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Multicenter Study of Anidulafungin and Micafungin MIC Distributions and Epidemiological Cutoff Values for Eight *Candida* Species and the CLSI M27-A3 Broth Microdilution Method

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Since epidemiological cutoff values (ECVs) using CLSI MICs from multiple laboratories are not available for *Candida* spp. and the echinocandins, we established ECVs for anidulafungin and micafungin on the basis of wild-type (WT) MIC distributions (for organisms in a species-drug combination with no detectable acquired resistance mechanisms) for 8,210 *Candida albicans*, 3,102 *C. glabrata*, 3,976 *C. parapsilosis*, 2,042 *C. tropicalis*, 617 *C. krusei*, 258 *C. lusitaniae*, 234 *C. guilliermondii*, and 131 *C. dubliniensis* isolates. CLSI broth microdilution MIC data gathered from 15 different laboratories in Canada, Europe, Mexico, Peru, and the United States were aggregated to statistically define ECVs. ECVs encompassing 97.5% of the statistically modeled population for anidulafungin and micafungin were, respectively, 0.12 and 0.03 μg/ml for *C. albicans*, 0.12 and 0.03 μg/ml for *C. glabrata*, 8 and 4 μg/ml for *C. parapsilosis*, 0.12 and 0.06 μg/ml for *C. tropicalis*, 0.25 and 0.25 μg/ml for *C. krusei*, 1 and 0.5 μg/ml for *C. lusitaniae*, 8 and 2 μg/ml for *C. guilliermondii*, and 0.12 and 0.12 μg/ml for *C. dubliniensis*. Previously reported single and multicenter ECVs defined in the present study were quite similar or within 1 2-fold dilution of each other. For a collection of 230 WT isolates (no *fks* mutations) and 51 isolates with *fks* mutations, the species-specific ECVs for anidulafungin and micafungin correctly classified 47 (92.2%) and 51 (100%) of the *fks* mutants, respectively, as non-WT strains. These ECVs may aid in detecting non-WT isolates with reduced susceptibility to anidulafungin and micafungin due to *fks* mutations.

The echinocandins anidulafungin and micafungin are widely recognized as first-line antifungal agents for the treatment of candidemia and other forms of invasive candidiasis (infections of normally sterile sites, tissues, and organs) (1–3). The Clinical and Laboratory Standards Institute (CLSI) Subcommittee on Antifungal Susceptibility Tests has standardized the broth microdilution reference method for testing the echinocandins against *Candida* spp. (4) and has developed new species-specific clinical breakpoints (CBPs) for the more prevalent species (*Candida albicans*, *C. glabrata*, *C. guilliermondii*, *C. krusei*, *C. parapsilosis*, and *C. tropicalis*) (5–7); epidemiological cutoff values (ECVs) for common and less prevalent species (e.g., *C. dubliniensis*, *C. guilliermondii*, and *C. lusitaniae*) have also been defined (6–8). Whereas CBPs are used to identify those isolates that are likely to respond to treatment with a given antimicrobial agent administered at the approved closing regimen for that agent, the ECV can be used as the most sensitive indicator of the emergence of strains with reduced susceptibility to a given agent (6, 9–12). An ECV is an MIC threshold value that allows the discrimination of wild-type (WT) strains (those without mutational or acquired resistance mechanisms) from non-WT strains (those harboring mutational or acquired resistance mechanisms) (6, 7, 9, 10, 12).

The species-specific ECVs defined previously for all three echinocandins (anidulafungin, caspofungin, and micafungin) were determined using CLSI MIC results from a single laboratory (8, 13). Because MIC distributions generated by a single laboratory may not be completely representative of WT MICs for all agents and species of *Candida* (14), we further validated these ECVs for anidulafungin and micafungin by gathering MIC data from multiple laboratories (15 different centers) in Canada, Europe, Mexico, Peru, and the United States. MIC data were also collected for caspofungin; however, due to excessive heterogeneity in the respective MIC distributions, the caspofungin results are presented separately (14). Although the number of isolates needed to calculate a representative ECV is not established, there is a working consensus among experts that recommends at least 50 strains (and, preferably, 100) from at least 3 to 5 different laboratories (15, 16).

