

Rare case of fungal keratitis caused by *Plectosporium tabacinum*

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Abstract: A rare case of fungal keratitis caused by *Plectosporium tabacinum* is reported. A 78-year-old female gardener presented with conjunctivitis and an oval infiltrate with irregular margins in the nasal half of the cornea in the right eye. Light microscopy of corneal scrapings revealed a filamentous fungus, and a diagnosis of fungal keratitis was made. The patient was admitted into our hospital on February 19, 2008. Treatment with topical miconazole, topical fluconazole, pimaricin ointment, intravenous miconazole, and corneal debridement was commenced. One week later, the infiltrate improved, but the central part of the infiltrate was still deep. Topical fluconazole was switched to topical voriconazole, and intravenous miconazole was switched to intravenous voriconazole. One month after admission, the causative organism was identified by morphology and molecular biological analysis as *Plectosporium tabacinum*. The corneal infiltrate resolved 3 months after admission. A stromal scar persisted for 3 months after the patient was discharged. This is the first detailed report of fungal keratitis caused by *P. tabacinum*. Voriconazole was effective in treating this refractory keratitis.

Keywords: fungal keratitis, *Plectosporium tabacinum*, voriconazole, filamentous fungi

Introduction

Filamentous fungi are the major etiologic agents of fungal keratitis, with *Fusarium* and *Aspergillus* species most frequently implicated as primary pathogens.¹ Here we report a refractory case of fungal keratitis caused by *Plectosporium tabacinum*, a filamentous fungus found in soil. To our knowledge, there have been only two reports of human infection caused by this species, including one case of fungal keratitis in Hungary² and one case of onychomycosis in Brazil.³

Case report

A 78-year-old female gardener presented to a local ophthalmologist in December 2007 complaining of blurred vision and a foreign body sensation in her right eye. Her local ophthalmologist treated her with topical antibiotics for corneal abrasion in the right eye. Two months later, treatment with topical corticosteroids was initiated because her symptoms remained unchanged. However, her blurred vision intensified. She was referred to our facility with a diagnosis of corneal ulcer on February 18, 2008. Her medical history was significant for hypertension. She denied a history of ocular trauma. She had undergone cataract surgery with intraocular lens implantation in both eyes in 2007. At initial presentation, her visual acuity was counting fingers in the right eye and 0.8 in the left eye. Intraocular pressure was not measured in

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the right eye, but was 9 mmHg in the left eye. Slit-lamp examination of the right eye revealed conjunctival injection, an oval infiltrate with irregular margins in the nasal half of the cornea, 2+ cells, keratic precipitates, and hypopyon in the anterior chamber (Figure 1A and B). Corneal opacity prevented visualization of the fundus in the right eye. Light microscopy of corneal scrapings taken from the right eye at the initial presentation showed uniformly septate hyphae. The patient was diagnosed as having keratitis caused by a filamentous fungus, and was admitted into our hospital on February 19, 2008. Treatment was initiated with topical miconazole 0.1% and fluconazole 0.1% hourly, pimaricin 1% ointment six times a day, intravenous miconazole 400 mg a day, and corneal debridement every 3 days. One week later, the infiltrate improved, but the central part of the infiltrate was still deep (Figure 1C). Topical fluconazole 0.1% was switched to topical voriconazole 1% hourly, and intravenous miconazole was switched to intravenous voriconazole 400 mg daily. Microscopic examination of corneal scrapings cultured on potato dextrose agar and stained with lactophenol cotton blue did not identify any specific pathogen. One month after admission, the causative organism was identified as *P. tabacinum*, based on its morphology and the sequence of the internal transcribed spacer (ITS1-5.8S-ITS2) region of the ribosomal RNA gene. Drug susceptibility testing showed that the 80% inhibitory concentration (IC₈₀) of voriconazole against this organism was 0.125 µg/mL, with good susceptibility (Table 1). Treatment with voriconazole

