99.3)	0.25 (96.3)	0.12 (97.8)
C. parapsilosis	1,238	4 (100.0)	1 (98.6)	4 (100.0)
C. guilliermondii	88	16 (100.0)	4 (95.5)	4 (98.9)

[a] The data are compiled from Pfaller et al. (31).
[b] ECV, epidemiological cutoff value (μg/ml).

cies seen with the Etest and CLSI comparison occurred with anidulafungin and *C. albicans* (7 discrepant results).

Regarding the individual *Candida* species, the EAs between the EUCAST and the CLSI BMD MIC results were >90% for all organism-drug combinations, with the exception of *C. krusei* and caspofungin (63.6% EA) and *C. guilliermondii* and caspofungin (63.2% EA). Likewise, the EAs for the Etest and CLSI comparison were >90% for all organism-drug combinations with the exception of *C. albicans* and anidulafungin (78.1% EA), *C. guilliermondii* and caspofungin (84.2% EA), and *C. guilliermondii* and micafungin (79.0% EA).

The ECVs for each echinocandin and the six *Candida* species are shown in Table 2. The ECVs were determined in a previous study of 8,271 isolates of *Candida* spp. tested against all three echinocandins using the CLSI BMD method (31). The application of these ECVs allowed both the assessment of the CA between methods and a means of discriminating WT strains (MIC less than or equal to the ECV) from those with acquired resistance mutations (MIC greater than the ECV) (2, 4, 31).

The CA between the results obtained with the EUCAST and Etest methods and those obtained by the CLSI method for each echinocandin and *Candida* species are shown in Table 3. Excellent CA was observed for all comparisons between EUCAST and CLSI, with the exceptions of *C. glabrata* and caspofungin (85.3% CA) and *C. krusei* and caspofungin (54.5% CA). In both instances, the discrepancies were due to EUCAST results that were less than or equal to the ECV when the CLSI results were greater than the ECV. A smaller number of VM discrepancies between the EUCAST and CLSI results were observed with *C. glabrata* and both anidulafungin and micafungin and with *C. parapsilosis* and caspofungin.

The CA between the Etest and CLSI results was also excellent for all comparisons, with the exception of *C. krusei* and caspofungin (81.8% CA). The only VM discrepancies between the Etest and CLSI results were seen with *C. glabrata* and both caspofungin and micafungin and with *C. guilliermondii* and caspofungin.

In our previous assessment of this collection of 133 clinical isolates of *Candida* spp. for the presence or absence of *fks* HS mutations, only 7 strains were found to have *fks* mutations (4). These strains are shown in Table 4 and included 2 strains of *C. tropicalis*, 1 of *C. albicans*, and 4 of *C. glabrata*. With the exception of one strain of *C. glabrata* with an L644W alteration in *FKS2*, these isolates all show elevated MICs to the three echinocandins, similar to those reported elsewhere (2, 3, 16, 25, 39).

The ability of each of the antifungal susceptibility test methods to differentiate those strains with acquired *fks* mutations from WT strains is shown in Table 5. As shown in Table 2, the ECVs for anidulafungin and *C. albicans*, *C. glabrata*, and *C. tropicalis* were 0.12 μg/ml, 0.25 μg/ml, and 0.12 μg/ml, respectively. Using these cutoffs, the CLSI method distinguished 5 of the 7 mutant strains from WT strains, whereas both EUCAST and Etest identified 6 of the 7 mutants as not WT. None of the three methods distinguished the strain of *C. glabrata* with the L644W *fks2* mutation from WT strains when tested against anidulafungin.

The ECVs for caspofungin and *C. albicans*, *C. glabrata*, and *C. tropicalis* were all 0.12 μg/ml (Table 2). With caspofungin as the test reagent, both the CLSI method and the Etest differentiated all 7 mutant strains from WT strains and the EUCAST method identified 6 of 7 mutant strains. Again, the mutant strain with the lowest MIC to caspofungin as determined by all three methods was the *C. glabrata* strain with the L644W *fks2* mutation.

