Germinated and Nongerminated Conidial Suspensions for Testing of Susceptibilities of *Aspergillus* spp. to Amphotericin B, Itraconazole, Posaconazole, Ravuconazole, and Voriconazole

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Received 14 August 2000/Returned for modification 5 October 2000/Accepted 20 November 2000

The role of the laboratory in the selection and monitoring of antifungal therapy has gained greater attention with the increased incidence of systemic fungal infections and the growing number of new antifungal agents. The National Committee for Clinical Laboratory Standards (NCCLS) has proposed standard conditions for molds (document M38-P) (7, 8, 19, 21). Although the pathogenic form of most opportunistic molds is the hyphae, document M38-P (19) describes the more convenient and standardized preparation of nongerminated conidial inoculum suspensions. Prior studies have compared MICs obtained by employing either germinated conidia or hyphal suspensions to those obtained with nongerminated conidia for dematiaceous fungi (13), *Aspergillus* spp., and other opportunistic monilaceous molds (2, 5, 13, 16–18, 22, 25). However, findings on the effect of hyphae on MIC determination (2, 5, 13, 17, 22, 25) have been more contradictory than those on the effect of germinated conidia (16, 18).

Although *Aspergillus fumigatus* is responsible for the majority (85 to 90%) of the different clinical manifestations of *Aspergillus* infections (4), other *Aspergillus* spp. also have been associated with severe infection in immunocompromised hosts (4, 21, 24, 25). The purpose of this study was to evaluate the effect of germinated and nongerminated conidia for dematiaceous fungi (13), *Aspergillus* spp., and other opportunistic monilaceous molds (2, 5, 13, 16–18, 22, 25). However, findings on the effect of hyphae on MIC determination (2, 5, 13, 17, 22, 25) have been more contradictory than those on the effect of germinated conidia (16, 18).

The in vitro fungicidal activities each agent were determined as previously described (9); the MFC was the lowest drug dilution that showed fewer than three colonies (99.9% killing). Overall, the MICs (0.12 to 4 μg/ml) and MFCs (0.5 to >8 μg/ml) of all of the agents tested with both inocula were the same or within 2 dilutions for the 72 isolates. Therefore, MICs and MFCs can be obtained with convenient and standardized nongerminated conidia.

The effect of germinated and nongerminated conidia of *Aspergillus* spp. on the fungistic (National Committee for Clinical Laboratory Standards document M38-P) and fungicidal activities (MICs and minimal fungicidal concentrations [MFCs] respectively) of amphotericin B, itraconazole, posaconazole (SCH56592), ravuconazole (BMS-207147), and voriconazole was evaluated. MFCs were the lowest drug dilutions that showed fewer than three colonies (99.9% killing). Overall, the MICs (0.12 to 4 μg/ml) and MFCs (0.5 to >8 μg/ml) of all of the agents tested with both inocula were the same or within 2 dilutions for the 72 isolates. Therefore, MICs and MFCs can be obtained with convenient and standardized nongerminated conidia.

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prepare, they have been traditionally employed for the antifungal susceptibility testing of molds. Because the measurement of conidial susceptibility could represent inhibition of conidial germination instead of hyphal growth by the antifungal agent, hyphae should be the fungal cells tested to evaluate antifungal activity.

The same or within a 2-dilution range (Table 1). In prior studies, germinated conidia had no effect, or no significant effect, on the MICs of itraconazole, amphotericin B (for 3 to 10 A. fumigatus and A. flavus strains) (16, 18), voriconazole, and posaconazole (for A. fumigatus) (18).

Organisms (no. of strains) and antifungal agent  | MIC (MIC<sub>90</sub>) [μg/ml] | MIC (MIC<sub>90</sub>) [μg/ml] |
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Aspergillus flavus (12) | Amphotericin B 0.2–2 (2) 0.5–2 (2) | Itraconazole 0.03–0.2 (0.2) 0.03–0.5 (0.2) |
| Voriconazole 0.12–0.5 (0.5) 0.12–0.5 (0.5) | Posaconazole 0.12–1.0 (0.5) 0.03–0.5 (0.5) |
| Ravuconazole 0.12–0.5 (0.5) 0.12–1.0 (1.0) | |
Aspergillus fumigatus (30) | Amphotericin B 0.2–2 (2) 0.5–2 (2) | Itraconazole 0.01–8 (0.5) 0.03–8 (0.2) |
| Voriconazole 0.12–1.0 (0.5) 0.06–1.0 (0.5) | Posaconazole 0.06–1.0 (0.12) 0.03–1.0 (0.2) |
| Ravuconazole 0.06–8 (0.5) 0.12–8 (1.0) | |
Aspergillus nidulans (10) | Amphotericin B 0.2–4 (1.0) 0.2–4 (1.0) | Itraconazole 0.01–0.5 (0.2) 0.01–0.5 (0.5) |
| Voriconazole 0.01–2 (0.2) 0.01–1.0 (0.2) | Posaconazole 0.03–0.5 (0.12) 0.06–0.2 (0.12) |
| Ravuconazole 0.03–2 (0.2) 0.06–2 (0.5) | |
Aspergillus sydowii (1) | Amphotericin B 0.12 (ND<sup>a</sup>) 0.12 (ND) | Itraconazole 0.2 (ND) 0.5 (ND) |
| Voriconazole 0.12 (ND) 0.12 (ND) | Posaconazole 0.5 (ND) 0.2 (ND) |
| Ravuconazole 0.12 (ND) 0.2 (ND) | |
A. terreus (12) | Amphotericin B 1.0–2 (2) 0.5–2 (4) | Itraconazole 0.01–0.5 (0.2) 0.03–0.5 (0.2) |
| Voriconazole 0.12–1.0 (1.0) 0.06–1.0 (1.0) | Posaconazole 0.03–0.5 (0.5) 0.01–0.5 (0.5) |
| Ravuconazole 0.2–1.0 (1.0) 0.12–1.0 (2) | |

