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Multicenter Study of Isavuconazole MIC Distributions and Epidemiological Cutoff Values for the *Cryptococcus neoformans-Cryptococcus gattii* Species Complex Using the CLSI M27-A3 Broth Microdilution Method

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Epidemiological cutoff values (ECVs) of isavuconazole are not available for *Cryptococcus* spp. The isavuconazole ECVs based on wild-type (WT) MIC distributions for 438 *Cryptococcus neoformans* nongenotyped isolates, 870 isolates of genotype VNI, and 406 *Cryptococcus gattii* isolates from six laboratories and different geographical areas were 0.06, 0.12, and 0.25 μg/ml, respectively. These ECVs may aid in detecting non-WT isolates with reduced susceptibilities to isavuconazole.

Infections caused by members of the *Cryptococcus neoformans-Cryptococcus gattii* species complex are considered the second most common severe mycoses among immunocompromised and nonimmunocompromised hosts in certain regions. Although infections caused by *C. neoformans* var. *grubii* (serotype A) and, to a lesser degree, by *C. neoformans* var. *neoformans* (serotype D) are seen worldwide, those by *C. gattii* (serotypes B and C) are more geographically restricted. Irrespective of the species, cryptococcal disease is associated with high mortality rates (≥12.7%) (1–4). *C. neoformans* var. *grubii* comprises genotypes VNI, VNII/VNB, VNII (AFLP1, AFLP1A, AFLP1B), *C. neoformans* var. *neoformans* comprises genotypes VNIV (AFLP2) and VNIII (AFLP3), and *C. gattii* comprises genotypes VGI (AFLP4), VGII (AFLP6), VGIV (AFLP10), VGIII (AFLP5), and VGIV (AFLP7) (1, 5–7). Amphotericin B, its lipid formulations, and fluconazole are recommended as the primary alternative induction treatments and/or as salvage consolidation therapies for infections caused by *C. neoformans* and *C. gattii* (3, 8). Isavuconazole (BAL4815, codeveloped by Basilea Pharmaceutica International Ltd. [Basel, Switzerland] and Astellas Pharma [Tokyo, Japan]) is a new water-soluble triazole with favorable pharmacodynamic and pharmacokinetic parameters and is under clinical evaluation (phase III) for the treatment of invasive aspergillosis and other mycoses. Isavuconazole's mode of action is the inhibition of ergosterol biosynthesis (enzymes 14-α-sterol demethylases A and B, which are encoded by the *cyp51* [*ERG11*] gene), similar to that of the other azoles. The *in vitro* activity of isavuconazole is also similar to that of most licensed triazoles against *Aspergillus* spp., *Candida* spp., and *Cryptococcus* spp.; it also has activity comparable to that of posaconazole and, to a certain extent, itraconazole against some *Mucorales* species (9).

The Clinical and Laboratory Standards Institute (CLSI) has established standard conditions for testing the susceptibilities of *Candida* spp. and *Aspergillus* spp. to isavuconazole (10). In the absence of clinical breakpoints (CBPs) (the ultimate means of differentiating treatable and nontreatable isolates), epidemiological cutoff values (ECVs) have been defined for the more prevalent *Candida* spp. and the *C. neoformans-C. gattii* species complex versus those of the four licensed triazoles (11, 12). The ECV (also known as the ECOFF or CO_{WT}) is the highest wild-type (WT)-susceptible value; it is based on MIC distributions where there are two distinct populations, (i) the WT population of isolates/MICs with no detectable acquired or mutational resistance to the drug being evaluated and (ii) the non-WT population or isolates that harbor one or more resistance markers (13). The ECV based on pooled data from multiple laboratories serves as an early indication of emerging changes in the patterns of organism susceptibilities to the agent being evaluated or separates WT from non-WT isolates. Although the *in vitro* activity of isavuconazole against members of the *C. neoformans-C. gattii* species complex has been evaluated by CLSI M27-A3 methodology (14), ECVs based on data from multiple laboratories, as well as different geographical areas, have not been established for this complex. While data for the present study were submitted from a total of seven laboratories, the distribution data from one of the laboratories were abnormal (mode at the lower concentration tested) and were not included in the analysis. In addition, MICs were not submitted by each laboratory for each of the cryptococcal groups evaluated. Therefore, the purpose of this study was to define isavuconazole ECVs for three groups within the *C. neoformans-C. gattii* species complex for which MICs originated from three or four of the six collaborating laboratories.

Each isolate originated from a unique clinical specimen. The

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TABLE 1 Pooled MIC distributions and ECVs for the *Cryptococcus neoformans-Cryptococcus gattii* species complex and isavuconazole using the CLSI M27-A3-RPMI microdilution method[a]

Species	No. of isolates	No. of isolates with an MIC[b] (μg/ml) of:							MIC range (μg/ml)	Mode (μg/ml)[d]	ECV (μg/ml)[c]	
		0.008	0.016	0.03	0.06	0.12	0.25	0.5			95%	97.5%
C. neoformans VNI[e] (AFLP1)	870	68	270	274	213	37	7	1	0.008–0.5	0.03	0.12	0.12
C. neoformans (nongenotyped)	438	10	135	170	96	19	6	2	0.008–0.5	0.03	0.06	0.12
C. gattii[e]	406	23	78	106	87	87	25	7	0.008–0.5	0.03	0.25	0.25

[a] MICs were pooled from three or four of the six collaborating laboratories that submitted qualifying data for each cryptococcal group.
[b] MICs as determined by the CLSI broth microdilution method using standard RPMI 1640 broth.
[c] ECVs comprising ≥95% and ≥97.5% of the statistically modeled MIC population.
[d] Most frequent MIC.
[e] *C. gattii* comprises the VGI through VGIV (also known as AFLP4 through AFLP7 and AFLP10) genotypes.

