Invasive Apophysomyces variabilis Infection in a Burn Patient

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Apophysomyces variabilis is an emerging fungal pathogen that can cause significant infections in immunocompetent patients. We report a case of A. variabilis invasive wound infection in a 21-year-old male after a self-inflicted burn injury.

CASE REPORT

We report a case of a 21-year-old active-duty U.S. Marine with a known psychiatric history who suffered 90% total body surface area (TBSA) burns after self-immolation with gasoline while stationed in Okinawa, Japan. After the flames were doused by immersion in freshwater, he was taken immediately to a local emergency department, where he was intubated and underwent full-body escharotomies. He was transferred to a military hospital in Hawaii, where he underwent aggressive fluid resuscitation and received fresh frozen plasma for coagulopathy. Fiberoptic bronchoscopy was performed, which showed no inhalational injury. He was then transferred to the U.S. Army Institute of Surgical Research Burn Center in San Antonio, TX, approximately 4 days after the burning. Upon arrival, he underwent fascial bilateral excision of the lower and upper extremities with tangential excision of the anterior-posterior torso. A 4:1 mesh sandwich was applied to the anterior/posterior torso, and an allograft was applied to 80% of the TBSA. On postburn day 8, he was found to have suspected fungal growth on the allograft on his lower back. Fascial excision was performed, and histopathology of the excised tissue showed numerous large-diameter aseptate hyphae located mostly in the nonviable adipose tissue but with evidence of microinvasion of blood vessels (Fig. 1). Due to the clinical concern of fungal invasion beyond the burn wound, the patient was initially empirically treated with voriconazole and liposomal amphotericin B. Deep-wound cultures subsequently grew a fungus that was tentatively identified as a species within the order Mucorales. Blood cultures were negative for the fungus. Amphotericin B was continued to cover for the mucoralean species, while voriconazole was continued to empirically cover other possible fungal pathogens that might have been infecting this patient. Despite aggressive debridement of infected tissue and systemic antifungal administration, fungal infection persisted. The patient eventually died of extensive burn wounds and multiorgan failure syndrome. The fungal isolate from lateral back tissue was referred to the Fungus Testing Laboratory, University of Texas Health Science Center at San Antonio, where it was identified as Apophysomyces variabilis by phenotypic and genotypic testing.

Fungal identification. The isolate submitted on Mycobiotic agar (Remel, Lenexa, KS) was submitted to the Fungus Testing Laboratory collection as UTHSCC 11-1354. Colonies were white and woolly, filling the agar slant tube, but failed to form any fruiting structures. Given the rapid growth and sterile nature of the isolate, the initial impression suggested either an Apophysomyces or a Saksenaea species. A carnation leaf agar (CLA) plate (16) and a water agar plate (18) both prepared in house, were inoculated and incubated at 25°C and 35°C, respectively, to induce sporangiospore formation. On CLA, the culture produced brown, unbranched sporangiophores, prominent apophyses, and pyriform sporangia similar to those observed in A. ossiformis and A. trapeziformis (2). However, a notable phenotypic difference in our isolate was the sporangiospore size and shape variability (Fig. 2A and B). A darkened area below the apophysis was also present—a feature described in other species within the genus (Fig. 2C). Temperature studies on potato flake agar slants (16) prepared in house and incubated for 7 days indicated 4+ growth at 37°C, 2+ growth at 40°C, and no growth at either 45 or 50°C. On the basis of the above features, the isolate was identified as Apophysomyces sp. and molecular characterization was initiated.

Molecular identification of this isolate was performed as described previously (19). Briefly, genomic DNA was prepared from a 24-h potato dextrose agar plate grown at 30°C using the Prepman Ultra reagent (Applied Biosystems, Foster City, CA). PCR assays were performed using the ITS1 and NL4 primers (19). The amplicon was purified with the Qiagen PCR purification kit and incubated at 40°C, and no growth at either 45 or 50°C. The sequences were assembled using MacVector software (MacVector, Inc., Cary, NC), and the individual internal transcribed spacer (ITS) and D1/D2 regions were used to perform BLASTn searches of the NCBI database (http://www.ncbi.nlm.nih.gov/BLAST/). Individual sequence results were considered significant at identities of ≥97%. The three top hits in the ITS search were A. variabilis (accession number FJ556442.1; identity, 708/708 [100%]), A. variabilis (accession number F813492.1; identity, 701/701 [100%]), and A. variabilis (accession number F813491.1; identity, 701/701 [100%]). The results of the D1/D2 search were similar, with the top three hits being A. variabilis (accession number FJ556455.1; identity, 679/680 [99%]), A. variabilis (accession number FN556455.1; identity, 679/680 [99%]), and A. variabilis (accession number FJ556455.1; identity, 679/680 [99%]).