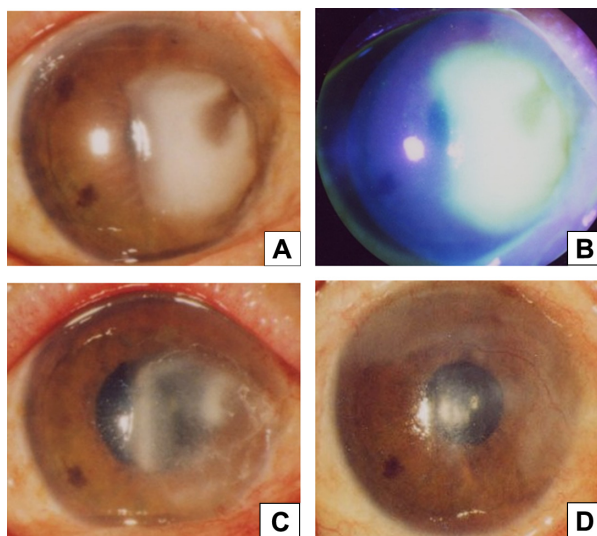


Figure 1 Slit-lamp photographs. (A) Oval infiltrate with irregular margins in the nasal half of the cornea at initial presentation. (B) Fluorescein staining at initial presentation. The lesion was not stained with fluorescein. (C) One week after admission, the infiltrate improved, but in the central part was still deep. (D) A stromal scar remained 3 months after the patient was discharged.

Table 1 Sensitivity of the isolate to various antifungal agents

Medicine	IC ₈₀ (µg/mL)
Amphotericin B	0.5*
Flucytosine	>64
Fluconazole	64
Itraconazole	0.25
Miconazole	1
Micafangin	0.25
Voriconazole	0.125

Note: *100% inhibitory concentration.

Abbreviation: IC₈₀, 80% inhibitory concentration.

was continued, and the infiltrate gradually reduced in size. However, due to the slow regression of the infiltrate, oral itraconazole 200 mg a day was added to the treatment 2 months after admission. The IC₈₀ of itraconazole was 0.25 µg/mL. Liver function was evaluated regularly during administration of systemic voriconazole and itraconazole. Three months after admission, the corneal infiltrate resolved, and the patient was discharged. At the time of discharge, her right visual acuity was counting fingers, and intraocular pressure was 9 mmHg. Topical and systemic treatment with antifungal agents was continued after discharge. A stromal scar persisted for 3 months after discharge, and visual acuity was 0.02 (Figure 1D). The treatment course in the right eye is shown in Figure 2.

Identification of causative organism

The causative agent was initially searched for at the Central Clinical Laboratory of Kurume University Hospital but could not be identified. The isolate was then sent to First Laboratories Co, Ltd, where it was re-examined morphologically and analyzed using molecular biology techniques. The fungus produced dull salmon-pink, flat and smooth colonies, with fine aerial hyphae at the marginal area, after culture on potato dextrose agar at 25°C in the dark for 13 days (Figure 3A). Slide culture preparations showed conidiophores that were hyaline and hypha-like, unbranched or branched, and forming a scanty cluster (Figure 3B). Conidiogenous cells were phialides occurring on the tips of conidiophores or as side branches of hyphae. These were hyaline, hypha-like, and tapered toward the tops, with an open end and collarette (Figure 3B and C, arrows). Phialides were straight or occasionally twisted (Figure 3B and D, asterisks). Phialoconidia formed in masses from the open ends. These were hyaline, cylindrical, pointed at both ends, straight or slightly curved at the basal portion, with 0–3 septa (mostly uniseptate), and were 7.0–12.9 × 1.8–3.6 µm (average 10.0 × 2.6 µm) in size. Based on these morphological characteristics, the isolate was identified as *P. tabacinum*.⁴

