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Comparison of Visual and Spectrophotometric Methods of Broth Microdilution MIC End Point Determination and Evaluation of a Sterol Quantitation Method for In Vitro Susceptibility Testing of Fluconazole and Itraconazole against Trailing and Nontrailing *Candida* Isolates

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Visual determination of MIC end points for azole antifungal agents can be complicated by the trailing growth phenomenon. To determine the incidence of trailing growth, we performed testing of in vitro susceptibility to fluconazole and itraconazole using the National Committee for Clinical Laboratory Standards broth microdilution M27-A reference procedure and 944 bloodstream isolates of seven *Candida* spp., obtained through active population-based surveillance between 1998 and 2000. Of 429 *C. albicans* isolates, 78 (18.2%) showed trailing growth at 48 h in tests with fluconazole, and 70 (16.3%) showed trailing in tests with itraconazole. Of 118 *C. tropicalis* isolates, 70 (59.3%) showed trailing growth in tests with fluconazole, and 35 (29.7%) showed trailing in tests with itraconazole. Trailing growth was not observed with any of the other five *Candida* spp. tested (*C. dubliniensis*, *C. glabrata*, *C. krusei*, *C. lusitaniae*, and *C. parapsilosis*). To confirm whether or not isolates that showed trailing growth in fluconazole and/or itraconazole were resistant in vitro to these agents, all isolates that showed trailing growth were retested by the sterol quantitation method, which measures cellular ergosterol content rather than growth inhibition after exposure to azoles. By this method, none of the trailing isolates was resistant in vitro to fluconazole or itraconazole. For both agents, a 24-h visual end point or a spectrophotometric end point of 50% reduction in growth relative to the growth control after 24 or 48 h of incubation correlated most closely with the result of sterol quantitation. Our results indicate that MIC results determined by either of these end point rules may be more predictive of in vivo outcome for isolates that give unclear visual end points at 48 h due to trailing growth.

Antifungal drug susceptibility testing has become more important due to the increase in serious fungal infections and the concomitant emergence of resistance to antifungal agents (18). In 1997, the National Committee for Clinical Laboratory standards (NCCLS) published an approved reference procedure (document M27-A) for the in vitro testing of five antifungal agents against *Candida* spp. and *Cryptococcus neoformans* (8). The NCCLS document describes a broth macrodilution method and its microdilution modifications, specifies a defined test medium as well as a standardized inoculum, and recommends the visual determination of the MIC end points after incubation at 35°C for 48 h for *Candida* spp. Although both methods are essentially the same with the exception of medium volume, the description of the MIC end point determination step for the azoles is somewhat ambiguous for the microdilution procedure. By this method the end point is defined as the lowest drug concentration at which a "prominent decrease in turbidity" is observed compared with the growth in the control drug-free medium (8). For the macrodilution procedure, the end point is the lowest concentration at which growth is reduced to 20% of the growth control. However, it has become apparent that the specified decrease in turbidity in the microdilution test more closely corresponds to a 50% reduction in growth as assessed by spectrophotometric reading (7, 9, 10, 14).

Another problem yet to be resolved is the proper interpretation of trailing growth in broth dilution MIC tests with azole antifungal agents, such as fluconazole and itraconazole. The term "trailing" has been used to describe the reduced but persistent growth that some isolates of *Candida* spp. exhibit over an extended range of drug concentrations. Estimated as occurring in about 5% of isolates (2), this trailing growth can be so great as to make an isolate that appears susceptible after 24 h appear resistant at 48 h. Two in vivo studies of this

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phenomenon that employed murine models of invasive candidiasis have shown that the 24-h result was consistent with the response to treatment in vivo (2, 16). This suggests that isolates that demonstrate the trailing phenomenon should be classed as susceptible rather than resistant. This concept has been supported by a clinical demonstration that oropharyngeal candidiasis due to such isolates responds to a low dose of fluconazole used to treat typical susceptible isolates (15).

Over a 2-year period between 1 October 1998 and 30 September 2000, the Centers for Disease Control and Prevention (CDC) conducted population-based active surveillance for candidemia in two metropolitan areas of the United States. Over the course of this surveillance we collected 944 incident bloodstream isolates of *Candida* spp. To determine the incidence of trailing growth among these isolates, as well as the effect of different methods of visual and spectrophotometric end point determination on apparent rates of in vitro resistance to fluconazole and itraconazole, we performed antifungal susceptibility testing using the NCCLS broth microdilution M27-A reference procedure. In addition, isolates that exhibited trailing growth in tests with either fluconazole or itraconazole were retested using a sterol quantitation method (1) that measures cellular ergosterol content rather than growth inhibition after exposure to azole drugs.

