



Subcutaneous phaeohyphomycosis due to *Phaeoacremonium venezuelense*: The first clinical case report in Costa Rica

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ABSTRACT

Phaeoacremonium is a genus of dematiaceous fungi that rarely causes human infections. We describe a case of subcutaneous infection in a 70-year-old diabetic man with lesions on the dorsum of the one foot. The agent was isolated, and for the final identification we performed matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) and DNA sequencing. After diagnosis, the patient underwent curettage of the cyst and received 100mg of Itraconazole, twice daily for 6 months. Clinical resolution of the lesion was observed after treatment. This is the first case of infection by *Phaeoacremonium venezuelense* reported in Costa Rica.

1. Introduction

Phaeohyphomycoses are a group of infections caused by dematiaceous fungi such as *Exophiala*, *Phialophora*, *Cladophialophora*, *Cladosporium*, *Curvularia*, and *Alternaria* [1,2] that are frequently present in soils and associated with plant detritus in warm and humid climates [3–5]. Phaeohyphomycoses can present as cutaneous, corneal, subcutaneous, and systemic infections after traumatic implantation of artifacts like thorns and wood splinters contaminated with fungal elements [1,3,6]. The lesion starts with a small papule that evolves into a large subcutaneous nodule with abundant purulent and necrotic material, some cases present verrucous plaques. In the affected extremity, there is significant inflammation and limitation of mobility [5]. The most common clinical manifestation worldwide is the subcutaneous phaeohyphomycosis or phaeohyphomycosis cyst, corresponding to a single abscess or granuloma, without lymphatic invasion and chronic evolution [6]. As weeks or months may pass before the lesion appears [3,5], in some cases, patients do not remember the event that caused the lesion [6]. This infection occurs in both immunosuppressed and immunocompetent individuals and in equal proportions among men and women, with most cases concentrated in adults between 30 and 50 years of age who work in

agriculture [3].

Here, we present the first confirmed case of phaeohyphomycosis in Costa Rica.

2. Case presentation

A 70-year-old non-insulin-dependent diabetic male, working as a farmer, was seen in the Emergency Department of the Escalante Pradilla Hospital, located in the southern region of Costa Rica. He had a 6-month history of pain and inflammation on the dorsum of the left foot, where a subcutaneous nodule of 4 cm in diameter, undefined margins, soft to palpation, and accompanied by edema and perilesional erythema was observed (Fig. 1A). The patient did not remember any traumatic event that could have caused the lesion. He had previously undergone drainage of the abscess and prescription of several courses of antibiotics with recurrence of the lesion. On day zero, the abscess once more was drained and the extracted material was sent to the clinical laboratory. On day 2 a mycelial fungus was isolated. On the 9th the isolation was referred to the Mycology Laboratory of Instituto Costarricense de Investigación y Enseñanza en Nutrición y Salud (INCIENSA) for identification. At INCIENSA, the isolate was cultured on day 10 in Malt Extract

