



## Implantation subcutaneous phaeohyphomycosis caused by *Rhytidhysteron rufulum*: A case report

Tanis C. Dingle<sup>a,b,\*</sup>, Bradley Jansen<sup>a</sup>, Christopher Walker<sup>c</sup>, Medica Sam<sup>d</sup>, Bob Verity<sup>e,f</sup>, Daniel Purdy<sup>e</sup>, Paulose Paul<sup>g</sup>, Ilan S. Schwartz<sup>h</sup>

<sup>a</sup> Alberta Precision Laboratories – Public Health Laboratory, Edmonton, AB, Canada

<sup>b</sup> Department of Pathology and Laboratory Medicine, University of Calgary, Calgary, AB, Canada

<sup>c</sup> Alberta Precision Laboratories, Edmonton, AB, Canada

<sup>d</sup> Department of Diagnostic Imaging, Misericordia Hospital, Edmonton, AB, Canada

<sup>e</sup> Department of Laboratory Medicine and Pathology, University of Alberta, Edmonton, AB, Canada

<sup>f</sup> DynaLIFE Medical Labs, Edmonton, AB, Canada

<sup>g</sup> Department of Surgery, University of Alberta, Edmonton, AB, Canada

<sup>h</sup> Department of Medicine, University of Alberta, Edmonton, AB, Canada

### ARTICLE INFO

#### Keywords:

Phaeohyphomycosis  
Fungal infection  
Mycology  
*Rhytidhysteron rufulum*

### ABSTRACT

Subcutaneous phaeohyphomycosis is caused by traumatic implantation of melanized environmental fungi. The majority of cases occur in tropical areas of the world or are associated with travel from these regions. Herein, we describe a rare case of subcutaneous phaeohyphomycosis caused by *Rhytidhysteron rufulum* in an immunocompetent Somalia-born patient. The use of molecular diagnostics as an essential tool for identification of rare fungal pathogens is highlighted.

### 1. Introduction

Phaeohyphomycosis englobe several clinical manifestations, including superficial, cutaneous, subcutaneous and systemic infections, caused by melanized fungi, observed in tissue as hyphae, yeast cells, vesicular elements or a combination of fungal elements. The fungi causing phaeohyphomycosis are generally not highly virulent, save for a few species, and cause infection in both immunocompetent and immunocompromised hosts. Most infections are acquired by traumatic implantation, as in the case report described here. Herein, we describe a case of phaeohyphomycosis caused by the fungus *Rhytidhysteron rufulum* in a foreign-born Canadian patient. While this organism is found readily in the environment in tropical regions of the world, it is a rare cause of subcutaneous phaeohyphomycosis though becoming more frequently described in the literature.

### 2. Case presentation

A 38-year-old woman presented with a chronic nodule on her left leg. The lesion had been present for approximately 2 years, and there was no clear antecedent trauma. The lesion fluctuated in size, and was non

painful.

She was originally from Somalia, and had lived there until approximately a decade prior to presentation. She had also traveled to Kenya. The patient had a history of diabetes mellitus for approximately 7 years, managed with insulin. She had recently completed a 9-month course of treatment for culture-proven tuberculosis involving lungs and cervical lymph nodes.

On examination (Day 0), there was a fluctuant, non-tender mass at the anterior left shin. There was no fistula or drainage. An ultrasound on Day 0 demonstrated a complex cystic lesion with vascularity and calcification, which was intimately associated with the fascia of the tibialis anterior muscle. An MRI at day +28 showed a lobulated mass within the subcutaneous fat on the anterior aspect of the left shin. The lesion measured 2.2 cm craniocaudal, 2.2 cm transverse and 1.1 cm AP. It was peripherally enhancing, and T2 hyperintense. There mass did not invade into muscle. The lesion was followed clinically, and another MRI was performed 16 months later (Day +512) (Fig. 1). At that time, the lesion had increased in size to 2.7 cm craniocaudal, 2.4 cm transverse, and 1.2 cm AP. Given the increase in size, it was considered concerning for sarcoma.

An excisional biopsy was performed on Day +547. Tissue was sent to

\* Corresponding author. Alberta Precision Laboratories – Public Health Laboratory, Edmonton, AB, Canada.

E-mail address: [tanis.dingle@aplabs.ca](mailto:tanis.dingle@aplabs.ca) (T.C. Dingle).

