AMERICAN SOCIETY FOR MICROBIOLOGY | Antimicrobial Agents and Chemotherapy

International Evaluation of MIC Distributions and Epidemiological Cutoff Value (ECV) Definitions for *Fusarium* Species Identified by Molecular Methods for the CLSI Broth Microdilution Method

A. Espinel-Ingroff,[a] A. L. Colombo,[b] S. Cordoba,[c] P. J. Dufresne,[d] J. Fuller,[e] M. Ghannoum,[f] G. M. Gonzalez,[g] J. Guarro,[h] S. E. Kidd,[i] J. F. Meis,[j] T. M. S. C. Melhem,[k] T. Pelaez,[l] M. A. Pfaller,[m] M. W. Szeszs,[n] J. P. Takahaschi,[o] A. M. Tortorano,[p] N. P. Wiederhold,[q] J. Turnidge[r]

VCU Medical Center, Richmond, Virginia, USA[a]; Universidade Federal de São Paulo, São Paulo, Brazil[b]; Instituto Nacional de Enfermedades Infecciosas "Dr. C. G. Malbrán," Buenos Aires, Argentina[c]; Institut National de Santé Publique du Québec, Laboratoire de Santé Publique du Québec, Sainte-Anne-de-Bellevue, Quebec, Canada[d]; Provincial Laboratory, Alberta Health Services, Edmonton, Canada[e]; University Hospitals Case Medical Center and Case Western Reserve University, Cleveland, Ohio, USA[f]; Universidad Autónoma de Nuevo León, Monterrey, Nuevo León, Mexico[g]; Facultat de Medicina, IISPV, URV, Reus, Spain[h]; National Mycology Reference Centre, SA Pathology, Adelaide, Australia[i]; Department of Medical Microbiology and Infectious Diseases, Canisius Wilhelmina Hospital and Radboud University Medical Center, Nijmegen, The Netherlands[j]; The Instituto Adolfo Lutz Reference Center, São Paulo, Brazil[k]; Hospital General Universitario Gregorio Marañón, School of Medicine—Universidad Complutense, Madrid, Spain[l]; JMI Laboratories, North Liberty, Iowa, USA[m]; Mycology Department, The Instituto Adolfo Lutz Reference Center, São Paulo, Brazil[n]; Grupo Fleury, São Paulo, Brazil[o]; Department of Biomedical Sciences for Health, Università degli Studi di Milano, Milan, Italy[p]; University of Texas Health Science Center, San Antonio, Texas, USA[q]; University of Adelaide, Adelaide, Australia[r]

The CLSI epidemiological cutoff values (ECVs) of antifungal agents are available for various *Candida* spp., *Aspergillus* spp., and the Mucorales. However, those categorical endpoints have not been established for *Fusarium* spp., mostly due to the difficulties associated with collecting sufficient CLSI MICs for clinical isolates identified according to the currently recommended molecular DNA-PCR-based identification methodologies. CLSI MIC distributions were established for 53 *Fusarium dimerum* species complex (SC), 10 *F. fujikuroi*, 82 *F. proliferatum*, 20 *F. incarnatum-F. equiseti* SC, 226 *F. oxysporum* SC, 608 *F. solani* SC, and 151 *F. verticillioides* isolates originating in 17 laboratories (in Argentina, Australia, Brazil, Canada, Europe, Mexico, and the United States). According to the CLSI guidelines for ECV setting, ECVs encompassing ≥97.5% of pooled statistically modeled MIC distributions were as follows: for amphotericin B, 4 μg/ml (*F. verticillioides*) and 8 μg/ml (*F. oxysporum* SC and *F. solani* SC); for posaconazole, 2 μg/ml (*F. verticillioides*), 8 μg/ml (*F. oxysporum* SC), and 32 μg/ml (*F. solani* SC); for voriconazole, 4 μg/ml (*F. verticillioides*), 16 μg/ml (*F. oxysporum* SC), and 32 μg/ml (*F. solani* SC); and for itraconazole, 32 μg/ml (*F. oxysporum* SC and *F. solani* SC). Insufficient data precluded ECV definition for the other species. Although these ECVs could aid in detecting non-wild-type isolates with reduced susceptibility to the agents evaluated, the relationship between molecular mechanisms of resistance (gene mutations) and MICs still needs to be investigated for *Fusarium* spp.