The main objectives of the present study included (i) definition of the WT MIC distributions of anidulafungin and micafungin for five of the most common and three less common *Candida* species causing invasive candidiasis by using aggregated CLSI MIC results from 15 different laboratories (131 to 8,210 MICs, according to

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species and antifungal agent), (ii) proposal of the ECV for each species-drug combination for the 24-h CLSI method, (iii) comparison of the ECVs obtained from this multicenter study with those previously proposed by a single laboratory and those recently determined in a multicenter study for the Sensititre YeastOne method (17), and (iv) demonstration of the ability of these ECVs to discriminate WT from non-WT strains of *Candida* using a collection of 230 WT isolates (no *fks* mutations) and 51 isolates with *fks* mutations.

MATERIALS AND METHODS

Isolates. Each isolate was recovered from a unique clinical specimen at 15 different centers or reference laboratories: The University of Iowa, Iowa City, IA; JMI Laboratories, North Liberty, IA; VCU Medical Center, Richmond, VA; Instituto de Medicina Tropical Alexander von Humboldt-Universidad Peruana Cayetano Heredia, Lima, Peru; Hospital Universitario La Fe, Valencia, Spain; University of Texas Health Science Center, San Antonio, TX; University of Alberta, Edmonton, Alberta, Canada; Universidad Autónoma de Nuevo León, Monterey, Nuevo León, Mexico; Facultat de Medicina, IISPV, URV, Reus, Spain; The Innsbruck Medical University, Innsbruck, Austria; Centers for Disease Control and Prevention, Atlanta, GA; Hospital Universitario de Valme, Seville, Spain; Canisius Wilhelmina Hospital and Radboud University, Nijmegen Medical Center, Nijmegen, Netherlands; University of Texas Health Science Center, Houston, TX; Hospital General Universitario Gregorio Marañón, Facultad de Medicina-Universidad Complutense, Madrid, Spain; and Laboratoire de Santé Publique du Québec, Institut National de Santé Publique du Québec, Quebec, Quebec, Canada. These laboratories were coded 1 to 20 (for several studies), but because some laboratories were excluded from the study or did not provide echinocandin data, we used data from the remaining 15 laboratories (Table 1). Isolates were identified and stored at each medical center using standardized nonmolecular methodologies; isolates were not characterized for mutations. The total numbers of aggregated available CLSI MICs from the 15 laboratories per species were as follows: 8,210 for *C. albicans*, 3,102 for *C. glabrata*, 3,976 for *C. parapsilosis*, 2,042 for *C. tropicalis*, 617 for *C. krusei*, 258 for *C. lusitaniae*, 234 for *C. guilliermondii*, and 131 for *C. dubliniensis*.

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.

In addition to these isolates, we included the anidulafungin and micafungin MIC distributions from an earlier collection of 281 isolates (5 species; 230 WT and 51 non-WT) all tested for the presence (non-WT) and absence (WT) of mutations in *fks1* and *fks2* (*C. glabrata* only) (6, 10) in order to assess the ability of the various anidulafungin and micafungin ECVs to discriminate non-WT from WT strains of *Candida* at the molecular level.

Antifungal susceptibility testing. Broth microdilution testing was performed in each laboratory 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 in air at 35°C. MIC values were determined visually after 24 h of incubation as the lowest concentration of drug that caused a significant diminution (≥50% inhibition) 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.

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 QC MICs were within the established reference range were used in the study.

Definitions. The definitions of the WT population and ECV were those reported previously (6, 8, 9, 16). A WT population was the subpopulation of isolates/MICs for a species-drug combination with no acquired detectable resistance mechanisms (12, 17).

