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In Vitro Antifungal Susceptibility of Cladophialophora carrionii, an Agent of Human Chromoblastomycosis

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A global collection of Cladophialophora carrionii strains (n = 81) was tested against nine antifungal drugs. MIC90 values of all strains were as follows in increasing order: itraconazole and posaconazole, 0.063 μg/ml; terbinafine, 0.125 μg/ml; isavuconazole and voriconazole, 0.25 μg/ml; caspofungin, 2 μg/ml; micosafungin, 4 μg/ml; amphotericin B, 8 μg/ml; and fluconazole, 64 μg/ml.

Chromoblastomycosis is a chronic, progressive, polymorphic implantation mycosis. Lesions are limited to cutaneous and subcutaneous tissues, causing hyperproliferation leading to verrucous or nodular clinical features (1–3). Two genera of melanized hyphomycetes, Cladophialophora and Fonsecaea, both belonging to the family Herpotrichiellaceae in the order Chaetothyriales, are common causes. They have in common that a pathogenic invasive phase is formed in skin with the expression of mucin-like cells. Occasional cases have been reported due to species of <i>Phialophora</i>, <i>Exophiala</i>, and <i>Rhinocladiella</i>, which also belong to this family (4). The disease is encountered worldwide in subtropical and tropical climate zones, with a clear distinction between the vicarious species of <i>Cladophialophora</i> in arid climates and <i>Fonsecaea</i> and <i>Rhinocladiella</i> in humid, tropical climates (5).

<i>Cladophialophora carrionii</i> is a relatively frequent etiologic agent of chromoblastomycosis in arid and semiarid climate zones of South and Central America (6, 7), Australia (8), and Asia (9, 10). The infection is very difficult to treat. Several therapies have been applied, but there is no standard for treatment (3). Small series of <i>in vitro</i> susceptibility studies with itraconazole, voriconazole, and terbinafine have been published showing considerable variation between and within genera and species (11, 12).

The aim of the present study was to determine the susceptibility profiles of a large collection of <i>C. carrionii</i> strains to nine antifungal agents, including isavuconazole (13). Isolates were taken from the reference collections of the CBS-KNAW Fungal Biodiversity Centre (CBS, Utrecht, The Netherlands) or the Institute Pasteur (CNRMA/IP, Paris, France). The set comprised isolates from Venezuela (n = 46), China (n = 20), Madagascar (n = 9), and Australia (n = 6). Seventy-five clinical isolates originated from patients with chromoblastomycosis, and six environmental isolates were from dry plant debris in Venezuela (Table 1). All strains were identified to the species level by sequencing of the internal transcribed spacer of the ribosomal DNA (rDNA) region and partial translation of the elongation factor 1-α and β-tubulin genes (S. Deng, A. H. G. Gerrits van den Ende, L. Yang, H. Badali, M. J. Najafzadeh, R. Y. Li, C. H. Klaassen, F. Hagen, J. F. Meis, B. Papierok, J. Sun, W. D. Liu, G. S. De Hoog, submitted for publication). <i>In vitro</i> activities of nine antifungal agents were determined with the reference guideline M38-A2 (14). Three reference strains, <i>Paecilomyces variotii</i> (ATCC 22319), <i>Candida parapsilosis</i> (ATCC 22019), and <i>Candida krusei</i> (ATCC 6258) were included as quality controls. Kruskal-Wallis and Mann-Whitney U tests were used for comparison of the MICs of all antifungal agents among strains from four groups (Latin America, Asia, Africa, and Australia).

