First report of subcutaneous phaeohyphomycosis caused by Ochroconis tshawytschae in an immunocompetent patient

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We report the first case and clinical course of a case of human subcutaneous phaeohyphomycosis caused by Ochroconis tshawytschae, a rare fish pathogen. The diagnosis was based upon histopathological and mycological examinations of clinical samples. Identification of the etiologic agent was assessed on its phenotypic characteristics and subsequently, confirmed by molecular data. In vitro antifungal susceptibility of the isolate was investigated and a comparison was prepared of all of its features to those of its two most relevant related species, O. gallopava and O. humicola.

Keywords melanized fungi, subcutaneous phaeohyphomycosis, Ochroconis tshawytschae

Introduction

Phaeohyphomycosis refers to infections caused by melanized or dematiaceous, filamentous fungi which may be histologically observed as dark-pigmented hyphae or yeast-like cells. Over 150 species and 70 genera of melanized fungi have been implicated in human and animal diseases, among which Ochroconis spp. are rarely indicated as human pathogens [1].

Ochroconis tshawytschae is a member of the Ochroconis genus which was first isolated in 1946 from kidney mycoses of Chinook salmon (Oncorhynchus tshawytschae) [2]. It was initially referred to as Heterosporium tshawytschae, later transferred to the genus Scolecobasidium in 1974 by McGinnis and Ajello [3], and finally reassigned as O. tshawytschae in 1977 by Kirilenko and All-Achmed [4]. O. tshawytschae is an established dematiaceous pathogen for fish [2] but no human infections have been reported associated with the organism. Here we describe the first occurrence of O. tshawytschae as an agent of human subcutaneous phaeohyphomycosis in an immunocompetent patient.

Case report

The patient was a 19-year-old male student from East China’s Zhe Jiang Province. He presented with painless, erythematous and indurated plaques on his face and left forearm when referred to our hospital. The patient recalled a facial bruise on his right cheek after a fall which occurred approximately 12 years prior to his present clinical conditions. The wound soon healed through the use of simple topical antibiotic treatment, but one month later, a painless erythematous lesion was found to be slowly enlarging and becoming indurated on the traumatized skin. Seven years before the present hospitalization, histopathologic studies indicated that the patient had a subcutaneous mycosis and the individual was empirically given oral itraconazole, 200 mg per day. Unfortunately, no specimens were obtained at this time to attempt to recover the fungus in culture. The lesion was mostly healed with scarring after one month of antifungal therapy. The patient himself discontinued the
medication when he felt that he was ‘in control’. Five years ago, the lesion reappeared on his right cheek, later involving the left cheek and left forearm. During the subsequent years, he received several short courses of treatment with itraconazole or terbinafine, but no cure was obtained. Basically the patient was otherwise healthy with no unidentifiable conditions which might have led to impaired immunity. The results of routine hematological and urine examinations were normal. Multiple erythematous, indurated plaques and nodules were observed on physical examination of both cheeks and the nose, as well as several superficial ulceration and crusted lesions (Fig. 1). In addition, similar lesions were also seen on his left forearm.

Direct examination of 10% potassium hydroxide wet mounts of the ulcer exudates and crust material revealed abundant light-brown, branched and septate hyphae. Portions of both materials were inoculated onto culture media to attempt to recover the etiologic agent. While antifungal therapy was initiated with oral itraconazole, 200 mg twice per day, the patient was lost to follow-up as he did not return for re-evaluation.

**Histological examination**

Histopathological examination revealed hyperkeratosis, hypokeratosis and pseudoepitheliomatous hyperplasia in the epidermis. Studies of the deep dermis showed a mixed chronic inflammation with a granulomatous component. Microscopic evaluation of periodic acid-Schiff (PAS) stained tissue sections revealed abundant branched and septate hyphae, as well as several moniliiform hyphae.

**Mycological study**

Primary isolation of the fungus was performed on agar slants of Sabouraud glucose agar (SGA) containing chloramphenicol (CMP, 0.125 g/l) which were incubated at 26°C. Physiological tests included the growth of the fungus on SGA containing cycloheximide (CHX, 0.5 g/l) at 26°C and 37°C (CHX tolerance test), and on SGA at 26, 37, 40 and 45°C. Slide culture was prepared on potato dextrose agar (PDA) medium and incubated at 26°C for two weeks.

All clinical specimens grown on SGA containing CMP produced olivaceous-brown, velvety colonies with a reddish brown diffusible pigment. Colonial growth was evident by the 5th day on SGA and reached approximately 1 cm in diameter after two weeks at both 26 and 37°C. No visible growth was observed at 40 and 45°C. The strain was tolerant to CHX and grew well at both 26 and 37°C on CHX-containing SGA. Microscopically, branched, hyaline and septate hyphae were observed. Conidia were cylindrical, 4-celled, verrucose, thin-walled and were formed on frilled denticles in the apical region of conidiophores (Fig. 2). Based on these morphological characteristics [5], the fungus was identified as *Ochroconis tshawytschae*.