<https://doi.org/10.1016/j.mmcr.2022.03.002>

Received 15 December 2021; Received in revised form 14 January 2022; Accepted 2 March 2022

Available online 5 March 2022

2211-7539/© 2022 The Authors. Published by Elsevier B.V. on behalf of International Society for Human and Animal Mycology. This is an open access article

under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

pathology and to the microbiology laboratory for fungal and mycobacterial culture. On pathology review, necrotizing granulomatous inflammation was observed with fungal elements on both the PAS and GMS staining (Fig. 2, lower right and left, respectively). Some areas of hyphal thickening were visible and no muriform cells were seen. The features seen were considered consistent with a deep fungal infection.

The microbiology laboratory set up bacterial, fungal and mycobacterial culture of the lesion biopsy. All culture media grew the same fungus. The isolate was slow-growing (>2 weeks) on phyto agar. The fungal culture grew a furry, dark brown colony with dark reverse suggesting a dematiaceous fungus (Fig. 2, upper left). On microscopy, hyphae were primarily observed with some areas of thickening (Fig. 2, upper right). Few loose oval conidia were also observed but were not enough to allow for microscopic identification. The isolate was subsequently sent for ITS sequencing [1] and identified as *Rhytidhysterion rufulum*.

Susceptibility testing was performed on the isolate per CLSI M38 guidelines [2]. Minimum inhibitory/effective concentrations were as follows: Amphotericin B 0.5 µg/mL, voriconazole 1.0 µg/mL, posaconazole 0.06 µg/mL, itraconazole 0.12 µg/mL and micafungin 0.03 µg/mL. There are currently no clinical breakpoints or epidemiologic cutoff values available to interpret susceptibility results for this species of fungus.

### 3. Discussion

Phaeohyphomycosis is a fungal infection involving subcutaneous or occasionally systemic disease caused by environmental melanized moulds. The primary causes of subcutaneous phaeohyphomycosis include *Fonsecaea pedrosoi*, *Phialophora verrucosa*, *Exophiala* species and *Cladophialophora carrionii*. Rarely, other environmental melanized fungi cause subcutaneous phaeohyphomycosis. Here we report a case of subcutaneous phaeohyphomycosis caused by *Rhytidhysterion rufulum* in an immunocompetent female with diabetes mellitus with no apparent trauma prior to development of the subcutaneous nodule two years prior.

*Rhytidhysterion* species are melanized saprophytic fungi in the Ascomycetes phylum associated with plant disease found primarily on the wood of citrus and mangrove plants [3]. *Rhytidhysterion* has a worldwide distribution but is primarily found in tropical and subtropical climates of the world [4]. Of the four known *Rhytidhysterion* species, only *R. rufulum* has been associated with human disease, albeit rarely [3]. There have been 8 reported cases of *R. rufulum* phaeohyphomycosis in the literature originating in India [1][3][4] [–] [9]. More recently, a single case of *R. rufulum* phaeohyphomycosis was reported in an Ethiopian-born

patient in Tennessee, USA [10]. Of the 10 reported cases, including ours, 8 were male and two were female. It is unclear whether the unequal sex distribution represents differences in exposure risks or susceptibility to disease, or diagnostic bias.

*R. rufulum* infection is acquired by traumatic implantation of contaminated soil or plant material, and occupational exposure (farming, walking barefoot) is the most common place of acquisition. Subcutaneous phaeohyphomycosis is the most common clinical presentation though a single case of subcutaneous *R. rufulum* chromoblastomycosis has also been reported in a renal transplant patient [4]. Reported cases have occurred in patients with diabetes [5,8,10] and kidney transplantation [4,10], however cases in persons with no underlying comorbidities have also been described [3,6,7,9]. Patients present with painless, swollen, soft, slow-growing, ulcerative or non-ulcerative nodules, most commonly on the feet or legs. Many patients present years after initial nodule observation and do not recall a specific trauma that incited their lesion. Diagnosis can be made by pathologic examination and fungal culture on nodule biopsy or fine needle aspirate of the lesion.

Upon histopathologic investigation, biopsies typically show irregular branching, septate hyphae which may be pigmented or non-pigmented. On direct KOH exam (if performed), thick brown septate hyphae are observed [3]. In culture, *R. rufulum* takes 2–3 weeks to grow from a clinical specimen, but grows well on routine fungal culture media. Initially the colony is light grey and velvety, becoming dark and cottony over time, with a darkly pigmented reverse. By microscopy, *R. rufulum* has dark, septate, smooth-walled hyphae [4] with occasional chlamydospores. Discoidal ascospores and bitunicate asci may be observed [4]. Ascospores are thick-walled, darkly pigmented and elliptical or fusiform shaped. The ascospores have 3 horizontal or longitudinal septa [3,5]. Due to lack of sporulation in culture, isolates require molecular identification, typically by ITS sequencing of the 18S rRNA gene, to definitively identify the organism. MALDI-TOF mass spectrometry has also proven reliable when databases are augmented with in-house generated spectra [11].

