**Actinomucor elegans as an Emerging Cause of Mucormycosis**

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We report an invasive mucormycosis caused by *Actinomucor elegans* in a patient with refractory aplastic anemia. The organism was isolated from a necrotic skin lesion on the patient’s left arm and demonstrated angioinvasive features on histopathology examination. In contrast to three cases described previously, we describe the first case of *A. elegans* invasive fungal infection in an immunocompromised patient. This report, along with the three previously reported cases, is convincing evidence that *A. elegans* is an emerging fungal pathogen capable of causing invasive mucormycosis in humans.

**CASE REPORT**

A 58-year-old man was diagnosed with aplastic anemia 9 years prior to this presentation. He was initially treated with high-dose cyclophosphamide, but achieved only a partial remission; he remained transfusion dependent for several years. He remained neutropenic (absolute neutrophil count, <500 cells/mm$^3$) over the last 2 years. Three months prior to this presentation, he was hospitalized with possible fungal pneumonia based on the presence of multiple subcentimeter lung nodules, some of which had central cavitations, on a computed tomography (CT) of his chest. Bronchoalveolar lavage cultures for bacteria, fungi, and acid-fast organisms at the time were negative. He was empirically treated with voriconazole for an unknown duration.

Three weeks prior to this presentation, the patient presented to an outside hospital with fever and a necrotic lesion on the dorsal surface of the left forearm. His fever persisted, and the lesion continued to progress despite a 2-week course of oral trimethoprim-sulfamethoxazole and amoxicillin-clavulanate. Subsequently, he presented to the outside hospital with rigors and a temperature of 38.9°C. Treatment with intravenous vancomycin and piperacillin-tazobactam was initiated, and the patient was transferred to the Johns Hopkins Hospital for further evaluation and treatment.

On admission, he was afebrile (36.4°C) and had normal vital signs. Physical examination revealed a raised 10-cm by 8-cm erythematosus area on the dorsal surface of the left forearm distal to the elbow, with a central, irregular, black eschar measuring approximately 5 cm in diameter. Broad-spectrum antibiotic agents were continued, and treatment with fluconazole (400 mg orally once daily) was started. Seven days later, fluconazole was transitioned to voriconazole (400 mg orally every 12 h), based on the finding of a pleural-based consolidative mass in the posterior-medial right lower lobe, measuring 4.0 by 3.0 by 4.2 cm with surrounding ground glass opacity on a chest CT. Sinus CTs did not demonstrate any evidence of concomitant sinusitis. Magnetic resonance imaging of his left upper extremity demonstrated skin ulceration with extensive edema. Multiple sets of blood cultures were negative for fungi and mycobacteria. The patient underwent surgical debridement of the lesion on his left arm, and tissues were sent for histopathology and microbiology examination. Histopathology revealed invasion of underlying blood vessels and deeper tissues by aseptate hyphae (Fig. 1). A fungus like the one in the order Mucorales grew from tissue cultures in 4 days that was further identified as *Actinomucor elegans*. Antifungal therapy was switched to liposomal amphotericin B (7.5 mg/kg of body weight once daily) and micafungin (150 mg once daily). Ten days later, the patient underwent a second surgical debridement due to persistent necrosis and inflammation extending to the entire peripheral and deep edges of the resection. After 2 weeks of combination antifungal therapy, due to deteriorating renal function, oral posaconazole (200 mg orally four times daily) was added to his regimen. Due to severe nausea and vomiting, posaconazole was discontinued after a week. His course was unfortunately further complicated by central-line-associated bacteremia with *Escherichia coli* and *Klebsiella pneumoniae*. Due to his overall poor prognosis, the patient was eventually discharged to hospice care and expired shortly thereafter.

**Histopathology.** An ellipse of hair-bearing skin with a centrally located black ulcerated lesion collected from the necrotic left arm lesion was sent for histopathology examination. Routine hematoxylin and eosin stains revealed ulcerative and necrotic skin with numerous invasive fungal hyphae in the soft tissue extending to the level of the subcutis and which demonstrated angioinvasion (Fig. 1). These fungal forms were broad with ribbon-like hyphal structures, irregular thickness, and irregular branching. These features were histologically characteristic of members of the order Mucorales. Gomori methenamine silver stain further confirmed the fungal morphology.