While the genus *Fusarium* and its teleomorphic (sexual) forms encompass a variety of species, only some have been associated with human disease. Identification of *Fusarium* isolates to the accepted phylogenetic species complex (SC) or species level is essential (1–4) but challenging, since important taxonomic changes have been made, and the taxonomy is still in a state of flux for some genera. Following the results of DNA-sequencing studies, well-known prevalent fungal genera were divided into several new genera. By 2013, the consensus was to continue using certain well-known generic names and to have a single name for each fungal species, including those in the genus *Fusarium* (5). In addition, the names of the well-known *Fusarium* anamorphs, as they have been used in the present paper, ought to be used instead of those of the known teleomorphs (e.g., *Haemonectria* and *Gibberella*) (1–3). However, the perception is that new generic changes may be suggested, such as the establishment of the genus *Bisifusarium* to include the more commonly known members of the *Fusarium dimerum* SC and the name *Neocosmospora solani* to replace *Fusarium solani* (6). The most frequent causes of fungal infections are members of three complexes, the *F. solani* species complex (SC), the *F. oxysporum* SC, and the *Fusarium* (*Gibberella*) *fujikuroi* SC (which includes, among others, *F. verticillioides* and *F. proliferatum*), and the next most frequent causes belong to the *F. dimerum* SC and *F. incarnatum-F. equiseti* SC; their distribution could be region dependent (4, 7–10). Common clinical presentations are onychomycosis, keratitis, allergic disease (sinusitis and bronchopulmonary disease) for nonimmunocompromised patients and disseminated disease, as well as other severe invasive infections, in immunocompromised hosts (e.g., patients with prolonged neutropenia and T-cell immunodeficiency) (1, 4, 7–13). Amphotericin B lipid formulations, voriconazole, posaconazole, and, to a lesser extent, itraconazole have been recommended or used for the treatment and prophylaxis of *Fusarium* infections, in addition to surgical debridement and reversal of immunosuppression (14–

Received 8 October 2015 **Returned for modification** 12 November 2015
Accepted 23 November 2015
Accepted manuscript posted online 7 December 2015
Citation Espinel-Ingroff A, Colombo AL, Cordoba S, Dufresne PJ, Fuller J, Ghannoum M, Gonzalez GM, Guarro J, Kidd SE, Meis JF, Melhem TMSC, Pelaez T, Pfaller MA, Szeszs MW, Takahaschi JP, Tortorano AM, Wiederhold NP, Turnidge J. 2016. International evaluation of MIC distributions and epidemiological cutoff value (ECV) definitions for *Fusarium* species identified by molecular methods for the CLSI broth microdilution method. Antimicrob Agents Chemother 60:1079–1084. doi:10.1128/AAC.02456-15.
Address correspondence to A. Espinel-Ingroff, avingrof@vcu.edu.

19). The survival rate is low, with some reports suggesting 30% or less, for fusariosis, especially among patients with persistent neutropenia (16–22). Successful therapeutic treatment of invasive disease is usually associated with neutrophil recovery, a major factor in making the setting of clinical breakpoints so challenging. The new formulations of itraconazole and posaconazole have improved bioavailability and reduced variability in exposure among subjects (23, 24). However, the efficacy of these formulations in the treatment of fusariosis has not been established.

A reproducible procedure for testing the antifungal susceptibilities of *Fusarium* spp. is described by the Clinical and Laboratory Standards Institute (CLSI) Subcommittee on Antifungal Susceptibility Tests in document M38-A2 (25). However, neither species-specific clinical breakpoints (BPs) nor epidemiological cutoff values (ECVs) have been established for this fungal group. The main reason, as for other, less-prevalent fungal species, is the lack of both clinical trials and knowledge regarding molecular resistance mechanisms for *Fusarium* spp. As a consequence, information on the relationships between resistance mechanisms, low and high MICs, and clinical response to therapy is not available. However, it is still possible to define ECVs. These are calculated on the basis of MIC distributions (≥100 MIC results per species and antifungal agent) from multiple (≥3) independent laboratories (26, 27; CLSI documents on ECVs under development). ECVs can identify non-wild-type (non-WT [often harboring molecular mechanisms of resistance]) isolates or isolates that are less susceptible to the antifungal agent being evaluated. Although amphotericin B and triazole MIC data have been reported for a variety of *Fusarium* spp., most available data were obtained for isolates identified only to the genus level or by nonmolecular methods, or the number of isolates evaluated was small (2, 4, 28–31). Therefore, there was a need to pool data from multiple laboratories in order to define ECVs for *Fusarium* spp.