The ECV is the highest MIC value of the WT population. It is calculated by taking into account the MIC distribution, the modal MIC of each distribution, and the inherent variability of the test (usually within 1 doubling dilution) and should encompass ≥95% of isolates (7).

Data analysis. The MIC distributions of each of the eight species tested in each participant laboratory were first screened for evidence of grossly skewed distributions that precluded statistical fitting, and the modal MICs for each laboratory were determined (14, 16); however, their pseudomodes were the same as those observed in the laboratories included in the analysis. Grossly skewed distributions and distributions which had a modal MIC at the lowest concentration tested were excluded. Next, the aggregate WT distributions for each antifungal agent and species of *Candida* were obtained by pooling qualifying MIC distributions from participant laboratories, and the ECV was then estimated by the statistical method of Turnidge et al. (18). For this study, a minimum of 3 laboratories and 100 data points was required to establish a reasonable estimated ECV for a given agent and species. In the statistical method, the modeled WT population is based on fitting a lognormal distribution at the lower end of the MIC range, calculating the mean and standard deviation of that normal distribution, and using those values to estimate the MIC (ECV) that captured at least 95%, 97.5%, and 99% of the modeled WT population, rounded up to the nearest 2-fold dilution. The modes for each agent and species and the inherent variability (within approximately 1 doubling dilution) of susceptibility testing were also considered, and a search for outlier laboratories in each distribution was performed (19).

RESULTS AND DISCUSSION

A total of 17 laboratories submitted MIC data for anidulafungin and micafungin; data for 2 laboratories were omitted due to the use of 2.0% glucose (rather than the 0.2% glucose prescribed by CLSI) in the test medium (4). In addition, MICs for some species and antifungal agent combinations from 1 and 5 laboratories, respectively, were not included in the final analysis due to truncated distributions (modal MIC at the lowest concentration tested). The remaining aggregated CLSI MIC distributions from the 15 laboratories for the various species versus anidulafungin and micafungin were 8,210 and 7,874, respectively, for *C. albicans*, 2,680 and 3,102, respectively, for *C. glabrata*, 3,976 and 3,484, respectively, for *C. parapsilosis*, 2,042 and 1,605, respectively, for *C. tropicalis*, 322 and 617, respectively, for *C. krusei*, 234 and 258, respectively, for *C. lusitaniae*, 222 and 234, respectively, for *C. guilliermondii*, and 131 and 117, respectively, for *C. dubliniensis* (Tables 1 to 3).

The data in Table 1 include the number of MIC results and the range and modal MIC values for each laboratory that contributed qualifying data for the different drug-organism combinations. These results demonstrate the comparability of the MIC distributions contributed by the various laboratories for each antifungal agent and species of *Candida*. With few exceptions, the modal MICs for each laboratory were within 1 2-fold dilution of one another within the different drug-organism pairs.

The pooled WT MIC distributions for anidulafungin and micafungin and each of the eight species of *Candida* are shown in Table 2. The *in vitro* activities of the 2 antifungal agents tested were similar to those observed by other authors using the CLSI method (20–22). All of the MIC distributions were typical for WT organisms and covered 3 to 5 2-fold dilution steps surrounding the modal MIC.

Table 3 depicts the proposed anidulafungin and micafungin ECVs (using ≥95%, ≥97.5%, and ≥99% of the modeled MIC population) as well as the modal MICs for each of the eight species

TABLE 1 MIC distributions of anidulafungin and micafungin for eight species of *Candida* and each contributing laboratory determined using CLSI M27-A3 broth microdilution methods