**Mycology.** Culture from the skin biopsy tissue grew the organism on both a 5% sheep blood agar plate and a Sabouraud agar plate within 4 days. The organism grew at 37°C but not at 42°C. On potato dextrose agar, the colonies displayed brown beige color on both surface and reverse sides and a wooly texture (Fig. 2A). Lactophenol cotton blue stain revealed aseptate aerial hyphae and branched sporangiophores terminating at sporangia; no sexual structure was seen (Fig. 2B and C). Rhizoids were also present (not shown) in a patient with refractory aplastic anemia. The organism was isolated from a necrotic skin lesion on the patient’s left arm and demonstrated angioinvasive features on histopathology examination. In contrast to three cases described previously, we describe the first case of *A. elegans* invasive fungal infection in an immunocompromised patient. This report, along with the three previously reported cases, is convincing evidence that *A. elegans* is an emerging fungal pathogen capable of causing invasive mucormycosis in humans.
shown in the figures). The organism was deposited in the University of Alberta Microfungus Collection & Herbarium (UAMH) and assigned strain no. UAMH 11617.

DNA sequencing identification of this organism was also performed. After DNA extraction using the Zymo Research ZR fungal DNA kit (Zymo Research, Orange, CA), the internal transcribed spacer (ITS) region (ITS1-5.8S-ITS2) and the D1/D2 region of 28S ribosomal DNA were amplified and sequenced. PCRs were conducted using the Taq polymerase and with the following amplification conditions: for ITS (ITS 1 primer, 5'-TCC GTA GGT GAA CTT GCG G-3'; ITS 4 primer, 5'-TCC TCC GCT TAT TGA TAT GC-3'), there was an initial hold at 95°C for 5 min followed by 30 cycles of 95°C for 30 s, 55°C for 1 min, 72°C for 1 min, and a final extension at 72°C for 6 min (12); for D1/D2 (NL-1 primer: 5'-GCA TAT CAA TAA GCG GAG GAA AAG-3'; NL-4 primer, 5'-GGT CCG TGT TTC AAG ACG G-3'), there was an initial hold at 94°C for 2 min followed by 30 cycles of 94°C for 15 s, 55°C for 30 s, 68°C for 2 min, and a final extension at 68°C for 5 min (7). PCR amplicons were sequenced using an ABI Prism 3100 genetic analyzer (Applied Biosystems, Foster City, CA). Sequence results were analyzed by SmartGene (SmartGene, Inc., Raleigh, NC) software and library and also blasted using the NCBI database. The sequence results from both ITS and D1/D2 regions matched 100% with Actinomucor elegans in both the SmartGene library and the NCBI database. Based on the results from phenotypic and DNA sequence analysis, the fungal isolate was identified as Actinomucor elegans.

The isolate was sent to the Fungus Testing Laboratory, Department of Pathology, University of Texas Health Sciences Center (San Antonio, TX) after the patient expired to further determine the antifungal drug susceptibility pattern as part of this case study. The reference laboratory performed in vitro antifungal susceptibility of the isolate using the broth microdilution method recommended by the Clinical and Laboratory Standards Institute (CLSI) (1). The MIC results were as follows: amphotericin B, 0.25 μg/ml; itraconazole, 0.25 μg/ml; voriconazole, 8 μg/ml; posaconazole, 0.125 μg/ml; and micafungin, >8 μg/ml. The susceptibility pattern of A. elegans was only determined previously in two cases. In the paper by Davel et al. (2), the MICs were as follows: amphotericin B, 2 μg/ml; and itraconazole, 1 μg/ml. In the paper by Khan et al. (4), the MICs were as follows: amphotericin B, 1 μg/ml; voriconazole, 8 μg/ml; posaconazole, 0.25 μg/ml; and caspofungin, ≥32 μg/ml. Compared to the MIC results from the two case studies, our A. elegans isolate did not show any elevated MICs against these antifungal drugs.

Conclusions. Actinomucor elegans belongs to the order Mucorales and was previously isolated from soil samples after anaerobic incubation in Russia (5). It is also known for its association with the production of soy-based products, providing flavor and texture to food (11).

Human infection caused by A. elegans has only been described previously in three cases (Table 1). The first case was reported in Argentina in 2001 (2). An 11-year-old female without any underlying conditions presented with maxillary sinusitis. She underwent surgical debridement, and her surgical cultures grew A. elegans, which was identified based on morphology and temperature studies. The patient was cured with surgery and intravenous amphotericin B treatment. The second case was reported in Kuwait in 2008 (4). Swabs from a wound containing necrotic tissue...
from the left foot of a diabetic male patient grew *A. elegans*, identified based on phenotypic and genotypic methods. The etiologic role of the organism in this case was unclear because further information about histopathology evidence, clinical presentations, treatment, and outcome of this patient was not available. The third case was reported in 2009 (10). A 30-year-old American soldier fighting in Iraq suffered extensive battle injuries to his right side, including his torso, arm, and leg, resulting in extensive necrosis. Despite debridement, the infection became disseminated and ultimately led to his death. Postmortem surgical cultures grew *A. elegans* identified based on phenotypic and genotypic methods.

