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Wild-Type MIC Distributions and Epidemiological Cutoff Values for Amphotericin B, Flucytosine, and Itraconazole and *Candida* spp. as Determined by CLSI Broth Microdilution

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Clinical breakpoints (CBPs) and epidemiological cutoff values (ECVs) have been established for several *Candida* spp. and the newer triazoles and echinocandins but are not yet available for older antifungal agents, such as amphotericin B, flucytosine, or itraconazole. We determined species-specific ECVs for amphotericin B (AMB), flucytosine (FC) and itraconazole (ITR) for eight *Candida* spp. (30,221 strains) using isolates from 16 different laboratories in Brazil, Canada, Europe, and the United States, all tested by the CLSI reference microdilution method. The calculated 24- and 48-h ECVs expressed in μg/ml (and the percentages of isolates that had MICs less than or equal to the ECV) for AMB, FC, and ITR, respectively, were 2 (99.8)/2 (99.2), 0.5 (94.2)/1 (91.4), and 0.12 (95.0)/0.12 (92.9) for *C. albicans*; 2 (99.6)/2 (98.7), 0.5 (98.0)/0.5 (97.5), and 2 (95.2)/4 (93.5) for *C. glabrata*; 2 (99.7)/2 (97.3), 0.5 (98.7)/0.5 (97.8), and 05. (99.7)/0.5 (98.5) for *C. parapsilosis*; 2 (99.8)/2 (99.2), 0.5 (93.0)/1 (90.5), and 0.5 (97.8)/0.5 (93.9) for *C. tropicalis*; 2 (99.3)/4 (100.0), 32 (99.4)/32 (99.3), and 1 (99.0)/2 (100.0) for *C. krusei*; 2 (100.0)/4 (100.0), 0.5 (95.3)/1 (92.9), and 0.5 (95.8)/0.5 (98.1) for *C. lusitaniae*; −/2 (100.0), 0.5 (98.8)/0.5 (97.7), and 0.25 (97.6)/0.25 (96.9) for *C. dubliniensis*; and 2 (100.0)/2 (100.0), 1 (92.7)/−, and 1 (100.0)/2 (100.0) for *C. guilliermondii*. In the absence of species-specific CBP values, these wild-type (WT) MIC distributions and ECVs will be useful for monitoring the emergence of reduced susceptibility to these well-established antifungal agents.

The Clinical and Laboratory Standards Institute (CLSI) Subcommittee on Antifungal Testing has recently established species-specific clinical breakpoints (CBPs) for broth microdilution (BMD) susceptibility testing of the five most common *Candida* spp. (*Candida albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, and *C. krusei*) and the currently available systematically active triazole (fluconazole and voriconazole) and echinocandin (anidulafungin, caspofungin, and micafungin) antifungal agents (23, 24, 30). These CBPs were established by considering the MIC distributions of each agent and species, as well as the most recent and comprehensive molecular, biochemical, pharmacodynamic, and clinical data as they relate to MIC values. In lieu of CBPs for posaconazole and these five *Candida* spp., Pfaller et al. (25) established epidemiological cutoff values (ECVs) to differentiate wild-type (WT) strains (those without mutational or acquired resistance mechanisms) from non-WT strains (those having mutational or acquired resistance mechanisms) as a means of tracking the emergence of reduced susceptibility to posaconazole among *Candida* spp. Subsequently, ECVs were established for the triazoles and echinocandins and a total of 11 *Candida* spp. (25, 26, 28). ECVs provide a sensitive means for detecting emerging resistance and may be used to identify isolates that are less likely to respond to contemporary therapy due to acquired resistance mechanisms when limited clinical data preclude the development of CBPs (13, 23–26, 28, 30, 39).

