Isolation, identification and susceptibility of *Pyrenochaeta romeroi* in a case of eumycetoma of the foot in the UK

Sir,

Mycetoma, caused by fungi or filamentous bacteria, is a local, granulomatous, slowly progressive inflammatory disease with tumefaction and sinuses draining grain-filled pus primarily affecting the foot. The majority of the disease occurs between the latitudes of 15° south and 30° north [1]. In Europe, only a few cases, presumably imported, have been reported [2]. If untreated, this disease may lead to severe local tissue destruction with bone involvement, requiring surgical amputation [1].

The large numbers of causative organisms and the complexities of in vitro differentiation of fungi that cause mycetoma complicate the identification process. Molecular tests improve the quality of diagnosis [3].

A previously healthy 56-year-old male moved from Pakistan to the UK in 1967. He had worked barefoot in cotton processing, extensively exposed to raw cotton imported from various parts of the world. The patient first noticed localised swelling on the lateral side of his right foot in 1978. In 1979 and 1989 histology of a lesion biopsy revealed fungal hyphae.

The patient did not receive antifungal therapy until 1995, when itraconazole 200 mg twice a day (b.i.d.) was introduced, followed by the addition of flucytosine 1 g three times daily in combination. After 9 months of therapy, no improvement was observed and the antimycotics were discontinued. Magnetic resonance imaging (MRI) displayed appearances in keeping with fungal osteomyelitis of the right talus and calcaneum. Subsequently the patient's condition deteriorated with increased foot pain, localised swelling and formation of sinuses.

In October 1996, fungus was cultured from a sinus drain and was identified as *Madurella grisea* by macromorphology and micromorphology (confirmed by Bristol Reference Laboratory). The isolate is held in the Regional Mycology Laboratory Manchester's culture collection (isolate no. F4174). Susceptibility testing was performed with shaking at 30 °C by the macrobroth method using yeast nitrogen base (Difco, Oxford, UK) broth with 0.5% glucose as medium [4]. Inocula were prepared to a turbidity of between McFarland standards 4 and 5, then diluted 1:100. Minimum inhibitory concentrations (MICs) were read at 48 h with a no-growth endpoint. Itraconazole and voriconazole MICs [and minimum fungicidal concentration (MFCs)] were >32 mg/L and 0.25 mg/L, respectively.

In April 2002 the patient received voriconazole 200 mg b.i.d., which was discontinued after 7 weeks owing to elevated liver function tests, but was reintroduced at a reduced dose (150 mg b.i.d.) in July 2002 until October 2006. Minimal improvement was seen over this time. Random voriconazole serum levels were 2.9–5.3 mg/L.

In 2006, the isolate from 1996 was retrieved from storage and re-tested by a modified European Committee on Antimicrobial Susceptibility Testing (EUCAST) microbroth method (the modification being the inoculum concentration, prepared as previous). Voriconazole and posaconazole MICs were >8 mg/L and 0.25 mg/L and MFCs were >8 mg/L and 1 mg/L respectively; itraconazole was not tested.

The ITS sequence of isolate F4174 (GenBank accession no. FJ528674) was compared both with National Center for Biotechnology Information (NCBI) and local databases using BLAST. A phylogenetic tree was constructed (Fig. 1) using the most closely

