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International and Multicenter Comparison of EUCAST and CLSI M27-A2 Broth Microdilution Methods for Testing Susceptibilities of *Candida* spp. to Fluconazole, Itraconazole, Posaconazole, and Voriconazole

A. Espinel-Ingroff,[1]* F. Barchiesi,[2] M. Cuenca-Estrella,[3] M. A. Pfaller,[4] M. Rinaldi,[5] J. L. Rodriguez-Tudela,[3] and P. E. Verweij[6]

VCU Medical Center, Richmond, Virginia[1]; Public Health, Ancona, Italy[2]; Instituto de Salud Carlos III, Majadahonda, Spain[3]; University of Iowa College of Medicine, Iowa City, Iowa[4]; University of Texas Health Science Center, San Antonio, Texas[5]; and Medical Center Nijmegen, Nijmegen, The Netherlands[6]

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The aim of this study was to compare MICs of fluconazole, itraconazole, posaconazole, and voriconazole obtained by the European Committee on Antibiotic Susceptibility Testing (EUCAST) and CLSI (formerly NCCLS) methods in each of six centers for 15 *Candida albicans* (5 fluconazole-resistant and 4 susceptible-dose-dependent [S-DD] isolates), 10 *C. dubliniensis*, 7 *C. glabrata* (2 fluconazole-resistant isolates), 5 *C. guilliermondii* (2 fluconazole-resistant isolates), 10 *C. krusei*, 9 *C. lusitaniae*, 10 *C. parapsilosis*, and 5 *C. tropicalis* (1 fluconazole-resistant isolate) isolates. CLSI MICs were obtained visually at 24 and 48 h and spectrophotometric EUCAST MICs at 24 h. The agreement (within a 3-dilution range) between the methods was species, drug, and incubation time dependent and due to lower EUCAST than CLSI MICs: overall, 94 to 95% with fluconazole and voriconazole and 90 to 91% with posaconazole and itraconazole when EUCAST MICs were compared against 24-h CLSI results. The agreement was lower (85 to 94%) against 48-h CLSI endpoints. The overall interlaboratory reproducibility by each method was ≥92%. When the comparison was based on CLSI breakpoint categorization, the agreement was 68 to 76% for three of the four species that included fluconazole-resistant and S-DD isolates; 9% very major discrepancies (≤8 μg/ml versus ≥64 μg/ml) were observed among fluconazole-resistant isolates and 50% with voriconazole (≤1 μg/ml versus ≥4 μg/ml). Similar results were observed with itraconazole for seven of the eight species evaluated (28 to 77% categorical agreement). Posaconazole EUCAST MICs were also substantially lower than CLSI MIC modes (0.008 to 1 μg/ml versus 1 to ≥8 μg/ml) for some of these isolates. Therefore, the CLSI breakpoints should not be used to interpret EUCAST MIC data.

Candida spp. and *Aspergillus* spp. are responsible for the majority (80 to 90%) of fungal infections. During the last two years, two new antifungal agents (the echinocandin caspofungin and the triazole voriconazole) have been licensed for the systemic treatment of fungal infections. Other triazoles (posaconazole and ravuconazole) and echinocandins (anidulafungin and micafungin) are undergoing phase III clinical trials. The increasing number of fungal infections and new antifungal agents has underscored the need for testing the antifungal susceptibilities of fungal pathogens to these agents. The Clinical and Laboratory Standards Institute (CLSI; formerly the National Committee for Clinical Laboratory Standards [NCCLS]) has developed a reference method (CLSI [formerly NCCLS] M27-A2 document) for antifungal susceptibility testing of *Candida* spp. and *Cryptococcus neoformans* (6). Agreement has been demonstrated between CLSI results and those obtained by a broth microdilution method with the following testing guidelines (1–3, 8–10): (i) RPMI 1640 with 2% dextrose medium to enhance the growth of yeast cells; (ii) an inoculum size of 0.5×10^5 to 2.5×10^5 CFU/ml; (iii) flat-bottom microdilution trays; and (iv) 24-h spectrophotometric MICs. Based on those studies, the European Committee on Antibiotic Susceptibility Testing (EUCAST) has proposed a broth microdilution method for testing fermentative yeasts that incorporates the above testing guidelines (4). However, the utility of CLSI fluconazole, voriconazole, and itraconazole breakpoints for interpreting EUCAST MICs has not been evaluated.