Whereas the use of the newer triazoles and echinocandins for the treatment of invasive candidiasis (IC) continues to increase (20), the older, established agents, amphotericin B (both conventional and lipid formulations), flucytosine, and itraconazole, also serve as additional systemically active agents for empirical, prophylactic, and adjunctive therapy (21, 41). Although abundant MIC data are available for each of these older antifungal agents (7, 15, 19, 22, 27, 31, 35, 37), CBPs are either flawed (flucytosine and itraconazole) or nonexistent (amphotericin B), and ECVs have not been described for these agents and any *Candida* species. The continued use of these agents, and other members of their respective classes, will produce an ever-increasing selection pressure for resistance, and thus, it is prudent to develop criteria, such as an ECV, to provide the means for tracking the emergence of reduced susceptibility to these clinically available antifungal agents. For these reasons, we considered that the determination of 24- and 48-h WT MIC distributions and ECVs would be useful in surveillance for emergence of reduced susceptibility for the three older

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TABLE 1 WT MIC distributions of amphotericin B for eight *Candida* species using CLSI BMD methods

Species	Incubation time (h)	No. of isolates tested	No. of isolates with MIC (μg/ml) of:								
			≤0.03	0.06	0.12	0.25	0.5	1	2	4	≥8
C. albicans	24	9,252	27	59	278	663	4,263	3,777	169	16	
	48	11,554	27	49	330	809	2,362	7,297	584	86	10
C. glabrata	24	3,117	6	10	57	244	780	1,926	81	13	
	48	3,451	5	3	11	131	534	2,249	474	44	
C. parapsilosis	24	3,107	26	52	144	372	1,020	1,411	74	8	
	48	3,391	6	12	44	216	369	2,215	437	92	
C. tropicalis	24	2,062	1	5	30	91	511	1,362	57	5	
	48	2,373	4	2	19	67	267	1,660	336	17	1
C. krusei	24	577	9	10	12	29	86	331	96	4	
	48	611		9	4	22	46	282	240	8	
C. lusitaniae	24	71		1	3	8	17	30	12		
	48	93			1	8	28	18	31	7	
C. guilliermondii	24	47				8	22	9	8		
	48	83		1	6	16	29	21	10		
C. dubliniensis	48	75	1	7	15	11	16	16	9		

antifungal agents among *Candida* spp. Furthermore, this may be considered a necessary step toward the development, or revision, of useful, species-specific CBPs (23, 24, 29, 30). In the present study, we analyzed aggregated CLSI MIC data gathered in 16 laboratories in Brazil, Canada, Europe, and the United States (41 to 14,716 MICs, according to species and antifungal agent); we used these data to propose amphotericin B, flucytosine, and itraconazole ECVs for each of eight (*C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, *C. krusei*, *C. lusitaniae*, *C. guilliermondii*, and *C. dubliniensis*) *Candida* spp. In the absence of CBPs, ECVs could help to characterize the susceptibility of *Candida* spp. to amphotericin B (and its lipid formulations), flucytosine, and itraconazole and to monitor the emergence of strains with mutations that could lead to reduced susceptibility or resistance to these agents.

MATERIALS AND METHODS

Isolates. Each isolate was recovered from a unique clinical specimen at 16 different medical centers or referral laboratories: the University of Iowa, Iowa City, IA; JMI Laboratories, North Liberty, IA; VCU Medical Center, Richmond, VA; the University of Texas Health Science Center, San Antonio, TX; the University Hospitals of Cleveland, Cleveland, OH; the University of Texas Health Science Center, Houston, TX; the Centers for Disease Control and Prevention, Atlanta, GA; Hospital Universitario La Fe, Valencia, Spain; Unidad de Microbiologia Experimental, Valencia, Spain; Hospital Universitario de Valme, Seville, Spain; Hospital General Unversitario Gregorio Maranon, Madrid, Spain; Servicio de Micologia, Centro Nacional de Microbiologia, Instituto de Salud Carlos III, Majadahonda, Spain; the Adolfo Lutz Institute Public Health Reference Center, Rio Claro, Brazil; Mycology Department, Adolfo Lutz Institute, São Paulo, Brazil; the University of Alabama at Birmingham, Birmingham, AL; and the University of Alberta, Edmonton, Canada. Isolates were identified and stored at each medical center using standardized methodologies; isolates were not characterized for mutations. The total numbers of aggregated available CLSI MICs from the 16 laboratories per species were as follows: 14,716 for *C. albicans*, 5,846 for *C. glabrata*, 4,894 for *C. parapsilosis*, 3,624 for *C. tropicalis*, 809 for *C. krusei*, 119 for *C. lusitaniae*, 130 for *C. dubliniensis*, and 83 for *C. guilliermondii*.

Whereas these isolates generally represented the incident isolate for each episode of infection and were likely WT strains, the extent of prior exposure to antifungal therapy is not known. This must be recognized as a possible limitation of the study, as prior exposure may result in acquired antifungal resistance, skewing the results.

