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Fungal keratitis caused by *Metarhizium anisopliae complex*

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ABSTRACT

Purpose: To report a case of fungal keratitis caused by Metarhizium anisopliae complex
Methods: Case report
Results: Our patient presented with a central corneal infiltrate. Fungal culture yielded a Metarhizium species. She was started on antifungal agents with no significant improvement. A therapeutic corneal transplant was performed after perforation. At two years follow up, she was free of infection.
Conclusion: Metarhizium anisopliae is a very rare cause of keratitis. Although previous reported cases showed clinical improvement with antifungal agents, this case required surgical treatment to control the infection.

1. Introduction

Metarhizium anisopliae is a fungus commonly used as an agricultural pesticide in many countries around the world. We are reporting a case of fungal keratitis caused by *M. anisopliae* in a patient who is an office worker and had no history of participation in agricultural work. Four cases have been published in the literature describing ocular involvement of *M. anisopliae*. Three patients had keratitis alone and had good response to medical therapy. One patient had sclerokeratitis and required a therapeutic corneal transplant. Our patient had keratitis unresponsive to medical therapy. She ultimately required therapeutic corneal transplant to control the infection. At two years follow up, her cornea was clear with no evidence of recurrence of the infection.

2. Case

A 52-year-old female was seen at the emergency department for evaluation of right eye pain, decreased vision and photophobia (Day 0). She saw her ophthalmologist one week prior to her presentation (Day −7) and was started on moxifloxacin 0.5% (Vigamox; Alcon Canada, Mississauga, ON) eye drops for a corneal abrasion in her right eye. She wears monthly contact lenses and denies any history of trauma or agricultural work. Her past medical history is non-contributory. On examination, her visual acuity was counting fingers in the right eye and 20/25 in the left eye. Anterior segment examination of the right eye showed marked conjunctival injection, central corneal infiltrate with feathery borders, an epithelial defect that measured 3.6 mm x 3.8 mm, and a deep anterior chamber with trace cells. Anterior segment examination was within normal limits in the left eye. Dilated fundus examination showed normal maculae and optic nerves in both eyes. Corneal scraping of the right eye was performed (Day 0) and the patient was started on fortified vancomycin and tobramycin eye drops every hour. Later that day, fungal hyphae were observed by microscopic examination of the corneal scraping, so oral fluconazole 200 mg/day and topical voriconazole 1% eye drops were started (natamycin eye drops were not available). The patient was seen daily for follow up; her pain was improving and vision was stable at counting fingers. However; one week later (Day +7), her vision dropped to hand motions and she developed a total epithelial defect. There were inflammatory cells in the anterior chamber with no change in the infiltrate size and no evidence of vitritis or retinitis. Corneal biopsy was performed which showed neutrophilic collections with no fungal elements seen. Three days later (Day +11), she developed a central corneal perforation and flat anterior chamber. Cyanoacrylate gluing of the perforation was attempted without success. The patient underwent an emergency therapeutic penetrating keratoplasty with severing of iridocorneal adhesions. The crystalline lens was not visibly present and had dislocated into the vitreous. An anterior vitrectomy was performed. A 9.0 mm corneal donor button was sutured in place. The patient's cornea was sent for histopathologic examination which showed granulation tissue and mixed acute inflammatory cells. All intraoperative cultures were negative. At Day +21, the fungus that grew from the corneal scraping was

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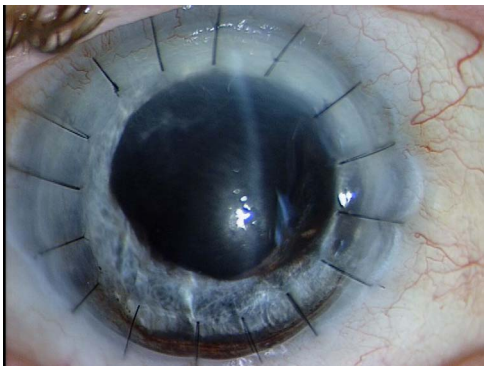

Fig. 1. Slit lamp photograph of the right eye 3 months after full thickness corneal transplant.

reported as *Metarhizium* species.

