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Multicenter Study of Isavuconazole MIC Distributions and Epidemiological Cutoff Values for *Aspergillus* spp. for the CLSI M38-A2 Broth Microdilution Method

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Epidemiological cutoff values (ECVs) were established for the new triazole isavuconazole and *Aspergillus* species wild-type (WT) MIC distributions (organisms in a species-drug combination with no detectable acquired resistance mechanisms) that were defined with 855 *Aspergillus fumigatus*, 444 *A. flavus*, 106 *A. nidulans*, 207 *A. niger*, 384 *A. terreus*, and 75 *A. versicolor* species complex isolates; 22 *Aspergillus* section *Usti* isolates were also included. CLSI broth microdilution MIC data gathered in Europe, India, Mexico, and the United States were aggregated to statistically define ECVs. ECVs were 1 μg/ml for the *A. fumigatus* species complex, 1 μg/ml for the *A. flavus* species complex, 0.25 μg/ml for the *A. nidulans* species complex, 4 μg/ml for the *A. niger* species complex, 1 μg/ml for the *A. terreus* species complex, and 1 μg/ml for the *A. versicolor* species complex; due to the small number of isolates, an ECV was not proposed for *Aspergillus* section *Usti*. These ECVs may aid in detecting non-WT isolates with reduced susceptibility to isavuconazole due to *cyp51A* (an *A. fumigatus* species complex resistance mechanism among the triazoles) or other mutations.

Fungal infections caused by the *Aspergillus fumigatus* species complex and other *Aspergillus* spp. are common and are usually associated with high morbidity and mortality rates, especially in immunocompromised hosts (1–4). The triazoles itraconazole, voriconazole, and posaconazole have a broad spectrum of *in vitro* activity against molds and are important therapeutic agents for the systemic treatment and prevention of aspergillosis (5). Isavuconazole (BAL4815; codeveloped by Basilea Pharmaceutica International Ltd., Basel, Switzerland, and Astellas Pharma, Tokyo, Japan) is a newer water-soluble triazole, with favorable pharmacodynamic and pharmacokinetic (PK/PD) parameters, that is under clinical evaluation (phase III) for the treatment of invasive aspergillosis and candidiasis (6–8). Similar to the other azoles, isavuconazole's mode of action is the inhibition of ergosterol biosynthesis (the enzymes 14-α-sterol demethylases A and B, which are encoded by the *cyp51A* and *cyp51B* genes, respectively). Isavuconazole has *in vitro* and *in vivo* activities similar to those of licensed triazoles against *Aspergillus* spp.; other molds, including the mucormycetes (zygomycetes); as well as *Candida* spp. (9–13). Acquired azole resistance in *Aspergillus* spp., associated with mutations of the *cyp51* gene, has been documented since the1990s, and it appears to have increased in recent years, especially in Europe (14–20).

Although individual laboratories have evaluated the *in vitro* activity of isavuconazole against both yeasts and filamentous fungi by Clinical and Laboratory Standards Institute (CLSI) and European Committee on Antimicrobial Susceptibility Testing (EUCAST) methodologies, epidemiological cutoff values (ECVs), based on MIC data from multiple laboratories (at least three laboratories) as well as different geographical areas, have not been established for this agent and *Aspergillus* spp. Because of this, we have defined isavuconazole ECVs for four of the six species complexes evaluated (*A. fumigatus*, *A. flavus*, *A. niger*, and *A. terreus*) and tentative susceptibility cutoffs for the *A. nidulans* species complex and the *A. versicolor* species complex. Sufficient MICs for the latter two species were not available to calculate a more definite ECV. Wild-type (WT) distributions of each species were calculated by using aggregated MIC data gathered from three to eight laboratories in Europe, India, Mexico, and the United States (75 to 855 MICs according to species); ECVs were not defined for *Aspergillus* section *Usti* (22 isolates comprising four species), because at least 100 MIC data points originating from three or more laboratories are recommended (CLSI Establishing ECOFFs Workshop, Tampa, FL, January 2013).

