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ORIGINAL ARTICLE

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 yeastsincluding Exophiala species are increasingly recognized as agents of humaninfection. 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) dermatitidisin 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 heterogenous group of moulds that cause a wide range of diseases including phaeohyphomycosis, chromoblastomycosis and eumycoticmycetoma[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 speciesindeed 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 rRNA 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 ℃ are reached on a daily basis, but was much less common in adjacent localities, which are about 25℃ [7, 9]. In this study we report an onychomycosisdue to*Exophialadermatitidis*in an Iranian healthy woman.

CASE REPORT

We present a case of onychomycosis caused by *Exophialadermatitidis* 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.

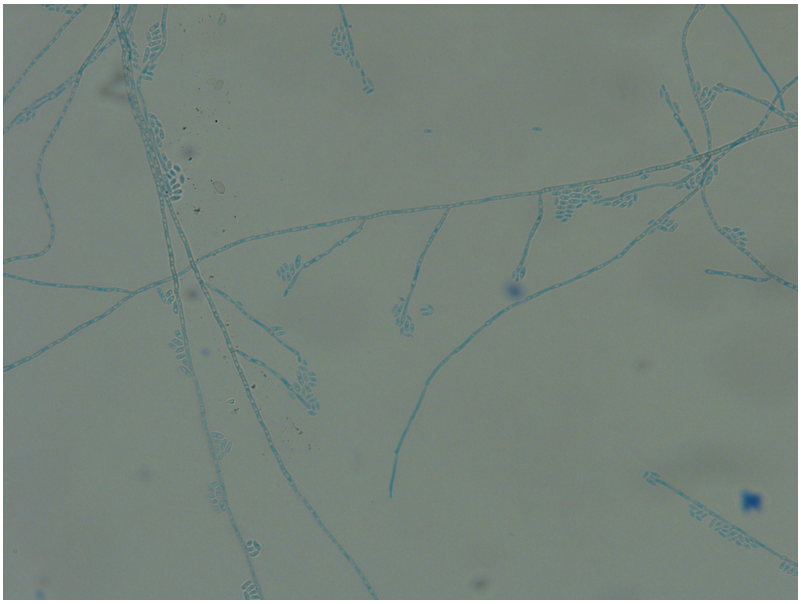

Fig 2. Microscopic appearance of Exophiala dermatitidis (CBS 130575)

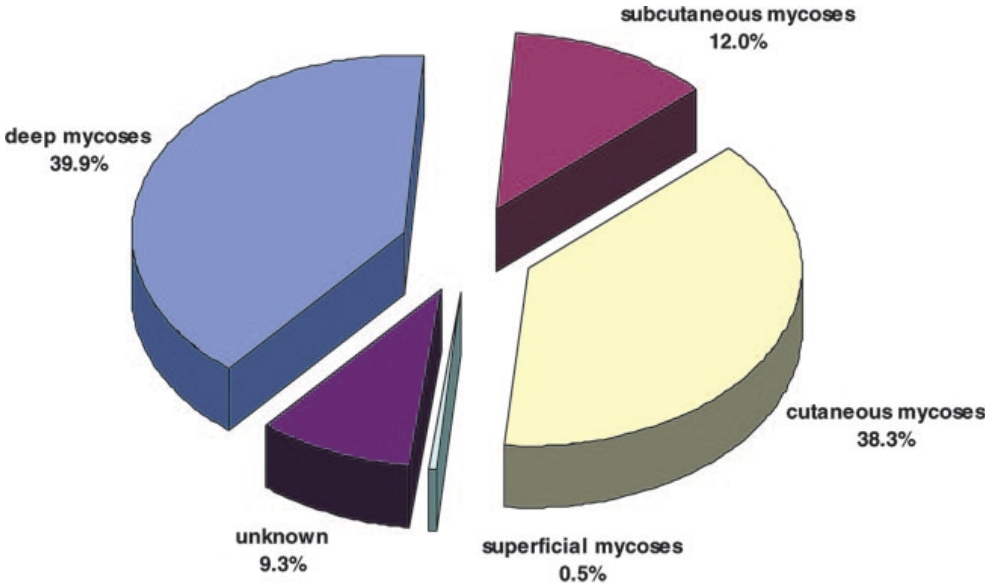

Fig 3.Localization of infections caused by Exophiala species in the United States

