Case Report to JCM 20.11.09

First Autochthonous Case of *Rhinocladiella mackenziei* Cerebral Abscess outside the Middle East

Hamid Badali, Jagdish Chander, Shaifali Bansal, Atul Aher, Surendra S. Borkar, Jacques F. Meis, and G. Sybren De Hoog

1CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands, 2Institute of Biodiversity and Ecosystem Dynamics, University of Amsterdam, Amsterdam, The Netherlands, 3Department of Medical Mycology and Parasitology, School of Medicine/Molecular and Cell Biology Research Centre, Mazandaran University of Medical Sciences, Sari, Iran, 4Department of Microbiology, Government Medical College Hospital, Chandigarh, India, 5Department of Medicine, 6Department of Microbiology and Pathology, People’s College of Medical Sciences & Research Centre, Bhopal, Madhya Pradesh, 7Department of Medical Microbiology and Infectious Diseases, Canisius Wilhelmina Hospital, Nijmegen, The Netherlands, 8Peking University Health Science Center, Research Center for Medical Mycology, Beijing, China.

*Correspondence: G.S. de Hoog, CBS-KNAW Fungal Biodiversity Centre, P.O. Box 85167, NL-3508 AD, Utrecht, The Netherlands. Tel: +31-30-2122663; fax: +31-30-2512097; E-mail: de.hoog@CBS.KNAW.nl.
Cerebral phaeohyphomycosis due to *Rhinocladiella mackenziei* is a severe infection in the Middle East, with nearly 100% mortality despite the application of combined surgical and antifungal therapy and occasionally occurring in otherwise healthy patients. We report the first case of brain infection in an elderly male in India, where *R. mackenziei* is not endemic.

**CASE REPORT**

A 50-year-old Indian male who had type two diabetes mellitus for the last four years and suffered from psychiatric illness was admitted to the Department of Neurology, Peoples College of Medical Sciences, Bhopal, India, and presented with a one-day history of frontal headache, dizziness, slurring speech and weakness over the left half of his body. Ten days previously he had become inattentive due to his uncontrolled diet and irregular anti-diabetic treatment. First computed tomography (CT) scan of the brain demonstrated a mass lesion and patient underwent indigenous (Ayurvedic) treatment for 5-6 days. The chest X-ray was normal. Laboratory investigations of full blood analysis revealed a hemoglobin level of 16.8 g/dl; white blood cells, serum electrolytes and liver function tests were within normal range. Blood cultures were sterile and urine cultures became positive with *Escherichia coli*. Later, a second CT scan revealed a large (around 5-6 cm in diam), discrete, irregular, peripheral ring-enhancing necrotic lesion in the right frontoparietal region causing significant mass effects and midline shift with perifocal oedema (Fig. 1A-B). Neurosurgical intervention consisted of a right frontoparietal craniotomy for decompression of the space-occupying lesion. The necrotic material was encapsulated and approximately 8 ml thick viscous black fluid, was aspirated which was predominately caseous. The material was analyzed by two mycological and histopathological laboratories involved. The lesions were multiple ring-enhancing, greyish-white, soft, measuring 1.5 × 0.4 cm. Microscopic examination of biopsy sections (pus or necrotic tissue) stained with hematoxylin and eosin revealed necrosis with dense and diffuse mixed inflammatory infiltrates. There were several foreign bodies and Langhans’ type of giant cells with granuloma formation and the presence of numerous septate hyphae surrounded by a dense inflammatory response (Fig. 2A-B). Following this the biopsy specimens were also stained with Ziehl-Neelsen and revealed many moniliform septated hyphal elements (Fig 2C). The tentative diagnosis of chronic granulomatous inflammation with fungal infection was made. The clinical specimens were cultured on Sabouraud dextrose agar (SDA, Difco) and SDA supplemented with chloramphenicol (0.5 mg/


3 ml) and incubated at 30–35 °C for up to 10 days, as well as on brain-heart infusion agar with 5% sheep blood at 37 °C (Oxoid LTD., Basingstoke, Hampshire, England). Growth of melanized fungi after one week was observed and these were morphologically classified as *Rhinocladiella mackenziei* (formerly, *Ramichloridium mackenziei*). Stock cultures were maintained on slants of 2 % Malt extract agar (MEA, Difco) and Oat meal agar (OA, Difco) at 30 °C, and a voucher strain was deposited into the CBS-KNAW culture collection and preserved as CBS 125089.