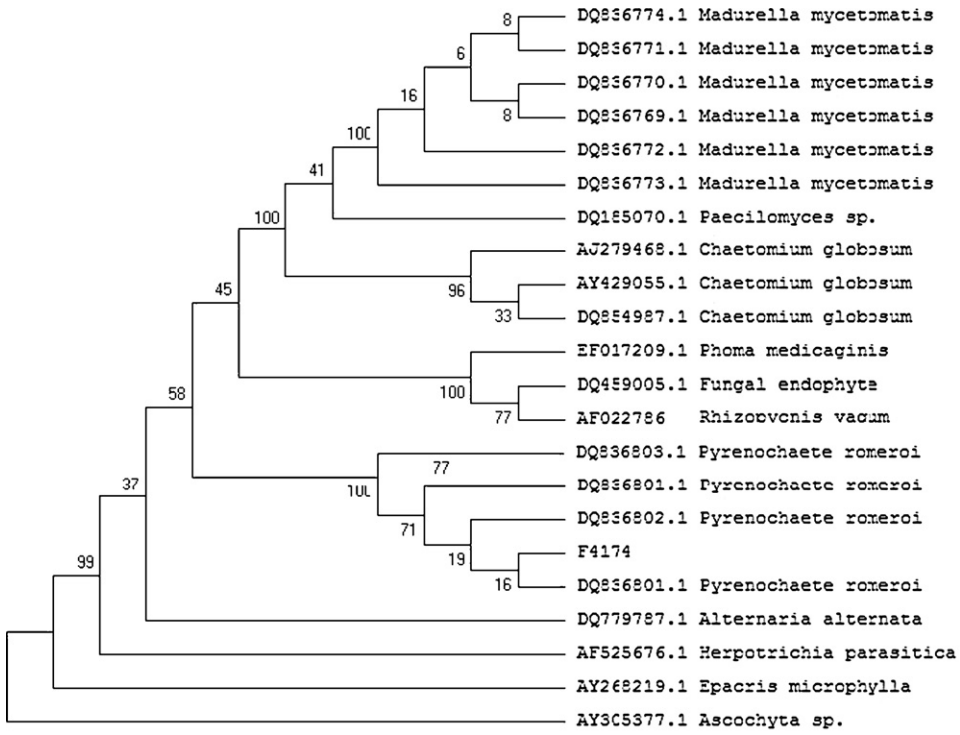

Fig. 1. Phylogenetic tree of the ITS sequences of isolate F4174 and closely related fungi, including members of the *Madurella* and *Pyrenochaeta* genera. Sequences were cropped to 433 bp and aligned using CLUSTALW (GO = 15, GE = 1, IUB DNA matrix, transition weight 0.5). Evolutionary history was inferred using the maximum parsimony (MP) method. The bootstrap consensus tree inferred from 2000 replicates is taken to represent the evolutionary history of the taxa analysed. Branches corresponding to partitions reproduced in <50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (2000 replicates) is shown next to the branches. The MP tree was obtained using the Close-Neighbor-Interchange algorithm with search level 3 in which the initial trees were obtained with the random addition of sequences (10 replicates). All positions containing gaps and missing data were eliminated from the data set (Complete Deletion option). There was a total of 433 positions in the final data set, out of which 172 were parsimony informative. Phylogenetic analyses were conducted in MEGA4. F4174 lies within the *Pyrenochaeta romeroi* clade.

related sequences plus a number of randomly chosen ascomycete outgroups. This showed the isolate to be within the *Pyrenochaeta romeroi* clade.

In April 2007, treatment with posaconazole 400 mg b.i.d. was started. Random posaconazole serum levels measured by bioassay were 0.9–2.1 mg/L. Following 17 months of posaconazole therapy there was nearly complete disappearance of local pain and decreased local swelling. MRI in August 2008 of the patient's foot showed extensive bony and soft tissue infection. Posaconazole was discontinued in March 2009 and further swelling was observed over the subsequent 6 months.

Pyrenochaeta romeroi is widely distributed worldwide, where it grows on soil and vegetation, particularly in tropical climates. It has been reported as a rare cause of mycetoma in humans [5,6].

Clinical trials have shown successful treatment of eumycetoma with ketoconazole and itraconazole [1]. However, our patient responded poorly or not at all to itraconazole, corroborating in vitro results. There are no reports on the use of voriconazole in *P. romeroi* infections. In our patient, voriconazole was minimally effective and response was best classed as a failure. The difference in voriconazole susceptibility results observed is presumably due to the change in methodology, as on both occasions the same isolate was tested.

Unresponsiveness to treatment with voriconazole in our patient corroborates the susceptibility results using the modified EUCAST method. Posaconazole has been shown to have comparable or improved in vitro activity compared with itraconazole against agents causing mycetoma [7]. A literature search revealed no reports on the clinical efficacy of posaconazole in *Pyrenochaeta* infections. A modest response at best was seen in our patient with posaconazole initially, with clinical evidence of breakthrough more recently.

Considering the fact that our patient had worked barefoot in direct contact with cotton imported from mycetoma-endemic countries with possible microscopic injuries to his feet, as well as the absence of any known preceding injuries, this seems the probable route of infection. We believe that this case is the first description of this mode of mycetoma acquisition.