Two quality control (QC) isolates, *C. parapsilosis* ATCC 22019 and *C. krusei* ATCC 6258, were used on each day of testing by the participant laboratories, as recommended by CLSI (4, 5). Only those results for which the QC MICs were within the established reference range were used in the study.

Antifungal susceptibility testing. Broth microdilution (BMD) testing was performed in accordance with the guidelines in CLSI document M27-A3 (4), using RPMI 1640 medium with 0.2% glucose, an inoculum of 0.5×10^3 to 2.5×10^3 cells/ml, and incubation at 35°C. MIC values were determined visually, after 24 and 48 h of incubation, as the lowest concentration of drug that caused complete inhibition (amphotericin B) or a significant diminution (≥50% inhibition; flucytosine and itraconazole) of growth relative to that of the growth control (4). In all instances, MIC trays were prepared using reagent grade powders, as directed by CLSI (4). Specifically, lipid formulations of amphotericin B were not tested.

Data analysis and definitions. The 24- and 48-h MIC ($MIC_{24/48}$) distributions for each species and antifungal agent from each laboratory were reviewed for outlier results, and modal MICs were determined for each species and antifungal agent from each laboratory; the aggregated data were then used to establish the WT MIC distributions and ECVs. It should be noted that some of the laboratories submitted MIC results that represented both 24- and 48-h readings, whereas others submitted data for only 24-h or only 48-h readings; thus, the number of results for each organism and drug differ for the two reading times. The definitions of WT organisms and ECVs were those outlined previously (13, 23–26, 28, 30, 38). A WT organism is defined as a strain that does not harbor any acquired or mutational resistance mechanisms to the particular antimicrobial agent being examined. The typical MIC distribution for WT organisms covers three to five doubling dilutions surrounding the modal MIC (1, 13, 38).

The ECVs for amphotericin B, flucytosine, and itraconazole and the eight *Candida* spp. were obtained, as described previously (23–26, 30), by considering the WT MIC distribution, the modal MIC for each distribution, and the inherent variability of the test (usually within one doubling dilution). In general, the ECV is determined to be at approximately two dilutions above the modal MIC and encompasses 95% of the results in the WT distribution (23–26, 30, 38). Organisms with acquired or mutational resistance mechanisms may be included among those for which the MIC results are higher than the ECV (1, 13, 30).

RESULTS AND DISCUSSION

The 24- and 48-h WT MIC distributions for amphotericin B, flucytosine, and itraconazole and each of eight *Candida* spp. are shown in Tables 1 to 3, respectively. The ECVs for each agent and

TABLE 2 WT MIC distributions of flucytosine for eight *Candida* species using CLSI BMD methods

Species	Incubation time (h)	No. of isolates tested	No. of isolates with MIC (μg/ml) of:										
			≤0.06	0.12	0.25	0.5	1	2	4	8	16	32	≥64
C. albicans	24	8,007	3,295	2,496	1,218	530	246	93	31	22	18	10	48
	48	7,241	409	3,147	1,633	721	708	297	154	37	30	15	90
C. glabrata	24	3,387	1,508	1,710	80	21	12	15	17	6	6	2	10
	48	2,981	982	1,682	206	36	16	11	13	15	10		10
C. parapsilosis	24	3,165	1,310	1,615	166	33	20	10	7	2	1	1	
	48	2,274	315	1,160	615	133	32	6	5	4			4
C. tropicalis	24	2,046	745	920	198	38	6	4	5	14	15	38	63
	48	1,588	140	455	566	224	52	27	3	9	6	18	88
C. krusei	24	499	2	17	6	6	13	39	69	176	163	5	3
	48	420	2	16	2	5	8	20	27	95	219	23	3
C. lusitaniae	24	85	10	66	1	4		1			1		2
	48	98	22	64	2		3	1		1		2	3
C. dubliniensis	24	84		61	9	13	1						
	48	44		39	2	2	1						
C. guilliermondii	24	41		30	3	3	2			3			

species are shown in Table 4. Whereas both 24- and 48-h ECVs ($ECV_{24/48}$) are provided for most species and antifungal agents, only 48-h ECVs are provided for *C. dubliniensis* and amphotericin B and only 24-h ECVs are provided for *C. guilliermondii* and flucytosine due to insufficient data (less than 30 results) at the 24-h and 48-h reading times, respectively.

