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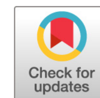

Multicenter, International Study of MIC/MEC Distributions for Definition of Epidemiological Cutoff Values for *Sporothrix* Species Identified by Molecular Methods

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ABSTRACT Clinical and Laboratory Standards Institute (CLSI) conditions for testing the susceptibilities of pathogenic *Sporothrix* species to antifungal agents are based on a collaborative study that evaluated five clinically relevant isolates of *Sporothrix schenckii sensu lato* and some antifungal agents. With the advent of molecular identification, there are two basic needs: to confirm the suitability of these testing conditions for all agents and *Sporothrix* species and to establish species-specific epidemiologic cutoff values (ECVs) or breakpoints (BPs) for the species. We collected available CLSI MICs/minimal effective concentrations (MECs) of amphotericin B, five triazoles, terbinafine, flucytosine, and caspofungin for 301 *Sporothrix schenckii sensu stricto*, 486 *S. brasiliensis*, 75 *S. globosa*, and 13 *S. mexicana* molecularly identified isolates. Data were obtained in 17 independent laboratories (Australia, Europe, India, South Africa, and South and North America) using conidial inoculum suspensions and 48 to 72 h of incubation at 35°C. Sufficient and suitable data (modal MICs within 2-fold concentrations) allowed the proposal of the following ECVs for *S. schenckii* and *S. brasiliensis*, respectively: amphotericin B, 4 and 4 μg/ml; itraconazole, 2 and 2 μg/ml; posaconazole, 2 and 2 μg/ml; and voriconazole, 64 and 32 μg/ml. Ketoconazole and terbinafine ECVs for *S. brasiliensis* were 2 and 0.12 μg/ml, respectively. Insufficient or unsuitable data precluded the calculation of ketoconazole and terbinafine (or any

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other antifungal agent) ECVs for *S. schenckii*, as well as ECVs for *S. globosa* and *S. mexicana*. These ECVs could aid the clinician in identifying potentially resistant isolates (non-wild type) less likely to respond to therapy.

KEYWORDS ECVs, *Sporothrix*, antifungal resistance, molecular methods

Sporotrichosis is considered a relatively uncommon granulomatous infection of the cutaneous and subcutaneous tissue, although dissemination to other deep-seated organs has been reported (1, 2). The first case of sporotrichosis was documented in the United States in the late 1800s by Benjamin Schenck (3, 4). This case was followed by worldwide reports, as well as numerous outbreaks (e.g., in the South African mines in the 1920s and 1930s, among children in relatively remote areas of Peru, some Brazilian case clusters, and in the United States) (5–8). In addition, several feline outbreaks caused by *Sporothrix brasiliensis* with transmissions from cat to human to cat have been reported in Brazil (7, 8). Most other outbreaks or infections have been associated with traumatic inoculation of vegetative materials and/or soil. Until recently, all cases were attributed to *Sporothrix schenckii*, according to phenotypic identification (macro- and microscopic studies, carbohydrate assimilation, and conversion to the yeast phase). The advent of molecular methodologies and the use of internal transcribed spacer (ITS) region sequence analysis of chitin synthase, β-tubulin, and calmodulin (CAL) genes indicated that there were various cryptic species nested in the medically relevant clade. The taxon was considered the *Sporothrix schenckii* species complex (8–12). Therefore, sporotrichosis is caused by different pathogenic species, including the three clinically relevant species evaluated in the present study: *S. schenckii sensu stricto* (referred to from here on as *S. schenckii*), *S. brasiliensis*, and *Sporothrix globosa*. We also evaluated one rare species in the environmental clade, *Sporothrix mexicana* (10, 11).

The recommended therapeutic agents for the treatment of human sporotrichosis are itraconazole, amphotericin B and its lipid formulations (invasive/disseminated disease), terbinafine, and fluconazole; a saturated solution of potassium iodide has been an alternative choice for lymphocutaneous/cutaneous infections (2, 13–18). Ketoconazole is not used as much given its low efficacy and potentially severe side effects (13, 16). Among the newer triazoles, *in vivo* and *in vitro* activity has been reported with posaconazole in combination with amphotericin B, while voriconazole has not been considered a therapeutic choice for these infections due to its high MICs (19, 20).

