Fatal *Actinomucor elegans* var. *kuwaitiensis* Infection following Combat Trauma

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We report the first case of invasive mucormycosis secondary to *Actinomucor elegans* infection. A severely injured soldier with a fatal *A. elegans* var. *kuwaitiensis* infection is described. The identification of this fungus was performed by classical and molecular methods, and this report documents the pathogenicity of the recently described variety *Actinomucor elegans* var. *kuwaitiensis*.

CASE REPORT

A previously healthy 30-year-old male was injured by an improvised explosive device in Iraq, sustaining extensive wounds to his right side. He was evacuated to a military hospital in Iraq and taken immediately to the operating room for complex pelvic fracture debridement and fixation, right lower extremity disarticulation, right through-the-elbow amputation, and an exploratory laparotomy. He was stabilized and evacuated to a military medical center in Germany. After further evaluation and stabilization, including washing out of his right flank, hip, and forearm and washing out of his abdomen without evidence of bowel injury, a wound vacuum-assisted closure device was placed over his open abdomen, and he was transferred to Brooke Army Medical Center (BAMC) for further care.

The patient was febrile upon arrival to BAMC. A left subclavian central venous catheter that was placed while the patient was in the field was replaced over a wire, and blood, urine, and respiratory cultures were performed. Rare fungal elements noted in the respiratory sample were later identified as an *Aspergillus* species. This fungus was not recovered subsequently, and blood and urine cultures remained negative. Broad-spectrum antibiotics were started for empirical antibacterial therapy, and the patient was taken to surgery for wound exploration and debridement. The pelvic wound was noted to extend into the peritoneal cavity, exposing the right lobe of the liver, right kidney, and small bowel, all of which appeared healthy. Gram stains of wound cultures taken from the right ilium and the iliac vein were unremarkable, and cultures ultimately grew no organisms. On day 4 postinjury, the patient was again febrile, and urine, blood, and sputum samples were sent for Gram staining and culture. Evaluation of the sputum revealed yeast, identified 3 days later as *Arthrographis kahae*. This fungus was not isolated again, and its identification was not further confirmed. On day 5, the patient’s temperature increased to 103°F, and his left subclavian central venous catheter was removed and cultured, revealing no growth. Devitalized tissue in the hip and hamstring and a portion of the rectus muscles on the right were debrided. A wound vacuum-assisted closure device was placed over the right hip wound, which communicated with the abdomen. Given the patient’s persistent fever on day 6, blood and urine cultures were sent for testing. The patient’s clinical status continued to deteriorate, with worsening hypotension, oliguria, and evidence of rhabdomyolysis. On day 7, the patient was once again taken to surgery for debridement. He was noted to have areas of necrosis involving multiple abdominal and gluteal muscles, with skin necrosis that extended across the midline. Extensive debridement of these areas was performed, and tissue from the right hip was submitted for culture. On day 8, the patient again underwent extensive debridement of necrotic tissue. Muscles surrounding the pelvis and extending into the abdomen were further debrided. Wound necrosis was widespread and too substantial to fully remove. Necrotic tissue extended up the anterior wall, involving the intercostals, the psoas, and paraspinal muscles, and extended throughout the bowel (including the stomach), liver, and right kidney. Sections from bowel and muscle were sent for examination intraoperatively, and touch prep of tissue was remarkable for fungal elements. He was given a dose of caspofungin in the operating room and subsequently started on fluconazole for treatment of candidemia (identified as *Candida tropicalis*) found on blood cultures obtained on day 6. The health care team felt that the patient’s wounds and extent of tissue necrosis were beyond survivability. The prognosis was discussed in detail with his family, who opted to transition to comfort measures on the afternoon of day 9. The patient expired later that evening.

Tissue from debridement of the right hip collected on day 7 was suspicious for a mucormycosis by histopathology—later confirmed by culture as an *Actinomucor elegans* infection. Specimens submitted for histopathology on day 8 were later noted to have diffuse invasive fungal infection with frequent lymphovascular invasion. Gomori methenamine silver (GMS) staining revealed fungal structures consistent with a mixed...
mucormycosis/candidiasis (Fig. 1). At autopsy, postmortem tissue examination showed intravascular and parenchymal fungal elements that were consistent with a dual population of *Candida* and *Mucor* species-like mold, and cultures ultimately grew *Candida tropicalis* and *Actinomucor elegans*, the latter of which was identified 18 to 21 days postmortem. Involved organs included lungs, stomach, small and large bowels, liver, spleen, pancreas, adrenal glands, kidneys, prostate, and bladder. Pathology determined the cause of death to be a dual-population, disseminated, invasive fungal infection of *Candida* and *Mucorales* complicating multiple blast injuries.