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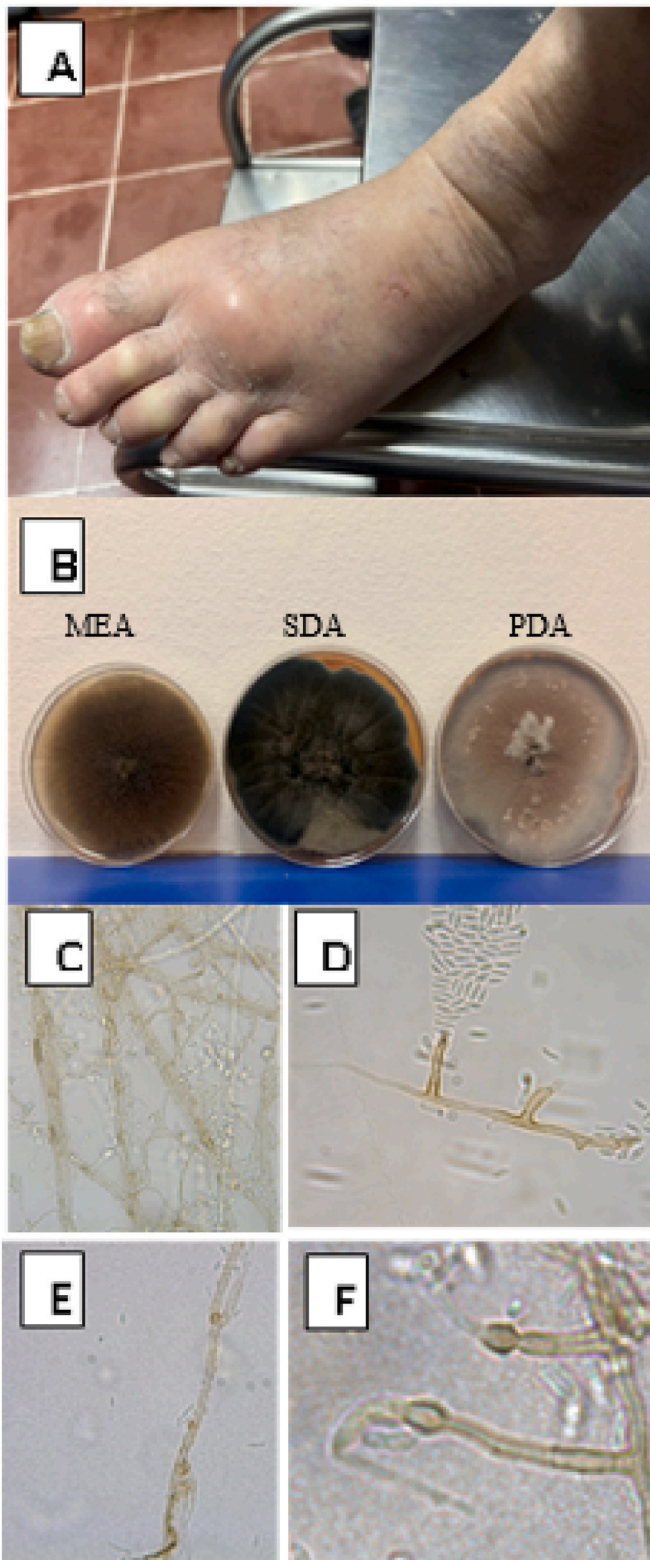


Fig. 1. A) Phaeohyphomycotic cyst. B) Growth of isolated colonies in MEA, SDA, and PDA. C) Fuliginous hyphae in fascicles. D) Phialides with the basal septum. E) Verrucous hyphae F) Phialides with percurrent proliferation..

agar (MEA), Sabouraud Dextrose agar (SDA) and Potato Dextrose agar (PDA) at 30 °C for 14 days. There was no pigment production in any of the media used (Fig. 1B).

Microscopic analysis showed darkly pigmented hyphae, rough in appearance, grouped in fascicles. Conidiophores of different sizes were

observed, mainly medium and short, with some branching. The phialides presented different shapes: cylindrical at the base and conical at the tip, or navicular in shape. Some phialides presented basal septum and percurrent proliferation was observed in some of these. After 4 weeks of incubation, on day 30, warts were observed in some of the hyphae from the original culture (Fig. 1C–F). On day 52, morphological characteristics described by Mostert et al. in 2006 [7] were used for preliminary isolate identification.

On day 60, the isolate was processed for identification using matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) from Bruker Daltonics (Bremen, Germany). Protein extraction with formic acid was performed according to the protocol recommended by the commercial company [8]. Using the Bruker database (MBT Filamentous Fungi IVD Library 3.0, 2019), it was not possible to identify the agent. Therefore, the spectra obtained (Fig. 2) were analyzed with the MSI (Mass Spectra Identification) online platform (<https://msi.happy-dev.fr>) developed by the Assistance Publique-Hôpitaux de Paris, the Sorbonne University of Paris, and the Belgian Coordinated Collections of Microorganisms (BCCM), and the agent was identified as *Phaeoacremonium venezuelense*.

Three months later the identification obtained was confirmed by DNA sequencing at the Mycotic Diseases Branch laboratory at the U.S. Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia. The nucleic acid was extracted using the kit DNeasy® Blood and tissue (Qiagen; Valencia, CA, USA) according to the manufacturer's instructions. Conventional PCR targeting the internal transcribed spacer 1 (ITS1) and ITS2 regions of ribosomal DNA (rDNA) and the D1/D2 region of 28S rDNA, were performed as described by White *et al* [9] and Gade *et al* [10] respectively. Products were purified and sequenced according to Gade *et al*. The final identification was made by comparing the sequence with known sequences using the BLAST algorithm from the National Center for Biotechnology Information (NCBI). Sequences for the ITS regions was deposited in the NCBI database under accession number OR263957. The blastn of these loci confirmed the speciation of *Phaeoacremonium venezuelense* with 100 % identity.

