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An environmental *Sporothrix* as a cause of corneal ulcer

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**A B S T R A C T**

In this case we introduce *Sporothrix pallida*, a non-pathogenic environmental *Sporothrix* species as a cause of infectious keratitis in a corneal transplant recipient. Human infections caused by *S. schenckii* are well-known but human infection with *Sporothrix pallida* has not been previously reported.

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1. Introduction

Fungi are opportunistic agents of infections of the cornea. Although rare, especially in temperate climates, fungal infection typically occurs in corneas that are compromised by underlying disease or trauma. Fungal keratitis is often a diagnostic and therapeutic challenge, especially in geographic areas where these infections are not commonly seen. The associated stromal inflammation is not unique to fungal keratitis and management is restricted to the availability of topical antifungal agents and effectiveness depends on susceptibility of the causative agent and the extent to which the antifungal penetrates into corneal tissue. The predominant etiologic agents of fungal keratitis such as *Fusarium*, *Aspergillus*, *Curvularia*, *Alternaria*, *Candida* etc. are also known to cause localized or systemic infections in other parts of the body. Some fungi that are considered nonpathogenic to humans have been isolated as rare causes of infectious keratitis, usually following corneal injury or the long term use of topical corticosteroids [1,2]. Non-pathogenic molds such as *Phoma*, *Dichotomopithorium*, *Rhzoctonia*, *Cephalosporum*, *Lasiodiplodia*, *Colletotrichum*, *Cladophorium*, *Metrarhizium* and *Pheosaria* have been reported [1,2]. We describe a case of fungal keratitis caused by *Sporothrix pallida*, a non-pathogenic environmental mold closely related to the known pathogen *Sporothrix schenckii*. *S. pallida* has not been previously described as causing pathologic infection in humans. Reporting of cases like this that are caused by unusual organisms not only adds to the cumulative literature but provides some measure of the relative frequency of organisms likely to be encountered. This case also provides the results of susceptibility testing and their use in the management of antifungal therapy.

2. Case

The patient is a 75 year old woman who presented to the Cornea service in January 2012 complaining of two weeks of decreased vision in her right eye. She has a history of bilateral Fuchs corneal dystrophy and underwent a combined cornea transplant and cataract extraction in the right eye in 2006. She has worn a rigid gas permeable contact lens in the right eye to improve visual acuity following the cornea transplant. She had an episode of graft rejection in 2008 and has been maintained on prednisilone acetate eye drops once per day since. Medications also included latanaprost and brimonidine eye drops for treatment of glaucoma. She denied any history of recent trauma. At presentation, her uncorrected visual acuity was 20/70 in the right eye. Examination of her cornea revealed central irregular anterior stromal infiltrates in a branching pattern with an overlying epithelial defect. The patient’s cornea was scraped and specimens were submitted for Gram stain and bacterial, mycobacterial and fungal culture. Gram stain of the corneal scrapings showed numerous branching septate hyphal elements consistent with fungus. The results of the culture as detailed below identified *Sporothrix pallida* as the causative organism. The patient was started on topical voriconazole every hour and the prednisilone acetate was discontinued. Following six weeks of treatment with voriconazole, she had a small, residual central scar. Her best corrected visual acuity returned...
to 20/30. She resumed her prednisolone acetate and contact lens wear.

Three months later she developed new symptoms of pain, redness and decreased vision. A new infiltrate was noted adjacent to the previous scar. Due to her previous history, repeat cultures were taken and she was restarted on topical voriconazole. *S. pallida* was again isolated from her right cornea, and susceptibility testing was done, which showed an elevated minimum inhibitory concentration (MIC) to voriconazole. The patient had an incomplete response to treatment and developed a persistent epithelial defect with a central infiltrate. Her best corrected visual acuity was 20/40. She underwent a repeat penetrating keratoplasty to remove the infected graft.

Laboratory diagnosis was made by inoculating the corneal scrapings onto sheep blood agar, chocolate agar, and Sabouraud dextrose agar (SAB) with chloramphenicol and gentamicin (Remel, Lenexa, KS) and submitted to the Microbiology Laboratory for routine bacterial and fungal culture. After four days of incubation at 30 °C, several flat, glabrous, cream-colored colonies that developed a wrinkled and folded surface with maturation were seen on the fungal culture. A wet mount demonstrated oval yeast forms. A germ tube test was negative, an API 20C (bioMérieux, Durham, NC) yeast identification panel resulted in no identification, and a urea slant was positive for urease production. Culture at 25 °C on corn meal agar yielded tan colonies consisting of oval yeast, long pseudohyphae, and septate hyphae with terminal obovoid conidia with short denticles growing sympodially on a conidiogenous cell. The culture did not darken with age.

