Subcutaneous Hy alophy homycosis Caused by
Colletotrichum gloeosporioides

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The coelomycete Colletotrichum gloeosporioides was isolated in pure culture from subcutaneous nodules of the
left forearm and elbow of a farmer after traumatic injury. To our knowledge, we report the first case involving
this fungus as an etiological agent of subcutaneous infection. The in vitro inhibitory activities of amphotericin
B, itraconazole, ketoconazole, miconazole, fluocytosine, and fluconazole were studied.

Colletotrichum Corda is a complex form genus of the form
class Coelomycetes, asexual fungi producing conidia within
fruit bodies, named conidiomata. The structures are spherical-
(pycnidia), with conidiogenous cells lining the inner cavity
wall, or are cup-shaped (acervuli), in which case, the conidiog-
ogenous cells form a palisade on the surface of the conidioma.
This genus comprises several hundred species, mostly plant
pathogens, which have been described mainly on the basis of
their conidial morphology and the presence or absence of
setae. The genus Colletotrichum was monographed by von Arx
(16), and only a restricted number of species was accepted.
Although only rarely pathogenic to humans, Colletotrichum
spp. have been reported as almost exclusively causing keratitis
(6–8, 11, 12), usually after an eye injury. In this report, we
describe a diabetic man with a history of trauma who developed
a subcutaneous infection caused by Colletotrichum gloeospo-
rioides (Pers.) Sacc.

Case report. A 56-year-old male farmer and resident of
Maringá in the state of Paraná, Brazil, presented himself to the
Serviço de Dermatologia do Hospital Universitário Regional
de Maringá in May 1996 because of the presence of nodular
lesions on his left forearm and elbow. Past medical history
revealed that he was diabetic and hypertensive and that he was
receiving prednisone (20 mg/day) by autoprescription. He re-
ported a previous traumatic injury of his left hand by rotten
wood that had required local suturing. He told of the appear-
ance, approximately 1 year later, of nonpruritic nodules in the
same trauma site. He denied having suffered fever and loss of
weight. On examination, he presented tuberose-nodular, ery-
thematosus, violaceous, solitary or confluent lesions measuring
1 to 3 cm in diameter and localized on the left forearm and
elbow. In addition, several vinaceous, macular lesions of ca.1.5-
cm diameter were observed, also on the dorsum of the forearm
(Fig. 1). The results of routine laboratory investigations of
blood and urine were within normal limits. Radiography of
the thorax showed cardiomegaly with left ventricular hypertrophy
and ectasia of the aortic arch. Radiography of the left elbow
showed only soft-tissue thickening without evidence of bone or
joint lesions. A computerized tomography scan showed the
presence of an expansive, hypodense, and multilocular lesion
in the soft tissue of the left elbow region. Biopsy of the lesions
was performed, and the contents of some of the nodules were
aspirated for examination. Direct examination of all specimens
sampled revealed the presence of sepsate, acute angled
branching, irregular, hyaline hyphae. A histologic section of
the biopsy material stained with periodic acid-Schiff and Go-
mori methenamine silver stains showed a hyperplasic epider-
mis with a psoriasiform pattern. The dermis showed an exten-
sive granulomatous reaction with central necrosis and multiple
foci of microabscesses. Throughout the tissue, numerous irreg-
ularly shaped, hyaline, septate, branched hyphae were present
(Fig. 2 and 3). All of the tissue samples and the contents of the
nodules were cultured on Sabouraud’s glucose agar (SGA) and
potato dextrose agar (PDA). Fungal colonies grew out in all
the cases in both media. Microscopic examination of the cul-
tures demonstrated that all of them presumably belonged to
the same species. Routine bacteriological cultures and cultures
for mycobacteria were negative. While the diagnostic proce-
dures were being performed, and before establishing treat-
ment, the patient unfortunately died as a result of a car acci-
dent. An autopsy was not performed.

