Actinomortierella wolfii: Identity and pathology

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ABSTRACT

Actinomortierella wolfii (Mortierellales), formerly Mortierella wolfii, is a causative agent of bovine systemic infection and abortion. Human infections caused by this species are extremely rare. Here, we present a case of a patient with B-Cell Acute Lymphoblastic Leukemia (B-ALL) who was diagnosed with a rhinocerebral infection caused by this fungus. Amphotericin treatment of the patient proved unsuccessful. This type of disease is otherwise narrowly exclusively limited to members of the order Mucorales. The taxonomy of the causative agent is discussed.

1. Introduction

Severe opportunistic human infection by members of early diverging fungal lineages has been observed in four orders: Basidiobolales, Entomophthorales, Mortierellales, and Mucorales [1]. Species of Rhizopus, Rhizomucor, Lichtheimia and Mucor in Mucorales are by far the most frequent etiologic agents. Other, less commonly represented mucoralean genera include Cunninghamamella, Apophysomyces, Sakasenea, Syncpha-

lastrum, and Cokeromyces [2,3]. Despite the large phylogenetic distances between these genera, infections are often clinically similar, with an acute and destructive course. Rhino-cerebral mucormycosis is a life-threatening opportunistic fungal infection, affecting severely compromised individuals with poorly controlled diabetes mellitus, on prolonged immunosuppressive therapy, or with haematological malignancies [2]. Recently, COVID-19 infection has become an additional risk factor for severe disease [4]. The rhinocerebral form is the most common clinical manifestation of mucormycosis (39%) [5], followed by pulmonary, gastrointestinal, disseminated, and cutaneous presentations.

Mortierella is a genus of subclass Mortierellomycotina [7] and has mainly been reported as a causative agent of bovine mycotic abortion [8, 9] and other systemic infections in cattle [10,11], while human infections are extremely rare [11–13]. The Mortierellomycotina contain a single order, Mortierellales, with a single family, Mortierellaceae, which in a recent taxonomic overview were described with 136 species in fourteen genera [6]. The genera comprise lipid-accumulating organisms, such as M. alpina, and some show effective chitin degration [14]. Others are biotechnologically significant as industrial producers of polyunsaturated fatty acids, such as arachidonic acid or eicosapentaenic acid [15]. In addition, Mortierellaceae have been reported to support phosphate uptake in plants, although the exact mechanism of this beneficial exchange is still unclear [16]. Species of Mortierella in a broader sense are widely distributed in the environment and are extremely diverse ecologically and physiologically. Infectious strains in this genus are rare and pathogenicity does not seem to be an ecological strategy [7]. Thus far, only two species, Actinomortierella wolfii (as M. wolfii) and M. polycyphala have been reported from vertebrate infections; however, future studies are needed to confirm that the latter species is a vertebrate opportunist [1,18].

The type species of Mortierella, M. polycyphala, was described in 1863 by Coemans [17]. As observed in most Mortierellales, phylogenetic distances between genera and species are significant. This led Vandepol et al. [6] to recognize 14 genealogically exclusive genera within the Mortierellaceae, i.e. Actinomortierella, Aquamortierella, Bermiella, Dis-
sophora, Entomortierella, Gamsiella, Gryganskiella, Linnemannia, Lobosporangium, Lunasporangiospora, Modicella, Mortierella, Necromortierella and Podilla. Of the two species known from vertebrate infections, M. polycyphala was retained in the genus as the type, whereas M. wolfii was reclassified in Actinomortierella. Wagner et al. [7] and Vandepol et al. [6] noted that ribosomal markers rDNA were unable to resolve phylogenetic relationships within the Mortierellaceae because the ITS spacer is too divergent to align across the family, while the nuclear ribosomal large subunit (LSU) and small subunit (SSU) regions are too
conserved. For this reason, additional non-ribosomal markers such as RNA polymerase subunit B (rbp1 and rpb2) and translation elongation factor 1-alpha (TEF1) were used as a basis for taxonomic revision of Mortierellaceae. In this classification, Mortierella wolfii was reclassified as A. wolfii [6]. *Actinomortierella wolfii* is the only species of *Actinomortierella* known to cause mammal infection, possibly enhanced by its thermotolerance [6]. *Mortierella polycephala* was reported from cattle in an older, unconfirmed paper [18]. Below a rare case of a patient with B-Cell Acute Lymphoblastic Leukemia with a rhinocerebral infection caused by *Actinomortierella wolfii* is described.