The ECVs for micafungin and *C. albicans*, *C. glabrata*, and *C. tropicalis* were 0.03 μg/ml, 0.03 μg/ml, and 0.12 μg/ml, respectively (Table 2). Using these ECVs, the CLSI method identified all 7 mutant strains and the EUCAST and Etest methods identified 6 of the 7 mutant strains.

The *C. glabrata* strain possessing the *fks2* HS1 L644W substitution had lower MIC values for all three echinocandin agents, similar to or at the ECV (Table 4). The presence of an *fks1* HS1 mutation leading to the L644W alteration was previously detected in one strain of *C. tropicalis* and one of *C. krusei*, which displayed caspofungin MICs of 1 μg/ml and 8 μg/ml, respectively (12). The *C. tropicalis* strain possessed only the L644W alteration and had low caspofungin MIC results, whereas the *C. krusei* strain showed an additional alteration that was not located within the HS region but could be involved in the higher MIC values for caspofungin. The *C. glabrata* strain harboring the L644W mutation in the present study showed only a modest elevation of the caspofungin MIC result (0.25 μg/ml) but had lower micafungin and anidulafungin MIC values (0.06 μg/ml and 0.12 μg/ml, respectively). The role of this mutation in the resistance to different echinocandins should be further investigated.

This study both confirms and extends the observations of Arendrup et al. (2). Like Arendrup et al. (2), we found that all three methods were comparable in their ability to differentiate WT strains from those with *fks* HS mutations. Although the degree of separation between WT and mutant strains was only one dilution for two of the seven mutants, the data provide additional support for the usefulness of the previously defined ECVs. Although Arendrup et al. (2) voiced some concern regarding the use of caspofungin as a test reagent to identify mutant versus WT strains of *Candida* species, we found that caspofungin provided good separation between WT and mutant strains. To bolster these findings, we have combined the caspofungin data of Arendrup et al. (2) with those of the present study for *C. albicans*, *C. glabrata*, and *C. tropicalis* (Table 6). This provides a total of 136 isolates, 31 of which contain *fks* mutations (11 *C. albicans*, 14 *C. glabrata*, and 6 *C. tropicalis* strains), tested by all three methods. The ECV for

TABLE 3. Categorical agreement between the results of the CLSI and EUCAST broth microdilution methods and the Etest method for anidulafungin, caspofungin, and micafungin and *Candida* spp. using epidemiological cutoff values[a]

