Lagenidium sp. ocular infection mimicking ocular pythiosis

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Keywords: Lagenidium sp., lagenidiosis, Pythium insidiosum, pythiosis, keratitis

Running Title: ocular lagenidiosis mimicking pythiosis

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This manuscript is an original article that has not been published and is not under consideration elsewhere. All authors participated in the preparation of the manuscript. The final manuscript has been seen and approved by all authors. All authors do not have conflict of interest to declare.
Abstract

This is the report of a Lagenidium sp in a Thai patient was diagnosed with severe keratitis unresponsive to antibacterial and antifungal drugs. Corneal biopsy confirmed the presence of aseptate hyphae. The ITS DNA sequence of this strain showed 97% identity with Lagenidium giganteum and other Lagenidium species.
Case Presentation

A 43-year-old Thai housewife was referred to King Chulalongkorn Memorial Hospital with lid swelling, pain, redness and itching of the left eye for 3 weeks. Her clinical symptoms began two days after she flushed her eyes with tap water due to eye irritation during house cleaning. She was treated with systemic and topical antibiotics, but her condition worsened. The initial diagnosis was presumed to be a fungal corneal ulcer unresponsive to both topical and systemic antifungal agents. Patient had been given topical cefazolin, topical amikacin, topical natamycin, topical voriconazole, oral acyclovir and oral itraconazole prior to transfer. Her past medical history was unremarkable.

Initial examination revealed visual acuity of her left eye was finger-counting at 1 foot. Slit-lamp examination showed ciliary injection and 5×5.6 mm yellowish, mid-stromal, reticular-pattern corneal infiltration with feathery edge (Fig. 1A). A 4×3-mm-in-diameter corneal epithelial defect over the infiltrated area was detected. Posterior eye segment evaluation by ocular ultrasound demonstrated no sign of endophthalmitis. The right eye appeared normal.

In vivo confocal microscopy identified numerous branching fungal-like elements with interlocking and hyper-refractive thin lines in the corneal stroma (Fig. 1B). Direct examination of a left corneal scraping specimen revealed aseptate hyphae (Fig. 1C). Corneal biopsy stained with hematoxylin and eosin (H&E) and Grocott's methenamine silver (GMS) staining showed longitudinal and transverse aseptate broad hyphae (Fig. 1D). Rapid growth of translucent submerged colonies was found in 2% dextrose Sabouraud agar at 37°C within 48 hours (Fig. 2A). The strain was deposited at the American Type Culture Collection (ATCC) accession number ATCC MYA-4932. The microscopic features of recovered strain after 48 hours of
incubation at 37°C showed the development of broad, sparsely septate hyaline, 9 to 15 µm in diameter branched hyphae (Fig. 2B). Fruiting bodies could not be found on any of the 2% dextrose Sabouraud agar plates. Zoospores were induced in Sabouraud dextrose agar pH 7.0 and corn meal agar (Difco) then transferred to a 2% water agar pH 6.9 with boiled grass blade (1) (Fig. 2C). Based on these results and the relative higher frequency of ocular pythiosis in Thailand, *P. insidiosum* keratitis was suspected.

She received oral terbinafine, itraconazole and topical natamycin as antifungal agents. Immunotherapy with an in-house *Pythium* antigen was also used. This vaccine was modified from the original vaccine formulation (patent number: US 5,948,413 and US 6,287,573 B1) (2,3). The treatment consisted of 3 subcutaneously injections of a 100 µL (2 mg/ml) dose of antigen applied at 7-day intervals.