The purposes of the present study were (i) to compare EUCAST and reference CLSI MICs of fluconazole, itraconazole, posaconazole, and voriconazole for 71 *Candida* isolates; (ii) to assess the reproducibility among six laboratories of MIC results obtained by each method; (iii) to determine the utility of CLSI fluconazole, itraconazole, and voriconazole breakpoints for EUCAST MIC data; and (iv) to identify substantial differences (>3 dilutions) between the methods for posaconazole MICs. Five to 15 isolates of *Candida albicans*, *C. dubliniensis*, *C. glabrata*, *C. guilliermondii*, *C. krusei*, *C. lusitaniae*, *C. parapsilosis*, and *C. tropicalis* were evaluated in each center.

* Corresponding author. Mailing address: VCU Medical Center, Medical Mycology Research Laboratory, 1101 E. Marshall St., Sanger Hall Room 7049, P. O. Box 980049, Richmond, VA 23298-0049. Phone: (804) 828-9711. Fax: (804) 828-3097. E-mail: avingrof@hsc.vcu.edu.

MATERIALS AND METHODS

Study design. The study was designed to compare MICs obtained by the proposed EUCAST microdilution method (4) with those obtained by the CLSI

TABLE 1. Agreement between EUCAST and CLSI MICs for *Candida* spp. in six laboratories[a]

Fungus (no. of values)	Antifungal agent	% Agreement between:		% Interlaboratory agreement for:	
		E and N at 24 h	E and N at 48 h	E	N
C. albicans (90)	Fluconazole	92	92	94	97
	Itraconazole	84	80	93	81
	Posaconazole	89	85	91	92
	Voriconazole	90	87	94	90
C. dubliniensis (58)	Fluconazole	98	98	95	95
	Itraconazole	95	92	98	93
	Posaconazole	93	90	98	97
	Voriconazole	98	98	98	100
C. glabrata (42)	Fluconazole	92	81	95	98
	Itraconazole	90	77	86	93
	Posaconazole	90	86	88	95
	Voriconazole	92	83	90	86
C. guilliermondii (30)	Fluconazole	92	90	97	93
	Itraconazole	90	82	96	97
	Posaconazole	95	100	96	97
	Voriconazole	100	100	100	97
C. krusei (60)	Fluconazole	97	97	93	100
	Itraconazole	90	86	88	100
	Posaconazole	88	84	90	98
	Voriconazole	93	92	85	95
C. lusitaniae (53)	Fluconazole	98	98	98	100
	Itraconazole	93	89	96	100
	Posaconazole	95	93	100	98
	Voriconazole	98	98	98	100
C. parapsilosis (60)	Fluconazole	98	98	98	97
	Itraconazole	94	94	94	98
	Posaconazole	96	98	98	100
	Voriconazole	100	100	95	97
C. tropicalis (28)	Fluconazole	93	93	89	90
	Itraconazole	92	67	96	100
	Posaconazole	83	79	96	100
	Voriconazole	89	89	89	97
All organisms (421)	Fluconazole	95	94	95	97
	Itraconazole	90	85	93	93
	Posaconazole	91	90	92	96
	Voriconazole	94	92	93	95

[a] Percent agreements of MICs between the methods within a 3-dilution range (e.g., 0.25, 0.5, and 1 μg/ml) in six laboratories, regardless of categorization, and agreements of MIC results by each method among the six centers are given. N, CLSI reference method for yeasts (M27-A2 document); E, EUCAST method (7.1 document). CLSI and EUCAST broth microdilution methods were performed according to their respective testing guidelines.

(formerly NCCLS) reference M27-A2 broth microdilution method (6) in six independent laboratories. Each laboratory tested the same panel of 71 coded isolates of *Candida* spp. (Tables 1, 2, and 3) and the two quality control (QC) isolates with four agents by each broth microdilution method following a standard protocol. This protocol included the susceptibility testing guidelines described in the CLSI (formerly NCCLS) M27-A2 document and those recommended by the EUCAST Subcommittee (document 7.1) (4, 6). Two MIC readings were performed by the reference method, i.e., at 24 and 48 h. Each first and second day, CLSI MICs for each isolate-drug combination were compared to EUCAST MICs (24 h). In addition, the interlaboratory reproducibility of EUCAST and reference MIC results as well as categorical differences of MIC endpoints between the methods were determined.

