



## Pythium Keratitis- A Mini-Overview

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### Abstract

Pythium is an *oomycete* of Phylum Straminopila (green algae), Class Oomycetes, Order Pythiales and Family Pythiaceae that causes a devastating infection of the cornea but was first associated with various systemic infections. It lacks ergosterol in the cytoplasmic membrane and doesn't have chitin in the cell wall like fungus; instead contains beta glucans and cellulose. This article we are reviewing about brief taxonomy with various investigation and management of pythium keratitis till date. Risk factors include dust, foreign body, vegetative matter, dirty water, insect injury, idiopathic, improper previous treatment with prolonged non-compliance. Common signs observed are tentacle like infiltrate, hypopyon, endothelial exudates, dot like infiltrate, superficial plaque, total corneal ulcer, peripheral furrowing and perforated corneal ulcer. Microbiological examination helps to establish a definitive diagnosis with corneal scrapping subjected to 10% KOH wet mount, Gram stain, Potassium iodide-sulfuric acid showing thin septate hyphae and culture with 5% sheep blood agar at 37 degree shows flat, feathery edged, partially submerged, colourless colonies. Polymerase chain reaction (PCR) with DNA sequencing is very useful diagnostic method showing vesicles with zoospores which develop after incubation just before zoospore release. Confocal microscopy shows presence of hyper-reflective, double-walled filaments varying in size. Histopathological examination is with GMS, PAS and H & E stains. Various new methods have come for diagnosis like proteomic analysis, molecular analysis, Loop mediated isothermal amplification (LAMP). Treatment comprises of medical and surgical intervention. In medical intervention started with topical natamycin, combination of natamycin and voriconazole eyedrops, itraconazole 1% eyedrops, topical linezolid (0.2%) hourly and 1% azithromycin eye ointment twice daily with oral azithromycin 500mg once daily for 2 weeks and minocycline eye ointment 4 times per day, oral linezolid 1200mg twice daily and topical chloramphenicol eyedrops hourly combination. Triple drug regimen with topical (0.2%) and oral linezolid and topical 1% azithromycin eye ointment has showed good outcome. In surgical intervention we have seen either Therapeutic keratoplasty (TPK) either alone or with Nitrous oxide Cryotherapy with alcohol swab. If infection is too aggressive it may require evisceration or enucleation. There were reported cases with immunotherapy with PIA vaccine and topical ethanol showing some improvement but require larger study to comment on its efficacy.

**Keywords:** Pythium; Keratitis; Culture; Insidiosum

**Abbreviations:** PCR: Polymerase Chain Reaction; LAMP: Loop Medicated Isothermal Amplification; IVCN: In

Vivo Confocal Microscopy; TPK: Therapeutic Penetrating Keratoplasty; SA: Surgical Adjunct; ITS: Internal Transcribed Spacer.

## Introduction

Pythium insidiosum is an oomycete with broad branching and ribbon-like septate or aseptate filaments resembling zygomycetes fungus like features. Pythium species is classified in the Phylum Straminopila (green algae), Class Oomycetes, Order Pythiales and Family Pythiaceae [1,2]. This organism was first found in 1884 causing pythiosis, which affects various sites leading to different forms of the disease i.e a cutaneous, vascular, ocular, gastrointestinal and a systemic form, which is rarely seen. P. insidiosum lacks ergosterol in the cytoplasmic membrane and doesn't have chitin in the cell wall like fungus; instead contains beta glucans and cellulose. Asexual reproduction in the oomycetes occurs in the form of sporangia containing biflagellate zoospores [3] and direct exposure of the cornea to the zoospores of P.insidiosum, can result in pythium keratitis [4]. Systemic infections in humans with Pythium are also well documented with pythiosis being a life threatening disease having increased morbidity and mortality [5]. Pythiosis insidiosii of the subcutaneous tissues and intestine is characterized by granulomatous lesions or ulcers which are fast growing [6,7]. History of exposure to water and other aquatic environments such as agricultural fields and also soil is very imperative to raise the suspicion of Pythium keratitis [8,2]. An outbreak of Pythium keratitis was reported from Thailand in 2009 however, cases have also been reported from other parts of the world [9-16]. Nearly 80% of the patients lose their vision [17-19]. Pythium keratitis is an emerging dreaded infection of the cornea due to its poor response to traditional antifungal medication, protracted course and ocular morbidity [16]. Initially thought as fungal keratitis but many studies have reported 10% to 23% of fungal isolates from patients with fungal keratitis as unidentified because of lack of sporulation in culture [20]. The major challenge of Pythium keratitis are diagnostic and treatment modalities. In this review we have compiled recent

information on the diagnosis and treatment of this disease.

