

Necrotizing fasciitis caused by *Apophysomyces variabilis* in an immunocompetent patient



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ABSTRACT

Mucormycosis caused by *Apophysomyces variabilis* is rarely reported in humans. A case of *A. variabilis* infection in an immunocompetent men after friction burns in a car accident is described. The infection presented as a rapidly progressive necrotizing infection of the skin and soft tissue, which required extensive surgical debridement and total colonic defunctioning colostomy associated with prolonged antifungal therapy. *A. variabilis* infection should be considered as a differential diagnosis of rapidly progressive necrotizing skin and soft tissue infections in immunocompetent individuals.

1. Introduction

Mucormycosis are uncommon infections that usually affect patients with comorbidities such as diabetes mellitus, immunosuppression, neutropenia and metabolic acidosis, with high morbidity and mortality rates. Species from the genera *Rhizopus*, *Mucor* and *Lichtheimia* are responsible for 80% of the mucormycosis in humans, while *Apophysomyces spp.* is responsible for less than 3% of these infections [1]. The genus *Apophysomyces* (phylum Mucoromycota, Subphylum Mucoromycotina) was described by Misra et.al in 1979, isolated from soil samples in northern India [2]. *A. elegans* was considered the only member of this genus until 2010, when Alvarez et al., described three new species based on morphological and physiological characteristics, and genetic differences (*A. variabilis*, *A. trapeziformis* and *A. ossiformis*) [3]. In 2014 Bonifaz et al. described a fifth new species (*A. mexicanus*) [4]. We present here a case of a patient with necrotizing fasciitis caused by *A. variabilis* after a motor vehicle collision in the northern region of Colombia (Valledupar, Cesar).

2. Case

A previously healthy 35-year-old man suffered from polytrauma with blunt chest trauma, multiple open fractures in the left tibia and femur associated with complex wounds in the left hand, and 10 ×

10 cm friction burns in the right lumbosacral region. Upon admission (**day 0**), resuscitation and antibiotic therapy with cefazolin 1 g i.v. every 8 h plus gentamicin 160 mg i.v. daily was initiated, the patient was taken to surgery for femur and tibia osteosynthesis, and a skin flap in the left hand, requiring intensive care unit (ICU) management after the intervention due to the need of non-invasive ventilatory support and multiple transfusions. The patient had an adequate clinical evolution with weaning off ventilatory support and stabilization of hemodynamic parameters and was transferred into the general ward. However, on **day 10**, the patient reported intense and progressive pain in the right gluteal region with a 15 × 15 cm hardening area, with heat and redness. Soft tissue ultrasonography ruled out collection, and the blood count showed high levels of leukocytes and neutrophilia (leukocytes 25,000 cells/mm³, neutrophils 85%) with elevated C-reactive protein (CRP) (120 mg/L). The antibiotics were changed to piperacillin tazobactam and the patient was assessed by plastic surgery department who discarded surgical treatment. 72 h later (**day 13**) the patient had a poor evolution with increased pain in the gluteal region, skin sloughing and the appearance of a central necrotic area. The patient was taken to surgical drainage where a sample of purulent secretion (5 ml) was collected. This sample was sent together with tissue samples for histopathology staining and microbiological cultures.

On **day 16**, due to the appearance of surgical wound necrosis with persistent pain, hardening area, heat and redness in the right gluteus,