After the preliminary identification of the fungus using MALDI-TOF MS, the patient was admitted to the Dermatology Department of the Escalante Pradilla Hospital, underwent drainage of the abscess and curettage of the cyst. During surgery, a foreign body similar to a splinter was removed. Subsequently, oral treatment with itraconazole (100 mg, every 12 hours, for 6 months) was started. At the end of the treatment, the patient attended a follow-up appointment with an evident clinical cure.

3. Discussion

The genus *Phaeoacremonium* is widely distributed in the environment; isolates have been cultured from woody plants, humans, and beetle larvae [11]. Historical studies of *Phaeoacremonium* have focused on species that cause grapevine disease [11]. It was not until 1974 that the first case of infection in humans was described in a renal transplant patient. Since then, a few cases have been reported in the literature worldwide [12–16]. The scarcity of reported cases may be due to challenges with the identification of the *Phaeoacremonium* spp., which often requires molecular methods to confirm genus and species. Until recently, the identification of *Phaeoacremonium* was performed using only morphological keys whose distinguishing characteristics included minor differences between species, since their structure is very simple with few morphological features to differentiate them [12]. In addition, some of the main species causing human infections were not described until Mostert *et al*. [16] used sequencing of genes coding for β -tubulin to identify these new species and associate them with previously undescribed morphological characteristics [12]. However, in many Latin American countries, especially in Central America and the Caribbean, access to molecular techniques is limited; therefore, sometimes it is necessary to use other methodologies to achieve a timely identification

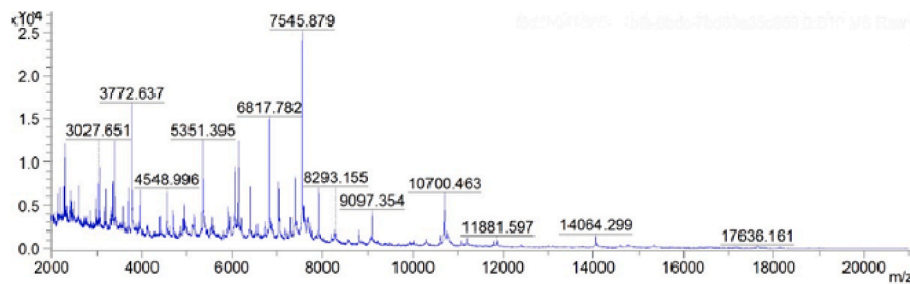


Fig. 2. Mass Spectra obtained from *Phaeoacremonium venezuelense*.

of this type of pathogens. In this case, morphological characterization of the isolate and MALDI-TOF were used for the preliminary identification of the fungus. This preliminary result allowed the patient to receive the appropriate treatment after months of waiting for a diagnosis. It should be noted that preliminary identification was not possible in this case using the currently available MALDI-TOF Bruker's library. It was necessary to access the MSI database to achieve a satisfactory result, which was subsequently confirmed by sanger sequencing.

Notably, this case corresponds to a phaeohyphomycotic cyst, frequent in immunocompetent individuals, where the lesions appear as an asymptomatic solitary subcutaneous abscess. This clinical presentation is different from the nodular subcutaneous form (limited or disseminated), less frequent than the previous one, which occurs mostly in immunosuppressed patients. The nodular lesion usually starts as a papule and continues with single or multiple confluent nodules of warty or granulomatous appearance, occasionally producing pruritus and pain [3].

To the best of our knowledge, this is the first case of *Phaeoacremonium venezuelense* infection reported in Costa Rica. Given the difficulty of properly identifying *Phaeoacremonium* spp., we do not rule out underreporting of cases, both in our country and in the rest of the Central American and the Caribbean region. Some causes of underreporting could include lack of commercially available molecular techniques and personnel well trained to correctly identify the causal agents.

In case an organism suspected of being *Phaeoacremonium* is isolated, we recommend referring it to a specialized mycology laboratory, where morphological and molecular techniques can help provide an accurate identification.

Disclaimer

The findings and conclusions of this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control (CDC).

CRediT authorship contribution statement

Mariamalia Cob: Writing – original draft, Methodology, Conceptualization. **Luisa F. López:** Writing – review & editing, Methodology. **D. Joseph Sexton:** Writing – review & editing. **Adrián Fallas:** Resources. **Juan David Muñoz:** Resources. **Ricardo Gutiérrez:** Writing – review & editing.

Declaration of competing interest

We have no personal or financial interests that could influence this work.

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