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Comparison of Visual 24-Hour and Spectrophotometric 48-Hour MICs to CLSI Reference Microdilution MICs of Fluconazole, Itraconazole, Posaconazole, and Voriconazole for *Candida* spp.: a Collaborative Study

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A multicenter (six-center) study evaluated the performance (interlaboratory reproducibility, compatibility with reference methods, and categorical agreement) of 24-h visual and 48-h spectrophotometric MICs. MICs of fluconazole, itraconazole, voriconazole, and posaconazole were compared to reference 48-h microdilution broth visual MICs (CLSI [formerly NCCLS] M27-A2 document) for 71 isolates of *Candida* spp. that included 10 fluconazole-resistant strains. Twenty readings (5%) were reported as showing no growth at 24 h, mostly for *Candida dubliniensis* and from a single center. The overall interlaboratory agreement of 24-h visual readings and 48-h spectrophotometric MICs, as well their compatibility with reference values, were excellent with the four triazoles for most of the species (93 to 99%, within 3 dilutions). The categorical agreement between the investigational reading conditions and reference values was good with fluconazole and voriconazole (93 to 97%) but lower with itraconazole (86 to 88%), due primarily to minor errors. There were only 0 to 3% very major errors with these three triazoles; the number of substantial errors (more than three dilutions) was also low (<2%) with posaconazole. These data suggest that the performance of both investigational MIC readings gives results similar to those of reference MICs. Since spectrophotometric MICs are more objective and the 24-h time period would shorten the MIC determination of azoles, the description of either of these two reading conditions in the M27-A2 document should be considered by the CLSI subcommittee in addition to or instead of the longer, less practical, and more subjective 48-h visual MIC reading.

The Clinical and Laboratory Standards Institute (CLSI; formerly the NCCLS) has developed a reproducible broth microdilution procedure for antifungal susceptibility testing of *Candida* spp. and *Cryptococcus neoformans* with various new and established antifungal agents (M27-A2 document). The CLSI document (9) recommends the determination of visual MICs, with the azole MIC being defined as the lowest concentration at which a prominent decrease in turbidity is observed. It has been demonstrated that this decrease in growth (turbidity) corresponds to approximately 50% inhibition in growth as determined by a spectrophotometer (1, 4, 14). Although 24-h MIC endpoints are more practical and efficient results and spectrophotometric MICs are less subjective, the reproducibility and suitability of these MIC endpoints have not been addressed in multicenter studies. The aim of this collaborative (six-center) study was to evaluate the performance of 24-h visual and 48-h spectrophotometric MIC readings by three different criteria: (i) compatibility with 48-h reference visual MICs (CLSI M27-A2 document) regardless of breakpoint agreement, (ii) interlaboratory reproducibility among the six centers, and (iii) categorical agreement according to NCCLS interpretive breakpoints for fluconazole, itraconazole, and voriconazole. The number of substantial differences (more than 3 dilutions) was also determined for posaconazole MICs.

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MATERIALS AND METHODS

Study design. Each laboratory tested the same panel of 71 coded isolates of *Candida* spp. and the two quality control (QC) isolates with four agents by following a standard protocol; the set of isolates included 15 isolates of *Candida albicans*; 10 isolates each of *Candida dubliniensis*, *Candida krusei*, and *Candida parapsilosis*; 9 isolates of *Candida lusitaniae*; 7 isolates of *Candida glabrata*; and 5 isolates each of *Candida guilliermondii* and *Candida tropicalis*. The standard protocol included the susceptibility testing guidelines described in the CLSI M27-A2 document. Two MIC readings were performed in addition to the 48-h visual reading (reference MIC) as follows: visually at 24 h and spectrophotometrically at 48 h. Each of the two investigational MIC readings for each isolate-drug combination was compared to the corresponding 48-h reference visual MIC in order to assess their compatibility as well as their categorical agreement with CLSI breakpoints for fluconazole, itraconazole, and voriconazole. In the same manner, the number of substantial discrepancies was determined with posaconazole. The interlaboratory reproducibility of MICs obtained by each reading condition (including the 48-h reference visual reading) was also assessed for the four triazoles.