The Clinical and Laboratory Standards Institute (CLSI) has described testing conditions for the "filamentous phase of the *S. schenckii* species complex" because the initial CLSI collaborative evaluation predated molecular studies, which included only five isolates that were documented as "*S. schenckii*" (21, 22). Therefore, the species of *Sporothrix* are not mentioned in the CLSI M38-A2 document (21). In addition, interpretive MIC/minimal effective concentration (MEC) categories, either formal breakpoints (BPs) or epidemiological cutoff values (ECVs), have not been established for any of the *Sporothrix* species. Method-dependent and species-specific ECVs should identify the non-wild-type (non-WT) isolates with reduced susceptibility to the agent being evaluated due to acquired mutational or other resistance mechanisms (23, 24). While ECVs would not predict the clinical success of therapy, these endpoints could identify the isolates less likely to respond to the specific agents. We collected available MICs/MECs for nine antifungal agents from 17 laboratories for molecularly identified isolates of four *Sporothrix* species. These MIC/MEC values represent the antifungal susceptibilities of the two more prevalent species (*S. schenckii* and *S. brasiliensis*), as well those of *S. globosa* and *S. mexicana*, to the different agents as determined by the CLSI M38-A2 method (21). Although the *in vitro* data were obtained in 17 laboratories, the isolates originated from different geographical areas (Australia, Europe, India, South Africa, and both South and North American countries).

The purposes of the present study were (i) to pool available MIC/MEC data determined by the broth microdilution M38-A2 method originating from 17 independent laboratories for *S. schenckii*, *S. brasiliensis*, *S. globosa*, and *S. mexicana*; (ii) to define the

WT susceptibility MIC/MEC distributions of amphotericin B, five triazoles, terbinafine, flucytosine, and caspofungin; (iii) to assess the suitability of these distributions for ECV calculation (including interlaboratory modal agreement); and (iv) to propose CLSI ECVs for two of the species (*S. schenckii* and *S. brasiliensis*) when the agent-species combination comprised >100 MICs that originated in 3 to 9 laboratories. MICs of *S. globosa* and *S. mexicana* that originated in 3 or 4 laboratories were also listed when the distribution comprised at least 10 isolates from ≥3 centers; caspofungin, flucytosine, and fluconazole data are summarized below.

RESULTS AND DISCUSSION

CLSI BPs, which reliably predict clinical response to therapy, are not available for any filamentous (mold) species, including the *Sporothrix* species. While the establishment of BPs requires, in addition to other parameters, the clinical correlation of both high and low *in vitro* results with *in vivo* data, ECVs are based solely on *in vitro* data obtained in multiple laboratories (24, 25). ECVs or BPs are needed in order to identify the potential *in vitro* resistance to the agent under evaluation. Although the scarcity of clinical data has precluded the establishment of CLSI BPs for mold testing, several ECVs (e.g., for certain species of *Aspergillus*, *Fusarium*, and the Mucorales) are available (23, 24, 26, 27). ECVs should distinguish the two populations (WT and non-WT) that are present in the MIC/MEC distribution of a species and agent combination. ECVs for *S. brasiliensis* and some agents were recently reported using data from a single laboratory (28). However, the definition of ECVs using data from multiple laboratories allows the evaluation of modal (more frequent values in each MIC/MEC distribution) compatibility among the individual distributions included in the pool (a CLSI requirement) (24). To our knowledge, ECVs have not been defined for any other *Sporothrix* species; therefore, we collected available MIC/MEC data for *S. schenckii*, *S. brasiliensis*, *S. globosa*, and *S. mexicana* from 17 laboratories worldwide in order to propose ECVs for several antifungal agents.

Another requirement for the definition of ECVs is that the MIC/MEC data must be accompanied by results for at least one of the quality control (QC) or reference strains (23, 24). Examination of the results for QC or reference isolates in our study demonstrated that discrepant MICs for the QC and reference strains (21), although uncommon, were obtained in some laboratories as follows: (i) amphotericin B, itraconazole, and posaconazole MICs lower than the expected limits for the QC *Candida krusei* ATCC 6258 strain from one laboratory; (ii) lower amphotericin B and posaconazole MICs for the QC isolate *Paecilomyces variotii* ATCC MYA-3630 and the reference *Aspergillus flavus* ATCC 204304 strain, respectively, from another laboratory. As far as we know, MIC limits have not been established for terbinafine and any fungal strain. However, the laboratories that provided terbinafine MICs used as their internal controls some of the QC or reference isolates. Terbinafine MICs ranged from 0.25 to 1 μg/ml and 0.25 to 0.5 μg/ml for *A. fumigatus* ATCC MYA-3626 and *Aspergillus flavus* ATCC 204304, respectively. Nevertheless, the MIC ranges for *C. krusei* ATCC 6258 (2 to 64 μg/ml) and to a certain extent for *Candida parapsilosis* ATCC 22019 (0.01 to 0.5 μg/ml) were wider than the approved ranges for QC or reference isolates (21). These results indicated that both *Candida* QC strains could be unsuitable as either QC or reference isolates for terbinafine, but future collaborative studies should establish control guidelines for the agent.

