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Comparison of the Broth Microdilution (BMD) Method of the European Committee on Antimicrobial Susceptibility Testing with the 24-Hour CLSI BMD Method for Testing Susceptibility of *Candida* Species to Fluconazole, Posaconazole, and Voriconazole by Use of Epidemiological Cutoff Values▽

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The antifungal broth microdilution (BMD) method of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) was compared with CLSI BMD method M27-A3 for fluconazole, posaconazole, and voriconazole susceptibility testing of 1,056 isolates of *Candida*. The isolates were obtained in 2009 from more than 60 centers worldwide and included 560 isolates of *C. albicans*, 175 of *C. glabrata*, 162 of *C. parapsilosis*, 124 of *C. tropicalis*, and 35 of *C. krusei*. The overall essential agreement (EA) between EUCAST and CLSI results ranged from 96.9% (voriconazole) to 98.6% (fluconazole). The categorical agreement (CA) between methods and species of *Candida* was assessed using previously determined epidemiological cutoff values (ECVs). The ECVs (expressed as μg/ml) for fluconazole, posaconazole, and voriconazole, respectively, were as follows: 0.12, 0.06, and 0.03 for *C. albicans*; 32, 2, and 0.5 for *C. glabrata*; 2, 0.25, and 0.12 for *C. parapsilosis*; 2, 0.12, and 0.06 for *C. tropicalis*; 64, 0.5, and 0.5 for *C. krusei*. Excellent CA was observed for all comparisons between the EUCAST and CLSI results for fluconazole, posaconazole, and voriconazole, respectively, for each species: 98.9%, 93.6%, and 98.6% for *C. albicans*; 96.0%, 98.9%, and 93.7% for *C. glabrata*; 90.8%, 98.1%, and 98.1% for *C. parapsilosis*; 99.2%, 99.2%, and 96.8% for *C. tropicalis*; 97.1%, 97.1%, and 97.1% for *C. krusei*. We demonstrate high levels of EA and CA between the CLSI and EUCAST BMD methods for testing of triazoles against *Candida* when the MICs were determined after 24 h and ECVs were used to differentiate wild-type (WT) from non-WT strains. These results provide additional data in favor of the harmonization of these two methods.

The triazole class of antifungal agents includes fluconazole, posaconazole, and voriconazole. Each of these agents has good *in vitro* and clinical activity against most species of *Candida* (3, 32). Despite the broad utilization of these agents in the prevention and treatment of invasive candidiasis (2, 6, 16, 34), longitudinal surveillance studies have documented the sustained potency of all three triazoles since the introduction of fluconazole in 1990 (8, 9, 18, 21, 25, 28, 31). Although resistance to the triazoles remains relatively uncommon among cases of invasive candidiasis (IC) (19, 23, 25), numerous examples of clinical failure associated with elevated MICs to one or more of these agents have been reported (1, 17, 20, 22, 23, 27). Indeed, one of the pressing concerns surrounding this class of antifungal agents is the emergence of cross-resistance within the class, particularly involving IC due to *C. glabrata* (1, 17, 19, 20, 23, 24, 35).

Currently, there are two independent standards for broth microdilution (BMD) antifungal susceptibility testing of the triazoles against *Candida* species: the Clinical and Laboratory Standards Institute (CLSI) method (5) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) method (30). The two methods are similar in that both use BMD, RPMI 1640 broth, 35 to 37°C incubation temperatures, and a prominent inhibitory (50% relative to the growth control) MIC endpoint. 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]), duration of incubation (24 and 48 h [CLSI] versus 24 h [EUCAST]), round-bottom (CLSI) versus flat-bottom (EUCAST) microdilution wells, and visual (CLSI) versus spectrophotometric (EUCAST) end point readings. Studies have shown that the two methods produce very similar fluconazole MICs, especially when both are read after 24 h of incubation, with an essential agreement (EA; ± two dilutions) of 95% and an intraclass correlation coefficient of 0.954 (4, 7, 10, 29). Very little comparative data currently exist for testing of voriconazole and posaconazole by both methods (4, 10).

