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Comparison of EUCAST and CLSI Broth Microdilution Methods for the Susceptibility Testing of 10 Systemically Active Antifungal Agents against Candida spp.

AMENDED ABSTRACT

Background: The need for reproducible, clinically relevant antifungal susceptibility testing of *Candida* spp. has been promoted by the increasing number of infections, the expanding use of new and established antifungal agents, and recognition of antifungal resistance or mechanism as an important clinical problem. Currently, there are two independent standards for clinical susceptibility testing of *Candida*: the Clinical and Laboratory Standards Institute (CLSI) method and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) method. These methods have been harmonized so that there is a close agreement between MIC results obtained when testing fluconazole (FLC) against Candida.

Methods: Due to the international importance of these two methods for surveillance of antifungal resistance there is a need to continue the process of harmonization for the testing of other new and established antifungal agents. The present study examines the essential agreement (EA; $\pm \log_2$ dilutions) between the methods for testing 10 antifungal agents (amphotericin B, flucytosine [5FC], anidulafungin [ANF], caspofungin [CSF], micafungin [MCF], FLC, itraconazole, posaconazole [PSC], voriconazole [VRC], and isavuconazole [ISA]) against a collection of 357 clinical isolates of Candida spp.

Results: C. albicans (CA; 114 isolates), C. glabrata (CG, 73 isolates), C. parapsilosis (CP, 76 isolates), C. tropicalis (CT, 60 isolates) and C. krusei (CK, 34 isolates) were concurrently tested by both methods. Excellent EA between CLSI and EUCAST MIC results was observed. The overall EA between EUCAST and CLSI results ranged from 78.9% [PSC] to 99.6% [5FC]. The EA was >90% for 5FC (99.6%), CSF (96.4%), MCF (98.8%), FLC (99.2%), VRC (98.9%) and ISA (93.1%).

Conclusions: These results suggest that the CLSI and EUCAST methods provide comparable results for a wide range of antifungal agents and may be used effectively in resistance surveillance and clinical testing of the five most common species of Candida.

INTRODUCTION

The Clinical and Laboratory Standards Institute (CLSI) Subcommittee on Antifungal Susceptibility Tests has standardized the broth microdilution (BMD) reference method for testing amphotericin B, flucytosine, the triazoles (including the investigational agent isavuconazole), and the echinocandins, against Candida spp.; and most recently has validated 24-h MIC readings for all agents and has developed new species-specific clinical breakpoints (CBPs) and epidemiological cutoff values (ECVs) for these agents and several species of *Candida*. In addition to the CLSI BMD method, the only other international standard method for antifungal susceptibility testing of yeasts is that of the European Committee on Antimicrobial Susceptibility Testing (EUCAST). The similarities and (minor) differences between the two BMD methods have been discussed previously. The two methods have been harmonized so that there is a close agreement between MIC results when testing fluconazole and voriconazole against *Candida* to the extent that there are common CBPs for the two methods for some species of *Candida*.

In the present study, we examine the essential agreement (EA; MIC ± 2 log₂ dilutions) between the two methods for testing 10 antifungal agents (amphotericin B, flucytosine, anidulafungin, caspofungin, micafungin, fluconazole, isavuconazole, itraconazole, posaconazole, and voriconazole) against a collection of 357 clinical isolates of *Candida* selected to provide both WT and non-WT MIC phenotypes (using CLSI methods and ECVs) for most agents and species. We also provide an estimate of categorical agreement (CA; susceptibility results that fall within the same interpretive category) between the two methods by using the ECVs previously determined for each antifungal agent and species of Candida to categorize the isolates as WT (MIC, ≤ECV) or non-WT (MIC > ECV) as determined by each method.