MATERIALS AND METHODS

Isolates. A total of 944 incident bloodstream isolates of *Candida* spp. were collected as part of a population-based active surveillance program in Baltimore, Md., and the state of Connecticut. Species identification was confirmed at the CDC using standard methods, including characteristic growth on Chromagar Candida plates, API 20C biochemical profiles, and morphological appearance on Dalmau cornmeal agar plates. Prior to antifungal susceptibility testing, each isolate was subcultured at least twice on Sabouraud dextrose agar plates to ensure purity and optimal growth. *C. parapsilosis* ATCC 22019 and *C. krusei* ATCC 6258 were used as quality control organisms and were included each time that a set of isolates was tested.

Antifungal drugs. Standard powders of fluconazole and itraconazole were supplied by Pfizer Pharmaceuticals Group (Central Research Division, Groton, Conn.) and Janssen Research Foundation (Beerse, Belgium). Stock solutions were prepared in water (fluconazole) or dimethyl sulfoxide (itraconazole). Further dilutions of each antifungal agent were prepared with RPMI 1640 medium (Sigma Chemical Co., St. Louis, Mo.) which had been buffered to pH 7.0 with 0.165 M morpholinopropanesulfonic acid (Sigma), as outlined in NCCLS document M27-A (8). The drug dilutions were dispensed into 96-well microdilution plates that were then sealed and frozen at −70°C until needed.

Broth microdilution susceptibility test method. MICs of fluconazole and itraconazole were determined by the NCCLS broth microdilution method (8). The final concentrations of the antifungal agents ranged from 0.125 to 64 μg/ml for fluconazole and 0.015 to 8 μg/ml for itraconazole. The yeast inoculum was adjusted to a concentration of 0.5×10^3 to 2.5×10^3 CFU/ml in RPMI 1640 medium, and an aliquot of 0.1 ml was added to each well of the microdilution plate. The plates were incubated at 35°C. The MIC end points were read visually following 24 and 48 h of incubation and were defined as the lowest concentration that produced a prominent decrease in turbidity compared with that of the drug-free growth control. The plates were agitated prior to reading at 48 h to ensure the contents of each well were resuspended. This was not done at 24 h to avoid contamination. In addition to visual end point readings, the optical density of each microplate well was measured after 48 h of incubation with a microplate spectrophotometer set at 405 nm. Spectrophotometric MICs were calculated based on the density of the growth control and were the lowest drug concentrations that resulted in a 50 or 80% reduction in growth compared with that of the drug-free growth control.

Sterol quantitation method. Total cellular ergosterol from isolates of *C. albicans* and *C. tropicalis* that exhibited trailing growth was quantified as previously described (1, 2). Briefly, for each strain, a colony from an overnight Sabouraud dextrose agar plate culture was used to inoculate 50 ml of Sabouraud dextrose broth (Difco, Detroit, Mich.) containing 0, 4, 16, or 64 μg of fluconazole per ml or 0, 0.06, 0.5, or 4 μg of itraconazole per ml. The cultures were incubated at 35°C for16 h with shaking at 135 rpm. The stationary-phase cells were harvested by centrifugation at 1,500 × *g* for 5 min and washed once with sterile distilled water. The wet weight of the cell pellet was determined. Cells were saponified by adding 3 ml of 25% alcoholic potassium hydroxide solution (25 g KOH and 36 ml of sterile distilled water, brought to 100 ml with 100% ethanol) to each pellet and vortex mixing for 1 min. Cell suspensions were then incubated in an 85°C water bath for 1 h. Following incubation, tubes were allowed to cool to room temperature. Sterols were then extracted by addition of a mixture of 1 ml of sterile distilled water and 3 ml of *n*-heptane followed by vigorous vortex mixing for 3 min. The heptane layer was transferred to a clean borosilicate glass screw-cap tube. A 200-μl aliquot of sterol extract was diluted fivefold in 100% ethanol and scanned spectrophotometrically between 200 and 300 nm. The presence of ergosterol and the late sterol intermediate 24(28) dihydroergosterol (DHE) in the extracted sample resulted in a characteristic four-peak curve. The absence of detectable ergosterol in extracts was indicated by a flat line. A dose-dependent decrease in the height of the absorbance peaks was evident and corresponded to decreased ergosterol concentration in the sample.