Table 2 summarizes the MIC results in terms of the MIC ranges, geometric mean (GM) MIC, and MIC90 and MIC90 values of nine antifungal agents for 81 <i>C. carrionii</i> strains. All strains had low MICs of itraconazole, voriconazole, posaconazole, isavuconazole, and terbinafine, while the highest MICs were consistently found with fluconazole, amphotericin B, micafungin, and caspofungin. The MIC90 of fluconazole, amphotericin B, micafungin, and caspofungin were 64 μg/ml, 8 μg/ml, 4 μg/ml, and 2 μg/ml, respectively. These data are in agreement with previously reported findings for <i>Cladophialophora</i> (11, 15), <i>Rhinocladiella</i> (16), and <i>Fonsecaea</i> (17). No difference was found in the activities between voriconazole and isavuconazole against <i>C. carrionii</i> (MIC range, 0.016 to 1 μg/ml; GM, 0.148/0.136 μg/ml; MIC90, 0.25 μg/ml). The MIC range and MIC90 of voriconazole were 2 log2-dilution steps more active than values found in <i>C. bantiana</i> (range, 0.125 to 4 μg/ml; MIC90, 2 μg/ml) (15) and in <i>Phialophora</i> and <i>Cyphellophora</i> (MIC range, 0.125 to 4 μg/ml; MIC90, 1 μg/ml) (18). Table 3 shows rare <i>Cladophialophora</i> species causing (sub)cutaneous disorders but which are related to <i>Fonsecaea</i> (19) and to <i>C. yegeesi</i>, an environmental sibling of <i>C. carrionii</i>. The values were in the same range, with the exception of lower MICs of caspofungin and micafungin in the cutaneous species <i>C. immunda</i> and <i>C. saturnica</i> and of voriconazole in <i>C. yegeesi</i> and <i>C. samoensis</i>.

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TABLE 1 Cladophialophora strains used in this study

<table>
<thead>
<tr>
<th>Species</th>
<th>Accession no.</th>
<th>Source</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cladophialophora</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>yegresii</td>
<td>CBS 114403</td>
<td>Plant, Cactaceae</td>
<td>Venezuela</td>
</tr>
<tr>
<td></td>
<td>CBS 114404</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CBS 114405</td>
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<td></td>
<td>CBS 114406</td>
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<tr>
<td></td>
<td>CBS 114407</td>
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</tr>
</tbody>
</table>

TABLE 2 MIC values of nine antifungal agents against 81 C. carrionii strains

<table>
<thead>
<tr>
<th>Strain (n) and drug</th>
<th>GM ± Range (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flucytosine</td>
<td>0.016 – 0.5</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>0.016 – 0.5</td>
</tr>
<tr>
<td>Itraconazole</td>
<td>0.016 – 0.5</td>
</tr>
<tr>
<td>Voriconazole</td>
<td>0.016 – 0.5</td>
</tr>
<tr>
<td>Isavuconazole</td>
<td>0.016 – 0.5</td>
</tr>
<tr>
<td>Caspofungin</td>
<td>0.008 – 0.25</td>
</tr>
<tr>
<td>Miconazole</td>
<td>0.008 – 0.25</td>
</tr>
<tr>
<td>Terbinafine</td>
<td>0.008 – 0.25</td>
</tr>
</tbody>
</table>

The activities of itraconazole and posaconazole against C. carrionii were comparable (Table 2) and similar to those of C. bantiana and of Fonsecaea species (15, 17). Phialophora and Cyphellophora (18) had responses to posaconazole (MIC<sub>90</sub> 0.063 µg/ml) similar to those found in C. carrionii, but the itraconazole value was different (MIC<sub>90</sub> 0.5 µg/ml). Terbinafine varied considerably in its activity against strains of C. carrionii (MIC range of 0.008 to 1 µg/ml). MIC ranges and MIC<sub>90</sub>s of posaconazole, isavuconazole, voriconazole, and terbinafine showed potent activity against C. carrionii (Table 2). Posaconazole was the drug with the best overall in vitro activity. The latter also holds true in an animal model of C. carrionii infection (20).

For miconafungin, most C. carrionii isolates from Venezuela had low MICs. The range was 0.016 to 8 µg/ml, the GM was 0.26 µg/ml, and the MIC<sub>90</sub> was 0.5 µg/ml. Some strains deviated sig-
nificantly (Table 2), and all nine strains from Madagascar had 3 \log_{10}\text{-dilution-step}-higher MICs than the majority of Venezuelan strains (range, 0.125 to 8 \mu g/mL; GM, 1.47 \mu g/mL; MIC\(_{50}\) 4 \mu g/mL) (P < 0.01). The activities against Chinese and Australian strains were intermediate. For amphotericin B, the MIC range (0.5 to 8 \mu g/mL) and MIC\(_{90}\) (0.5 \mu g/mL) were intermediate. For amphotericin B, the MIC range (0.5 to 8 \mu g/mL) and MIC\(_{90}\) (0.5 \mu g/mL) were intermediate. For amphotericin B, the MIC range (0.5 to 8 \mu g/mL) and MIC\(_{90}\) (0.5 \mu g/mL) were intermediate.

**REFERENCES**