After the corneal transplantation, oral fluconazole was discontinued and the patient was kept on topical voriconazole along with topical antibiotic drops. The patient had a pars plana vitrectomy plus lensectomy at day +45 leaving her aphakic. Bacterial and fungal cultures of vitreous fluid were negative and topical voriconazole was stopped immediately after. She then developed uncontrolled glaucoma, and underwent trabeculectomy. At two years follow-up, her visual acuity is 20/400 in her right eye. She is comfortable and the graft remains clear with no signs of rejection or recurrence of infection (Fig. 1).

2.1. Identification

Corneal scrapings were inoculated onto Chocolate, Sheep Blood and Sabouraud dextrose agar (SDA) in the clinic (Day 0). After 5 days of incubation a small white filamentous fungus was observed on the SDA plates, however it was not readily identifiable and reincubated. On Day 10 an identification could still not be made so the culture was forwarded to the reference laboratory for identification (Public Health Ontario Laboratories). There the mold was subcultured onto SDA containing chloramphenicol, cycloheximide and gentamicin and Leonian's agar and incubated at 28 °C for 7 days. The subcultured fungus resembled the original culture; macroscopically and microscopically (Fig. 2) the features of the fungus were characteristic of a member of the *Metarhizium anisopliae* complex. Macroscopically the fungus initially appeared to be wooly white but over time the center of the colony took on a pale yellow colour and green conidia developed (Fig. 2A). Upon microscopic examination phialides producing elongated, or ellipsoidal, conidia could be seen (Fig. 2B), as well as aggregates of these ellipsoidal conidia in regular chains or columns; this formation is characteristic of *M. anisopliae* (Fig. 2C). As this was thought to be the causative agent of the infection, species level identification was warranted; PCR of the internal transcribed spacer region (ITS2), and the 5′ end of the 28S rRNA gene (D1D2 hypervariable region) was performed. Amplicons of approximately 350 base pairs (bp) and 570 bp respectively were generated and sequenced. Sequences were compared to the NCBI nucleotide nr/nt database using BLAST. [1] The ITS2 sequence was 100% homologous over 323 bp to strains of *M. anisopliae*, *Metarhizium robertsii*, *Metarhizium pingshaense*, and *Metarhizium guizhouense*, while the D1D2 sequence was 99–100% homologous to strains of *M. anisopliae* and *M. guizhouense* over 571 bp. All of the homologous sequences belong to species within the *Metarhizium anisopliae* complex [2], therefore although a definitive species identification could not be made based on these sequences, phenotypically and genotypically an identification of *M. anisopliae* complex is supported. Susceptibility testing was not performed.

3. Discussion

Metarhizium anisopliae, is an environmental fungus commonly found in the soil throughout the world [3]. It was first described in 1879 by Metschnikoff and called *Entomophthora anisopliae*, but later renamed *M. anisopliae* by Sorkin in 1883 [4]. *M. anisopliae* has been used as a biological control agent of insects in many countries globally [5]. It was not believed to adversely affect humans because it is unable to grow at human body temperature [6]; however, it has been recently reported as pathogenic to humans. There have been four reported cases of *M. anisopliae* causing ocular infection [5–8]. The first report of mycotic keratitis caused by *M. anisopliae* was in 1997, in an 18-year-old Colombian man [7]. Another case was reported 4 years later in a healthy 36-year-old female in the United States [8]. Both patients were treated successfully with natamycin eye drops. A third case of ocular involvement was reported in a 52-year-old Australian woman who developed sclerokeratitis [6]. She did not respond to multidrug therapy which