The MFCs of the three new triazoles, amphotericin B, and itraconazole obtained with both types of conidia are listed in Table 2. Overall, both types of inocula also yielded similar fungicidal results. A prior study found no significant difference in the killing ability (killing curve experiments) of four antifungal agents against germinated and nongerminated conidia.

Organisms (no. of strains) and antifungal agent  | MFC<sup>a</sup> (MFC<sub>90</sub>) [μg/ml] | MFC<sup>a</sup> (MFC<sub>90</sub>) [μg/ml] |
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Aspergillus flavus (12) | Amphotericin B 0.5–2 (2) 0.5–2 (2) | Itraconazole 0.2–8 (1.0) 0.06–8 (0.5) |
| Voriconazole 0.12–8 (2) 0.2–8 (2) | Posaconazole 0.06–2 (1.0) 0.12–1.0 (1.0) |
| Ravuconazole 0.5–8 (>8) 0.2–8 (>8) | |
Aspergillus fumigatus (30) | Amphotericin B 0.2–8 (2) 0.5–8 (4) | Itraconazole 0.2–8 (8) 0.12–8 (8) |
| Voriconazole 0.06–8 (4) 0.06–8 (2) | Posaconazole 0.12–8 (>8) 0.06–8 (>8) |
| Ravuconazole 0.5–8 (>8) 0.2–8 (>8) | |
Aspergillus nidulans (10) | Amphotericin B 0.2–8 (1.0) 0.5–8 (1.0) | Itraconazole 0.2–8 (>8) 0.12–8 (>8) |
| Voriconazole 0.06–8 (0.5) 0.06–8 (0.5) | Posaconazole 0.03–2 (1.0) 0.06–2 (1.0) |
| Ravuconazole 0.06–2 (2) 0.06–2 (1.0) | |
Aspergillus sydowii (1) | Amphotericin B 0.5–2 (1.0) 1.0–2 (1.0) | Itraconazole 0.5–8 (2) 0.2–4 (0.5) |
| Voriconazole 0.5–2 (2) 0.2–2 (1.0) | Posaconazole 0.2–1.0 (0.5) 0.2–0.5 (0.5) |
| Ravuconazole 2–8 (>8) 2–8 (>8) | |
A. terreus (12) | Amphotericin B 1.0–4 (4) 1.0–8 (>8) | Itraconazole 0.06–8 (2) 0.12–8 (>8) |
| Voriconazole 1.0–8 (>8) 1.0–8 (>8) | Posaconazole 0.06–4 (2) 0.12–4 (2) |
| Ravuconazole 4–8 (>8) 4–8 (>8) | |

<sup>a</sup> Fewer than three colonies.
<sup>b</sup> ND, not determined.
of *A. fumigatus* (18). Although standard conditions are not available for determination of fungicidal activities against fungi, the fungicidal activities of voriconazole (3, 15, 23, 24), posaconazole (9, 20), and ravuconazole (10) against *Aspergillus* spp. have been evaluated. Although prior data have been obtained by nonstandardized MFC measurement procedures, the amphotericin B and voriconazole MFC90s for *A. terreus* were higher (≥8 μg/mL) than those for the other species tested (0.5 to 4 μg/mL) in this and other studies (20, 23). MFC ranges of the other agents similar to those listed in Table 2 have been published for *Aspergillus* spp. (3, 9, 10, 15, 20).

In conclusion, the data obtained in this and other studies indicate that MICs for isolates of *Aspergillus* spp. can be obtained by using a nongerminated conidial inoculum. Preparations of in vitro results with a hyphal inoculum. Aspergillus spp. can be obtained in this and other studies also suggest that interlaboratory evaluations are warranted to investigate the reliability and clinical usefulness of the determination of MFCs of both established and investigational agents for molds.

### REFERENCES