METHODS

Organisms. A total of 357 clinical isolates of *Candida* spp. were selected from global surveillance collections to represent both WT and non-WT MIC results for the azoles (12.6% of fluconazole results were non-WT) and the echinocandins (6.4% of anidulafungin and micafungin results were non-WT). The study collection encompassed five species of *Candida*, including 114 isolates of *C*. albicans, 73 of C. glabrata, 76 of C. parapsilosis, 60 of C. tropicalis, and 34 of *C. krusei*. Species identification was established by conventional reference methods, and 28S and internal transcribed spacer (ITS) sequencing as described elsewhere. The isolates were stored as water suspensions until used in the study. Prior to testing, each isolate was passaged at least twice onto potato dextrose agar (Remel) and CHROMagar Candida medium (Becton Dickinson and Company, Sparks MD) to ensure purity and viability.

Antifungal susceptibility testing. All isolates were tested for *in vitro* susceptibility to amphotericin B, flucytosine, anidulafungin, caspofungin, micafungin, fluconazole, isavuconazole, itraconazole, posaconazole and voriconazole using the CLSI and EUCAST BMD 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 CLSI method compared to the EUCAST method.

CLSI BMD testing was performed exactly as outlined in the CLSI document M27-A3 by using round-bottom trays and RPMI 1640 medium with 0.2% glucose, inocula of 0.5 x 10^3 to 2.5 x 10^3 cells/ml, and incubation at 35°C. MIC values were determined visually after 24-h incubation as the lowest concentration of drug that caused complete inhibition (amphotericin B) or a significant diminution (≥50% inhibition; all other agents) of growth relative to that of the growth control.

EUCAST BMD testing was performed exactly as outlined in document EDef 7.2 by using flat-bottom trays and RPMI1640 medium with 2.0% glucose, inocula of 0.5×10^5 to 2.5 x 10^5 cells/ml. and incubation at 35°C. MIC values were determined spectrophotometrically (at 490 nm), after 24-h incubation, as the lowest concentration of drug that resulted in complete (100%, amphotericin B) or in \geq 50% (all other agents) inhibition of growth relative to that of the growth control.

<u>Quality control</u>. Quality control was performed as recommended in CLSI document M27-A3 using *C. krusei* ATCC 6258 and *C.* parapsilosis ATCC 22019.

Analysis of results. The MIC results for each triazole obtained with the EUCAST method 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. Discrepancies of more than two dilutions among MIC results were used to calculate the EA. The recently described CLSI ECVs for each agent and species (see **Table 1**) were used to obtain CA percentages between the MIC values determined with the EUCAST method and those determined by the CLSI method. The ECVs were determined as described elsewhere and can be used as the most sensitive measure of the emergence of strains with reduced susceptibility to a given agent. 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 MIC was less than or equal to the ECV. Major (M) discrepancies were identified when the isolates BMD MIC was greater than the ECV by the EUCAST method and less than or equal to the ECV by the CLSI method.

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RESULTS

- The overall EA between the EUCAST and CLSI methods ranged from 78.9% (posaconazole) to 99.6% (flucytosine).
- MIC values generated by the CLSI method were higher than those obtained by the EUCAST method for 60% (fluconazole) to 100.0% (amphotericin B flucytosine, anidulafungin, micafungin, itraconazole) of results for all agents with the exception of caspofungin where 15 of 16 discrepancies (93.8%) were due to EUCAST MICs that were higher than CLSI MIC results.
- The largest number of discrepancies observed with the EUCAST and CLSI comparison occurred with *C. glabrata* tested against anidulafungin (40 discrepant results), with C. parapsilosis tested against posaconazole (25 discrepant results) and with C. albicans and C. parapsilosis tested against itraconazole (21 and 22 discrepant results, respectively).
- EAs for individual *Candida* species between the EUCAST and CLSI BMD results were >90% for flucytosine, caspofungin, micafungin, fluconazole and voriconazole (Table 2).
- EA was >90% for both amphotericin B and anidulafungin tested against C. parapsilosis, C. tropicalis, and C. krusei, the agreement between methods was poor for both of these agents tested against *C. albicans* and *C.* glabrata.
- Overall, CA using ECVs (Table 1) between the EUCAST and CLSI methods was 95.0% with 2.5% VM and M discrepancies (data not shown). The CA was >93% for all antifungal agents tested with the exception of caspofungin (84.6%) where 10% of the results were categorize as non-WT by the EUCAST method and WT by the CLSI method.
- CAs between the EUCAST and CLSI BMD MIC result for individual Candida species were >90% for all organism-drug combinations with the exception of *C. albicans* and flucytosine (88.8%) *C. parapsilosis* and caspofungin (86.8%), *C. tropicalis* and caspofungin (73.3%), posaconazole (87.3%), and voriconazole (85.0%), and C. krusei and caspofungin (61.8%; Table 3).
- The rate of VM discrepancies was <2.0% for 25 of the 36 organism-drug combinations and that of M discrepancies was <2.0% for 33 of the 36 combinations.