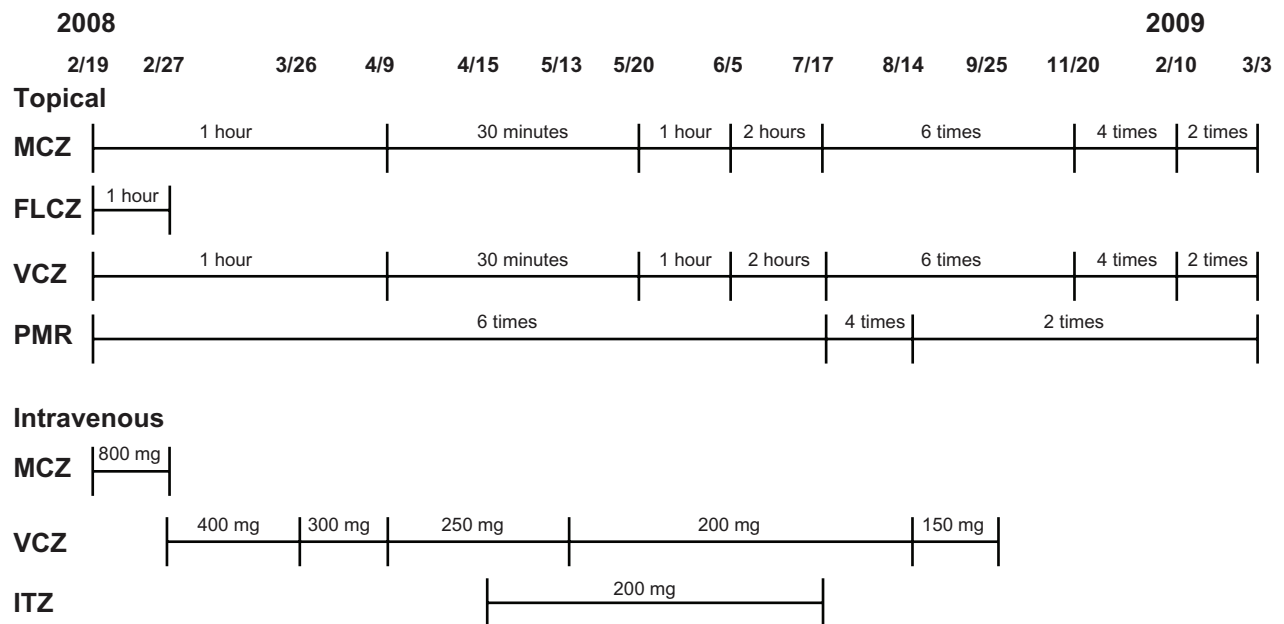


Figure 2 Treatment course for the right eye.

Abbreviations: MCZ, miconazole; FLCZ, fluconazole; VCZ, voriconazole; PMR, pimaricin; ITZ, itraconazole.

A Basic Local Alignment Search Tool (BLAST) search showed that the sequence on the ITS region had 100% similarity with six sequences (AM408781, AB264787, AB264786, AB264782, AB266251, AB264785) on the ITS regions of *Plectosphaerella cucumerina* (the teleomorphic

name of *P. tabacinum*) registered with the National Center for Biotechnology Information, National Institutes of Health, Bethesda, MD. This confirmed the morphological identification. The sequence was registered at the DNA Data Bank of Japan, National Institute of Genetics, Japan,

