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


A global collection of Cladophialophora carrionii strains \( (n = 81) \) was tested against nine antifungal drugs. MIC\textsubscript{90}s of all strains were as follows in increasing order: itraconazole and posaconazole, 0.063 \( \mu \)g/ml; terbinafine, 0.125 \( \mu \)g/ml; isavuconazole and voriconazole, 0.25 \( \mu \)g/ml; caspofungin, 2 \( \mu \)g/ml; micafungin, 4 \( \mu \)g/ml; amphotericin B, 8 \( \mu \)g/ml; and fluconazole, 64 \( \mu \)g/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 muriform cells. Occasional cases have been reported due to species of Phialophora, Exophiala, and Rhinocladiella, 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 Cladophialophora in arid climates and Fonsecaea and Rhinocladiella in humid, tropical climates \((5)\).

Cladophialophora carrionii 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 in vitro 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 C. carrionii 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 (CNRM/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). In vitro activities of nine antifungal agents were determined with the reference guideline M38-A2 \((14)\). Three reference strains, Paecilomyces variotii (ATCC 22319), Candida parapsilosis (ATCC 22019), and Candida krusei (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 MIC\textsubscript{90} and MIC\textsubscript{95} values of nine antifungal agents for 81 C. carrionii 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 MIC\textsubscript{90}s of fluconazole, amphotericin B, micafungin, and caspofungin were 64 \( \mu \)g/ml, 8 \( \mu \)g/ml, 4 \( \mu \)g/ml, and 2 \( \mu \)g/ml, respectively. These data are in agreement with previously reported findings for Cladophialophora \((11, 15)\), Rhinocladiella \((16)\), and Fonsecaea \((17)\). No difference was found in the activities between voriconazole and isavuconazole against C. carrionii (MIC range, 0.016 to 1 \( \mu \)g/ml; GM, 0.148/0.136 \( \mu \)g/ml; MIC\textsubscript{90}, 0.25 \( \mu \)g/ml). The MIC range and MIC\textsubscript{90} of voriconazole were 2 log₂-dilution steps more active than values found in C. bantiana (range, 0.125 to 4 \( \mu \)g/ml;MIC\textsubscript{90}, 2 \( \mu \)g/ml) \((15)\) and in Phialophora and Cyphello- phora (MIC range, 0.125 to 4 \( \mu \)g/ml; MIC\textsubscript{90}, 1 \( \mu \)g/ml) \((18)\). Table 3 shows rare Cladophialophora species causing (sub)cutaneous disorders but which are related to Fonsecaea \((19)\) and to C. yegeesi, an environmental sibling of C. carrionii. The values were in the same range, with the exception of lower MICs of caspofungin and micafungin in the cutaneous species C. immunda and C. saturnica and of voriconazole in C. yegeesi and C. samoensis.
A table and a paragraph extracted from the document:

**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 yegresii</td>
<td>CBS 114405</td>
<td>Plant, Cactaceae</td>
<td>Venezuela</td>
</tr>
<tr>
<td></td>
<td>CBS 114406</td>
<td>Plant, Cactaceae</td>
<td>Venezuela</td>
</tr>
<tr>
<td></td>
<td>CBS 114407</td>
<td>Plant, Cactaceae</td>
<td>Venezuela</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Strain (n) and drug</th>
<th>GM</th>
<th>Range</th>
<th>50%</th>
<th>90%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>All Cl. carrionii strains (81)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>2.643</td>
<td>0.5–8</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>25.04</td>
<td>4–64</td>
<td>32</td>
<td>64</td>
</tr>
<tr>
<td>Itraconazole</td>
<td>0.03</td>
<td>0.008–0.125</td>
<td>0.031</td>
<td>0.063</td>
</tr>
<tr>
<td>Voriconazole</td>
<td>0.016–0.063</td>
<td>0.125</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td>Posaconazole</td>
<td>0.025</td>
<td>0.016–0.063</td>
<td>0.063</td>
<td>0.25</td>
</tr>
<tr>
<td>Isavuconazole</td>
<td>0.136</td>
<td>0.016–1</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td>Caspofungin</td>
<td>1.367</td>
<td>0.25–4</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Micafungin</td>
<td>0.296</td>
<td>0.016–8</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td>Terbinafine</td>
<td>0.049</td>
<td>0.008–1</td>
<td>0.125</td>
<td></td>
</tr>
</tbody>
</table>

**C. carrionii, Venezuela (46)**

- Amphotericin B: 2.767, 0.5–8, 2, 8
- Fluconazole: 31.07, 8–64, 32, 64
- Itraconazole: 0.038, 0.016–0.125, 0.031, 0.063
- Voriconazole: 0.181, 0.031–1, 0.125, 0.25
- Posaconazole: 0.029, 0.016–0.063, 0.063, 0.25
- Isavuconazole: 0.168, 0.016–1, 0.125, 0.5
- Caspofungin: 1.365, 0.25–4, 2
- Micafungin: 0.206, 0.016–8, 0.25
- Terbinafine: 0.053, 0.016–1, 0.031

