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# Mucormycosis Caused by *Rhizopus microsporus* var. *microsporus*: Cellulitis in the Leg of a Diabetic Patient Cured by Amputation

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**Mucormycosis accompanied the development of bacterial infection in the leg of a diabetic African-American man. Local injury, diabetic ketoacidosis, renal insufficiency, and antimicrobial therapy were factors that contributed to the pathogenesis of the mucormycosis. The cellulitis was caused in part by *Rhizopus microsporus* var. *microsporus* and was cured by amputation. We report this unusual case of mucormycosis to emphasize the value of fungal identification, to illustrate a dramatic and successful clinical result, and to draw attention to an apparent role for bacterial infection and its treatment in the pathogenesis of mucormycosis. It is the third case report of mucormycosis in a human in which *R. microsporus* var. *microsporus* was definitively identified as the etiologic agent.**

Mucormycosis remains a frequently fatal infectious disease. However, in aggressively managed cases in which surgery and intravenous amphotericin B are used immediately, survival is now a reasonable expectation (2, 3, 9, 10, 16, 18, 22). The fatality rate is unknown since the disease is not reportable. Mucormycosis most commonly develops in patients with diabetes mellitus, malignant hematologic disorders, severe leukopenia, or a history of deferoxamine therapy. However, why so few persons even with these serious predisposing conditions get mucormycosis when people are routinely exposed to decayed vegetative matter containing conidia is an unanswered epidemiologic question. Anecdotal cases are not a substitute for incidence or attack rates after exposures or among high-risk groups. The disease is caused by species of the genera *Rhizopus*, *Mucor*, *Rhizomucor*, *Absidia*, *Cunninghamella*, *Saksenaea*, and *Apophysomyces*. Since the diagnosis is made in part from histologic sections, initial specimens may not be submitted for fungal culture, and the same or comparable surgical biopsy specimens are often not available for culture when the histologic diagnosis is reported to the clinician. Since the infection is often fulminant, the opportunity for obtaining a second specimen for culture may be lost. Many patients have died without benefit of microbiological diagnosis. Furthermore, if a fungus is isolated, the identification of the species of fungus is difficult in many clinical microbiology laboratories, for they have neither the expertise to carry the identification to the species level nor an incentive to send the isolate to a reference laboratory. Hence, only the genus is reported. Without full identification of the fungus responsible for the infection, the assessment of pathogenicity and the interpretation of the results of therapy are not possible. We report here an unusual case of mucormycosis to emphasize the value of identification of the fungus, to illustrate a dramatic and successful clinical result, and to draw attention to an apparent role for bacterial infection in mucormycosis.

**Report of a case.** A 42-year-old man was admitted to University Hospital (Shreveport, La.) for a necrotic ulcer of the proximal right leg. It measured 7 by 7 cm and was surrounded by cellulitis that extended above the knee. A week earlier, he had fallen down stairs injuring his leg. Five days before admission, inflammation developed and led to a malodorous ulcer. He denied fever and chills, but he had a poor appetite. Nausea and vomiting resulted in his not taking insulin on the day of admission. He had had insulin-dependent diabetes mellitus for 18 years. Six months earlier his serum creatinine level had been 240 μmol/liter (2.7 mg/dl); it had risen to 420 μmol/liter (4.7 mg/dl) 1 month prior to admission. Clonidine had been prescribed, but it was not taken.

On physical examination, his temperature was 37.6°C, supine blood pressure was 190/104 mm Hg, supine pulse was 108 beats per min, sitting blood pressure was 154/80 mm Hg, sitting pulse was 120 beats per min, and respirations were 16/min. He was thin and in mild distress. In addition to the right leg ulcer, abnormalities were a 3-by-3-cm neuropathic, noninflamed ulcer of the second right metatarsal head, nontender right femoral nodes, a healed amputation of the left fifth toe, flat neck veins when supine, and a systolic ejection murmur. Chest and sinus roentgenograms and a renal ultrasound were normal.

