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Two cases of fungal keratitis caused by *Metarhizium anisopliae*

Abigail L. Goodman[a], Shawn R. Lockhart[b], Colleen B. Lysen[b], Lars F. Westblade[a,c], Carey-Ann D. Burnham[d], Eileen M. Burd[a,*]

[a] Department of Pathology and Laboratory Medicine, Emory University School of Medicine, Atlanta, GA, USA
[b] Mycotic Diseases Branch, Centers for Disease Control and Prevention, Atlanta, GA, USA
[c] Department of Pathology and Laboratory Medicine, Weill Cornell Medicine, New York, NY, USA
[d] Department of Pathology & Immunology, Washington University School of Medicine, St. Louis, MO, USA

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ABSTRACT

We present two cases of keratitis due to Metarhizium anisopliae in geographically separated areas of the United States. The isolates were microscopically similar but morphologically different and were identified by ribosomal DNA sequencing. Both isolates had low minimum inhibitory concentration (MIC) values to caspofungin and micafungin, but high MIC values to amphotericin B. The morphologic and antifungal susceptibility differences between the two isolates indicate possible polyphylogeny of the group.

1. Introduction

Metarhizium anisopliae is an entomopathogenic filamentous fungus that is a plant symbiont and is found in soil throughout the world [1]. It causes disease in a wide variety of insects and other arthropods and is used commercially as an agricultural pesticide in many countries, including the United States (US) [1]. Previously, *M. anisopliae* was not considered to be pathogenic to humans due to its optimal growth temperature of 25 °C [2], however, human infection has been reported in the literature. Most of the cases have been ophthalmic infections with additional cases of fungal sinusitis and disseminated fungemia. The first reported case occurred in Columbia, South America in 1997 and was a fungal keratitis in an 18-year-old man [3]. There have now been four reported cases of fungal keratitis [3–6] and two cases of sclerokeratitis [7,8]. Here we report two additional cases of *Metarhizium* keratitis in soft contact lens wearers.

2. Case

The first case is a 47-year-old woman from Georgia, US, who presented to Emory University Hospital (Day 0) with severe pain, foreign body sensation, and decreased visual acuity in her left eye that started 10 days prior and got progressively worse. The patient was a soft contact lens wearer who reports poor lens hygiene and that she routinely wore her contact lenses overnight. The patient had no history of ocular disease or underlying systemic disease.

On examination (Day 0), the affected eye had an approximately 2 × 2 mm fluffy white infiltrate slightly superior and to the left of the visual axis surrounded by scattered stromal subepithelial infiltrates, 1 + edema, 1 + scleral injection and a large (10 mm) epithelial defect. Visual acuity was 20/20 in the right eye and 20/200 in the left eye.

The differential diagnosis was herpes simplex virus, *Acanthamoeba*, or bacterial infection, but because of the appearance of the corneal infiltrates the infection was presumed to be fungal. Treatment with topical voriconazole eye drops (10 mg/mL, not commercially available, prepared by compounding pharmacy in-house) every hour plus gatifloxacin eye drops (5 mg/mL, Zymaxid®, Allergan, Irvine, CA) four times per day was started on Day 0 along with oral acyclovir (1 g twice daily, Zovirax®, GlaxoSmithKline, Philadelphia, PA). Scrapings from the corneal lesion were taken and were directly inoculated onto tryptic soy agar with sheep blood (Remel, Lenexa, KS) and Sabouraud dextrose agar (Remel) plates and sent to the Clinical Microbiology Laboratory for bacterial and fungal culture. A Gram stain was not performed but corneal scrapings evaluated in the Ophthalmic Pathology Laboratory showed epithelial cells and septate fungal elements.

The culture plates were incubated at 30 °C in ambient air and within three days the plates began to show growth of compact velvety colonies along the inoculation streaks. The colonies were initially white and gradually turned dark green on the surface and brownish orange on the reverse (Fig. 1A, B). Tease preparations and slide culture showed numerous microconidia (Fig. 2). Based on morphologic and growth characteristics, the isolate was presumptively identified as *Metarhizium* species (isolate B10964).

After three weeks (Day 21), oral voriconazole (200 mg every 12 h)

* Corresponding author.
E-mail address: eburd@emory.edu (E.M. Burd).