Antifungal agent	Species (total no. of isolates tested)	Laboratory	No. of isolates tested	MIC (μg/ml)	
				Range	Mode
Anidulafungin	*C. albicans* (8,210)	1	336	0.008–0.5	0.03
		2	138	0.015–0.25	0.03
		3	363	0.008–2	0.015
		9	447	0.008–2	0.03
		12	1,246	0.008–1	0.03
		18	1,397	0.008–2	0.015
		19	4,283	0.008–1	0.03
	C. glabrata (2,680)	1	106	0.015–2	0.06
		2	121	0.015–2	0.03
		3	98	0.015–4	0.03
		6	40	0.015–0.12	0.06
		18	1,079	0.008–4	0.03
		19	1,236	0.015–4	0.06
	C. parapsilosis (3,976)	1	28	0.12–4	1
		2	81	0.12–4	0.5
		3	246	0.015–4	2
		9	159	0.015–4	2
		10	391	0.03–4	1
		11	275	0.03–8	2
		12	878	0.03–4	1
		14	44	0.03–2	1
		17	31	0.12–2	1
		18	599	0.008–8	1
		19	1,238	0.015–4	2
		20	6	0.03–2	1
	C. tropicalis (2,042)	1	36	0.015–1	0.06
		2	49	0.015–0.25	0.03
		3	55	0.015–2	0.03
		6	45	0.015–0.25	0.03
		9	72	0.015–0.25	0.03
		12	425	0.008–2	0.06
		18	364	0.008–0.5	0.015
		19	996	0.008–2	0.03
	C. krusei (322)	1	20	0.03–1	0.06
		2	29	0.03–0.25	0.12
		3	19	0.03–2	0.03
		6	2	0.06–2	0.06
		9	25	0.06–0.25	0.12
		11	79	0.03–2	0.06
		12	83	0.03–2	0.06
		18	54	0.015–0.25	0.03
		20	11	0.015–0.12	0.03
	C. lusitaniae (234)	1	20	0.06–0.5	0.25
		2	12	0.06–1	0.25
		9	35	0.06–1	0.25
		12	9	0.06–1	0.25
		18	59	0.008–1	0.5
		19	99	0.06–1	0.5
	C. guilliermondii (222)	1	9	0.25–4	2
		6	2	1	1
		9	6	0.5–2	2
		11	8	1–2	1
		12	91	0.03–4	1
		14	3	0.06–1	1
		18	15	0.25–4	1
		19	88	0.06–4	1
	C. dubliniensis (131)	1	38	0.015–4	0.06
		5	11	0.015–0.25	0.06
		9	14	0.015–0.12	0.03

(Continued on following page)

TABLE 1 (Continued)

Antifungal agent	Species (total no. of isolates tested)	Laboratory	No. of isolates tested	MIC (μg/ml)	
				Range	Mode
		11	5	0.015–0.03	0.03
		18	63	0.015–1	0.06
Micafungin	*C. albicans* (7,874)	1	95	0.008–0.5	0.015
		3	366	0.008–2	0.015
		9	447	0.015–4	0.03
		12	1,246	0.008–1	0.03
		18	1,398	0.008–2	0.015
		19	4,282	0.008–0.5	0.015
		20	40	0.008–1	0.015
	C. glabrata (3,102)	1	52	0.008–0.12	0.03
		2	284	0.015–4	0.03
		3	97	0.008–1	0.015
		9	164	0.015–0.12	0.03
		12	176	0.008–2	0.015
		18	1,079	0.008–32	0.015
		19	1,236	0.008–2	0.015
		20	14	0.015–4	0.015
	C. parapsilosis (3,484)	1	32	0.06–4	1
		2	239	0.06–2	0.5
		3	251	0.015–4	1
		4	27	0.015–1	0.5
		9	159	0.25–4	1
		11	55	0.03–2	1
		12	878	0.015–4	1
		18	599	0.015–4	1
		19	1,238	0.015–2	1
		20	6	0.015–2	1
	C. tropicalis (1,605)	1	39	0.008–0.12	0.015
		3	53	0.008–4	0.015
		8	45	0.015–8	0.03
		9	72	0.008–0.25	0.06
		11	32	0.008–0.06	0.03
		18	363	0.008–1	0.03
		19	996	0.008–1	0.015
		20	5	0.008–0.06	0.03
	C. krusei (617)	1	41	0.03–1	0.12
		2	72	0.015–0.25	0.12
		3	21	0.015–1	0.12
		4	60	0.03–0.5	0.12
		11	5	0.03–0.06	0.06
		12	83	0.015–0.25	0.12
		18	54	0.06–0.12	0.12
		19	270	0.015–0.25	0.06
		20	11	0.03–0.12	0.06
	C. lusitaniae (258)	2	56	0.015–16	0.12
		9	35	0.03–0.5	0.25
		12	9	0.015–0.5	0.12
		18	59	0.015–2	0.25
		19	99	0.008–1	0.12
	C. guilliermondii (234)	1	13	0.03–0.5	0.25
		2	27	0.06–0.25	0.25
		11	91	0.015–8	0.5
		18	14	0.25–2	0.25
		19	88	0.015–8	0.5
		20	1	0.25	0.25
	C. dubliniensis (117)	2	27	0.015–2	0.03
		3	1	0.015	0.015
		9	10	0.03–0.06	0.03
		17	63	0.008–1	0.06
		18	16	0.015–8	0.06