Phenotypic identification of *Actinomucor*, *Actinomucor elegans* was identified by morphological features and molecular characterization. An isolate from the right leg stump was forwarded to the Fungus Testing Laboratory, University of Texas Health Science Center at San Antonio (UTHSCSA), and accessioned into the stock collection as UTHSC 07-831. There, the sterile, woolly, white isolate was subcultured onto 60-mm Czapek Dox agar plates (prepared in-house) at 25°C and subjected to a water culture at 37°C in an effort to induce sporulation, as the frequently sterile *Mucorales* *Apophysomyces elegans* and *Saksenaea vasiformis* were under consideration. It was also subcultured on potato flake agar (prepared in-house) tubes at 25, 37, 40, and 45°C for temperature studies. The isolate grew rapidly at 25°C, filling the 60-mm Czapek Dox agar plate in 2 days. Colonies were initially white and woolly, becoming yellowish brown after 4 days of incubation, and displayed a pale-yellow diffusing pigment. The isolate also grew rapidly in the 37°C water culture. Temperature studies revealed growth up to 40°C but not at 45°C. Salient microscopic features included verticillately branched sporangiothores of various lengths (Fig. 2), commonly delicately roughened below the septum (Fig. 3), with secondary branches being borne beneath and to the side of septa below the primary sporangium (Fig. 2 and 4). At maturity, sporangia were covered with prominent spine-like projections on their outer walls, and small, barely visible collarettes were present. Sporangiospores were smooth to very delicately spiny as observed by light microscopy, were nearly globose, and measured 6 to 8 μm in diameter (Fig. 2 to 4). Rhizoids were present. On the basis of these features, the isolate was identified as *Actinomucor elegans* and deposited into the University of Alberta Microfungus Collection and Herbarium as strain UAMH 10999.

Genotypic identification of *Actinomucor*. To determine the etiologic agent, a sequence-based approach using both the internal transcribed spacer (ITS) and D1/D2 ribosomal DNA regions as targets for the molecular identification of 07-831 was performed, followed by a TA cloning step to yield a plasmid that would serve as a sequencing template.

The case isolate was grown for 18 h at 30°C on potato dextrose agar (Difco, Detroit, MI). A small number of hyphae were scraped off each plate and suspended in 50 μl of Prepman Ultra reagent (Applied Biosystems, Foster, CA) in a 0.5-ml microcentrifuge tube. The suspension was vortexed initially for 45 s to 1 min and then heated for 15 min at 100°C. The suspension was then pelleted for 5 min at maximum speed in a
microcentrifuge according to the manufacturer’s instructions. The supernatant was transferred to a new tube and stored on ice until PCRs could be set up.

PCRs were performed directly on 3 µl of the Prepman supernatant in a 50-µl reaction mixture using high-fidelity PfX50 DNA polymerase (Invitrogen, Carlsbad, CA) according to the manufacturer’s instructions. ITS amplicons were obtained using primers (ITS1 and ITS4) and PCR conditions as previously described (20). D1/D2 PCR amplicons were obtained using primers (NL-1 and NL-4) and PCR conditions as described previously (14, 16). Amplifications were performed in a PTC-100 thermocycler (MJ Research, Watertown, MA) using the preprogrammed three-step protocol as the standard program for all reactions and consisted of 35 cycles using an annealing temperature of 59°C and a 1-min extension time. Since the PCR product was going to be used in TA cloning, an additional A-tailing procedure was performed due to the blunt-ended fragments generated by PfX50 amplification. Tailing was performed at the end of the PfX50 cycling program by adding 1 µl of Taq DNA polymerase (Invitrogen, Carlsbad, CA) to the PfX50 reaction mixture and incubating at 72°C for 30 min. A 5-µl aliquot of the PCR mixture was run on a 0.7% agarose gel and stained with ethidium bromide to confirm amplification, and then the remaining PCR mixture (45 µl) was purified using the Wizard SV gel and PCR cleanup system (Promega, Madison, WI). Three microliters of the purified PCR product was used for TA cloning into pGEM-T Easy (Promega, Madison, WI), which was performed according to the manufacturer’s instructions. TA-cloned plasmid DNA was obtained using a Qiaprep spin miniprep kit (Qiagen, Valencia, CA) and then sequenced at the UTHSCSA advanced nucleic acids core facility using vector-derived primers (T7 and SP6) in order to identify the insert sequence. Sequences were then used to perform individual nucleotide-nucleotide searches of the ITS and D1/D2 regions using the BLASTn algorithm at the NCBI website (http://www.ncbi.nlm.nih.gov/BLAST/). Identifications were calculated based on percentages of identity from the top three BLAST searches for both the ITS and D1/D2 regions.