Susceptibility testing. MICs of fluconazole (Pfizer Central Research, New York, N.Y.), itraconazole (Janssen, Beerse, Belgium), posaconazole (Schering-Plough Research Institute, Kenilworth, N.J.), and voriconazole (Pfizer Central Research) were determined simultaneously in each of six centers by following the broth microdilution M27-A2 guidelines for a total of 71 isolates (Tables 1 to 4) from the culture collection of the University of Iowa College of Medicine. MICs were also determined visually at 24 h and by a spectrophotometer at 48 h. Because categorical agreement was to be evaluated, the set of isolates included 10 fluconazole-resistant isolates; these 10 isolates also have different susceptibil-

TABLE 1. Comparison of 24-h visual and 48-h spectrophotometric MICs with visual 48-h reference MICs for 71 *Candida* spp. isolates in six laboratories[a]

Organism (no. of values)	Antifungal agent	% Agreement between:		% Interlaboratory agreement for:		
		NV48 and V24	NV48 and S48	V24	NV48	S48
Candida albicans (90)	Fluconazole	99	96	96	97	99
	Itraconazole	96	96	93	81	94
	Posaconazole	96	94	90	92	92
	Voriconazole	97	94	90	90	92
C. dubliniensis (59)	Fluconazole	100	100	100	95	92
	Itraconazole	94	97	96	93	95
	Posaconazole	98	98	98	97	93
	Voriconazole	100	98	100	100	98
C. glabrata (42)	Fluconazole	92	98	100	98	95
	Itraconazole	87	95	87	93	93
	Posaconazole	97	95	95	95	90
	Voriconazole	89	95	92	86	95
C. guilliermondii (30)	Fluconazole	100	100	96	93	97
	Itraconazole	100	97	93	97	87
	Posaconazole	96	100	88	97	100
	Voriconazole	100	100	92	97	97
C. krusei (60)	Fluconazole	100	100	97	100	100
	Itraconazole	98	98	97	100	98
	Posaconazole	100	100	93	98	97
	Voriconazole	100	100	95	95	98
C. lusitaniae (53)	Fluconazole	100	100	100	100	98
	Itraconazole	100	100	100	100	96
	Posaconazole	100	98	95	98	94
	Voriconazole	100	100	100	100	100
C. parapsilosis (60)	Fluconazole	100	100	97	97	98
	Itraconazole	95	97	91	98	98
	Posaconazole	95	98	88	100	98
	Voriconazole	96	97	91	97	97
C. tropicalis (29)	Fluconazole	100	97	96	90	97
	Itraconazole	100	97	97	100	93
	Posaconazole	97	97	100	100	97
	Voriconazole	93	97	97	97	97
All values (423)	Fluconazole	99	99	98	97	98
	Itraconazole	97	97	94	93	94
	Posaconazole	97	97	93	96	95
	Voriconazole	97	97	94	95	97

[a] Percent agreement 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 breakpoint categorization. NV48, CLSI visual reference MICs at 48 h (M27-A2 document); V24, visual MICs at 24 h; S48, spectrophotometric MICs at 48 h.

ity patterns to voriconazole and posaconazole. In addition, QC *C. parapsilosis* ATCC 22019 and *C. krusei* ATCC 6258 isolates were included as controls. MICs for these isolates were within the range (2, 9).

Stock inoculum suspensions were obtained from 24-h cultures on Sabouraud dextrose agar at 35°C. The turbidity of each yeast suspension was adjusted by the spectrophotometric method to match the turbidity of a 0.5 McFarland standard and diluted to a concentration of 1.0×10^3 to 5.0×10^3 CFU/ml or 2× the final testing inoculum (9). The microdilution trays containing the drug dilutions were prepared at a central facility (Trek Diagnostics Systems, Cleveland, OH) and shipped frozen to each laboratory. Following inoculation of the microdilution plates, the plates were incubated at 35°C in a non-CO_2 incubator and MICs were determined visually at both 24 and 48 h and by a spectrophotometer at 48 h. A spectrophotometric reading was not performed at 24 h to avoid contamination. A blank plate (only medium and drug concentrations) was also incubated each time testing was performed. For the spectrophotometric reading, microdilution plates were agitated until homogeneity in the wells was achieved (~5 min at 50 to 60 rpm with a microdilution shaker) and read at a wavelength in the range of 530 to 550 nm using microdilution readers. An optical density of ≥0.5 was observed in the growth control well. The mean optical density value for the 96 wells of the blank microdilution trays was obtained, and the resulting mean was subtracted from the value for each MIC well to calculate the percentage of growth relative to the growth in the control well (drug-free well). Visual and spectrophotometric MICs were considered to be the lowest drug concentrations that resulted in 50% (or more) growth reduction. QC isolates were tested in the same manner in each center.