For identification, fungal colonies from the biopsy material
and from the nodules’ contents were inoculated into SGA and
other routine mycological media, such as PDA, cornmeal,
malt extract, and oatmeal agars and incubated at room tempera-
ture. All of the media gave rise to white to grayish, loosely textured
colonies with similar characteristics. Colonies on PDA grew
very quickly, occupying the whole surface of the Petri dish in 10
days. They were greenish gray with pinkish to salmon patches,
powdery to velutine, profusely sporulated, and with abundant
production of conidiomata; the reverse was grayish (Fig. 4).
The colonies on oatmeal agar also grew very quickly. They had
a floccose texture with abundant production of white aerial
mycelium. The production of conidiomata was mainly re-
stricted to the central areas, and the reverse was uncolored.
The conidia were borne on elongated phialides in acervular
conidiomata, or, in the early stages of development, on solitary
fertile hyphae. The conidia were straight, cylindrical to slightly
clavate, hyaline, obtuse at the apex, extremely variable in
length, and measured 6 to 26 by 4 to 7 μm (Fig. 5 and 6).
Numerous appressoria were also present. They were clavate,
triangular or irregular, dark pigmented, and measured 8 to 15
by 5 to 8 μm (Fig. 7). The isolate was identified as C. gloeosporioides. The isolate was subcultured under various conditions and maintained in our mycology laboratory at the Medical School, University Rovira i Virgili, as no. FMR 6273. A living culture of this isolate has been deposited in the Centraalbureau voor Schimmelcultures of The Netherlands.

**Antifungal susceptibility testing.** The case isolate and four additional isolates of C. dematium (Pers. ex Fr) Grove, five isolates of C. coccodes (Wallr.) Hughes, and seven isolates of C. gloeosporioides from very diverse sources were tested to determine their susceptibility to antifungal drugs (Table 1). Tests were accomplished by a previously described microdilu-
tion method (10) performed mainly according to the National Committee for Clinical Laboratory Standards’ guidelines for yeasts, by using RPMI 1640 medium (buffered to pH 7.0 with 0.165 M morpholinepropanesulfonic acid [MOPS]), an inoculum of $4.4 \times 10^4$ to $2.8 \times 10^5$ CFU/ml, a temperature of incubation of 30°C, a second-day reading (48 h), and an additive drug dilution procedure.

**Discussion.** *Colletotrichum* spp. are typical fungi pathogenic for plants and causing anthracnosis, necrosis, leaf spot, and fruit rot. The diseases caused are commonly seed borne. Some species cause latent infections on woody plants, and the infected plants often show poor growth, and their fruits may rot (17). The species are anamorphs of the genus *Glomerella* Spauld. & H. Schrenk, classically considered as belonging to the order Phyllachorales, although some evidence exists about their relationship with the Sordariales (15), both orders of the Ascomycota. von Arx (16) delimited the most important species of *Colletotrichum*, considering their most distinctive characteristics to be the shape and size of the conidia and their specific hosts. However, further studies have enlarged the genus (13). The taxonomy of the genus is still unclear, and a comprehensive review of the numerous species described is needed. Sutton (14) pointed out that in vitro studies are required to determine the nature of the most representative morphological features, such as sclerotia, setae, and appressoria, in order to compare them with those shown in the host plant, because some differences have been reported. *C. gloeosporioides* is one of the commonest plant-pathogenic fungi to occur in the tropics and subtropics and is found worldwide. It constitutes a very heterogeneous taxon. von Arx (16) has given more than 600 synonyms for this species and has recognized nine forms, but probably many more can be differentiated by a combination of cultural characteristics, morphology, host range, and pathogenicity. Several molecular techniques have been used for a better characterization of the plant-pathogenic strains of this fungus (1, 5). Up to now, four species of *Colletotrichum* were known to have caused infections in humans or other animals (2). They are *C. coccodes*, *C. dematium*, *C. gloeosporioides* and *C. graminicola* (Ces.) Wilson. These species had been associated exclusively with keratitis (6–8, 11, 12), but recently Midha et al. (9) described a case of disseminated infection in a neutropenic patient probably caused by an unidentified *Colletotrichum* sp. Hence, the case of infection re-
ported here is the second one concerning an extraocular infection caused by a member of this genus. This change in the spectrum of the infection has also been noted in other fungi, first associated with keratitis, such as *Fusarium* (3) and *Acremonium* (4) spp. among others. The species pathogenic for humans were recently described and illustrated, and a key for their identification was also included (2). The other three pathogenic species can be easily differentiated from *C. gloeosporioides* by their setose conidiomata. In particular, *C. dematium* and *C. graminicola* are clearly distinguished by their falcate conidia, similar to those of *Fusarium* spp., although unicellular. The ascigerous state of *C. gloeosporioides, Glomerella cingulata* (Stoneman) Spauld. & H. Schrenk has been reported as developing on PDA (8).