Microscopic studies using slide culture techniques with potato dextrose agar (PDA) were conducted. This medium was selected because it readily induces sporulation and suppresses growth of aerial hyphae (9). After two weeks, slides were prepared from these cultures in lactic acid or lactophenol cotton blue under BSL-3 safety regulations and light micrographs were taken using Nikon Eclipse 80i microscope with a Nikon digital sight DS-Fi1 camera. After 2 weeks growth at 27 °C on MEA in darkness the moderately rapidly growing colonies, were velvety and olivaceous-brown; colony reverse was olivaceous-black (Fig. 3A). Conidiophores arose at right angles from creeping hyphae, were stout, thick-walled, brown, 3.0–4.5 µm wide, cells 10–25 µm long, apically with short cylindrical denticles and brown conidia which were ellipsoidal, 8.5–12.0 x 4–5 µm, with prominent, 1 µm wide basal scars (Fig. 3B-C). Cardinal growth temperatures of strain CBS 125089 were between 9 and >40 °C, with an optimum at 30 °C and still some growth at 40 °C. Morphologically *R. mackenziei* resembles *Pleurothecium obovoideum* (Mats.) Arzanlou & Crous from dead wood, but *P. obovoideum* has distinct conidiophores, and the ascending hyphae are thick-walled with cylindrical denticles up to 1.5 µm long. In contrast, *R. mackenziei* has only slightly prominent denticles. *Pleurothecium obovoideum* clusters in the order *Chaetosphaeriales* (3).

DNA was extracted using an Ultra Clean Microbial DNA Isolation Kit (Mobio, Carlsbad, CA, U.S.A.) according to the manufacturer’s instructions. ITS rDNA was amplified using primers V9G (5’-TTA CGT CCC TGC CCT TTG TA-3’) and LS266 (5’-GCAT TCC CAAACA ACT CGA CTC-3’) and sequenced with the internal primers ITS1 (5’-TCC GTA GGT GAA CCT GCG G-3’) and ITS4 (5’-TCC TCC GCT TAT TGA TAT GC-3’). PCR amplification and sequencing were according to Badali *et al.* (4). Sequences were compared with GenBank and through local blast with a molecular database maintained for research purposes at the CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands. The isolate (CBS 125089 =
GQ863214) was identified as *R. mackenziei* having 99.5% sequence identity with the ex-type isolate of that species (CBS 650.93 = AY857540) which had originally been isolated from a case of cerebral phaeohyphomycosis from Saudi Arabia. The molecular results confirmed the mycological diagnosis, and histopathological observation led to the diagnosis of a cerebral phaeohyphomycosis due to *Rhinocladiella mackenziei*.

For the management of the case insulin infusion was given along with intravenous amphotericin B deoxycholate (0.6 mg/kg/day). Simultaneously empiric anti-tuberculous and antibacterial therapy was started, consisting of sequential beta-lactam antibiotics (ceftriaxone, piperacillin, cefoperazone) combined with metronidazole and amikacin. During follow-up there was no clinical improvement. Therefore, successive CT scans after 8 days were obtained, showing decreases in size of the lesions (around 4 cm); however, an increase in parietal edema, enlargement of mass effects and multiple, coalescing ring-enhancing, cerebral lesions were seen (Fig 1C). Despite amphotericin B therapy the patient’s condition continued to deteriorate and eventually he expired two weeks after diagnosis of the disease. The in vitro antifungal susceptibilities of this strain (CBS 125089) were determined by microbroth dilution according to the Clinical and Laboratory Standard Institute document M38-A2 (13). Methods for sporulation and preparation of suspensions were according to Badali *et al.*, 2009 (5). *Paecilomyces variotii* (ATCC 22319), *Candida parapsilosis* (ATCC 22019) and *Candida krusei* (ATCC 6258) were used as quality control (13). The MICs of eight antifungal drugs were as follows: amphotericin B, 16 µg/ml; fluconazole 32 µg/ml, itraconazole 0.063 µg/ml, voriconazole 0.5 µg/ml, posaconazole 0.031 µg/ml and isavuconazole 0.5 µg/ml. The two echinocandin agents yielded MECs of 8 µg/ml and 4 µg/ml for caspofungin and anidulafungin, respectively.

**DISCUSSION**

Cerebral phaeohyphomycosis by melanized fungi is a rare, but highly significant disease due to the regional prevalence and high mortality of up to 70% despite combined surgical and antifungal therapy (10). The majority of reported central nervous system infections caused by dematiaceous fungi were found to be brain abscesses in patients with no predisposing factors or immunodeficiency; symptoms included headache, seizures cerebral irritation, fever and neurological deficits (12). Binford *et al.* (6) described one of the first cases of brain abscess due
to *Cladosporium trichoides* (= *Cladophialophora bantiana*) and Campbell & Al-Hedaithy (7) reported on a similar case by *Ramichloridium mackenziei* (= *Rhinocladiella mackenziei*).

The latter review listed 8 cases of this infection, all occurring in countries of the Middle East. *Exophiala dermatitidis* (8), *Cladophialophora bantiana* (11) and *Rhinocladiella mackenziei* (7, 16), all members of the order *Chaetothyriales*, are notorious agents of cerebral infections. One of the most striking features of these organisms is their neurotropism in humans.

Arzanlou *et al.*, 2007 studied the phylogenetic and morphotaxonomic revision of *Ramichloridium* and allied genera, because *Rhinocladiella* was in the past frequently confused with the genus *Ramichloridium*. *Rhinocladiella* species were shown to cluster in the order *Chaetothyriales*, while *Ramichloridium* clustered in the order of *Capnodiales* (3).

