In recent decades, fungal infections due to Aspergillus species have become a major cause of morbidity and mortality among immunocompromised patients (1–3). Aspergillus fumigatus is the most frequently isolated species, although there has been an increase in the incidence of other species, including Aspergillus flavus, A. niger, and A. terreus (1, 4, 5).

A. terreus is considered an emerging opportunistic fungus which can produce superficial to serious invasive infections (4–8). Invasive infections are often treated empirically with amphotericin B, a widely used broad-spectrum drug. However, most A. terreus isolates are resistant in vivo and in vitro to this drug (9–13).

Voriconazole has proved to be most effective, in vivo and in vitro, against this species (14–16), although some publications (9, 17–19) have already reported clinical isolates of A. terreus with higher MICs than the established epidemiologic cutoff values (ECVs) for itraconazole and voriconazole (20).

The aim of this study was to determine the antifungal susceptibility profile of clinical and environmental isolates of A. terreus for amphotericin, terbinafine, and triazole derivatives and monitor the possible emergence of strains with reduced antifungal triazole activity.

A total of 40 isolates of A. terreus complex—19 clinical and 21 environmental—were studied. Environmental isolates were obtained from outdoor and indoor hospital environments and from soils and trees in Resistencia (27°27′05″S, 58°59′12″W) and Corrientes (27°30′00″S, 58°48′00″W) (cities located in northeastern Argentina). Clinical isolates were obtained from skin and soft tissues samples, bronchoalveolar lavage samples, fingernails, and toenails.

All of isolates were identified as A. terreus complex according to general taxonomical keys (21–24).

For molecular identification, DNA extraction was performed according to the method described by Bosco Borgetat et al. (25) The partial sequence of the calmodulin (CalM) gene was amplified under conditions described by Peterson (26), using primers CF1F (5′ GCCGACTCTTTGAGYGARGAR) and CF4R (5′ TTTTTGTGAC TCA TRAGYTGGAC). PCR products were purified using Pure-Link quick PCR purification kit (Invitrogen, Germany) following the supplier’s protocol. PCR products were sent for sequencing to the Department of Ecology, Genetics and Evolution Sequencing and Genotyping Service, University of Buenos Aires, Buenos Aires, Argentina, and to the Division of Hygiene and Medical Microbiology Medical University of Innsbruck, Innsbruck, Austria.


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and environmental isolates was determined by the Student t test (unpaired, unequal variance). A P value of <0.05 was considered significant.

The MIC ranges, geometric means, modes, MIC₉₀ₛ and MIC₉₀ₙ obtained are summarized in Table 1.

* A. terreus* is a cosmopolitan fungus frequently isolated from indoor and outdoor environments in northeast Argentina (28). In addition, it is one of the more frequently opportunistic agents of onychomycosis isolated in these regions (22).

Clinical breakpoints have not been established for mold testing. However, epidemiologic cutoff values (ECVs) of amphotericin B, itraconazole, posaconazole, and voriconazole are available for five *Aspergillus* spp. (among them *A. terreus*). The ECV of amphotericin B for *A. terreus* was defined as 4 μg/ml, encompassing 97.5% of the modeled wild-type population (29), and the ECVs of itraconazole and voriconazole for *A. terreus* were defined as 1 μg/ml (20).

The use of voriconazole for the treatment of invasive aspergillosis caused by *A. terreus* improved clinical response of patients (15, 16, 29, 39). These *in vivo* results correlate with our *in vitro* data; all voriconazole MICs were lower than the ECV (20) for both isolates and environmental isolates was determined by the Student t test (unpaired, unequal variance). A P value of <0.05 was considered significant.

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Reports on susceptibility testing of terbinafine have increased since this antifungal has shown a high activity *in vitro* against a broad spectrum of pathogenic fungi (34). This drug showed potent *in vitro* activity against all isolates of *A. terreus* tested, with MICs lower than triazole derivatives. These data are consistent with values published by Moore and Walls, who reported a MIC₉₀ of 0.25 μg/ml with a range of 0.125 to 1 μg/ml (11). Garcia-Effron et al. reported higher values (MIC₉₀, 1 μg/ml; range, 0.03 to 4 μg/ml) (34), although these differences may be due to the different methods used.