Amphotericin B WT distribution and ECVs. Table 1 shows the 24- and 48-h MIC distributions for amphotericin B and the eight *Candida* spp. Whereas MIC data from clinical trials (18, 33, 35, 37) and population-based surveillance (22) show a restricted range of 0.12 to 1 μg/ml, the combined data from 11 laboratories span a range from ≤0.03 to 4 μg/ml for 24-h results and ≤0.03 to ≥8 μg/ml for 48-h results. The modal MICs were either 0.5 μg/ml (*C. albicans* at 24 h and *C. guilliermondii* at 24 and 48 h of incubation) or 1 μg/ml for each species, with the exception of *C. lusitaniae* (48-h mode, 2 μg/ml) (Tables 1 and 4); 93% (16,960/18,233) of the results at 24 h and 86% (18,689/21,631) at 48 h of incubation were within the 3-dilution range from 0.25 to 1 μg/ml.

The ECVs for amphotericin B and each species are shown in Table 4. The ECV at both 24 and 48 h was 2 μg/ml for each species, with the exception of *C. krusei* and *C. lusitaniae* (48-h ECV, 4 μg/ml). An amphotericin B ECV of 2 μg/ml at either 24 or 48 h of incubation encompasses 97% to 100.0% of the results for the indicated species, and the 48-h ECV of 4 μg/ml encompasses 100.0% of the results for *C. krusei* and *C. lusitaniae*.

In the literature, a default breakpoint for resistance or nonsusceptibility to amphotericin B is variously cited to be an MIC of either >0.5 μg/ml or >1 μg/ml (22, 34, 37). This cutoff is loosely based on the attainment of peak serum concentrations of 2 μg/ml and the pharmacodynamic correlate of a peak-concentration-to-MIC ratio of 2 as predictive of near-maximal activity (34, 37). Analyses of both clinical trial data (35) and clinical and microbiological data from population-based surveillance (22) have failed to establish any clinical correlation between amphotericin B MICs and clinical outcome. Park et al. (22) specifically addressed the predictive value of a CBP of >1 μg/ml using the CLSI method and found a distinct lack of prediction of clinical outcome; however, the limited database of 107 cases treated with amphotericin B did

TABLE 3 WT MIC distributions of itraconazole for eight *Candida* species using CLSI BMD methods

Species	Incubation Time (h)	No. of isolates tested	No. of isolates with MIC (μg/ml) of:										
			≤0.008	0.016	0.03	0.06	0.12	0.25	0.5	1	2	4	≥8
C. albicans	24	14,716	1,406	3,882	4,429	3,082	1,174	405	162	92	39	20	25
	48	14,298	1,212	2,855	4,273	3,636	1,303	366	221	159	73	119	81
C. glabrata	24	5,769	17	77	203	330	463	1,162	1,932	973	362	128	122
	48	5,846	5	16	32	73	196	593	1,726	1,871	618	335	381
C. parapsilosis	24	4,894	105	524	883	1,081	1,366	773	145	14	1	1	1
	48	4,643	83	239	563	937	1,377	1,063	310	57	5	5	4
C. tropicalis	24	3,624	15	236	732	1,072	965	391	133	44	16	16	4
	48	3,231	31	150	383	703	889	607	270	118	18	34	28
C. krusei	24	809	3	6	13	45	86	315	289	44	8		
	48	793	3	2	7	19	46	186	363	140	27		
C. lusitaniae	24	119	2	2	16	29	32	17	16	5			
	48	104		5	10	20	29	21	17	2			
C. dubliniensis	24	85	1	3	37	21	14	7	2				
	48	130	33	10	31	19	25	8	4				
C. guilliermondii	24	61		1	4	8	2	17	24	5			
	48	40		1		3	3	9	20	3	1		

TABLE 4 ECVs for amphotericin B, flucytosine, and itraconazole and eight *Candida* species