Statistical analysis. Both on-scale (e.g., 0.12 and 128 μg/ml) and off-scale (e.g., <0.12 and > 128 μg/ml) MICs were included in the analysis. MICs of each drug-organism combination obtained at either 24 h or by a spectrophotometer in the six laboratories were compared to corresponding reference MICs. For comparisons of compatibility with reference MICs as well as for interlaboratory agreement of each reading condition, agreement was defined when MIC results were within a 3-dilution range. The CLSI interpretive breakpoints for fluconazole (susceptible [S], ≤8 μg/ml; susceptible-dose dependent [S-DD], 16 to 32 μg/ml; resistant [R], ≥64 μg/ml), itraconazole (S, ≤ 0.12 μg/ml; S-DD, 0.25 to 0.5 μg/ml; R, ≥1 μg/ml) (9), and voriconazole (S, ≤1 μg/ml; S-DD, 2 μg/ml; R, ≥ 4 μg/ml) (CLSI subcommittee, January 2005 meeting) were used to obtain

TABLE 2. Categorical agreement between 24-h visual and 48-h spectrophotometric MICs and visual 48-h reference fluconazole MICs for 71 *Candida* spp. in six laboratories

Organism (no. of values)	Parameter[a]	% of MICs by category[b]			% Errors			% Categorical agreement (no. of discrepant results)[c]
		S	S-DD	R	Minor	Major	Very major	
Candida albicans (90)	48 h	43	20	37				
	24 h	46	20	34	3	0	0	97 (3)
	S48	43	18	39	4	0	0	96 (4)
C. dubliniensis (59)[d]	48 h	98	2	0				
	24 h	100	0	0	2	0	0	98 (1)
	S48	98	2	0	0	0	0	100 (0)
C. glabrata (42)	48 h	71	0	29				
	24 h	66	8	26	5	0	0	95 (2)
	S48	69	0	31	0	2	0	98 (1)
C. guilliermondii (30)[d]	48 h	54	3	43				
	24 h	65	0	35	4	0	0	96 (1)
	S48	57	3	40	7	0	0	93 (2)
C. krusei (60)	48 h	2	73	25				
	24 h	17	77	6	37	0	0	63 (22)
	S48	0	63	37	22	0	0	78 (13)
C. lusitaniae (53)[d]	48 h	100	0	0				
	24 h	100	0	0	0	0	0	100 (0)
	S48	100	0	0	0	0	0	100 (0)
C. parapsilosis (60)[d]	48 h	100	0	0				
	24 h	100	0	0	0	0	0	100 (0)
	S48	100	0	0	0	0	0	100 (0)
C. tropicalis (29)	48 h	83	0	17				
	24 h	83	0	17	0	0	0	100 (0)
	S48	79	0	21	3	0	0	97 (1)
All values (423)	48 h	67	15	18				
(403)	24 h	69	16	15	7	0	0	93 (29)
(423)	S48	66	13	21	5	.2	0	95 (21)

[a] Twenty-four hour and 48 h MICs were visually determined at those incubation times; S48, spectrophotometric MICs were determined at 48 h.
[b] Percentages of MICs (visual 24-h and 48-h spectrophotometric MICs) that were within the CLSI breakpoint categorization.
[c] Percentages of MIC pairs (each visual 24-h and 48-h spectrophotometric MIC compared with the reference MIC from each lab) that were in agreement with the CLSI breakpoint classification. The number of discrepant results is the number of MIC values per species/parameter that fell into a different CLSI breakpoint category.
[d] There were one to eight determinations of "no growth or missing report" at 24 h for these species.

categorical agreement percentages between either visual 24-h or 48-h spectrophotometric MICs and reference MICs, including percentages of either minor, major, or very major errors. Very major errors were identified when the reference MIC indicated resistance and the result was susceptibility according to either of the two investigational readings. Minor errors represented categorical shifting between the three MIC reading conditions from susceptibility or resistance to S-DD. Major errors were identified when the isolate was classified as resistant by either of the two investigational readings and as susceptible by the reference reading. Tentative interpretive breakpoints are not available for posaconazole. Therefore, the number of substantial differences (e.g., 4 or more dilutions from the reference mode MIC) between the methods was also identified for posaconazole.