Table 1. Epidemiological cutoff values (ECVs) for systemically active antifungal agents and Candida spp. determined by 24-h CLSI broth microdilution methods^a.

	Antifungal	ECV (µg/ml) ^b			Antifungal		ECV (µg/ml) ^b			
Species	agent	WT	non-WT	– Species	agent	WT	non-WT			
C. albicans	Amphotericin B	≤2	>2	C. tropicalis	Amphotericin B	≤2	>2			
	Flucytosine	≤0.5	>0.5		Flucytosine	≤0.5	>0.5			
	Anidulafungin	≤0.12	>0.12		Anidulafungin	≤0.12	>0.12			
	Caspofungin	≤0.12	>0.12		Caspofungin	≤0.12	>0.12			
	Micafungin	≤0.03	>0.03		Micafungin	≤0.12	>0.12			
	Fluconazole	≤0.5	>0.5		Fluconazole	≤2	>2			
	Itraconazole	≤0.12	>0.12		Itraconazole	≤0.5	>0.5			
	Posaconazole	≤0.06	>0.06		Posaconazole	≤0.12	>0.12			
	Voriconazole	≤0.03	>0.03		Voriconazole	≤0.06	>0.06			
C. glabrata	Amphotericin B	≤2	>2	C. krusei	Amphotericin B	≤2	>2			
	Flucytosine	≤0.5	>0.5		Flucytosine	≤32	>32			
	Anidulafungin	≤0.25	>0.25		Anidulafungin	≤0.12	>0.12			
	Caspofungin	≤0.12	>0.12		Caspofungin	≤0.25	>0.25			
	Micafungin	≤0.03	>0.03		Micafungin	≤0.12	>0.12			
	Fluconazole	≤32	>32		Fluconazole	≤64	>64			
	Itraconazole	≤2	>2		Itraconazole	≤1	>1			
	Posaconazole	≤2	>2		Posaconazole	≤0.5	>0.5			
	Voriconazole	≤0.5	>0.5		Voriconazole	≤0.5	>0.5			
C. parapsilosis	Amphotericin B	≤2	>2	a. Data comp	mpiled from references (Pfaller et al					
	Flucytosine	≤0.5	>0.5	2010a; Pfaller et al 2010c; Pfaller et al 2011d;						
	Anidulafungin	≤4	>4		and Pfaller et al, 2012a).					
	Caspofungin	≤1	>1		 ECV, epidemiological cutoff value; WT, wild- type; non-WT, non-wild-type. 					
	Micafungin	≤4	>4	type; non-						
	Fluconazole	≤2	>2							
	Itraconazole	≤0.5	>0.5							
	Posaconazole	≤0.25	>0.25							
	Voriconazole	≤0.12	>0.12							

