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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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1 **Multicenter and international study of MIC/MEC distributions for definition of**
2 **epidemiological cutoff values (ECVs) for species of *Sporothrix* identified by molecular**
3 **methods**
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5 5/22-7/16
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37
38
39
40
41 **Abstract**
42
43 Clinical and Laboratory Standards Institute (CLSI) conditions for testing the
44 susceptibilities of pathogenic *Sporothrix* species to antifungal agents are based on a
45 collaborative study that evaluated five clinically relevant isolates of *Sporothrix schenckii sensu*
46 *lato* and some antifungal agents. With the advent of molecular identification, there are two basic
47 needs: to confirm the suitability of these testing conditions for all agents and *Sporothrix* species
48 and to establish species-specific epidemiologic cutoff values (ECVs) or breakpoints (BPs) for
49 these species. We collected available CLSI MICs/MECs of amphotericin B, five triazoles,
50 terbinafine, flucytosine and caspofungin for 301 *Sporothrix schenckii sensu stricto*, 486 *S.*
51 *brasiliensis,* 75 *S. globosa* and 13 *S. mexicana* molecularly identified isolates. Data were
52 obtained in 17 independent laboratories (Australia, Europe, India, South Africa, South and North
53 America) using conidial inoculum suspensions and 48-72 h of incubation at 35°C. Sufficient and
54 suitable data (modal MICs within 2-fold concentrations) allowed the proposal of the following
55 ECVs for *S. schenckii* and *S. brasiliensis*, respectively: amphotericin B 4 and 4 µg/ml,
56 itraconazole 2 and 2 µg/ml; posaconazole 2 and 2 µg/ml; and voriconazole 64 and 32 µg/ml;
57 ketoconazole and terbinafine ECVs for *S. brasiliensis* were 2 and 0.12 µg/ml, respectively.
58 Insufficient or unsuitable data precluded the calculation of ketoconazole and terbinafine ECVs
59 for *S. schenckii* as well as ECVs for *S. globosa* and *S. mexicana* or any other antifungal agent.
60 These ECVs could aid the clinician in identifying potentially resistant isolates (non-wild type)
61 less likely to respond to therapy.
62 245
63 **Introduction**
64
65 Sporotrichosis is considered a relatively uncommon granulomatous infection of the
66 cutaneous and subcutaneous tissue, although dissemination to other deep-seated organs has
67 been reported (1,2). The first case of sporotrichosis was documented in the United States in the

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68 late 1800s by Benjamin Schenck (3,4). This case was followed by worldwide reports as well as
69 numerous outbreaks (e.g., in the South African mines in the 1920s and 1930s, among children
70 in relatively remote areas of Peru, the Brazilian case clusters, and in the USA (5-8). In addition,
71 several feline outbreaks caused by *Sporothrix brasiliensis* with transmissions from cat to human
72 to cat have been reported in Brazil (7,8). Most other outbreaks or infections have been
73 associated with traumatic inoculation of vegetative materials and/or soil. Until recently, all cases
74 were attributed to *S. schenckii*, according to phenotypic identification (macro and microscopic
75 studies, carbohydrate assimilations, and conversion to the yeast phase). The advent of
76 molecular methodologies and the use of internal transcribed spacer (ITS), region sequence
77 analysis of chitin-synthase, ß-tubulin and calmodulin (CAL) genes indicated that there were
78 various cryptic species nested in the medically relevant clade. The taxon was considered as the
79 *Sporothrix schenckii* species complex (8-12). Therefore, sporotrichosis is caused by different
80 pathogenic species, including the three clinically relevant species evaluated in the present
81 study*: S. schenckii* sensu stricto (referred from now only as *S. schenckii)*, *S. brasiliensis*, and *S.*
82 *globosa.* We also evaluated one rare species in the environmental clade, *S. mexicana* (10,11).
83
84 The recommended therapeutic agents for the treatment of human sporotrichosis are
85 itraconazole, amphotericin B and its lipid formulations (invasive/disseminated disease),
86 terbinafine, and fluconazole; the saturated solution of potassium iodide has been an alternative
87 choice for lymphocutaneous/cutaneous infections (2,13-18). Ketoconazole is not used as much
88 given its low efficacy and potentially severe side effects (13,16). Among the newer triazoles, *in*
89 *vivo* and *in vitro* activity has been reported with posaconazole in combination with amphotericin
90 B, while voriconazole has not been considered a therapeutic choice for these infections due to
91 its high MICs (19,20).
92
93 The Clinical and Laboratory Standards Institute (CLSI) has described testing conditions
94 for the "filamentous phase of the *S. schenckii* species complex", because the initial CLSI
95 collaborative evaluation predated molecular studies, which only included five isolates that were
96 documented as "*S. schenckii*" (21,22). Therefore, the species of *Sporothrix* are not mentioned in
97 the CLSI M38-A2 document (21). In addition, interpretive MIC/MEC categories, either formal
98 breakpoints (BPs) or epidemiological cutoff values (ECVs), have not been established for any of
99 *Sporothrix* species. Method-dependent and species-specific ECVs should identify the non-wild
100 type (non-WT) isolates with reduced susceptibility to the agent being evaluated due to acquired
101 mutational or other resistance mechanisms (23,24). Whilst ECVs would not predict the clinical