Although we received MIC/MEC data from 17 laboratories for the four *Sporothrix* species evaluated in the present study, distributions for each species/agent combination were not collected from each center. In addition, the following unsuitable distributions were excluded: (i) aberrant distributions (mode at the lowest or highest concentration tested) or distributions where the mode was not obvious (e.g., distributions having two or more modes), (ii) distributions where MICs for the QC isolate(s) were outside the recommended limits, and (iii) distributions for which the mode was more than 1 concentration/dilution from the global mode (23, 24). In addition, we incorporated only data obtained by the same, unmodified M38-A2 testing parameters according to responses to the survey sent to each laboratory (described below) as follows: (i)

TABLE 1 Pooled MIC distributions of four *Sporothrix* species from 2 to 9 laboratories determined by the CLSI M38-A2 broth microdilution method

Agent	Species	No. of laboratories	Total no. of isolates	No. of isolates with MIC (μg/ml) of[a]:										
				≤0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	≥32
Amphotericin B	*S. schenckii*[b]	9	263	2		5	9	29	**100**	78	33	3	1	3
	S. brasiliensis	9	486	6		10	64	112	**175**	100	15	4		
	S. globosa	4	75			3	5	8	19	**29**	6	3		2
	S. mexicana	ID												
Itraconazole	*S. schenckii*	8	194		4	5	22	**71**	56	17	9	3	2	5
	S. brasiliensis	8	306	2	2	12	19	60	**146**	38	6		5	16
	S. globosa	4	53			5	10	**17**	10	9	1		1	3
	S. mexicana	3	13				3	**4**	2	1				
Ketoconazole	*S. schenckii*	2	92		1	11	12	**32**	17	16	3			
	S. brasiliensis	5	338	6	13	45	64	**126**	71	13				
	S. globosa	ID												
	S. mexicana	ID												
Posaconazole	*S. schenckii*	8	301		1	10	15	67	**114**	55	13	14	8	4
	S. brasiliensis	5	200	2	1	6	13	32	**128**	14	1			3
	S. globosa	3	59				12	**25**	12	5	1		2	2
	S. mexicana	ID												
Voriconazole	*S. schenckii*	6	252					3	1	6	17	42	**108**	75
	S. brasiliensis	7	200					1	9	17	32	**79**	56	6
	S. globosa	3	41						2	5	10	**14**	9	1
	S. mexicana	3	11						2	1	2	4	2	
Terbinafine	*S. schenckii*	2	118	2	18	23	26	**43**	6					
	S. brasiliensis	3	368	131	**151**	75	7	2	2					
	S. globosa	3	35	5	**16**	6	3	4	1					
	S. mexicana	ID												

[a]The highest number in each row (showing the most frequently obtained MIC or mode) is indicated in boldface.
[b]*S. schenckii* refers to *S. schenckii sensu stricto*. ID, insufficient data with comparable mode.

MIC distributions that were obtained using conidial suspensions as the inoculum, (ii) MICs obtained after 48 to 72 h of incubation at 35°C, and (iii) MICs obtained by the standard growth inhibition criteria for each agent. Those are essentially the M38-A2 testing guidelines for obtaining *in vitro* data for a variety of nondermatophyte mold species and agents; the exception is terbinafine (evaluated in multicenter studies only for dermatophytes by the CLSI reference method) (21). However, regarding the *Sporothrix* species, the testing guidelines were based on the multicenter evaluation that included five isolates of *S. schenckii sensu lato* and four (amphotericin B, fluconazole, itraconazole, and ketoconazole) of the nine agents evaluated in the present study (21, 22). Since collaborative studies have not been conducted with molecularly identified isolates and QC data are not available for terbinafine, the present collaborative study provides important corroboration of the testing conditions that could yield the most comparable values for six of the nine agents (the best interlaboratory modal agreement). These parameters could serve as the basis for further related studies for evaluating other agents and species, e.g., *S. globosa* and *S. mexicana*.

