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Comparison of the Broth Microdilution Methods of the European Committee on Antimicrobial Susceptibility Testing and the Clinical and Laboratory Standards Institute for Testing Itraconazole, Posaconazole, and Voriconazole against *Aspergillus* Isolates[▽]

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We compared EUCAST and CLSI antifungal susceptibility testing methods for itraconazole, posaconazole, and voriconazole by testing 245 *Aspergillus* clinical isolates. The essential agreement (EA) between methods was excellent: 100% (itraconazole), 98.4% (posaconazole), and 99.6% (voriconazole) assessing EA at ±2 dilutions and 99.6% (itraconazole), 87.7% (posaconazole), and 96.3% (voriconazole) at ±1 dilution.

The triazole antifungals include the mold-active agents itraconazole, posaconazole, and voriconazole (2). Each of these agents has good *in vitro* and *in vivo* activity against most species of *Aspergillus* (1, 9, 12, 16, 17, 22, 29). Although resistance (R) to triazoles is uncommon, increased R has been noted in several regions of the world since 1999 (11, 12, 23, 25, 27, 28). These observations suggest that triazole resistance among *Aspergillus* spp. may be more common than acknowledged and that clinical microbiology laboratories should determine the *in vitro* susceptibility of clinically relevant isolates of *Aspergillus* spp. (9, 11, 12, 17, 22, 28).

There are two independent standards for broth microdilution (BMD) antifungal susceptibility testing of triazole activity against *Aspergillus* species: the Clinical and Laboratory Standards Institute (CLSI) method (5) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) method (7, 13, 15, 24). The two methods are similar in that both use BMD, RPMI 1640 broth, incubation at 35 to 37°C for 48 h, and a complete (100%) inhibition visual MIC endpoint. They differ in their values for inoculum density (0.4 to 5×10^4 CFU/ml [CLSI] versus 2 to 5×10^5 CFU/ml [EUCAST]) and glucose content of the medium (0.2% [CLSI] and 2.0% [EUCAST]) and in the use of round-bottom (CLSI) versus flat-bottom (EUCAST) microdilution wells (15). Whereas numerous studies have shown that the two methods produce similar triazole (fluconazole, posaconazole, and voriconazole) MIC results when testing against *Candida* species (3, 6, 8, 20, 21), very few such comparisons exist for these methods as applied to *Aspergillus* spp. (4, 10). Gomez-Lopez et al. (10) demonstrated that itraconazole MICs obtained by the CLSI method were comparable to those obtained by the EUCAST method when applied to Spanish isolates of *Aspergillus* spp. More recently, Chryssanthou and Cuenca-Estrella (4) determined the susceptibilities to posaconazole and voriconazole of 40 clinical isolates of *Aspergillus* spp. by both the CLSI and EUCAST BMD methods. They found that, overall, the level of essential agreement (EA [defined as agreement within ±2 $\log_2$ dilutions]) was 92.5% and the intraclass correlation coefficient was >0.9.

In an effort to further pursue the harmonization of the CLSI and EUCAST BMD methods for testing the triazoles and *Aspergillus* spp., we have utilized our 2009 global antifungal surveillance database (18, 19) to determine the EA between the CLSI and EUCAST MICs for 245 clinical isolates of *Aspergillus* species tested against itraconazole, posaconazole, and voriconazole. This report represents the most extensive comparison of these two BMD methods for the testing of *Aspergillus* spp. to date. Given the important role that both methods currently play in antifungal resistance surveillance, it is important to demonstrate the comparability of the results (11, 14–17, 22, 28).

A total of 245 clinical isolates of *Aspergillus* spp. obtained from 20 medical centers worldwide during 2009 were tested against itraconazole, posaconazole, and voriconazole. The collection included 160 isolates of *A. fumigatus*, 32 of *A. flavus*, 40 of *A. niger*, and 13 of miscellaneous species, including 8 of *A. terreus*, 3 of *A. versicolor*, and 1 each of *A. nidulans* and *A. glaucus*. The isolates were obtained from a variety of sources, including sputum, bronchoscopy, and tissue biopsy specimens, and represented individual infectious episodes. The isolates were collected at individual study sites and sent to the University of Iowa (Iowa City) for identification and susceptibility testing as described previously (17, 18). All isolates were identified by standard microscopic morphology (26) and were stored as spore suspensions in sterile distilled water at room temperature. Before testing, each isolate was subcultured at least twice on potato dextrose agar (Remel, Lenexa, KS) to ensure viability and purity. As a screen for detection of cryptic species within the *A. fumigatus* complex (e.g., *A. lentulus*), all *A. fumigatus* isolates were tested for growth at 50°C. All isolates screened grew at 50°C, confirming that they were likely to be *A. fumigatus.*

All isolates were tested for *in vitro* susceptibility to itraconazole, posaconazole, and voriconazole by the use of the CLSI

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TABLE 1. *In vitro* susceptibilities of *Aspergillus* isolates to itraconazole, posaconazole, and voriconazole as determined by the CLSI and EUCAST broth microdilution methods

