Case Report

Subcutaneous phaeohyphomycosis caused by *Exophiala equina*, with susceptibility to eight antifungal drugs

Mohammad Javad Najafzadeh,1 Moo Kyu Suh,2 Myung Hoon Lee,2 Gyoung Yim Ha,3 Jung Ran Kim,4 Tae Heung Kim,5 Hyo Jin Lee,6 Jong Soo Choi,6 Jacques F. Meis7 and G. Sybren De Hoog8,9,10

**Correspondence**
G. S. De Hoog
dehoog@cbs.knaw.nl

1Department of Parasitology and Mycology, and Cancer Molecular Pathology Research Center, Ghaem Hospital, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
2Departments of Dermatology, Dongguk University, Gyeongju, South Korea
3Laboratory Medicine, Dongguk University, Gyeongju, South Korea
4Pathology, College of Medicine, Dongguk University, Gyeongju, South Korea
5White Skin Clinic, Changwon, South Korea
6Department of Dermatology, College of Medicine, Yeungnam University, Daegu, South Korea
7Department of Medical Microbiology and Infectious Diseases, Canisius-Wilhelmina Hospital, Nijmegen, The Netherlands
8CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands
9Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, The Netherlands
10Peking University Health Science Center, Research Center for Medical Mycology, Beijing, PR China

A case of subcutaneous phaeohyphomycosis caused by *Exophiala equina* is reported in a 75-year-old female, who showed subcutaneous abscesses on both forearms for 8 months. A lesion was initiated by inoculation with a spine from a tree. Histopathologically, suppurative granulomatous inflammation was present and short hyphal elements were observed. Upon culture greyish–black, velvety colonies of a black yeast were obtained after 3 weeks. The strain grew well at 25°C, but poorly at 37°C. After sequencing the internal transcribed spacer domain and the partial β-tubulin gene, the fungus was identified as *E. equina*. The patient was successfully treated with fluconazole for 3 months.

**Introduction**
Phaeohyphomycosis is a general term for disorders caused by melanized fungi with hyphal histopathology. It covers a wide variety of clinical forms, including cutaneous and subcutaneous, central nervous system and disseminated infections. The mycoses should be distinguished from mycetoma and chromoblastomycosis, which are also caused by dematiaceous fungi (Revankar, 2007) but have muriform cells or grains as invasive forms, respectively. The disease is usually found in immunocompetent hosts. The number of dematiaceous moulds that have been documented as aetiological agents of phaeohyphomycosis continues to increase (Walsh et al., 2004).

*Exophiala*, characterized by yeast–hypha dimorphism and annellidic conidiogenesis (De Hoog et al., 2000), is the main genus of opportunistic and pathogenic black yeasts. The most serious pathogens in the genus are *Exophiala dermatitidis* (Sudhadham et al., 2008), *E. spinifera* (Li et al., 2008) and *E. asiatica* (Li et al., 2009). The presence of melanin and the ability to assimilate alkyl benzenes have been suggested to play an important role in pathogenicity and in evasion from the host defence (De Hoog et al., 2011). Species of *Exophiala* have been difficult to identify, but in recent years diagnostics have expanded with molecular tools, particularly the sequencing data of rDNA internal transcribed spacer (ITS) regions. With these data many strains that were originally identified as *Exophiala jeanselmei* on the basis of morphology are now
known to belong to other *Exophiala* species (Zeng *et al.*, 2007).

The type strain of *Exophiala equina* was isolated as the probable aetiological agent of a subcutaneous infection of a horse’s lower leg by Pollacci (1923). Most existing strains sequenced belonging to this species, however, originate from cold water or watery environments, such as drinking water, a cooling system of a packaging machine, the tubing of a gelly installation, silica gel, washings of *Tilia* roots, etc. (De Hoog *et al.*, 2011). Cases are uncommon in humans and are mainly observed in cold-blooded vertebrates (De Hoog *et al.*, 2011). Below, we report a case of subcutaneous phaeohyphomycosis caused by the species *E. equina*.

**Case report**

The patient was a 75-year-old female living in South Korea, presenting with asymptomatic erythematous skin lesions (20–25 cm in diameter) on both forearms (Fig. 1a, b). The initial lesion started 8 months earlier, caused by the inoculation of a spine from a tree. Although she received treatment at a local clinic, the lesions on both arms increased in size and became suppurative. There was nothing of note in the patient’s family history or past medical history. All laboratory tests were non-specific or within normal range, except the liver function test (aspartate aminotransferase 57 U ml\(^{-1}\)/alanine aminotransferase, 89 U ml\(^{-1}\)).

Examination of KOH mounts from the lesions revealed many brownish hyphae. Histopathology with haematoxylin and eosin staining of purulent lesions showed a granulomatous response with histiocytes, polymorphonuclear cells and giant cells (Fig. 1c). With periodic acid Schiff staining short hyphal fragments were found in abscess samples; muriform cells were not observed (Fig. 1d). Fungal culture from skin biopsy specimens grown on Sabouraud’s dextrose agar yielded olivaceous–black, slimy colonies with velvety, olivaceous–grey centres and flat margins, which later became umbonate, felt, greyish–black, with velvety, grey centres (Fig. 2a). After incubation at 25 °C for 3 weeks the colony reverse was greyish–black. The fungus grew poorly on malt extract agar (MEA) at 37 °C, and did not grow at 40 °C. Microscopic morphology was indistinguishable from that of *E. jeanselmei* (Fig. 2b).