BLASTn results for the 07-831 ITS region returned the three highest percentages of identity with (i) Actinomucor elegans var. kuwaitiensis accession no. AJ849551.1, 99.4% (713 of 717 bp); (ii) Actinomucor elegans accession no. FJ176396.1, 99.0% (709 of 716 bp); and (iii) Actinomucor elegans accession no. AM745429.1, 99.0% (709 of 716 bp). The three highest percentages of identity for the D1/D2 region were for (i) Actinomucor elegans var. kuwaitiensis accession no. AJ849552.1, 100% (726 of 726 bp); (ii) Actino-
**Discussion.** The Mucorales are an order of fungi ubiquitous in soil and known to sometimes cause life-threatening infections, termed mucormycosis (zymomycosis), in immunocompromised patients. Mucormycosis has also been reported to occur in immunocompetent victims following trauma (1, 3, 4, 6, 7, 9, 12, 17, 19). Previously reported genera known to cause invasive mucormycoses include *Absidia*, *Rhizopus*, *Mucor*, and *Rhizomucor*, as well as *Apophysomyces*, *Saksenaea*, *Cunninghamella*, *Syncephalastrum*, and *Cokeromyces* (18). Mucormycosis carries a high risk of morbidity and mortality due to the propensity of the fungi to produce vascular invasion and spread hematogenously, leading to dissemination and tissue necrosis (1). As demonstrated with our patient, a high degree of suspicion for invasive fungal infections is needed for early recognition and proper antifungal therapy in order to decrease morbidity and mortality.

*Mucormycosis* is generally acquired through inhalation of sporangiospores, leading to rhinocerebral and pulmonary disease in immunocompromised hosts (8). In trauma and burn victims, mucormycosis usually starts with direct inoculation of an open wound with contaminated soil rich in organic material (8) or colonization of open wounds via contaminated dressings or during surgical procedures in a hospital setting (18). Trauma and burn patients in intensive care units commonly have multiple associated comorbidities (including organ dysfunction, contaminated wounds, bacterial sepsis, and prolonged courses of broad-spectrum antibiotics), many of which also contribute to compromised immune function, placing the patient at higher risk for developing mucormycosis (3). The levels of virulence of the Mucorales appear to vary, with diseases ranging from contamination of open wounds to production of severe systemic infection (3). Cutaneous mucormycosis is the most common presentation in trauma patients, often...
characterized by indurated, dusky-appearing nodules surrounded by a pale halo (3). In a comprehensive review of 929 patients with zygomycosis (mucormycosis), penetrating trauma accounted for 34% of those presenting with cutaneous involvement. Of those with cutaneous involvement, 24% had extension into surrounding muscle or bone and 20% experienced hematogenous dissemination from the skin to other organs (17). Invasive infection associated with vascular invasion may result in thrombosis and infarction of surrounding tissues (3). The type and location of injury are significant in predicting the morbidity and mortality of trauma patients. Of trauma and burn patients infected with *Saksenaea*, two-thirds of patients with abdominal wounds and 100% of those with sinusitis died despite surgery (19). Another study of mucormycosis in trauma patients found a 50% mortality rate among patients with head or trunk involvement, and two-thirds of patients with limb involvement required amputation (3). Aggressive treatment of mucormycosis is required in cases of trauma and burns, with removal of contaminated and necrotic tissue and concurrent treatment with antifungal agents, typically intravenous amphotericin B (3, 4, 9, 19).