Abnormal initial laboratory results included the following: leukocyte count, $22.7 \times 10^9$/liter (22,700/μl), with 0.875 (87.5%) neutrophils, 0.08 (8%) lymphocytes, and 0.045 (4.5%) monocytes; hematocrit, 31% with microcytic erythrocyte indices; platelet count, $701 \times 10^9$/liter (701,000/μl); urea nitrogen concentration, 32.5 mmol/liter (91 mg/dl); creatinine concentration, 660 μmol/liter (7.5 mg/dl); glucose concentration, 32.1 mmol/liter (579 mg/dl); sodium concentration, 129 mmol/liter; potassium concentration, 5.6 mmol/liter; and carbon dioxide content, 9 mmol/liter. Arterial blood gas values were pH 7.26, partial $O_2$ pressure was 98 mm Hg, and partial $CO_2$ pressure was 28 mm Hg.

**Initial hospital course and clinical microbiology.** Intravenous therapy with clindamycin at 900 mg every 8 h, nafcillin at 2 g every 4 h, and amikacin at 350 mg (one dose) was administered in the emergency room. A short time later, smears and cultures were done. Gram stain showed no organisms; swabbed specimens from the ulcer were cultured aerobically and anaerobically and demonstrated moderate growth of *Staphylococcus aureus* (β-lactamase negative) and a few colonies of *En-*

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*terobacter cloacae* but no anaerobes. Two sets of blood for cultures were drawn into bottles containing resin to bind antimicrobial agents; each set consisted of one aerobic bottle and one anaerobic bottle (Bactec 660; bottles 16 and 17; Johnston Laboratories, Cockeysville, Md.); they yielded no growth. On day 2 the patient was taken to the operating room for debridement. The operative culture grew *S. aureus* (β-lactamase negative) (moderate), *E. cloacae* (numerous), viridans group streptococci (a few organisms), and a non-*Clostridium perfringens Clostridium* sp.

Histopathology of the debrided tissue showed colonies of bacteria amid acute and chronic inflammation and necrosis. Fungi having wide nonseptate hyphae with acute or 90-degree branching were seen invading blood vessels and nerves. This histology made the nosocomial acquisition of mucormycosis unlikely.

On day 3, the incision, which extended from the medial thigh to the lower leg, was indurated, showed dark necrosis, and was covered with a white fuzzy film. Four specimens from the ulcer were obtained. Gram stain of two swabbed specimens showed numerous gram-negative bacilli. Two of the four specimens of freshly swabbed clinical material, i.e., the fuzzy white film were examined in 20% KOH preparations by direct microscopy and revealed broad nonseptate hyphae morphologically consistent with zygomycetes in tissue (6). Two of the four specimens were negative on KOH preparation, Gram stain, and culture, including fungal culture. Three of the four specimens were plated for bacterial growth, one of which showed growth of *E. cloacae* (numerous), alpha streptococci (*Enterococcus* spp.), and *Rhizopus* spp.

**Histology of the amputated limb.** Progressive gangrene prompted amputation above the knee on day 3. The tissue sections stained with periodic acid-Schiff stain revealed numerous nonseptate hyphae measuring 7 to 14 μm in diameter. Although hyphal morphology in the tissue was typical for a member of the order *Mucorales* (Fig. 1A and B), considerable numbers of hyphae had unusually thick walls. The cross sections of the thick-walled hyphae are shown in Fig. 1C. The variability in size and the thick walls in tissue sections are notable.

**Mycology.** Two of the four fungal cultures yielded growth. Growth was visible by the third day of incubation at 30°C on a battery of standard selective fungus culture media (Emmons' modification of Sabouraud dextrose agar with gentamicin, SabHi agar with chloramphenicol, Mycosel with chloramphenicol and cycloheximide [BBL], and birdseed agar with chloramphenicol) and filled the space in each culture tube by the fifth day. The cottony aerial mycelia were at first white and then acquired a gray, speckled appearance. Microscopically, the colonies were composed of broad, predominantly coenocytic hyphae with stolons which bore rhizoids and fascicles of unbranched brown sporangiophores at the same intervals on the stolons but on opposite sides, in a manner consistent with members of the genus *Rhizopus*. The sporangiophores bore columellate, brown sporangia filled with sporangiospores. Mature sporangiospores were rhomboidal and striated with a tinge of golden pigment (Fig. 1D). Identification of the fungus isolated from this patient as *Rhizopus microsporus* var. *microsporus* was conducted by one of us (K.J.K.-C). This fungus produced a colony with fruiting structures most closely resembling those described for *R. microsporus* van Tieghem. It grew well at 45°C, but it failed to do so at 50°C. The species identification was confirmed by M. A. A. Schipper at Baarn, The Netherlands.