MATERIALS AND METHODS

Isolates. Each isolate was recovered from unique clinical specimens at the following medical centers: VCU Medical Center, Richmond, VA; Vallabhbhai Patel Chest Institute, University of Delhi, Delhi, India; Universidad Autónoma de Nuevo León, Monterrey, Nuevo León, México; The Innsbruck Medical University, Innsbruck, Austria; Hospital Universitario de Valme, Seville, Spain; Canisius Wilhelmina Hospital, Nijmegen, Netherlands; Hospital General Universitario Gregorio Marañón, Faculty of Medicine, Universidad Complutense, Madrid, Spain; and JMI Laboratories, North Liberty, IA. The total aggregated maximum available MIC

Received 3 April 2013 Returned for modification 7 May 2013
Accepted 21 May 2013
Published ahead of print 28 May 2013
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doi:10.1128/AAC.00636-13

TABLE 1 Pooled MIC distributions of isavuconazole for *Aspergillus* spp. from two to eight laboratories, using the CLSI M38-A2 microdilution method[a]

Species complex or section	No. of isolates/no. of laboratories	No. of isolates with MIC (μg/ml) of:								
		0.03	0.06	0.125	0.25	0.5	1.0	2.0	4.0	≥8.0
A. fumigatus	855/8		6	31	149	**508**	113	33	4	11
A. flavus	444/7			2	29	**253**	146	13	1	
A. nidulans	106/3		7	**51**	19	17	12			
A. niger	207/6		1	4	11	52	**75**	55	7	2
A. terreus	384/4		4	32	**171**	162	14	1		
A. versicolor	75/3	5	3	20	**24**	17	4	1		1
Aspergillus section *Usti*[b]	22/2				2		**10**	10		

[a] Wild-type MIC distributions for each species were obtained by using the CLSI broth microdilution method (M38-A2) and according to the recently identified testing conditions for *Aspergillus* spp. and isavuconazole: 48 h of incubation and 100% growth inhibition (24, 25). Values in boldface type indicate modes or most frequent MICs for each species. The following are species complexes, as per nonmolecular identification (21): *A. fumigatus*, *A. flavus*, *A. nidulans*, *A. niger*, *A. terreus*, and *A. versicolor*.
[b] *Aspergillus* section *Usti* comprised the following species, as per molecular identification: *A. calidoustus* (17 isolates), *A. pseudodeflectus* (2 isolates), *A. ustus* (2 isolates), and *A. insuetus* (1 isolate) (22, 23; Peláez et al., unpublished).

data for each species complex were obtained for 855 isolates of *A. fumigatus*, 444 of *A. flavus*, 106 of *A. nidulans*, 207 of *A. niger*, 384 of *A. terreus*, 22 of *Aspergillus* section *Usti* (comprising *A. calidoustus* [17 isolates], *A. pseudodeflectus* [2 isolates], *A. ustus* [2 isolates], and *A. insuetus* [1 isolate]) (T. Peláez, P. Escribano, C. Padilla, B. Gama, J. Guinea, A. Espinel-Ingroff, and E. Bouza, unpublished data), and 75 of *A. versicolor*. Identification of most of the isolates to the species level was performed in each laboratory by using conventional methods (both macroscopic and microscopic characteristics on Sabouraud and Czapek-Dox agars) (21), but the species listed for *Aspergillus* section *Usti* were identified by partially amplifying and sequencing the β-tubulin (*benA*) gene, using βtub1/2 primers (22, 23; Peláez et al., unpublished). We had at least six *A. fumigatus* species complex isolates with documented acquired resistance to triazoles (MICs of itraconazole, voriconazole, and isavuconazole of ≥4 μg/ml) and confirmed mechanisms of resistance (14, 17). Data for at least one of four quality control (QC) isolates, *Candida parapsilosis* ATCC 22019, *Candida krusei* ATCC 6258, *Paecilomyces variotii* ATCC MYA-3630, and *A. flavus* ATCC 204394 (24, 25), were reported by the participant laboratories.

Antifungal susceptibility testing. Isavuconazole MIC results for each available isolate in the total set (Tables 1 and 2) were obtained by each center according to the CLSI M38-A2 broth microdilution method (standard RPMI 1640 broth [0.2% dextrose] and final inoculum concentrations that ranged from 0.4×10^4 to 5×10^4 CFU/ml); MICs were the lowest drug concentrations that produced complete growth inhibition (100%) at 48 h. MICs of licensed triazoles for the isolates for which isavuconazole MICs were high (>4 μg/ml) were determined in the same manner. Isavuconazole MICs for the QC *Candida* strains were obtained after 48 h by using 50% growth inhibition criteria. These are the optimal testing conditions recently identified for isavuconazole and *Aspergillus* spp. and for *Candida* spp. after 48 h of incubation (24, 25).