Accurate identification of the causative fungus in subcutaneous phaeohyphomycosis is essential to ensure appropriate patient management as significant species-specific antifungal susceptibility differences have been described. Due to its rarity, *Rhytidhysterion*-specific treatment protocols do not exist. ECMM guidelines on the management of phaeohyphomycosis indicate that surgery alone may be effective for treatment of subcutaneous nodules caused by melanized fungi [12]. Cryotherapy and laser therapy have also been successful. Oral antifungal agents are recommended as co-adjunctive therapy in immunocompromised patients and to prevent disseminated disease. For patients with

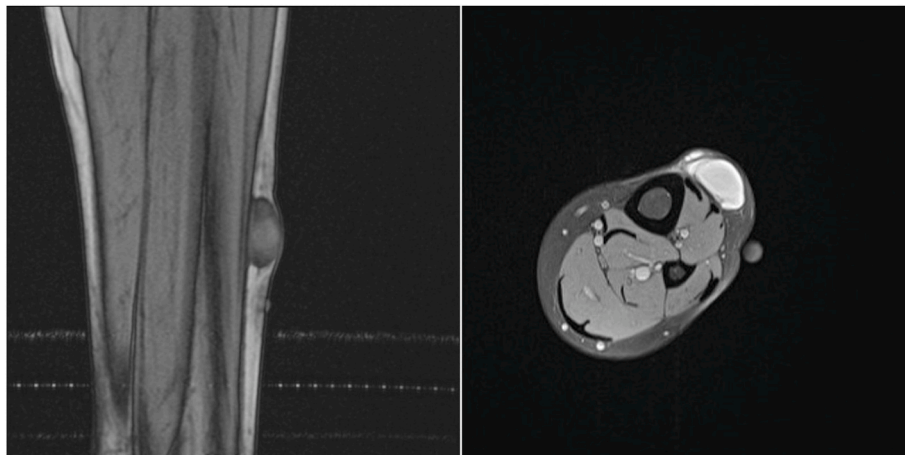


Fig. 1. Coronal (left) and axial (right) MRI of the left lower extremity. There is a non-specific T1 hypointense and T2 hyperintense oval mass in the subcutaneous fat left lower extremity along the anterolateral shin with broad base contact of the tibialis anterior muscle.

