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In Vitro Activities of Amphotericin B and Voriconazole against Aleurioconidia from *Aspergillus terreus*

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This study used aleurioconidia as inoculum and compared the MICs of amphotericin B and voriconazole to those obtained for conidia of 31 *Aspergillus terreus* strains. For conidia and aleurioconidia, the MIC at which 90% of strains were inhibited was 2.5 μg/ml and 5 μg/ml with amphotericin B and 1 μg/ml and 2 μg/ml with voriconazole.

Invasive aspergillosis is an important cause of morbidity and mortality in immunocompromised patients (2, 4, 12). *Aspergillus fumigatus* accounts for the majority of these cases (19). There have also been reports of infection due to *Aspergillus terreus*, which is often refractory to treatment with amphotericin B (6, 7, 11, 12, 13, 14, 17). For this reason, voriconazole (VRZ) is recommended for the treatment of aspergillosis; it shows a higher response rate in comparison to amphotericin B and is more active against *A. terreus* (3, 8, 16). Little is known about the pathogenesis and treatment of *A. terreus* infections. Whether its refractoriness to amphotericin B is caused by intrinsic resistance or to profound immunosuppression is not well understood. *A. terreus* is unique among aspergilli in producing lateral cells directly on hyphae. These aleurioconidia (also referred to as accessory conidia) are globose, significantly larger than the conidia produced in fruiting heads, usually solitary but occasionally occur in pairs, and are produced both in vitro and in vivo. The relevance of aleurioconidia in the clinical setting is not clear; we hypothesized that aleurioconidia might be the source of amphotericin B resistance. The present study used aleurioconidia as inoculum and compared the MICs of amphotericin B and VRZ to that for *A. terreus* conidia and investigated the susceptibility of *A. terreus* hyphae.

The MICs of amphotericin B (Squibb, Vienna, Austria) and VRZ (Pfizer, Sandwich, United Kingdom) for 33 clinical *A. terreus* isolates were determined as outlined in the NCCLS M38-A document (10) and as described elsewhere (5). Final concentrations of VRZ and amphotericin B were 0.03 μg/ml to 8 μg/ml and 0.07 to 10 μg/ml.

For aleurioconidia formation conidia were harvested (1 to 5 × 10^5 CFU/ml) in phosphate-buffered saline, and 1,500 μl was transferred to a tube containing 40 ml Sabouraud 2% dextrose broth (Merck, Vienna, Austria). These solutions were vortexed at 4,000 × *g* for 5 min and incubated at 28°C for 3 to 4 days to allow growth of hyphae and aleurioconidia. To avoid fungal sporulation, the tubes were centrifuged once a day at 4,000 × *g* for 5 min. Tubes containing aleurioconidia were vortexed and filtered through a sterile filter (size, 0.45 μm) to remove hyphal mats. Filtrates containing more than 98% aleurioconidia were counted and diluted in a hemocytometer to an inoculum of 5 × 10^4 CFU/ml. The MIC endpoint was the lowest drug concentration showing 80% reduction in growth for VRZ and no visible growth for amphotericin B. The broth microdilution assay for hyphae and the FUN 1 viability staining for selected experiments (Molecular Probes, Eugene, The Netherlands) were determined as described elsewhere (8). All tests were performed in duplicate and were repeated at least three times.

The MIC ranges and MICs at which 90% of strains were inhibited for amphotericin B and VRZ for conidia, aleurioconidia, and hyphae are given in Table 1.

The present study shows that for amphotericin B, aleurioconidia and conidia of *A. terreus* do not have significantly different MICs; for VRZ, the study showed consistently low MICs for both types of conidia. Twenty-six and 29 of 31 isolates were within twofold dilutions for amphotericin B and VRZ MICs.

Amphotericin B is considered standard first line therapy for the treatment of invasive aspergillosis in patients with neutropenia (4, 15). However, the survival rate for immunocompromised hosts with invasive aspergillosis caused by *A. terreus* is dismal, and a review of the literature documents that therapy with amphotericin B frequently fails to eradicate the organism (1, 2, 6, 11, 17). It is suggested that aleurioconidia produced in vivo possibly play an important role in this amphotericin B refractoriness of *A. terreus.* However, in our study the MICs of *A. terreus* conidia and aleurioconidia did not differ dramatically and were beyond safely achievable amphotericin B concentrations (1 μg/ml) (2). Also, the MICs for *A. terreus* aleurioconidia and conidia for amphotericin B were only slightly higher than those for hyphae. In general, hyphae of *Aspergillus* spp. are more resistant to antifungals than are conidia (8). Two isolates did not produce aleurioconidia and the reason for this is unknown; the role of these accessory conidia remains to be further investigated.