Ergosterol content was calculated as a percentage of the wet weight of the cells by the following equations: % ergosterol + % 24(28) DHE = $[(A_{281.5}/290) \times F]$/pellet weight, % 24(28) DHE = $[(A_{230}/518) \times F]$/pellet weight, and % ergosterol = [% ergosterol + % 24(28) DHE] − % 24(28) DHE, where F is the factor for dilution in ethanol and 290 and 518 are the E values (in percentages per centimeter) determined for crystalline ergosterol and 24(28) DHE, respectively.

The MIC of fluconazole and itraconazole was defined as the concentration of drug which caused an 80% reduction in the total cellular ergosterol content compared to that in the drug-free control. MICs which fell between two drug concentrations (i.e., less than 80% reduction at one concentration but more than 80% reduction at the next-higher concentration) were mathematically extrapolated based on the amount of reduction at the drug concentration which gave results closest to an 80% reduction end point.

Analysis of results. To permit comparisons between the results of the different methods of end point interpretation, the interpretive breakpoints proposed by NCCLS for fluconazole and itraconazole were used. According to the NCCLS criteria (8, 17), isolates for which fluconazole MICs are ≤8 μg/ml are classed as susceptible, while those for which MICs are ≥64 μg/ml are classed as resistant. Isolates for which MICs are 16 or 32 μg/ml are termed susceptible-dose dependent (S-DD). Isolates for which itraconazole MICs are ≤0.125 μg/ml are classed as susceptible, while those for which MICs are ≥0.25 to 0.5 μg/ml are classed as S-DD, and those for which MICs are ≥1 μg/ml are classed as resistant.

RESULTS

Table 1 summarizes the in vitro susceptibilities of the 944 isolates of *Candida* spp. to fluconazole and itraconazole as determined by the broth microdilution procedure with four different methods of end point determination: visual reading after 24 and 48 h of incubation and spectrophotometric reading after 48 h with calculation of 50% and 80% growth inhibition end points. The data are reported as MIC ranges and the MICs at which 50 and 90% of the isolates are inhibited (MIC_{50}s and MIC_{90}s, respectively). For *C. albicans*, marked differences in MIC_{90}s (more than 4 doubling dilutions) were noted for both drugs between the 24- and 48-h visual end points, as well as between the 50 and 80% spectrophotometric end points. For *C. tropicalis*, marked differences in MIC_{50}s and MIC_{90}s were noted for both drugs between the two visual end points, as well as between the two spectrophotometric end points. Less marked differences in MIC_{90} values were noted in tests with *C. glabrata*. For *C. parapsilosis*, *C. krusei*, *C. lusitaniae* or *C. dubliniensis*, MIC_{50}s and MIC_{90}s for both drugs did not differ by more than 2 doubling dilutions for any of the four different methods of end point determination. In each experiment, the visual MICs at 48 h for the two quality control strains were within the accepted limits as established by the NCCLS document M27-A method (data not shown).

Table 2 details the susceptibility interpretations for the 429 isolates of *C. albicans* and 118 isolates of *C. tropicalis* derived

TABLE 1. In vitro susceptibilities of 944 isolates of *Candida* species to fluconazole and itraconazole as determined by broth microdilution testing with four different methods of end point determination