The purposes of the present study were (i) to define the WT susceptibility endpoint MIC distributions of the three most prevalent species/species complexes (the *F. oxysporum* SC, the *F. solani* SC, and *F. verticillioides*) using aggregated CLSI M38-A2 broth microdilution MIC data originating from 16 of the 17 participating laboratories and (ii) to propose ECVs for amphotericin B, voriconazole, posaconazole, and itraconazole based on combinations of antifungal agents and species or complexes for which ≥113 isolates originating from ≥7 independent laboratories were used. Pooled distributions of MICs of amphotericin B, voriconazole, posaconazole, and itraconazole for 10 to 82 isolates belonging to less-prevalent species/complexes (e.g., *F. dimerum* SC, *F. fujikuroi*, *F. incarnatum-F. equiseti* SC, *F. proliferatum*) were also collated. We aggregated a total of 10 to 608 MICs (species and antifungal agent dependent) obtained in the 17 participating laboratories (in Argentina, Australia, Brazil, Canada, Europe, Mexico, and the United States).

MATERIALS AND METHODS

Isolates. Each isolate was recovered from unique clinical specimens from patients most of whom had eye, skin (sometimes both a cutaneous infection and infection of a nail or other organ), sinus, or pulmonary infections or invasive disease (blood, lymph nodes). Antifungal susceptibility testing was performed according to the CLSI broth microdilution method (M38-A2) at the following medical centers: VCU Medical Center, Richmond, VA; Hospital São Paulo, Escola Paulista de Medicina—UNIFESP, São Paulo, Brazil; Instituto Nacional de Enfermedades Infecciosas "Dr. C. G. Malbrán," Buenos Aires, Argentina; Institut National de Santé Publique du Québec, Laboratoire de Santé Publique du Québec, Sainte-Anne-de-Bellevue, Quebec, Canada; Provincial Laboratory, Alberta Health Services, Edmonton, Canada; University Hospitals Case Medical Center and Case Western Reserve University, Cleveland, OH; Universidad Autónoma de Nuevo León, Monterrey, Nuevo León, Mexico; Facultat de Medicina, IISPV, URV, Reus, Spain; National Mycology Reference Centre, SA Pathology, Adelaide, Australia; Canisius Wilhelmina Hospital, Nijmegen, The Netherlands; The Instituto Adolfo Lutz Reference Center, São Paulo, Brazil; Hospital General Universitario Gregorio Marañón, Madrid, Spain; JMI Laboratories, North Liberty, IA; Mycology Department, The Instituto Adolfo Lutz Reference Center, São Paulo, Brazil; Grupo Fleury, São Paulo, Brazil; Department of Biomedical Sciences for Health, Università degli Studi di Milano, Milan, Italy; and the University of Texas Health Science Center, San Antonio, TX.

The isolates were identified in each laboratory using conventional methods (both macroscopic and microscopic characteristics on potato dextrose agar) (1, 32) and were confirmed by DNA-PCR-based molecular assays (e.g., sequencing and amplification of β-tubulin [BenA], translation elongation factor 1α [TEF], or the largest and/or second largest subunit of RNA polymerase [RPB1 and/or RPB2, respectively], as well as analysis of the internal transcribed spacer 1 [ITS1] and ITS2 regions) (1, 4, 10, 33, 34). The CLSI MICs of each of the four antifungal agents were aggregated for 53 *F. dimerum* SC (including 1 *F. delphinoides* isolate), 10 *F. fujikuroi*, 82 *F. proliferatum*, 20 *F. incarnatum-F. equiseti* SC, 226 *F. oxysporum* SC, 608 *F. solani* SC (including 11 *F. falciforme* isolates), and 151 *F. verticillioides* isolates originating from 3 to 16 of the 17 independent laboratories (see Table 1). Additionally, insufficient MIC data (<10 isolates from 2 to 3 laboratories) were provided for other members of the *F. fujikuroi* SC, identified as *F. sacchari*, *F. subglutinans*, and *F. thapsinum* (data not shown). Since molecular resistance mechanisms have not been elucidated for *Fusarium* spp. and any antifungal agent, none of the isolates were evaluated for gene mutations.

