Isolation and identification of *Rhizomucor pusillus* from rhinofacial mucormycosis in a diabetic patient

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**Introduction:** Mucormycosis is usually an invasive mycotic disease caused by fungi in the class Mucormycetes. It often occurs in immunocompromised patients but sporadic cases without apparent immune impairment have been described.

**Case presentation:** Here we report a case of rhinofacial mucormycosis due to *Rhizomucor pusillus* in a 55-year-old diabetic female. She presented with diabetic ketoacidosis and nasal obstruction, nasal discharge and right-sided cheek swelling following sinus surgery, which had been performed at a private hospital 1 month previously. Endoscopic biopsy was performed and the sample was sent for histopathological examination and KOH wet mount, which showed broad, pauci-septate hyphae with right-angle branching. The tissue was inoculated onto Sabouraud dextrose agar and white mycelial growth was obtained which turned grey with age. Morphological identification confirmed it as *Rhizomucor pusillus*. In vitro antifungal susceptibility testing was performed by means of the microbroth dilution method according to CLSI Approved Standard M38-A. The isolate was found to be susceptible to amphotericin B, itraconazole and posaconazole but resistant to voriconazole and echinocandins. Functional endoscopic sinus surgery was performed and local necrotic tissue was debrided. The patient was put on liposomal amphotericin B, with a successful outcome.

**Conclusion:** Early diagnosis is critical in prevention of morbidity and mortality associated with disease.

**Keywords:** amphotericin B; diabetes mellitus; mucormycosis; rhinofacial mucormycosis; *Rhizomucor pusillus*.

**Introduction**

Mucormycosis is an invasive fungal infection caused by members of the order Mucorales. Rhino-orbito-cerebral mucormycosis accounts for one-third to one-half of all cases of mucormycosis (Roden *et al.*, 2005). Various risk factors contribute to the development of mucormycosis, the most common being diabetes mellitus with ketoacidosis (Dökmetaş *et al.*, 2002). Genus *Rhizopus* is most commonly isolated from rhino-orbito-cerebral mucormycosis cases. *Rhizomucor pusillus* belongs to the genus *Rhizomucor* and family Mucoraceae. It is a thermophilic saprobic fungus mainly causing infection in immunocompromised patients (Ribes *et al.*, 2002). Worldwide there were only 22 cases of *R. pusillus* infection reported before 2013. Only one case of *R. pusillus* infection in India was reported during 1990–2007 (Gomes *et al.*, 2011). Rhino-orbito-cerebral mucormycosis caused by *R. pusillus* was found only in 9% of cases. Immunocompromising conditions were present in 91% of mucormycosis cases due to *R. pusillus*, haematological disorders and malignancies being the most common (73%). Diabetes mellitus was present only in one instance. We here report the case of a 55-year-old diabetic female suffering from rhino-orbito-cerebral mucormycosis caused by *R. pusillus*.

**Case report**

A 55-year-old female patient presented with a 1 month history of nasal blockage, nasal discharge, watering of eyes, right-sided facial swelling and loosening of teeth. On examination, she had purulent discharge in the right nasal cavity with small polyps. Her right side upper teeth were mobile. The swelling was accompanied with pain and intermittent fever. But there was no history of nasal bleeding, facial weakness and numbness, vision loss or periorbital pain. There was also history of right-sided
molar tooth pain 1 month previously. She underwent a Caldwell Luc operation at Omni hospital, Chandigarh. But 1 month later she developed swelling of the right cheek. She had recently been diagnosed with type II diabetes mellitus but was not having any treatment for diabetes mellitus. Her father and brother also had type II diabetes mellitus. She also had hypertension. There was no history of any corticosteroid intake, iron therapy, haematological malignancy, trauma, transplantation, dialysis or intravenous drug intake. Her blood sugar level (fasting) was 310 mg dl\(^{-1}\), haemoglobin 12.6 g dl\(^{-1}\), urea 12 mg dl\(^{-1}\), creatinine 0.93 mg dl\(^{-1}\) and bicarbonate level 7 mmol l\(^{-1}\). Her HIV status was negative. An NCCT scan of the paranasal sinus showed complete fracture of the right lateral part of both anterior and posterior right maxilla at the level of the alveolar recess. Opacification and hypertrophy of inferior turbinates was seen on both sides. There was also opacification of the left maxillary antrum and ethmoidal sinus. Endoscopic biopsy was performed and the material was sent to the Departments of Microbiology and Pathology of our hospital (Government Medical College Hospital, Chandigarh).

