Antifungal Susceptibilities of *Sporothrix albicans*, *S. brasiliensis*, and *S. luriei* of the *S. schenckii* Complex Identified in Brazil

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Received 4 February 2011/Returned for modification 25 April 2011/Accepted 25 May 2011

We studied 40 strains of the species complex formerly classified as the single species *Sporothrix schenckii* to identify new species within this complex and evaluate their antifungal susceptibility profiles. Based on phenotypic tests (ability to grow at 37°C, colony diameters, and pigmentation of the colonies, as well as assimilation of sucrose and raffinose) and molecular assays (amplification of a fragment of the calmodulin gene), here we report the identification of *S. albicans*, *S. brasiliensis*, *S. luriei*, and *S. schenckii*; two isolates of these species were detected as itraconazole-resistant strains.

Sporotrichosis is a subcutaneous mycosis affecting humans and animals caused by *Sporothrix schenckii*. It has a worldwide distribution, especially in tropical and subtropical areas of Latin America, where areas of endemicity have been recognized (1, 3, 4, 12). Recently, Marimon et al. (9, 11), proposed that *Sporothrix schenckii* is a complex encompassing six cryptic species that had been previously identified by others (4). In this context, variation in the antifungal susceptibility profiles among these new species was hypothesized. The aim of this study was to explore a collection of 40 isolates formerly classified as *Sporothrix schenckii* in order to identify new species and evaluate their susceptibility to antifungal agents.

The isolates were from cases of human (*n = 31*) and animal (*n = 9*) sporotrichosis diagnosed in the hinterlands of Rio Grande do Sul (Brazil) and were maintained in the Department of Microbiology of the Universidade Federal de Santa Maria (UFSM), Santa Maria, Brazil. Among the human-derived strains, 18 (58.06%) were from fixed cutaneous sporotrichosis, 13 (41.9%) were from the lymphocutaneous form of the mycosis. Of the strains isolated from animals (*n = 9*), eight were from cats and one (*S. luriei*) was isolated from a dog with sporotrichosis. As proposed by Marimon et al. (9, 11), the phenotypic tests included the ability/ inability to grow at 37°C on potato dextrose agar (PDA; HiMedia, Mumbay, India), different colony diameters (mm) after 21 days of incubation at 30°C on PDA, the pigmentation of the colonies on cornmeal agar (CMA), and the assimilation of sucrose and raffinose. The susceptibility tests were conducted according the procedures proposed for the M38-A2 technique (2). For molecular identification, a fragment of the calmodulin (CAL) gene was amplified from genomic DNA using the degenerate primers CL1 [5′-GA(GA)T(AT)CAAAGGAGGCCTTCTC-3′] and CL2A [5′-TTTTTGACATGACTTGGAC-3′] (9). DNA sequencing was performed on the purified amplicons using a MegaBace 500 automatic sequencer. The sequences were aligned against sequences available in GenBank with ClustalX (version 1.8) followed by manual adjustments with a text editor. The phylogenetic analysis was performed with MEGA (Molecular Evolutionary Genetic Analysis) software version 4.0 (17).

Based on recently proposed procedures (9, 11), we phenotypically identified four species: *Sporothrix schenckii* (*n = 37*), *Sporothrix brasiliensis* (*n = 1*), *Sporothrix luriei* (*n = 1*), and *Sporothrix albicans* (*n = 1*). The phenotypic identification was easy to perform and was concordant with the molecular findings. The molecular sequencing confirmed 37 isolates as *Sporothrix schenckii*. The GenBank search also revealed three new species: (i) strain PG1 (*Sporothrix brasiliensis*) (GenBank accession no. HQ404315), (ii) strain PG2 (*Sporothrix schenckii* var. *luriei*) (GenBank accession no. HQ404316), and (iii) strain PG3 (*Sporothrix albicans*) (GenBank accession no. HQ404317), all showing high bootstrap support values (Fig. 1) and corresponding with the species identified in the phenotypic tests. Based on studies by Marimon et al. (9) and reinforced by Romeo et al. (13), only the sequencing of highly informative genetic loci such as the calmodulin-encoding gene is, at present, useful for elucidating relationships and differentiating among cryptic species of the *S. schenckii* complex. The new species, *S. brasiliensis*, *S. luriei*, and *S. albicans*, were isolated from feline, canine, and feline sporotrichosis, respectively. To date, *S. brasiliensis* has been associated only with human sporotrichosis (9). Table 1 describes the susceptibility parameters, highlighting terbinafine as the most active drug. By restricting the comparisons to studies employing the same susceptibility tests, our results are in accordance with those of Marimon et al. (10) and Silveira et al. (15). Terbinafine, ketoconazole, and amphoterine B showed the best activities, while fluconazole (MIC range, 32 to 128 μg/ml) and caspofungin (MIC range, 8 to 32 μg/ml) were less active. The results of our tests with itraconazole against *S. shroneckii*. Of the strains isolated from animals (*n = 9*), eight were from cats and one (*S. luriei*) was isolated from a dog with sporotrichosis. As proposed by Marimon et al. (9, 11), the phenotypic tests included the ability/ inability to grow at 37°C on potato dextrose agar (PDA; HiMedia, Mumbay, India), different colony diameters (mm) after 21 days of incubation at 30°C on PDA, the pigmentation of the colonies on cornmeal agar (CMA), and the assimilation of sucrose and raffinose. The susceptibility tests were conducted according the procedures proposed for the M38-A2 technique (2). For molecular identification, a fragment of the calmodulin (CAL) gene was amplified from genomic DNA using the degenerate primers CL1 [5′-GA(GA)T(AT)CAAAGGAGGCCTTCTC-3′] and CL2A [5′-TTTTTGACATGACTTGGAC-3′] (9). DNA sequencing was performed on the purified amplicons using a MegaBace 500 automatic sequencer. The sequences were aligned against sequences available in GenBank with ClustalX (version 1.8) followed by manual adjustments with a text editor. The phylogenetic analysis was performed with MEGA (Molecular Evolutionary Genetic Analysis) software version 4.0 (17).