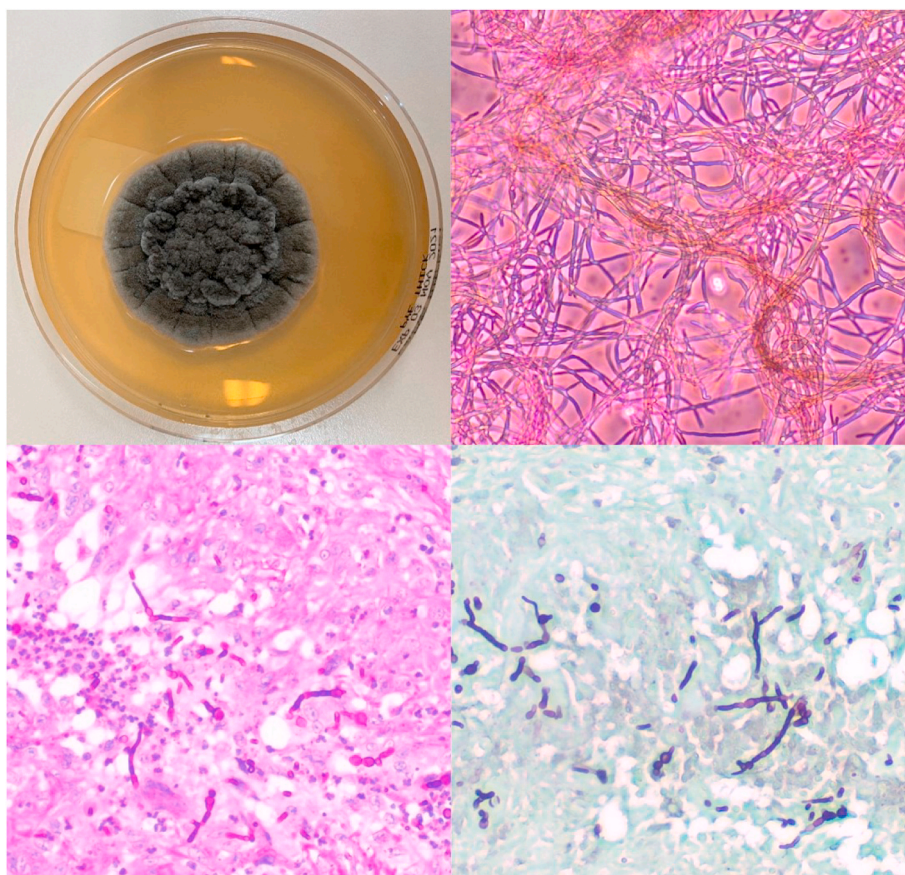


Fig. 2. Upper Left, *Rhytidhysterion rufulum* growth on a phytone plate. Upper right, *Rhytidhysterion rufulum* tape mount, 200X magnification. Lower left, PAS stain of lower leg lesion biopsy section, 200X magnification. Lower right, GMS stain of lower leg lesion biopsy section, 200X magnification.

multiple subcutaneous nodules, systemic antifungal therapy is recommended [12]. Of the reported cases of *R. rufulum* phaeohyphomycosis, the majority were treated with a combination of surgical excision and itraconazole. Combination therapy with terbinafine has also been described [8]. Our isolate had minimum inhibitory/effective concentrations less than or equal to 1.0 µg/mL for itraconazole, voriconazole, posaconazole, micafungin and amphotericin B.

In summary, we present a case of phaeohyphomycosis caused by *R. rufulum* in a Somalia-born patient in Canada. The patient was successfully managed by surgical excision of her lesion. This report, along with the others reported in the literature, highlight that molecular identification of rare melanized fungi is an asset to diagnosis and management of patients with subcutaneous phaeohyphomycosis.

#### Funding

This research did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors.

#### Declaration of competing interest

There are none.

#### References

- [1] Clinical and Laboratory Standards Institute, MM18: Interpretive Criteria for Identification of Bacteria and Fungi by Targeted DNA Sequencing, second ed., 2018.
- [2] Clinical Laboratory Standards Institute, M38: Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeast, third ed., 2017.
- [3] J. Chander, N. Singla, R. Kundu, U. Handa, A. Chowdhary, Phaeohyphomycosis caused by *Rhytidhysterion rufulum* and review of literature, *Mycopathologia* 182 (3–4) (2017) 403–407.
- [4] A. Chowdhary, J. Guarro, H.S. Randhawa, et al., A rare case of chromoblastomycosis in a renal transplant recipient caused by a non-sporulating species of *Rhytidhysterion*, *Med. Mycol.* 46 (2) (2008) 163–166.
- [5] A. Tejashree, C. Suchetha, K. Visakha, M. Krishna Karthik, A rare case of subcutaneous phaeohyphomycosis caused by *Rhytidhysterion rufulum*, *Indian J. Microbiol. Res.* 4 (4) (2017) 468.
- [6] Y. Sarita, A. Ruchi, C. Jagdish, C. Anuradha, A rare case report of subcutaneous mycoses by *Rhytidhysterion rufulum*, *Int. J. Med. Res. Health Sci.* 6 (4) (2017) 12–15.
- [7] K. Mishra, S. Das, S. Goyal, et al., Subcutaneous mycoses caused by *Rhytidhysterion* species in an immunocompetent patient, *Med Mycol Case Rep* 5 (1) (2014) 32.
- [8] V.K. Mahajan, V. Sharma, N. Prabha, et al., A rare case of subcutaneous phaeohyphomycosis caused by a *Rhytidhysterion* species: a clinico-therapeutic experience, *Int. J. Dermatol.* 53 (12) (2014) 1485–1489.
- [9] N. Mudhigeti, R. Patnayak, U. Kalawat, S.R.C. Yeddula, Subcutaneous *Rhytidhysterion* infection: a case report from South India with literature review, *Cureus* 10 (4) (2018).
- [10] J.A. Braue, R.W. Larue, A.S. Boyd, J.D. Fine, Phaeohyphomycosis caused by *Rhytidhysterion rufulum*, *Clin. Exp. Dermatol.* 45 (4) (2020) 524–526.
- [11] M. Fraser, A.M. Borman, E.M. Johnson, Rapid and robust identification of the agents of black-grain mycetoma by matrix-assisted laser desorption/ionization-time of flight mass spectrometry, *J. Clin. Microbiol.* 55 (8) (2017) 2521–2528.
- [12] A. Chowdhary, J.F. Meis, J. Guarro, et al., ESCMID and ECMM joint clinical guidelines for the diagnosis and management of systemic phaeohyphomycosis: diseases caused by black fungi, *Clin. Microbiol. Infect.* 20 (S3) (2014) 47–75.