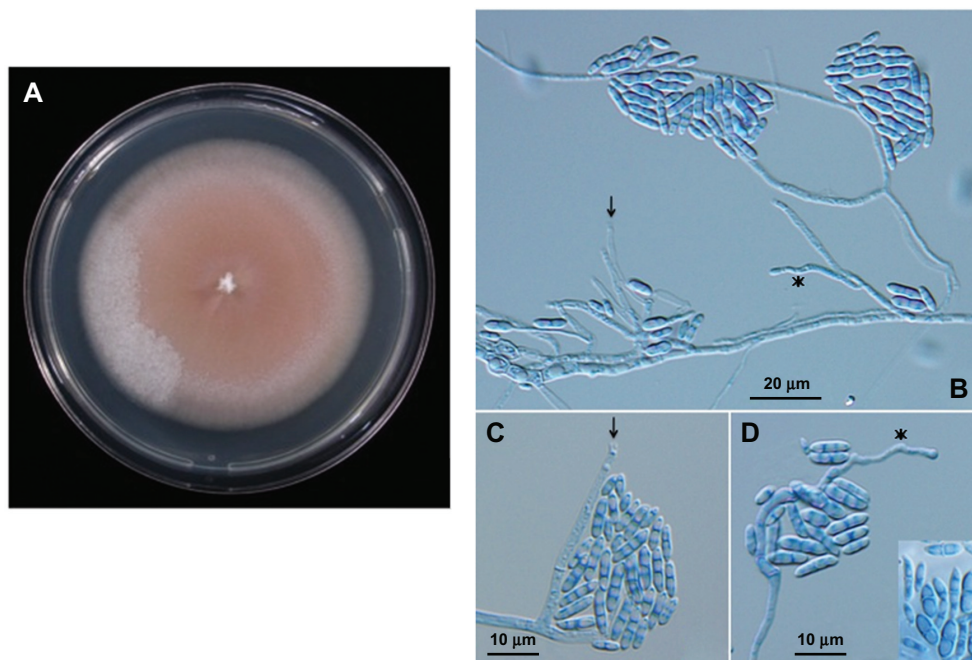


Figure 3 Morphological findings of the isolate. **(A)** Colony incubated on potato dextrose agar at 25°C for 13 days. **(B, C, D)** Microscopic findings on slide cultures grown on potato dextrose agar. Phialides arise from conidiophore apices or hyphae as side branches. They are straight or twisted (asterisks), tapering toward the tops, with an open end and collar (arrows). Phialoconidia are cylindrical with pointed ends, or narrow spindle-shaped, straight, or slightly curved, and mostly uniseptate. Lactophenol cotton blue stain. **(B)** ×400; **(C and D)**, and inset ×600.

as accession number AB469880, and a living culture was deposited at the Medical Mycology Research Center (originally the Institute for Food Microbiology), Chiba University, Japan, as IFM 58012.

Discussion

P. tabacinum is a soil-borne fungus and a saprophyte commonly found in decaying plants. It is ubiquitously found in soil throughout the world and is also known as a plant pathogen. A number of plant diseases, including diseases of sunflower and basil, have been reported.⁵ Some strains cause blight or spot diseases in vegetables and flowers in Japan.⁴ Interestingly, the ITS sequence of the isolate from our patient was 100% identical with those from some vegetables.

According to Palm et al,⁵ the teleomorph (sexual form) of *P. tabacinum* was first isolated from cucumber in 1919 and was named *Venturia cucumeria* by Lindfors. In 1933, the anamorph (asexual form) was isolated from diseased tobacco and was designated as *Cephalosporium tabacinum*. In 1968, the anamorph was later placed within the genus *Fusarium* and was then transferred in 1984 to the genus *Microdochium*, based on the morphology of the conidium. However, the characteristics of the conidia and the manner of conidiogenesis differ from those of *Microdochium* species, where conidia are blastic rather than phialidic. The name *Plectosporium tabacinum* was established by Palm et al in 1995, and has since been widely accepted.

P. tabacinum is a rare human pathogen. To our knowledge, only two cases have been reported, including one of fungal keratitis² and one of onychomycosis.³ The previous report of fungal keratitis came from Hungary, and was presented in abstract form at the 14th International Society for Human and Animal Mycology World Congress, Buenos Aires, Argentina, in 2000. According to this abstract, a 57-year-old man had developed a corneal ulcer after grass struck his eye while he was weeding. Despite the lack of a pronounced inflammatory reaction, the clinical picture was highly suggestive of a fungal etiology. Corneal scrapings stained with Gomori-Grocott methenamine silver and Periodic acid Schiff showed fungal elements. Cultures on Sabouraud glucose agar yielded mold colonies. The fungus was identified as *P. tabacinum* at the Centraalbureau voor Schimmelcultures in The Netherlands.