Antifungal agent	Species (no. tested)	Test method	MIC (µc		— EA (%)
Amphotericin B	C. albicans (114)	CLSI	Range 0.12 -1	Mode 0.5	83.3
	C. glabrata (73)	EUCAST CLSI	0.03 - 2 0.12 - 2	0.12 0.5	
	C. parapsilosis (76)	EUCAST	0.03 - 1 0.25 - 1	0.12 1	75.3
		EUCAST	0.12 - 1	0.25	98.7
	C. tropicalis (60)	CLSI EUCAST	0.25 - 2 0.12 - 1	1 0.25	93.3
	C. krusei (34)	CLSI	0.5 - 2	1	91.2
lucytosine	C. albicans (89)	EUCAST CLSI	0.12 - 2 0.25 - 1	0.5	100.0
-	C. glabrata (45)	EUCAST CLSI	0.5 - 2 0.5 - 8	0.5 0.5	
		EUCAST	0.5 - 8	0.5	100.0
	C. parapsilosis (48)	CLSI EUCAST	0.5 0.5	0.5 0.5	100.0
	C. tropicalis (31)	CLSI EUCAST	0.5 - 32 0.5 - 32	0.5 0.5	100.0
	C. krusei (17)	CLSI	8 - 32	16	94.1
Anidulafungin	C. albicans (114)	EUCAST CLSI	<u>4 - 8</u> 0.008 - 2	<u> </u>	
Ū.	C. glabrata (73)	EUCAST CLSI	0.008 - 0.5 0.015 - 4	0.008 0.12	82.5
		EUCAST	0.008 - 2	0.008	45.2
	C. parapsilosis (76)	CLSI EUCAST	0.12 - 4 0.25 - 4	2 1	97.4
	C. tropicalis (60)	CLSI EUCAST	0.008 - 2 0.008 - 0.5	0.03 0.008	93.3
	C. krusei (34)	CLSI	0.03 - 2	0.06	91.2
aspofungin	C. albicans (114)	EUCAST CLSI	0.008 - 1 0.008 - 16	0.03	
		EUCAST	0.008 - 8 0.03 - 16	0.06	95.6
	C. glabrata (73)	CLSI EUCAST	0.06 - 16	0.03 0.12	94.5
	C. parapsilosis (76)	CLSI EUCAST	0.06 - 4 0.12 - 2	0.25 1	98.7
	C. tropicalis (60)	CLSI	0.015 - 4	0.03	91.7
	C. krusei (34)	EUCAST CLSI	0.06 - 4 0.06 - 8	0.12 0.25	97.1
licafungin	C. albicans (93)	EUCAST CLSI	0.12 - 16 0.008 - 0.06	0.5 0.015	
	ζ, γ	EUCAST	0.008 - 0.06	0.008	100.0
	C. glabrata (53)	CLSI EUCAST	0.008 - 2 0.008 - 0.5	0.015 0.008	100.0
	C. parapsilosis (51)	CLSI EUCAST	0.25 - 2 0.25 - 4	1 0.5	100.0
	C. tropicalis (36)	CLSI	0.015 - 1	0.03	94.4
	C. krusei (34)	EUCAST CLSI	0.008 - 0.5 0.015 - 0.25	0.008 0.12	100.0
luconazole	C. albicans (114)	EUCAST CLSI	0.03 - 0.25 0.06 - >64	0.06	
Ideonazoic		EUCAST	0.06 - >64	0.25	99.1
	C. glabrata (73)	CLSI EUCAST	0.5 - >64 0.25 - >64	8 4	95.9
	C. parapsilosis (76)	CLSI EUCAST	0.12 - >64 0.12 - >64	0.5 0.5	98.7
	C. tropicalis (60)	CLSI	0.12 - >64	0.25	100.0
	C. krusei (34)	EUCAST CLSI	0.25 - >64 16 - >64	0.5 32	
	、 <i>,</i>	EUCAST	16 - >64	32	100.0
savuconazole	C. albicans (88)	CLSI EUCAST	0.008 - 0.12 0.008 - 0.06	0.015 0.015	100.0
	C. glabrata (58)	CLSI EUCAST	0.03 - 8 0.008 - 8	0.5 0.12	69.0
	C. parapsilosis (47)	CLSI	0.008 - 0.25	0.03	100.0
	C. tropicalis (28)	EUCAST CLSI	0.008 - 0.25 0.008 - 16	0.06 0.03	
	C. krusei (16)	EUCAST CLSI	0.008 - 16 0.12 - 0.5	0.06 0.25	89.3
	、 <i>,</i>	EUCAST	0.25 - 1	0.5	100.0
Itraconazole	C. albicans (114)	CLSI EUCAST	0.008 - 1 0.008 - 0.5	0.03 0.008	81.6
	C. glabrata (73)	CLSI EUCAST	0.06 - 8 0.008 - 8	1 0.25	83.6
	C. parapsilosis (76)	CLSI	0.008 - 1	0.12	71.1
	C. tropicalis (60)	EUCAST CLSI	0.015 - 0.25 0.015 - 8	0.03 0.06	
		EUCAST	0.008 - 8	0.03	88.3
	C. krusei (34)	CLSI EUCAST	0.12 - 2 0.06 - 0.5	0.25 0.12	85.3
Posaconazole	C. albicans (110)	CLSI EUCAST	0.008 - 1 0.008 - 0.5	0.03 0.008	87.3
	C. glabrata (65)	CLSI	0.06 - 4	1	75.4
	C. parapsilosis (73)	EUCAST CLSI	0.008 - 8 0.015 - 0.5	0.25 0.06	65.8
	C. tropicalis (55)	EUCAST CLSI	0.008 - 0.12 0.015 - 8	0.015 0.03	
	,	EUCAST	0.008 - 8	0.03	89.1
	C. krusei (29)	CLSI EUCAST	0.12 - 2 0.03 - 0.5	0.25 0.06	69.0
oriconazole	C. albicans (114)	CLSI	0.008 - 2	0.008	99.1
	C. glabrata (73)	EUCAST CLSI	0.008 - 2 0.008 - 4	0.008 0.25	97.3
	C. parapsilosis (76)	EUCAST CLSI	0.008 - 8 0.008 - 1	0.12 0.015	
	/	EUCAST	0.008 - 1	0.015	100.0
	C. tropicalis (60)	CLSI EUCAST	0.008 - 16 0.015 - 8	0.03 0.03	93.3
	C. krusei (34)	CLSI EUCAST	0.06 - 1 0.12 - 1	0.25	100.0

