Multicenter and international study of MIC/MEC distributions for definition of epidemiological cutoff values (ECVs) for species of *Sporothrix* 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 these species. We collected available CLSI MICs/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, South and North America) using conidial inoculum suspensions and 48-72 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 ECVs for *S. schenckii* as well as ECVs for *S. globosa* and *S. mexicana* or any other antifungal agent. These ECVs could aid the clinician in identifying potentially resistant isolates (non-wild type) less likely to respond to therapy.

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Introduction

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, the Brazilian case clusters, and in the USA (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 *S. schenckii*, according to phenotypic identification (macro and microscopic studies, carbohydrate assimilations, 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, ß-tubulin and calmodulin (CAL) genes indicated that there were various cryptic species nested in the medically relevant clade. The taxon was considered as 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 from now only as *S. schenckii)*, *S. brasiliensis*, and *S. globosa.* We also evaluated one rare species in the environmental clade, *S. 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; the 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 only included 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/MEC categories, either formal breakpoints (BPs) or epidemiological cutoff values (ECVs), have not been established for any of *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). Whilst ECVs would not predict the clinical

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success to therapy, these endpoints could identify those isolates less likely to respond to the specific agents. We have collected available MICs/MECs of nine antifungal agents from 17 laboratories for molecularly identified isolates of four *Sporothrix* species. These MIC/MEC values represent the antifungal susceptibility of the two more prevalent species (*S. schenckii* and *S. brasiliensis*) as well of 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 purpose of the present study was (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 those 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 to 4 laboratories were also listed when the distribution comprised at least 10 isolates from ≥3 centers; caspofungin, flucytosine and fluconazole data were summarized in the text.

Results and Discussion

CLSI BPs, which reliably predict clinical response to therapy, are not available for any filamentous (mould) 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 mould 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 value 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) lower amphotericin B, itraconazole and posaconazole MICs 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 strains, 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 both *A. fumigatus* ATCC MYA-3626 and *A. flavus* ATCC 204304, respectively. Nevertheless, the MIC ranges for the *C. krusei* ATCC 6258 (2 to 64 µg/ml) and to certain extent for *C. 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 this 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 (mode at the lowest or highest concentration tested) or distributions where the mode is not obvious (e.g., distributions having two or more modes), (ii) when MICs for the QC isolate(s) were outside the recommended limits, or (iii) the mode of a particular distribution was more than one concentration/dilution than the global mode (23,24). In addition, we only incorporated data obtained by the same and unmodified M38-A2 testing parameters as per responses to the survey sent to each laboratory (described below) as follows: (i) 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) 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 non-dermatophyte mould species and agents; the exception is terbinafine (only evaluated in multicenter studies 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 about the testing conditions that could yield the most comparable values for six of the nine agents (best interlaboratory modal agreement). These parameters could serve as the basis for further and 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 modes for *S. brasiliensis* and *S. globosa* (0.06 µg/ml). Flucytosine, fluconazole and caspofungin data were also collected for *S. schenckii*, *S. brasiliensis* and *S. globosa* from two to five laboratories. Although most of those 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 addition to those summarized in Table 1, these studies (i) predated the advent of molecular identification, (ii) 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%], 30°C incubation, 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 antifungal susceptibility trend 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 yield the most reproducible results. Our collaborative study provides such corroboration at least for the two more prevalent species and clinically relevant

204 therapeutic agents. In addition, our results suggest that the incubation time for *S. globosa* needs
205 to be longer and that further evaluation is needed for *S. mexicana,* among other species.
206
207 Table 2 summarizes MIC ranges, modes and more importantly our proposed ECVs for
208 the species and agents with sufficient data to fulfill the current criteria (≥ 100 MICs of each
209 agent and species obtained in ≥ 3 independent laboratories) for establishing method-and
210 species-dependent ECVs by the iterative statistical method (23,24). The CLSI has selected the
211 97.5% over the 95% ECVs, both values were calculated and documented. As expected, the
212 highest ECVs were for voriconazole versus *S. schenckii* and *S. brasiliensis* (64 and 32 µg/ml,
213 respectively) and the lowest value for terbinafine and *S. brasiliensis* (0.12 µg/ml). Sufficient and
214 suitable terbinafine MIC data were not available to calculate the terbinafine ECV for *S. schenckii*
215 according to the current criteria; this species/agent combination needs to be further evaluated.
216 We are also proposing ECVs of 4 µg/ml for amphotericin B and ECVs of 2 µg/ml for three
217 triazoles and both *S. schenckii* and *S. brasiliensis*. The high ECVs for these two species (e.g.,
218 amphotericin B and voriconazole ECVs above expected and achievable serum levels) indicate
219 their resistant nature, as was the case for certain species among the Mucorales and *Fusarium*
220 spp. (26,27). Although the ECV is not a predictor of clinical response to therapy, the high values
221 suggest that isolates of these species could be unresponsive to therapy with these agents. On
222 the other hand, categorization of an isolate as WT does not necessarily signify that it is
223 susceptible to or treatable by the agent under evaluation.
224
225 Unfortunately, among the moulds, genetic information concerning the mechanisms of
226 resistance is mostly available for *A. fumigatus* and the triazoles. To our knowledge that is not
227 the case for the clinically relevant *Sporothrix* species. In addition, limited data have been
228 documented regarding the possible correlation between MICs for the *Sporothrix* infective isolate
229 and the outcome of therapy with the specific agent, including amphotericin B, itraconazole or
230 terbinafine (17,33). In one of those two studies, five patients who responded to oral itraconazole
231 (pulse, 400 mg/day one week with a three week break) for lymphangitic and fixed cutaneous
232 sporotrichosis, the itraconazole MICs for 4 of the 5 infecting *S. schenckii* isolates were either
233 0.25 or 0.5 µg/ml (17). Those itraconazole MICs were below our proposed ECV of 2 µg/ml for
234 this species and those strains could be considered WT strains (Table 2). In the other report,
235 seven patients with various and persistent *S. brasiliensis* infections (including disseminated
236 disease) were treated for ≥ 13 weeks as follows: itraconazole 100 mg (3 patients), terbinafine
237 200 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 cured/infection free); terbinafine between 0.03 and 0.12 µg/ml (1 of 3 patients cured); posaconazole 1 µg/ml and amphotericin B between 2 and 4 µg/ml (patient died). Our proposed ECVs for *S. brasiliensis* and those four agents were: 2, 0.12, 2 and 4 µg/ml, respectively, and thus, those 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 therapeutic outcome, but to identify the non-WT strains that could be less likely to respond to therapy.