Internal transcribed spacer (ITS) amplicons, obtained by PCR using genomic DNA of the cultured strain and the clinical samples and the Universal primers ITS-1 and ITS4 amplified smaller PCR products than those expected for the ITS sequences of *P. insidiosum* (4). The ITS amplicon was cloned into PCR 2.1-TOPO plasmid vector (Invitrogen, Carlsbad, CA. USA), purified and sequenced using BigDye Terminator chemistry in an ABI Prism 310 genetic analyzer (Perkin-Elmer, Foster City, CA. USA). The sequence was then analyzed using Basic Local Alignment Search Tool (BLAST) available at the National Center for Biotechnology Information (NCBI). The analysis placed the isolated strain closely to *Lagenidium giganteum* (97% identity) and to the other *Lagenidium* species available in the database and away from *P. insidiosum*. Based on this analysis, the strain was identified as *Lagenidium* sp. (accession number: JX646749)
Despite aggressive treatment with immunotherapy, topical and oral antifungal agents, the infection progressed rapidly. Additional intracameral amphotericin B given twice, 1 week apart, was implemented. However, the ulcer worsened, showing thinning and impending perforation of the cornea. Therapeutic corneal transplantation was then performed. Despite the new approach, the infection recurred, thus repeat penetrating keratoplasty (PK) was required. The infection was clinically controlled with no worsening visual acuity, and no corneal infiltration. After 10 months follow-up post-secondary surgery no recurrence of the infection was reported. A third surgical procedure to improve patient’s vision due to a scarred corneal graft was also scheduled.

The pathogenic oomycete *Pythium insidiosum* has been known as the only oomycete causing infection in mammals and birds (5-8). This notion was recently challenged by the finding of at least two different *Lagenidium* strains causing subcutaneous infections in cats and dogs (9-11). Grooters (11) introduced the term lagenidiosis to describe these infections along with a detailed description of their clinical, pathological and diagnostic features. However, a detailed description of the etiologic agents involved in this unusual infection has not been published. The lack of information on the taxonomy and phylogenetics of this novel group of pathogenic oomycetes was recently highlighted (13,14).

So far, the infections caused by *Lagenidium* species have been reported only in lower animals (9-11). However, at least one unpublished human case was recently mentioned (12). We have had the opportunity to evaluate a case of human keratitis in Thailand caused by a filamentous oomycete that we have identified by molecular tools as a *Lagenidium* sp.
Although the diagnosis of *Lagenidium* keratitis was confirmed, the appropriate treatment to manage the infection could not be found in the literature. Brown et al. (9) showed the in vitro susceptibility of animal pathogenic *Lagenidium* sp. to terbinafine, caspofungin and mafenoxam, but not to itraconazole, posaconazole and voriconazole. Also, it seems that a previous subcutaneous *Lagenidium* infection in a human patient responded only to posaconazole treatment (9). Other published data on the management of 6 *Lagenidium*-infected dogs could also provide additional information on the treatment of this infection in mammalian hosts; however, treatment in both studies did not show satisfactory outcomes (10,11).

Initially she was treated with oral terbinafine, oral itraconazole and topical natamycin as antifungal agents in combination with PIA (*Pythium insidiosum*-Vaccice), PK (penetrating keratoplasty), and secondary PK. Systemic and topical antifungal agents were tapered in 3 months postoperatively and the patient was discharged after 52-days. There has not been recurrence of the infection in more than 10-month follow up period. We could not conclude, however, whether adequate surgical removal of the affected tissue was the only factor in the successful response. In conclusion, we report the first case of ocular lagenidiosis in humans successfully treated with the same management protocols used in ocular pythiosis (4,5,15,16). The differentiation between *P. insidium* and *Lagenidium* spp. is required for an appropriate management, since the latter pathogen is more aggressive with poor prognosis (9-11).

**Conflict of Interest**

All authors do not have any financial interests or connections that might raise the question of bias in the manuscript or the conclusions, implications, or opinions.

**References**


Fig. 1. Panel A shows corneal infiltration with reticular pattern and feathery edge. Panel B demonstrates numerous fungal elements as branching with interlocking and hyper-refractive thin line in the corneal stroma via *in vivo* confocal microscopy. Panel C shows broad, rare-septate hyphae (magnification: 40x) in KOH preparation. Panel D shows longitudinal and transverse broad hyphae in GMS staining (magnification: 40x).
Panel A shows a 48 hours *Lagenidium* sp. culture on 2% sabouraud dextrose agar recovered from the case in this study. In this medium the strain developed submerged glabrous white/yellowish colonies in less than 24 hours. Panel B (Bar= 18 µm) depicts the presence of broad rarely septate hyaline hyphae. Note that the strain does not develop fruiting bodies in this medium. Panel C (Bar= 45 µm) shows the development of several sporangia with zoospores in liquid induction medium (arrows). Several swimming zoospores can be observed around the sporangia. Although the diameter of the *Lagenidium* hyphae from this case is broader, the hyphae shares several phenotypic characteristics in common with *Pythium insidiosum*.