Species (no. of isolates)	Method of end point determination[a]	MIC[b] (μg/ml) of:					
		Fluconazole			Itraconazole		
		Range	50%	90%	Range	50%	90%
C. albicans (429)	Visual, 24-h incubation	<0.125–>64	0.25	0.5	<0.015–>8	0.03	0.125
	Visual, 48-h incubation	<0.125–>64	0.5	>64	<0.015–>8	0.06	>8
	Spectrophotometric, 50%	<0.125–>64	<0.125	0.5	<0.015–>8	0.03	0.125
	Spectrophotometric, 80%	<0.125–>64	0.5	>64	<0.015–>8	0.06	>8
C. tropicalis (118)	Visual, 24-h incubation	<0.125–>64	0.5	2	<0.015–>8	0.125	>8
	Visual, 48-h incubation	0.25–>64	>64	>64	0.03–>8	>8	>8
	Spectrophotometric, 50%	<0.125–>64	0.5	4	<0.015–>8	0.125	0.5
	Spectrophotometric, 80%	0.25–>64	>64	>64	0.03–>8	>8	>8
C. glabrata (229)	Visual, 24-h incubation	<0.125–>64	4	16	<0.015–>8	0.25	0.5
	Visual, 48-h incubation	1–>64	8	>64	0.06–>8	0.5	>8
	Spectrophotometric, 50%	<0.125–>64	4	32	<0.015–>8	0.25	1
	Spectrophotometric, 80%	1–>64	8	>64	0.03–>8	0.5	4
C. parapsilosis (125)	Visual, 24-h incubation	<0.125–4	0.5	1	<0.015–0.5	0.06	0.125
	Visual, 48-h incubation	0.25–16	0.5	1	<0.015–1	0.125	0.25
	Spectrophotometric, 50%	<0.125–4	0.5	1	<0.015–1	0.06	0.25
	Spectrophotometric, 80%	<0.125–16	0.5	2	<0.015–1	0.125	0.5
C. krusei (20)	Visual, 24-h incubation	4–64	16	32	0.125–1	0.5	1
	Visual, 48-h incubation	16–>64	32	64	0.25–>8	0.5	1
	Spectrophotometric, 50%	2–>64	32	64	0.125–1	0.25	1
	Spectrophotometric, 80%	16–>64	64	64	0.25–>8	0.5	2
C. lusitaniae (15)	Visual, 24-h incubation	0.25–1	1	1	<0.015–0.25	0.06	0.125
	Visual, 48-h incubation	0.25–>64	1	2	<0.015–>8	0.125	0.25
	Spectrophotometric, 50%	<0.125–1	0.5	1	<0.015–0.5	0.06	0.125
	Spectrophotometric, 80%	0.25–>64	1	2	0.03–>8	0.125	0.25
C. dubliniensis (8)	Visual, 24-h incubation	<0.125–8	<0.125	<0.125	<0.015–0.25	0.03	0.06
	Visual, 48-h incubation	<0.125–8	<0.125	0.25	<0.015–0.25	0.06	0.06
	Spectrophotometric, 50%	<0.125–8	<0.125	0.25	<0.015–0.25	0.03	0.06
	Spectrophotometric, 80%	<0.125–8	0.25	0.25	<0.015–0.25	0.06	0.125

[a] Spectrophotometric end points (with calculation of 50 and 80% growth inhibition) determined after a 48-h incubation.
[b] 50% and 90%, MIC_{50} and MIC_{90}, respectively

from the four different methods of end point determination. Marked differences in the numbers of isolates classed as resistant were evident. The incidence of in vitro resistance was highest for both organisms and both drugs when visual MICs were determined after 48 h of incubation or when a spectrophotometric end point of 80% growth inhibition at 48 h was used. These differences in the apparent rates in drug resistance were due, in major part, to the presence of trailing growth in a substantial number of tests with *C. albicans* and *C. tropicalis* isolates following incubation for 48 h. Trailing growth was not observed with any of the other five *Candida* spp. tested.

Altogether 78 *C. albicans* (18.2%) and 70 *C. tropicalis* (59.3%) isolates showed trailing growth at 48 h in tests with fluconazole (defined as MICs of ≤8 μg/ml at 24 h and of ≥64 μg/ml at 48 h),

TABLE 2. Interpretation of in vitro susceptibilities of 429 isolates of *C. albicans* and 118 isolates of *C. tropicalis* to fluconazole and itraconazole as determined by broth microdilution testing with four different methods of end point determination

Method of end point determination[a]	No. of isolates in susceptibility category											
	C. albicans (*n* = 429)						*C. tropicalis* (*n* = 118)					
	Fluconazole			Itraconazole			Fluconazole			Itraconazole		
	S	S-DD	R	S	S-DD	R	S	S-DD	R	S	S-DD	R
Visual, 24-h incubation	424	1	4	404	19	6	108	1	9	61	34	23
Visual, 48-h incubation	345	2	82	325	24	80	35	3	80	16	20	82
Spectrophotometric, 50% inhibition	422	2	5	408	17	4	108	3	7	77	34	7
Spectrophotometric, 80% inhibition	346	3	80	292	62	75	48	3	67	20	25	73

[a] Spectrophotometric end points (with calculation of 50 and 80% growth inhibition) determined after a 48-h incubation.