Based on the above features, the infection was provisionally diagnosed as a subcutaneous phaeohyphomycosis caused by *E. jeanselmei*. In an effort to provide a more definitive identification, subcultures were referred to the Centraalbureau voor Schimmelcultures (CBS) Fungal Biodiversity Centre, Utrecht, The Netherlands, for DNA sequencing. The fungus was grown on MEA and was transferred to a 2 ml Eppendorf tube containing 400 μl TEX-buffer (Tris 1.2 %, w/v, Na–EDTA 0.38 %, w/v, pH 9.0) with glass beads (Sigma G9143) and was homogenized by Mobio vortexing for 5–10 min. DNA was extracted according to methods described previously (Najafzadeh *et al.*, 2011b) and was subjected to routine methods of molecular identification (Najafzadeh *et al.*, 2011a).

The entire sequence of the rDNA ITS and a partial sequence of β-tubulin (*BT2*) domains were aligned with voucher strains maintained at the CBS, including type strains of all hitherto described *Exophiala* species. The isolate showed close sequence similarity with CBS 119.23, the type strain of *E. equina* (De Hoog *et al.*, 2011). The sequence data for the isolate were deposited in GenBank with accession numbers JQ797584 and JQ797585 for ITS and *BT2*, respectively. The isolate was deposited in the reference collection of the CBS-KNAW Fungal Biodiversity Centre, accession number CBS 128222.

![Fig. 1.](image1.png) **Fig. 1.** (a) Erythematous plaques with abscesses and crusts on both forearms. (b) Close-up view of the lesion on the left forearm. (c) Granulomatous inflammation with abscess in the dermis (haematoxylin and eosin stain, ×100). (d) Short hyphae within a dermal abscess (periodic acid Schiff stain, ×400).

![Fig. 2.](image2.png) **Fig. 2.** (a) Macroscopic morphology of *E. equina*. (b) Microscopic appearance of *E. equina* (CBS 128222). Conidia at the apices of annelidic conidiogenous cells, having pointed tips, are visible in the slide culture of *E. equina* (lactophenol–cotton blue stain, ×400).
The skin lesions were too extensive to be removed surgically. Itraconazole could not be used due to the abnormal liver function test. With oral fluconazole (50 mg day$^{-1}$ for 3 months), the skin lesions improved. There was no recurrence for 3 months after the treatment was completed.

**In vitro antifungal susceptibility testing**

The *in vitro* antifungal susceptibilities of *E. equina* were determined by microbroth dilution according to the Clinical and Laboratory Standards Institute document M38-A2 (CLSI, 2008). Methods for sporulation and preparation of suspensions were those of Najafzadeh et al. (2010). *Paecilomyces variotii* (ATCC 22319), *Candida parapsilosis* (ATCC 22019) and *Candida krusei* (ATCC 6258) were used as quality controls. The MICs of six of the eight antifungal drugs used in this study were: 1 µg ml$^{-1}$, amphotericin B; 64 µg ml$^{-1}$, fluconazole; 0.063 µg ml$^{-1}$, itraconazole; 2 µg ml$^{-1}$, voriconazole; 2 µg ml$^{-1}$, isavuconazole; and 0.031 µg ml$^{-1}$, posaconazole. The two echinocandins caspofungin and micafungin yielded minimum effective concentrations of >8 and 0.25 µg ml$^{-1}$, respectively.

**Discussion**

Dematiaceous fungi are an extremely heterogeneous group of opportunistic and potentially pathogenic species. A large number of the fungal species classified in this order are potential agents of human disease. The yeast-like representatives of this group belong to the genus *Exophiala*. Infections due to these fungi are uncommon, but have become increasingly significant because they concern a wide variety of clinical syndromes and because infections mostly occur in immunocompetent patients.

Microscopically, *Exophiala* species are very similar. Some species can be differentiated by physiological features such as temperature tolerance and nitrate assimilation. For numerous taxa molecular characterization is required (Revankar & Sutton, 2010); sequencing of the ITS rRNA region is usually sufficient for routine species distinction in the genus *Exophiala* (Revankar & Sutton, 2010). *Paecilomyces variotii* (ATCC 22319), *Candida parapsilosis* (ATCC 22019) and *Candida krusei* (ATCC 6258) were used as quality controls. The MICs of six of the eight antifungal drugs used in this study were: 1 µg ml$^{-1}$, amphotericin B; 64 µg ml$^{-1}$, fluconazole; 0.063 µg ml$^{-1}$, itraconazole; 2 µg ml$^{-1}$, voriconazole; 2 µg ml$^{-1}$, isavuconazole; and 0.031 µg ml$^{-1}$, posaconazole. The two echinocandins caspofungin and micafungin yielded minimum effective concentrations of >8 and 0.25 µg ml$^{-1}$, respectively.

**Acknowledgements**

The authors wish to thank Bert Gerrits van den Ende for his kind cooperation and assistance. The work of M. J. N. was financially supported by the School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.

**References**


Molecular epidemiology of Fonsecaea species. Emerg Infect Dis 17, 464–469.


