**Mucor ramosissimus** Samutsevitsch Isolated from a Thigh Lesion

IRENE WEITZMAN,¹* PHYLIS DELLA-LATTA,¹ GERARD HOUSEY,² AND GLADYS REBATTÀ¹

Clinical Microbiology Service, Columbia-Presbyterian Medical Center,¹ and Department of Pathology, Columbia University, College of Physicians and Surgeons,² 622 West 168th Street, New York, New York 10032-3784

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*Mucor ramosissimus* Samutsevitsch is presented for the first time as an etiologic agent of cutaneous zygomycosis in a patient with aplastic anemia on immunosuppressive therapy. This report also represents the third case caused by this species reported in the literature. A biopsy taken from a lesion on the patient's thigh revealed broad, nonseptate, nonbranching hyphae compatible in morphology with a Zygomycete; *M. ramosissimus* was cultured twice from the thigh lesion. The patient was treated successfully with amphotericin B. Identifying features of *M. ramosissimus* include the following: numerous sporangia lacking columellae and resembling those of *Mortierella* spp., short, erect sporangiophores repeatedly branching sympodially; tough, persistent, and diffused sporangial walls; numerous oidea in chains; extremely low colonies; and restricted growth at 36°C. This paper describes the isolate and strives to alert the clinical microbiologist to this rarely reported pathogen.

Zygomycosis is an umbrella term encompassing all mycotic diseases caused by fungi in the class Zygomycetes. Some prefer the term mucormycosis, because most of the fungi pathogenic to humans are found in the order Mucorales and produce a distinctive disease in the debilitated patient (4). Risk factors include diabetic ketoacidosis, neutropenia, immunosuppressive therapy, severe trauma, protein-calorie malnutrition, and iron overload with or without deferoxamine (4, 5, 9). Clinical manifestations include rhinocerebral, pulmonary, cutaneous, gastrointestinal, central nervous system, disseminated, and miscellaneous disorders (9). The most frequently encountered etiologic agent for humans is *Rhizopus oryzae* (arrhizus) (4). Species of *Mucor* are rarely agents of zygomycosis (5, 6).

*Mucor ramosissimus* is reported here, for the first time, as an etiologic agent of cutaneous zygomycosis in a patient with aplastic anemia on immunosuppressive therapy, and this represents the third case caused by this species reported in the literature. Mucocutaneous and rhinocerebral zygomycosis were previously reported in 1974 and 1964, respectively (1, 10). The purpose of this paper is to present an expanded description of this isolate of *M. ramosissimus* and to familiarize clinical microbiologists with this potential pathogen, which may be reported only rarely because of incorrect identification (since it resembles *Mortierella* spp.) or because of a lack of sufficient and accessible identifying information (2–4, 7).

Case report. A 31-year-old Hispanic female was admitted to the Columbia-Presbyterian Medical Center complaining of frequent episodes of bleeding from the gums and epistaxis. Her medical history was significant for aplastic anemia first diagnosed in 1986, which presumably followed infection with varicella-zoster virus. She had been treated subsequently at another medical center with antithymocyte globulin, which produced no response, and bone marrow transplantation was considered. However, none of the family members who were tested for bone marrow compatibility at the time were found to be an appropriate match. Since that time, the patient's management had included multiple blood transfusions and immunosuppressive therapy and was complicated by episodes of profuse menstrual bleeding, epistaxis, and oozing of blood from the gums. Furthermore, the patient reported many episodes of fever, chills, and general malaise, presumably due to undiagnosed infections secondary to her decreased leukocyte count.

Her medical history was significant for pyelonephritis, pneumonia, hepatitis A infection, hepatitis B infection, and Epstein-Barr virus exposure. Her current medications included prednisone (50 mg administered orally four times a day), aminoacaproic acid (Amikar; 1 g administered four times a day, every 6 h), and cyclosporine (200 mg administered orally four times a day).

During hospitalization, the patient developed a well-circumscribed lesion of the thigh, which was evaluated as described below. The patient was treated with amphotericin B (Fungizone), responded favorably, and was discharged.

Laboratory studies. The thigh lesion was biopsied and the specimen was sent to the dermatopathology laboratory. The hematoxylin-and-eosin-stained preparation revealed broad, nonseptate, nonbranching hyphal fragments in the dermis compatible in morphology with a Zygomycete. Two swabs were taken from the biopsy site at different times on the same day and sent to the mycology laboratory for culture to rule out aspergillosis. The swabs were streaked onto homemade Sabouraud dextrose agar (SDA) plus 0.1% yeast extract, Mycosel (BBL Microbiology Systems, Cockeysville, Md.), and brain heart infusion agar (BBL). Direct examination of the swabs by using 10% NaOH plus glycerol showed them to be negative for fungi. Cultures were incubated at 30°C. A rapidly spreading gray colony suggestive of a Zygomycete was observed within 6 days on all media except Mycosel. Microscopic examination of teased mounts in lactophenol cotton blue from the SDA culture revealed sporangiophores which were short, mostly erect, hyaline, septate, smooth, and branching; numerous small, dark brown, globose sporangia lacking columellae; and an absence of rhizoids. All of these features were suggestive of a *Mortierella* species. In addition, numerous chains of thin-

* Corresponding author.
and thick-walled oidia and smooth-walled, globose, hyaline sporangiospores were observed.