**Hospital course.** Immediately postamputation, amphotericin B was administered intravenously as a 1-mg test dose, and then 20 mg was infused over several hours. The daily dose of amphotericin B was increased to 60 mg, which was discontinued on day 13, after the administration of a total of 477 mg. Clindamycin and nafcillin were discontinued on days 5 and 8, respectively. An expected amount of edema in the stump gradually resolved. There was no clinical evidence of inflammation, lymphadenitis, or local or disseminated mucormycosis. The patient received conservative management of renal impairment, the creatinine value being 410 μmol/liter (4.6 mg/dl) at discharge, 47 days following admission.

**Discussion.** The taxonomy of the genus *Rhizopus* has undergone revision. *R. microsporus* now includes four varieties, including, for example, *R. microsporus* var. *microsporus* and *R. microsporus* var. *rhizopodiformis*, the only two varieties known to cause disease in humans. Since *R. microsporus* denotes the type variety of the species, its varietal name is often omitted. *R. microsporus* var. *oligosporus* and *R. microsporus* var. *chinensis* have not been reported to cause disease in humans. The four varieties are not separate species because they mate with each other, despite distinguishing morphologic features and abilities to grow at various temperatures (17, 20). All four varieties grow at 45°C (20). *R. microsporus* var. *microsporus* can be differentiated from *R. microsporus* var. *rhizopodiformis* by the formation of angular and striated sporangiospores and the lack of growth at 50°C. Identification is best handled by a *Rhizopus* expert.

Two humans with infections caused by *R. microsporus* var. *microsporus* with definitive identification have been reported in the literature (9, 10). While *R. microsporus* var. *microsporus* is pathogenic for mice upon intravenous injection (21), its virulence in humans appears to be limited either in fact or because full identification has not often been done. The identities of organisms labelled *R. microsporus* in a reference collection were not substantiated in two cases, one being a report of an isolate from human gastrointestinal mucormycosis (15), identified later by Scholar et al. (21) as *R. microsporus* var. *rhizopodiformis*. Similarly, Gitter and Austwick (5) reported the fungus from a case of gastrointestinal mucormycosis in swine in 1959, but Scholar et al. (21) identified it as *R. microsporus* var. *rhizopodiformis*. A fatal infection in an adolescent boy on chronic ambulatory peritoneal dialysis who received deferoxamine was *R. microsporus* var. *rhizopodiformis* (14). Kerr et al. (9) reported a case of infection caused by *R. microsporus* var. *microsporus* in the limb of a patient who was receiving hemodialysis and who survived with aggressive treatment and amputation. It has also been isolated from a lung biopsy specimen from a leukemic patient with pneumonia, although the case was not fully reported (10).

As our patient's infection progressed, his diabetes became uncontrolled. The association of mucormycosis with diabetes and other immunocompromising conditions is established (1–5, 7, 9–16, 18, 19, 22). The deterioration of his diabetes to ketoacidosis contributed to the host defect and the severity of the mucormycosis. Similarly, renal failure contributed to host compromise through an unknown mechanism, as in other patients (2, 9, 14, 22).

The pathogenesis in our patient also relates to the mixed infection at the site of his injury, unequivocally consistent with mixed synergistic bacterial gangrene. The ulcer was an aerobic and anaerobic bacterial infection that appears to have preceded or coincided with the mucormycosis. Judging from the smell and the extent of the necrosis, there probably were more anaerobic bacteria present than the non-*C. perfringens Clostridium* sp., but not *C. perfringens*, that was isolated. Other anaerobes presumably were not isolated because antimicrobial agents were begun before specimens for culture were obtained.