In the only international multicenter (six-laboratory) study to compare CLSI and EUCAST methods for testing fluconazole, posaconazole, and voriconazole, Espinel-Ingroff et al. (10) used a well-defined panel of 71 clinical isolates of *Candida* spp. and found excellent intra- and interlaboratory reproducibility for both methods and all three triazoles and an EA between MICs read after 24 h of incubation with both methods of 95% (range, 92% to 98% by species) for fluconazole, 91% (range, 83% to 96% by species) for posaconazole, and 94% (range, 89% to 100% by species) for voriconazole. Unfortu-

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nately, due to the lack of 24-h MIC breakpoints for the CLSI method, categorical agreement (CA) at 24 h was not determined. Subsequently, the CLSI has established 24-h MIC clinical breakpoints (CBPs) for fluconazole and *Candida* spp. that are identical to those of EUCAST (susceptible [S], MIC $\leq$ 2 μg/ml; susceptible dose dependent [SDD], MIC = 4 μg/ml; resistant [R], MIC $\geq$ 8 μg/ml) for *C. albicans*, *C. tropicalis*, and *C. parapsilosis* (27).

In the interest of developing a sensitive measure to detect the emergence of resistance to both fluconazole and voriconazole, both the CLSI (25, 28) and EUCAST (11, 12) have defined the 24-h wild-type (WT) MIC distributions and epidemiological cutoff values (ECVs or ECOFFs) for the five most common species of *Candida*: *C. albicans*, *C. tropicalis*, *C. parapsilosis*, *C. glabrata*, and *C. krusei*. ECVs (24 h) for posaconazole and *Candida* have been defined by the CLSI (28) but are not yet available for the EUCAST method. The WT MIC distribution for a species is defined as the MIC distribution for isolates that exhibit no acquired or mutational resistance to the drug in question, whereas the non-WT isolates may possess acquired or mutational resistance mechanisms (11, 12, 14, 15, 25, 26, 36, 37). The upper limit to the WT distribution is defined as the ECV. Organisms with acquired resistance mechanisms may be included among those for which the MICs are higher than the ECVs (14, 15, 25, 26).

In an effort to further pursue the harmonization of the CLSI and EUCAST BMD methods for testing the triazoles and *Candida* spp., we have utilized our 2009 ARTEMIS global antifungal surveillance database (8, 28) to determine the EA between 24-h EUCAST and CLSI MICs for 1,056 clinical isolates of *Candida* species tested against fluconazole, posaconazole, and voriconazole. We also provide an estimate of the CA between the two methods by using the ECVs previously determined for each antifungal agent and species of *Candida* (25, 28). Finally, we have reanalyzed the 24-h fluconazole, posaconazole, and voriconazole MIC data from the earlier multicenter study of Espinel-Ingroff et al. (10), using the CLSI ECVs to demonstrate further the comparability of the two methods.

MATERIALS AND METHODS

Organisms. A total of 1,056 clinical isolates of *Candida* species were obtained in 2009 from more than 60 medical centers worldwide. The collection included 560 isolates of *C. albicans*, 175 of *C. glabrata*, 162 of *C. parapsilosis*, 124 of *C. tropicalis*, and 35 of *C. krusei*. All isolates were obtained from blood or other normally sterile body sites and represented individual infectious episodes. The isolates were collected at individual study sites and were sent to the University of Iowa (Iowa City, IA) for central reference laboratory identification and susceptibility testing as described previously (22, 24, 25). The isolates included in the multicenter study of Espinel-Ingroff et al. (10) were *C. albicans* (15 isolates, 90 replicates), *C. glabrata* (7 isolates, 42 replicates), *C. parapsilosis* (10 isolates, 60 replicates), *C. tropicalis* (5 isolates, 35 replicates), and *C. krusei* (10 isolates, 60 replicates). The isolates were identified by standard methods (13) and 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 fluconazole, posaconazole, and voriconazole using the CLSI and EUCAST BMD methods. The isolates included in the study of Espinel-Ingroff et al. (10) were each tested once against the three triazoles by both methods in each of six laboratories. 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 document M27-A3 (5) 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 ($\geq$50% inhibition) of growth below control levels (5, 25, 28).