The MIC distributions of the four *Sporothrix* species and six of the nine agents evaluated are depicted in Table 1. The modal MICs ranged between 0.5 and 2 μg/ml for most of the species and agent combinations; the exceptions were the higher voriconazole (8 to 16 μg/ml) and the lower terbinafine (0.06 μg/ml) modes for *S. brasiliensis* and *S. globosa*. Flucytosine, fluconazole, and caspofungin data were also collected for *S. schenckii*, *S. brasiliensis*, and *S. globosa* from two to five laboratories. Although most of the distributions were either abnormal or unsuitable for ECV definition, both fluconazole and flucytosine modes were consistently at the upper end of the distribution (≥32 μg/ml) for *S. brasiliensis* and *S. schenckii*, while caspofungin modes were ~1 μg/ml (data not listed in Table 1). While abundant *in vitro* data are found in the literature in

TABLE 2 CLSI-ECVs for *S. schenckii sensu stricto* and *S. brasiliensis* based on MICs from 3 to 9 laboratories determined by the CLSI broth microdilution method

Species	Antifungal agent	No. of isolates tested	MIC (μg/ml)		ECV[b]	
			Range	Mode[a]	≥95%	≥97.5%
S. schenckii	Amphotericin B	263	0.03 to 32	1	4	4
	Itraconazole	194	0.06 to ≥32	0.5	2	2
	Ketoconazole	ND[c]				
	Posaconazole	301	0.06 to 16	1	2	4
	Voriconazole	252	0.5 to >32	16	64	64
	Terbinafine	ND				
S. brasiliensis	Amphotericin B	486	0.03 to 8	1	4	4
	Itraconazole	306	0.01 to 32	1	2	2
	Ketoconazole	338	0.01 to 2	0.5	2	2
	Posaconazole	200	0.01 to 4	1	2	2
	Voriconazole	200	0.5 to 32	8	32	32
	Terbinafine	368	≤0.01 to 1	0.06	0.12	0.25

[a]Mode, most frequent MIC.
[b]Calculated CLSI ECVs comprising ≥95% and ≥97.5% of the statistically modeled population; the values are based on MICs determined by the CLSI M38-A2 broth dilution method (21).
[c]ND, not determined, due to an insufficient number of isolates or laboratories for ECV calculation.

addition to those summarized in Table 1, (i) these studies predated the advent of molecular identification, (ii) the studies reported MIC/MEC data mostly for *S. schenckii* and *S. brasiliensis*, and (iii) MICs were obtained for either the yeast or filamentous phase or by modified versions of the CLSI reference method (e.g., supplemented RPMI broth [2% dextrose], 30°C incubation, or longer incubation times) (29–32). Although some MIC ranges in Table 1 were wider than those in prior studies, owing perhaps to the larger number of isolates (e.g., ≥200 versus <100) and different testing conditions, the trend of antifungal susceptibility of those species to the various agents is similar. When MICs that were obtained using both the yeast and conidial phases of *S. schenckii* were compared, the yeast phase yielded lower amphotericin B and itraconazole MICs, while terbinafine MICs were similar or the same (30). There was a need to ascertain which testing conditions yielded the most reproducible results. Our collaborative study provides such information, at least for the two more prevalent species and clinically relevant therapeutic agents. In addition, our results suggest that the incubation time for *S. globosa* needs to be longer and that further evaluation is needed for *S. mexicana*, among other species.

Table 2 summarizes MIC ranges and modes and, more importantly, our proposed ECVs for the species and agents with sufficient data to fulfill the current criteria (≥100 MICs of each agent and species obtained in ≥3 independent laboratories) for establishing method- and species-dependent ECVs by the iterative statistical method (23, 24). The CLSI has selected the 97.5% over the 95% ECVs; both values were calculated and documented. As expected, the highest ECVs were for voriconazole versus *S. schenckii* and *S. brasiliensis* (64 and 32 μg/ml, respectively), and the lowest value was for terbinafine and *S. brasiliensis* (0.12 μg/ml). Sufficient and suitable terbinafine MIC data were not available to calculate the terbinafine ECV for *S. schenckii* according to the current criteria; this species-agent combination needs to be further evaluated. We are also proposing ECVs of 4 μg/ml for amphotericin B and ECVs of 2 μg/ml for three triazoles and both *S. schenckii* and *S. brasiliensis*. The high ECVs for these two species (e.g., amphotericin B and voriconazole ECVs above expected and achievable serum levels) indicate their resistant nature, as was the case for certain species among the Mucorales and *Fusarium* spp. (26, 27). Although the ECV is not a predictor of clinical response to therapy, the high values suggest that isolates of these species could be unresponsive to therapy with these agents. On the other hand, categorization of an isolate as WT does not necessarily signify that it is susceptible to or treatable by the agent under evaluation.