RESULTS AND DISCUSSION

To our knowledge, this is the first multicenter (six-center) study where the interlaboratory reproducibility of 24-h visual and 48-h spectrophotometric MICs as well as their compatibility with reference MICs (48-h visual M27-A2 MICs) have been evaluated. The CLSI microdilution method for yeasts is reproducible, and the correlation of reference MICs with in vivo treatment outcome (interpretative breakpoints) has been demonstrated for fluconazole and itraconazole versus *Candida* spp. (9) and more recently with voriconazole (CLSI subcommittee, January 2005 meeting). However, the performance of the M27-A2 microdilution method could be improved by either shortening the time of incubation from 48 to 24 h or by the determination of spectrophotometric instead of visual MIC endpoints. Because of this, we evaluated the performance of these two investigational MIC reading methods in three different ways: (i) their interlaboratory reproducibility, (ii) their compatibility with reference MICs regardless of breakpoint categorization, and (iii) the categorical agreement for the three agents for which breakpoints are available. For posaconazole, substantial differences were identified.

Of the 423 readings reported by the six centers for each agent, 20 readings (5%) were reported as no growth at 24 h for *Candida* non-*albicans*; 54% of them were from one of the six centers, and *C. dubliniensis* was the most common species. Therefore, the standard RPMI 1640 appears to adequately support the growth of *Candida* spp. for the determination of

TABLE 3. Categorical agreement between 24-h visual and 48-h spectrophotometric MICs and visual 48-h reference voriconazole MICs for 71 *Candida* spp. in six laboratories

Organism (no. of values)	Parameter[a]	% of MICs by category[b]			% Errors			% Categorical agreement (no. of discrepant results)[c]
		S	S-DD	R	Minor	Major	Very major	
Candida albicans (90)	48 h	83	7	10				
	24 h	85	8	7	1	0	2	97 (3)
	S48	83	7	10	7	0	0	93 (6)
C. dubliniensis (59)[d]	48 h	100	0	0				
	24 h	100	0	0	0	0	0	100 (0)
	S48	98	2	0	0	0	0	100 (0)
C. glabrata (42)	48 h	74	7	19				
	24 h	78	11	11	13	0	3	84 (6)
	S48	69	12	19	5	0	0	95 (2)
C. guilliermondii (30)[d]	48 h	77	20	3				
	24 h	85	12	3	4	0	0	96 (1)
	S48	74	23	3	10	0	0	90 (3)
C. krusei (60)	48 h	100	0	0				
	24 h	100	0	0	0	0	0	100 (0)
	S48	100	0	0	0	0	0	100 (0)
C. lusitaniae (53)[d]	48 h	100	0	0				
	24 h	100	0	0	0	0	0	100 (0)
	S48	100	0	0	0	0	0	100 (0)
C. parapsilosis (60)[d]	48 h	100	0	0				
	24 h	100	0	0	0	0	0	100 (0)
	S48	100	0	0	0	0	0	100 (0)
C. tropicalis (29)	48 h	83	0	17				
	24 h	86	0	14	0	0	3	97 (1)
	S48	79	0	21	0	3	0	97 (1)
All values (423)	48 h	92	3	5				
(403)	24 h	93	3	4	1.7	0	1	97 (11)
(423)	S48	90	4	6	2.6	0.5	0	97 (11)

[a] Twenty-four hour and 48 h MICs were visually determined at those incubation times; S48, spectrophotometric MICs were determined at 48 h.
[b] Percentages of MICs (visual 24-h and 48-h spectrophotometric MICs) that were within the CLSI breakpoint categorization.
[c] Percentages of MIC pairs (each visual 24-h and 48-h spectrophotometric MIC compared with the reference MIC from each lab) that were in agreement with the CLSI breakpoint classification. The number of discrepant results is the number of MIC values per species/parameter that fell into a different CLSI breakpoint category.
[d] There were one to eight determinations of "no growth or missing report" at 24 h for these species.

MICs at 24 h, as has been demonstrated in this and other studies (1, 3, 4, 10, 14).

