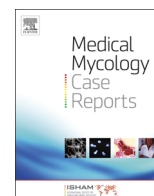




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Subcutaneous infection by *Ochroconis mirabilis* in an immunocompetent patient

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ABSTRACT

Recently, the taxonomy of *Ochroconis* (*Ascomycota*, *Pezizomycotina*, *Venturiales*, *Symptoventuriaceae*) has been revised with the recognition of an additional genus, *Verruconis*. *Ochroconis* comprises mesophilic saprobes that occasionally infect vertebrates which mostly are cold-blooded, while *Verruconis* contains thermophilic species which is a neurotrope in humans and birds. On the basis of molecular data it is noted that only a single *Ochroconis* species regularly infects immunocompetent human hosts. Here we report a subcutaneous infection due to *Ochroconis mirabilis* in a 50-year-old immunocompetent female patient. *In vitro* antifungal susceptibility tests revealed that terbinafine was the most effective drug. The patient was successfully cured with oral administration of terbinafine 250 mg daily in combination with 3 times of topical ALA-photodynamic therapy for 9 months.

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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 *Symptoventuriaceae* [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 infection, 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 *Scolecobasidium 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

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Fig. 1. (A) Erythematous, nodular lesions in the face. (B) Skin lesions markedly recovered after 3 months of oral administration of terbinafine combined with 3 times of topical ALA-photodynamic therapy. (For interpretation of the references to color in this figure, the reader is referred to the web version of this article.)

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, 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.

The *in vitro* susceptibility of the strain to eight antifungal agents was determined using the microdilution method in accord with the guidelines of the Clinical and Laboratory Standards Institute (CLSI) M38A **at day +28** [11]. The minimum inhibitory concentrations (MICs) were defined as the lowest concentration at which no growth occurred which led to the following results: itraconazole 4 µg/ml; ketoconazole, > 8 µg/ml; fluconazole, > 64 µg/ml; micконаazole 2 µg/ml; voriconazole 2 µg/ml; anidulafungin, > 4 µg/ml; amphotericin B, > 4 µg/ml and terbinafine 0.06 µg/ml.

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

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