Clinical isolates. A total of 71 isolates from the culture collection of the University of Iowa College of Medicine included 15 *Candida albicans* (5 fluconazole-resistant and 4 susceptible-dose-dependent [S-DD] isolates), 10 *C. dubliniensis*, 7 *C. glabrata* (2 fluconazole-resistant isolates), 5 *C. guilliermondii* (2 fluconazole-resistant isolates), 10 *C. krusei*, 9 *C. lusitaniae*, 10 *C. parapsilosis*, and 5 *C. tropicalis* (1 fluconazole-resistant isolate) isolates. The CLSI QC isolates, *Candida krusei* ATCC 6258 and *Candida parapsilosis* ATCC 22019, also were tested each time a set of clinical isolates was evaluated by the two procedures (4, 6). The set of isolates also included strains with different susceptibility patterns to voriconazole and posaconazole. Each isolate represented a unique strain from a single patient and was maintained in sterile water and subcultured onto antimicrobial-free medium to ensure viability and purity prior to testing.

Antifungal agents. EUCAST and CLSI reference microdilution plates containing serial drug dilutions of posaconazole (Schering-Plough Research Institute, Kenilworth, N.J.), voriconazole (Pfizer Central Research, New York, N.Y.), and the established triazoles fluconazole (Pfizer Central Research) and itraconazole (Janssen, Beerse, Belgium) were prepared by TREK Diagnostic Systems (Cleveland, OH) following the CLSI M27-A2 and EUCAST guidelines (4, 6), shipped frozen to each laboratory, and stored at −70°C until the day of the test. Voriconazole and itraconazole drug dilutions ranged from 0.008 to 16 μg/ml, posaconazole from 0.004 to 8 μg/ml, and fluconazole from 0.12 to 128 μg/ml in both reference and EUCAST microdilution plates.

Stock inoculum preparation. Stock inoculum suspensions of the yeasts were prepared in sterile saline (8.5 g/liter) NaCl from 24-h cultures on Sabouraud dextrose agar at 35°C. The turbidity of each yeast suspension was adjusted by the spectrophotometric method (6).

CLSI broth microdilution method (M27-A2 document). U-bottom microdilution plates containing 100 μl of the twofold serial dilutions of the antifungal drugs in standard RPMI 1640 medium (0.2% glucose) were inoculated with 100 μl of inoculum containing between 1.0×10^3 and 5×10^3 CFU/ml. Following inoculation of the reference microdilution plates, they were incubated at 35°C in a non-CO_2 incubator, and MICs were determined after 24 and 48 h. Reference MICs corresponded to the lowest drug dilution that showed prominent growth inhibition (50% or more) (6). QC isolates were tested in the same manner in each participant laboratory.

Proposed EUCAST broth microdilution method. Flat-bottom microdilution plates containing 100 μl of the twofold serial dilutions of the antifungal drugs in double-strength RPMI 1640 medium (2% glucose) were inoculated with 100 μl of inoculum containing between 0.5×10^5 and 2.5×10^5 CFU/ml. The microdilution plates were incubated at 35°C in a non-CO_2 incubator, and MICs were determined after 24 h; microdilution plates were reincubated if the optical density was ≤0.5 (indicative of poor growth) and read after the second day of incubation. MICs were determined with a spectrophotometer at a wavelength in the range of 530 to 550 nm. EUCAST MICs corresponded to the lowest drug dilution that showed a reduction of growth of 50% or more compared with the growth control (4). QC isolates were tested in the same manner in each participant laboratory.