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102 success to therapy, these endpoints could identify those isolates less likely to respond to the
103 specific agents. We have collected available MICs/MECs of nine antifungal agents from 17
104 laboratories for molecularly identified isolates of four *Sporothrix* species. These MIC/MEC
105 values represent the antifungal susceptibility of the two more prevalent species (*S. schenckii*
106 and *S. brasiliensis*) as well of those of *S. globosa* and *S. mexicana* to the different agents as
107 determined by the CLSI M38-A2 method (21). Although the *in vitro* data were obtained in 17
108 laboratories, the isolates originated from different geographical areas (Australia, Europe, India,
109 South Africa, and both South and North American countries).

110

111 The purpose of the present study was (i) to pool available MIC/MEC data determined by
112 the broth microdilution M38-A2 method originating from 17 independent laboratories for *S.*
113 *schenckii*, *S. brasiliensis, S. globosa* and *S. mexicana*; (ii) to define the WT susceptibility
114 MIC/MEC distributions of amphotericin B, five triazoles, terbinafine, flucytosine, and
115 caspofungin; (iii) to assess the suitability of these distributions for ECV calculation (including
116 interlaboratory modal agreement); and (iv) to propose CLSI ECVs for two of those species (*S.*
117 *schenckii* and *S. brasiliensis*) when the agent/species combination comprised >100 MICs that
118 originated in 3 to 9 laboratories. MICs of *S. globosa* and *S. mexicana* that originated in 3 to 4
119 laboratories were also listed when the distribution comprised at least 10 isolates from ≥3
120 centers; caspofungin, flucytosine and fluconazole data were summarized in the text.

121

122 **Results and Discussion**

123

124 CLSI BPs, which reliably predict clinical response to therapy, are not available for any
125 filamentous (mould) species including the *Sporothrix* species. While the establishment of BPs
126 requires, in addition to other parameters, the clinical correlation of both high and low *in vitro*
127 results with *in vivo* data, ECVs are based solely on *in vitro* data obtained in multiple laboratories
128 (24,25). ECVs or BPs are needed in order to identify the potential *in vitro* resistance to the agent
129 under evaluation. Although the scarcity of clinical data has precluded the establishment of CLSI
130 BPs for mould testing, several ECVs (e.g., for certain species of *Aspergillus*, *Fusarium* and the
131 Mucorales) are available (23,24,26,27). ECVs should distinguish the two populations (WT and
132 non-WT) that are present in the MIC/MEC distribution of a species and agent combination.
133 ECVs for *S. brasiliensis* and some agents were recently reported using data from a single
134 laboratory (28). However, the definition of ECVs using data from multiple laboratories allows the
135 evaluation of modal (more frequent value in each MIC/MEC distribution) compatibility among the

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136 individual distributions included in the pool (a CLSI requirement) (24). To our knowledge, ECVs
137 have not been defined for any other *Sporothrix* species; therefore, we collected available MIC/
138 MEC data for *S. schenckii*, *S. brasiliensis, S. globosa* and *S. mexicana* from 17 laboratories
139 worldwide in order to propose ECVs for several antifungal agents.
140