MIC data for at least one of the three quality control (QC) isolates *Candida parapsilosis* ATCC 22019, *C. krusei* ATCC 6258, and *Paecilomyces variotii* ATCC MYA-3630 and/or for the reference isolate *Aspergillus flavus* ATCC 204304 were reported by the participating laboratories (25).

Antifungal susceptibility testing. The MICs of amphotericin B and the three triazoles (defined as the lowest drug concentrations that produced complete growth inhibition [100%] at 48 h [25]) for each available isolate in the total set (see Tables 1 and 2) were determined in each center by the CLSI broth microdilution method (with standard RPMI 1640 broth [0.2% dextrose] and final inoculum concentrations ranging from 0.4×10^4 to 5×10^4 CFU/ml). MICs for the *Candida* QC strains were determined after 48 h by using the 50% (triazoles) and 100% (amphotericin B) growth inhibition criteria (25). These MICs were within the recommended MIC limits with the following exceptions: discrepant MICs for both the *C. krusei* and *C. parapsilosis* QC isolates and the triazoles were observed, but the agreement (97.7 to 99.7%) was similar to or higher than those listed in the M38-A2 document (25); the modes were within 1 dilution.

Definitions. As defined in the introduction, the WT is the population of strains in a species-drug combination with no detectable acquired resistance mechanisms. The ECV (or WT cutoff value [CO_{WT}]) is the highest MIC that would categorize an isolate as WT (without known mechanisms of resistance) or, alternatively, the critical drug concentration value that may identify those strains that have decreased susceptibility to the agent being evaluated (non-WT isolates) or are potentially resistant (26, 27, 35).

Data analysis. The data were analyzed as reported previously in various studies, by following the CLSI guidelines set forth for this purpose (26, 27, 35; CLSI documents on ECVs under development). Briefly, after the MIC distributions for each combination of an antifungal agent and a species or species complex from each laboratory were listed in an Excel spreadsheet, they were reviewed for skewed/abnormal distributions (e.g., the mode at the lowest concentration tested and/or bimodal [two

TABLE 1 Pooled MIC distributions of amphotericin B and three triazoles for species of *Fusarium* from 3 to 16 laboratories as determined by the CLSI broth microdilution method[a]

Agent	Species or SC[b]	No. of labs	No. of isolates tested	No. of isolates[c] with a MIC (μg/ml) of:							
				≤0.25	0.5	1	2	4	8	16	>16
Amphotericin B	*F. dimerum* SC	8	50	3	7	**16**	13	5	5	1	
	F. fujikuroi	3	10		1	**6**	3				
	F. proliferatum	10	82	1	5	16	**31**	22	5	1	1
	F. verticillioides	9	151		1	27	**84**	28	6	5	
	F. incarnatum-F. equiseti SC[d]	6	20		3	3	5	**6**	3		
	F. oxysporum SC	14	226	1	10	37	**107**	61	8	2	
	F. solani SC	15	608	8	46	120	**265**	125	29	15	
Itraconazole	*F. dimerum* SC	7	45			3	1		15	**25**	1
	F. fujikuroi	3	10							1	**9**
	F. proliferatum	10	60			1		4	14	**21**	20
	F. verticillioides	7	96			2	4	5	27	**41**	17
	F. incarnatum-F. equiseti SC	6	20			1	1	2	**8**	6	2
	F. oxysporum SC	9	148			2	2	4	29	**87**	24
	F. solani SC	11	338		2	1	7	5	90	**220**	13
Posaconazole	*F. dimerum* SC	7	48		1	2	3	5	**25**	11	1
	F. fujikuroi	3	10		2	3	**4**	1			
	F. proliferatum	9	49			7	**16**	6	8	5	7
	F. verticillioides	7	113	15	**43**	33	9	3			10
	F. incarnatum-F. equiseti SC	6	19		3	2	5	**6**	2	1	
	F. oxysporum SC	10	148		1	20	**53**	37	13	22	2
	F. solani SC	8	357			8	15	42	**163**	113	16
Voriconazole	*F. dimerum* SC	7	53			3	9	15	**24**	2	
	F. fujikuroi	3	10				2	**5**	1	2	
	F. proliferatum	10	74			3	10	**29**	24	6	2
	F. verticillioides	8	143		1	25	**70**	35	2	2	8
	F. incarnatum-F. equiseti SC	6	20		1	2	5	**8**	3		1
	F. oxysporum SC	13	200		5	10	36	**94**	47	5	3
	F. solani SC	16	555		3	9	51	123	**243**	119	7