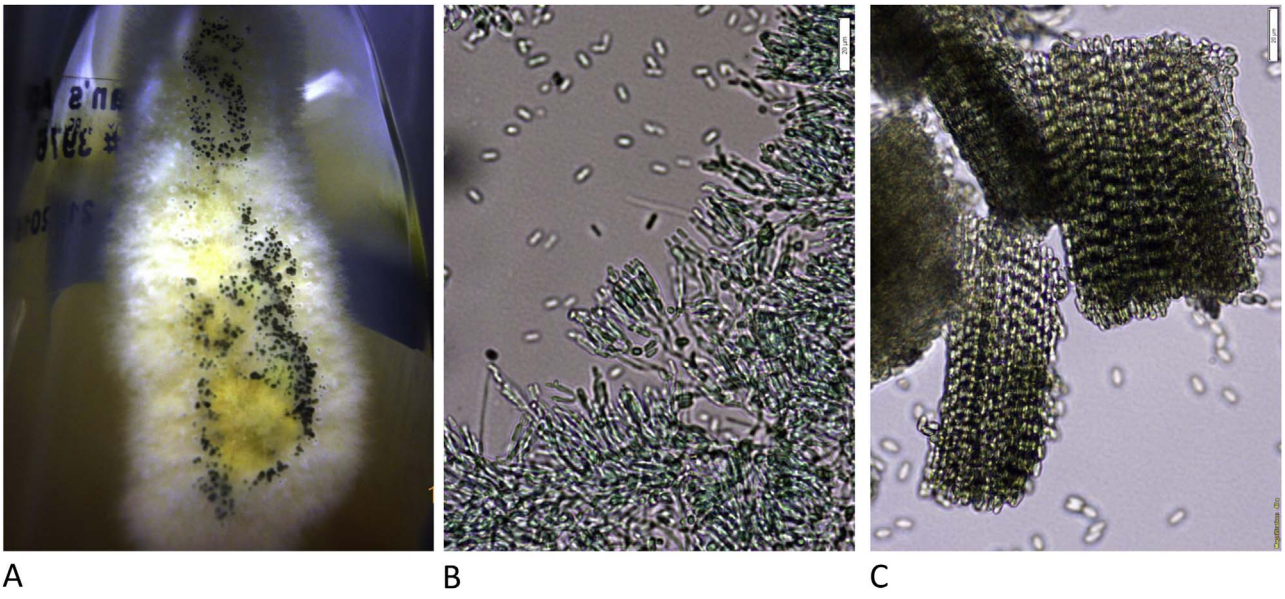

Fig. 2. (A) Macroscopic growth of *M. anisopliae* complex on Leonean's agar after 7 days demonstrating green conidia. (B and C) Mold prepared with lactophenol cotton blue demonstrating mature conidiogenous cells and conidia. White bar = 20 µm. Magnification 40X. (B) Characteristic *Metarhizium anisopliae* complex phialides with developing conidia. (C) Distinctive ellipsoidal conidia in regular chains or columns.

include natamycin eyes drops, topical and systemic voriconazole and eventually required a therapeutic penetrating keratoplasty. The fourth case was reported in a 12-year-old girl who responded well to topical antifungal therapy [5]. To our knowledge, this is the first reported case of fungal keratitis caused by a member of the *M. anisopliae* complex in Canada and only the fifth ocular case worldwide. An attempt was made to obtain natamycin eye drops but it was not available at our institution. Our patient did not respond well to topical voriconazole and oral fluconazole and ultimately required a therapeutic corneal transplant. The possibility that the *M. anisopliae* was a contaminant cannot be completely ruled out as the fungus grew from only the initial corneal scraping; however, the direct microscopic examination of the corneal scraping revealed fungal elements, and no other fungi, or bacteria, were cultured from the specimens. Therefore, although a rare human pathogen, *M. anisopliae* was determined to be the infectious agent in this case.

Fungal keratitis is often found in agricultural workers or people who participate in outdoor activities. Our case of fungal keratitis caused by *M. anisopliae* appeared in a 52-year-old woman who is an office worker with no history of trauma or participation in agricultural work. The patient's contact lens was never cultured and may have been the source of the infection. Two of the previously reported cases had a history of contact lens wear, however the other two cases did not. In terms of management, most of the previous reported cases responded well to topical natamycin drops, which is not available at our institution. For this reason, topical voriconazole and systemic fluconazole were used. The patient responded initially but ultimately required a therapeutic penetrating keratoplasty because of corneal perforation. The fungus was not able to be cultured subsequent to the initiation of topical and systemic antifungal therapy, however, the patient's clinical condition continued to worsen. This could be secondary to the inflammatory process associated with the infection. After therapeutic corneal transplantation, the patient was followed for two years and there was no evidence of recurrence of the infection or inflammation.

Though somewhat rare, fungal keratitis is a possibility in patients with contact lens keratitis that is not improving despite medical therapy. Re-culturing and, if necessary, corneal biopsy should be performed in order to obtain a diagnosis and to provide optimal treatment. In some cases, like our patient, therapeutic corneal transplantation might be the only option to cure the infection and save the patients' vision.

Conflict of interest

There are none.

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There are none.

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