EUCAST BMD testing was performed exactly as outlined in document EDef 7.1 (30) 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 $\geq$50% inhibition of growth relative to that of the growth control.

Quality control. Quality control was performed as recommended in CLSI document M27-A3 (5) 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 (25, 28) were used to obtain CA percentages between the MIC values determined with the EUCAST method and those determined by the CLSI method. The ECV for each triazole and each species of *Candida* was obtained 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 (25–28). In general, the ECV encompasses at least 95% of isolates in the WT distribution (36). The ECV can be used as the most sensitive measure of the emergence of strains with reduced susceptibility to a given agent (14, 15, 33). 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 isolate's triazole MIC was greater than the ECV by the EUCAST method and less than or equal to the ECV by the CLSI method.

The previously published study of Espinel-Ingroff et al. (10) was reanalyzed in order to compare the CA between EUCAST and CLSI for the three triazoles in the context of a multicenter study. CA between CLSI and EUCAST was assessed subsequent to the original analysis by first assigning a consensus MIC for each organism and antifungal pair based on the mode of six MIC values for each isolate (88% to 92% of MICs were with 1 $\log_2$ dilution of the mode for each isolate) as determined by each method and using the CLSI BMD ECVs to determine WT and non-WT populations.

RESULTS AND DISCUSSION

Table 1 summarizes the *in vitro* susceptibilities of 1,056 isolates of *Candida* spp. to fluconazole, posaconazole, and voriconazole as determined by the CLSI and EUCAST BMD methods read after 24 h of incubation. MIC values were achieved after 24 h of incubation for all organisms by both methods. The MIC results for each agent were typical of those for each species of *Candida* (18, 24, 31). The EUCAST MIC results tended to be one 2-fold dilution higher than those determined by the CLSI method for most agents and species. Although a one-dilution difference between methods is well within the acceptable variation for BMD methods, it should be recognized that for organism groups with MICs that tend to cluster around a breakpoint this difference could impact categorization and clinical decision making.

The overall EA between the EUCAST and CLSI methods ranged from 96.9% (voriconazole) to 98.6% (fluconazole) (Table 1). Of the discrepancies noted between the EUCAST and CLSI BMD results, the MIC values generated by EUCAST method were higher than those obtained by the CLSI method in 64 of 73 (87.7%) instances (15 of 15 with fluconazole, 16 of 25 with posaconazole, and 33 of 33 with voriconazole). The largest number of discrepancies observed with the EUCAST and CLSI comparison occurred with *C. albicans* tested against

TABLE 1. *In vitro* susceptibilities of *Candida* isolates to fluconazole, posaconazole, and voriconazole as determined by the 24-h CLSI and EUCAST broth microdilution methods