**Molecular analysis of D1-D2 region and the Internal Transcribed Spacer (ITS) rRNA gene**

The D1/D2 region of the large subunit ribosomal RNA was amplified from genomic DNA by PCR using universal primers NL1/NL4 [6]. The amplification was performed at 94°C for 10 min, followed by 30 cycles at 94°C for 30 s, 57°C for 30 s, and 72°C for 1 min, with
a final extension at 72°C for 7 min. The ITS region was also amplified using primers ITS 1 and ITS4 as previously described [7]. The PCR products were sequenced via commercial service (Shanghai Sangon Biological Engineering Technology & Services) which demonstrated that both D1-D2 and the ITS sequences (GenBank nos. GU 328005 and JN974456, respectively) displayed 99% similarity to the entries of Ochroconis tshawytschae deposited in GenBank (accession nos. AB161066 and FJ914694, respectively). The strain was later referred to the Centraalbureau voor Schimmelcultures (The Netherlands) where the identification was confirmed and the isolate included in their collection as CBS 129970.

**Antifungal susceptibility test**

The in vitro susceptibility of the strain to six antifungal agents was determined using the microdilution method in accord with the guidelines of the Clinical and Laboratory Standards Institute (CLSI) M38A [8]. The minimum inhibitory concentrations (MICs) were defined as the lowest concentration at which no growth occurred which lead to the following results: itraconazole, 0.5 μg/ml; ketoconazole, 2 μg/ml; voriconazole 0.125 μg/ml; fluconazole, > 64 μg/ml; caspofungin, 0.25 μg/ml and amphotericin B, 4 μg/ml.

**Discussion**

Phaeohyphomycosis refers to local or systemic tissue infection caused by members of the melanized, filamentous fungi. According to Revankar et al., the disease is divided into five main categories, i.e., allergic superficial infection, deep local infection, pulmonary infection, central nervous system infection, and disseminated infection [1]. Deep local phaeohyphomycosis represents a heterogeneous group of infection syndromes, among which subcutaneous lesions are the most commonly seen. Although it is generally considered to be an opportunistic infection, a significant fraction has been noted in healthy individuals. Minor trauma is often the inciting factor and since dematiaceous fungi are universally distributed in the natural environment, patients from a rural background, i.e., farmers or gardeners, are at an increased risk. As was shown in our case, the patient was also likely to acquire the infection via skin trauma.

Ochroconis tshawytschae is a member of Chaetothyriales [1,5]. Within Ochroconis, O. gallopava and O. humicola are more frequently associated with human and animal infections [5,9–20], with O. gallopava being the most medically significant member of the genus. Just like several other species in the order Chaetothyriales, O. gallopava is notorious as a neurotropic pathogen, causing brain infections and peritonitis in immunocompromised populations, especially organ transplant recipients [1,14–20], as well as pulmonary infections in immunocompetent individuals [13]. However, cutaneous or subcutaneous infection caused by O. gallopava is rare, with only one historical case of localized subcutaneous abscesses being reported [11]. O. humicola, by contrast, is clinically insignificant, causing rare phaeohyphomycosis of cold-blooded vertebrates, tortoise and cat [9,10,12].

This report describes the first human case of subcutaneous phaeohyphomycosis due to O. tshawytschae. Although data concerning the ecological distribution of O. tshawytschae is limited, a recent survey indicated its presence of in soil in Qinghai Province of China [21]. The infection was likely transmitted through contact with contaminated dirt or soil. The patient’s profile was distinctly different from those with infections caused by the more clinically significant and deadly O. gallopava. First, the present patient was apparently immunocompetent, having no documented risk factors such as hematological malignancies, bone marrow or solid organ transplantations, AIDS or of having received immunosuppressive therapeutics. Second, the infection was chronic and localized to the skin, indicating that the species was much less invasive than O. gallopava. The spread of lesions to his nose and forearm might be attributed to the self-implantation caused by scratching. It is noteworthy that the infection remained inactive for as long as two years before the lesions relapsed, suggesting that the initial recovery after short-term treatment could be rather misleading. A prolonged therapy with oral antifungals, therefore, is reasonable for the management of subcutaneous phaeohyphomycosis, with premature discontinuation of therapy discouraged until the clearance of the infection is confirmed.

Identification of the O. tshawytschae was based on microscopic and physiological characteristics, and further confirmed by molecular data. Differentiation from O. gallopava and O. humicola was based on the presence of 4-celled conidia, growth tolerance on CHX-containing agar, and development at 37°C but not 45°C (summarized in Table 1). Molecular data, both the D1–D2 region and the ITS region provide sufficient nucleotide variations for the reliable separation of three Ochroconis species.

In summary, we present the first case of subcutaneous phaeohyphomycosis caused by O. tshawytschae in an immunocompetent individual. This case highlights the diagnostic and therapeutic challenges associated with subcutaneous infections caused by rare melanized, filamentous fungi. A prolonged antifungal therapy may be required for the clearance of infections caused by O. tshawytschae.
Table 1  Morphological and physiological characteristics of *Ochroconis tshawytschae*, *O. gallopava* and *O. humicola*.

<table>
<thead>
<tr>
<th>Species</th>
<th><em>O. tshawytschae</em></th>
<th><em>O. gallopava</em></th>
<th><em>O. humicola</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Conidial morphology</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of cells</td>
<td>4</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Shape</td>
<td>Cylindrical</td>
<td>Clavate</td>
<td>Cylindrical</td>
</tr>
<tr>
<td>Constriction</td>
<td>None</td>
<td>Present (not at the septum)</td>
<td>Present (at the septum)</td>
</tr>
<tr>
<td>Physiological features</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tolerance to CHX</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Tolerance to 37°C</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Tolerance to 45°C</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
</tbody>
</table>

CHX, cycloheximide; +, Positive; –, Negative.

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**References**


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