Statistical analysis. Both on-scale (e.g., 0.12 and 128 μg/ml) and off-scale (i.e., <0.12 and >128 μg/ml) MICs were included in the analysis. For the comparison between the two methods, MICs of each drug-organism combination obtained by each method in the six laboratories were compared as follows: (i) 24-h MIC pairs by both methods and (ii) EUCAST MICs versus CLSI 48-h MICs. Values were considered in agreement when the discrepancies between the methods were no more than 2 $\log_2$ dilutions. The reproducibility of the results obtained by the six laboratories was evaluated by determining the percent agreement between MICs that were within 3 dilutions (e.g., 0.25, 0.5, and 1 μg/ml) as well by intraclass correlation coefficients (ICCs) for converted $\log_2$ MICs. In addition, because the EUCAST Subcommittee has not yet established breakpoints, the interpretive CLSI criteria (M27-A2 document) were used to evaluate the agreement between EUCAST and CLSI results for fluconazole and itraconazole data regarding these interpretative criteria (6). Tentative interpretive breakpoints have recently been established for voriconazole (personal communication, CLSI Subcommittee, January 2005 meeting) but are not available for posaconazole. Therefore, substantial differences (4 or more dilutions) between the methods were also identified for the latter agent.

RESULTS

Three readings were obtained for each isolate with the four antifungal agents in each of the six centers. A total of 852 readings in each center for the 71 isolates, or 5,112 readings

TABLE 2. Agreement between EUCAST and CLSI fluconazole and itraconazole MICs according to CLSI breakpoint categorization for *Candida* spp. in six laboratories[a]

Fungus (no. of values)	Antifungal	Method	% of MICs by CLSI classification[b]			% Agreement[c] between the methods
			S	S-DD	R	
C. albicans (90)	Fluconazole	CLSI	43	20	37	74
		EUCAST	61	12	27	
	Itraconazole	CLSI	40	37	23	59
		EUCAST	64	35	1	
C. dubliniensis (58)	Fluconazole	CLSI	98	2	0	96
		EUCAST	98	0	2	
	Itraconazole	CLSI	98	2	0	97
		EUCAST	100	0	0	
C. glabrata (42)	Fluconazole	CLSI	71	0	29	76
		EUCAST	76	19	5	
	Itraconazole	CLSI	36	33	31	55
		EUCAST	64	19	17	
C. guilliermondii (30)	Fluconazole	CLSI	53	3	43	90
		EUCAST	60	7	33	
	Itraconazole	CLSI	23	33	43	77
		EUCAST	13	50	37	
C. krusei (60)	Fluconazole	CLSI	2	73	25	68
		EUCAST	10	82	8	
	Itraconazole	CLSI	2	81	17	28
		EUCAST	70	28	2	
C. lusitaniae (53)	Fluconazole	CLSI	100	0	0	100
		EUCAST	100	0	0	
	Itraconazole	CLSI	68	34	0	66
		EUCAST	96	2	2	
C. parapsilosis (60)	Fluconazole	CLSI	100	0	0	98
		EUCAST	100	2	0	
	Itraconazole	CLSI	68	32	0	67
		EUCAST	98	2	0	
C. tropicalis (28)	Fluconazole	CLSI	83	0	17	96
		EUCAST	89	0	11	
	Itraconazole	CLSI	52	31	17	64
		EUCAST	86	4	10	
All organisms (421)	Fluconazole	CLSI	66	17	17	78.5
		EUCAST	73	18	8	
	Itraconazole	CLSI	48	38	14	58.5
		EUCAST	76	18	6	

[a] The CLSI (formerly NCCLS) reference method for yeasts (M27-A2 document) and the EUCAST 7.1 method were used.
[b] Percentages of MICs by each method that were within the CLSI breakpoint categorization.
[c] Percentages of EUCAST MICs that were in agreement regarding the CLSI breakpoint classification.

from the six centers, were reported. All isolates had sufficient growth for MIC determination using the EUCAST medium, but 20 of 421 laboratory reports (for each drug) were documented as no growth at 24 h with the standard medium. However, 35% of these documentations originated from the same laboratory, and nine of them were for *Candida dubliniensis*.

