Subcutaneous phaeohyphomycosis caused by *Wallemia sebi* in an immunocompetent host

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This is a case of subcutaneous phaeohyphomycosis due to *Wallemia sebi* in a 43-year-old-female, the first case described since 1950. The lesion presented as a non-healing ulcer on the dorsum of the left foot. Diagnosis was based on histological demonstration of the fungus and its recovery in culture.

**Case report.** A 43-year-old house-wife, resident of Varanasi in the State of Uttar Pradesh in North India, presented with a non healing ulcer, with a ragged margin and was covered with slough, on the dorsum of left foot (Fig. 1). The lesion was erythematous at its base, mildly warm (to touch), minimally tender and of eight months duration; it had started as an itchy papule that gradually progressed to its present size (8 by 6 cm). She could not recall any injury on the foot prior to the development of the lesion. Smears of scrapings from the lesion stained by Gomori methenamine silver and Gram stains were negative for fungal structures and bacteria; a few polymorphonuclear neutrophils were seen. Ziehl-Neelsen (Z-N) stained smears did not show any acid-fast bacilli. The patient was non-diabetic and HIV negative. The patient had no other systemic or underlying disease. Routine haematological examination and chest X-ray were normal; X-ray of the foot did not reveal any bone erosion. A provisional clinical diagnosis of cutaneous tuberculosis/deep mycosis was made. A biopsy was taken from the lesion. Histopathological examination showed hyperplastic epidermis with ulceration. Deep dermis showed dense acute or chronic inflammatory granulation tissue lining an abscess cavity filled with necrotic material. Grocott stained tissue sections revealed septate hyphae (Fig. 2). No acid-fast bacilli were seen in tissue
sections stained with Z-N stain. A portion of the biopsy was minced into tiny pieces, which were cultured on multiple slopes of Sabouraud dextrose agar (SDA; Difco Laboratories, Detroit, Mich.) containing chloramphenicol (0.05 mg/ml) and slopes of SDA with chloramphenicol and cycloheximide (0.5 mg/ml).

Cultures on SDA without cycloheximide yielded several colonies of a slow growing dematiaceous mould, which was identified as *Wallemia sebi* on the basis of a detailed study of its colonial and microscopic morphology (4). The colonies attained a diameter of 2 to 4 mm after 2 weeks at 25ºC (Fig. 3 A). They were elevated, irregular in shape and orange-brown to blackish-brown. Microscopic examination of fungus growth in lactophenol cotton blue mounts showed slender, cylindrical and usually unbranched conidiophores. Conidiophores were smooth, subhyaline and with a swollen upper part from which a cylindrical, verrucose fertile hypha emerges and fragments to form conidia. Conidia were catenate, cubical, up to 2.5 µm diam., becoming spherical or subspherical, finely verruculose, subhyaline and brown in mass (Fig. 3 B). This isolate did not grow at 37ºC and growth at 35ºC was extremely slow (up to 1.5 mm diam after 2 weeks).

A living culture of the isolate has been deposited in the Faculty of Medicine (Reus, Spain) as FMR 8645 and in the Centraalbureau voor Schimmelcultures (Utrecht, the Netherlands) as CBS 121954.

An *in vitro* antifungal susceptibility test of the isolate, performed according to CLSI guidelines (12) with exception of the incubation temperature (30ºC in our case), and with *Candida parapsilosis* ATCC 23019 and *C. krusei* ATCC 62258 as quality controls, showed the following MICs (in µg/ml) for the different
antimycotics: itraconazole 0.125, micafungin 0.06, flucytosine >64, voriconazole 0.03, posaconazole 0.25, terbinafine <0.03, ketoconazole <0.03, and amphotericin B 2. Due to the extremely slow growth of the fungus, MICs could only be read after 7 days of incubation.

The patient was put on itraconazole 100 mg twice daily and asked to report after three weeks for evaluation. The patient did not return and was regrettably lost to follow-up.

Phaeohyphomycosis refers to infections of skin, subcutaneous tissues and internal organs caused by dematiaceous (melanized) fungi that produce pigmented hyphae and/or yeast like cells in culture, and frequently in the infected tissue. Species of several genera of dematiaceous fungi, e.g. Alternaria, Bipolaris, Curvularia, Cladophialophora, Cladosporium, Exophiala, Exserohilum, Phaeoacremonium or Phialophora, are commonly reported as agents of phaeohyphomycosis (4, 11). Wallemia sebi, another dematiaceous anamorphic fungus and a common causative agent of farmer’s lung disease (10,13-15,18), is a rarely known agent of human infection reported in earlier literature between 1909-1950 (2, 3, 6, 9). Thus it is considered of interest to report here a case of subcutaneous phaeohyphomycosis caused by W. sebi.

The class Wallemiomycetes and the order Wallemiales has been recently erected and considered as a sister group of the Basidiomycota to accommodate the single genus Wallemia (8, 19). This genus comprises three xerophilic species, which are phenotypically mainly distinguished by the size range of
conidia and by the degree of their xerophily (19). *Wallemia sebi* is the only species capable of growth on media such as malt extract agar without additional solutes (NaCl, glucose) and shows the smallest conidia (1.5-2.5 µm diam). It is the only species of *Wallemia* that has been involved in human infections.

*Wallemia sebi* is a mould with a world-wide distribution. It is common in indoor environment and has been isolated from jams, dates, bread, cakes, salted beans, maize flour, crystalline sugar, fish, bacon, fruits, soil, hay and textiles. This fungus is also commonly found in agricultural environments in many parts of the world (1, 5, 7, 16, 20). There are only a few known cases of cutaneous or subcutaneous infections in humans due to *W. sebi*, reported in the earlier literature, without specified clinical features (2, 3, 6, 9). The infections caused by this fungus were called “hemisporiosis” named after the synonymous species *Hemispora stellata*. Subsequent to 1950, *W. sebi* has not been reported as an agent of human infection. The present case provides further evidence of pathogenic role of this rarely known fungus.

One intriguing issue related to *W. sebi* infections is the fact that they have not been described since the fifties of the last century. One explanation could be that these infections were previously under diagnosed mainly due to the extremely slow growth of this fungus, which possibly led to discarding the isolates as laboratory contaminants. More difficult to explain is why so far this fungus has only infected immunocompetent patients. Other known pathogen moulds such as *Fusarium, Acremonium, Scedosporium*, etc., before the emergence of immunocompromised patients also caused localized infections in
immunocompetent patients but nowadays their spectra of action have changed substantially affecting mainly the immunosuppressed hosts. Unfortunately, due to the rarity of the *W. sebi* infections scarce data on their clinical features and treatment exist. In conclusion, *W. sebi* should be added to the relatively short list of basidiomycetous fungi that are known to cause infections in humans.
References


Fig. 1. Ulcer with ragged margin due to *Wallemia sebi* on the dorsum of the left foot; peripheral areas show partial healing.

Fig. 2. Groccot stain showing a septate hypha. Bar = 10 μm.

Fig. 3. *Wallemia sebi*. (A) Colonies on Sabouraud dextrose agar at 25 °C after 2 weeks. (B) Conidia. Bar = 50 μm.
Fig. 2