Mycology. A culture from SDA was subcultured onto plates of Czapek agar (Remel Laboratories, Lenexa, Kans.), potato dextrose agar (PDA) (Remel), and SDA with Emmons modifications (Remel). Slide cultures were prepared from PDA and cornmeal dextrose agar (Remel), and all cultures were incubated for 7 days at 23°C. Slants of PDA were incubated at 23, 30, 36, 40, 42, and 48°C to determine the optimum and maximum temperatures of growth; a PDA butt was inoculated on the surface and incubated at 23°C to determine the height of growth. In addition, nitrate and carbon assimilation tests were conducted with media available in the mycology laboratory rather than media specialized for the Zygomycetes in which the nitrogen source is ammonium or asparagine and the carbon source is a 1% liquid solution (8). The nitrate assimilation test was performed with Salkin’s nitrate agar (BBL) to determine the isolate’s ability to utilize KNO$_3$ as the sole source of nitrogen. The ability to assimilate sucrose and lactose as the sole sources of carbon was tested by the auxanographic method with Noble agar (Difco, Detroit, Mich.), yeast nitrogen base (Difco), and sucrose, glucose, and lactose disks (BBL).

Our cultures grew rapidly on SDA and PDA at 23°C, covering the entire surface of the agar plates in 7 days. Colonies were flat and light gray on SDA and charcoal gray with a light yellow-gray reverse on PDA. Growth on Czapek agar was sparse but sporulating. PDA butt cultures were 1 to 2 mm in height (Fig. 1). Cultures showed optimal growth at 30°C, restricted growth at 36°C, and no growth at 40°C or above. Sporangiophores arose directly from the substrate mycelium and were up to 14 μm in diameter, hyaline, smooth to slightly roughened, short, and erect. Branching of the sporangiophores was sympodial (Fig. 2), with successive branches often shorter and smaller in diameter. Sporangiospores were frequently septate below the sporangium and somewhat constricted, becoming progressively narrower as they grew upwards and infrequently exhibiting swollen, racket-shaped regions towards the top. Sporangia (16 to 58 μm long by 16 to 64 μm wide) were globose, ranged from having few to many spores and from being yellow-brown to brown, and were often devoid of observable columellae (Fig. 3). Sporangial walls were frequently persistent, and sporangia were often observed intact. Columellae (14 to 28 μm long by 14 to 22 μm wide) were smooth, hyaline, annulate, subglobose, conical, and cylindrical and frequently had collars (Fig. 4). Sporangiospores (4 to 8 μm in diameter) were ovoid to globose, smooth, hyaline, thin walled, and homogeneous. Oidia (4 to 14 μm in diameter) were numerous and hyaline and were observed in terminal or intercalary chains. They were globose to elongate and thin walled when young and thick walled with a dense cytoplasm when mature. Our biochemical test results indicated that nitrate (KNO$_3$) was assimilated and that sucrose was not assimilated, in agreement with the findings of Scholer et al. (8). In addition, glucose was assimilated but lactose was not.

The first reported case of zygomycosis caused by *M. ramosissimus* occurred in a 39-year-old Caucasian woman with no obvious underlying disease who developed chronic destructive mucocutaneous disease lasting 24 years (10). The second case discussed in the literature involved a 39-year-old, alcohol-abusing, diabetic black male who developed rhinocerebral disease (1). Both cases, as well as the case we studied, subsequently responded to amphotericin B therapy; the first patient also required surgery. Hesseltine and Ellis provided a detailed description of the fungus in the first case and designated it the neotype strain (3). They concluded that this isolate represented a species described as a saprophyte many years ago in Russia (7). No description was provided for the second isolate (1).

In general, our isolate resembles that described by Hes-
FIG. 3. Sporangia lacking observable columellae (revealed by lactophenol cotton blue; magnification, ×400).

selting and Ellis (3). Identifying features in agreement are (i) numerous sporangia devoid of a columella and hence resembling a Mortierella species; (ii) short, erect sporangiophores which repeatedly branch sympodially; (iii) tough, persistent sporangial walls (but also numerous deliquescent sporangia); (iv) extremely low colonies; (v) numerous oidia in chains; and (vi) restricted growth at 36°C.

Our findings differ in the following minor features. (i) The occurrence of racket-shaped enlargements in the sporangiophores of our isolate was infrequently observed and was not an identifying feature. (ii) The sporangiophores were mostly smooth, not typically roughened. (iii) The columellae in our isolate were smaller (14 to 28 μm long by 14 to 22 μm wide versus 20 to 37 μm long by 17 to 30 μm wide) and more varied in shape.

The microbiologist should consider M. ramosissimus in the differential identification when the following are initially observed: (i) persistent (not breaking or diffuent), small, brownish sporangia lacking columellae; (ii) short, erect sporangiophores repeatedly branched sympodially (Fig. 2); (iii) numerous chains of oidia (arthrospores); and (iv) optimum growth at 30°C, restricted growth at 36 to 37°C, and no growth at 40°C. A tentative identification may be reached by (i) thoroughly searching 7-day-old teased mounts for columellae (if necessary, tapping the preparation to dislodge or break up the larger sporangia and uncover the columellae) and (ii) preparing butt (deep) cultures in PDA and observing them for low grayish colonies after incubation for 7 days at 23°C (Fig. 1). A Mucor isolate meeting these criteria should be sent to a reference laboratory for confirmation.

This culture was confirmed as M. ramosissimus at the Centraalbureau voor Schimmelcultures, Baarn, The Netherlands, and is on deposit in their collection under the name

FIG. 4. Cylindrical columella with collar (top) and conical columella (bottom).

Mucor aff. ramosissimus Samutsevitsch CBS 144.93. It will not be mentioned in their list of cultures because of some structures that are atypical compared with those of typical M. ramosissimus NRRL 3042, "mainly by the shape of the columellae and the more or less diffuent sporangium walls instead of the tough persistent walls." The culture is also on deposit at the Centers for Disease Control and Prevention, Atlanta, Ga. (B5335), and at the American Type Culture Collection (ATCC 90286).

REFERENCES