[a] MICs were determined by CLSI method M38-A2 (25).
[b] As identified by molecular methods (1, 4, 10, 33, 34). SC, species complex. *F. fujikuroi*, *F. proliferatum*, and *F. verticillioides* are members of the *Fusarium* (*Gibberella*) *fujikuroi* species complex.
[c] The highest number in each row (showing the most frequently obtained MIC, or mode) is in boldface.
[d] A synonym for the *Fusarium incarnatum-F. equiseti* species complex is *Fusarium semitectum*.

modes in the same distribution]), which were not included in the statistical analysis. According to CLSI recommendations (CLSI documents on ECVs under development) and following the examination of global WT modal MIC variability, distributions for each antifungal agent and species or species complex were pooled with the qualifying data (abnormal distributions not included). ECVs were calculated for each pooled distribution by the previously reported iterative statistical technique that captured at least 95%, 97.5%, and 99% of the modeled WT population (not the observed MICs) (35). In addition, we evaluated the inherent variability (within approximately 1 doubling dilution) of susceptibility testing and the presence of outlier laboratories in each pooled distribution.

RESULTS AND DISCUSSION

Susceptibility testing should aid in predicting patient response to therapy, which is the specific role of the BP (36, 37). The CLSI has established species-specific BPs only for testing the susceptibilities of some *Candida* spp. to echinocandins, fluconazole, and voriconazole (38). The reason for this dearth of BPs is that their establishment requires particular steps: (i) WT MIC distributions and ECVs for each species and agent being evaluated, (ii) the pharmacokinetic and pharmacodynamic (PK/PD) parameters of the agent, (iii) knowledge of the relationship between mechanisms of resistance and MICs, and, most importantly, (iv) the correlation of MICs with clinical response to treatment with the specific agent in clinical trials (35–37). Data for these steps are not available for *Fusarium* spp. However, we have gathered MIC distributions for the *F. oxysporum* SC, the *F. solani* SC, and *F. verticillioides* (the three species or complexes most commonly associated with human disease) with three triazoles and amphotericin B. Although ECVs were not proposed for the other species evaluated due to insufficient data, their pooled MIC distributions are listed in Table 1; the CLSI criteria require a minimum of 100 MICs/species (MICs for 100 species-agent combinations) from at least three laboratories and ECVs calculated by the iterative statistical method (CLSI documents on ECVs under development). It is expected that the proposed ECVs would separate the two populations (WT and non-WT) that are present in the MIC distribution of a species-agent combination. Although they would not distinguish between susceptible (treatable) and resistant (nontreatable) isolates as BPs do, our proposed ECVs can help to identify those isolates that are more likely to harbor acquired molecular muta-

tions conferring microbial resistance (non-WT isolates). This is important in the absence of BPs for *Fusarium* spp.

Table 1 depicts the pooled MIC distributions for the four agents and the *Fusarium* complexes/species evaluated. In general, the MIC distributions were typical for each antifungal agent and species, where 2 to 5 2-fold concentrations surround the modal MIC. The exceptions were itraconazole and some voriconazole distributions, which were skewed to the right. In addition, the distributions from the different laboratories were comparable, since their modal MICs for each combination of a species or species complex with an agent were within 1 2-fold dilution of one another, with three exceptions. The amphotericin B mode for *F. oxysporum* SC was 1 dilution higher in one of the contributing laboratories (4 μg/ml versus 1 to 2 μg/ml in the other laboratories), while the posaconazole and voriconazole modes were 1 dilution lower for *F. oxysporum* SC and *F. verticillioides* (1 μg/ml versus 2 to 4 μg/ml in the other laboratories) (data not shown in Tables 1 and 2). Most amphotericin B modes were 2 μg/ml; the exceptions were the lower modes for *F. dimerum* SC and *F. fujikuroi* and the higher mode for *F. incarnatum-F. equiseti* SC (Table 1). Among the triazoles, the highest values were observed when itraconazole was tested (modes, 8 to ≥16 μg/ml). Posaconazole and voriconazole modes ranged from 0.5 to 8 μg/ml and 2 to 8 μg/ml, respectively, with the lowest modes for *F. verticillioides* and the highest for both the *F. solani* SC and the *F. dimerum* SC. The MIC data (agent dependent) for 2 to 11 isolates of *F. falciforme* were similar to those of their *F. solani* SC with one exception: the eight posaconazole MICs for this species were >16 μg/ml. The same applied to the 4 to 8 isolates of the other three members of the *F. fujikuroi* SC (*F. sacchari*, *F. subglutinans*, and *F. thapsinum*), for which all itraconazole MICs were >16 μg/ml (data not shown in Table 1). Although some of the distributions for the less prevalent species are small, these results underline the need for identification to the species or complex level in addition to antifungal susceptibility testing.