Species (no. of isolates)	Antifungal agent	Test method	MIC (μg/ml)		Essential agreement (%)
			Range	Mode	
C. albicans (560)	Fluconazole	EUCAST	0.12–32	0.25	99.3
		CLSI	0.12–16	0.12	
	Posaconazole	EUCAST	0.015–0.5	0.06	97.9
		CLSI	0.007–0.5	0.03	
	Voriconazole	EUCAST	0.007–16	0.015	98.7
		CLSI	0.007–0.25	0.007	
C. glabrata (175)	Fluconazole	EUCAST	2–128	8	97.7
		CLSI	1–256	4	
	Posaconazole	EUCAST	0.03–16	0.5	99.4
		CLSI	0.06–16	0.5	
	Voriconazole	EUCAST	0.03–16	0.25	93.1
		CLSI	0.015–8	0.12	
C. parapsilosis (162)	Fluconazole	EUCAST	0.25–128	0.5	97.5
		CLSI	0.12–128	0.5	
	Posaconazole	EUCAST	0.015–2	0.06	95.1
		CLSI	0.007–0.25	0.06	
	Voriconazole	EUCAST	0.007–2	0.015	96.9
		CLSI	0.007–4	0.007	
C. tropicalis (124)	Fluconazole	EUCAST	0.12–16	0.25	98.4
		CLSI	0.12–4	0.12	
	Posaconazole	EUCAST	0.015–0.25	0.06	98.4
		CLSI	0.015–0.12	0.06	
	Voriconazole	EUCAST	0.007–0.5	0.03	91.1
		CLSI	0.007–0.12	0.015	
C. krusei (35)	Fluconazole	EUCAST	16–128	32	97.1
		CLSI	4–32	16	
	Posaconazole	EUCAST	0.03–0.25	0.12	94.3
		CLSI	0.03–1	0.25	
	Voriconazole	EUCAST	0.12–1	0.25	94.3
		CLSI	0.06–0.25	0.12	
Total (1,056)	Fluconazole	EUCAST	0.12–128	0.25	98.6
		CLSI	0.12–256	0.12	
	Posaconazole	EUCAST	0.015–16	0.015	97.6
		CLSI	0.007–16	0.03	
	Voriconazole	EUCAST	0.007–16	0.015	96.9
		CLSI	0.007–8	0.007	

TABLE 2. ECVs for fluconazole, posaconazole, and voriconazole and five species of *Candida* based on the the 24-h CLSI and EUCAST broth microdilution methods[a]

Species	Antifungal agent	Test method	No. tested	MIC mode (μg/ml)	ECV (% ≤ECV)
C. albicans	Fluconazole	EUCAST	15,991	0.25	1 (91.9)
		CLSI	8,059	0.12	0.5 (98.1)
	Posaconazole	EUCAST	NA[b]	NA	NA
		CLSI	8,059	0.015	0.06 (98.5)
	Voriconazole	EUCAST	13,630	0.016	0.12 (97.3)
		CLSI	8,057	0.007	0.03 (98.9)
C. glabrata	Fluconazole	EUCAST	5,018	16	32 (89.7)
		CLSI	2,240	4	32 (91.5)
	Posaconazole	EUCAST	NA	NA	NA
		CLSI	2,240	0.5	2 (96.2)
	Voriconazole	EUCAST	4,836	0.25	1 (91.4)
		CLSI	2,240	0.06	0.5 (90.4)
C. parapsilosis	Fluconazole	EUCAST	2,536	0.5	2 (92.6)
		CLSI	2,117	0.5	2 (93.2)
	Posaconazole	EUCAST	NA	NA	NA
		CLSI	2,116	0.06	0.25 (99.3)
	Voriconazole	EUCAST	2,571	0.016	0.12 (95.3)
		CLSI	2,117	0.007	0.12 (97.9)
C. tropicalis	Fluconazole	EUCAST	2,229	0.5	2 (93.7)
		CLSI	1,771	0.25	2 (98.4)
	Posaconazole	EUCAST	NA	NA	NA
		CLSI	1,771	0.03	0.12 (97.6)
	Voriconazole	EUCAST	2,958	0.3	0.12 (91.4)
		CLSI	1,771	0.015	0.06 (97.2)
C. krusei	Fluconazole	EUCAST	673	32	128 (98.4)
		CLSI	473	16	64 (99.8)
	Posaconazole	EUCAST	NA	NA	NA
		CLSI	473	0.25	0.5 (99.8)
	Voriconazole	EUCAST	1,289	0.25	1 (96.8)
		CLSI	472	0.12	0.5 (99.4)

[a] EUCAST data were compiled from references 11 and 12; CLSI data were compiled in an earlier study by Pfaller et al. (28).
[b] NA, data not available.

posaconazole (12 discrepant results) and *C. glabrata* tested against voriconazole (12 discrepant results).

