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, 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 × 10⁴ CFU/ml [CLSI] versus 2 to 5 × 10⁴ 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 (itraconazole, 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 log₂ 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...
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 $2 \times 10^5$ to $5 \times 10^5$ 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 $2 \times 10^5$ to $5 \times 10^5$ CFU/ml, and incubation at 35°C. MIC values were determined visually, after 48 h of incubation, as the lowest concentration of drug that caused 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 log₂ dilutions and more than ±2 log₂ 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).

The overall EA determined as the percentage of results within ±1 log₂ 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 log₂ dilutions was used (Table 1). Of the discrepancies (≥±2 log₂ 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 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).
posaconazole (80.0%), by the use of the ± 1 log₂ dilution criterion and were >97% for all comparisons by the use of the ± 2 log₂ dilution criterion (Table). Among the 8 discrepancies for *A. niger* and posaconazole noted using the ± 1 log₂ 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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