**Mycological and histopathological investigations**

A KOH wet mount of the biopsy specimen showed sparsely septate, broad, ribbon-like hyphae with right-angle branching suggestive of mucormycosis (Fig. 1a). A portion of biopsy material was cut into small pieces without homogenization and inoculated onto Sabouraud dextrose agar either supplemented with chloramphenicol and gentamicin or without antibiotics. One set of tubes was incubated at 22°C and another at 37°C. After 24 h of incubation, initially white floccose growth with low aerial tufts was obtained, which on further incubation turned grey within 2–3 days. The growth of *R. pusillus* has low aerial tufts compared with most other mucoraceous moulds. On lactophenol cotton blue examination, sporangiophores were branched irregularly and extensively. Internodal poorly formed rhizoids were present. Columellae were slightly pyriform to subglobe, measuring 20 to 45 μm in diameter (Fig. 1b).

Histopathological examination of the biopsy material from the right maxillary sinus after haematoxylin and eosin (Fig. 2a), periodic acid–Schiff (Fig. 2b) and Gomori methenamine silver staining showed large areas of necrosis, inflammatory exudate and many pauci-septate broad fungal hyphae with right-angle branching conforming to the morphology of mucormycetes.

**In vitro antifungal susceptibility testing**

*In vitro* antifungal sensitivity was determined by the microbroth dilution method according to CLSI (2005). Inoculum suspensions were prepared from 7 day potato dextrose agar (PDA; Difco) cultures by adding sterile saline solution and lightly scraping the surface of mature colonies with a sterile cotton swab. The homogeneous conidial suspensions were then transferred to sterile tubes and the supernatants were adjusted spectrophotometrically to OD\(_{530}\) 0.15–0.17. The inoculum suspensions, which consisted primarily of non-germinated conidia, were diluted 1:50 in RPMI 1640 medium. Microdilution plates were incubated at 35°C and examined visually after 24 and 48 h. *Candida parapsilosis* (ATCC 22019) was used as quality control. The MIC values obtained with various antifungal drugs at 24 and 48 h, respectively, were amphotericin B (0.03 and 0.03 mg l\(^{-1}\)), itraconazole (0.03 and 0.5 mg l\(^{-1}\)), voriconazole (0.25 and >8 mg l\(^{-1}\)), posaconazole (0.015 and 0.25 mg l\(^{-1}\)) and echinocandins (>16 mg l\(^{-1}\)). The strain was susceptible to amphotericin B, itraconazole and posaconazole, with MIC values <1 mg l\(^{-1}\).

**Treatment**

The patient’s blood sugar was controlled with human insulin twice a day. Her hypertension was controlled with 25 mg atenolol twice a day. Functional endoscopic sinus surgery...
surgery was performed and necrotic tissue was debrided. The patient was put on an intravenous infusion of liposomal amphotericin B [5 mg (kg body weight)\(^{-1}\) day\(^{-1}\)] for 4 weeks. Renal function was continuously monitored by serum urea and creatinine levels. There was no adverse effect of the antifungal treatment during the entire course.

**Outcome and follow-up**

This treatment led to significant reduction in facial swelling and the patient’s condition improved. She was discharged in good condition with advice for regular follow-up.