Regarding the individual species, the EAs between the EUCAST and the CLSI BMD MIC results were >90% for all organism-drug combinations and were >95% for all, with the exception of *C. glabrata* and voriconazole (93.1% EA), *C. tropicalis* and voriconazole (91.1% EA), and *C. krusei* and both posaconazole and voriconazole (94.3% EA each).

The ECVs for each triazole and the five species of *Candida* are shown in Table 2. The ECVs using the CLSI method were determined in a previous study of more than 16,000 isolates tested against all three agents (25, 28). For purposes of comparison we also show the ECVs for fluconazole and voriconazole, determined using the EUCAST method as reported previously (11, 12). This comparison demonstrates that both the WT MIC distributions and ECVs of the EUCAST method for the triazoles and each species of *Candida* are essentially the same as those determined by the CLSI BMD method read after 24 h of incubation, further showing the comparability of the two methods for susceptibility testing of the triazole antifungal agents. Although ECVs for posaconazole determined by the EUCAST method have not yet been published, analysis of the EUCAST data in the present study demonstrates values very close to those determined using the CLSI method: *C. albicans* ECV, 0.06 μg/ml (96.4% of results were less than or equal to the ECV), *C. glabrata* ECV, 2 μg/ml (94.3% of results were less than or equal to the ECV), *C. parapsilosis* ECV, 0.12 μg/ml (95.7% of results were less than or equal to the ECV), *C. tropicalis* ECV, 0.06 μg/ml (95.2% of results were less than or equal to the ECV), and *C. krusei* ECV, 0.25 μg/ml (100% of results were less than or equal to the ECV). The application of these ECVs allows both the assessment of the CA between methods and a means of discriminating WT strains (MICs less than or equal to the ECV) from those likely to have acquired resistance mechanisms (MIC greater than the ECV).

The CA between the results obtained with the EUCAST method and those obtained by the CLSI method for each triazole and species of *Candida* was determined by applying the CLSI ECVs shown in Table 2. Excellent CA was observed for all comparisons between the EUCAST and CLSI methods (Table 3). The only comparisons with a CA of <95% were *C. albicans* and posaconazole (93.6% CA, 3.0% VM discrepancies), *C. glabrata* and voriconazole (93.7% CA, 0.0% VM discrepancies), and *C. parapsilosis* and fluconazole (90.8% CA, 0.6% VM discrepancies). A small number of VM discrepancies were observed with voriconazole and *C. tropicalis* (0.8%) and posaconazole and *C. krusei* (2.9%).

TABLE 3. CA between the results of the 24-h CLSI and EUCAST broth microdilution methods for fluconazole, posaconazole, and voriconazole and *Candida* spp. based on ECVs