Herein, we report a fatal case of a biopsy-proven and invasive mucormycosis caused by *A. elegans* in a patient with refractory aplastic anemia. Although the fungus was isolated from his necrotic skin lesion on his left arm, it was not clear whether the skin lesion could have been the primary site of inoculation or could have been a metastatic focus after disseminated infection originating from the lung, blood, or other sites. We speculate that direct skin inoculation with the organism was the mostly likely cause of this presentation, albeit there was no definitive exposure history. The presence of cavitating nodular lung lesions in the setting of persistent and profound neutropenia and concomitant angioinvasive skin lesion raise concerns that the infection might have hematogenously spread to involve the patient’s lungs. However, a bronchoscopy was not performed, and respiratory samples were not obtained to confirm dissemination.

Treatment with an amphotericin B product along with prompt and aggressive surgical debridement is currently the mainstay for managing patients with mucormycosis (9). However, outcomes remain poor, with overall 12-week mortality among hematopoietic stem cell recipients with mucormycosis higher than 70% (6). Although echinocandins generally have no activity against fungus in the order Mucorales, at least one report provides clinical data suggesting that combination therapy of amphotericin B with caspofungin may have a beneficial clinical outcome in patients infected with a fungus in the order Mucorales (the majority of culture isolates in that study being *Rhizopus* species) (8). Our patient did not respond well clinically to the initial antifungal treatment with voriconazole. This may be explained by the high MICs against voriconazole. He was switched to combination therapy with liposomal amphotericin B and micafungin, along with aggressive surgical debridement. Despite the fact that the chest CT scan showed some improvements in the lung lesions after the combination therapy, amphotericin B and micafungin were discontinued because the patient developed renal insufficiency. He was switched to posaconazole, but his clinical response may have been suboptimal due to his poor oral intake and diarrhea. Furthermore, his underlying immune function may have also contributed to his fatal outcome. (The patient had remained profoundly neutropenic for years prior to this presentation.)

Although DNA sequencing was applied in this case study to confirm the identification, the organism can be identified by routine morphology examination and microscopic characteristics. *A. elegans* is differentiated from *Mucor* species by having rhizoids and branch sporangiophores; it is differentiated from *Rhizopus* species and *Lichtheimia* species by verticillately branched sporangiophores of various lengths (Fig. 2B and C).

Invasive mucormycosis caused by uncommon fungus in the order Mucorales has been increasingly seen in immunocompromised patients (3). Here we report an uncommon Mucorales fun-
gus, *Actinomucor elegans*, causing invasive and fatal mucormycosis in an immunocompromised patient. Our case report, together with the three previously reported cases, emphasizes that *Actinomucor elegans* is an emerging fungal pathogen capable of causing invasive mucormycosis in humans.

**Nucleotide sequence accession numbers.** ITS and D1/D2 sequence results have been deposited in GenBank under accession no. JQ002627 and JQ002628, respectively.

**REFERENCES**


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**TABLE 1 Chronological summary of case reports of mucormycosis caused by *Actinomucor elegans* **

<table>
<thead>
<tr>
<th>Case (reference)</th>
<th>Patient age (yr)/sex</th>
<th>Country</th>
<th>Risk factor(s)</th>
<th>Symptoms</th>
<th>Site involved</th>
<th>Treatment</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (2)</td>
<td>11/female</td>
<td>Argentina</td>
<td>None</td>
<td>Left-eye epiphora, nasal discharge</td>
<td>Maxillary sinus</td>
<td>Debridement, AmB</td>
<td>Cure</td>
</tr>
<tr>
<td>2 (1)</td>
<td>NA</td>
<td>Kuwait</td>
<td>DM</td>
<td>NA</td>
<td>Necrotic wound on left foot</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>3 (9)</td>
<td>30/male</td>
<td>Iraq/United States</td>
<td>None</td>
<td>Sustained extensive war injury to entire right side of body, progressive necrosis</td>
<td>Bowel, muscle (abdomen, right hip)</td>
<td>Debridement, caspofungin</td>
<td>Death</td>
</tr>
<tr>
<td>4 (this report)</td>
<td>58/male</td>
<td>United States</td>
<td>Aplastic anemia, DM</td>
<td>Neutropenic fever, necrotic lesion on left arm</td>
<td>Left arm, lung</td>
<td>Debridement, AmB micafungin, posaconazole</td>
<td>Death</td>
</tr>
</tbody>
</table>

**a** NA, not available; AmB, amphotericin B; DM, diabetes mellitus.