TABLE 2 Pooled MIC distributions of anidulafungin and micafungin for eight species of *Candida* from 5 to 12 laboratories using CLSI M27-A3 method

Antifungal agent	Species (no. of isolates tested)	No. of isolates with MIC (μg/ml) of[a]:											
		0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	≥16
Anidulafungin	*C. albicans* (8,210)	752	2,582	2,823	1,410	413	132	71	21	6			
	C. glabrata (2,680)	2	66	728	1,316	456	40	23	16	22	11		
	C. parapsilosis (3,976)	1	15	66	77	109	196	541	1,202	1,523	244	2	
	C. tropicalis (2,042)	152	449	784	419	140	47	32	11	8			
	C. krusei (322)	7	14	95	119	46	20	7	10	4			
	C. lusitaniae (234)	1		1	15	32	95	77	13				
	C. guilliermondii (222)			1	8	12	19	31	84	54	13		
	C. dubliniensis (131)		21	47	47	9	3	1	1	1	1		
Micafungin	*C. albicans* (7,874)	746	4,223	1,999	465	184	146	82	21	6	2		
	C. glabrata (3,102)	318	1,931	596	124	40	26	20	16	18	12		
	C. parapsilosis (3,484)		23	8	15	50	289	811	1,581	674	33		
	C. tropicalis (1,605)	71	598	588	270	54	14	2	6		1	1	
	C. krusei (617)		18	52	297	198	47	3	2				
	C. lusitaniae (258)	1	7	14	24	91	101	13	1	4			2
	C. guilliermondii (234)		5	8	19	25	60	67	38	7	3	2	
	C. dubliniensis (117)	1	5	43	55	5	1		1	1		5	

[a] Shaded values indicate the modes (the most frequent MIC).

of *Candida*. Very little difference between the ECVs encompassing 95%, 97.5%, or 99% of the modeled MIC populations was seen. While previous studies have reported ECVs to be the MIC encompassing at least 95% of the population defined using both the eyeball method and statistical methods for *Candida* spp. (7, 13) and *Aspergillus* spp. (16), for comparison purposes we used the ECV that encompassed 97.5% of the modeled population; these ECVs appeared to relate better to those defined by the eyeball method (18). In general, the ECVs for each antifungal agent and species of *Candida* were within 1 or 2 2-fold dilutions of the modal MIC values. Likewise, the ECVs for anidulafungin and micafungin and each species of *Candida* were within 1 to 2 2-fold dilutions of one another, with those of anidulafungin usually being higher than those of micafungin.