141 Another requirement for the definition of ECVs is that the MIC/MEC data must be
142 accompanied by results for at least one of the quality control (QC) or reference strains (23,24).
143 Examination of the results for QC or reference isolates in our study demonstrated that
144 discrepant MICs for the QC and reference strains (21), although uncommon, were obtained in
145 some laboratories as follows: (i) lower amphotericin B, itraconazole and posaconazole MICs
146 than the expected limits for the QC *Candida krusei* ATCC 6258 strain from one laboratory; (ii)
147 lower amphotericin B and posaconazole MICs for the QC isolate *Paecilomyces variotii* ATCC
148 MYA-3630 and the reference *Aspergillus flavus* ATCC 204304 strains, respectively, from
149 another laboratory. As far as we know, MIC limits have not been established for terbinafine and
150 any fungal strain. However, the laboratories that provided terbinafine MICs used as their internal
151 controls some of the QC or reference isolates. Terbinafine MICs ranged from 0.25 to 1 µg/ml
152 and 0.25 to 0.5 µg/ml for both *A. fumigatus* ATCC MYA-3626 and *A. flavus* ATCC 204304,
153 respectively. Nevertheless, the MIC ranges for the *C. krusei* ATCC 6258 (2 to 64 µg/ml) and to
154 certain extent for *C. parapsilosis* ATCC 22019 (0.01 to 0.5 µg/ml) were wider than the approved
155 ranges for QC or reference isolates (21). These results indicated that both *Candida* QC strains
156 could be unsuitable as either QC or reference isolates for terbinafine, but future collaborative
157 studies should establish control guidelines for this agent.
158

159 Although we received MIC/MEC data from 17 laboratories for the four *Sporothrix* species
160 evaluated in the present study, distributions for each species/agent combination were not
161 collected from each center. In addition, the following unsuitable distributions were excluded: (i)
162 aberrant (mode at the lowest or highest concentration tested) or distributions where the mode is
163 not obvious (e.g., distributions having two or more modes), (ii) when MICs for the QC isolate(s)
164 were outside the recommended limits, or (iii) the mode of a particular distribution was more than
165 one concentration/dilution than the global mode (23,24). In addition, we only incorporated data
166 obtained by the same and unmodified M38-A2 testing parameters as per responses to the
167 survey sent to each laboratory (described below) as follows: (i) MIC distributions that were
168 obtained using conidial suspensions as the inoculum; (ii) MICs obtained after 48 to 72 h of
169 incubation at 35°C; and (iii) by the standard growth inhibition criteria for each agent. Those are

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170 essentially the M38- A2 testing guidelines for obtaining in vitro data for a variety of non-
171 dermatophyte mould species and agents; the exception is terbinafine (only evaluated in
172 multicenter studies for dermatophytes by the CLSI reference method) (21). However, regarding
173 the *Sporothrix* species, the testing guidelines were based on the multicenter evaluation that
174 included five isolates of *S. schenckii sensu lato* and four (amphotericin B, fluconazole,
175 itraconazole and ketoconazole) of the nine agents evaluated in the present study (21,22). Since
176 collaborative studies have not been conducted with molecularly identified isolates and QC data
177 are not available for terbinafine, the present collaborative study provides important corroboration
178 about the testing conditions that could yield the most comparable values for six of the nine
179 agents (best interlaboratory modal agreement). These parameters could serve as the basis for
180 further and related studies for evaluating other agents and species, e.g., *S. globosa* and *S.*
181 *mexicana*.
182
183 The MIC distributions of the four *Sporothrix* species and six of the nine agents evaluated
184 are depicted in Table 1. The modal MICs ranged between 0.5 and 2 µg/ml for most of the
185 species and agent combinations; the exceptions were the higher voriconazole (8 to 16 µg/ml)
186 and the lower terbinafine modes for *S. brasiliensis* and *S. globosa* (0.06 µg/ml). Flucytosine,
187 fluconazole and caspofungin data were also collected for *S. schenckii*, *S. brasiliensis* and *S.*
188 *globosa* from two to five laboratories. Although most of those distributions were either abnormal
189 or unsuitable for ECV definition, both fluconazole and flucytosine modes were consistently at
190 the upper end of the distribution (≥32 µg/ml) for *S. brasiliensis* and *S. schenckii*, while
191 caspofungin modes were ~1 µg/ml (data not listed in Table 1). While abundant in vitro data are
192 found in the literature in addition to those summarized in Table 1, these studies (i) predated the
193 advent of molecular identification, (ii) reported MIC/MEC data mostly for *S. schenckii* and *S.*
194 *brasiliensis*, and (iii) MICs were obtained for either the yeast or filamentous phase or by
195 modified versions of the CLSI reference method (e.g., supplemented RPMI broth [2%], 30°C
196 incubation, longer incubation times) (29-32). Although some MIC ranges in Table 1 were wider
197 than those in prior studies, owing perhaps to the larger number of isolates (e.g., ≥ 200 versus <
198 100) and different testing conditions, the antifungal susceptibility trend of those species to the
199 various agents is similar. When MICs that were obtained using both the yeast and conidial
200 phases of *S. schenckii* were compared, the yeast phase yielded lower amphotericin B and
201 itraconazole MICs, while terbinafine MICs were similar or the same (30). There was a need to
202 ascertain which testing conditions yield the most reproducible results. Our collaborative study
203 provides such corroboration at least for the two more prevalent species and clinically relevant