Species	Antifungal agent	Incubation time (h)	No. of isolates tested	MIC μg/ml		ECV (μg/ml) (%[a])
				Range	Mode	
C. albicans	Amphotericin B	24	9,252	≤0.03–4	0.5	2 (99.8)
		48	11,554	≤0.03–≥8	1	2 (99.2)
	Flucytosine	24	8,007	≤0.06–≥64	0.06	0.5 (94.2)
		48	7,241	≤0.06–≥64	0.12	1 (91.4)
	Itraconazole	24	14,716	≤0.008–≥8	0.03	0.12 (95.0)
		48	14,298	≤0.008–≥8	0.03	0.12 (92.9)
C. glabrata	Amphotericin B	24	3,117	≤0.03–4	1	2 (99.6)
		48	3,451	≤0.03–4	1	2 (98.7)
	Flucytosine	24	3,387	≤0.06–≥64	0.12	0.5 (98.0)
		48	2,981	≤0.06–≥64	0.12	0.5 (97.5)
	Itraconazole	24	5,796	≤0.008–≥8	0.5	2 (95.7)
		48	5,846	≤0.008–≥8	1	4 (97.9)
C. parapsilosis	Amphotericin B	24	3,107	≤0.03–4	1	2 (99.7)
		48	3,391	≤0.03–4	1	2 (97.3)
	Flucytosine	24	3,165	≤0.06–32	0.12	0.5 (98.7)
		48	2,274	≤0.06–≥64	0.12	0.5 (97.8)
	Itraconazole	24	4,894	≤0.008–≥8	0.12	0.5 (99.7)
		48	4,643	≤0.008–≥8	0.12	0.5 (98.5)
C. tropicalis	Amphotericin B	24	2,062	≤0.03–4	1	2 (99.8)
		48	2,373	≤0.03–≥8	1	2 (99.2)
	Flucytosine	24	2,046	≤0.06–≥64	0.12	0.5 (93.0)
		48	1,588	≤0.06–≥64	0.25	1 (90.5)
	Itraconazole	24	3,624	≤0.008–≥8	0.06	0.5 (97.8)
		48	3,231	≤0.008–≥8	0.12	0.5 (93.9)
C. krusei	Amphotericin B	24	577	≤0.03–4	1	2 (99.3)
		48	611	0.06–4	1	4 (100.0)
	Flucytosine	24	499	≤0.06–≥64	8	32 (99.4)
		48	419	≤0.06–≥64	16	32 (99.3)
	Itraconazole	24	809	≤0.008–2	0.25	1 (99.0)
		48	793	≤0.008–2	0.5	2 (100.0)
C. lusitaniae	Amphotericin B	24	71	0.06–2	1	2 (100.0)
		48	93	0.12–4	2	4 (100.0)
	Flucytosine	24	85	≤0.06–≥64	0.12	0.5 (95.3)
		48	98	≤0.06–≥64	0.12	1 (92.9)
	Itraconazole	24	119	≤0.008–1	0.12	0.5 (95.8)
		48	104	0.016–1	0.12	0.5 (98.1)
C. dubliniensis	Amphotericin B	48	75	≤0.03–2	0.5–1	2 (100.0)
	Flucytosine	24	84	0.12–1	0.12	0.5 (98.8)
		48	44	0.12–1	0.12	0.5 (97.7)
	Itraconazole	24	85	≤0.008–0.5	0.03	0.25 (97.6)
		48	130	≤0.008–0.5	NM[b]	0.25 (96.9)
C. guilliermondii	Amphotericin B	24	47	0.25–2	0.5	2 (100.0)
		48	83	0.06–2	0.5	2 (100.0)
	Flucytosine	24	41	0.12–8	0.12	1 (92.7)
	Itraconazole	24	61	0.016–1	0.5	1 (100.0)
		48	40	0.016–2	0.5	2 (100.0)

[a] Percentage of isolates at less than or equal to the ECV (epidemiological cutoff value; μg/ml).
[b] NM, no mode.

not contain an episode of IC for which the amphotericin B MIC was >1 μg/ml.

The results of the present study, while not taking into consideration any pharmacokinetic/pharmacodynamic or clinical data, suggests that an ECV of 2 μg/ml should be used to determine whether a clinical isolate of *Candida* should be considered WT or non-WT with respect to amphotericin B susceptibility. This cutoff would encompass all of the isolates reported by Rex et al. (35) and by Park et al. (22). Notably, these WT strains of *Candida* were associated with a 50% (22) to 79% (33, 35) success rate when treated with amphotericin B. Similar response data of 65% favorable response was seen in the amphotericin B arm (115 patients) of a study reported by Mora-Duarte et al. (18), where the MIC range was 0.25 to 2 μg/ml. Thus, an amphotericin B MIC greater than 2 μg/ml should be considered to be distinctly unusual for the vast majority of *Candida* spp., suggesting that treatment with this agent alone may not be optimal (34, 37).