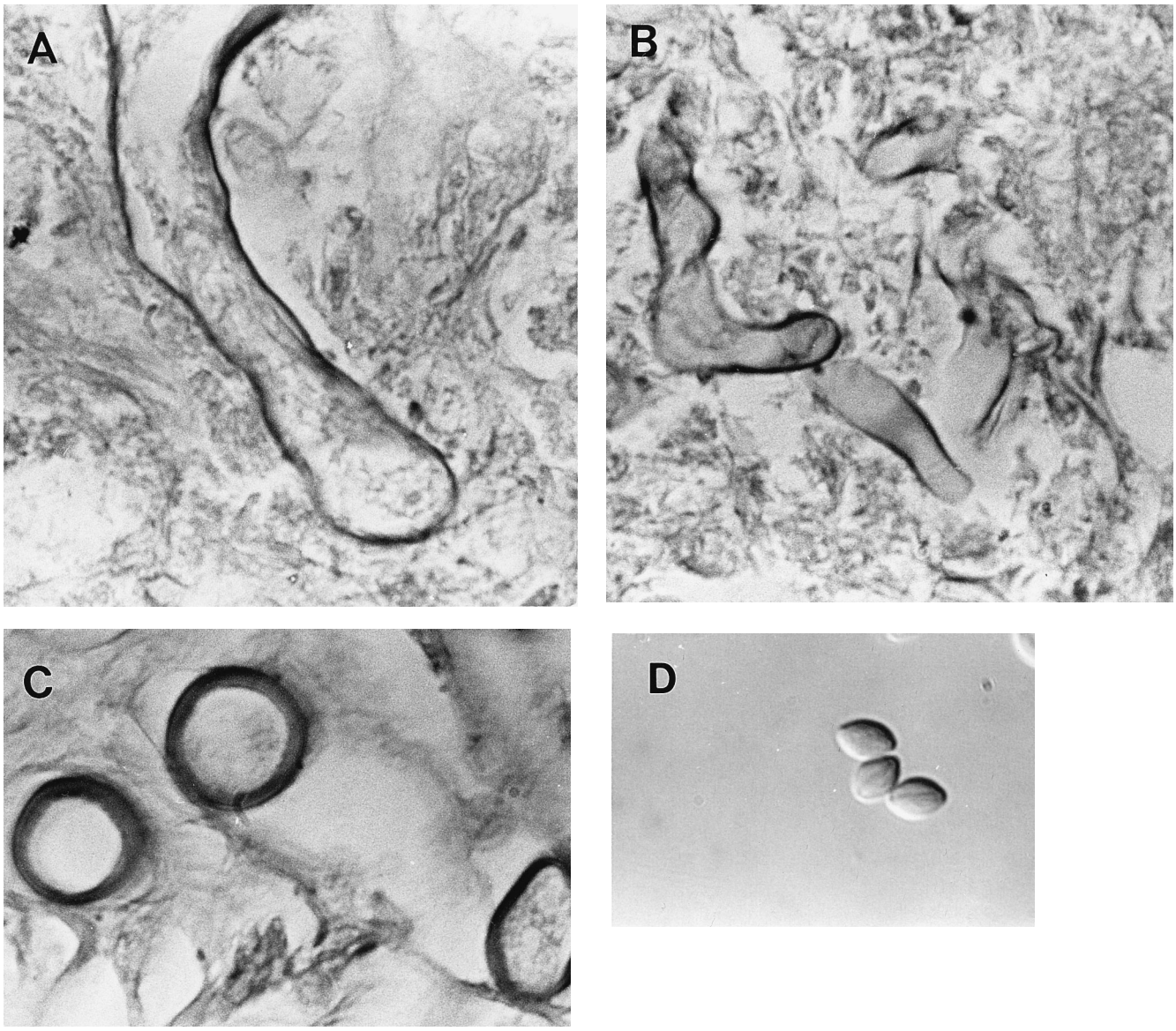


FIG. 1. *R. microsporus* var. *microsporus* in tissue. Histopathological sections stained by periodic acid-Schiff stain showing nonseptate wide hyphae (A and B) typical of mucormycotic agents and unusually thick-walled hyphae seen in cross-sections (C). (A to C) Magnifications, ×1,200. Rhomboidal and striated sporangiospores produced by the fungal isolate grown on Sabouraud's agar (D). (D) Magnification, ×1,000.