Species (no. tested)	Antifungal agent (ECV [μg/ml])	Test method	No. of isolates (%) with indicated result:		% CA	% of isolates with discrepant results that were:	
			≤ECV	>ECV		VM	M
C. albicans (560)	Fluconazole (0.5)	EUCAST	551 (98.4)	9 (1.6)	98.9	0.0	1.1
		CLSI	557 (99.5)	3 (0.5)			
	Posaconazole (0.06)	EUCAST	540 (96.4)	20 (3.6)	93.6	3.0	3.4
		CLSI	542 (96.8)	18 (3.2)			
	Voriconazole (0.03)	EUCAST	557 (99.6)	2 (0.4)	98.6	0.0	1.4
		CLSI	558 (99.8)	1 (0.2)			
C. glabrata (175)	Fluconazole (32)	EUCAST	158 (90.3)	17 (9.7)	96.0	0.6	3.4
		CLSI	163 (93.1)	12 (6.9)			
	Posaconazole (2)	EUCAST	165 (94.3)	10 (5.7)	98.9	0.0	1.1
		CLSI	167 (95.4)	8 (4.6)			
	Voriconazole (0.05)	EUCAST	148 (84.6)	27 (15.4)	93.7	0.0	6.3
		CLSI	159 (90.9)	16 (9.1)			
C. parapsilosis (162)	Fluconazole (2)	EUCAST	133 (82.1)	29 (17.9)	90.8	0.6	8.6
		CLSI	146 (90.1)	16 (9.9)			
	Posaconazole (0.25)	EUCAST	159 (98.1)	3 (1.9)	98.1	0.0	1.9
		CLSI	162 (100.0)	0 (0.0)			
	Voriconazole (0.12)	EUCAST	152 (93.8)	10 (6.2)	98.1	0.0	1.9
		CLSI	155 (95.7)	7 (4.3)			
C. tropicalis (124)	Fluconazole (2)	EUCAST	122 (98.4)	2 (1.6)	99.2	0.0	0.8
		CLSI	123 (99.2)	1 (0.8)			
	Posaconazole (0.12)	EUCAST	123 (99.2)	1 (0.8)	99.2	0.0	0.8
		CLSI	124 (100.0)	0 (0.0)			
	Voriconazole (0.06)	EUCAST	119 (96.0)	5 (4.0)	96.8	0.8	2.4
		CLSI	121 (97.6)	3 (2.4)			
C. krusei (35)	Fluconazole (64)	EUCAST	34 (97.1)	1 (2.9)	97.1	0.0	2.9
		CLSI	35 (100.0)	0 (0.0)			
	Posaconazole (0.5)	EUCAST	35 (100.0)	0 (0.0)	97.1	2.9	0.0
		CLSI	34 (97.1)	1 (2.9)			
	Voriconazole (0.5)	EUCAST	34 (97.1)	1 (2.9)	97.1	0.0	2.9
		CLSI	35 (100.0)	0 (0.0)			

A reanalysis of the 24-h MIC data for each agent and test method from the multicenter study of Espinel-Ingroff et al. (10) is shown in Table 4. We used the consensus MIC (consensus of six individual determinations) for each method and organism-antifungal agent combination and the CLSI ECVs shown in Table 2 to assess the CA between the two methods in the context of a multicenter study to support our single-center results, as shown in Table 3. In this analysis the CA was 100.0% for all comparisons, with the exceptions of *C. albicans* and posaconazole (87.0% CA) and *C. glabrata* and fluconazole (71.4% CA). The only VM discrepancies between the EUCAST and CLSI results were seen with two isolates of *C. albicans* for which the posaconazole MICs determined with the EUCAST method were were less than or equal to the ECV (WT) and those determined with the CLSI methods were greater than the ECV (non-WT) and with two isolates of *C. glabrata* for which the fluconazole MICs determined by EUCAST were were less than or equal to the ECV (WT) and those determined by CLSI were greater than the ECV (non-WT). With respect to the latter two isolates of *C. glabrata*, 4/6 and 5/6 laboratories, respectively, participating in the multicenter study reported WT fluconazole MICs by the EUCAST method and non-WT MICs by the CLSI method. No trailing growth was reported. These results provide additional support for the data shown in Tables 1 and 3 and indicate excellent quantitative and qualitative agreement between the two methods when testing all three triazoles against *Candida* spp.