Definitions. The wild type (WT) is defined as the population of strains in a species-drug combination with no detectable acquired resistance mechanisms, and the ECV is the highest WT MIC. The ECV categorizes an isolate as either WT or non-WT, and it has been described as either the WT cutoff value (CO_{WT}) or the epidemiological cutoff value (ECOFF or ECV) (26).

We use the ECV term throughout the present report because it has been previously used in similar fungal studies (26–29).

Data analysis. The MIC distributions of each of the seven species tested in each participant laboratory were first screened for evidence of grossly abnormal distributions, and modal (most frequent) MICs for each laboratory were determined (28, 29). Grossly skewed distributions and distributions which had a modal MIC at the lowest concentration tested were excluded. Next, the WT distributions were obtained by pooling qualifying MIC distributions from participant laboratories for each species, and the ECV was then estimated by statistical methods (30). A minimum of 3 laboratories and 100 data points were required to establish a reasonable estimated ECV for a species. In the statistical method, the modeled WT population is based on fitting a normal distribution at the lower end of the MIC range, calculating the mean and standard deviation of that normal distribution, and using those values to estimate the MIC that

TABLE 2 Isavuconazole ECVs based on MICs from three to eight laboratories and as determined by the CLSI M38-A2 broth microdilution method

Species complex or section[a]	No. of isolates/no. of laboratories	MIC range (μg/ml)	Mode (μg/ml)[b]	ECV[c]			% observed above ECV		
				95%	97.5%	99%	ECV 95%	ECV 97.5%	ECV 99%
A. fumigatus	855/8	0.06–8	0.5	1	1	1	5.6	5.6	5.6
A. flavus	444/7	0.06–2	0.5	1	1	2	3.2	3.2	0.2
A. nidulans[d]	106/3	0.06–1	0.12	0.25	0.25	0.25	27.4	27.4	27.4
A. niger	207/6	0.06–>8	1	4	4	4	1.0	1.0	1.0
A. terreus	386/5	0.06–2	0.25	1	1	1	0.3	0.3	0.3
A. versicolor[d]	75/3	0.03–>8	0.25	1	1	2	2.7	2.7	1.3
Aspergillus section *Usti*[e]	22/2	0.25–2	1	ND	ND	ND	ND	ND	ND

[a] The following are species complexes, as per nonmolecular identification (21): *A. fumigatus*, *A. flavus*, *A. nidulans*, *A. niger*, *A. terreus*, and *A. versicolor*. *Aspergillus* section *Usti* comprised the following species, as per molecular identification: *A. calidoustus* (17 isolates), *A. pseudodeflectus* (2 isolates), *A. ustus* (2 isolates), and *A. insuetus* (1 isolate) (22, 23; Peláez et al., unpublished).
[b] Most frequent MIC.
[c] Calculated ECVs comprising ≥95, ≥97.5, or ≥99% of the statistically modeled MIC population.
[d] Tentative values until more data are obtained.
[e] ND, not determined due to insufficient numbers of laboratories and isolates/species.

captured at least 95%, 97.5%, and 99% of the modeled WT population, rounded up to the nearest 2-fold dilution. The modes for each species, the inherent variability (± 1 $\log_2$ dilution) of susceptibility testing, as well as a search for outlier laboratories in each distribution were also considered (31).

RESULTS AND DISCUSSION

For the MIC result to be clinically useful, it should categorize the isolate as either susceptible (treatable), intermediate (possibly treatable), or resistant (nontreatable) to the specific agent; this result is the clinical breakpoint (CBP) or the predictor of clinical outcome (26, 32). CBPs are established based on clinical trial data, global surveillance and ECVs, resistance mechanisms, and PK/PD parameters from model systems. The CLSI has not defined CBPs for molds due to the insufficient number of resistant isolates recovered during clinical trials; most isolates are WT strains. ECVs for *Aspergillus* spp. and the licensed triazoles (itraconazole, posaconazole, and voriconazole), caspofungin, and amphotericin B were recently defined according to current criteria for ECV definition (CLSI data from multiple laboratories [at least three] and for ≥100 isolates). In the present study, ECVs of isavuconazole for four *Aspergillus* species complexes as well as tentative values for two other species were established. It is expected that these susceptibility cutoffs may aid in the evaluation of clinical isolates by detecting those strains with reduced isavuconazole susceptibility due to *cyp51* mutations and may serve as an early warning of emerging changes in the susceptibility patterns of these organisms.