DNA was prepared from the isolate as previously described [3]. Sequence analysis of the internal transcribed spacer (ITS) and large subunit (LSU) sequence identified 100% matches to isolates of *Sporothrix palida* and *S. styloides*. Additional sequence analysis of the β-tubulin gene [4] clearly distinguished the isolate as *S. palida* with 100% sequence identity across ITS, LSU and β-tubulin (1448 base pairs total) to *S. palida* isolate CBS131.56.

Antifungal susceptibility testing on the second isolate was done using the methodology of the Clinical and Laboratory Standards Institute, document M38-A2 [5]. Minimum inhibitory concentration (MIC) and minimum effective concentration (MEC) values were determined following 48 h of growth at 25 °C. MICs for the azoles were notably elevated and ranged from 4 μg/ml to > 256 μg/ml (Table 1). MICs for quality control strains were within reference range. Slow growth prevented determination of yeast phase susceptibilities with no growth in the drug-free well following 72 h of incubation at 35 °C.

### 3. Discussion

The pathogenic dimorphic fungus *Sporothrix schenckii* has been recovered from many clinical specimens worldwide and is well-known as the causative agent of sporotrichosis. Infection usually involves the skin and subcutaneous tissues but can occasionally disseminate or occur in other sites, primarily in immunocompromised patients [6]. Although only one clinically relevant species of *Sporothrix* was classically identified, recent molecular and phenotypic data demonstrate that what had been known as *Sporothrix schenckii* is actually a complex of species of different genetic lineages that tend to cluster in distinctive geographic regions [4,7]. Accordingly, *Sporothrix schenckii* is now named the *S. schenckii* complex and is comprised of a number of newly recognized *Sporothrix* species [7,8].

*S. schenckii* complex organisms live as saprophytes on wood, decaying vegetation, animal excreta and soil, and some species are considered to be associated with human infection. Other *S. schenckii* complex species that are also found in the environment are clearly distinct from human isolates and are non-pathogenic in animal studies [9]. Phylogenetic studies based on 28S rDNA and internal transcribed spacer ITS sequences have been used to make comparisons at the genus level and separate the group of clinical strains from the environmental strains but are not informative enough to distinguish relationships within each group [10]. More information has been gained by sequencing the beta-tubulin and calmodulin genes. This information, along with morphological and cultural differences have lead to the recognition of distinct taxa within the environmental strains now described as *S. palida*, *S. styloides*, *S. humicola* and *S. ligniivorax* [4]. The previously described species *S. albicans* and *S. nivea* have been found to be synonymous with *S. palida*, which was retained as the accepted name since it was the first of the three to be described [4]. There are no reported cases of human infection by *S. palida*, but that does not eliminate the possibility of infections that may have been erroneously ascribed to *S. schenckii*.

The source of the infection in the present case is unclear. The patient had recently returned from a vacation in Texas, but denied any recent trauma. She was on long term topical corticosteroids due to her previous history of graft rejection and was at least focally immunocompromised. Despite an initial good response to topical therapy, she ultimately required a repeat keratoplasty to eradicate the infection.

In vitro antifungal susceptibility profiles have been reported for *S. schenckii* complex isolates but not for the non-pathogenic *Sporothrix* species. Although a standardized method for antifungal susceptibility testing is established, no interpretive breakpoints exist for *Sporothrix* sp. and correlation with clinical outcome remains to be determined [5]. Reported studies have shown that *Sporothrix* susceptibility patterns are somewhat variable and depend on the species tested [11,12]. *S. brasiliensis* had generally low MICs to all of the antifungal agents tested and *S. mexicana* had high MICs, except against terbinafine. Terbinafine had low geometric mean MICs for all of the species tested and was found to be the most active antifungal agent. Ketoconazole generally also had very low geometric mean MICs except against *S. mexicana* and *S. albicans*. Posaconazole was the most active of the systemic antifungal agents. Fluconazole and micafungin were not active against any of the species. Although itraconazole has been effectively used clinically, *in vitro* activity was poor for itraconazole and ravuconazole, except against *S. brasiliensis*. MICs against eberconazole, ravuconazole and amphoteracin B were moderately high and amphotericin B has been used clinically with variable results. MIC ranges were wider for voriconazole and fluconazole and high geometric mean MICs indicate that these agents would generally not be effective. Itraconazole is currently considered to be the treatment of choice for cutaneous infections, while amphotericin B is the treatment of choice for systemic or disseminated infections [13].

For the *S. palida* isolate obtained in this case, anidulafungin and micafungin appeared to have the most favorable *in vitro* activity. Caspofungin, voriconazole and posaconazole had elevated MICs above 2 μg/ml. Very high MICs were obtained for fluconazole,
itraconazole, and flucytosine which were suggestive of a low level of susceptibility to these drugs.

Conflict of interest statement

There are none.

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