The first case of Onychomycosis due to *Exophiala dermatitidis* in Iran: Molecular identification and Antifungal Susceptibility

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ABSTRACT

Onychomycosis are a relatively common disorder. Black yeasts including *Exophiala* species are increasingly recognized as agents of human infection. *Exophiala* is the main genus of black yeast. They are often found in soil and generally distributed worldwide. Black yeasts fungi are rare case of onychomycosis. We report the first case of onychomycosis due to *Exophiala* (Wangiella) dermatitidis Iran. The fungus was identified by its morphological characteristics and through DNA sequencing of the internal transcribed spacer (ITS) region of rDNA. In vitro antifungal susceptibility has shown that itraconazole and posaconazole (0.063 μg/ml) had the highest activity against *E. dermatitidis*. 

Keywords: Phaeohyphomycosis; *Exophiala dermatitidis*; Black yeast; Onychomycosis

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INTRODUCTION

Melanized or dematiaceous fungi are a large and heterogeneous group of moulds that cause a wide range of diseases including phaeohyphomycosis, chromoblastomycosis and eumycotic mycetoma [1-2]. Dematiaceous fungi which also include members of the genus *Exophiala*, involve skin or soft tissue, or may be systemic infections, which have a significant mortality rate and are widely distributed in the environment, especially in soil, wood and plant matter [3]. Several species indeed have marked phenetic characteristics, such as the large conidiophores of *E. spinifera*, or the thermo-tolerance and absence of nitrite assimilation in *E. dermatitidis*. The majority of species, however, are morphologically variable, due to their passage through complicated life cycles where diagnostic features are variably expressed [21] and, conversely, very similar microscopic structures can be expressed in phylogenetically remote species. In recent years diagnostic approaches have been supplemented by molecular tools, particularly sequence data of the RNA internal transcribed spacer (ITS) regions [22-24]. *Exophialadermatitidis* was first described from Japan by Kano[4], from a severe cutaneous infection in an adolescent patient without
any known immune disorder. Severe, fatal and disseminated cases were exclusively reported from healthy, mainly adolescent patients in East Asia, where the fungus became known as a major pathogen [5-7]. Furthermore it is particularly known as an asymptomatic colonizer of the lung of the patients with cystic fibrosis [8]. The route of infection is still a mystery. The species is known to occur in the environment and was recently proven to be particularly abundant on the tile and other insert surface of public Turkish steam baths of European sauna complexes, where temperatures of over 60 °C are reached on a daily basis, but was much less common in adjacent localities, which are about 25°C [7, 9]. In this study we report an onychomycosis due to *Exophiala dermatitidis* in an Iranian healthy woman.

**CASE REPORT**

We present a case of onychomycosis caused by *Exophiala dermatitidis* in a 54-year-old Iranian female without history of immunodeficiency and underlying disease who presented in June 2011 to Razi hospital in Tehran, Iran. The patient was mountaineer, she has blackish pigmentation in toenail and distal area of the nail was empty. The other nails and skin of the soles and interdigital webs were normal. Scrapings were collected deeply from hyperkeratotic distal areas. Examination of potassium hydroxide mounts from the samples revealed brown, septate, branching hyphae. The scrapings were cultured on malt extract agar at 25°C. The colony was initially moist and gray, becoming black or dark-green with dull surface after 7 days (Fig 1). Based on microscopy morphology it was identified as *E. dermatitidis* (Fig 2).

![Fig 1.Macroscopic appearance of *Exophiala dermatitidis* (CBS 130575) on MEA after incubating at 25 °C for 7 days.](image1)

![Fig 2. Microscopic appearance of *Exophiala dermatitidis* (CBS 130575)](image2)
For molecular verification, subcultures of the isolate were referred to the Central bureau voor Schimmel cultures Fungal Biodiversity Centre, Utrecht, The Netherlands, for DNA sequencing. The accession number assigned to our strain by Centraalbureau voor Schimmelcultures (CBS) (Utrecht, the Netherlands) is CBS 130575. The isolate was subjected to routine methods of molecular identification involving the ribosomal Internal Transcribed Spacer (ITS) domain[10]. Briefly, Mycelia were grown on 2% MEA plates for one week at 24°C. 1 cm² of fungal growth were then 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) and glass beads (Sigma G9143) to be homogenized by Mobiovortexing for 5-10 min. Aliquots of the homogenate were incubated with 120 μl SDS 10% and 10 μl proteinase K to which 120 μl of 5 M NaCl and 1/10 vol CTAB 10% (cetyltrimethylammonium bromide) buffer were added and mixed with 700 μl SEVAG (24:1, chloroform:isoamylalcohol). A total of 225 μl of 5 M NH₄-acetate was added and the solution was centrifuged. The resulting supernatant was transferred to 0.55 vol isopropanol and the pellet washed with ice cold 70% ethanol. After drying at room temperature, it was resuspended in 100 μl TE buffer (Tris 0.12% w/v, Na-EDTA 0.04% w/v) plus 1.5 μl RNAse 20 U/ml. ITS rDNA was amplified using primers V9 (5′-ATTACGTCCCTGCCCCTGTA-3′) and LS266 (5′-GCATTCCCCAAACACTCGACTC-3′) and sequenced with the internal primers ITS1 (5′-CCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-
Onychomycosis was considered as a fungal nail infection mainly caused by dermatophytes, sometimes yeasts and rarely caused by nondermatophyte molds such as dematiaceous fungi. Clinical features may include a history of trauma, involvement of only one or two toenails. Alternaria spp. [16], Curvularialunata[17], Chaetomiumglobosum[18], and Neoscytalidium[19] have been reported as an agent of onychomycosis. In our study we report a case of nail infection of a immunocompetent woman, recently isolated from a handrail. Subcutaneous infections in humans were less common (12.0%, involving sinusitis, mycetoma, and subcutaneous cysts), whereas strains were exceptional as commensals (0.5%, involving hair) (Fig. 3, 4) [25].Pathogenetic mechanisms of Exophialadermatitidis is unclear, a probable virulence factors are presence of melanin in cell wall, able to grow at temperatures above 37 °C and produces extracellular polysaccharide capsules[7].

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DISCLOSURE OF INTEREST
The authors declare that they have no conflicts of interest concerning this article.

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REFERENCES

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