The ECVs defined in the present study are similar to those reported previously from a single laboratory using the CLSI broth microdilution method as well as those reported by Canton et al. (17) using the Sensititre YeastOne method. In each case, the method for calculating the ECVs was that of Turnidge et al. (18); however, the single-laboratory CLSI study and the multicenter YeastOne study used the 95% threshold, while in the present study, ECVs encompassing 97.5% of the modeled population were also defined (Table 3). In general, 100% of the ECVs from both the single-laboratory CLSI study and the YeastOne study

TABLE 3 Anidulafungin and micafungin ECVs for eight species of *Candida* based on MICs from 5 to 12 laboratories determined by the CLSI M27-A3 broth microdilution method

Antifungal agent tested	Species	No. of isolates	MIC (μg/ml)		ECV (μg/ml)[a]		
			Range	Mode[b]	≥95%	≥97.5%	≥99%
Anidulafungin	*C. albicans*	8,210	0.008–2	0.03	0.06	0.12	0.12
	C. glabrata	2,680	0.008–4	0.06	0.12	0.12	0.25
	C. parapsilosis	3,976	0.008–8	2	4	8	8
	C. tropicalis	2,042	0.008–2	0.03	0.12	0.12	0.12
	C. krusei	322	0.008–2	0.06	0.12	0.25	0.25
	C. lusitaniae	234	0.008–1	0.25	1	1	1
	C. guilliermondii	222	0.03–4	1	4	8	8
	C. dubliniensis	131	0.015–4	0.03	0.12	0.12	0.12
Micafungin	*C. albicans*	7,874	0.008–4	0.015	0.03	0.03	0.03
	C. glabrata	3,102	0.008–4	0.015	0.03	0.03	0.03
	C. parapsilosis	3,484	0.015–4	1	2	4	4
	C. tropicalis	1,605	0.008–8	0.015	0.06	0.06	0.12
	C. krusei	617	0.015–1	0.06	0.25	0.25	0.25
	C. lusitaniae	258	0.008-≥16	0.25	0.5	0.5	1
	C. guilliermondii	234	0.015–8	0.5	2	2	4
	C. dubliniensis	117	0.008–8	0.06	0.12	0.12	0.12

[a] Calculated ECVs comprising ≥95%, ≥97.5%, or ≥99% of the statistically modeled MIC population.
[b] The most frequent MIC.

TABLE 4 Application of ECVs to MIC distributions of anidulafungin and micafungin for *Candida* strains tested for the presence of *fks1* and *fks2* mutations using the CLSI broth microdilution method[a]

Species (no. of isolates tested)	Antifungal agent	ECV (μg/ml)	No. of isolates by ECV category (no. of isolates showing mutations)	
			MIC ≤ ECV	MIC > ECV
C. albicans (52)	Anidulafungin	0.12	42 (1)	10 (10)
	Micafungin	0.03	31 (0)	21 (11)
C. glabrata (169)	Anidulafungin	0.12	135 (2)	34 (28)
	Micafungin	0.03	124 (0)	45 (30)
C. tropicalis (31)	Anidulafungin	0.12	25 (1)	6 (5)
	Micafungin	0.06	15 (0)	16 (6)
C. krusei (27)	Anidulafungin	0.25	24 (0)	3 (3)
	Micafungin	0.25	23 (0)	4 (3)
C. dubliniensis (2)	Anidulafungin	0.12	1 (0)	1 (1)
	Micafungin	0.12	1 (0)	1 (1)

[a] Data were compiled from references 6, 10, and 25 to 27.

were equal to or within 1 2-fold dilution of those reported in the present multicenter study (ECVs comprising 97.5% of the population), except for the higher YeastOne ECV of anidulafungin for *C. tropicalis* (1 μg/ml versus 0.12 μg/ml). Previous comparison of MICs obtained by the YeastOne and CLSI methods provided comparable results (23, 24). Regarding the European Committee on Antimicrobial Susceptibility Testing (EUCAST-AFST) cutoff values for *Candida* and the echinocandins, this organization has established breakpoints for anidulafungin and *C. albicans* (≤0.03 μg/ml), *C. glabrata* (≤0.06 μg/ml), *C. krusei* (0.06 μg/ml), and *C. tropicalis* (0.06 μg/ml) and, more recently, for micafungin and *C. albicans* (≤0.01 μg/ml), *C. glabrata* (≤0.03 μg/ml), and *C. parapsilosis* (0.002 μg/ml) (http://www.EUCAST.org).