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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

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238 patient) (33). MICs for the serial infective isolates and the clinical response to therapy were as
239 follows: itraconazole 1 or 2 µg/ml (patients cured/infection free); terbinafine between 0.03 and
240 0.12 µg/ml (1 of 3 patients cured); posaconazole 1 µg/ml and amphotericin B between 2 and 4
241 µg/ml (patient died). Our proposed ECVs for *S. brasiliensis* and those four agents were: 2, 0.12,
242 2 and 4 µg/ml, respectively, and thus, those infecting isolates also could be considered WT
243 (Table 2). However, other factors related to the patient immune response or the use of adjuvant
244 treatments (cryosurgery/curettage) could interfere with meaningful *in vitro* versus *in vivo*
245 correlations. On the other hand, the combination of posaconazole and amphotericin B was
246 effective in murine models of disseminated disease caused by *S. schenckii* or *S. brasiliensis*
247 (34). The infective isolates for the murine model were WT according to our proposed ECVs.
248 Furthermore, the role of the ECV is not to predict therapeutic outcome, but to identify the non-
249 WT strains that could be less likely to respond to therapy.
250
251 In conclusion, the main role of the ECV is to distinguish between WT and non-WT
252 isolates and aid the clinician in identifying the non-WT isolates that are potentially refractory to
253 therapy with the agent evaluated. This is important when BPs are not available for the
254 species/agent being evaluated, which is the case for the *Sporothrix* species. Based on CLSI
255 MICs from multiple laboratories, we are proposing the following species-specific CLSI ECVs for
256 *S. schenckii* and *S. brasiliensis*, respectively: amphotericin B, 4 and 4 µg/ml; itraconazole, 2 and
257 2 µg/ml; posaconazole, 2 and 2 µg/ml; and voriconazole, 64 and 32 µg/ml. Our proposed
258 ketoconazole and terbinafine ECVs for *S. brasiliensis* are 2 and 0.12 µg/ml, respectively.
259 Insufficient data precluded the calculation of ketoconazole and terbinafine ECVs for *S.*
260 *schenckii*, as well as ECVs for *S. globosa* and *S. mexicana* versus any antifungal agent. More
261 importantly, we have corroborated that the susceptibility testing conditions described in the CLSI
262 M38-A2 document could yield the most reliable or reproducible results for the two most
263 prevalent species, which were based on our examination of modes from multiple laboratories.
264

265 Materials and methods

266
267 **Isolates.** The isolates evaluated were recovered from clinical specimens (mostly
268 lymphocutaneous, cutaneous [including disseminated disease] or subcutaneous lesions [>90%])
269 and to a lesser extent pulmonary lesions or other disseminated infections. In addition, we
270 received *S. brasiliensis* isolates (cutaneous lesions) of feline origin from 4 of the 17 laboratories.
271 MIC/MEC data for each agent were determined in each of the following centers: VCU Medical

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272 Center, Richmond VA, USA; Universidade Federal Rural do Rio de Janeiro, Seropédica, Brasil;
273 Fundação Oswaldo Cruz-Fiocruz, Instituto Nacional de Infectologia Evandro Chagas,
274 Laboratório de Micologia and Laboratório de Pesquisa Clínica em Dermatozoonoses em
275 Animais Domésticos , Rio de Janeiro, RJ, Brasil; Specialized Medical Mycology Center, Federal
276 University of Ceará, Fortaleza-CE, Brazil; Department of Medical Microbiology, Postgraduate
277 Institute of Medical Education & Research, Chandigarh, India; Department of Medical Mycology,
278 Vallabhbhai Patel Chest Institute, University of Delhi, Delhi, India; Canisius Wilhelmina Hospital,
279 Centre of Expertise in Mycology, Nijmegen, The Netherlands; Departamento Micologia, Instituto
280 Nacional de Enfermedades Infecciosas “Dr. C. G. Malbrán”, Buenos Aires, Argentina;
281 Universidad Autonóma de Nuevo León, Monterrey, Nuevo León, México; National Institute for
282 Communicable Diseases and University of the Witwatersrand, Johannesburg, South Africa;
283 Mycology Unit Medical School, Universitat Rovira i Virgili, Reus, Spain; Mycology Reference
284 Laboratory, Public Health England, Bristol, UK; National Mycology Reference Centre, SA
285 Pathology, Adelaide, Australia; Universidade Federal de São Paulo, São Paulo, Brasil; Instituto
286 de Biofísica, Universidade Federal do Rio de Janeiro, Brasil; and Instituto Adolfo Lutz, São
287 Paulo, Araçatuba, and Rio Claro Laboratories, Brasil.

