Case Report

A case of infection caused by the basidiomycete *Phellinus undulatus*

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We present a case of soft tissue infection caused by the basidiomycete *Phellinus undulatus*. To our knowledge, this is the first reported case of human infection caused by this fungus. Definitive identification was only possible through molecular analysis as the isolate failed to produce any distinct morphological features *in vitro*.

Introduction

Infections caused by filamentous basidiomycetes are rare, with most reports citing *Schizophyllum commune* or *Coprinus* species as the most common aetiological agents (Gené *et al.*, 1996; Sigler & Abbott, 1997; González *et al.*, 2001). The clinical spectrum of infections can include allergic bronchopulmonary disease (Kamei *et al.*, 1994), fungal ball of the lung (Sigler *et al.*, 1995), sinusitis (Rosenthal *et al.*, 1992; Sigler *et al.*, 1999), endocarditis (Speller & MacIver, 1971), brain abscess (Rihs *et al.*, 1996) and fatal pneumonia (Verweij *et al.*, 1997). We report a case of cutaneous infection caused by the basidiomycete *Phellinus undulatus*.

Case report

A 57-year-old type 2 diabetic female presented to the renal outpatient clinic (Middlemore Hospital, Auckland, New Zealand) in September 2008, with a gradually expanding mass on the medial aspect of her right knee. There was no history of recent trauma to the knee. Six years previously, she had been diagnosed with end-stage kidney failure secondary to diabetic nephropathy, and had commenced haemodialysis at that time. Her dialysis regimen consisted of three-times-weekly treatments through a left brachiocephalic fistula. Her other active medical co-morbidity included treated hypertension and ischaemic heart disease. She had a history of a previous central venous catheter-related blood stream infection secondary to *Staphylococcus aureus* in 2002.

On examination, the mass was non-tender and fluctuant, and was initially thought to be a lipoma. Magnetic resonance imaging examination of the knee revealed a well-circumscribed round mass within the subcutaneous tissue at the medial aspect of the right knee, measuring approximately

5 × 5 × 4 cm, with no involvement of deep structures or the knee joint. Further examination of the mass by ultrasound showed it to be compressible and hypoechoic, with no internal vascularity. Referral was made to the orthopaedic team (Middlemore Hospital) for surgical excision, which was undertaken in December 2008. The operative findings were of a large pus-filled cyst, which was completely excised.

Histological examination revealed a pseudocyst, containing thick creamy material. Microscopy showed chronic inflammatory changes, and staining with periodic acid-Schiff stain revealed the presence of non-branching, septate fungal hyphae within the wall of the pseudocyst. No organisms were seen on Gram staining, and there was no evidence of malignancy. Routine bacteriological cultures taken from the lesion perioperatively did not show growth; however, after incubation at 37 °C for 4 days, a few pure colonies of a fungus were seen on a horse blood agar plate. This was subcultured onto plain Sabouraud dextrose agar, and incubated at 25 and 37 °C. After 7 days at 25 °C, a yellow/cream-coloured, fluffy thallus with a yellow/brown reverse was seen (Fig. 1). The isolate did not grow on media containing cycloheximide (0.05 %), but was tolerant of benomyl (2 µg ml⁻¹). Microscopic examination revealed hyaline, septate hyphae without any particular features.

The isolate remained sterile in culture, so definitive identification was carried out by molecular analysis. Fungal chromosomal DNA was extracted from fungal culture using the PrepMan Ultra sample preparation reagent, as per the manufacturer's instructions. The D1, D2 and D3 region of the 25–28S (large subunit rRNA) was amplified with primers LROR and LR16 (Chen *et al.*, 2002). Amplification was performed in a 0.2 ml PCR tube with a total reaction volume of 50 µl using a GeneAmp PCR System 9700 thermocycler (Applied Biosystems). Each reaction tube contained 2.5 U *Taq* DNA polymerase,

Fig. 1. A fluffy, yellow/brown thallus was observed after 7 days at 25 °C on a plain Sabouraud dextrose agar plate.

250 µM (each of) dATP, dCTP, dGTP and dTTP, 20 mM Tris/HCl, 50 mM KCl, 2.0 mM MgCl₂, 0.1 µg primers µl⁻¹ and 5 µl sample DNA. A round of PCR was conducted with an initial 2 min denaturation step at 94 °C, coupled to a repeating cycle of 45 s at 94 °C, 45 s at 50 °C and 45 s at 72 °C for 40 cycles, followed by a 2 min final extension step at 72 °C.

The oligonucleotide primer LROR (5 µM) was used for sequencing. The PCR products used as templates for sequencing were purified using a High Pure PCR purification kit (Roche Diagnostics). Sequencing reactions were performed by using an ABI Prism BigDye terminator cycle sequencing kit in a GeneAmp PCR system 9700 thermal cycler and were electrophoresed using an ABI Prism 310 genetic analyzer. The sequence data were edited using DNASTAR software. A BLAST search was performed using the National Center for Biotechnology Information database. This revealed 99% identity with *P. undulatus*. The patient was followed up in the outpatient clinic (Middlemore Hospital), and at 9 months post-excision, there was no recurrence of infection, and no anti-fungal therapy was ever administered.

Discussion

The filamentous basidiomycetes are ubiquitous fungi that are commonly isolated from clinical specimens. However, their pathogenic role is often unclear, and cases of actual infection caused by basidiomycetes remain relatively rare (González *et al.*, 2001). Furthermore, identification is difficult, as many isolates do not sporulate and remain sterile in culture. In this patient, however, there was significant corroborative evidence that *P. undulatus* was the true cause of her infection (pure growth in culture, absence of any other bacterial or mycobacterial agents and, most

importantly, demonstration of fungal hyphae in histopathology specimens). Antifungal susceptibility testing of this isolate was not performed, as clinically it was felt that surgical excision of the lesion would be curative, and this has proved correct to October 2010. However, in one of the few past reports of an infection with *Phellinus* spp., it was necessary to utilize triple antifungal therapy with intravenous liposomal amphotericin B, intravenous caspofungin and oral voriconazole (Davis *et al.*, 2007).

Filamentous fungi are often agents of opportunistic infection in immunodeficient patients (Richardson & Lass-Flörl, 2008). Although our patient was not frankly immunocompromised, uraemia does result in a variable degree of impairment to host defences, and can be expected to increase susceptibility to novel and opportunistic pathogens (Meier *et al.*, 2002). The portal of entry for infection in our patient was not obvious, although the circumscribed nature of her infection is suggestive of local traumatic inoculation. However, the patient had no recollection of any notable trauma at that site.

The genus *Phellinus* belongs to the family *Hymenochaetaceae*, as does the closely related genus *Inonotus*. Most filamentous basidiomycetes such as these are obligate phytopathogens or wood-decaying fungi, and are ubiquitous in the environment. Approximately 220 species of *Phellinus* are recognized to date, many of which are known to occur throughout New Zealand on a variety of tree hosts (<http://nzfungi.landcareresearch.co.nz>).

Phellinus spp. produce numerous potential medicinal metabolites – the bioactivity of these is the subject of ongoing research (Dai *et al.*, 2010). However, to our knowledge there have been few documented infections with *Phellinus* spp. and none citing *P. undulatus* as the aetiological agent (Sutton *et al.*, 2005; Davis *et al.*, 2007).

The failure of many basidiomycetes to yield diagnostic characteristics means that identification is possible only by molecular analysis, as for our isolate. It is likely that the future increased use of molecular identification tools will expand the identification of basidiomycetes as agents of infection.

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