1. Introduction

The taxonomy of the Ochroconis lineage was recently revised by combining molecular phylogeny, morphology, and ecology. Two new genera were recognized: Ochroconis and Verruconis. Both Ochroconis and Verruconis were found to belong to the order Venturiales, family Sympoventuriaceae [1,2], and thus have a unique position as human opportunists, remote from all other melanized agents known to date. The genera show remarkably high phylogenetic diversity despite high degrees of phenotypic similarity. Verruconis contains a single clinically relevant species V. gallopava [3–6], which is thermophilic and causes deep, mostly neurotropic infections, probably after inhalation. By contrast, Ochroconis species are mesophilic, and are traditionally considered as non-pathogenic to humans or animals because of their inability to grow at 37 °C and are often treated as laboratory contaminants. However, Ochroconis species are occasionally able to cause mild infections in cold- as well as in warm-blooded hosts, although the taxonomy of species reported in the literature has not been clarified and exact species affiliations may be erroneous. Ochroconis humicola was described from recurrent infections in fish and was occasionally involved in mycoses of cats [7,8]. O. tshawytschae is also known from infections in fish and was involved in a human subcutaneous infection in immunocompetent human [9].

After taxonomic rearrangements it was realized that O. musae was actually the most common species of Ochroconis involved in vertebrate infection [2]. The species was introduced as Scolecosidium musae [10] from a banana plant just a few months prior to the description of the novel species O. mirabilis from animal infections; the two taxa proved to be identical. Ochroconis musae might have a natural habitat in relatively warm water, and re-identification of archived specimens proved that the fungus is an opportunist on humans [2]. Here, we describe involvement of O. Musae in a subcutaneous infection in an immunocompetent patient.

2. Case

A 50-year-old female patient from East China’s Shandong Province presented with painless or itching, erythematous and indurated plaques on her left cheek when she was referred to our hospital on 20 Sep, 2014 (at day 0). The lesion started at day 6 years as a painless red papule on the left cheek and was found to...
slowly enlarge. Several small satellite lesions were found at day – 3 years, and then the patient sought for treatment in a local hospital. During the subsequent two years, the patient has received different diagnoses for the lesions, and has received several incoherent treatments with oral or topical unknown drugs, but no cure was obtained. At day – 15 months, the patient was diagnosed with a subcutaneous mycosis through histopathological examination in a local hospital. Consequently, the patient received empirical treatment with oral itraconazole, 200 mg twice per day for 3 months (during day – 15 months to day – 13 months). The patient did not respond well to the treatment, so she discontinued the medication and was referred to our hospital. At day 0, clinical examination showed multiple erythematous and plaques, as well as superficial scales on her left cheek (Fig. 1A). The patient did not remember to have a history of any antecedent injury on her face, and denied long-term use of steroids or other drugs. She also denied fever and weight loss. Results of routine hematological examination and urine analysis were within normal limits. Serological tests for HIV and anti-nuclear antibodies (e.g., anti-dsDNA antibody and anti-ssDNA antibody) were negative and chest radiography was unremarkable at day +0.

Light microscopy of a skin biopsy showed dermal pigmented hyphae and spherical structures, suggestive for a mycosis at day +0 (Fig. 2A). Direct examination of 10% potassium hydroxide wet mounts of the scales of the lesions revealed abundant, pale-brown, branched and septate hyphal elements at day +0 (Fig. 2B).

Mycological culture was performed at day +0. Portions of both skin biopsies and skin debris were inoculated onto culture media attempting to recover the etiologic agent. Primary isolation of the fungus was performed on agar slants of Sabouraud’s glucose agar (SGA) containing chloramphenicol (CMP, 0.125 g/l) and incubated at 25 °C. Physiological tests included the growth of the fungus on SGA containing cycloheximide (CHX, 0.5 g/l) at 25 °C and 37 °C (CHX tolerance test), and on SGA at 15 °C, 20 °C, 25 °C, 30 °C, 35 °C, 37 °C and 40 °C. Slide culture was prepared on potato dextrose agar (PDA) medium and incubated at 25 °C for two weeks. All clinical specimens grown on SGA containing CMP produced grayish brown to dark brown, velvety colonies with a reddish brown diffusible pigment. Colonial growth was attained approximately 5 cm in diameter at day +28 at both 25 °C (Fig. 2C, D). No visible growth was observed at 37 °C, and 40 °C. The strain was tolerant to CHX and grew well at 25 °C on CHX-containing SGA. Microscopically, branched, pale brown septate hyphae were observed. Conidia were cylindrical, 2-celled, smooth-walled to verrucose, subhyaline to pale brown and were formed on frilled denticles in the apical region of conidiophores (Fig. 2E, F). Based on these morphological characteristics, the fungus was identified as *Ochroconis musae*.