Unfortunately, among the molds, genetic information concerning the mechanisms

of resistance is available mostly for *A. fumigatus* and the triazoles. To our knowledge, that is not the case for the clinically relevant *Sporothrix* species. In addition, limited data have been documented regarding the possible correlation between MICs for the *Sporothrix* infective isolate and the outcome of therapy with a specific agent, including amphotericin B, itraconazole, and terbinafine (17, 33). In one of the two studies, five patients who responded to oral itraconazole (pulse, 400 mg/day for 1 week with a 3-week break) for lymphangitic and fixed cutaneous sporotrichosis, the itraconazole MICs for 4 of the 5 infecting *S. schenckii* isolates were either 0.25 or 0.5 μg/ml (17). Those itraconazole MICs were below our proposed ECV of 2 μg/ml for the species, and the strains could be considered WT strains (Table 2). In the other report, seven patients with various and persistent *S. brasiliensis* infections (including disseminated disease) were treated for ≥13 weeks as follows: itraconazole, 100 mg (3 patients); terbinafine, 200 mg (3 patients); and amphotericin B followed by 800 mg of posaconazole (1 HIV-infected patient) (33). MICs for the serial infective isolates and the clinical response to therapy were as follows: itraconazole, 1 or 2 μg/ml (patients were cured/infection free); terbinafine, between 0.03 and 0.12 μg/ml (1 of 3 patients was cured); posaconazole, 1 μg/ml, and amphotericin B, between 2 and 4 μg/ml (the patient died). Our proposed ECVs for *S. brasiliensis* and the four agents were 2, 0.12, 2, and 4 μg/ml, respectively, and thus, the infecting isolates also could be considered WT (Table 2). However, other factors related to the patient immune response or the use of adjuvant treatments (cryosurgery/curettage) could interfere with meaningful *in vitro* versus *in vivo* correlations. On the other hand, the combination of posaconazole and amphotericin B was effective in murine models of disseminated disease caused by *S. schenckii* or *S. brasiliensis* (34). The infective isolates for the murine model were WT according to our proposed ECVs. Furthermore, the role of the ECV is not to predict a therapeutic outcome but to identify the non-WT strains that could be less likely to respond to therapy.

In conclusion, the main roles of the ECV are to distinguish between WT and non-WT isolates and to aid the clinician in identifying the non-WT isolates that are potentially refractory to therapy with the agent evaluated. This is important when BPs are not available for the species/agent being evaluated, which is the case for the *Sporothrix* species. Based on CLSI MICs from multiple laboratories, we propose the following species-specific CLSI ECVs for *S. schenckii* and *S. brasiliensis*, respectively: amphotericin B, 4 and 4 μg/ml; itraconazole, 2 and 2 μg/ml; posaconazole, 2 and 2 μg/ml; and voriconazole, 64 and 32 μg/ml. Our proposed ketoconazole and terbinafine ECVs for *S. brasiliensis* are 2 and 0.12 μg/ml, respectively. Insufficient data precluded the calculation of ketoconazole and terbinafine ECVs for *S. schenckii*, as well as ECVs for *S. globosa* and *S. mexicana* versus any antifungal agent. More importantly, we have corroborated that the susceptibility testing conditions described in the CLSI M38-A2 document could yield the most reliable or reproducible results for the two most prevalent species, which was based on our examination of modes from multiple laboratories.