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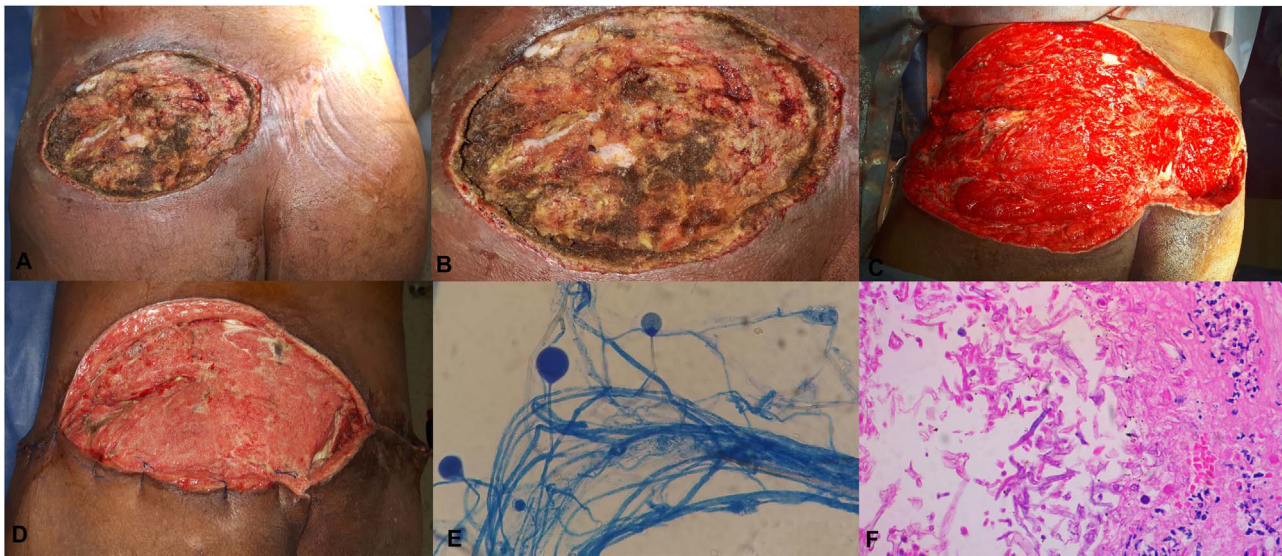


Fig. 1. A: Surgical wound in the right lumbosacral region with skin necrosis, subcutaneous cellular tissue necrosis and partial gluteus major necrosis. B: Previous picture zoom. C: Surgical wound after extensive debridement initiating in the upper and lateral region of thigh and right gluteus to the upper region of the left gluteus. D: 40× lactophenol blue. E: Surgical wound after 12 weeks of evolution, multiple surgical washes and antibiotic and antimetabolic treatment. F: Histopathological study with hematoilin eosin staining at 40×.

the patient was taken to surgery again, where skin necrosis, subcutaneous cellular tissue and partial necrosis of the gluteus maximus muscle were observed (Fig. 1A and B). Extensive debridement of necrotic tissues was performed and a support of negative pressure system was implemented (Negative Pressure Wound Therapy (NPWT) systems, Genadyne's Multi-Canister System from New York, USA). During the next 48 h (day 18), there was an increase in the hardening area and necrosis with involvement of the entire gluteus and back right thigh, persistent elevation of CRP levels and fever. The patient was taken to surgery again for total colonic defunctioning colostomy and lumbosacral lesion debridement (Fig. 1C), and transferred to the ICU again due to the need of ventilatory and hemodynamic support.

Testing results showed persistently negative blood cultures, microbiological examination of the tissue sample did not indicate bacterial growth, Gram and Ziehl-Neelsen staining of the secretion were negative. After 96 h of incubation, white and wooly colonies and hyaline aseptate hyphae were observed on agar-blood culture. Suspecting mucormycosis infection, tissue samples were cultured on malt extract agar, Sabouraud agar and water agar to induce the formation of sporangiospores. After 96 h, an overgrowing on all fungi cultures media was observed (white and wooly colonies), but only straight sporangiospores, prominent apophysis and piriform sporangium with evidence of abundant oblong sporangiospores were observed on water agar (Fig. 1D). Taken together, all these characteristics allowed the identification of *Apophysomyces* sp., and a sample was used for additional molecular identification. The determination of sensitivity by E-test was not possible due to poor growth.

On day 18, with the diagnosis of necrotizing fasciitis secondary to cutaneous mucormycosis a combined treatment with amphotericin B 50 mg i.v. daily, plus posaconazole 300 mg i.v daily, plus tigecycline 50 mg i.v. every 12 h, plus cefepime 2 g i.v. every 8 h, and surgical washings every 72 h was initiated. On day 39, after 21 days of treatment with amphotericin B + posaconazole and 6 surgical washings, reconstruction by plastic surgery with skin grafts was performed (Fig. 1E). On day 84, the patient was discharged with posaconazole 300 mg daily for 3 months. At 6 months follow-up post hospital discharge, 100% healing of the wound was evident, and the procedures for colostomy closure were initiated (day 264).