Most investigations show that *A. terreus* has intrinsic resistance to amphotericin B, with elevated MICs (4, 9, 14, 32, 34, 35). In our study, 95% (38/40) of all isolates exhibited amphotericin B MICs of ≥4 μg/ml. Only two isolates (one clinical and one environmental) showed amphotericin B MICs of 8 μg/ml, above the proposed ECV (29). On the other hand, some studies have reported strains with low MICs (<1 μg/ml) for this drug (18, 30, 31, 36). Only 4/19 clinical isolates and 8/21 environmental isolates showed amphotericin B MICs of 1 μg/ml in our study. These findings suggest that there may be *A. terreus sensu stricto* strains that are susceptible to amphotericin B, but more research is needed to know if these isolates represent variants with susceptibility to amphotericin B.

No statistically significant differences between the susceptibility data obtained for clinical and environmental isolates were observed, as reported by other authors (30, 37), although Araujo et al. (38) found environmental isolates with significantly higher MICs than clinical isolates for amphotericin B.

Antifungal susceptibility testing is essential in patient management and surveillance of resistance. Little is known about the susceptibility profile of *A. terreus* worldwide. The present study is a contribution to the knowledge of the susceptibility of this opportunistic fungus and shows that *A. terreus sensu stricto* isolates obtained in this region have low MICs for itraconazole, voriconazole, and terbinafine and exhibit high amphotericin B MICs.

**Nucleotide sequence accession numbers.** Sequences of the CalM genes of the 40 *A. terreus* isolates have been submitted to the European Nucleotide Archive (ENA) and assigned the accession numbers LN734824 to LN734863 (http://www.ebi.ac.uk/ena/data/view/LN734824-LN734863).

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We have no conflict of interest to declare.

**REFERENCES**


**TABLE 1 MICs of amphotericin B, terbinafine, andazole drugs obtained by broth microdilution for 40 Aspergillus terreus sensu stricto isolates**

<table>
<thead>
<tr>
<th>Isolate type (no. tested)</th>
<th>Drug a</th>
<th>MIC (μg/ml)</th>
<th>Mode</th>
<th>MIC₉₀</th>
<th>MIC₉₀</th>
<th>% ≤ ECV b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Range</td>
<td>GM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clinical (19)</td>
<td>AMB</td>
<td>1–8</td>
<td>2.17</td>
<td>2</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>VRC</td>
<td>0.125–0.5</td>
<td>0.30</td>
<td>0.25</td>
<td>0.25</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>ITC</td>
<td>≤0.03–0.5</td>
<td>0.26</td>
<td>0.5</td>
<td>0.125</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>TER</td>
<td>≤0.03–0.25</td>
<td>0.14</td>
<td>0.25</td>
<td>0.125</td>
<td>0.25</td>
</tr>
<tr>
<td>Environmental (21)</td>
<td>AMB</td>
<td>1–8</td>
<td>2.24</td>
<td>2</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>VRC</td>
<td>0.125–0.5</td>
<td>0.41</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>ITC</td>
<td>≤0.03–0.5</td>
<td>0.21</td>
<td>0.25</td>
<td>0.25</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>TER</td>
<td>≤0.03–0.25</td>
<td>0.09</td>
<td>0.125</td>
<td>0.06</td>
<td>0.125</td>
</tr>
</tbody>
</table>

a AMB, amphotericin B; VRC, voriconazole; ITC, itraconazole; TER, terbinafine.

b Percentage of MICs less than or equal to than the ECV (ECV = 1 μg/ml for itraconazole and voriconazole and 4 μg/ml for amphotericin B). ND, not determined (no ECVs were available for TER).

c GM, geometric mean.
