First Human Case of Fungal Keratitis Caused by a Putatively Novel Species of Lophotrichus

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We report an aggressive fungal keratitis caused by a putatively novel species of Lophotrichus in a patient with traumatic injury to the cornea from a dog paw. The organism was isolated from the patient’s necrotic cornea, which perforated despite coverage with hourly fortified broad-spectrum topical antibiotic therapy. This report represents the first case of human infection caused by this species.

CASE REPORT

A 50-year-old woman from rural Maryland, USA, presented to the Johns Hopkins Emergency Department in April 2011 5 days after her dog stepped on her right eye during sleep. She experienced foreign body sensation and blurry vision at the time but experienced a rapid decrease in vision on day 4. When she presented on day 5, she could see only light and dark in the right eye. She had no known history of contact lens use. She had undergone cataract surgery in both eyes 1 year prior to presentation and did not wear glasses except for reading.

The physical examination conducted 5 days after the injury revealed uncorrected visual acuity of light perception in the right eye and 20/25 at distance in the left eye. There was no afferent pupillary defect present. Slit-lamp examination of the right eye revealed upper and lower eyelid erythema and edema. The conjunctiva was + injected, and the cornea demonstrated a large central ulceration measuring 6 mm in diameter with yellow-green discharge (Fig. 1A). The anterior chamber demonstrated a robust fibrinous reaction, with no view of the lens or posterior segment. The patient immediately started treatment with a fortified topical 25-mg/ml vancomycin and 14-mg/ml tobramycin ophthalmic solution hourly to the right eye. Corneal scrapings were obtained and inoculated onto three culture media: blood agar containing 25-mg/ml vancomycin and 14-mg/ml tobramycin ophthalmic solution hourly to the right eye. Corneal scrapings were obtained and inoculated onto three culture media: blood agar containing 5% sheep blood, chocolate agar, and Sabouraud dextrose agar with gentamicin.

On day 6 after the injury, the patient added oral doxycycline at 100 mg twice daily and ciprofloxacin ointment nightly to her regimen. Ultrasonography revealed no choroidal or retinal detachment. When the patient returned on day 10, she complained of severe pain. Despite frequent drop use, the infiltrate and ulceration persisted, with formation of significant corneal neovascularization and inferior thinning. The eye was soft to palpation, and 360-degree shallow choroidal detachments were noted, consistent with microperforation of the cornea.

At this point, 4 days after corneal scrapings and cultures, preliminary results demonstrated the presence of a filamentous fungus (on the chocolate agar) and very light growth (on the first quadrant of the culture plate) of Corynebacterium macginleyi. Topical voriconazole at 1% was started hourly in the right eye, in addition to continuing fortified vancomycin and tobramycin. She was taken to the operating room, where a corneal biopsy was conducted and an Ambio5 (IOP Ophthalmics, Costa Mesa, CA, USA) amniotic membrane patch was grafted onto the ocular surface.

On day 17 after the initial injury, the infiltrate began to clear superiority and the cornea had partially epithelialized, at which point drops were reduced in frequency to four times daily. Visual acuity was light perception in the right eye. On day 31, the right cornea had epithelialized, but a 3.5-mm hyphema was present with engorged iris vessels, consistent with underlying background herpetic infection. Accordingly, she was started on acyclovir at 800 mg five times daily by mouth and, 2 days later, topical prednisolone acetate 1% ophthalmic suspension four times daily. Vancomycin and tobramycin were discontinued at 6 weeks, and voriconazole eye drops were discontinued at 1 year after surgery. A large inferior paracentral scar remained (Fig. 1B). Significant iridocorneal adhesions remained, and the patient was maintained on dorzolamide-timolol for intraocular pressure control and 800 mg of oral acyclovir daily to prevent recurrence of herpetic infection. The right eye remained stable in appearance, with no new ulceration or infiltrate, at 1 year.