Species (no. of isolates tested)	Antifungal agent (ECV [μg/ml])	Test	No. of isolates (%) with results:		% CA	% of isolates with discrepant results that were:	
			≤ECV	>ECV		VM	M
C. albicans (32)	Anidulafungin (0.12)	CLSI	32 (100.0)	0 (0.0)			
		EUCAST	31 (96.9)	1 (3.1)	96.9	0.0	3.1
		Etest	31 (96.9)	1 (3.1)	96.9	0.0	3.1
	Caspofungin (0.12)	CLSI	31 (96.9)	1 (3.1)			
		EUCAST	31 (96.9)	1 (3.1)	100.0	0.0	0.0
		Etest	31 (96.9)	1 (3.1)	100.0	0.0	0.0
	Micafungin (0.03)	CLSI	31 (96.9)	1 (3.1)			
		EUCAST	31 (96.9)	1 (3.1)	100.0	0.0	0.0
		Etest	31 (96.9)	1 (3.1)	100.0	0.0	0.0
C. glabrata (34)	Anidulafungin (0.25)	CLSI	28 (82.4)	6 (17.6)			
		EUCAST	29 (85.3)	5 (14.7)	97.1	2.9	0.0
		Etest	27 (79.4)	7 (20.6)	97.1	0.0	2.9
	Caspofungin (0.12)	CLSI	20 (58.8)	14 (41.2)			
		EUCAST	25 (73.5)	9 (26.5)	85.3	14.7	0.0
		Etest	17 (50.0)	17 (50.0)	91.2	5.9	2.9
	Micafungin (0.03)	CLSI	25 (73.5)	9 (26.5)			
		EUCAST	28 (82.4)	6 (17.6)	91.2	8.8	0.0
		Etest	27 (79.4)	7 (20.6)	94.1	5.9	0.0
C. parapsilosis (25)	Anidulafungin (4)	CLSI	25 (100.0)	0 (0.0)			
		EUCAST	25 (100.0)	0 (0.0)	100.0	0.0	0.0
		Etest	25 (100.0)	0 (0.0)	100.0	0.0	0.0
	Caspofungin (1)	CLSI	23 (92.0)	2 (8.0)			
		EUCAST	25 (100.0)	0 (0.0)	92.0	8.0	0.0
		Etest	24 (96.0)	1 (4.0)	96.0	0.0	4.0
	Micafungin (4)	CLSI	25 (100.0)	0 (0.0)			
		EUCAST	25 (100.0)	0 (0.0)	100.0	0.0	0.0
		Etest	25 (100.0)	0 (0.0)	100.0	0.0	0.0
C. tropicalis (12)	Anidulafungin (0.12)	CLSI	10 (83.3)	2 (16.7)			
		EUCAST	10 (83.3)	2 (16.7)	100.0	0.0	0.0
		Etest	10 (83.3)	2 (16.7)	100.0	0.0	0.0
	Caspofungin (0.12)	CLSI	10 (83.3)	2 (16.7)			
		EUCAST	10 (83.3)	2 (16.7)	100.0	0.0	0.0
		Etest	10 (83.3)	2 (16.7)	100.0	0.0	0.0
	Micafungin (0.12)	CLSI	10 (83.3)	2 (16.7)			
		EUCAST	10 (83.3)	2 (16.7)	100.0	0.0	0.0
		Etest	10 (83.3)	2 (16.7)	100.0	0.0	0.0
C. krusei (11)	Anidulafungin (0.12)	CLSI	11 (100.0)	0 (0.0)			
		EUCAST	11 (100.0)	0 (0.0)	100.0	0.0	0.0
		Etest	10 (90.9)	1 (9.1)	90.9	0.0	9.1
	Caspofungin (0.25)	CLSI	6 (54.5)	5 (45.5)			
		EUCAST	11 (100.0)	0 (0.0)	54.5	45.5	0.0
		Etest	4 (36.4)	7 (63.6)	81.8	0.0	18.2
	Micafungin (0.12)	CLSI	11 (100.0)	0 (0.0)			
		EUCAST	11 (100.0)	0 (0.0)	100.0	0.0	0.0
		Etest	11 (100.0)	0 (0.0)	100.0	0.0	0.0
C. guilliermondii (19)	Anidulafungin (16)	CLSI	19 (100.0)	0 (0.0)			
		EUCAST	19 (100.0)	0 (0.0)	100.0	0.0	0.0
		Etest	19 (100.0)	0 (0.0)	100.0	0.0	0.0
	Caspofungin (4)	CLSI	18 (94.7)	1 (5.3)			
		EUCAST	17 (89.5)	2 (10.5)	94.7	0.0	5.3
		Etest	19 (100.0)	0 (0.0)	94.7	5.3	0.0
	Micafungin (4)	CLSI	19 (100.0)	0 (0.0)			
		EUCAST	19 (100.0)	0 (0.0)	100.0	0.0	0.0
		Etest	19 (100.0)	0 (0.0)	100.0	0.0	0.0

[a] ECV, epidemiological cutoff value; CA, categorical agreement; VM, very major discrepancy; M, major discrepancy.