The identification was confirmed by sequencing rDNA, ITS 1 and ITS 2 regions, and phylogenetically analyzed with the reference sequences deposited in GenBank (Fig. 3) at day +0. The strain was enlisted in the reference collection of the Centraalbureau voor Schimmelcultures, Fungal Biodiversity Centre, Utrecht, The Netherlands, under accession number CBS 140,760.

In correspondence with the results of antifungal susceptibility testing, the patient was treated with oral terbinafine 250 mg daily associated with terbinafine cream twice per day from day +35. Over a period of three months of treatment, the skin lesions showed marked improvement with reduction in the lesion area (Fig. 1B). The drug was well-tolerated by the patient without showing any side-effects. The therapeutic regimen was continued for six subsequent months before the lesions healed completely. Because the agent was not thermo-tolerant, the patient received 3 times of ALA-photodynamic therapy during the treatment and responded well to the therapy (at day +35, day +65 and day +95). However, the patient gave up photodynamic therapy due to inconvenience. No recurrence of cutaneous lesions was seen at a 3-month follow-up visit.

3. Discussion

*Ochroconis musae* probably has a natural ecology as a water-borne species which is found in relatively warm environments. Several confirmed strains were derived from tropical ocean water, while another recurrent habitat was moist localizations in bathrooms, such as bath edges, sinks or shower cabins [12]. The single report from flyspecks on bananas [10] is probably exceptional. It is generally hypothesized that the ability to grow at 37 °C is...
necessary for infection of the human host, but exposed skin has a lower temperature and the maximum growth temperature of 33 °C. Remarkably, *O. musae* has a relatively low in vitro maximum of 30 °C [10] and we have to suppose that the fungus is able to temperature stress and loses this ability quickly when no longer needed. Our isolate is the case that grew well in 30 °C but not higher than 30 °C.

The subcutaneous lesions of the present case were in the face. Although the patient could not recall any history of trauma, a possibility of mini-traumata could not be ruled out because...
patient liked to use rubbing bath towels with some force, enhancing entrance of fungi from the bathroom. The skin may have become softened and more susceptible as a result of showering. Patient was immunocompetent, having no documented disorders such as HIV infection, hematological malignancies, solid organ or bone marrow transplantations, type II diabetes or receiving immunosuppressive therapeutics. Patient presented with localized skin lesions without significant extension despite the chronic nature of the infection.

The pathogenic agent induced mild infection which remained confined to the skin. Virulence of Ochroconis species is low; Samerpitak et al. [10] listed mainly cases of skin and nail infection. It is nevertheless possible that cases have been overlooked because the species was repeatedly misdiagnosed in the literature. Also in the present case the nature of the infection remained enigmatic for more than five years. Mycotic lesions appeared not typical and may mimic other diseases. Today, confirmation diagnosis is unambiguous with sequencing of ribosomal markers.

Mycosis caused by members of the fungal order Venturiales is poorly known, and limited knowledge on antifungal susceptibility is available. Amphotericin B, 5-fluorocytosine, ketoconazole, miconazole and itraconazole have been used with varying success. Our patient did not respond well to 3-month empirical treatment with oral itraconazole, 200 mg twice daily. Judging from results of in vitro antifungal susceptibility testing, we used terbinafine 250 mg daily to treat this patient. During the first 3 months the lesions showed marked improvement, but during subsequent months the lesions relapsed slowly. Therefore, treatment should be adjusted according to antifungal drug sensitivity monitoring over prolonged periods to prevent recurrence. Because the agent is mesophilic, local heat therapy, ALA-photodynamic treatment, was used for three times as adjuvant treatment with good response.

Conflict of interest

There are none.

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