Flucytosine WT distribution and ECVs. The 24- and 48-h MIC distributions for flucytosine and eight *Candida* spp. are shown in Table 2. The modal MICs were 0.12 μg/ml for five of the

eight species tested: the modal MIC was 0.06 μg/ml at 24 h for *C. albicans* and was 0.25 μg/ml at 48 h for *C. tropicalis*. These data are consistent with those reported previously (2, 6, 11, 14, 31) and document the excellent potency and spectrum of flucytosine against most *Candida* spp. Notably, the modal MIC of flucytosine against *C. krusei* was more than 64-fold higher ($MIC_{24/48}$, 8 and 16 μg/ml) than that seen with other species, documenting the decreased susceptibility of *C. krusei* to the agent.

The ECVs for flucytosine and the various *Candida* spp. are shown in Table 4. The 24- and 48-h ECVs were 0.5 to 1 μg/ml for all species, with the exception of *C. krusei* ($ECV_{24/48}$, 32 and 32 μg/ml). Aside from *C. krusei*, these ECVs approximate the susceptible breakpoint of ≤1 μg/ml established by the British Society for Mycopathology (3) and are well below the CBP for susceptibility of ≤4 μg/ml described by CLSI (4, 5, 37). This CBP was based on a combination of historical data and results from animal studies, with little or no consideration of clinical data or mechanisms of resistance (37). The finding that the majority of *Candida* sp. isolates have an MIC for flucytosine of ≤0.5 μg/ml raises concern that the CLSI CBPs of ≤4 μg/ml (susceptible), 8 to 16 μg/ml (intermediate), and ≥32 μg/ml (resistant) are entirely too high and are likely to be insensitive to the development of decreased susceptibility to flucytosine among the more highly susceptible species.

Resistance mechanisms for flucytosine are well described among various *Candida* spp. and include mutations in the *FCY2*, *FCY1*, and *FUR1* genes encoding cytosine permease, cytosine deaminase, and uracil phosphoribosyltransferase, respectively. In studies of *C. albicans* (8, 12, 32), *C. glabrata* (9, 40), *C. dubliniensis* (17), and *C. lusitaniae* (10), various patterns of reduced susceptibility to flucytosine have been elucidated, depending on the mutations present. In general, mutations in *FCY2* result in MICs that are somewhat elevated (>0.5 μg/ml but <8 μg/ml), whereas mutations in *FCY1* and *FUR1* result in MICs that are ≥32 μg/ml, depending on the heterozygosity of the organism (8–10, 12, 32, 40). In *C. albicans*, Dodgson et al. (8) found that isolates of *C. albicans* representing clade I for which *FUR1* was WT in both alleles all had flucytosine MICs of <0.5 μg/ml, those with a mutation in one allele had MICs of 0.5 to 8 μg/ml, and those with mutations in both alleles all had MICS of >16 μg/ml. This was confirmed by Hope et al. (12), who also showed that a *C. albicans* isolate with a mutation in *FCY1* exhibited an intermediate level of flucytosine resistance, with an MIC of 4 μg/ml. In the related species *C. dubliniensis*, McManus et al. (17) found that isolates with a homozygous mutation in *FCY1* (which encodes the deaminase) demonstrated high-level resistance (MIC, ≥128 μg/ml) and those without a mutation all had flucytosine MICs of ≤0.25 μg/ml. Edlind and Katiyar (9) demonstrated that the haploid yeast *C. glabrata* exhibited high-level flucytosine resistance (MIC, ≥32 μg/ml) associated with mutations in either *FCY1* or *FUR1* and moderately elevated MICs (MIC, 1 μg/ml) with mutations in *FCY2* (permease). Similar findings were also reported with the haploid yeast *C. lusitaniae* by Florent et al. (10). Taken together, these findings show that normally flucytosine-susceptible *Candida* spp. exhibit MICs of ≤0.5 μg/ml and do not have mutations in *FCY1*, *FCY2*, or *FUR1*, whereas isolates with MICs between 1 and 8 μg/ml may have mutations in *FCY2* or are heterozygous for mutations in *FUR1* and those with homozygous mutations in *FUR1* or in *FCY1* are highly resistant, with MICs of ≥32 μg/ml. These data provide support for the ECVs reported in Table 4 in that WT strains for which the flucytosine MIC is ≤0.5 μg/ml are unlikely to contain a flucytosine resistance mutation, whereas those for which the MIC is more than the ECV (i.e., >0.5 μg/ml) are likely to be either homozygous or heterozygous for a flucytosine resistance mutation.