TABLE 4. Summary of *fks* alterations detected in *Candida* spp. strains[a]

Species	Place of isolation	Mutation		MIC (μg/ml) of indicated antifungal agent in:								
				CLSI BMD			EUCAST BMD			Etest		
		fks1	*fks2*	ANF	CSF	MCF	ANF	CSF	MCF	ANF	CSF	MCF
C. tropicalis	Akron, OH	F641S		1	4	0.5	1	2	0.5	0.38	6	0.75
	Akron, OH	F641S		1	4	0.5	0.5	2	0.5	1	2	0.5
C. albicans	New York, NY	F641Y		0.12	1	0.25	0.25	1	0.25	0.25	2	0.008
C. glabrata	Osaka, Japan	NM	L644W	0.12	0.25	0.06	0.12	0.06	0.03	0.25	0.25	0.19
	Cleveland, OH	NM	S645P	4	8	2	4	8	4	1	0.75	0.5
	Akron, OH	S645P		4	8	4	2	8	2	1	16	1.5
	Detroit, MI	NM	S645P	4	8	4	4	8	4	8	>32	6

[a] The data were compiled from Castanheira et al. (4). CLSI, Clinical and Laboratory Standards Institute; EUCAST, European Committee on Antimicrobial Susceptibility Testing; BMD, broth microdilution; ANF, anidulafungin; CSF, caspofungin; MCF, micafungin; NM, no mutation.

caspofungin and all three of these species is ≤0.12 μg/ml (Table 2). Using this ECV, all 31 mutant strains are differentiated from WT strains using the CLSI and Etest methods and 30 of 31 are differentiated using the EUCAST method. Whereas the majority of strains for which the caspofungin MIC values are greater than the ECV contain *fks* mutations, there are several that do not. The nature of this decreased susceptibility to caspofungin remains to be determined. It is notable that, whereas 97 to 100% of the mutant strains are identified using a caspofungin ECV of 0.12 μg/ml, only 42 to 48% would be classified as "nonsusceptible" using the CLSI CBP for susceptibility of ≤2 μg/ml. These data are consistent with the findings of Garcia-Effron et al. (16–18) and Wiederhold et al. (41), indicating that a MIC of 0.25 to 0.5 μg/ml identified those strains with resistant GS for anidulafungin, caspofungin, and micafungin.

This study expands upon the findings of Arendrup et al. (2) in that, in addition to examining the ability of the three test methods to detect strains with *fks* mutations, we determined both the EA and CA among the methods for all three echinocandins and six species of *Candida*. Similar to that seen with the azoles (7, 9, 35), we have demonstrated excellent EA and

TABLE 5. MIC distributions of three echinocandins using the CLSI and EUCAST broth microdilution methods and the Etest method *versus* *Candida* spp. strains tested for the presence of *fks1* and/or *fks2* mutations

Antifungal agent	Species (no. of isolates tested)	Test	No. of isolates at MIC (no. of isolates showing mutation)[a]								
			≤0.03	0.06	0.12	0.25	0.5	1	2	4	≥8
Anidulafungin	*C. albicans* (32)	CLSI	**26**	**4**	**2 (1)**						
		EUCAST	**25**	**5**	**1**	1 (1)					
		Etest	**13**	**15**	**3**	1 (1)					
	C. glabrata (34)	CLSI	**7**	**15**	**4 (1)**	**2**		3		3 (3)	
		EUCAST	**22**	**3**	**3 (1)**	**1**		2	1 (1)	2 (2)	
		Etest	**24**		**2**	**1 (1)**	3	2 (2)	1		1 (1)
	C. tropicalis (12)	CLSI	**10**					2 (2)			
		EUCAST	**5**	**2**	**3**		1 (1)	1 (1)			
		Etest	**10**				1 (1)	1 (1)			
Caspofungin	*C. albicans* (32)	CLSI	**14**	**17**				1 (1)			
		EUCAST	**30**	**1**				1 (1)			
		Etest	**18**	**10**	**3**				1 (1)		
	C. glabrata (34)	CLSI	**1**	**19**		2 (1)	1	5	1	2	3 (3)
		EUCAST	**20**	**3 (1)**	**2**	1	1	1	2	1	3 (3)
		Etest	**2**	**8**	**7**	6 (1)	4	2 (1)	2	1	2 (2)
	C. tropicalis (12)	CLSI	**8**	**2**						2 (2)	
		EUCAST	**7**	**2**	**1**				2 (2)		
		Etest	**2**	**4**	**4**				1 (1)		1 (1)
Micafungin	*C. albicans* (32)	CLSI	**31**			1 (1)					
		EUCAST	**31**			1 (1)					
		Etest	**31**			1 (1)					
	C. glabrata (34)	CLSI	**25**	1 (1)	2	2	1		1 (1)	2 (2)	
		EUCAST	**28 (1)**	2	1				1 (1)	2 (2)	
		Etest	**27 (1)**	4			1 (1)		1 (1)		1 (1)
	C. tropicalis (12)	CLSI	**9**	**1**			2 (2)				
		EUCAST	**9**	**1**			2 (2)				
		Etest	**10**				1 (1)	1 (1)			