**Discussion**

In 1978, the genus *Rhizomucor* was established for *Mucor*-like fungi forming stolons and rudimentary rhizoids and expressing thermophilism (Ribes et al., 2002). Molecular phylogenetic studies based on nuclear small and large subunit rRNA gene sequences support the distinction of *R. pusillus* from *Mucor* species. *Rhizomucor* is commonly found contaminating air, soil and organic matter. Various species of *Rhizomucor* are known. *R. pusillus*, *Rhizomucor miehei* and *Rhizomucor variabilis* with two subspecies (*R. variabilis* var. *variabilis* and *R. variabilis* var. *regularior*) can cause mucormycosis in humans. *R. variabilis* was recently shown to be phylogenetically nested far from *R. pusillus* but within the clade *Mucor* (Gomes et al., 2011). *R. variabilis* var. *variabilis* has now been renamed as *Mucor irregularis*. *R. pusillus* is the most common species seen and has been detected in a variety of food items, including grains, seeds, nuts and beans. It is a thermophilic saprobic mucormycete with a wide distribution but it is not commonly associated with human disease (Hernanz et al., 1983). Its mode of transmission is by inhalation of spores and percutaneous introduction of spores into a susceptible host (Lu et al., 2009). Various studies in the literature have shown this species to be mostly associated with patients who are severely immunocompromised, especially those undergoing therapy for leukaemia and those who have uncontrolled diabetes mellitus (Germain et al., 1993). The pathogenicity of *R. pusillus* is due to its angioinvasive nature and thermosterility, which presumably allow it to grow in febrile patients (De pauw, 2008). Various presentations include primary cutaneous disease with or without dissemination, pulmonary disease and sinusitis with or without orbital participation or involvement of the brain. The majority of cases of *R. pusillus* infection present as pulmonary or disseminated disease. Lungs were the most commonly affected organs; pulmonary lesions were seen in 16 cases (Kimura et al., 2009). In five of these cases infection was restricted to the lung, whereas in another ten cases lung involvement was a part of disseminated, cardiopulmonary, sino-pulmonary infection. In one of these cases, initial rhino-orbito-sinus infection spread to the brain and lung. Sinus-only involvement was seen in two cases and cutaneous infections were only found in two cases. Patients with isolated pulmonary infection had a better prognosis than patients with disseminated infection (survival rates 80 and 22 %, respectively). Nine cases of disseminated infection due to *R. pusillus* have been reported in the literature (Germain et al., 1993). The underlying diseases in these cases were acute leukaemia (n=7) and non-Hodgkin’s lymphoma with renal transplantation and aplastic anaemia (n=1). The rhino-facial and rhino-orbito-facial *R. pusillus* infections were reported for an 11-year-old boy with acute leukaemia and a 38-year-old woman with diabetic ketoacidosis, respectively (Iwen et al., 2005). *R. pusillus* infection may also initially appear as a sino-orbital infection that rapidly invades the brain in patients with haematological malignancies. In our case report, the patient was diabetic and suffered from rhino-facial mucormycosis due to *R. pusillus*.

**Fig. 2.** (a) Haematoxylin and eosin staining (H&E) of biopsied material revealed a granulomatous and suppurative inflammatory lesion with irregular, long, pauci-septate fungal hyphae with right-angle branching. (Magnification × 40.) (b) Periodic acid–Schiff staining (PAS) of biopsied material revealed a granulomatous and suppurative inflammatory lesion with irregular, ribbon-like fragments of pauci-septate fungal hyphae. (Magnification × 100.)
Early diagnosis of *R. pusillus* infection often is feasible only in patients with accessible lesions who can tolerate a biopsy, which is required for histopathological identification and culture. *R. pusillus* has a temperature growth range of 20 to 60 °C. Morphological and biochemical properties help to differentiate *R. pusillus* from other mucormycetes. Mating studies are considered best for morphological identification of mucormycete species but they require maintenance of a library of testing strains. Zygospore production is useful for differentiating *R. pusillus* from *R. miehei*, as the former produces heterothallic zygospores. The higher tolerance of *R. miehei* to lovastatin can be useful for differentiating it from other species. DNA sequencing for comparison with known type strains in international databases is currently considered the best way to identify and classify mucormycete species. PCR and RFLP or other molecular methods have been used for *R. pusillus* identification in culture and tissue, and these techniques may be more rapid and reliable than standard mycological identification (De Hoog et al., 2000).

The overall mortality rate for *Rhizomucor* infections is significantly lower than that seen with other mucoraceous moulds. Among 18 out of 22 patients who received antifungal treatment, 11 survived (Gomes et al., 2011). Surgical debridement in addition to liposomal amphotericin B at a minimum dose of 5 mg kg⁻¹ day⁻¹ remains the treatment of choice for mucormycosis (Cornely et al., 2014). Our patient was managed by surgical treatment and liposomal amphotericin B treatment. Timely diagnosis and treatment aided in her survival.

**References**