TABLE 3. Comparison of interpretation of in vitro susceptibilities of trailing isolates of *C. albicans* and *C. tropicalis* to fluconazole and itraconazole as determined by broth microdilution testing with six different methods of end point determination and by sterol quantitation

Method of end point determination	No. of isolates in susceptibility category											
	C. albicans						*C. tropicalis*					
	Fluconazole (n = 78)			Itraconazole (n = 70)			Fluconazole (n = 70)			Itraconazole (n = 35)		
	S	S-DD	R	S	S-DD	R	S	S-DD	R	S	S-DD	R
Visual, 24-h incubation	78	0	0	70	0	0	70	0	0	35	0	0
Visual, 48-h incubation	0	0	78	0	0	70	0	0	70	0	0	35
Spectrophotometric, 24-h incubation, 50% growth inhibition	78	0	0	70	0	0	70	0	0	35	0	0
Spectrophotometric, 48-h incubation, 50% growth inhibition	74	1	3	66	4	0	66	2	2	33	1	1
Spectrophotometric, 24-h incubation, 80% growth inhibition	74	0	4	66	1	3	70	0	0	35	0	0
Spectrophotometric, 48-h incubation, 80% growth inhibition	18	1	59	14	7	49	17	1	52	4	4	27
Sterol quantitation	78	0	0	70	0	0	68	2	0	35	0	0

while 70 *C. albicans* (16.3%) and 35 *C. tropicalis* (29.7%) isolates showed trailing growth in itraconazole (defined as MICs of $\leq$0.125 μg/ml at 24 h and of $\geq$1 μg/ml after 48 h). All isolates that showed trailing growth were retested by the sterol quantitation method and by broth microdilution with visual and spectrophotometric reading after 24 h of incubation. The results (Table 3) indicate that none of the trailing isolates were resistant to fluconazole or itraconazole by the sterol quantitation method. Isolates of *C. albicans* and *C. tropicalis* that had fluconazole MICs of $\geq$16 μg/ml at 24 h and of $\geq$64 μg/ml at 48 h and/or itraconazole MICs of $\geq$0.25 μg/ml at 24 h and of $\geq$1 μg/ml at 48 h were also retested by the sterol quantitation method. All were found to be resistant to one or both drugs. Complete agreement between the results of sterol quantitation and broth microdilution MIC testing was seen when either a 24-h visual or a 24-h 50% spectrophotometric inhibition end point was applied to the latter method. Close agreement between the results of sterol quantitation and those of broth microdilution testing was also seen when a spectrophotometric end point, based on an 80% reduction in growth at 24 h or a 50% reduction at 48 h, was used.

DISCUSSION

In the past, in vitro testing of azole antifungal agents was regarded as problematic. The development of reliable and reproducible broth macrodilution and microdilution reference procedures for the in vitro testing of fluconazole and itraconazole against *Candida* spp. has, however, enabled MICs to be correlated with clinical outcomes and has permitted interpretive breakpoints to be proposed for these drugs (17, 18). The NCCLS document M27-A (8) specifies visual reading of MIC end points after 48 h of incubation, which is derived from the work of Espinel-Ingroff et al (5, 6). With fungicidal agents, such as amphotericin B, growth ceases upon exposure to the drug, and this results in clear-cut end points. However, with fungistatic agents, such as fluconazole and itraconazole, the onset of drug action is delayed, and this results in limited, but persistent, growth over a range of drug concentrations. Often this makes visual determination of end points difficult.

Spectrophotometric reading of broth microdilution tests with azole antifungal agents provides a more objective measurement of fungal growth and eliminates the subjective judgements that can confound visual assessment of MIC end points. However, published comparisons of visual and spectrophotometric MICs have demonstrated differences between them and have shown that a visual end point definition may not translate directly into an appropriate spectrophotometric end point. It has been reported that the visual end point specified in the NCCLS broth microdilution test with fluconazole or itraconazole best corresponds with a spectrophotometric end point of 50% reduction in growth, rather than the 80% reduction that might be inferred from the end point definition for the broth macrodilution test (7, 9,10, 14).