In conclusion, the main role of the ECV is to distinguish between WT and non-WT isolates and 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 are proposing 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 were 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, Brasil; 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, Brasil; Specialized Medical Mycology Center, Federal University of Ceará, Fortaleza-CE, Brazil; Department of Medical Microbiology, Postgraduate Institute of Medical Education & 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, UK; National Mycology Reference Centre, SA Pathology, Adelaide, Australia; Universidade Federal de São Paulo, São Paulo, Brasil; Instituto de Biofísica, Universidade Federal do Rio de Janeiro, Brasil; and Instituto Adolfo Lutz, São Paulo, Araçatuba, and Rio Claro Laboratories, Brasil.

Although data were received from 17 independent laboratories (coded 1 to 17), some MIC distributions were excluded from the study for previously discussed reasons. The isolates were identified using phenotypic and genetic approaches (e.g., temperature and nutritional tests, yeast conversion, species specific PCR and PCR-RFLP calmodulin and ß-tubulin sequencing) (10-12,35). The MIC data used for ECV definition were as follows: 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*, 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 isolates *A. flavus* ATCC 204304 or *A. fumigatus* ATCC MYA-3626. MICs were only pooled or used for the calculation of ECVs 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 $0.4x10^4$ to $5x10^4$ CFU/ml and an incubation at 35°C between 48 to 72 h (*S. schenckii, S. brasiliensis,* and *S. mexicana*) or ≥72 h for *S. globosa*. MICs were the lowest drug concentrations that produced either complete growth inhibition (100%: amphotericin B, itraconazole, posaconazole and voriconazole) or partial growth inhibition as follows: (terbinafine [80%], fluconazole, ketoconazole and flucytosine [50%]), or morphological changes (caspofungin MECs).

Data analysis. Data were analyzed by the 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 when (i) the distribution had a modal MIC at the lowest or highest concentration tested or were bimodal or when (ii) unusual modal variation (modes that were more than one dilution/concentration from the global mode) (24). Data for each species and agent were only included for the final calculation of ECVs when the total pooled distribution had ≥ 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 as described in the CLSI M38-A2 document (21), a survey was sent to the 17 participant laboratories requesting the following information: (i) the source of the agents used; (ii) the formulation of the RPMI medium as described in the CLSI document; (iii) the cells (conidia versus yeasts) and count 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 percentage of 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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478 **Table 1.** Pooled MIC distributions of four *Sporothrix* species from between 2 and 9 laboratories determined by CLSI M38-A2 broth microdilution method

Agent	Species*	No. labs	No. 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**	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	
	S. mexicana	3	13				3	**4**	2	1				3
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												

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480 [a]The highest number in each row (showing the most frequently obtained MIC or the mode) is indicated in boldface.

481 ***I**t refers to *Sporothrix schenckii sensu stricto*. ID: insufficient data with comparable mod

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

Species	Antifungal agent	No. of isolates tested	MIC (µg/ml)[a]		ECV[b]	
			Range	Mode	≥ 95 %	≥97.5 %
S. schenckii	Amphotericin B	263	0.03-32	1	4	4
	Itraconazole	194	0.06-≥32	0.5	2	2
	Ketoconazole	ND[c]				
	Posaconazole	301	0.06-16	1	2	4
	Voriconazole	252	0.5->32	16	64	64
	Terbinafine	ND[c]				
S. brasiliensis	Amphotericin B	486	0.03-8	1	4	4
	Itraconazole	306	0.01-32	1	2	2
	Ketoconazole	338	0.01-2	0.5	2	2
	Posaconazole	200	0.01-4	1	2	2
	Voriconazole	200	0.5-32	8	32	32
	Terbinafine	368	≤0.01-1	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; values based on MICs determined by the CLSI M38-A2 broth dilution method (21).

[c] ND, Not determined, due to insufficient number of isolates or laboratories for ECV calculation.