FIG. 6. Conidia of *C. gloeosporioides*. Magnification by Nomarski optics, ×1,440.
Of the nine previously reported clinical cases of infection attributed to *Colletotrichum* spp., one was caused by *C. dematioides* (6), one was caused by *C. graminicola* (11), three were caused by *C. coccodes* (reported as *C. atramentarium*) (7), three were caused by *C. gloeosporioides* (8, 12), and one was caused by a *Colletotrichum* sp. (9). Only the last one was extraocular; the other eight were typical cases of keratitis. All of these cases followed an ocular injury, except one in which the patient had been treated with general and topical corticosteroid therapy for 3 years because of uveitis (8). The data concerning these cases are very scanty, and the results from the different treatments applied were variable. One patient was successfully treated with topical amphotericin B (6). In another case, the same treatment together with general therapy with flucytosine also resolved the infection (8). However, in a third case, the use of a combination of an amphotericin B suspension and miconazole nitrate eye ointment was ineffective (12). In the invasive case, the patient died despite treatment with amphotericin B and itraconazole (9). In one case, the patient was cured after topical treatment with 4% piramicin (8), and in

![FIG. 7. Appressoria of *C. gloeosporioides*. Stain, lactophenol cotton blue. Magnification, ×440.](image)

**TABLE 1. Antifungal susceptibility of *Colletotrichum* strains**

<table>
<thead>
<tr>
<th>Strain</th>
<th>MIC (µg/ml) at 48 h</th>
<th>MIC (µg/ml) at 72 h</th>
<th>MLC (µg/ml) at 48 h</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>C. gloeosporioides</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CBS 465.83</td>
<td>0.06/0.06 (0.06)</td>
<td>16/64 (16)</td>
<td>4/64 (64)</td>
</tr>
<tr>
<td>CBS 170.59</td>
<td>0.06/0.06 (0.06)</td>
<td>16/64 (16)</td>
<td>4/64 (64)</td>
</tr>
<tr>
<td>FMR 3383</td>
<td>0.06/0.06 (0.06)</td>
<td>16/64 (16)</td>
<td>4/64 (64)</td>
</tr>
<tr>
<td>FMR 6273</td>
<td>0.125/0.25 (4)</td>
<td>&gt;128/128 (&gt;128)</td>
<td>&gt;64/64 (&gt;64)</td>
</tr>
<tr>
<td>CBS 147.28</td>
<td>0.125/0.25 (0.5)</td>
<td>16/64 (16)</td>
<td>4/64 (64)</td>
</tr>
<tr>
<td>CBS 572.97</td>
<td>0.125/0.25 (0.5)</td>
<td>16/64 (16)</td>
<td>4/64 (64)</td>
</tr>
<tr>
<td>CBS 160.50</td>
<td>0.125/0.25 (0.5)</td>
<td>16/64 (16)</td>
<td>4/64 (64)</td>
</tr>
<tr>
<td>CBS 527.77</td>
<td>0.25/0.25 (0.25)</td>
<td>8/16 (16)</td>
<td>4/16 (16)</td>
</tr>
<tr>
<td>CBS 953.97</td>
<td>0.25/0.25 (0.25)</td>
<td>8/16 (16)</td>
<td>4/16 (16)</td>
</tr>
</tbody>
</table>

| **C. coccodes** | | | |
| IMI 136601 | 0.06/0.06 (0.06) | >128/128 (>128) | 32/64 (>64) |
| CBS 122.25 | 0.06/0.06 (0.06) | >128/128 (>128) | 8/16 (64) |
| CBS 125.57 | 0.06/0.06 (0.06) | >128/128 (>128) | 8/16 (64) |
| CBS 6273 | 0.06/0.06 (0.06) | >128/128 (>128) | 8/16 (64) |
| CBS 572.97 | 0.06/0.06 (0.06) | >128/128 (>128) | 8/16 (64) |
| CBS 953.97 | 0.25/0.25 (0.25) | >128/128 (>128) | >64/64 (>64) |
| CBS 527.77 | 0.25/0.25 (0.25) | >128/128 (>128) | >64/64 (>64) |
| CBS 528.77 | 0.25/0.25 (0.25) | >128/128 (>128) | >64/64 (>64) |
| CBS 167.49 | 0.125/0.25 (0.25) | >128/128 (>128) | 8/16 (64) |
| CBS 170.59 | 0.125/0.25 (0.25) | >128/128 (>128) | 8/16 (64) |
| CBS 351.73 | 0.125/0.25 (0.25) | >128/128 (>128) | >64/64 (>64) |
| CBS 714.95 | 0.125/0.25 (0.25) | >128/128 (>128) | >64/64 (>64) |