Despite the considerable effort that went into defining and standardizing the NCCLS M27-A procedure, several reports have indicated that, for tests with *Candida* spp., a 24-h incubation time with a spectrophotometric end point is more reproducible than the recommended 48-h visual end point (7, 9, 15). The European Committee on Antibiotic Susceptibility Testing (EUCAST) Subcommittee for Antifungal Susceptibility Testing has recently recommended spectrophotometric reading of broth microdilution tests with end points of 50% growth inhibition after 24 h of incubation as part of a proposed standard method for testing of *Candida* spp. against azole antifungal agents (3, 19). The EUCAST procedure also specifies the use of a higher glucose concentration in the test medium, as well as a larger inoculum than the NCCLS method recommends (3, 19). An initial comparison has shown good agreement between the results of the two procedures, a finding that, if confirmed in larger multicenter studies, would obviate the need to develop separate interpretive breakpoints for the EUCAST procedure (M. Cuenca-Estrella, W. Lee, M. A. Ciblak, B. A. Arthington-Skaggs, D. W. Warnock, and J. L. Rodriguez-Tudela, Abstr. 41st Intersci. Conf. Antimicrob. Agents. Chemother., abstr. J570, p. 373, 2001).

The most important source of variation in the determination of visual MIC end points in tests with fluconazole and itraconazole is the trailing growth phenomenon. We have previously estimated that approximately 5% of *C. albicans* isolates display trailing growth when tested against fluconazole (2). The results of the present study indicate that trailing growth may be a more common problem than previously thought. Of the 429 *C. albicans* isolates tested, 78 (18.2%) showed trailing in tests with fluconazole, and 70 showed trailing with itraconazole (16.3%). Of the 118 *C. tropicalis* isolates tested, 70 (59.3%) showed trailing with fluconazole and 35 (29.7%) showed trailing with

itraconazole. However, none of the 397 isolates of *C. glabrata*, *C. parapsilosis*, *C. krusei*, *C. lusitaniae*, or *C. dubliniensis* tested showed signs of trailing growth.

In previous work, we have shown that the sterol quantitation method is more predictive of in vivo outcome than the M27-A broth microdilution procedure for *C. albicans* isolates that give unclear MIC end points due to trailing growth in fluconazole (2). Together with other studies, this work indicates that isolates of *C. albicans* and *C. tropicalis* that demonstrate the trailing growth phenomenon should be classed as susceptible rather than resistant (1, 2, 15, 16). In the present study, none of the large group of trailing isolates was found to be resistant to either fluconazole or itraconazole when retested by the sterol quantitation method. In contrast, the much smaller group of *C. albicans* and *C. tropicalis* isolates for which the fluconazole MICs were $\geq$16 μg/ml and/or for which the itraconazole MICs were $\geq$0.25 μg/ml after 24 h of incubation were found to be resistant to one or both drugs by the sterol quantitation method.

The high rates of apparent in vitro resistance to fluconazole and itraconazole that we observed among bloodstream isolates of *C. albicans* and *C. tropicalis*, derived from visual reading of MIC end points after 48 h of incubation (Table 2), stand in marked contrast to other published reports (4, 11–13). If, however, isolates that demonstrated trailing growth in tests with these agents are reclassified as susceptible, based on spectrophotometric MIC end points derived from 50% reduction in growth at 48 h, the overall rates of resistance are low and are similar to those reported from other population-based and sentinel surveillance programs (4, 11–13). Of the 429 *C. albicans* isolates tested, 5 (1.2%) were resistant to fluconazole and 4 (0.9%) were resistant to itraconazole. Of the 118 *C. tropicalis* isolates tested, 7 (5.9%) were resistant to fluconazole or itraconazole.

Our results demonstrate that duration of incubation and method of end point determination can have a significant impact on the outcome of in vitro susceptibility tests with azole antifungal agents and *Candida* spp. For compliance with the M27-A document, results for nontrailing isolates of *C. albicans* and *C. tropicalis*, as well as for other *Candida* spp., should continue to be reported on the basis of visual reading of MIC end points at 48 h and the corresponding published breakpoints. Our results also demonstrate that if the visual MIC of fluconazole or itraconazole is determined after 48 h of incubation, without regard to the result at 24 h, trailing isolates can be misinterpreted as resistant. Should this occur, reported rates of resistance to these drugs among *C. albicans* and *C. tropicalis* will be falsely high. One solution to this problem would be to report visual MICs of fluconazole and itraconazole after 24 h of incubation, although this might well necessitate adjustment of interpretive breakpoints. Given the close agreement between the results of sterol quantitation and in vivo outcome in a murine model of candidiasis (2), and between sterol quantitation and spectrophotometric MICs based on 50% reduction in growth at 48 h seen in the present study, a better option for trailing isolates would be to report the latter result as the final MIC.

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