Despite standardized testing conditions, variability is usually evident during comparison of MIC results from multiple laboratories, including studies where single strains are evaluated in order to select QC isolates (24, 25). The performance of antimicrobial susceptibility testing is monitored by the introduction of at least one QC or control strain for which MIC ranges of acceptable reproducibility (within 3 to 4 dilutions) have been established. Recent collaborative studies have identified the optimum parameters for testing isavuconazole and *Aspergillus* spp., and two yeasts and two molds were selected as QC strains and MIC limits for these isolates were proposed (25). In the present study, each participating laboratory reported isavuconazole MIC data for at least one of these four QC isolates in the following ranges: 0.015 to 0.06 μg/ml in three laboratories for *C. parapsilosis* ATCC 22019, 0.12 to 0.5 μg/ml in four laboratories for *C. krusei* ATCC 6258, 0.5 to 2 μg/ml in four laboratories for *A. flavus* ATCC 204304, and 0.06 to 0.5 μg/ml in two laboratories and 0.12 to 2 μg/ml in another laboratory for *Paecilomyces variotii* ATCC 3630. Therefore, most values were within the 3- to 4-dilution MIC range for the QC strains (25). Reproducibility was similar to that observed in the collaborative study proposing isavuconazole QC MIC ranges.

Table 1 depicts isavuconazole aggregated MIC distributions for the seven *Aspergillus* spp. The graphs indicated that the distributions of most species were symmetrical; the exception was the MIC distribution for the *A. nidulans* species complex. The skewed distribution of the *A. nidulans* species complex may have been due to the smaller numbers of isolates and laboratories (three laboratories and 106 isolates [Table 1]). It is noteworthy that most modal MICs for individual contributing laboratories for all the species evaluated were equal to or within one 2-fold dilution, including the modes (0.12 and 0.25 μg/ml) from the three laboratories that provided MICs for the *A. nidulans* species complex.

Table 2 depicts the proposed isavuconazole ECVs (using ≥95%, ≥97.5%, and ≥99% of the modeled MIC population) as well as the range and modal MICs for each of the seven *Aspergillus* spp. The lowest modal MIC (0.12 μg/ml) was for the *A. nidulans* species complex, and the highest was for the *A. niger* species complex and *Aspergillus* section *Usti* (1 μg/ml). Data from a previous study in which ECVs for *Aspergillus* spp. and the three other triazoles were defined allowed for the following comparisons between the data sets (27). The isavuconazole modal MIC for the *A. fumigatus* species complex was higher than those of posaconazole and voriconazole (0.5 μg/ml versus 0.06 and 0.25 μg/ml, respectively) but was the same as that of itraconazole; a similar pattern was evident for the *A. flavus* species complex. The isavuconazole mode for the *A. terreus* species complex was the same as those of itraconazole and posaconazole (0.25 μg/ml); a mode of 0.5 μg/ml has been reported for voriconazole. As for itraconazole, the isavuconazole mode for the *A. niger* species complex was higher than those for the other common species (1 μg/ml versus 0.25 to 0.5 μg/ml) as well as those for the *A. nidulans* species complex and the *A. versicolor* species complex (0.12 and 0.25 μg/ml, respectively). To our knowledge, aggregated MIC data from multiple laboratories have not been published for the *Aspergillus* section *Usti* group of species to allow modal MIC comparisons, but high triazole MICs have been reported for this group (33). Most isavuconazole modal MICs ±1 2-fold dilution comprised similar percentages of the populations for five of the seven species: 87.9% of *A. niger* species complex, 89.7% of *Aspergillus* section *Usti*, 90% of *A. fumigatus* species complex, 95.1% of *A. terreus* species complex, and 96.4% of *A. flavus* species complex isolates. Percentages were lower for *A. versicolor* species complex (81.3%) and *A. nidulans* species complex (72.6%) isolates. Since we have aggregated MIC data for our definition of isavuconazole ECVs from a wide geographical range, as in previous ECV studies (27–29), and little modal variation was observed among the laboratories contributing the MIC data for the majority of the species evaluated, we are confident in the validity of the MIC data in the present study.