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brasiliensis and S. albicans agreed with the results reported by Marimon et al. (10), but in general, our S. schenckii strains were more susceptible. S. schenckii has been reported to show a high MIC to itraconazole by several authors (6, 7, 10). Although breakpoints have not been established for S. schenckii, document M38-A3 (2) suggests that, for analytical purposes, a MIC of \( \geq 4.0 \) g/ml for itraconazole may be considered resistant for some filamentous fungi. In keeping with this finding, the itraconazole-resistant strains (S. albicans and S. luriei) showed cross-resistance with all other azoles. Kohler et al. (7) and Meinerz et al. (12), prior to the studies of Marimon et al. (9), reported that isolates from animals were more resistant to itraconazole than isolates from humans. This observation was supported by our results because, among 9 strains from animals, 2 showed itraconazole resistance, and among 31 strains from human cases of sporotrichosis, none showed itraconazole resistance. In addition, our results indicated the presence of the greater proportion of itraconazole-resistant species in animal sporotrichosis (2/9) than in human sporotrichosis (0/31). Although the Mann-Whitney test did not show differences between the two groups, the geometric mean showed that in general S. schenckii animal-derived isolates were more sensitive to azoles than human-derived isolates. The MIC values for am-

TABLE 1. Parameters of susceptibilities of the new Sporothrix species to antifungal agents

<table>
<thead>
<tr>
<th>Antifungal agent</th>
<th>Humans (S. schenckii; n = 31)</th>
<th>Animals (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC (µg/ml) for isolates from</td>
<td></td>
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<tr>
<td></td>
<td>Range 50% 90% GM</td>
<td>Range 50% 90% GM</td>
</tr>
<tr>
<td>ITZ</td>
<td>0.06–1</td>
<td>0.25</td>
</tr>
<tr>
<td>KTZ</td>
<td>0.125–2</td>
<td>0.5</td>
</tr>
<tr>
<td>MCZ</td>
<td>0.5–4</td>
<td>1</td>
</tr>
<tr>
<td>VCZ</td>
<td>2–16</td>
<td>8</td>
</tr>
<tr>
<td>FLZ</td>
<td>16–128</td>
<td>128</td>
</tr>
<tr>
<td>TRB</td>
<td>0.03–0.25</td>
<td>0.125</td>
</tr>
<tr>
<td>AMB</td>
<td>0.03–2.0</td>
<td>0.25</td>
</tr>
<tr>
<td>CAS</td>
<td>8–32</td>
<td>32</td>
</tr>
</tbody>
</table>

a ITZ, itraconazole; KTZ, ketoconazole; MCZ, miconazole; VCZ, voriconazole; FLZ, fluconazole; TRB, terbinafine; AMB, amphotericin B; CAS, caspofungin.

b 50% and 90%, MIC<sub>50</sub> and MIC<sub>90</sub>, respectively; GM, geometric mean.
photericin B and terbinafine were similar for both groups of strains. Due to the low number of animal isolates included here, these observations require further studies.

Finally, our findings emphasize two main points. (i) *S. luriei* had a remarkable azole resistance, as reported here for the first time. (ii) The recent studies focusing on the susceptibility of the former species *S. schenckii* (8, 15) or the new *Sporothrix* species (9, 10) included strains from different countries. However, here we included only strains isolated in the central region of Rio Grande do Sul State. Even in this limited area, we found a varied susceptibility profile to antifungal agents and detected four of the six new *Sporothrix* species. Therefore, our findings reinforce the importance of identifying *Sporothrix* isolates as proposed by Marimon et al. (9) and of evaluating their susceptibility patterns to better determine the best therapeutic option for each case of sporotrichosis.

We report that we have no conflicts of interest.

REFERENCES