*Rhinocladiella mackenziei* affects only the central nervous system and the integuments, with nearly 100% mortality in both immunocompetent and immunocompromised individuals. This species is the most common agent of brain infection and was thought to be restricted to the Middle East and the Persian Gulf region (7). Cases diagnosed in the U.K. or U.S.A. concerned immigrants from Saudi Arabia and Kuwait (16). Until now *R. mackenziei* has never been isolated from the environment. The natural niche of this organism thus remains unknown. To the best of our knowledge this patient represents the first case of *R. mackenziei* outside the arid climate zone of the Middle East. In contrast to all cases reported thus far, this strain (CBS 125089) originated from a humid sub-tropical climate, infecting a patient who claimed he had never travelled outside of India.

Cerebral infections due to *R. mackenziei* have thus far been diagnosed after CT-guided needle aspiration and were proven by positive histopathology and cultures. The mortality is almost 100% for all reported cases of infection despite surgical resection and antifungal therapy. There is no standard therapy for this disorder. Treatment as presented in the literature mostly involved (high-dose lipid) amphotericin B, itraconazole and 5-flucytosine, or a combination of these drugs (14). However strains of *R. mackenziei* isolated from infected patients are in vitro resistant to amphotericin B, which is often used as the gold standard of treatment. Only a single patient is reported to have survived an *R. mackenziei* cerebral infection, with pronounced radiological and clinical improvement after switching therapy from a combination of liposomal amphotericin B, 5-flucytosine, and itraconazole to posaconazole (2). This is supported by in vitro
results and data from a murine infection model (1), in which posaconazole prolonged the survival of mice and reduced the brain fungal burden compared to itraconazole and amphotericin B.

Previous in vitro antifungal susceptibility testing of 10 strains of *R. mackenziei* (5) has shown that the widest range (2 to 16 µg/ml) and the highest MICs were recorded for amphotericin B (MIC$_{50}$ 8 µg/ml and MIC$_{90}$ 16 µg/ml). In contrast, quite uniform patterns were obtained for susceptibility to itraconazole, posaconazole, and isavuconazole. Our results were in line with animal test data, but clinical experience and animal experiments have not been reported for the newer antifungal drugs. Animal studies have suggested that no benefit is achieved with amphotericin B in experimental infection due to poor penetration into the central nervous system (CNS) (1). Although liposomal amphotericin B likely has a better CNS penetration the only patient surviving a *R. mackenziei* cerebral infection (2) failed liposomal amphotericin and only improved on posaconazole an agent with good CNS penetration (15) and a low MIC for the pathogen (2) Delay in diagnosis, misidentification, poor standard therapy and limited data on effective alternative drugs are the main factors promoting the development of cerebral phaeohyphomycosis. With early diagnosis, when the lesion is singular, and with effective therapy with complete surgical excision of brain abscesses, patient’s outcome may be improved. Brain abscesses incited by *R. mackenziei* in patients from non-endemic areas have not been previously reported. Now that the fungus has been observed outside its endemic area, *R. mackenziei*, along with other neurotropic agents, should always be considered as a potential etiologic agent of cerebral phaeohyphomycosis in all patients, regardless of their area of residence.

**ACKNOWLEDGMENTS**

This work was supported by a grant (No. 13081) to H. Badali from the Ministry of Health and Medical Education of the Islamic Republic of Iran and the School of Medicine, Mazandaran University of Medical Sciences, Sari-Iran. The authors report no conflicts of interest. We thank Dr. Vichal Rastogi and Dr. V. K. Ramnani, Department of Microbiology, Dr. Sushil Jindal, Department of Medicine, Dr. Mridul Shai, Department of Surgery, People’s College of Medical Sciences & Research Centre, Bhopal, Madhya Pradesh, India for their contribution. As well as,
Dr. Hena Rani, Department of Microbiology, Government Medical College Hospital, Chandigarh, India is acknowledged for help in building up part of the microbiological work.

REFERENCES


FIG. 1. A–B. Initial computed tomography (CT) showing a mass lesion, discrete, large (around 5-6 cm in diam), irregular, peripheral ring-enhancing necrotic lesion in the right frontoparietal region with mass effects and midline shift with perifocal oedema. C. Third CT showing decreases in size of lesions (around 4 cm), however increasing of parietal edema, enlargement in mass effects and multiple, coalescing ring-enhancing, cerebral lesions were seen.

FIG. 2. A–B. Hematoxylin-eosin stain (H&E) revealed necrosis with dense and diffuse mixed inflammatory infiltrates with granuloma formation and the presence of numerous septate hyphae (arrows) surrounded by a dense inflammatory response. C. Biopsy specimens were also stained with Ziehl-Neelsen and revealed many moniliform branched septated hyphal elements.

FIG. 3. *Rhinocladiella mackenziei* (CBS 125089). A. Colony on malt extract agar (MEA, Difco) at 30 °C after 2 weeks in darkness. B–C. Semi-micronematous conidiophores and sympodially proliferating conidiogenous cells (arrows). Scale bar =10 µm