Table 3. Categorical agreement between the results of the CLSI and EUCAST broth microdilution methods for nine systemically active antifungal agents and *Candida* spp. using epidemiological cutoff values^a

			No. of isolates (9	%) with results:		% of isolates with discrepant results that were:	
Species (no. tested)	Antifungal agent (ECV [μg/ml])	Test method	≤ECV	>ECV	CA (%)	VM	М
C. albicans (114)	Amphotericin B (2)	CLSI EUCAST	114 (100.0) 114 (100.0)	0 (0.0) 0 (0.0)	100.0	0.0	0.0
	Flucytosine ^b (0.5)	CLSI EUCAST	81 (91.0) 77 (86.5)	8 (9.0) 12 (13.5)	88.8	3.3	7.9
	Anidulafungin (0.12)	CLSI EUCAST	109 (95.6) 111 (97.4)	5 (4.4) 3 (2.6)	98.2	1.8	0.0
	Caspofungin (0.12)	CLSI EUCAST	94 (82.5) 98 (86.0)	20 (17.5) 16 (14.0)	93.0	5.2	1.8
	Micafungin ^c (0.03)	CLSI EUCAST	89 (95.7) 89 (95.7)	4 (4.3) 4 (4.3)	100.0	0.0	0.0
	Fluconazole (0.5)	CLSI EUCAST	103 (90.4) 103 (90.4)	11 (9.6) 11 (9.6)	100.0	0.0	0.0
	Itraconazole (0.12)	CLSI EUCAST	103 (90.4) 112 (98.2)	11 (9.6) 2 (1.8)	92.1	7.9	0.0
	Posaconazole ^d (0.06)	CLSI EUCAST	100 (90.9)	10 (9.1)			0.0
	Voriconazole (0.03)	CLSI	106 (96.4) 103 (90.4)	4 (3.6) 11 (9.6)	92.7	6.4	
C. glabrata (73)	Amphotericin B (2)	EUCAST CLSI	102 (89.5) 73 (100.0)	12 (10.5) 0 (0.0)	99.1	0.0	0.9
	Flucytosine ^b (0.5)	EUCAST CLSI	73 (100.0) 44 (97.8)	0 (0.0) 1 (2.2)	100.0	0.0	0.0
	Anidulafungin (0.25)	EUCAST CLSI	44 (97.8) 66 (90.4)	1 (2.2) 7 (9.6)	100.0	0.0	0.0
	Caspofungin (0.12)	EUCAST CLSI	69 (94.5) 48 (65.8)	4 (5.5) 25 (34.2)	95.9	4.1	0.0
	Micafungin ^c (0.03)	EUCAST CLSI	41 (56.2) 45 (84.9)	32 (43.8) 8 (15.1)	90.4	0.0	9.6
	Fluconazole (32)	EUCAST	45 (84.9) 62 (84.9)	8 (15.1) 11 (15.1)	100.0	0.0	0.0
		EUCAST	62 (84.9)	11 (15.1)	91.8	4.1	4.1
	Itraconazole (2)	CLSI EUCAST	67 (91.8) 71 (97.3)	6 (8.2) 2 (2.7)	94.5	5.5	0.0
	Posaconazole ^d (2)	CLSI EUCAST	63 (96.9) 63 (96.9)	2 (3.1) 2 (3.1)	93.8	3.1	3.1
	Voriconazole (0.5)	CLSI EUCAST	57 (78.1) 56 (76.7)	16 (21.9) 17 (23.3)	90.4	4.1	5.5
C. parapsilosis (76)	Amphotericin B (2)	CLSI EUCAST	76 (100.0) 76 (100.0)	0 (0.0) 0 (0.0)	100.0	0.0	0.0
	Flucytosine ^b (0.5)	CLSI EUCAST	48 (100.0) 48 (100.0)	0 (0.0) 0 (0.0)	100.0	0.0	0.0