CLSI broth microdilution isavuconazole MICs used for ECV definition were obtained from the VCU Medical Center (Richmond, VA), the Vallabhbhai Patel Chest Institute, University of Delhi (Delhi, India), Universidad Autónoma de Nuevo León (Monterrey, Nuevo León, Mexico), the Department of Medical Microbiology and Infectious Diseases, Canisius-Wilhelmina Hospital (Nijmegen, The Netherlands), Department of Medical Microbiology, Radboud University Medical Center (Nijmegen, The Netherlands), and the University of California Davis Medical Center (Davis, CA). Species, serotype, and molecular type identifications were performed at each medical center using standard methodologies (15–19). We have aggregated CLSI MICs for 870 *C. neoformans* (VNI molecular genotype), 438 *C. neoformans* nontyped, and 406 *C. gattii* isolates (comprising the 5 molecular genotypes). We also received a set of isavuconazole MICs for *C. gattii* environmental isolates that were not incorporated in the analysis. The MIC ranges for the control isolates (*Candida parapsilosis* ATCC 22019 and *Candida krusei* ATCC 6258) were within 3 to 4 dilutions.

Our MICs were determined using CLSI testing parameters (with standard RPMI 1640 broth [0.2% dextrose], final inoculum concentrations that ranged from 0.4×10^3 to 5×10^3 CFU/ml, and 72 h of incubation); the MICs were the lowest drug concentrations that produced ≥50% growth inhibition compared to that of the growth control (14). MIC distributions obtained in at least three of the seven coded laboratories for each group were listed in Excel spread sheets and aggregated for the ECV statistical calculation by a previously described method (13); the MICs that captured at least 95% and 97.5% of the modeled WT population (instead of the observed values) were obtained. ECVs are not estimated when the distribution is grossly skewed, which precludes statistical fitting, and a laboratory's data are excluded when the distribution from a single laboratory is truncated (mode at the lower or upper end of the distribution). At least 100 MICs from three independent laboratories are required for each group to establish an ECV. Because of that, we pooled the MICs for five molecular genotypes of *C. gattii* and used only the distributions for the *C. neoformans* VNI genotype; data for the other genotypes ranged from 13 to 172 MICs for VGI to VGIV genotypes and from 13 to 46 for VNII to VNIV genotypes, mostly inadequate sample sizes for ECV calculation. In addition, 1 of the 4 *C. neoformans* nontyped distributions received was truncated and was not pooled for the analysis.

The ECV is the highest MIC of the WT population, while the CBP is the MIC that predicts the clinical outcome of therapy (13, 20, 21). Therefore, the ECV detects the non-WT strains with reduced susceptibility (due to mutations) to the agent being evaluated. Aggregated isavuconazole MIC distributions for *C. neoformans* (genotype VNI), *C. neoformans* nontyped, and *C. gattii* (pooled genotypes) isolates are shown in Table 1. Each of the three pooled distributions had the same MIC range (0.008 to 0.5 μg/ml) and mode (0.03 μg/ml), and these values reflect an earlier report of isavuconazole data for *C. neoformans* (22). Comparable results have been observed with a larger number of isolates and the licensed triazoles for the three groups evaluated in the present study: similar posaconazole and voriconazole ranges and modes 1 or 2 dilutions higher (0.06 to 0.12 μg/ml) (12). Our isavuconazole ECVs comprising ≥95% of the modeled population were 0.06 μg/ml (*C. neoformans* nontyped), 0.12μg/ml (genotype VNI), and 0. 25 μg/ml (*C. gattii*); the ECVs that comprised ≥97.5% of the population were either the same or 1 dilution higher (Table 1). Recently established itraconazole, posaconazole, and voriconazole ECVs for the same groups were 1 dilution higher (0.25 to 1 μg/ml), with the highest ECVs for *C. gattii* (12). It is noteworthy that the isavuconazole mode for the environmental *C. gattii* isolates was 0.12 μg/ml. Preliminary clinical data regarding isavuconazole treatment of cryptococcal infections was recently reported (F. Queiroz-Telles, O. A. Cornely, J. Perfect, L. Kovanda, B. Zeiher, and J. Vazquez, presented at the ICCAC Congress, Washington, DC, 5 to 9 September, 2014). Nine patients with either central nervous system or disseminated cryptococcal disease were treated with isavuconazole (200 mg three times daily for 2 days followed by 200 mg every day [intravenous or oral]; VITAL phase III, open-label, multicenter trial for the efficacy and safety of isavuconazole). Of the eight patients evaluated on the 84th day, six were treatment successes (including all *C. gattii* infections), and two were treatment failures. CLSI isavuconazole MICs for the seven available infecting *C. neoformans* and *C. gattii* isolates were ≤0.12 μg/ml, which may be considered WT according to our proposed ECVs (Table 1). These results underline the potential values of ECV data in the clinical setting.

In conclusion, data originating in three laboratories for each of the three groups enabled us to propose species-specific isavuconazole ECVs of 0.06 to 0.25 μg/ml for the *C. neoformans-C. gattii* species complex evaluated. The availability of standard CLSI parameters for testing isavuconazole against these species and the ECVs calculated in the present study will aid in monitoring isavuconazole resistance in cryptococcal species and distinguishing non-WT from WT isolates.

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