2. Case presentation

A 19-year-old newly diagnosed male patient with CD10 positive B-Cell Acute Lymphoblastic Leukemia (B-ALL) was started on day 0 induction therapy with daunorubicin (DNR), vincristine (VCR), peg-asparagine and dexamethasone. While on chemotherapy, he developed fever and his white blood cell count (WBC) was $20 \times 10^9/\mu L$ with 91% blast cells. Moreover, his absolute neutrophil count (ANC) was $0.204 \times 10^9/\mu L$. Since he got febrile neutropenia along with chemotherapy, intravenous (IV) meropenem was commenced.

On day 4 of chemotherapy, he complained of pain in the right eye and examination revealed periorbital cellulitis. His WBC was $0.95 \times 10^9/\mu L$ with neutrophils of $0.07 \times 10^3/\mu L$, Hb of $8 \ g/dL$, and platelets (Plt) of $3 \times 10^5/\mu L$. IV teicoplanin was started with the suspicion of bacterial origin. Later, the worsening of periorbital cellulitis and development of chemosis and exophthalmos warranted temporary withholding of chemotherapy and adding IV clindamycin. However, the patient’s condition deteriorated with reduced eye movements and reduced vision along with purulent nasal discharge. On examination, chemosis and exposure keratopathy in the right eye was observed. He was febrile and had WBC a level of less than $0.29 \times 10^3/\mu L$, ANC of $0.05 \times 10^9/\mu L$, Hb of $6 \ g/dL$, Plt of $14/\mu L$, and CRP of $336 \ mg/L$. Contrast Enhanced Computed Tomography (CECT) of the brain and orbit revealed a right pre-septal and orbital cellulitis, right-sided exophthalmos, intra-conveal fluid collection tracking along the posterior and medial margins of the orbit and, inflammatory sinus disease involving paranasal sinuses.

Orbital cavity debridement was performed on day 10 at the National Eye Hospital (NEH) and the biopsy of the right orbital cavity tissue was sent for pyogenic and fungal culture Fig. 1.

The positive direct smear result of the biopsy of the right orbital cavity tissue prompted the administration of IV amphotericin B deoxycholate at 1 mg/kg/day. However, by that time patient had a melting cornea with a poor visual prognosis. Clinically the lesion had extended to the orbital apex. MRI the of brain and orbit revealed right-sided maxillary sinusitis extending into bilateral ethmoid and frontal sinuses as well as the right orbit with evidence of orbital cellulitis. Inflammatory thickening in the media extra-conveal compartment was observed to cause right-sided proptosis.

At the Mycology Reference Laboratory, microscopy of the biopsy of the right orbital cavity tissue with 10% potassium Hydroxide (KOH) revealed infrequently septate, broad, ribbon like fungal filaments consistent with zygomycetous fungi. The remaining part of the biopsy specimen was used for culturing on Sabouraud’s Glucose Agar (SGA) with chloramphenicol, with the cultures incubated at 26 °C and 37 °C.

After 4 days of incubation, white, flat, floccose colonies with a zonate surface were observed in all four specimen cultures. A lactophenol cotton blue mount of a colony showed sterile, broad, hyaline, sparsely septate hyphae that branched at right angles, suggestive of a zygomycetous fungus. Although the culture did not sporulate on a slide culture and on special media it did produce spores with the use of a floating agar method. Diagnostic, tapering sporangiophores arising from rhizoids were observed. Deliquescent, globose sporangia rapidly disintegrated, leaving a collarette at the tip of the sporangiophore. Sporangiospores were small and ellipsoidal. These morphological features were suggestive of a Mortierella species (Fig. 2).

The isolate was subjected to direct DNA sequencing of the rDNA ITS region, which supported the identification as *Actinomortierella wolfii* based on 99.82% similarity to the type strain NRRL 62265 and CBS 611.70. In the original description of *M. wolfii* [24], the type strain derived from the dried type M-82 was deposited in the ARS Culture Collection by B.S. Mehrotra as A-12361 in 1963 and subsequently reaccessioned as NRRL 66265 in 2015. The ITS maximum likelihood tree generated with RAxML showed that the strain is grouped with the type and several reference strains of *A. wolfii* (99% bootstrap support, Fig. 3).

The ITS sequence of our strain was deposited in the GenBank with the accession number OL685369.

Based on the culture identification as *Actinomortierella wolfii*, IV amphotericin B administration was continued, and surgical debulking was repeated. This led to gradual improvement of the patient’s clinical condition. His fever settled and his inflammatory markers improved (CRP 84 mg/L). Subsequently, he recovered from neutropenia (WBC $6.29 \times 10^3/\mu L$, neutrophils $5.15 \times 10^5/\mu L$) and on day 59 of IV amphotericin B therapy, the direct smear and culture of debulked tissue became negative for fungi. Moreover, repeated CECT
cavernous sinus thrombosis. With the patient recovering, right orbital
exenteration was performed by the plastic surgery team. IV amphoter-
icin B deoxycholate (1mg/kg/day) was planned to continue until 2
weeks after the last negative culture, but unfortunately, the patient left
against medical advice prematurely.

