Case report

Successful medical management of *Pythium insidiosum* keratitis using a combination of minocycline, linezolid, and chloramphenicol

Sayo Maeno*ab, Yoshinori Oiea,*, Atsuko Sunadaa, Honami Tanibuchia, Shigehiro Hagiwarad, Koichi Makimurae, Kohji Nishidaa

*Department of Ophthalmology, Osaka University Graduate School of Medicine, Osaka, Japan
**Department of Ophthalmology, Osaka General Hospital of West Japan Railway Company, Osaka, Japan
†Department of Medical Technology, Osaka University Hospital, Osaka, Japan
‡Department of Clinical Laboratory Science, Graduate School of Medical Technology, Teikyo University, Tokyo, Japan
§Institute of Medical Mycology, Graduate School of Medicine, Teikyo University, Tokyo, Japan

**ARTICLE INFO**

**Keywords:**
*Pythium insidiosum* keratitis
Minocycline
Linezolid
Chloramphenicol

**ABSTRACT**

**Purpose:** To report successful medical management of *Pythium insidiosum* keratitis using an antibiotic combination of minocycline, linezolid, and chloramphenicol.

**Observations:** A 20-year-old Japanese man was referred for visual disturbance, hyperemia, and discharge from his right eye. Slit-lamp examination revealed a paracentral corneal hyphate ulcer. His visual acuity was 20/28. Smear examination of corneal scrapings revealed a filamentous fungus. Pimaricin ointment four times a day and voriconazole eye drops hourly were initially prescribed. Although intravenous liposomal amphotericin B 100 mg was added, the corneal infiltrates and ulcer worsened. The possibility of *P. insidiosum* keratitis was considered, and in vitro antifungal susceptibility testing were performed based on the disc diffusion method. The inhibition zones around each antibiotic disc revealed that the pathogen was susceptible to minocycline, linezolid, and chloramphenicol. Therefore, minocycline ointment four times a day, chloramphenicol eye drops hourly, and linezolid 1200 mg orally per day were also administered. Eventually, sequencing of ribosomal DNA confirmed the pathogen to be *P. insidiosum*. The triple regimen dramatically improved the patient's keratitis. Therapeutic penetrating keratoplasty for corneal perforation was successfully performed, and his visual acuity recovered from 20/2000 to 20/25.

**Conclusions and importance:** We have encountered a case of *P. insidiosum* keratitis that responded to a combination of minocycline, linezolid, and chloramphenicol. This triple combination should be considered in patients with *P. insidiosum* keratitis.

1. Introduction

*Pythium insidiosum* belongs to the kingdom *Stramenopila*, a fungus-like aquatic oomycota often found in tropical, subtropical, and temperate climates1. *P. insidiosum* keratitis has been reported in several countries, including Thailand, India, China, Australia, Haiti, and Israel1. Although *P. insidiosum* keratitis is often misdiagnosed as fungal keratitis clinically from corneal scrapings, it is recalcitrant to antifungal therapy2.

2. Case report

A 20-year-old Japanese man who presented with pain, hyperemia, and discharge in the right eye was referred to our hospital after one week of administration of moxifloxacin and fluorometholone eye drops without remission. He belonged to the water polo club of his university, and played with daily disposable contact lenses. His corrected distance visual acuity (CDVA) was 20/28, and slit-lamp examination revealed a paracentral corneal hyphate ulcer and inflammatory infiltrates with feathered margins involving inflammation in the anterior chamber (Fig. 1A, B). The contralateral eye was essentially normal. A set of corneal scrapings for smear including Fungiflora Y and microbial culture was performed immediately. Branching filamentous hyphae were observed in Fungiflora Y staining but were poorly stained on gram staining. Culture of the corneal scrapings revealed growth of a filamentous organism after a few days (Fig. 2A, B). The triple regimen dramatically improved the patient's keratitis. Treatment for fungal keratitis was initiated, including voriconazole eye drops and natamycin ointment. However, the symptoms and ulceration worsened, so
intravenous liposomal amphotericin B was introduced. The infiltrates extended progressively and a further corneal scraping with culture yielded the same results. After 2 weeks of outpatient treatment, the patient was hospitalized (Fig. 1C).

After 2 further weeks of intensive inpatient treatment, the possibility of *P. insidiosum* keratitis was considered, therefore a request was made for identification of the pathogen by polymerase chain reaction (PCR). There were no guidelines available from the Clinical and Laboratory Standards Institute for interpretation of tests for *P. insidiosum*, thus the antibiotic susceptibility of the isolates was interpreted by determining the inhibition zone around each antibiotic disc (Fig. 2D, E). The strains were characterized as sensitive if there was an inhibition zone around the disc or resistant if there was no inhibition zone. Minocycline and linezolid showed the largest inhibition zones, and chloramphenicol, erythromycin, and azithromycin showed inhibition zones of intermediate size. Tobramycin and quinolones did not show inhibition zones. While the PCR investigation was being performed, oral linezolid 1200 mg twice daily, topical chloramphenicol...
eye drops hourly, and minocycline ointment 4 times daily were initiated, with progressive regression of the corneal infiltration (Fig. 1D). The pathogen was eventually confirmed to be *P. insidiosum* by ribosomal DNA sequencing.

The patient was discharged from hospital when his corneal infection was noted to be settling after the change in regimen. Three weeks after discharge from hospital, he presented with a corneal perforation in his right eye accompanied by a very shallow anterior chamber and a decrease in his CDVA from 20/200 to 20/2000 (Fig. 1E). It was apparent at this time that although the patient’s infectious keratitis had been successfully managed by medical treatment, the perforation had not resolved. Penetrating keratoplasty (PK) was then performed, and the CDVA recovered to 20/25. There has been no recurrence of infection in the 11 months since his surgery (Fig. 1F).