The phagocytic host response and capacity for conidial and hyphal damage appear to be similar for *A. terreus* and *A. fumigatus* (18). *A. terreus* resistance is more likely related to its intrinsic polyene resistance than to any differences in host response; depletion of ergosterol may be a contributory factor

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TABLE 1. MIC ranges for conidia, aleurioconidia, and hyphae with amphotericin B and VRZ at 48 h

A. terreus strain	AMB MIC range (μg/ml)[b]			VRZ MIC range (μg/ml)		
	Conidia	Aleurio-conidia	Hyphae	Conidia	Aleurio-conidia	Hyphae
T2	1.25–2.5	5	2.5–5	0.5	1–2	1
T3	2.5–5	2.5–5	2.5	0.5–1	2	1
T4	2.5–5	5	2.5	1	1	1
T5	2.5–5	5	1.25–2.5	0.5–1	1	1–2
T6	1.25–2.5	2.5–5	1.25	0.25–0.5	1	1
T7	1.25–2.5	5	1.25–2.5	0.5	1	1
T8	1.25–2.5	5	1.25–2.5	0.5	1	1
T9	1.25	2.5–5	1.25	0.5–1	1	1
T10	1.25	5	2.5	0.25–0.5	1	1–2
T11	2.5	5	2.5	0.5–1	1–2	1
T12	5	2.5–5	2.5	0.5–1	1	1
T13	1.25–2.5	5	1.25–2.5	0.5–1	1	1
T14	1.25–2.5	1.25–2.5	1.25	0.5–1	1	1
T16	1.25–2.5	5	1.25–2.5	0.5–1	1	1
T17	1.25–2.5	5	1.25–2.5	1	1–2	1
T18	1.25–2.5	2.5–5	1.25–2.5	1	1	1
T19	1.25	5	1.25	0.5–1	1	1–2
T20	1.25	5	1.25–5	0.5	1	1
T21	1.25–2.5	5	1.25–2.5	0.5	1	1
T22	2.5–5	5	2.5–5	1	1–2	1
T23	2.5–5	5	5	0.5	1	1
T24	2.5–5	2.5–5	5	1	1	1
T25	2.5–5	5	2.5–5	0.5–1	2	1
T26	1.25	5	2.5	0.5	2	1
T27	1.25–2.5	1.25–2.5	1.25–2.5	1	1	1
T28	1.25–2.5	5	1.25–2.5	0.5	1	1
T29	1.25–2.5	5	1.25–2.5	0.5–1	1–2	1
T30	1.25	2.5–5	2.5	0.25–0.5	1	1
T31	1.25–2.5	5	1.25–2.5	1	1	1
T32	1.25–2.5	5	1.25–2.5	1	1	1
MIC_{90}[a]	2.5	5	2.5	1	2	2

[a] Data were calculated as the MIC at which 90% (MIC_{90}) of the isolates were inhibited.
[b] AMB, amphotericin B.

(18). *A. terreus*, with the highest MIC and the minimum lethal concentration of amphotericin B, had the lowest membrane ergosterol content.

The data for VRZ appear to be encouraging, because our in vitro findings are consistent with in vivo findings showing that azoles on *A. terreus* have greater efficacy than amphotericin B (6). The MICs indicate activity against aleurioconidia and hyphae at levels achievable with standard dosing regimens of VRZ. Serum concentrations of 4.56 ± 0.68 μg/ml for VRZ were achieved in a rat model of invasive aspergillosis and delayed or prevented mortality (9).

In conclusion, our data confirm that amphotericin B MICs from *A. terreus* aleurioconidia do not differ dramatically in comparison to MICs obtained from conidia. VRZ is more active against aleurioconidia and hyphae of *A. terreus*. More studies are warranted to clarify the amphotericin B resistance of *A. terreus.*

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