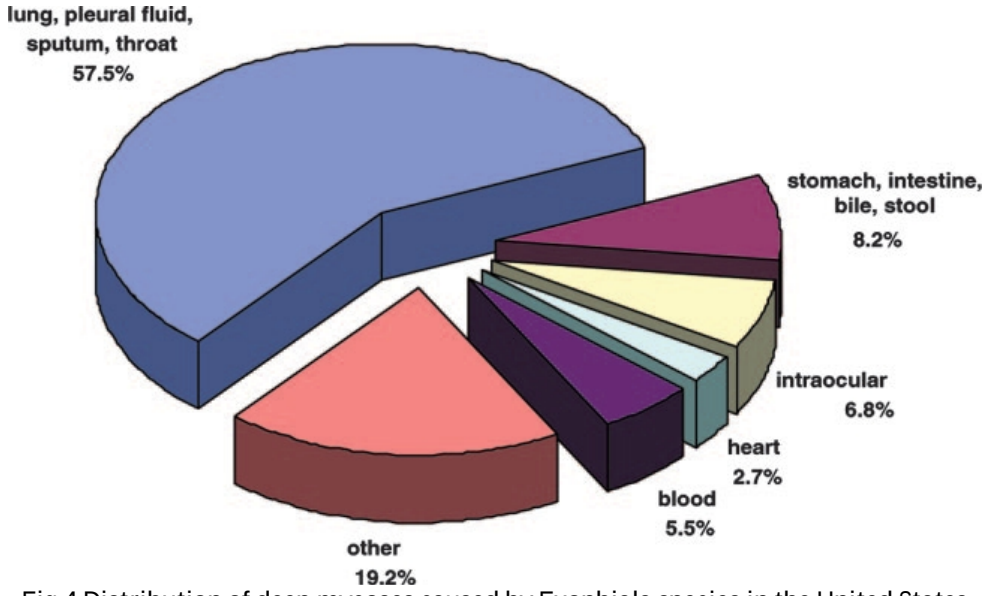

Fig 4.Distribution of deep mycoses caused by Exophiala species in the United States

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 CentraalbureauvoorSchimmelcultures (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 cm^2 of fungal growth were then transferred to a 2 ml Eppendorf tube containing 400 µ l 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 µ l SDS 10% and 10 µl proteinase K to which 120 µ l of 5 M NaCl and 1/10 vol CTAB 10% (cetyltrimethylammonium bromide) buffer were added and mixed with 700 µ l SEVAG (24:1, chloroform:isoamylalcohol). A total of 225 µ l of 5 M NH 4 -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 µ l TE buffer (Tris 0.12% w/v, Na- EDTA 0.04% w/v) plus 1.5 µ l RNAse 20 U/ml. ITS rDNA was amplified using primers V9G (5 ′-TTACGTCCCTGCCCTTTGTA-3 ′) and LS266 (5 ′-GCATTCCCAAACAACT CGACTC-3 ′) and sequenced with the internal primers ITS1 (5′-CCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-

TCCTCCGCTTATTGATATGC-3′). PCR amplification and sequencing were according to Najafzadeh*et al*[11]. Sequences were compared with Genbank and through local blast with a molecular database maintained for research purposes at the CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands. The obtained isolate was identified as *E. dermatitidis*by showing 99% similarity with the ex-type strain of that species (CBS 207.35, AF050269). rDNA ITS of *Exophialadermatitidis* CBS 130575 was deposited in Genbank as KC283188.

In vitro antifungal susceptibility testing

Minimal inhibitory concentrations (MICs) and minimum effective concentrations (MECs) for the Clinical isolate (CBS130575) towards eight antifungal agents were determined according to Clinical and Laboratory Standards Institute guidelines M38-A2 [12]. Methods for sporulation and preparation of suspensions were according to Najafzadeh*et al*[13]. The MICs of six of the eight antifungal drugs used in these studies were amphotericin B (0.250µg/ml), fluconazole [16 µg/ml), itraconazole (0.063µg/ml), voriconazole (0.125µg/ml),isavuconazole (0.500µg/ml), and posaconazole (0.063 µg/ml). The two echinocandin agents gave MECs for caspofungin (2µg/ml) and for micafungin (4µg/ml).

DISCUSSION

Phaeohyphomycosis is an amalgam of clinical disease caused by a wide variety of dematiaceous fungi. It is characterized by the presence of brown pigmented fungal element in tissue.[14]

Phaeohyphomycosis have wide spectrum of infections including superficial infections, onychomycosis, subcutaneous infections, keratitis, allergic disease, pneumonia, cerebral infections and disseminated disease [14-15]*Exophialadermatitidis*is one of themembers of the ascomycete order chaetothyriales in the family of herpotrichiellaceae, which has been reported as an agent of phaeohyphomycosis in the literatures and were repeatedly isolated from human systemic, single-organ infections (39.9%), particularly those involvingthe lungs (Fig. 3 and 4). More than 50% of the systemicstrains were isolated from the lungs, pleural fluid, or sputum, whereas isolation from the digestive system and feceswas uncommon. Cerebral infections were very rare. Strainsfrom human cutaneous infections, including skin, mucous membranes, nail, and corneal epithelium, were equally common as agents from deeplocalizations . Subcutaneousinfections 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].

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], *Chaetomiumglobusom*[18], and *Neoscytalidium*[19] have been reported as an agent of onychomycosis. In our study we report a case of nail infectiondue to*E.dermatitidis*in animmunocompetent woman, recently a case of onychomycosis caused by *E.dermatitidis*was reported by park *et al.*[3]From animmuno competent man. The results of MIC have indicated that amphotericin B, traconazole, voriconazole and posaconazole have good in vitro antifungal activities against *E. dermatitidis*; this issue was in agreement with the previous studies[20].

DISCLOSURE OF INTEREST

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

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