*Actinomucor* (order Mucorales) is a facultative anaerobe ubiquitous in nature (13) and has not previously been identified as a causative agent of invasive mucormycosis (zygomycosis). The genus *Actinomucor* was first described by Schostakowitsch in 1898 (17a) and then again by Benjamin and Hesseline in 1957 (2, 11). *Actinomucor* was noted to be similar to *Mucor*, *Rhizopus*, and *Absidia* species, with broad, irregular sparsely septate hyphae (15). *Actinomucor* is differentiated from *Mucor* by having branched stolons giving rise to rhizoids and sporangiophores and from *Rhizopus* and *Absidia* by the limited growth of stolons and by the arrangement of the columellae and sporangiophores. The genus *Actinomucor* was previously thought to contain two species, *A. elegans* (2) and *A. taiwanensis* (10). *Actinomucor elegans* was differentiated from *A. taiwanensis* by the smaller size of the sporangiospores, 5 to 8 μm versus 7 to 15 μm, and by a maximum growth temperature of 32°C versus 37°C. A study by Zheng and Liu in 2005 (21), however, characterizing 110 strains of *Actinomucor* by ITS region sequencing, demonstrated that the genera *Mucor* and *Actinomucor* are more closely related to one another than to the genus *Rhizopus* and that *Mucor meitauzae* (synonymous with *Actinomucor taiwanensis*) is actually a variety of *A. elegans*. Zheng and Liu, therefore, reduced *A. taiwanensis* to a varietal status of *A. elegans* now known as *A. elegans* var. *meitauzae*. Thus, the genus contained one species with two varieties, *A. elegans* var. *elegans* and *A. elegans* var. *meitauzae*. *Actinomucor* species are known for their association with the production of soy-based products, providing flavor and texture to food (11).

*Actinomucor elegans* has been shown to be possibly pathogenic for humans in only two cases reported in the literature. The first case, reported in 2001, was that of an immunocompetent 11-year-old girl diagnosed with maxillary sinusitis. *Actinomucor elegans* was recovered in a culture of maxillary sinus contents; however, invasive disease was not confirmed by histopathology, nor was eosinophilia reported (5). In 2008, an *Actinomucor* species was isolated from a swab of a foot ulcer of a patient with diabetes. Once again, invasive disease was not confirmed by histopathology; however, sequencing of the ITS and D1/D2 regions of this isolate determined that it was a new...
variety of *A. elegans, A. elegans* var. *kuwaitiensis* (Kw823), with the varietal name being based upon the geographic region of recovery. Although the D1/D2 region of Kw823 demonstrated nucleotide differences from *A. elegans* isolate ATCC 22814 of >1%, it also exhibited a nucleotide difference of <1% from the same region of other strains of *A. elegans* var. *elegans* and *A. elegans* var. *meitauzae*. As this isolate also did not demonstrate unequivocal phenotypic features, sequence data combined with morphology did not seem to support a separate species status. The isolate was also inoculated into immunocompetent mice to prove its in vivo pathogenicity, resulting in 100% mortality in the mouse model (11). Additional phenotypic studies determined that temperature maximums were not useful to separate varieties, as the reference strains *A. elegans* var. *elegans* and *A. elegans* var. *meitauzae* as well as the new variety demonstrated growth at up to 40°C.

Our patient was probably inoculated with *Actinomucor elegans* var. *kuwaitiensis* through his open skin at the time of injury. The concurrent *Candida tropicalis* infection was more likely nosocomial, and the other isolated fungi—*Arthrographis* and *Aspergillus* spp.—were most likely contaminants, as they were not further isolated. The patient’s comorbidities—which included acute kidney injury secondary to rhabdomyolysis, multiple fractures requiring surgical intervention, and prolonged use of broad-spectrum antibiotics—likely contributed to a degree of immune suppression, providing an adequate environment for the fungi to grow. As with other cases of mucormycosis in trauma or burn victims, diagnosis of our patient’s *Actinomucor* infection was not made in time to allow early initiation of antifungal therapy. It is clear that the medical team adequate cultures and remained abreast of the results. Unfortunately, mucormycosis was not in the differential, nor did the cultures suggest this fatal infection until too late. Mucormycosis should be considered a cause of rapidly progressive necrotic wounds in patients with the previously mentioned risk factors, as it may prove to be fatal unless treated early and adequately with surgical debridement and intravenous antifungal therapy.

**Nucleotide sequence accession numbers.** Sequences for *Actinomucor elegans* UTHSC 07-831 were deposited in GenBank under accession numbers FJ896015 (ITS) and FJ896016 (D1/D2).

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**REFERENCES**