3. Discussion

We have encountered a patient who was initially diagnosed to have fungal keratitis and treated accordingly but was eventually found to have *P. insidiosum* keratitis that was managed successfully by minocycline, linezolid, and chloramphenicol. *P. insidiosum* keratitis is often misdiagnosed as fungal keratitis based on slit-lamp examination findings and corneal scrapings. Dense grayish-white infiltrates with feathery margins, associated with tentacle-like lesions on slit-lamp examination and filaments resembling fungi on histochemical staining and confocal microscopy, are reported to be characteristics of *P. insidiosum* keratitis. When such observations are made in a patient who does not respond to treatment with antifungal agents, *P. insidiosum* should be considered as a causative pathogen. *P. insidiosum* does not contain ergosterol in the cell wall, and is recalcitrant to conventional antifungal therapy. Furthermore, accurate microbiological identification is based on formation of zoospores; in the absence of sporulation, DNA sequencing is needed for identification.

A large number of fungal species are known to cause keratitis. However, 10%–23% of fungal isolates from patients with fungal keratitis is reported as unidentified from lack of sporulation in culture. The prevalence of *P. insidiosum* among unidentified fungal isolates from keratitis is reported to be 5.5% in DNA sequencing of morphologically unidentified nonconsecutive fungal isolates from corneal scraping with keratitis, and 3.9% in zoospore formation of fungal colonies resembling those of *P. insidiosum* followed by DNA sequencing. Therefore, incidence of *P. insidiosum* keratitis is thought to be 1% or lower among fungal keratitis.

Currently, there is no standard protocol for *P. insidiosum* keratitis, and recent in vitro and in vivo susceptibility literature supports the use of antibacterial medications for this infection. Successful resolution of human *P. insidiosum* keratitis using a combination of linezolid, azithromycin, and atropine sulfate has been reported previously. Clinical comparisons of antifungal and antibacterial therapy have been reported, with a combination of topical linezolid and topical and oral azithromycin being found to be superior to topical natamycin, which might indicate the response of *P. insidiosum* to antibacterial agents. In vitro susceptibility of *P. insidiosum* to antibacterials including minocycline, tetracyclines, macrolides, and linezolid, as monotherapy and in combination with antifungal agents has been reported. Moreover, there is in vitro and in vivo evidence of a synergistic antibacterial effect between minocycline and other antibacterial agents when used to treat *P. insidiosum*. However, none of the previous studies have described the efficacy of minocycline and chloramphenicol in the management of human subjects. Administration of minocycline together with linezolid and chloramphenicol was effective for *P. insidiosum* keratitis in our case. Therefore, triple therapy consisting of minocycline, linezolid, and chloramphenicol, which act by inhibiting protein synthesis, may be a promising candidate treatment for *P. insidiosum*.

4. Conclusions

We have reported a case of *P. insidiosum* keratitis that responded to a triple regimen of minocycline, linezolid, and chloramphenicol. Treating clinicians should be aware of *P. insidiosum* as a potential causative agent when a patient presents with fungal keratitis refractory to antifungal therapies.

**Patient consent**

The patient who is the subject of this case report provided informed consent for his case to be published.

**Funding**

No funding or grant support

**Conflicts of interest**

None of the authors has any financial disclosures to make.

**Authorship**

All authors attest that they meet the current ICMJE criteria for authorship.

**Acknowledgments**

None.

**References**

1. Gaastra W, Limpman LJ, De Cock AW, et al. Pythium insidiosum: an overview. Vet Microbiol. 2010;146(1-2):1–16.
2. He H, Liu H, Chen X, Wu J, He M, Zhong X. Diagnosis and treatment of Pythium insidiosum corneal ulcer in a Chinese child: a case report and literature review. *Am J Case Rep.* 2016;17:982–988.
3. Ramappa M, Nagpal R, Sharma S, Chaurasia S. Successful medical management of presumptive Pythium insidiosum keratitis. *Cornea.* 2017;36(4):511–514.
4. Sharma S, Balne PK, Montokally SR, et al. Pythium insidiosum keratitis: clinical profile and role of DNA sequencing and zoospore formation in diagnosis. *Cornea.* 2015;34(4):438–442.
5. Gopinathan U, Sharma S, Garg P, Rao GN. Review of epidemiological features, microbiological diagnosis and treatment outcome of microbial keratitis: experience of over a decade. *Indian J Ophthalmol.* 2009;57:273–279.
6. Bagga B, Sharma S, Madhuri Guda SJ, et al. Leop forward in the treatment of Pythium insidiosum keratitis. *Br J Ophthalmol.* 2018;102(12):bjophthalmol-2017-311360.
7. Jesus FP, Ferreiro L, Loreto ES, et al. In vitro synergism observed with azithromycin, clarithromycin, minocycline, or tigecycline in association with antifungal agents against Pythium insidiosum. *Antimicrob Agents Chemother.* 2014;58(9):5621–5625.
8. Loreto ES, Tondolo JS, Piloto MB, Alves SH, Santorio JM. New insights into the in vitro susceptibility of Pythium insidiosum. *Antimicrob Agents Chemother.* 2014;58(12):7534–7537.
9. Loreto ES, Mario DAN, Denardi LB, et al. In vitro susceptibility of Pythium insidiosum to macrolides and tetracycline antibiotics. *Antimicrob Agents Chemother.* 2011;55:3588–3590.