Table 1 lists the percentages of agreement between the two methods for the eight *Candida* spp. evaluated as well as the percentages of interlaboratory reproducibility for each method. These percentages represent agreement in MIC results of no more than 2 $\log_2$ dilutions, regardless of breakpoint classification. The agreement between the methods was usually higher when testing fluconazole and voriconazole (92 to 95% overall agreement; ICCs, 0.73 to 0.95) than when testing the other two triazoles (85 to 91% overall agreement; ICCs, 0.56 to 0.93). The compatibility of EUCAST with CLSI MICs was slightly higher with 24-h results than with 48-h results (90 to 95% versus 85 to 94%, respectively). The lowest percentages of agreement were observed for isolates of *C. albicans*, *C. glabrata*, and *C. tropicalis*. The interlaboratory agreements among the six laboratories of results obtained by each method were similar (92 to 95% EUCAST versus 93 to 97% CLSI); the lowest percentages of interlaboratory reproducibility of EUCAST MICs were observed for isolates of *C. glabrata* and *C. krusei* (86 to 88%) when testing itraconazole and posaconazole and of the CLSI method when testing itraconazole and voriconazole against *C. albicans* and *C. glabrata* (81 and 86%, respectively).

Table 2 depicts the percentages of agreement between CLSI 48-h and EUCAST MICs regarding the categorical data established by the CLSI for fluconazole and itraconazole (6). Low rates of agreement were shown for four of the species that included either resistant or S-DD isolates (68 to 90% agreement with fluconazole and 28 to 77% with itraconazole), because EUCAST MICs were substantially and consistently lower than CLSI results. The agreement was good (96 to 100%) for *C. dubliniensis*, *C. lusitaniae*, *C. parapsilosis*, and *C. tropicalis*. In the majority of the cases, the EUCAST method categorized an isolate as either susceptible or S-DD, while by the CLSI method the isolate belonged to either the S-DD or resistant categories, respectively. However, major discrepancies (9%) were also observed where five fluconazole EUCAST susceptible values were obtained among 58 CLSI resistant MICs. Similar results were obtained with itraconazole, but the percentages of agreement were lower (32 to 73%) for most of the species.

With voriconazole, 18 susceptible MICs (≤1 μg/ml) were obtained by the EUCAST method among 36 resistant values (≥4 μg/ml) by the reference method. Substantially lower EUCAST than CLSI MICs were also documented for three isolates of *C. albicans*, *C. glabrata*, and *C. tropicalis* for which CLSI posaconazole mode MICs were >1 μg/ml (Table 3). The majority (90%) of posaconazole MICs were ≥1 μg/ml for 5 of the 10 fluconazole-resistant isolates compared to 9% by the EUCAST method.

Table 4 depicts the susceptibilities of the 71 isolates to the four agents in the six laboratories by both methods. In general, MICs by both methods appear to be similar, as demonstrated by the wide MIC range for certain species and drug combinations. However, in some instances for the species that included fluconazole-resistant isolates, fluconazole and itraconazole MICs at which 90% of the isolates tested were inhibited (MIC_{90}s) were in the S-DD category, while the CLSI corresponding results were in the resistant category. Although posaconazole and voriconazole MIC_{90}s were within 1 dilution between the two methods for most of the species, CLSI MIC_{90}s for *C. albicans*, *C. glabrata*, and *C. guilliermondii* tended to be ≥2 μg/ml, while EUCAST MICs were ≤1 μg/ml.

TABLE 3. Substantially discrepant posaconazole EUCAST MICs compared to CLSI results[a]

Species and isolate no.	EUCAST MIC in μg/ml (no. of results)[b]	CLSI mode
C. albicans 3	0.008 (1)	0.25–0.5
C. albicans 4	0.06–0.25 (6)	8
C. glabrata 7	0.25–0.5 (2)	4
C. tropicalis 10	0.5 (1)	8
Total	9 (63.5%)[c]	N/A

[a] CLSI M27-A2 and EUCAST broth microdilution methods. N/A, not applicable.
[b] The number of discrepant values/isolate is given.
[c] Percentage of agreement.