Histopathology. A total of three partial-thickness corneal biopsy specimens were acquired from the superior cornea; the inferior cornea was avoided due to thinning and microperforation. Histopathological examination revealed necrosis in regions of the deep cornea stroma where an intact epithelium remained (Fig. 2). Hematoxylin and eosin (H&E), periodic acid-Schiff, Gomori methenamine silver


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(GMS), Gram-Weigert, and Brown & Hopps stains were negative for bacteria, fungi, and *Acanthamoeba* organisms.

**Mycology and molecular identification.** Culture from the initial corneal scraping grew a filamentous fungus on the chocolate agar within 4 days at 37°C, and the fungus was then subcultured onto the potato dextrose agar for sporulation. Lactophenol cotton blue staining revealed an irregular and branched hyphal structure; no sexual structure or sporulation was seen. Growth of this fungus was inhibited by cycloheximide. DNA sequencing was performed to identify the organism by use of methods described previously (1). Briefly, after DNA extraction, the internal transcribed spacer (ITS) region was amplified and sequenced, and the results were analyzed by SmartGene (SmartGene, Inc., Raleigh, NC, USA) and used as a BLASTn query of the NCBI database. The ITS region showed 98% identity to *Lophotrichus fimeti* type strain CBS 129.78 (GenBank accession no. AY879799.1). Since *Lophotrichus* is very rarely isolated from clinical samples and the ITS sequence result showed only 98% identity, the isolate was sent to two reference laboratories for further investigation.

The fungus was referred to the Fungus Testing Laboratory (FTL) at the University of Texas Health Science Center at San Antonio (UTHSCSA) for identification by phenotypic characteristics (UTHSCSA DI14-343). A *Lophotrichus* species was confirmed on the basis of morphological features on a variety of media as illustrated in Fig. 3.

The fungus was also referred to the University of Alberta Microfungus Collection & Herbarium (UAMH) for species-level identification, where it was accessioned as UAMH 11809. Six *Lophotrichus* strains (five of which are type strains) (*Lophotrichus amplulus* UAMH 9122, *Lophotrichus bartlettii* UAMH 9287T, *L. fimeti* UAMH 4257T, *Lophotrichus macrosporus* UAMH 9258T, *L. martini* UAMH 8692T, and *Lophotrichus plumbeos* UAMH 8710T) were included in the phylogenetic analysis. Genomic DNA was extracted from mycelia of all seven isolates, and the ITS, large subunit (LSU), and beta-tubulin (BT2) genes were PCR amplified and sequenced using primer pairs comprising BMB-CR and ITS4 for the ITS region, BMB-CR and LR7 for the LSU region, and BT2a and BT2b for the BT2 region (2–4). Maximum parsimony (MP) analyses were performed individually for each locus. MP and Bayesian analyses were performed on the combined ITS-BT2 sequences by use of PAUP version 4.0b10 and MrBayes 3.1.2, respectively (5, 6). Clade support was assessed using the full heuristic search option for 2,000 bootstrap (BS) replications (7). Gaps were treated as missing data. Clades with BS values of ≥70% were considered strongly supported. The Bayes-
ian analysis used the general time-reversible (GTR) substitution model with estimation of invariant sites and an assumed discrete gamma distribution (GTR+I+G) as selected by ModelTest version 3.7 (8). Four Markov chains were run simultaneously, and trees were sampled at every 100th generation out of a total of 2 million, with the first 2,000 trees being discarded as “burn-in.” Inferences of posterior probabilities (PP) were calculated from 18,001 trees, and only clades with PP values of ≥95% were considered to be strongly supported. The consensus tree was visualized using PAUP. The topology of the MP trees for individual locus was congruent. The topology of the Bayesian tree was also congruent with the single most parsimonious tree for the concatenated BT2-ITS data set. Results from MP and Bayesian analyses indicated that the fungus was closely related to *Lophotrichus* species (Fig. 4). The BT2-ITS tree places UAMH 11809 in the *Lophotrichus* clade, which is supported by high BS and PP values (100 and 1.00, respectively), but there is insufficient support for conspecificity with any of the other *Lophotrichus* species. Thus, our case strain was identified as a member of a putatively novel species of *Lophotrichus* and will be described in a separate study.

