

Recurrent Phaeohyphomycosis due to *Phaeoacremonium alvesii* Identified with Internal Transcribed Spacer and Beta-tubulin Gene Sequencing

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Phaeoacremonium species that have been isolated from wood splinters and soil are known to be pathogens of phaeohyphomycosis in humans (1). We report here a rare case of recurrent cutaneous phaeohyphomycosis caused by *Phaeoacremonium alvesii* in an immunocompromised host.

CASE REPORT

A 93-year-old woman had been treated with prednisolone, 10 mg per day, for erythema multiforme for 4 years in a local clinic. She also had treatment with calcium channel blockers for hypertension. She was referred to our hospital due to a nodule on her left thigh. No significant skin trauma was mentioned. Physical examination revealed an intradermal nodule, 40×25 mm in size, with purulent discharge on her left thigh (Fig. 1a). The lesion was resected under local anaesthesia. Histopathological examination revealed a cystic lesion in the dermis to the subcutaneous tissue. The cavity was

lined with a mixed-cellular granulomatous wall without epithelial components (Fig. 1c). Histiocytes and multinucleated giant cells were observed in the granuloma (Fig. 1d). Periodic acid–Schiff stain and Grocott–Gomori’s methenamine silver stain showed many branching, septate hyphae (Fig. 1e, f). A fungal culture was not performed at the time of excision because the whole specimen was fixed in formalin fixative under the initial diagnosis of an epidermal cyst. DNA extracted from paraffin-embedded tissue was analysed using PCR. *P. alvesii* or *P. rubrigenum* was estimated from sequence analysis of the obtained internal transcribed spacer (ITS) regions of a ribosomal DNA gene. A wait-and-see approach was adopted because the cyst appeared to have been totally resected. Serum β-D-glucan was negative and no abnormality was seen on a chest X-ray. A tumour reappeared adjacent to the operation scar 1 year 11 months later, and the patient was admitted to our hospital. Physical examination revealed an intradermal nodule, 70×50 mm in size, under the surgical scar on her left thigh (Fig. 1b). Histopathological findings of a biopsy specimen were the same as previously. Culture of the pus resulted in formation of a circular velvety, pale-brown coloured colony on the surface, with a brown-coloured colony on the back, 48-mm in size, after 14 days of incubation on malt extract agar and potato-dextrose agar (Nissui Pharmaceutical Co. Ltd, Tokyo, Japan) at 27°C (Kanazawa Medical University [KMU] 10268; GenBank [DDBJ] accession number for ITS, LC508975; tubulin, LC508976) (Fig. S1a–d¹). The maximum growth temperature of the colony was 37°C on both media. A slide culture of the isolate revealed pale brownish branched conidiophores tapering towards the apex (Fig. S1e¹), suggesting *Phaeoacremonium* spp. Observation with a scanning electron microscope revealed phialide shapes with 12-μm long, elongate-ampulliform attenuated at the base (Fig. S1f¹), suggesting Type II phialides according to the classification of phialides by type (2). The conidia were also mostly ovoid, approximately 4 μm long. The percentage of identity of the clinical isolate (KMU10268; GenBank [DDBJ] accession number for ITS, LC508975; tubulin, LC508976) compared with the *P. alvesii* type strain was 99.8% and 99.5%. The percentage of identity of the clinical isolate compared with the *P. rubrigenum* type strain was 99.6% and 95.7%. Finally, the identification of *P. alvesii* was made. The patient was not able to stop taking corticosteroids due to the risk of adrenal crisis. Treatment with terbinafine was initiated at a dose of 125 mg daily in conjunction with localized hyperthermia because the patient declined surgical resection. The patient was lost to follow-up due to old age.

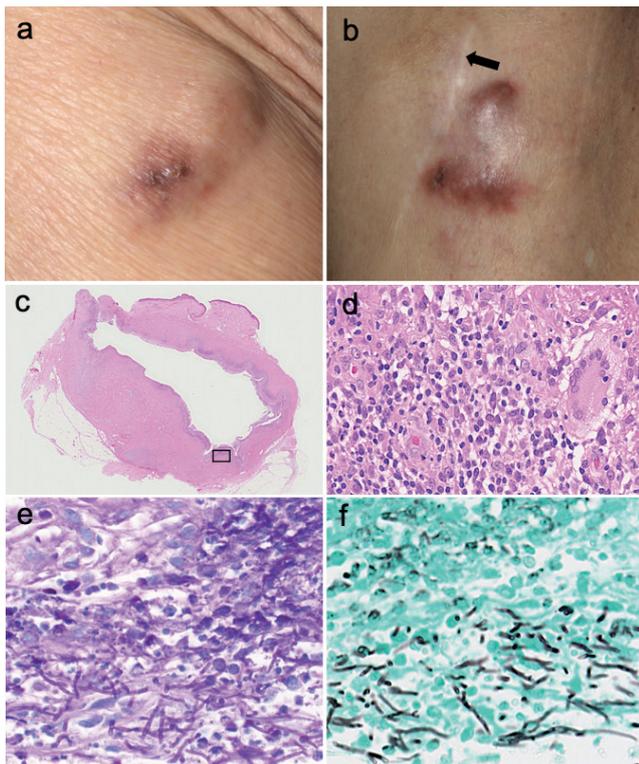


Fig. 1. (a) An intradermal nodule on the left thigh at the first visit. (b) Recurrence under a postoperative scar (arrows). (c) An excised specimen taken from the thigh (haematoxylin and eosin (HE), original magnification ×20). (d) Histiocytes and multinucleated giant cells in square of Fig. 2a (HE ×200). (e) Periodic acid–Schiff stain (×400). (f) Grocott–Gomori’s methenamine silver stain (×400).