<table>
<thead>
<tr>
<th>Strain</th>
<th>Amphotericin B</th>
<th>Fluconazole</th>
<th>Itraconazole</th>
<th>Ketoconazole</th>
<th>Miconazole</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBS 465.83</td>
<td>0.06/0.06 (0.06)</td>
<td>16/64 (16)</td>
<td>4/64 (64)</td>
<td>&lt;0.03/0.06 (0.25)</td>
<td>0.125/0.25 (1)</td>
</tr>
<tr>
<td>CBS 170.59</td>
<td>0.06/0.06 (0.06)</td>
<td>16/64 (16)</td>
<td>4/64 (64)</td>
<td>0.06/0.125 (0.25)</td>
<td>0.125/0.25 (1)</td>
</tr>
<tr>
<td>FMR 3383</td>
<td>0.06/0.06 (0.06)</td>
<td>16/64 (16)</td>
<td>4/64 (64)</td>
<td>0.06/0.125 (0.25)</td>
<td>0.125/0.25 (1)</td>
</tr>
<tr>
<td>FMR 6273</td>
<td>0.125/0.25 (4)</td>
<td>&gt;128/128 (&gt;128)</td>
<td>&gt;64/64 (&gt;64)</td>
<td>&gt;16/16 (&gt;16)</td>
<td>2/4 (&gt;16)</td>
</tr>
<tr>
<td>CBS 147.28</td>
<td>0.125/0.25 (0.5)</td>
<td>&gt;128/128 (&gt;128)</td>
<td>&gt;64/64 (&gt;64)</td>
<td>0.5/0.5 (16)</td>
<td>2/4 (16)</td>
</tr>
<tr>
<td>CBS 572.97</td>
<td>0.125/0.25 (0.5)</td>
<td>&gt;128/128 (&gt;128)</td>
<td>&gt;64/64 (&gt;64)</td>
<td>0.25/0.25 (1)</td>
<td>0.25/0.25 (1)</td>
</tr>
<tr>
<td>CBS 953.97</td>
<td>0.125/0.25 (0.5)</td>
<td>&gt;128/128 (&gt;128)</td>
<td>&gt;64/64 (&gt;64)</td>
<td>1/16 (&gt;16)</td>
<td>2/4 (16)</td>
</tr>
<tr>
<td>CBS 527.77</td>
<td>0.125/0.25 (0.5)</td>
<td>&gt;128/128 (&gt;128)</td>
<td>&gt;64/64 (&gt;64)</td>
<td>0.5/0.5 (16)</td>
<td>0.5/1 (&gt;16)</td>
</tr>
<tr>
<td>CBS 528.77</td>
<td>0.125/0.25 (0.5)</td>
<td>&gt;128/128 (&gt;128)</td>
<td>&gt;64/64 (&gt;64)</td>
<td>0.25/0.25 (16)</td>
<td>0.5/1 (&gt;16)</td>
</tr>
<tr>
<td>CBS 167.49</td>
<td>0.125/0.25 (0.25)</td>
<td>&gt;128/128 (&gt;128)</td>
<td>8/16 (64)</td>
<td>0.25/0.25 (8)</td>
<td>0.25/0.25 (2)</td>
</tr>
<tr>
<td>CBS 170.59</td>
<td>0.125/0.25 (0.25)</td>
<td>&gt;128/128 (&gt;128)</td>
<td>8/16 (64)</td>
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<tr>
<td>CBS 351.73</td>
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</tr>
<tr>
<td>CBS 714.95</td>
<td>0.125/0.25 (0.25)</td>
<td>&gt;128/128 (&gt;128)</td>
<td>8/16 (64)</td>
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<td>0.25/0.25 (2)</td>
</tr>
</tbody>
</table>

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the most recent case, the patient was also cured after combined treatment with topical natamycin, intracameral amphotericin B, and oral fluconazole (11).

The data obtained from the antifungal susceptibility testing of the isolate from the patient and 15 comparison isolates demonstrated, in general, that the MICs for the organisms were low, with the exception of those of flucytosine and, to a lesser degree, fluconazole. For only one isolate was the MIC of amphotericin B higher than 1 \( \mu \text{g/ml} \). For two isolates, the MICs of miconazole were higher than 4 \( \mu \text{g/ml} \), and for ketoconazole and itraconazole, there were only four isolates, in each case, for which the MICs exceeded such values. Differences between MICs read at 48 h and at 72 h were generally not important. Usually the values were the same after the two readings, and in only one case were the differences higher than 2 dilutions. Examination of the minimal lethal concentrations (MLCs) showed that the majority of isolates displayed a major degree of resistance. They were, however, mainly susceptible to amphotericin B. Only one strain (\( \text{C. coccodes CBS 125.57} \)) was clearly resistant to all of the drugs tested. No major differences were observed among the MICs and MLCs for the three species tested.

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