The isavuconazole ECV for the *A. fumigatus* species complex, the *A. flavus* species complex, and the *A. terreus* species complex, encompassing ≥95% of the modeled MIC population, was 1 μg/ml; ECVs encompassing either ≥97.5% or ≥99% of the population were either the same or 1 dilution higher (Table 2). An ECV of 1 μg/ml has been reported for these three species versus itraconazole and voriconazole, while posaconazole ECVs have been consistently lower (0.12 to 0.5 μg/ml) using both CLSI and EUCAST methods as well as other statistical approaches (27, 34–36). Of the 855 isolates, 5.6% had MICs exceeding the estimated ECV of 1 μg/ml, and two of the eight laboratories that contributed data had modal MICs at the ECV. Although *cyp51* gene mutations have been found in isolates with isavuconazole MICs of >2 μg/ml (14, 17), there is no genetic information for isolates for which MICs are 2 μg/ml. Such information and more MIC data would corroborate our estimated ECV for this species complex. In the meantime, the estimated ECV of 1 μg/ml would avoid misclassification of non-WT isolates as WT isolates. The frequency of isavuconazole MICs of >1 μg/ml (non-WT isolates) for *A. fumigatus* species complex isolates was 5.6% (48 isolates) (Table 2), which represents a higher rate than previously described for this species with the other three triazoles (2.2 to 3.1%) (27). However, we have a lower sample size in the present study (855 versus >1,600 isolates). Among the 855 *A. fumigatus* species complex isolates, 15

TABLE 3 Cross-resistance between isavuconazole (MICs of ≥4 μg/ml) and three licensed triazoles among 26 *Aspergillus* isolates, as determined by the CLSI M38-A2 microdilution method at 48 h[a]

Species complex (no. of isolates)	Range (mode)[b] (μg/ml) with[d]:			
	Itraconazole	Posaconazole	Voriconazole	Isavuconazole
A. fumigatus (15)[c]	1–≥16 (≥16)	0.5–8 (2)	0.5–8 (8)	4–8 (8)
A. flavus (1)	1 (NA)	1 (NA)	1 (NA)	4 (NA)
A. niger (9)	1–16 (2, 8)	0.5–1 (1)	1–≥16 (2)	4–≥16 (4)
A. versicolor (1)	8 (NA)	1 (NA)	4 (NA)	>8 (NA)

[a] Isavuconazole MICs of ≥4 μg/ml were not reported for the other species evaluated. Itraconazole, posaconazole, and voriconazole ECVs for the *A. fumigatus* species complex and the *A. flavus* species complex were 1, 0.25, and 1 μg/ml, respectively; those for the *A. niger* species complex were 2, 0.5, and 2 μg/ml, respectively; and those for the *A. versicolor* species complex were 2, 1, and 2 μg/ml, respectively (27).
[b] Most frequent MIC.
[c] Twelve of the 15 isolates contained a TR_{34} promoter duplication and an L98H mutation in the *cyp51A* gene (14, 17).
[d] NA, not applicable, because modes were not determined for single isolates.

isolates recovered from Europe (10 isolates), India (3 isolates), Mexico (1 isolate), and the United States (1 isolate) had isavuconazole MICs of ≥4 μg/ml. Twelve of the 15 isolates contained *cyp51A* with a TR_{34} promoter duplication and an L98H mutation that correlated with phenotypic triazole cross-resistance (Table 3) (14, 17). Modal MICs for these isolates are above ECVs defined for the *A. fumigatus* species complex and the licensed triazoles (27, 34, 35). Correlation between triazole MICs above the ECV (≥4 μg/ml) in the *A. fumigatus* species complex, single or multiple point mutations, and patient failure on licensed triazole treatment has been reported (14–20).