[a] Bold values indicate WT MICs.

TABLE 6. MIC distributions of caspofungin *versus Candida* spp. with and without *fks1* and/or *fks2* mutations using the CLSI and EUCAST broth microdilution methods and the Etest method[a]

Species (no. of isolates tested)	Test	No. of isolates at indicated MIC (no. of isolates showing mutation)[b]								
		≤0.03	**0.06**	**0.12**	0.25	0.5	1	2	4	≥8
C. albicans (52)	CLSI	**15**	**23**	**3**		2 (2)	1 (1)	3 (3)	2 (2)	3 (3)
	EUCAST	**30**	**1**		1	9	1 (1)	6 (6)	4 (4)	
	Etest	**22**	**15**	**4**	2 (2)			3 (3)	2 (2)	4 (4)
C. glabrata (53)	CLSI	**1**	**20**	**7**	3 (1)	1	8 (3)	5 (4)	2	6 (6)
	EUCAST	**20**	**3 (1)**	**2**	1	10	2 (1)	7 (5)	3 (2)	5 (5)
	Etest	**2**	**12**	**11**	7 (1)	4	5 (4)	5 (3)	4 (3)	3 (3)
C. tropicalis (31)	CLSI	**8**	**12**	**3**	3 (1)		1 (1)	2 (2)	2 (2)	
	EUCAST	**7**	**2**	**1**		11	4	3 (3)	3 (3)	
	Etest	**9**	**7**	**4**	5	1 (1)		2 (2)		3 (3)

[a] The data were compiled from Arendrup et al. (2) and Table 5 (this publication).
[b] MICs are in μg/ml. Bold values indicate WT MICs.

CA between the EUCAST and CLSI BMD methods for the testing of echinocandins against *Candida* species. The comparability of these results suggests that it should be possible to harmonize these approaches for testing echinocandins. Similarly, we confirm the results of previous studies regarding the excellent agreement between the Etest and CLSI methods for the testing of echinocandins (7, 26, 33). In contrast to the observations of others (1, 12, 13), we did not find the Etest to be any more sensitive than either the CLSI or EUCAST BMD method for the detection of *fks* mutants, nor did we find higher echinocandin MIC results for mutant strains tested by the Etest versus the CLSI or EUCAST method.

In conclusion, we have demonstrated high levels of EA and CA between the CLSI, EUCAST, and Etest methods for the testing of echinocandins against *Candida* species. We have confirmed the findings of Arendrup et al. (2) with regard to the ability of all three methods to discriminate strains of *Candida* species with acquired *fks* mutations from WT strains and have provided further validation of the ECVs for all three echinocandins and several species of *Candida.* These results suggest that the CLSI, EUCAST, and Etest methods may be used effectively in resistance surveillance and clinical testing of *Candida* species and echinocandins and provide additional data in support of lower CBPs for all three of these important antifungal agents.

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