The histopathological study showed abundant granulation tissue with foreign body reaction, multiple aseptate hyphae in intravascular space with right angle branching (Fig. 1F).

Fungal DNA was isolated using the MoBio PowerSoil® DNA isolation kit (MoBio Laboratories, Carlsbad, CA, US) following manufacturer's protocols. Amplification of the Internal transcribed spacer (ITS) gene region by PCR was performed using universal primers ITS4 and ITS5 as previously described [5]. The PCR fragments were purified by alcohol precipitation and sequenced (Sanger method) using the universal primers ITS1, ITS4 and ITS5. The assembled 774 bp sequence was compared against the NCBI (National Center for Biotechnology Information), UNITE and Warcup Fungal ITS RDP databases, showing a 99% identity percentage in 99% length with sequences reported for *Apophysomyces variabilis*. The corresponding bioinformatic analysis made it possible to assign this sample to species level. The nucleotide sequence obtained in this study has been submitted to GenBank under the accession number KY996810.

3. Discussion

Although *A. variabilis* is found in soil of tropical and subtropical areas with low nitrogen content [6], hereby we describe the first case of mucormycosis caused by *A. variabilis* in tropical areas of Latin America. Unlike other mucorales, this fungus affects mainly immunocompetent hosts. Infection usually occurs after traumatic inoculation of the microorganism, but can also occur after spore inhalation. The most common clinical presentation is skin and soft tissue infection (52.7%), followed by rhino-orbito-cerebral infection (25.7%), disseminated infection (10.8%) and isolated renal involvement (6.8%) [1].

There are few cases of *A. variabilis* infection reported in the literature, mostly from India [7–11,13, 15,16] (Table 1). The number of cases is likely to be underestimated because *A. variabilis* does not produce spores readily in traditional mycological culture media used in clinical laboratories, has special nutritional requirements, grows at high temperatures (37–42 °C) and after prolonged periods of incubation (7–10 days) [9].

Treatment of mucormycosis caused by *A. variabilis* requires an early diagnosis, together with an adequate control of predisposing factors and aggressive surgical debridement, associated with the administration of amphotericin B alone or combined with posaconazole. Minimal inhibitory concentration (MIC) of *A. variabilis* to amphotericin B ranged from 0.03 to 4.0 mg/l, and usually present low MIC to posaconazole (0.0625–2 mg/L). Infections with MIC to amphotericin B > 2 mg/L are associated with worse outcomes [6,8,10,12,13,14,15]. Although in the

Table 1
17 report of cases of *A. variabilis* infection.

^a Cases	Age (Y)	Type of infection	Area involved	Risk Factor	Primary disease	Outcome
1	9	Skin	Lower limb	Trauma	Without	Limb amputation [7]
6	39–50	Skin	4 Gluteal. 1 Upper limb. 1 Scapular.	4 IM IY. 1 Trauma. 1 Without.	1 DM. 5 Without.	4 Deaths [8]
1	ND	Rhino-orbito-cerebral.	Rhino-orbito-cerebral.	ND	DN	ND [9]
1	21	Skin	Dorsal	Burn 90% TBS	Without.	Death [10]
5	29–45	Skin	1 Thoracic wall. 3 Abdominal wall. 1 Upper limb.	2 Trauma. 2 IM IY. 1 Without.	2 DM. 3 Without.	4 Deaths [13]
1	45	Skin	Torso and limbs	Toxic epidermic necrosis.	Without	Death [11]
1	64	Skin	Abdominal wall.	Cutaneous zoster.	Chronic use of steroids.	Death [16]
1	74	Rhino-orbito-cerebral.	Rino orbital cerebral.	Without.	Without.	Death [15]

^a Cases: number of cases, Y: years, IM IY: intramuscular injections, ND: No data, TBS: Total Body Surface, DM: diabetes mellitus.

presented case antifungal sensitivity could not be realized, the combined treatment with amphotericin B and posaconazole was successful.

In conclusion, *A. variabilis* infection should be considered as a differential diagnosis of rapidly progressive necrotizing skin and soft tissue infections in immunocompetent individuals.

Conflict of interest

The authors have no conflicts of interest to declare and confirm that each one has made substantial contributions to the information or materials submitted for publication.

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