**Antifungal susceptibility testing.** Antifungal susceptibility testing on the case strain was performed by broth microdilution according to CLSI methods for filamentous fungi (M38-A2) (9). MICs for amphotericin B, fluconazole, itraconazole, and voriconazole were read as the lowest concentration of each agent that resulted in 100% inhibition of growth compared to the level for the growth control after 48 h of incubation. The MIC results were as follows: for amphotericin B, 2 μg/ml; for fluconazole, 64 μg/ml; for itraconazole, 1 μg/ml; and for voriconazole, 0.125 μg/ml.

*Lophotrichus* species (belonging to the family Microascaceae, class Sordariomycetes) have been isolated from soil, leaf litter, and
decaying wood as decomposers. Although members of this genus are ubiquitous in soil, they are rarely isolated from goat and rabbit dung (10).

Here, we describe the first case of fungal keratitis associated with a putatively novel species of *Lophotrichus* and successful treatment with voriconazole. Filamentous fungi are frequently implicated in fungal keratitis in humans, especially species of *Fusarium* and *Aspergillus* (11). However, numerous organisms have been associated with keratitis, especially with contact lens wear (12). In the present case report, the rapidity of progression to corneal perforation was consistent with rapid growth of *Lophotrichus* in vitro (10). Review of the Johns Hopkins Hospital (JHH) Microbiology Laboratory records revealed only one other putative infection caused by a *Lophotrichus* strain, which was isolated from bronchoalveolar lavage fluid (JHH accession no. 49-3R0949; unpublished data).

In the present case, the patient was initially started on fortified antibiotics due to the size and severity of the ulcer. The poor response to frequent topical antibiotics but good response to antifungal treatment suggests that bacterial infection was unlikely the principal cause of her ulcer. *Corynebacterium macginleyi* is typically isolated from conjunctival biota, as in our patient; however,
corneal ulceration associated with this organism is notably mild in severity (13).

*Lophotrichus* was isolated in culture but was not found in histological testing, which revealed necrotic tissue. However, a positive histological finding is largely dependent on the location and depth of tissue sampling. In a series of consecutive corneal biopsies conducted for microbial keratitis over 20 years, only 42% identified organisms (14). In our case, resection of tissue represents a balance between obtaining an adequate sample for diagnostic purposes and maintaining adequate tissue to retain the structural integrity of a necrotic cornea (the biopsy specimens were notably acquired from a thicker, less involved region of the cornea in order to avoid requiring a full-thickness corneal transplant in a cornea with extreme thinning). As such, a low yield would be expected.

In cases of fungal keratitis, entry of the organism into the cornea is often facilitated by an epithelial defect. Here, trauma from the dog’s paw could have directly inoculated the corneal stroma, or a herpetic epithelial infection may have provided an entry point for the fungus to invade the cornea.

Voriconazole was initially selected for its broad antifungal activity. In an *in vitro* study of the susceptibility of 381 filamentous ascomycetes to antifungals, voriconazole was active against the majority of those tested (14). Notably, among all isolates, members of Microascaceae consistently required the highest MICs of all drugs. Microascaceae tested were more susceptible to voriconazole than to amphotericin B and itraconazole. This is consistent with our *in vitro* susceptibility analyses as well as clinical reports in which infections by these organisms showed significant resistance to treatment (15).

In summary, we report a case of fungal keratitis associated with a putatively novel species of *Lophotrichus*. A severe ulcer progressed rapidly to perforation on topical fortified antibiotics but healed with the addition of frequent topical voriconazole and amniotic membrane grafting. This is to our knowledge the first report of this species associated with human infection; this organism should be considered in the differential of fungal keratitis in a rural setting.

**Nucleotide sequence accession numbers.** ITS and BT2 sequence data were deposited in GenBank under accession no. KM580494 and KM609216, respectively.

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**REFERENCES**