**C. carrionii, China (20)**

- Amphotericin B: 2.639, 0.5–8, 2, 4
- Fluconazole: 19.027, 8–32, 16, 32
- Itraconazole: 0.038, 0.016–0.125, 0.031, 0.063
- Voriconazole: 0.109, 0.016–0.5, 0.125, 0.25
- Posaconazole: 0.02, 0.016–0.063, 0.063, 0.25
- Isavuconazole: 0.181, 0.031–1, 0.25
- Caspofungin: 1.625, 0.25–4, 2
- Micafungin: 0.342, 0.063–4, 0.25
- Terbinafine: 0.037, 0.008–0.125, 0.031

**C. carrionii, Madagascar (9)**

- Amphotericin B: 3.175, 1–8, 4, 4
- Fluconazole: 18.664, 4–64, 16, 32
- Itraconazole: 0.022, 0.016–0.063, 0.031, 0.063
- Voriconazole: 0.109, 0.016–0.5, 0.125, 0.25
- Posaconazole: 0.02, 0.016–0.063, 0.063, 0.25
- Isavuconazole: 0.107, 0.031–0.5, 0.25
- Caspofungin: 1.361, 0.25–4, 1
- Micafungin: 0.342, 0.063–4, 0.25
- Terbinafine: 0.053, 0.008–0.125, 0.031, 0.063

**C. carrionii, Australia (6)**

- Amphotericin B: 1.414, 0.5–4, NC, NC
- Fluconazole: 17.96, 8–64, NC, NC
- Itraconazole: 0.02, 0.016–0.063, NC, NC
- Voriconazole: 0.125, 0.031–0.5, NC, NC
- Posaconazole: 0.022, 0.016–0.063, NC, NC
- Isavuconazole: 0.14, 0.063–0.5, NC, NC
- Caspofungin: 0.793, 0.5–1, NC, NC
- Micafungin: 0.281, 0.063–4, NC, NC
- Terbinafine: 0.07, 0.016–0.25, NC, NC

* GM, geometric mean; 50% and 90%, MIC50 and MIC90, respectively; NC, no comparison because there were <9 strains per species available.

The activities of itraconazole and posaconazole against C. carrionii were comparable (Table 2) and similar to those of C. bantiana and Fonsecaea species (15, 17). Philophora 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>50</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 micafungin, 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 log₂-dilution-step-higher MICs than the majority of Venezuelan strains (range, 0.125 to 8 μg/ml; GM, 1.47 μg/ml; MIC₉₀, 4 μg/ml) (P < 0.01). The activities against Chinese and Australian strains were intermediate. For amphotericin B, the MIC range (0.5 to 8 μg/ml) and MIC₉₀ (8 μg/ml) were much higher than those of C. bantiana (MIC range, 0.125 to 2 μg/ml; MIC₉₀, 1 μg/ml) (15) and Fonsecaea (MIC range, 0.5 to 2 μg/ml; MIC₉₀, 2 μg/ml) (17) and confirmed the results from a recent study (11).

The 81 investigated isolates of C. carrionii represented a worldwide collection from four continents: South America (n = 46), Asia (n = 20), Africa (n = 9), and Australia (n = 6). In a molecular phylogenetic analysis (Deng et al., submitted), three main populations were recognizable: an Asian group, a South American group, and a variable African/Australian group. The susceptibility against itraconazole, voriconazole, posaconazole, isavuconazole for the Latin American group was less than that of remaining groups (P < 0.05), and micafungin was active against most strains from Venezuela (GM, 0.206 μg/ml; MIC₉₀, 0.5 μg/ml), but inactive for strains from Madagascar (GM, 1.47 μg/ml; MIC₉₀, 4 μg/ml) and some scattered isolates from other continents. There was a significant difference (P < 0.01) in the MICs of micafungin between Madagascar and Venezuelan strains, but the activity of terbinafine among these three groups showed no difference (P > 0.05).

These results suggest that C. carrionii, the etiologic agent of chromoblastomycosis in arid climates, is particularly susceptible in vitro to the newer azoles and terbinafine, but resistant to amphotericin B, fluconazole, and caspofungin. This profile is similar to that of melanized fungi studied previously (12, 16, 17). The results for micafungin are variable because all strains from Madagascar (GM, 1.47 μg/ml) and some scattered isolates from other continents deviate significantly from the remaining strains. In general, these in vitro data still need to be verified by clinical studies.

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