While the *in vitro* activities of the four antifungal agents evaluated are similar to those reported previously for *Fusarium* isolates (both CLSI and EUCAST [European Committee on Antimicrobial Susceptibility Testing] MICs) (2, 4, 28–31), overall, our MIC ranges are wider (Tables 1 and 2). In addition to our aggregated itraconazole data, this was evident with amphotericin B MICs for both the *F. proliferatum* and *F. oxysporum* SCs and with voriconazole MICs for the *F. solani* SC. However, the number of isolates for each pooled distribution was higher than those tested in prior studies (2, 4, 28–31) (15 to 22 isolates for more-prevalent species) and perhaps better represented the range of susceptibilities to these agents. Nevertheless, the most frequent MICs (when provided) were similar to those in the present study. To our knowledge, pooled MIC data are not available for the less-prevalent species. Based on these data and the widespread geographical regions from which our pooled MIC data originated, we assume that our data are valid.

As mentioned above, the CLSI has set forth criteria for the calculation of species-specific ECVs based on unmodified CLSI methodologies for MIC determination (≥100 isolates originating in at least three independent laboratories per species-agent combination) and for the calculation of the ECV percentage (≥97.5% values) by the iterative statistical technique (CLSI documents on ECVs under development). Since ≥97.5% values risk classifying some isolates with acquired resistance mechanisms as WT, we have also provided the ≥95% and ≥99% ECVs. Either the values were the same or the ≥97.5 and ≥99% ECVs were separated by 1 dilution. Table 2 depicts the ECVs for the aggregated MIC distributions that met the CLSI criteria: amphotericin B, itraconazole, posaconazole, and voriconazole versus the *F. oxysporum* SC, the *F. solani* SC, and *F. verticillioides*. Insufficient data precluded the calculation of ECVs for the combination of itraconazole and *F. verticillioides* or any other species. The ECVs of amphotericin B were 4 μg/ml (*F. verticillioides*) and 8 μg/ml (*F. oxysporum* SC and *F. solani* SC); these values are actually above what is anecdotally considered the notional "breakpoint" for resistance among some *Aspergillus* spp. (2 μg/ml). Similarly high ECVs were observed among *Aspergillus* spp., *Mucor circinelloides*, and *Rhizopus arrhizus* (26, 39). As expected, the highest ECVs were those of the three triazoles for the *F. solani* SC (32 μg/ml). Lower posaconazole and voriconazole ECVs were calculated for *F. verticillioides* (2 and 4 μg/ml, respectively) and the *F. oxysporum* SC (8 and 16 μg/ml, respectively). These triazole ECVs are mostly higher than the expected maximal, variable, and dose-dependent trough levels of each of the agents (23, 24, 40) and highlight the intrinsically resistant nature of *Fusarium* spp. The same applies to amphotericin B values.

Although case series of *Fusarium* infections have been reported throughout the years (4, 7, 9, 16, 20–22), only in a recent report was an indication of a potential correlation between MICs for *Fusarium* spp. and response to treatment found (22), where CLSI MICs for seven *Fusarium* isolates identified by molecular methods, antifungal therapy (voriconazole or both voriconazole and amphotericin B), and clinical response were documented for patients with invasive fusariosis. Favorable clinical responses were reported for two of the seven patients infected with *F. verticillioides* (voriconazole MICs, 2 and 4 μg/ml, respectively); according to our voriconazole ECV for this species, both infecting strains

TABLE 2 Epidemiologic cutoff values of amphotericin B, itraconazole, posaconazole, and voriconazole for two clinically relevant *Fusarium* species complexes and *F. verticillioides* as determined by the CLSI broth microdilution method[a]