	Anidulafungin (4)	CLSI EUCAST	76 (100.0) 75 (98.7)	0 (0.0) 1 (1.3)	98.7	0.0	1.3
	Caspofungin (1)	CLSI	66 (86.8)	10 (13.2)			
	Micafungin ^c (4)	EUCAST CLSI	75 (98.7) 51 (100.0)	1 (1.3) 0 (0.0)	86.8	13.2	1.3
	Fluconazole (2)	EUCAST CLSI	51 (100.0) 66 (86.8)	0 (0.0) 10 (13.2)	100.0	0.0	0.0
	Itraconazole (0.5)	EUCAST CLSI	67 (88.2) 75 (98.7)	9 (11.8) 1 (1.3)	98.7	1.3	0.0
	Posaconazole ^d (0.25)	EUCAST CLSI	76 (100.0) 72 (98.6)	0 (0.0) 1 (1.4)	98.7	0.0	1.3
	Voriconazole (0.12)	EUCAST CLSI	73 (100.0) 72 (94.7)	0 (0.0) 4 (5.3)	98.6	1.4	0.0
C. tropicalis (60)	Amphotericin B (2)	EUCAST	<u>71 (93.4)</u> 60 (100.0)	<u>5 (6.6)</u> 0 (0.0)	98.7	0.0	1.:
C. tropicans (60)	,	EUCAST	60 (100.0)	0 (0.0)	100.0	0.0	0.0
	Flucytosine ^b (0.5)	CLSI EUCAST	27 (87.1) 27 (87.1)	4 (12.9) 4 (12.9)	100.0	0.0	0.0
	Anidulafungin (0.12)	CLSI EUCAST	51 (85.0) 53 (88.3)	9 (15.0) 7 (11.7)	96.7	3.3	0.0
	Caspofungin (0.12)	CLSI EUCAST	40 (66.7) 28 (46.7)	20 (33.3) 32 (53.3)	73.3	3.4	23.
	Micafungin ^c (0.12)	CLSI EUCAST	33 (91.7) 34 (94.4)	3 (8.3) 2 (5.6)	97.2	2.8	0.0
	Fluconazole (2)	CLSI EUCAST	48 (80.0) 48 (80.0)	12 (20.0) 12 (20.0)	96.6	1.7	1.7
	Itraconazole (0.5)	CLSI EUCAST	56 (93.3)	4 (6.7)	96.6	1.7	1.7
	Posaconazole ^d (0.12)	CLSI	56 (93.3) 45 (81.8)	4 (6.7) 10 (18.2)			
	Voriconazole (0.06)	EUCAST CLSI	50 (90.9) 48 (80.0)	5 (9.1) 12 (20.0)	87.3	10.9	1.8
C. krusei (34)	Amphotericin B (2)	EUCAST CLSI	39 (65.0) 34 (100.0)	21 (35.0) 0 (0.0)	85.0	0.0	15.
	Flucytosine ^b (32)	EUCAST CLSI	34 (100.0) 17 (100.0)	0 (0.0) 0 (0.0)	100.0	0.0	0.0
	Anidulafungin (0.12)	EUCAST CLSI	17 (100.0) 32 (94.1)	0 (0.0) 2 (5.9)	100.0	0.0	0.0
	Caspofungin (0.25)	EUCAST	32 (94.1) 24 (70.6)	2 (5.9) 10 (29.4)	94.0	3.0	3.0
	Micafungin ^c (0.12)	EUCAST	13 (38.2) 21 (95.5)	21 (61.8) 1 (4.5)	61.8	2.9	35.
		EUCAST	21 (95.5)	1 (4.5)	91.0	4.5	4.5
	Fluconazole (64)	CLSI EUCAST	33 (97.1) 33 (97.1)	1 (2.9) 1 (2.9)	94.0	3.0	3.0
	Itraconazole (1)	CLSI EUCAST	33 (97.1) 34 (100.0)	1 (2.9) 0 (0.0)	97.1	2.9	0.0
	Posaconazole ^d (0.5)	CLSI EUCAST	27 (93.1) 29 (100.0)	2 (6.9) 0 (0.0)	93.1	6.9	0.0
	Voriconazole (0.5)	CLSI EUCAST	33 (97.1) 31 (91.2)	1 (2.9) 3 (8.8)	94.1	0.0	5.9