The comparisons between either 24-h visual or 48-h spectrophotometric MICs and visual 48-h reference MICs are depicted in Table 1. These percentages represent agreement in MIC results within 3 dilutions, regardless of breakpoint classification. The agreement between 24-h MICs with reference values was excellent for most of the species with the four triazoles (97 to 99% overall agreement). Results were similarly good for all the species by the spectrophotometric reading. The percentages of interlaboratory reproducibility among the six centers for each of the three reading conditions are also listed separately in Table 1. The interlaboratory reproducibility among the six laboratories for 24-h results was excellent with fluconazole (98% overall agreement), slightly lower for the other triazoles (93 to 94% overall agreement), and similar to the interlaboratory reproducibility obtained with reference and spectrophotometric MICs (93 to 98% overall agreement for the four triazoles). Among the species, the lowest interlaboratory performance of 24-h visual readings was for *C. glabrata* versus itraconazole and for *C. guilliermondii* and *C. parapsilosis* versus posaconazole; also, itraconazole 48-h spectrophotometric readings did not perform well for *C. guilliermondii*. These results demonstrate good-to-excellent quantitative agreement between both investigational and reference readings for most of the species-drug combinations investigated. The assessment of reproducibility is the first step during the process of establishing standard guidelines. Our results indicate that the reproducibility of the three reading conditions is equitable.

After the principle of reproducibility had been validated, the next step was to address the correlation of these in vitro results with in vivo outcomes to therapy as well as to identify isolates that were potentially resistant to the agent being evaluated. This assessment was conducted by determining the qualitative categorical agreement between investigational and reference reading conditions. The overall categorical agreement was excellent with both fluconazole and voriconazole (Tables 2 and 3). Very major errors were absent with fluconazole, and the percentage of errors was only 1% (or four errors) with voriconazole. These very major errors were reported for 4 of the 10

TABLE 4. Categorical agreement between 24-h visual and 48 h spectrophotometric MICs and visual 48-h reference itraconazole MICs for 71 *Candida* spp. in six laboratories

Organism (no. of values)	Parameter[a]	% of MICs by category[b]			% Errors			% Categorical agreement (no. of Discrepant Results)[c]
		S	S-DD	R	Minor	Major	Very major	
Candida albicans (90)	48 h	40	37	23				
	24 h	41	43	16	10	0	1	89 (10)
	S48	39	36	25	12	1	1	86 (13)
C. dubliniensis (59)[d]	48 h	98	2	0				
	24 h	100	0	0	2	0	0	98 (1)
	S48	95	3	2	3	2	0	95 (3)
C. glabrata (42)	48 h	36	33	31				
	24 h	52	24	24	29	0	0	71 (11)
	S48	38	26	36	17	2	0	81 (8)
C. guilliermondii (30)[d]	48 h	23	33	44				
	24 h	27	42	31	8	0	0	92 (2)
	S48	20	43	37	10	0	0	90 (3)
C. krusei (60)	48 h	2	81	17				
	24 h	10	88	2	23	0	2	75 (15)
	S48	3	69	28	17	0	0	83 (10)
C. lusitaniae (53)[d]	48 h	68	32	0				
	24 h	73	27	0	10	0	0	90 (5)
	S48	77	23	0	13	0	0	87 (7)
C. parapsilosis (60)[d]	48 h	68	32	0				
	24 h	71	29	0	14	0	0	86 (8)
	S48	75	25	0	17	0	0	83 (10)
C. tropicalis (29)	48 h	52	31	17				
	24 h	69	14	17	17	0	0	83 (5)
	S48	52	28	20	14	3	0	83 (5)
All values (423)	48 h	49	36	15				
(403)	24 h	55	36	9	14	0	.2	86 (57)
(423)	S48	51	32	17	11	.7	.2	86 (59)

[a] Twenty-four hour and 48 h MICs were visually determined at those incubation times; S48, spectrophotometric MICs were determined at 48 h.
[b] Percentages of MICs (visual 24-h and 48-h spectrophotometric MICs) that were within the CLSI breakpoint categorization.
[c] Percentages of MIC pairs (each visual 24-h and 48-h spectrophotometric MIC compared with the reference MIC from each lab) that were in agreement with the CLSI breakpoint classification. The number of discrepant results is the number of MIC values per species/parameter that fell into a different CLSI breakpoint category.
[d] There were one to eight determinations of "no growth or missing report" at 24 h for these species.

fluconazole-resistant isolates of *C. albicans*, *C. glabrata*, and *C. tropicalis*. However, the categorical agreement was also low among the six centers by the reference readings for these isolates. Although the categorical agreement was rather poor for the combination of *C. krusei* versus fluconazole (63% categorical agreement, mostly due to minor errors), it was excellent (100%) with voriconazole for this species; there were only 0 to 5% minor errors for the other seven species evaluated with fluconazole (Table 2). Since *C. krusei* is resistant to fluconazole, there is no need to do susceptibility testing for this species versus fluconazole. The performance of the 24-h MIC was superior to those reported for either the fluconazole disk diffusion or YeastOne colorimetric method and the reference method (below 93% overall categorical agreement) (5, 6, 7, 8, 11, 12) but similar to results for voriconazole zone diameters versus *C. glabrata* (97%) (11).