Species or species complex	Antifungal agent[b]	MIC (μg/ml)		Calculated statistical ECV (μg/ml)[c]		
		Range	Mode[d]	≥95%	≥97.5%	≥99%
F. verticillioides	AMB	0.5–16	2	4	4	4
	ITR	1–≥16	16	ND	ND	ND
	POS	≤0.25–≥16	0.5	2	2	2
	VOR	0.5–≥16	2	4	4	8
F. oxysporum SC	AMB	≤0.25–16	2	4	8	8
	ITR	1–≥16	16	32	32	32
	POS	0.5–16	2	8	8	8
	VOR	0.5–≥16	4	8	16	16
F. solani SC	AMB	≤0.25–16	2	4	8	8
	ITR	0.5–≥16	16	16	32	32
	POS	1–≥16	8	32	32	32
	VOR	0.5–≥16	8	16	32	32

[a] ECVs were defined for pooled distributions for ≥100 isolates from ≥3 laboratories using the methodology of CLSI document M38-A2 (25, 35).
[b] AMB, amphotericin B; ITR, itraconazole; POS, posaconazole; VOR, voriconazole.
[c] Calculated ECVs comprising ≥95%, ≥97.5%, and ≥99% of the statistically modeled population. ND, not determined (due to insufficient data).
[d] MIC most frequently obtained for each distribution.

would be considered WT isolates (Table 2). Of the four patients infected with *F. solani*, the correlation was evident for only one (a favorable clinical response and a voriconazole MIC of 4 μg/ml, or another WT strain). The remaining three patients failed therapy; two of them were treated with both voriconazole and amphotericin B (amphotericin B MICs, 4 μg/ml; voriconazole MICs, >8 μg/ml or >16 μg/ml). These amphotericin B MICs could be considered WT, and both voriconazole MICs would more likely be considered non-WT, although the final MIC endpoint was not given (voriconazole ECV for the *F. solani* SC, 32 μg/ml). However, it is important to keep in mind that categorization of an isolate as WT does not indicate that the isolate is susceptible (treatable), given that ECVs do not predict clinical response to therapy. Similarly, other factors preclude correlations of *in vitro* and clinical responses to therapy in other studies, where cultures, species, and especially MICs are not reported and the response was influenced by the site of infection, the underlying disease, and/or the reversal of immunosuppression. In addition, the molecular mechanisms of resistance have not been evaluated for any *Fusarium* isolate causing human disease, as they have been for *Candida* and *Aspergillus*. As found with the Mucorales, the molecular biology of *Fusarium* sp. resistance needs to be investigated.

In conclusion, species-specific amphotericin B ECVs (comprising ≥97.5% of the modeled populations) of 4 μg/ml (*F. verticillioides*) and 8 μg/ml (*F. oxysporum* SC and *F. solani* SC), posaconazole ECVs of 2 μg/ml (*F. verticillioides*), 8 μg/ml (*F. oxysporum* SC), and 32 μg/ml (*F. solani* SC), voriconazole ECVs of 4 μg/ml (*F. verticillioides*), 16 μg/ml (*F. oxysporum* SC), and 32 μg/ml (*F. solani* SC), and itraconazole ECVs of 32 μg/ml (*F. oxysporum* SC and *F. solani* SC) have been proposed based on CLSI data from multiple laboratories. ECVs were mostly 1 dilution lower when ≥95% of the modeled populations was used, which could be more clinically relevant. Like the ECVs for *Candida* spp. and *Aspergillus* spp., the proposed ECVs for the more-prevalent *Fusarium* spp. may aid in the detection of strains with acquired mechanisms of resistance (non-WT) to the agents evaluated. However, ECVs are not BPs and cannot predict clinical response to therapy, and categorization of an isolate as WT does not mean that it is necessarily treatable or susceptible. Also, as for the Mucorales, knowledge regarding molecular mechanisms of resistance and their relationship with MICs is needed.

ACKNOWLEDGMENTS

We thank M. Castanheira (JMI Laboratories, North Liberty, IA), V. R. de Azevedo Bastos (Universidade Federal de São Paulo, São Paulo, Brazil), and A. Fothergill (University of Texas Health Science Center, San Antonio, TX) for their collaboration.

We declare no conflicts of interest.

FUNDING INFORMATION

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sector.

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