DISCUSSION

This is the first study that has compared in six laboratories EUCAST and CLSI MIC results for a large number of isolates with well-documented interpretative MIC endpoints. In addition, we compared EUCAST results to CLSI MICs obtained at both 24 and 48 h. The availability of the CLSI (formerly NCCLS) M27-A2 method (6) led to the development of more objective, practical, and faster alternative methods for use in the routine clinical laboratory. For this purpose, the EUCAST Subcommittee has proposed the supplementation of the reference RPMI 1640 medium with 2% of dextrose in order to obtain a superior turbidity in the growth control well and thus shorten the MIC determination to 24 h (2, 5, 8, 10). In our study, the reference medium also supported sufficient growth at 24 h, since MICs for most isolates were obtained from each center (92 to 100%) at this incubation time. Nguyen and Yu (7) have reported similar results. Among the species, the longer incubation time may be required for isolates of *C. dubliniensis*, because 35% of the no-growth reports were for this species; other than that, MICs were readily determined at 24 h by both methods. A shorter reporting time of MICs by the CLSI method represents a significant improvement in the performance of this method.

The agreement between the methods was dependent on the species, the antifungal agent, and the incubation time used to determine the CLSI results. The highest agreements were observed with fluconazole and voriconazole. Three previous studies have evaluated the compatibility between EUCAST and CLSI microdilution methods in one to two laboratories (1–3). Although similarly good reproducibility (overall, 92 to 99%) has been reported with fluconazole, itraconazole, and voriconazole (1, 2), in another study (3), the reproducibility was lower with itraconazole (78 to 81%) and for *C. tropicalis* with fluconazole (88%). In our study, itraconazole MICs also yielded the lowest agreement between the methods (Table 1). Results with posaconazole were adequate but had consistently lower agreement (79 to 100%) than that obtained with fluconazole and voriconazole, which is not surprising, since this antifungal is more closely related to itraconazole than to fluconazole. Comparisons of the two methods with posaconazole have not been reported. Among the species, the lowest percentages of reproducibility between the methods were observed for *C. albicans*, *C. krusei*, and *C. tropicalis* with either itraconazole, posaconazole, or both. Cuenca-Estrella et al. (3) also found low (88%) reproducibility between the methods for *C. tropicalis* versus fluconazole.

The interlaboratory reproducibility of each method among the six laboratories was mostly good to excellent (Table 1). Similar results were obtained among nine laboratories (4) with fluconazole for six of eight isolates of *Candida* spp. (98 to 100%) and for all isolates with itraconazole (85 to 93%). When testing fluconazole, the lowest interlaboratory agreement of EUCAST MICs (83%) was for the *C. glabrata* isolate (4), while in our study, the agreement was 95% for this species.

Antimicrobial susceptibility testing should not only provide reproducible data butalso identify isolates that are potentially resistant to the agent being evaluated. Because of that, we included 10 strains, among the different species, that have been classified by the CLSI method as resistant to fluconazole (MICs, ≥64 μg/ml). These fluconazole results were duplicated by the six laboratories by the CLSI method, but three EUCAST results were ≤8 μg/ml (susceptible values); the same applied for isolates that were categorized as itraconazole re-

TABLE 4. Fluconazole, itraconazole, posaconazole, and voriconazole EUCAST and CLSI MIC data for *Candida* isolates[a]