To our knowledge, there is only one report of triazole ECVs for non-*A. fumigatus* species (27); our comparison of non-WT rates for these species are based on results from that study. There were 14 isavuconazole MICs above the ECV for non-WT isolates for the *A. flavus* species complex (MICs of >1 μg/ml; 3.2%) (Tables 1 and 2); percentages of non-WT isolates have been variable for this species and the licensed triazoles (posaconazole, 5.6%; voriconazole, 2%; itraconazole, 0.7%). There were only a few isavuconazole non-WT isolates for the *A. terreus* species complex (MIC of >1 μg/ml; 0.3%); these results reflect the rate of non-WT isolates found among itraconazole and posaconazole distributions for this species, while the rate of isavuconazole non-WT values was lower than that for voriconazole (0.3 and 3%, respectively) (27). Our isavuconazole ECV for the *A. niger* species complex (an *Aspergillus* section *Nigri* group member) was 2 dilutions higher (4 μg/ml) than those for the other three more common species (Table 2); both itraconazole and voriconazole ECVs for this species have been higher than that for posaconazole (2 and 0.5 μg/ml, respectively) (27). There were only two isavuconazole non-WT isolates of the *A. niger* species complex (MIC > 4 μg/ml) (Table 1); however, cross-resistance was observed with both itraconazole and voriconazole for one isolate (Table 3). In general, isavuconazole MIC_{90}s for *A. niger* species complex isolates have been consistently 1 dilution higher than those for other *Aspergillus* spp. (7, 10), and reduced susceptibility to licensed triazoles (most itraconazole MICs, ≥4 μg/ml; voriconazole MICs, 2 μg/ml; posaconazole MICs, 0.12 to 0.5 μg/ml) have been documented in Europe for *A. tubingensis* and *A. foetidus* (*Aspergillus* section *Nigri* group members) (37, 38). These high triazole MICs are a concern because the *A. niger* species complex is more frequently involved in pulmonary aspergillosis. Molecular resistance mechanisms have not been identified for the *A. niger* species complex, but azole resistance was correlated with the multiplication of *cyp51A* in an engineered laboratory strain of this species (38–40). Two *cyp51* genes were reported in *A. terreus* species complex isolates, and more recently, a Cyp51Ap M217I alteration was found in isolates with elevated EUCAST itraconazole MICs (1 to 2 μg/ml), but such MICs were also found in the absence of alterations; isavuconazole was not evaluated in that study (41). Although the clinical relevance of mold testing remains uncertain in the absence of breakpoints, both CLSI and EUCAST methodologies can identify emerging triazole resistance (MICs above the ECV).

The number of isolates for which isavuconazole MICs were available was substantially smaller for the *A. nidulans* species complex, the *A. versicolor* species complex, and *Aspergillus* section *Usti* (Tables 1 and 2), because these species are less frequently associated with disease. Therefore, the required total numbers of isolates and laboratories allowed the definition of only a tentative ECV of 1 μg/ml for the *A. versicolor* species complex, with 2.7% of the isolates being above this tentative cutoff; cross-resistance was observed for one isolate (Table 3). Although an isavuconazole ECV for the *Aspergillus* section *Usti* members could not be proposed because this distribution contained several species and the number of isolates is small, the MIC distribution and mode, as listed in Tables 1 and 2, are important for future studies, since little information is available in the literature regarding the patterns of susceptibility of this fungal group to antifungal agents (33, 42). While the distribution of *A. nidulans* species complex isolates fulfilled the criteria for ECV definition, we are proposing a tentative isavuconazole ECV of 0.25 μg/ml, because as mentioned above, its MIC distribution was skewed. Therefore, the percentage of MICs above the ECV (27.4%) could be erroneously high (Table 2). This tentative ECV may change when more laboratories and MICs become available. Mechanisms of resistance have not been documented for these less common species, despite the reported reduced susceptibility of *A. versicolor* species complex isolates to the three licensed triazoles (38). Amplification or overexpression of the *cyp51* gene in an *A. nidulans* species complex laboratory mutant has been correlated with triazole resistance (43), which suggested that mechanisms of resistance in the non-*A. fumigatus* species could be similar to those in the *A. fumigatus* species complex.

In conclusion, data originating from three to eight laboratories enable us to propose species-specific isavuconazole ECVs of 1 μg/ml for the *A. fumigatus* species complex, the *A. flavus* species complex, and the *A. terreus* species complex and 4 μg/ml for the *A. niger* species complex. In addition, we have proposed tentative isavuconazole ECVs for the *A. nidulans* species complex (0.25 μg/

ml) and the *A. versicolor* species complex (1 μg/ml) and have provided a susceptibility MIC distribution for *Aspergillus* section *Usti*. The availability of CLSI standard parameters for testing of *Aspergillus* with isavuconazole in addition to our ECVs would aid in monitoring emerging isavuconazole resistance in *Aspergillus* spp. as well as to distinguish non-WT from WT isolates.

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