In our case, it took about one month after obtaining samples from the lesion to identify the pathogen as *P. tabacinum*. Cultures on potato dextrose agar produced creamy, moist colonies measuring 40–50 mm in diameter after 12 days. Light microscopy with lactophenol cotton blue staining revealed

regular septate hyphae with phialides that produced conidia. We initially suspected *Fusarium* as a pathogen, because this species is commonly isolated from fungal keratitis.¹ However, cultures from the corneal scrapings did not show the crescent-shaped macroconidia characteristic of *Fusarium* species. Cultures were continued, and uniseptate conidia were produced. Because the causative agent could not be identified at our facility, the fungus was sent to First Laboratories Co, Ltd, for identification. It was re-examined morphologically, analyzed with molecular biology techniques, and confirmed as *P. tabacinum*. A diagnosis was made of fungal keratitis caused by this fungus.

P. tabacinum is a ubiquitous fungus found in soil and plants, but rarely causes human infection. The patient in the report of Simon et al, who underwent eye trauma while weeding,² was a keen gardener, as was our patient. Both cases might have been infected with *P. tabacinum* through soil or agricultural products.

Theoulakis et al⁶ reported keratitis caused by a rare human pathogen, *Thielavia subthermophila*. This fungus is also ubiquitously found in soil and plants, and the patient had corneal trauma inflicted from contact with a palm tree. In cases of fungal keratitis caused by rare human pathogens, the pathogen is often difficult to detect. We verified the morphological identification of our pathogen by DNA analysis. The pathogen in the case of Simon et al was identified as *P. tabacinum* at the Centraalbureau voor Schimmelcultures, which maintains a world-renowned collection of fungi, but the method of fungal identification was not described in the abstract. The fungus in the case of Theoulakis et al was also identified by a DNA sequence analysis with a BLAST search. Therefore, the ITS sequence analysis is useful for identification or confirmation of uncommon fungal pathogens.

Drug susceptibility testing in our case showed low susceptibility to fluconazole and miconazole, but high susceptibility to voriconazole. Voriconazole, a triazole antifungal agent, has excellent intraocular penetration. Hariprasad et al⁷ reported that the aqueous concentration of voriconazole was 1.13 µg/mL and the vitreous concentration was 0.81 µg/mL when the plasma concentration was 2.13 µg/mL. The IC₈₀ of voriconazole against *P. tabacinum* was 0.125 µg/mL. The plasma voriconazole concentration of our patient was at least 2.16 µg/mL at all points in time during admission. Therefore, the aqueous voriconazole concentration of our patient was considered to be adequate. The infiltrate gradually reduced in size after initiation of voriconazole treatment. However, due to slow regression of

the infiltrate, oral itraconazole was added to the treatment 2 months after admission. The organism showed relatively good susceptibility to itraconazole and the infiltrate improved.

Although fungal keratitis caused by this organism is extremely rare, the finding that this organism is sensitive to treatment with voriconazole but unresponsive to treatment with fluconazole and miconazole should be kept in mind. We initially treated our patient with fluconazole and miconazole, but the infiltrate did not respond well. Fungal keratitis reported by Simon et al was also treated with fluconazole and miconazole, but the keratitis was unresponsive to this treatment.² The IC_{80} of itraconazole against *P. tabacinum* in our case was second only to that of voriconazole (Table 1). Thus, itraconazole may also be effective in the treatment of *P. tabacinum* keratitis. The choice of antifungal agents is important in the treatment of refractory fungal keratitis caused by *P. tabacinum*.

This is the first detailed report of *P. tabacinum* keratitis. Prompt and effective antifungal treatment is important in patients with refractory fungal keratitis. Sequencing of the ITS region is useful for fungal identification in patients with keratitis caused by a rare human pathogen.

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Disclosure

The authors have no financial interests to report in this work.

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