Species (no. tested)	Antifungal agent	Test method[a]	MIC (μg/ml)		% essential agreement[b]	
			Range	Mode	±1 dil	±2 dil
A. fumigatus (160)	Itraconazole	EUCAST	0.25 to >8	1	99.4	100.0
		CLSI	0.5 to >8	1		
	Posaconazole	EUCAST	0.06 to 1	0.5	90.0	97.5
		CLSI	0.25 to 2	0.5		
	Voriconazole	EUCAST	0.25 to 2	0.5	95.6	100.0
		CLSI	0.12 to 1	0.5		
A. flavus (32)	Itraconazole	EUCAST	0.25 to 2	0.5	100.0	100.0
		CLSI	0.5 to 1	1		
	Posaconazole	EUCAST	0.25 to 1	0.25	90.6	100.0
		CLSI	0.25 to 1	0.5		
	Voriconazole	EUCAST	0.5 to 2	1	96.9	100.0
		CLSI	0.25 to 1	0.5		
A. niger (40)	Itraconazole	EUCAST	0.25 to 4	2	100.0	100.0
		CLSI	0.5 to 8	2		
	Posaconazole	EUCAST	0.12 to 1	1	80.0	100.0
		CLSI	0.25 to 2	1		
	Voriconazole	EUCAST	0.25 to 2	1	100.0	100.0
		CLSI	0.25 to 2	0.5		
Total[c] (245)	Itraconazole	EUCAST	0.25 to >8	1	99.6	100.0
		CLSI	0.5 to >8	1		
	Posaconazole	EUCAST	0.06 to 4	0.5	87.7	98.4
		CLSI	0.25 to >8	0.5		
	Voriconazole	EUCAST	0.12 to 4	0.5	96.3	99.6
		CLSI	0.12 to 8	0.5		

[a] EUCAST, European Committee on Antimicrobial Susceptibility Testing; CLSI, Clinical and Laboratory Standards Institute.
[b] ±1 dil, % of results within plus/minus 1 $\log_2$ dilution of one another; ±2 dil, % of results within plus/minus 2 $\log_2$ dilutions of one another.
[c] In addition to listed species, the total number of isolates tested included *A. terreus* (8 isolates), *A. versicolor* (3 isolates), *A. nidulans* (1 isolate), and *A. glaucus* (1 isolate).

and EUCAST BMD methods. Reference powders of each agent were obtained from their respective manufacturers. Testing by personnel performing the *in vitro* susceptibility studies was conducted in a blinded manner with respect to the results of the CLSI method compared to those of the EUCAST method.

CLSI BMD testing was performed exactly as outlined in document M38-A2 (5) by using RPMI 1640 medium with 0.2% glucose, inocula of 0.4×10^4 to 5×10^4 CFU/ml, and incubation at 35°C for 48 h. MIC values were determined visually as the lowest concentration of drug that caused complete inhibition of growth (first clear well) relative to that of the growth control.

EUCAST BMD testing was performed exactly as detailed by EUCAST (24) by using RPMI 1640 medium with 2.0% glucose, flat-bottom microdilution trays, inocula of 2×10^5 to 5×10^5 CFU/ml, and incubation at 35°C. MIC values were determined visually, after 48 h of incubation, as the lowest concentration of drug that resulted in complete growth inhibition. Quality control was ensured by testing the following strains recommended by CLSI (5) and EUCAST (7): *C. parapsilosis* ATCC 22019, *C. krusei* ATCC 6258, and *A. flavus* ATCC 204304.

The MIC results for each triazole obtained with the EUCAST method were compared to those obtained with the CLSI BMD method. High off-scale MIC results were converted to the next highest concentration value, and low off-scale MIC results were left unchanged. Discrepancies of more than ±1 $\log_2$ dilutions and more than ±2 $\log_2$ dilutions among MIC results were used to calculate the EA.

Table 1 summarizes the *in vitro* susceptibilities of 245 isolates of *Aspergillus* spp. to itraconazole, posaconazole, and voriconazole as determined by both methods. The MIC results for each agent were typical of those for each species of *Aspergillus* (1, 9, 17, 19, 22, 28).

The overall EA determined as the percentage of results within ±1 $\log_2$ dilution ranged from 87.7% (posaconazole) to 99.6% (itraconazole) and improved to 98.4% (posaconazole) to 100.0% (itraconazole) when the more standard criterion of ±2 $\log_2$ dilutions was used (Table 1). Of the discrepancies (>±2 $\log_2$ dilutions) noted between the EUCAST and CLSI BMD results, the MIC values generated by the CLSI method were higher than those obtained by the EUCAST in 4 of 5 (80%) instances (4 of 4 with posaconazole and 0 of 1 with voriconazole). The largest number of discrepancies observed with the EUCAST and CLSI comparison occurred with *A. fumigatus* tested against posaconazole (4 discrepant results). Notably, 3 of the later discrepant results resulted in isolates of *A. fumigatus* being categorized as wild-type (WT) strains by EUCAST and as non-WT strains by CLSI according to the criteria published by Espinel-Ingroff et al. (9).

Regarding the individual species, the EAs between the EUCAST and CLSI BMD MIC results were ≥90% for all organism-drug combinations, with the exception of *A. niger* and

posaconazole (80.0%), by the use of the ± 1 $\log_2$ dilution criterion and were >97% for all comparisons by the use of the ± 2 $\log_2$ dilution criterion (Table). Among the 8 discrepancies for *A. niger* and posaconazole noted using the ± 1 $\log_2$ dilution criterion, only one resulted in what would be considered a very major discrepancy (WT by EUCAST and non-WT by CLSI). The remaining 7 discrepant results would still be categorized as WT by both methods.

These results confirm and extend those of Chryssanthou and Cuenca-Estrella (4), demonstrating that, as with *Candida* spp., susceptibility results obtained by the two methods are comparable when testing the triazoles against *Aspergillus* spp. As with those investigators, we found a higher level of intermethod agreement with itraconazole and voriconazole than with posaconazole. Both methods may be used with confidence for both clinical testing and in antifungal resistance surveillance. One limitation of this study is that, for most of the agents and species, the range of MICs is quite narrow, reflecting the fact that triazole resistance is uncommon in clinical isolates of *Aspergillus* from most geographic regions. Thus, this study does not address how the methods would compare with respect to the ability to detect isolates with elevated MICs. Further evaluation using a multicenter study design is warranted.

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