Fungus (no. of values)	Antifungal agent	MIC (μg/ml) by:					
		EUCAST			CLSI M27-A2		
		Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}
C. albicans (90)	Fluconazole	0.12–>64	8	>64	0.12–>64	16	>64
	Itraconazole	0.008–1	0.12	0.25	0.01–16	0.5	1
	Posaconazole	0.004–0.5	0.12	0.25	0.008–16	0.5	1
	Voriconazole	0.008–2	0.12	1	0.008–16	0.25	2
C. dubliniensis (58)	Fluconazole	0.12–8	0.25	4	0.12–16	0.25	2
	Itraconazole	0.008–0.06	0.008	0.03	0.008–0.25	0.06	0.12
	Posaconazole	0.004–0.06	0.008	0.03	0.004–0.12	0.06	0.06
	Voriconazole	0.008–0.06	0.008	0.03	0.008–0.06	0.008	0.03
C. glabrata (42)	Fluconazole	0.12–64	2	32	0.12–>64	4	>64
	Itraconazole	0.008–4	0.06	1	0.01–16	0.25	2
	Posaconazole	0.008–4	0.12	1	0.008–4	0.25	2
	Voriconazole	0.008–8	0.03	1	0.008–8	0.12	4
C. guilliermondii (30)	Fluconazole	0.12–>64	4	64	0.25–>64	4	>64
	Itraconazole	0.008–16	0.25	2	0.03–16	0.5	16
	Posaconazole	0.004–4	0.12	1	0.01–2	0.25	1
	Voriconazole	0.008–8	0.12	1	0.008–4	0.12	2
C. krusei (60)	Fluconazole	2–64	16	32	8–>64	32	64
	Itraconazole	0.008–0.5	0.12	0.25	0.12–1	0.5	1
	Posaconazole	0.008–0.5	0.12	0.5	0.06–1	0.25	0.5
	Voriconazole	0.008–1	0.12	0.5	0.06–1	0.25	0.5
C. lusitaniae (53)	Fluconazole	0.12–1	0.25	0.5	0.12–2	0.25	1
	Itraconazole	0.008–0.25	0.03	0.06	0.01–0.5	0.12	0.25
	Posaconazole	0.04–0.06	0.008	0.03	0.004–0.25	0.03	0.06
	Voriconazole	0.008–0.01	0.008	0.01	0.008–0.03	0.008	0.01
C. parapsilosis (60)	Fluconazole	0.12–4	1	2	0.12–8	1	4
	Itraconazole	0.008–0.25	0.03	0.12	0.03–0.5	0.12	0.25
	Posaconazole	0.008–0.12	0.03	0.06	0.01–0.25	0.06	0.12
	Voriconazole	0.008–0.5	0.01	0.06	0.008–0.25	0.03	0.12
C. tropicalis (28)	Fluconazole	0.12–>64	0.5	>64	0.12–>64	1	>64
	Itraconazole	0.008–16	0.03	0.5	0.06–16	0.12	8
	Posaconazole	0.008–8	0.01	8	0.03–8	0.06	8
	Voriconazole	0.008–16	0.03	16	0.008–16	0.06	16
All isolates (421)	Fluconazole	0.12–>64	4	32	0.12–>64	8	64
	Itraconazole	0.008–16	0.12	0.5	0.008–16	0.25	4
	Posaconazole	0.004–8	0.06	1	0.004–8	0.25	2
	Voriconazole	0.008–16	0.06	2	0.008–16	0.12	4

[a] CLSI M27-A2 and EUCAST broth microdilution methods.

sistant by the CLSI method. Similar categorical discrepancies between these two methods have been reported by Cuenca-Estrella et al. (3) with both fluconazole (*C. parapsilosis* and *C. glabrata*) and itraconazole (*C. glabrata*, *C. krusei*, and *C. tropicalis*). Because in our study major categorical discrepancies were not observed with 24-h CLSI MICs, the higher carbohydrate content in the RPMI 1640 could be responsible for the lowering of EUCAST results. Since EUCAST MICs were consistently the lower values and trays were not shaken prior to the spectrophotometric reading, it is possible that the unusually elevated EUCAST results (0.5%) were caused by the presence of air bubbles in the MIC wells, as has been demonstrated in our laboratory (personal communication).

Interpretative breakpoints are not available for posaconazole, but tentative MIC breakpoints of ≤1 μg/ml (susceptible) and ≥4 μg/ml (resistant) have been recently established for voriconazole (personal communication, CLSI Subcommittee, January 2005 meeting). In our study, 50% of voriconazole resistant MICs corresponded to susceptible values by the EUCAST method; these could be considered very major errors. EUCAST MICs substantially lower than CLSI MICs have been previously reported for *C. glabrata* with voriconazole (MIC_{90}s of 0.5 and 4 μg/ml, respectively) and for *C. parapsilosis* with caspofungin (MIC ranges, 0.5 to 2 and 0.5 to >16 μg/ml, respectively) (1). Although the two methods have not been compared for testing posaconazole, a similar trend was observed with this agent for some isolates (Table 4).

In conclusion, results of this study and previous comparisons of both methods indicate that EUCAST MICs of the triazoles are consistently lower than those obtained with the CLSI ref-

erence method. Because these lower EUCAST values also reflect substantial categorical shifting, including major discrepant results, the CLSI breakpoints do not appear to be useful for the categorical interpretation of EUCAST MIC data. Therefore, it is not recommended to provide interpretive MIC results when reporting EUCAST in vitro data until breakpoints have been established for EUCAST methodology.

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