a. CLSI, Clinical and Laboratory Standards Institute; EUCAST, European Committee on Antimicrobial Susceptibility Testing; ECV, epidemiological cutoff value; CA, categorical agreement; VM, very major discrepancy; M, major discrepancy. Flucytosine was tested against 230 isolates (89 C. albicans, 45 C. glabrata, 48 C. parapsilosis, 31 C. tropicalis, 17 C. krusei).

Micafungin was tested against 255 isolates (93 C. albicans, 53 C. glabrata, 51 C. parapsilosis, 36 C. tropicalis, 22 C. krusei). Posaconazole was tested against 332 isolates (110 C. albicans, 65 C. glabrata, 73 C. parapsilosis, 55 C. tropicalis, 29 C. krusei).

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CONCLUSIONS

 Overall, the EUCAST MIC results tended to be ≤1 2-fold dilution lower than those determined by the CLSI method for most agents and species with the exception of caspofungin where the EUCAST MIC results tended to be 1 dilution higher than the CLSI results.

- The VM and M discrepancy rates may seem higher than normally seen in methods comparison studies due in part to the use of ECVs to assess the CA where only two categories, WT and non-WT, were employed as opposed to the use of clinical breakpoints with other comparisons where the susceptible and resistant categories are buffered by the intermediate or susceptible dose dependent categories.
- The results indicate that the EUCAST and CLSI methods produce comparable results for testing the systemically active antifungal agents against the five most common species of Candida. Problem areas include testing of amphotericin B, anidulafungin, and isavuconazole against C. glabrata, itraconazole and posaconazole against most species, and caspofungin against *C. parapsilosis, C. tropicalis,* and *C.* krusei.

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