DISCUSSION

Ajello et al. first described a subcutaneous abscess caused by *Phialophora parasitica* as “Phaeohyphomycosis” (3). Approximately 65% of infectious diseases caused by *Phaeoacremonium* spp are soft tissue infections. Clinically,

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Table I. Reported cases of human infections due to *Phaeoacremonium alvesii*

Author	Source	Age, years/sex	Immunosuppression	History of trauma	Location	Clinical finding	Treatment
Padhye et al. (7)	USA	83/F	Unknown	No	Acrotarsium	Subcutaneous nodule	Excision
Guarro et al. (8)	Brazil	19/M	Non-existence	No	Ankle	Subcutaneous nodule	Excision, Itraconazole
Mostert et al. (2)	USA	Unknown	Unknown	Unknown	Synovial fluid, Cornea	Unknown	Unknown
Current case	Japan	93/F	Existence	No	Thigh	Subcutaneous nodule	Excision, Terbinafine

subcutaneous abscesses and cysts or chronic or acute arthritis are the most common features (4). Although phaeohyphomycosis is often caused by trauma, in our case the patient had no memory of trauma. Histopathologically, the characteristic lesion is a circumscribed cyst or chronic abscess located in the subcutis or lower dermis (5). The cyst wall is composed of dense fibrous tissue with a chronic granulomatous reaction facing the cavity (5). Regarding *Phaeoacremonium* species, *P. parasiticum* is the most frequent species (6). Only 2 cases of human subcutaneous infection due to *P. alvesii* have been reported (2, 7, 8) (Table I). The pathogenic fungi in those 2 previous cases were initially reported to be *P. inflatipes* and *P. aleophilum*; however, Mostert et al. corrected them to *P. alvesii* based on the results of actin and β -tubulin sequence analysis in 2005 (2). The first reported case was an 83-year-old woman with a soft mass on her foot. She had no past medical history. The lesion was cured by excision without complications (7). Histopathological examination of the mass showed proliferation of giant cells surrounded by a granuloma and phaeoid hyphal elements. The second reported case was a 19-year-old man in a non-immunocompromised condition with a fistulized nodule on his ankle (8). The nodule reappeared 6 times after only excision on all occasions and was finally cured by a combination of oral itraconazole and excision. Histopathology was not shown in the report.

There is no standard treatment for phaeohyphomycosis (2). Surgical removal has provided cure for localized skin lesions. In more invasive cases, however, systemic antifungals are usually required (9). Phaeohyphomycosis caused by *Phaeoacremonium* is less common, but some fatal cases directly related to the fungal infection have been reported in immunocompromised hosts (6). Even in localized cases, recurrence after resection, as in the previously reported case and the current case, should be considered and the cases should be treated with care. Minimum inhibitory concentration (MIC) was not done in our case. According to a previous case report of phaeohyphomycosis caused by *P. alvesii* (8), the MIC of antifungal drugs were: amphotericin B (2 μ g/ml), fluconazole (8 μ g/ml), itraconazole (>16 μ g/ml), terbinafine (2 μ g/ml) and voriconazole (1 μ g/ml). Terbinafine was chosen because of contraindications of co-administration with calcium channel blockers. It is important for clinicians to consider performing histopathological and microbiological examinations at the time of excision of a chronic subcutaneous nodule or cyst in patients who are immunocompromised or who have a history of trauma. In microbiological examination, conidiophores

of *Phaeoacremonium* species are erect, nearly cylindrical and slightly tapered, straight or flexuous, and variable in length (2). Phialide shapes that show elongate-ampulliform attenuated at the base can be confirmed by observation with a light microscope (2). Furthermore, molecular biological analysis including sequencing of β -tubulin has been established and contributes to the accurate identification of the species of causative pathogens (2). This implies that previously identified *Phaeoacremonium* species with ITS regions of a ribosomal DNA gene only may be found to be a different species by analysis of β -tubulin genes.

It is predicted that reports of *Phaeoacremonium* infection will increase due to the improvement in identification methods and the increase in immunocompromised conditions in the population. The current case and the previously reported case (8) indicate that attention should be paid to the possibility of recurrence and the need for long-term follow-up in cases of cutaneous *P. alvesii* infection. Further accumulation of cases, including isolation of pathogens and the determination of optimal treatment, are required.

The authors have no conflicts of interest to declare.

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