On the other hand, the categorical agreement between the 24-h and reference results was lower (86%) with itraconazole than with the other two triazoles (Tables 2, 3, and 4). The lower performance was due to minor errors (14%) or shifting between susceptibility and S-DD or between S-DD and resistance for most of the species, with 24-h results tending to be the lower results. Only 0.2% very major errors (two for *C. krusei* and one for *C. albicans*) and 0.7% major errors were observed with itraconazole (Table 4). Similarly, categorical discrepancies have been reported between the 24-h YeastOne colorimetric method and reference testing for itraconazole (5). With the advent of the newer, safer, and potentially more effective triazoles voriconazole and posaconazole and the echinocandin caspofungin, very little testing is expected to be required with itraconazole, because itraconazole would be less used for the treatment of invasive yeast infections. Posaconazole MICs were $\leq$0.5 μg/ml for 92% of the isolates in the six centers by the three reading conditions. Five substantially lower posaconazole MICs (0.004 to 0.25 μg/ml) were reported at 24 h for two *C. albicans* isolates, one *C. guilliermondii* isolate, and two *C. parapsilosis* isolates, while reference MIC modes were 4 to 7 dilutions higher (0.03 to 8 μg/ml).

The determination of 24-h MICs would improve the performance of the reference method by providing faster results.

Furthermore, it has been reported that the 24-h MIC endpoint correlated better than the 48-h endpoint with sterol quantitation (fluconazole and itraconazole) and with treatment outcome in a murine model of invasive candidiasis with fluconazole treatment (1, 14). Revankar et al. (13) has demonstrated that oropharyngeal candidiasis caused by heavy trailing isolates responded to the same dose of fluconazole used to treat susceptible isolates. The earlier MIC determination could then preclude the false classification of voriconazole- or posaconazole-susceptible *Candida* spp. isolates as resistant strains when heavy trailing is present at 48 h, as it has been reported with fluconazole (1, 14).

The categorical agreement in spectrophotometric MICs was superior to those determined visually at 24 h with fluconazole (93% versus 95%, respectively) and the same as that seen with itraconazole (88% and voriconazole (97%). The overall percentages of minor errors were similar (Tables 2 to 4). As for 24-h results with itraconazole, the lower categorical agreement was due to minor errors, because only 0.2% very major errors were reported by the six centers. Very major errors were not documented for fluconazole or voriconazole. In contrast to what was observed for 24-h results, major errors (false resistance) were observed with the three agents for which breakpoints are available, but the overall percentages were low (0.2 to 0.7%). Only six posaconazole MICs were substantially higher than reference mode values (0.25 to 8 μg/ml versus 0.01 to 1 μg/ml). It is noteworthy that these higher spectrophotometric MICs were the outlier results among the six centers for the particular isolate. Therefore, it is possible that, despite the agitation of the microdilution trays before the spectrophotometric reading, persistent air bubbles produced the aberrant results. Although spectrophotometric MICs have been reported in several studies, no data regarding categorical comparisons are available.

In summary, MICs of the four triazoles investigated can be determined at 24 h for most *Candida* spp., the exception being the few isolates that did not yield sufficient growth for a MIC determination at 24 h in all laboratories (especially for *C. dubliniensis*); the 48-h incubation is required when sufficient growth is not present for MIC determination at 24 h. All fluconazole- and most itraconazole- and voriconazole-resistant isolates were identified following 24 h of incubation and for all isolates by the spectrophotometric reading at 48 h. The same applied to isolates for which high posaconazole MICs were determined by the reference reading. Since spectrophotometric MICs appear to mimic reference data, this more objective tool can be used for obtaining in vitro data with the azoles if the choice is to continue reading at 48 h. However, the provision of faster data to the clinician warrants that the CLSI subcommittee consider shortening the incubation time for the determination of MICs of the four triazoles evaluated in the present study versus most *Candida* spp. The concern against reporting 24-h MICs was that the breakpoints needed to be adjusted. However, in the present study the categorical agreement between visual 24-h and reference MICs was good to excellent and suggests that such an adjustment is not necessary.

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