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References

[1] Ahmed AOA, van Leeuwen W, Fahal A, van de Sande W, Verbrugh H, van Belkum A. Mycetoma caused by *Madurella mycetomatis*: a neglected infectious burden. Lancet Infect Dis 2004;4:566–74.

[2] Hay RJ, Mackenzie DW. Mycetoma (madura foot) in the United Kingdom—a survey of forty-four cases. Clin Exp Dermatol 1983;8:553–62.

[3] Desnos-Ollivier M, Bretagne S, Dromer F, Lortholary O, Dannaoui E. Molecular identification of black-grain mycetoma agents. J Clin Microbiol 2006;44:3517–23.

[4] Denning DW, Hanson LH, Perlman AM, Stevens DA. In vitro susceptibility and synergy studies of *Aspergillus* species to conventional and new agents. Diagn Microbiol Infect Dis 1992;15:21–34.

[5] Maiti PK, Haldar PK. Mycetomas in two different trauma-prone parts of body: a study of 212 cases. Indian J Med Microbiol 1998;16:19–22.

[6] Mohanty JC, Mohanty SK, Sahoo A, Ghosh SK, Pattnaik KL. Eumycetoma caused by *Pyrenochaeta romeroi*—a case report. Indian J Dermatol 2000;45:76–7.

[7] Sabatelli F, Patel R, Mann PA, Mendrick CA, Norris CC, Hare R, et al. In vitro activities of posaconazole, fluconazole, itraconazole, voriconazole, and amphotericin B against a large collection of clinically important molds and yeasts. Antimicrob Agents Chemother 2006;50:2009–15.

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Propranolol inhibits *Candida albicans* adherence and biofilm formation on biotic and abiotic surfaces

Sir,

Candida albicans is associated with nosocomial infections partly due to its ability to adhere to a variety of biomaterials such as catheters and form biofilms. Biofilms have clinical repercussions, mainly due to their notorious resistance to antimicrobial agents and host immune defences [1]. A mature *C. albicans* biofilm is formed of a biphasic structure composed of a thin layer of yeast cells covered by hyphal elements embedded in a layer of extracellular material [2]. Hyphal formation is essential to the structural integrity of *C. albicans* biofilms [1,2]. Events that inhibit *C. albicans* filamentation probably impair biofilm formation. Here we describe the effects of propranolol on hyphae formation by planktonic *C. albicans* cells [3,4] as well as on biofilm development. Propranolol, a β-adrenergic receptor antagonist, probably binds to phosphatidic acid (PA), blocking diacylglycerol (DAG) synthesis [3].

Propranolol (0.5–1 mM) significantly reduced *C. albicans* biofilm formation (Fig. 1A). The inhibitory effect of propranolol was also observed after the onset of biofilm development, when the addition of 1 mM propranolol at 6 h after the onset significantly inhibited biofilm formation (Fig. 1C). However, the addition of propranolol 24 h after the onset showed only a slight inhibitory effect, suggesting that propranolol acts mainly in the early stages of *C. albicans* biofilm formation (Fig. 1D). Additionally, ultrastructural analyses revealed that biofilms grown in the presence of propranolol are fragile, resulting in biofilms formed by monolayers of elongated cells (Fig. 1B).

In addition to the importance of the morphological transition in mature biofilm formation, initial attachment of cells to a substrate is crucial in the establishment of this microbial community. Thus, we also evaluated the effect of propranolol on adherence of *C. albicans* to abiotic surfaces. The attachment step, required for the formation of biofilm, is greatly affected by this compound. This result, together with the inhibitory effect on germ tube formation, may explain the effect of propranolol on *C. albicans* biofilm development.

The ability of *C. albicans* cells to attach to host epithelial and endothelial cells is also an important factor involved in the initial stages of colonisation and subsequent development of fungal infection. Consequently, we evaluated the effect of propranolol on the adherence of *C. albicans* to HeLa cells. Adhesion of fungal cells to epithelial cells was significantly affected by propranolol. Thus, propranolol could prevent the early stages of *C. albicans* colonisation and biofilm formation on biological and non-biological surfaces.

Adherence of microorganisms to surfaces involves numerous factors such as cell hydrophobicity, electrostatic forces and spe-