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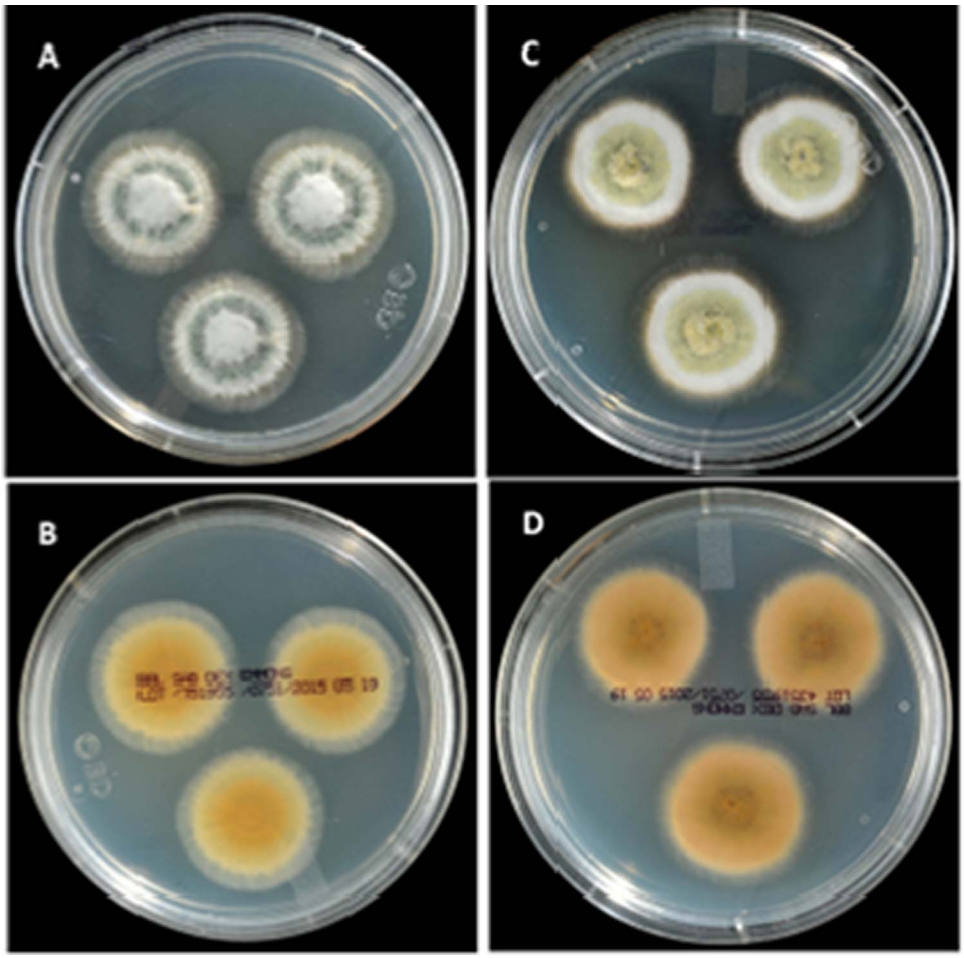

Fig. 1. Morphologically different colony types of the B10964 (A, B), Georgia isolate, and B11022 (C, D), Missouri isolate, *Metarhizium anisopliae* isolates after 7 days of growth on Sabouraud dextrose agar at 25 °C (BBL, Becton, Dickinson and Company, Franklin Lakes, NJ). Colonies are floccose with a dense heaped center that was either light with a dark green ring (B10964) or yellow-green (B11022), both with a white fringe. The reverse of both isolates was a brownish orange color.

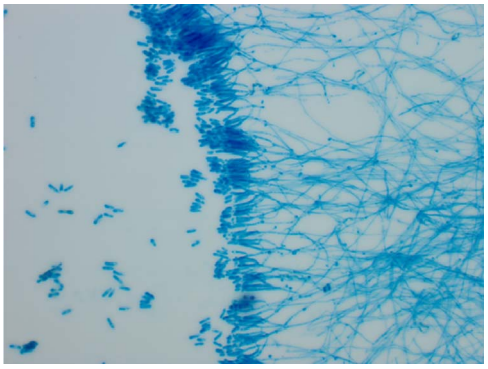

Fig. 2. Lactophenol cotton blue stain of slide culture of the Georgia *Metarhizium anisopliae* (B10964) isolate, showing slender conidiophores with verticilliate branching ending with clavate, parallel phialides and cylindrical microconidia in chains (original magnification, 400 ×).

was added and the frequency of voriconazole eye drops was decreased to four times daily. The acyclovir was discontinued due to determination of a fungal infection. Natamycin eye drops (50 mg/mL, NATACYN®, Alcon, Fort Worth, TX) every hour were administered early in the treatment regimen, however the patient was unable to tolerate them.

At 34 days after presentation, the patient developed inflammation-associated glaucoma with intraocular pressure as high as 50 mm Hg (normal range10–21 mm Hg) and visual acuity was reduced to light perception in the left eye. The inflammation-associated glaucoma was treated with trans-scleral diode laser cyclophotocoagulation.

Approximately 12 weeks after the initial visit (Day 84), superior thinning was noted, but the infiltrate was improving and the size of the epithelial defect had decreased to 4.5 × 2.5 mm. Approximately one week later new infiltrates were noted and corneal smears showed occasional fungal elements. Again, scrapings from the corneal lesion were taken and directly inoculated onto tryptic soy gar with sheep blood and Sabouraud dextrose agar plates and sent to the Clinical Microbiology Laboratory for bacterial and fungal culture, and once more a mould (isolate B11041) was identified with the same characteristics as the initial isolate (isolate B10964).

A corneal transplant was performed 13 weeks (Day 91) after the initial visit due to persistence of fungal keratitis. There were no signs of continued fungal infection but a cataract developed and was subsequently removed.

The second case is a 50-year-old man from rural Missouri, US, who presented to his primary care provider with left eye pain (Day 0). The pain was acute in onset (approximately one week in duration) and the patient experienced photophobia and vision loss in the left eye. On exam, the primary care provider noted a cloudy white area in the left eye suspicious for fungal infection, and referred the patient to Barnes-Jewish Hospital for further work up.

The patient reported using disposable contact lenses designed to be worn during the day each day for two weeks, and recounted sleeping with the lenses in place approximately three times per week. Of note, he had been in a motor vehicle accident approximately 30 years prior and