The ability of the ECVs encompassing 97.5% of the statistically modeled population to differentiate strains of *Candida* with acquired mechanisms of resistance to the echinocandins (e.g., mutations in *fks1* or *fks2*) may be seen in the data presented in Table 4. The isolates in the collection for which the results are depicted in Table 4 were selected from global and population-based surveillance and reference collections to represent both WT and non-WT isolates with available MIC results for anidulafungin and micafungin, and all isolates were previously characterized regarding the presence or absence of mutations in the hot-spot (HS) regions of *fks1* (*C. albicans*, *C. glabrata*, *C. tropicalis*, *C. krusei*, and *C. dubliniensis*) and *fks2* (*C. glabrata* only) (6, 10, 25–27). A total of 51 isolates harbored mutations in either *fks1* or *fks2*: 11 *C. albicans* isolates, 30 *C. glabrata* isolates, 6 *C. tropicalis* isolates, 3 *C. krusei* isolates, and 1 *C. dubliniensis* isolate. The ECVs for anidulafungin and *C. albicans*, *C. glabrata*, *C. tropicalis*, and *C. dubliniensis* were all 0.12 μg/ml, and the ECV for *C. krusei* was 0.25 μg/ml (Table 3 and Table 4). Using these cutoffs, the CLSI method correctly classified 47 (92.2%) of the 51 mutant strains as non-WT (for which the MIC was greater than the ECV) and 223 (97.0%) of 230 WT strains (with no *fks* mutation) as WT. The ECVs for micafungin and *C. albicans*, *C. glabrata*, *C. tropicalis*, *C. krusei*, and *C. dubliniensis* were 0.03 μg/ml, 0.03 μg/ml, 0.06 μg/ml, 0.25 μg/ml, and 0.12 μg/ml, respectively (Tables 3 and 4). Using these ECVs, the CLSI method with micafungin correctly classified all 51 mutant strains as non-WT and 194 (84.3%) of 230 WT strains (for which the MIC was less than or equal to the ECV) as WT. The reason for these misclassifications could be due to an alternative mechanism of resistance (WT as non-WT) or could simply be reflective of the crossing over of WT and non-WT MIC distributions; unfortunately, there is no enough clinical information about these strains.

In summary, MIC data originating in 5 to 12 laboratories in 7 different countries have enabled us to propose species-specific ECVs for anidulafungin and micafungin and eight different species of *Candida*. We present ECVs that encompass 95%, 97.5%, and 99% of the statistically modeled population and recommend that the ECVs encompassing 97.5% be used to differentiate WT from non-WT strains of *Candida* for both echinocandins. The robust nature of these ECVs is demonstrated by comparison with those generated in another multicenter study using the YeastOne method as well as those generated in a single-center study using the CLSI method. Furthermore, we demonstrate the ability of the species-specific ECVs for both echinocandins to identify *fks* mutant strains in a population of 281 well-characterized *Candida* species. Although either anidulafungin or micafungin testing proved very sensitive in detecting the *fks* mutant strains, testing with micafungin tended to misclassify some isolates of *C. albicans*, *C. glabrata*, and *C. tropicalis* that did not harbor *fks* mutations as non-WT. The ECVs for anidulafungin and micafungin and the CLSI broth microdilution method will help in monitoring the emergence of echinocandin resistance among target species of *Candida*.

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