There are several notable findings in this extensive comparison of the EUCAST and CLSI BMD methods for testing triazoles against *Candida* spp. First, we have demonstrated that the determination of MICs for all three triazoles after 24 h of incubation is feasible using the CLSI method. Second, we have confirmed the excellent EA between methods when testing fluconazole (24-h incubation) and extend this to include both posaconazole and voriconazole. Third, we demonstrate for the first time a strong CA between the two methods for testing fluconazole, posaconazole, and voriconazole against *Candida* spp. when the MICs are determined after 24 h of incubation. The availability of ECVs for each triazole and the five major species of *Candida* has facilitated this comparison and shows that both methods are comparable in discriminating WT from non-WT strains of *Candida*. The fact that we were able to show this relationship using data from both a single-center study and a multicenter study further strengthens the conclusion that both methods provide highly concordant results. These results

TABLE 4. Results of a multicenter evaluation of CA between the results of the 24-h CLSI and EUCAST broth microdilution methods for azoles and *Candida* spp. based on ECVs[a]

Species (no. tested)	Antifungal agent (ECV [μg/ml])	Test method	No. of isolates (%) with indicated result		% CA	% of isolates with discrepant results that were:	
			≤ECV	>ECV		VM	M
C. albicans (15)	Fluconazole (0.5)	EUCAST	6 (40.0)	9 (60.0)	100.0	0.0	0.0
		CLSI	6 (40.0)	9 (60.0)			
	Posaconazole (0.06)	EUCAST	8 (53.3)	7 (46.7)	87.0	13.0	0.0
		CLSI	6 (40.0)	9 (60.0)			
	Voriconazole (0.03)	EUCAST	6 (40.0)	9 (60.0)	100.0	0.0	0.0
		CLSI	6 (40.0)	9 (60.0)			
C. glabrata (7)	Fluconazole (32)	EUCAST	7 (100.0)	0 (0.0)	71.4	28.6	0.0
		CLSI	5 (71.4)	2 (28.6)			
	Posaconazole (2)	EUCAST	7 (100.0)	0 (0.0)	100.0	0.0	0.0
		CLSI	7 (100.0)	0 (0.0)			
	Voriconazole (0.05)	EUCAST	5 (71.4)	2 (28.6)	100.0	0.0	0.0
		CLSI	5 (71.4)	2 (28.6)			
C. parapsilosis (10)	Fluconazole (2)	EUCAST	10 (100.0)	0 (0.0)	100.0	0.0	0.0
		CLSI	10 (100.0)	0 (0.0)			
	Posaconazole (0.25)	EUCAST	10 (100.0)	0 (0.0)	100.0	0.0	0.0
		CLSI	10 (100.0)	0 (0.0)			
	Voriconazole (0.12)	EUCAST	10 (100.0)	0 (0.0)	100.0	0.0	0.0
		CLSI	10 (100.0)	0 (0.0)			
C. tropicalis (5)	Fluconazole (2)	EUCAST	4 (80.0)	1 (20.0)	100.0	0.0	0.0
		CLSI	4 (80.0)	1 (20.0)			
	Posaconazole (0.12)	EUCAST	5 (100.0)	0 (0.0)	100.0	0.0	0.0
		CLSI	5 (100.0)	0 (0.0)			
	Voriconazole (0.06)	EUCAST	4 (80.0)	1 (20.0)	100.0	0.0	0.0
		CLSI	4 (80.0)	1 (20.0)			
C. krusei (10)	Fluconazole (64)	EUCAST	10 (100.0)	0 (0.0)	100.0	0.0	0.0
		CLSI	10 (100.0)	0 (0.0)			
	Posaconazole (0.5)	EUCAST	10 (100.0)	0 (0.0)	100.0	0.0	0.0
		CLSI	10 (100.0)	0 (0.0)			
	Voriconazole (0.5)	EUCAST	10 (100.0)	0 (0.0)	100.0	0.0	0.0
		CLSI	10 (100.0)	0 (0.0)			

[a] Data were compiled from Espinel-Ingroff et al. (10).

indicate that the CLSI and EUCAST methods may be used effectively in resistance surveillance of *Candida* spp. and triazole antifungal agents and provide a major step toward eventual harmonization of the clinical breakpoints for the triazoles as determined by each method.

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