MATERIALS AND METHODS

Isolates. The isolates evaluated were recovered from clinical specimens (mostly lymphocutaneous, cutaneous [including disseminated disease], or subcutaneous lesions [>90%] and to a lesser extent pulmonary lesions or other disseminated infections). In addition, we received *S. brasiliensis* isolates (cutaneous lesions) of feline origin from 4 of the 17 laboratories. MIC/MEC data for each agent were determined in each of the following centers: VCU Medical Center, Richmond, VA, USA; Universidade Federal Rural do Rio de Janeiro, Seropédica, Brazil; Fundação Oswaldo Cruz-Fiocruz, Instituto Nacional de Infectologia Evandro Chagas, Laboratório de Micologia and Laboratório de Pesquisa Clínica em Dermatozoonoses em Animais Domésticos, Rio de Janeiro, RJ, Brazil; Specialized Medical Mycology Center, Federal University of Ceará, Fortaleza-CE, Brazil; Department of Medical Microbiology, Postgraduate Institute of Medical Education and Research, Chandigarh, India; Department of Medical Mycology, Vallabhbhai Patel Chest Institute, University of Delhi, Delhi, India; Canisius Wilhelmina Hospital, Centre of Expertise in Mycology, Nijmegen, The Netherlands; Departamento Micologia, Instituto Nacional de Enfermedades Infecciosas Dr. C. G. Malbrán, Buenos Aires, Argentina; Universidad Autonóma de Nuevo León, Monterrey, Nuevo León, México; National Institute for Communicable Diseases and University of the Witwatersrand, Johannesburg, South Africa; Mycology Unit Medical School, Universitat Rovira i Virgili, Reus, Spain; Mycology Reference Laboratory, Public Health England, Bristol, United Kingdom; National

Mycology Reference Centre, SA Pathology, Adelaide, Australia;. Universidade Federal de São Paulo, São Paulo, Brazil; Instituto de Biofísica, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil; and Instituto Adolfo Lutz, São Paulo, Araçatuba, and Rio Claro Laboratories, Brazil.

Although data were received from 17 independent laboratories (coded as 1 to 17), some MIC distributions were excluded from the study for reasons discussed previously. The isolates were identified using phenotypic and genetic approaches (e.g., temperature and nutritional tests, yeast conversion, and species-specific PCR and PCR-restriction fragment length polymorphism [RFLP] calmodulin and β-tubulin sequencing) (10–12, 35). The MIC data used for ECV definition were from 301 *S. schenckii* and 486 *S. brasiliensis* isolates. Among the 486 isolates of *S. brasiliensis*, 261 were isolated from cats. In addition, MIC/MEC data were collected for 75 *S. globosa* and 13 *S. mexicana* isolates, respectively. At least one of the QC isolates (*C. parapsilosis* ATCC 22019, *C. krusei* ATCC 6258, or *P. variotii* ATCC MYA-3630) was evaluated by the participant laboratories during testing; some laboratories also evaluated the reference isolate *A. flavus* ATCC 204304 or *A. fumigatus* ATCC MYA-3626. MICs were pooled or used for the calculation of ECVs only when MICs for the QC or reference isolates were consistently within the established MIC limits as approved by the CLSI (21).

***In vitro* susceptibility testing.** MIC data for each isolate in the set that was included for analysis or depicted in Tables 1 and 2 were obtained at each center according to the CLSI M38-A2 broth microdilution method (21) (standard RPMI 1640 broth [0.2% dextrose], final conidial suspensions that ranged from 0.4×10^4 to 5×10^4 CFU/ml, and incubation at 35°C for 48 to 72 h [*S. schenckii*, *S. brasiliensis*, and *S. mexicana*] or $\geq$72 h [*S. globosa*]). MICs were the lowest drug concentrations that produced either complete growth inhibition (100%: amphotericin B, itraconazole, posaconazole, and voriconazole), partial growth inhibition (terbinafine [80%]; fluconazole, ketoconazole, and flucytosine [50%]), or morphological changes (caspofungin MECs).

Data analysis. Data were analyzed by iterative statistical analysis as previously described in various ECV reports (24–27). MIC/MEC distributions of each species received from each center were listed in electronic spreadsheets. Individual distributions were not included in the final analysis (i) when the distribution had a modal MIC at the lowest or highest concentration tested or that was bimodal or (ii) when unusual modal variation (modes that were more than 1 dilution/concentration from the global mode) was present (24). Data for each species and agent were included for the final calculation of ECVs only when the total pooled distribution had $\geq$100 isolates and originated from at least three laboratories (Tables 1 and 2).

Surveys. To ascertain that the collected *in vitro* susceptibility data in our study were developed following the same testing conditions described in the CLSI M38-A2 document (21), a survey was sent to the 17 participant laboratories requesting the following information: (i) the source(s) of the agents used, (ii) the formulation of the RPMI medium as described in the CLSI document, (iii) the cells (conidia versus yeasts) and counts used to prepare the inoculum suspensions, and (iv) the growth inhibition criteria to determine MICs/MECs for each agent (including incubation temperature and length and percent growth inhibition). The laboratories were also requested to provide MIC/MEC data for at least one of the QC or reference isolates (21).

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