Perhaps it is as appropriate to focus on the isolation of *R. microsporus* var. *microsporus* from the ulcerated leg as it is important to develop the association of *R. microsporus* infection with a mixed aerobic and anaerobic necrotizing synergistic bacterial gangrene. Within 2 days of blunt and penetrating injury to the leg, infection was present, but the patient delayed seeking care. Which came first, the bacteria or the fungus, is not known, but evidence favors the bacterial process being present with or before the *R. microsporus* infection. One explanation for the mucormycosis is that the mixed bacterial infection established local and systemic, e.g., hyperglycemic, conditions for the growth of *R. microsporus*. The first evidence of gangrene was the failure of tissue to bleed on its cut surfaces. Debridement to normal tissue could not be accomplished, a characteristic of fungal infections which typically invade vessels, infarcting distal structures, a process which accompanied mixed bacterial cellulitis in this man's leg. Gross fungal growth was dramatic in the wound, which was covered with a white fuzzy film throughout the incision within 24 h of the debridement. A similar observation ("mycelia could be seen in the wound as a fluffy, white layer") was photographed previously (4). The microbial virulence of *R. microsporus* may be related to its occasional production of the mycotoxin rhizonin A (23), but no studies with humans are available.

The source of *R. microsporus* var. *microsporus* is unknown. Elastoplast bandages contaminated with *R. rhizopodiformis* and *Rhizopus* spp. (presumably *R. rhizopodiformis*) were shown to cause postoperative wound infections before this source was discovered and corrected (10, 18). Cutaneous mucormycosis such as the present case has been infrequently reported (7–11, 13, 15). Although the debridement occurred 24 h after admission, the histology showed fungi, making this case almost certainly not nosocomial.

Evidence that antibiotic treatment increases the risk for

mucormycosis and/or the severity of mucormycosis pertains to our patient. Whether mixed synergistic bacterial gangrene and mucormycosis were both present from the outset is unknown. Antimicrobial agents were instituted before the diagnosis of mucormycosis, as in many cited (3, 7–16, 18, 22) and uncited cases. For example, in a detailed report sinus infection with group B streptococci and *Veillonella* spp. accompanied *R. microsporus* var. *rhizopodiformis* infection (18). Antimicrobial therapy is evidence that the physician recognized an infection. The antimicrobial therapy may have aided the growth of *R. microsporus* in our patient and members of the order *Mucorales* in other patients in unknown ways, allowing mucormycosis to emerge as the dominant infection. So many cases of mucormycosis have been treated as bacterial infections, which then worsen rapidly, that to not at least consider a role for bacteria and/or antimicrobial agents in their pathogenesis is to avoid the obvious. Mechanisms of an influence of antimicrobial therapy must take into account the fact that preexisting, often mixed aerobic and anaerobic bacterial infections may damage tissue before mucormycosis begins. A mechanism for the progression of mucormycosis once the bacteria are killed may include the absence of bacteria (or bacterial products) or the absence of competition for nutrients.

The decision to amputate in our patient was made because of the extensive gangrene observed during debridement with worsening afterward, not because mucormycosis was diagnosed. The other well-documented case of *R. microsporus* var. *microsporus* was also treated successfully with amputation (9). Therapy with amphotericin B was administered until it was clear that no *Rhizopus* infection could be found in the stump or elsewhere.

In conclusion, a preexisting or concurrent, virulent, mixed aerobic and anaerobic bacterial infection occurred in our patient with mucormycosis. The fact that bacterial infection and/or the antibacterial treatment of such infections may favor the development and the pathogenesis of mucormycosis is supported by our observations. Amputation for progressive gangrene was curative in our patient, although the amphotericin B, administered after amputation, might have treated undetected infection outside of the primary site. *R. microsporus* var. *microsporus* was demonstrated for the third time to cause a significant clinical infection in a human.

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