Itraconazole WT distribution and ECVs. The 24- and 48-h MIC distributions for itraconazole and the eight *Candida* spp. are shown in Table 3. Overall, itraconazole was quite active against most *Candida* spp. (modal MICs, 0.03 to 0.12 μg/ml), with the exception of *C. glabrata* (modal MIC, 0.5 to 1 μg/ml), *C. krusei* (modal MIC, 0.25 to 0.5 μg/ml), and *C. guilliermondii* (modal MIC, 0.5 μg/ml). These patterns of *in vitro* susceptibility of the different *Candida* spp. are well known (16, 19, 27, 36). Although itraconazole has often been used as a comparator in surveys of the *in vitro* antifungal susceptibilities of opportunistic fungal pathogens, it has rarely been the primary focus of such studies (27); thus, its activity against opportunistic fungi is generally underappreciated (16, 21). In part, this may also be due to a perception of rather poor activity of itraconazole against *Candida* spp., given the very conservative CBPs (susceptible, MIC ≤ 0.12 μg/ml; susceptible dose-dependent, MIC = 0.25 to 0.5 μg/ml; resistant, MIC ≥ 1 μg/ml) assigned by the CLSI (36). These breakpoints were assigned based entirely on MICs for isolates of *Candida* spp. (90% of which were *C. albicans*) obtained from patients with oropharyngeal candidiasis who were treated with oral itraconazole (capsule and/or solution) and in whom serum concentrations of <0.5 μg/ml were common (36).

The ECVs for itraconazole and each species are shown in Table 4. The ECV was lowest for *C. albicans* (0.12 μg/ml at 24 and 48 h) and was 0.25 μg/ml (24 and 48 h) for *C. dubliniensis* and 0.5 μg/ml (24 and 48 h) for all other species, with the exception of *C. glabrata* ($ECV_{24/48}$, 2 and 4 μg/ml), *C. krusei* ($ECV_{24/48}$, 1 and 2 μg/ml), and *C. guilliermondii* ($ECV_{24/48}$, 1 and 2 μg/ml). Given these MIC distributions and ECVs, it is clear that the CLSI CBPs for itraconazole are inappropriate for any species other than *C. albicans*. Whereas the CBPs for itraconazole should provide an optimal means for detecting decreased susceptibility among isolates of *C. albicans*, the ECVs for all other species should be used for this purpose.

Summary and conclusions. The ECVs determined for *C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, *C. krusei*, *C. lusitaniae*, *C. dubliniensis*, and *C. guilliermondii* will be important in detecting the emergence of decreased susceptibility to amphotericin B, flucytosine, and itraconazole in ongoing surveillance efforts. The previous CBPs for flucytosine appear to be too insensitive to be of epidemiological value in monitoring the emergence of decreased susceptibility to the agent, especially among the more susceptible species (e.g., *C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, *C. lusitaniae*, *C. dubliniensis*, and *C. guilliermondii*). Likewise, the CBPs for itraconazole should be applied only to *C. albicans*, and the ECVs should be used to detect emerging resistance among the other *Candida* spp. Given the absence of CBPs for amphotericin B, the ECVs established here will prove useful in detecting the emergence of potential resistance, as the agent (conventional and lipid formulations) is employed in the management of IC.

Future studies must include molecular analysis of resistance mechanisms for the strains that fall outside the ECV to better understand the frequency and clinical importance of such strains and mechanisms. The establishment of the WT MIC distributions

and ECVs for amphotericin B, flucytosine, and itraconazole and each *Candida* spp. will be useful in resistance surveillance and may prove to be an important step in the development of species-specific CBPs for these well-established antifungal agents.

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