288

289 Although data were received from 17 independent laboratories (coded 1 to 17), some
290 MIC distributions were excluded from the study for previously discussed reasons. The isolates
291 were identified using phenotypic and genetic approaches (e.g., temperature and nutritional
292 tests, yeast conversion, species specific PCR and PCR-RFLP calmodulin and ß-tubulin
293 sequencing) (10-12,35). The MIC data used for ECV definition were as follows: 301 *S. schenckii*
294 and 486 *S. brasiliensis* isolates. Among the 486 isolates of *S. brasiliensis*, 261 were isolated
295 from cats. In addition, MIC/MEC data were collected for 75 *S. globosa* and 13 *S. mexicana*,
296 respectively. At least one of the QC isolates (*C. parapsilosis* ATCC 22019, *C. krusei* ATCC
297 6258, or *P. variotii* ATCC MYA-3630) was evaluated by the participant laboratories during
298 testing; some laboratories also evaluated the reference isolates *A. flavus* ATCC 204304 or *A.*
299 *fumigatus* ATCC MYA-3626. MICs were only pooled or used for the calculation of ECVs when
300 MICs for the QC or reference isolates were consistently within the established MIC limits as
301 approved by the CLSI (21).

302

303 ***In vitro* susceptibility testing.** MIC data for each isolate in the set that was included for
304 analysis or depicted in Tables 1 and 2 were obtained at each center according to the CLSI M38-
305 A2 broth microdilution method (21) (standard RPMI 1640 broth [0.2% dextrose], final conidial

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306 suspensions that ranged from $0.4x10^4$ to $5x10^4$ CFU/ml and an incubation at 35°C between 48
307 to 72 h (*S. schenckii, S. brasiliensis,* and *S. mexicana*) or ≥72 h for *S. globosa*. MICs were the
308 lowest drug concentrations that produced either complete growth inhibition (100%: amphotericin
309 B, itraconazole, posaconazole and voriconazole) or partial growth inhibition as follows:
310 (terbinafine [80%], fluconazole, ketoconazole and flucytosine [50%]), or morphological changes
311 (caspofungin MECs).
312
313 **Data analysis.** Data were analyzed by the iterative statistical analysis as previously
314 described in various ECV reports (24-27). MIC/MEC distributions of each species received from
315 each center were listed in electronic spreadsheets. Individual distributions were not included in
316 the final analysis when (i) the distribution had a modal MIC at the lowest or highest
317 concentration tested or were bimodal or when (ii) unusual modal variation (modes that were
318 more than one dilution/concentration from the global mode) (24). Data for each species and
319 agent were only included for the final calculation of ECVs when the total pooled distribution had
320 ≥ 100 isolates and originated from at least three laboratories (Tables 1 and 2).
321
322 **Surveys**. To ascertain that the collected in vitro susceptibility data in our study were
323 developed following the same testing conditions as described in the CLSI M38-A2 document
324 (21), a survey was sent to the 17 participant laboratories requesting the following information: (i)
325 the source of the agents used; (ii) the formulation of the RPMI medium as described in the CLSI
326 document; (iii) the cells (conidia versus yeasts) and count used to prepare the inoculum
327 suspensions; and (iv) the growth inhibition criteria to determine MICs/MECs for each agent
328 (including incubation temperature and length, and percentage of growth inhibition). The
329 laboratories were also requested to provide MIC/MEC data for at least one of the QC or
330 reference isolates (21).
331

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333
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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												

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

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485
486 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
487 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

488 [a] Mode, most frequent MIC.
489 [b]Calculated CLSI ECVs comprising ≥95 % and ≥ 97.5 % of the statistically modeled population; values based on MICs determined by the
490 CLSI M38-A2 broth dilution method (21).
491 [c]ND, Not determined, due to insufficient number of isolates or laboratories for ECV calculation.
492