Eight months later, the patient was readmitted with severe symp-
toms, including extensive skin lesion on the right side of his face. With
the suspicion of recurrence of the *Actinomortierella wolfii* infection, IV
amphotericin B deoxycholate (1mg/kg/day) therapy was recommenced,
and a biopsy was planned for mycological studies. Unfortunately, his

![Fig. 2. Growth of *Actinomortierella wolfii* on Sabouraud’s Glucose Agar (A), Lactophenol cotton blue preparation of the clinical isolate from floating agar culture showing sporangiophore and spores. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)](image1)

![Fig. 3. Evolutionary relationships among three species of *Actinomortierella* inferred from 25 nuclear ribosomal ITS sequences downloaded from NCBI GenBank. OL65369 (red font) is the NCBI GenBank accession obtained for the strain reported in the present paper. The phylogram was rooted on ITS sequences of three species of *Mortierella*. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)](image2)
condition deteriorated and he expired before interventions.

3. Discussion

Rhinocerebral mucormycosis is limited to individuals with pronounced risk factors such as diabetic ketoacidosis, malignancies, bone-marrow and solid organ transplantation, iron chelation therapy, or chemotherapy induced neutropenia [19]. Our patient had been on induction chemotherapy leading to neutropenia that predisposed him to mucocutaneous infection. *Actinomortierella*, until recently classified as *Mortierella* in the order *Mucorales* [24], was reclassified in the subphylum *Mortierellomycomycotina* (family *Mortierellaceae*, order *Mortierellales*) [7], and thus the clinical diagnosis should not be reported as mucormycosis. The rhinocerebral form observed in the present case, however, was clinically similar to mucocutaneous rhinocerebral infection in its rapid course, necrosis and cellulitis, affection of the orbital space and proptosis. This deviates significantly from chronic rhinocerebral and rhinocerebral infections caused by *Aspergillus* [20]. There is also strong similarity between clinical manifestations in humans caused by members of the *Mucorales* and *Mortierellales*, even though the agents are distantly related phylogenetically.

Given the significant phylogenetic distances among genealogically exclusive lineages within the *Mortierellaceae*, Vandepol et al. [6] elevated the clades to the genus level, which were previously recognized as groups within *Mortierella* [7]. Consequently, *M. wolfii* was reclassified in *Actinomortierella*. Nguyen et al. [21] also reported that *A. wolfii* (as *M. wolfii*) formed a genealogically distinct lineage within the *Mortierellales*. Vandepol et al. [6] noted that ITS rDNA was too variable to be aligned across the *Mortierellaceae* and LSU rDNA was too conserved; however, a comparative phylogenomic analysis revealed multiple phylogenetically distinct clades within *Mortierella sensu lato*. Three species have been identified in *Actinomortierella* [6], as shown in Fig. 3, of which only *A. wolfii* is reported as an animal opportunistic [22,23].

*Actinomortierella wolfii* is a saprophytic fungus that is naturally found on rotten silage [11]. Although the transmission route in the present case is unknown [12], transmission to humans and other animals may be via inhalation from environmental sources or ingestion of contaminated food [25]. This species is of veterinary importance, particularly in cattle abortion due to placentitis and endometritis [13,25], bovine neptitidis, pyrogallolmonatous pneumonia and melenogencephalitis [12,22,23]. Davies et al. [10] reported a bovine case of systemic infection following abortion, while Munday et al. [11] described a disseminated case of a neonatal calf, and Wada et al. [26], an equine eye infection.

There are only a few case reports of human infections caused by *A. wolfii*, and these include cutaneous infection, keratitis, and disseminated infection [1]. Two earlier cases of cutaneous infections by *Actinomortierella* (as *Mortierella*) species were judged erroneous due to misidentification of the etiological agent as *A. wolfii* [27]. Lily Therese et al. [13] described a human keratitis case caused by *A. wolfii* that was identified by PCR based DNA sequencing targeting the nuclear ribosomal ITS region. In addition, an invasive *A. wolfii* infection was described in a haematopoietic stem cell transplant chronic granulomatous disease (CGD) patient who was on voriconazole prophylaxis [12]. The fungus was isolated from a liver biopsy and the identification was confirmed by culture and molecular methods.

Early diagnosis of mucormycosis as well as infections by *Mortierellales* is important because of the often acute and destructive course of the infection. Deep tissue biopsy is the most appropriate specimen [28]. This species is of veterinary import, particularly in cattle food [25]. This species is of veterinary import, particularly in cattle.

Conflict of interest

There are none.

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