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

Received 8 January 2013
Accepted 20 February 2013

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

Abbreviations: CBS, Centraalbureau voor Schimmelcultures; ITS, internal transcribed spacer.

The GenBank/EMBL/DDBJ accession numbers for the ITS and BT2 sequences of the *E. equina* isolate are JQ797584 and JQ797585, respectively.
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, feltly, 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.
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* (Zeng & De Hoog, 2008). As an example, molecular studies have clearly shown that *E. jeanselmei*, previously thought to be a major agent of phaeohyphomycosis (Matsumoto et al., 1994), was a heterogeneous assemblage of several quite-different species (De Hoog et al., 2003; Vitale & De Hoog, 2002). In a recent study on the prevalence of clinical species in the USA, Zeng et al. (2007) reidentified strains by ITS sequencing and found that the prevalent species were *E. dermatitidis* (29%), *Exophiala xenobiotica* (20%) and *Exophiala oligosperma* (19%). The species *E. jeanselmei* made up only 8% of the isolates and is an agent of traumatic subcutaneous infection, eventually leading to eumycetoma (Badali et al., 2010), while other *Exophiala* species are isolated from either deep or superficial locations.

*E. equina* is a further segregate of *E. jeanselmei* (De Hoog et al., 2011). It is a member of a clade, mainly comprising waterborne *Exophiala* species, that is able to cause cutaneous or disseminated infections in cold-blooded water animals. Hosts mainly are fish, frogs, toads, turtles or crabs, while human infections are rare (De Hoog et al., 2011). The present case represents the first case, to the best of our knowledge, of subcutaneous phaeohyphomycosis in an elderly but immunocompetent individual. Inoculation of the agent was considered to have occurred from contact with contaminated plant material. *E. equina* was originally described from a subcutaneous infection of a horse’s lower leg (Pollacci, 1923). Among the human infections were a corneal ulcer (CBS 120905) and cases of onychomycosis (CBS 122267 and CBS 120387). The species was also noted on the skin of patients with diabetes, who had a relatively low temperature of the extremities due to impaired blood circulation, which allows invasion by species that are unable to grow at 37 °C. Further isolates were recovered from skin flakes (CBS 121285), stools (CBS 120906) and sputum (CBS 121286) (De Hoog et al., 2011). A transmission route involving bathing facilities, as hypothesized for domestic black yeast species (Lian & De Hoog, 2010), seems probable for this fungus as well.

The results of susceptibility testing have shown that itraconazole, posaconazole, amphotericin B and micafungin have high activities, while fluconazole has low activity against *E. equina*. The patient was treated with fluconazole (50 mg day\(^{-1}\) for 3 months), which seemed to be effective, but possibly poor growth at 37 °C and the immune system of the patient were sufficient to clear the infection within the 3 months of treatment.

**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.