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FN554254.1; identity, 655/656 [99%]), and A. variabilis (accession number FN554253.1; identity, 651/652 [99%]). Thus, sequencing and phenotypic characterization confirmed the isolate as A. variabilis.

Antifungal susceptibility testing of the case isolate was performed, with MICs (μg/ml) as follows: amphotericin B, \( \leq 0.03 \); posaconazole, 0.06; voriconazole, 8; itraconazole, 0.125; anidulafungin, >8; caspofungin, >8.

Discussion. Apophysomyces was first isolated from soil samples in India in 1979 (14). Morphologically, this fungus typically produces pyriform sporangia; conspicuous funnel- and/or a bell-shaped apophyses; and clear, thin, and smooth-walled sporangiospores that are mostly oblong with rounded ends. It is a thermotolerant fungus that grows rapidly between 26 and 42°C (5, 14, 20). While most isolates have been reported from India, it also has been isolated in Australia, Southeast Asia, the United States, and South America, suggesting a broad distribution which covers tropical and subtropical climates. In the United States, Apophysomyces represents only 0.5% of the clinically significant Mucorales isolates (3).

Although Apophysomyces is typically an environmental mold, there have been an increasing number of human infections reported. Human infections by Apophysomyces involve a wide range of patients, the majority of which were immunocompetent (4). This is in contrast to infections caused by other members of the order Mucorales that tend to involve immunocompromised individuals and are most commonly seen in patients with poorly controlled diabetes. The most common mode of infection is traumatic implantation of contaminated soil or water leading to cutaneous or subcutaneous infections, rhino-orbital infection after facial trauma (9, 10, 12), or osteomyelitis after breakdown of the overlying skin (7, 13, 20). Renal infection by Apophysomyces has been reported, suggesting a possible hematogenous mode of infection following traumatic implantation through the skin (17).

FIG 1 Histopathology (hematoxylin and eosin stained) of excised tissue from the left lower back showing numerous large-diameter aseptate hyphae located mostly in nonviable adipose tissue with evidence of microinvasion of blood vessels.

FIG 2 Features of A. variabilis fruiting structures produced on CLA after 7 days of incubation at 25°C (bright-field microscopy, lactophenol cotton blue mount). Shown are a sporangiophore, the apophysis, and variable sporangiospores (A); sporangiospores of various sizes and shapes (B); and a fruiting structure showing a darkened area below the apophysis (C).
Disseminated infection with *A. elegans* has also been reported after kidney transplantation from a donor who died by drowning (15). Recently, a cluster of cutaneous mucormycosis cases has been reported in the aftermath of the Joplin, MO, tornado, in which 13 of the confirmed cases yielded *A. trapeziformis* (8). In our patient, the mode of infection is unclear but could have been traumatic implantation by his immersion in water after the burn.

Until recently, it was believed that *Apophysomyces* comprised a single species, *A. elegans*. However, sequence analysis of several genes, combined with physiological and morphological characteristics, has led to the recognition of four distinct *Apophysomyces* species (*A. elegans*, *A. ossiformis*, *A. trapeziformis*, and *A. variabilis*) (2). The incidence of human infection due to *A. variabilis* is unknown. Most reports of infections due to *Apophysomyces* have been attributed to *A. elegans* prior to the identification of the four distinct species of *Apophysomyces*. The organisms responsible for the first reported cases of human *A. variabilis* infection were identified as *A. elegans* morphologically and subsequently identified as *A. variabilis* by ITS sequencing (11). It is likely that some of the reported cases of *A. elegans* infection were due to *A. variabilis*, although the extent to which these infections are due to *A. variabilis* is uncertain. This also underscores the difficulty of identification by morphology and that definitive identification may require study by ITS sequencing. The isolate described here has been deposited in the University of Alberta Microfungus Collection under accession number UAMH 11571. The sequences were deposited in GenBank under accession numbers JN980700 for the ITS sequence and JN980699 for the D1/D2 sequence.

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**REFERENCES**