## History and Taxonomy

Chandler et al in 1980 named this disease seen in plants and animals as "pythiosis", which was previously known as phycomycosis; although no species was identified [21]. In 1987, the organism causing the disease in humans was identified as Pythium insidiosum [22]. The organism is classified in kingdom protectistia, Phylum-oomycota, order- Peronosporales, Family- Pythiaceae, Genus-Pythium Pringsheim.

## Survey Methodology

Literature search was done using pubmed database with keywords, 'pythiosis', pythium insidiosum, Keratitis. Articles were extracted and summarized to identify the clinical information.

## Epidemiology, Pathogenesis and Risk Factors

The life cycle of Pythium insidiosum is seen in mammals (horses, dogs and humans) [23]. Infection occurs with entry of zoospores through cutaneous wound causing chemotaxis and encystment followed by formation of germ tube (hyphae) and blood vessel invasion [2]. Patients become infected after contact between a skin injury and zoospore-contaminated water [24]. Risk factors include dust, foreign body, vegetative matter, dirty water, insect injury, idiopathic, improper previous treatment with prolonged non-compliance [8]. Dirty water can be wastewater; rice field's wastewater, groundwater, rainwater, tap water, or river water, and many of patients in this series were engaged in farming occupations [25]. Infection occurs in tropical and subtropical regions (Table 1).

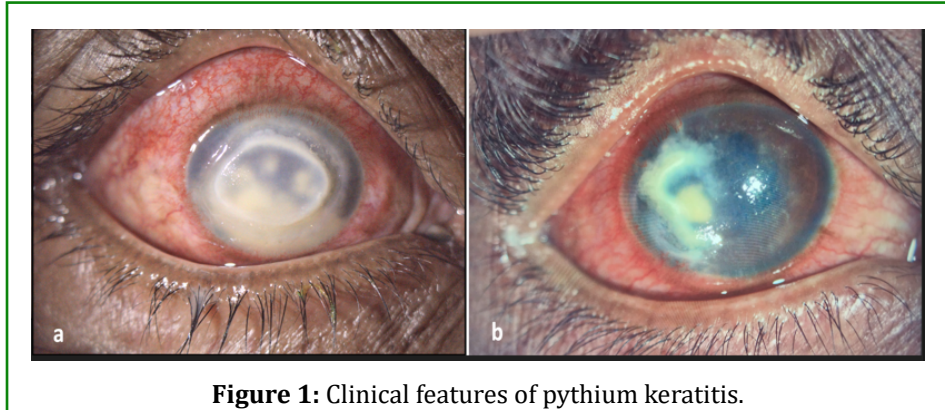
Host factors	Environmental Factors
Normal hosts	Contact lens
Immunocompromised individual (diabetes, Crohn's disease, treatment with immunotherapy)	Agricultural work
	Direct ocular contact with aquatic or nonaquatic region
	Rainy season
Clinical Features	Management
Linear, tentacle-like infiltrate or dot-like infiltrates in cornea	Medical -oral doxycycline or azithromycin with topical linezolid
Grayish white stromal infiltrate with feathery margins.	Triple therapy-topical linezolid (0.2%), oral linezolid linezolid(1200mg) and azithromycin1% eye ointment
Stromal infiltrate of reticular pattern	Surgical - early therapeutic penetrating keratoplasty
Radial keratoneuritis	Adjunct therapy-perioperative cryotherapy.
Rapidly progressive infiltrate	

**Table 1:** Essential Information for Diagnosis and Management.

## Clinical Features

Patients generally present with symptoms like pain, redness, loss of vision, conjunctival discharge and headache. On examination, common signs observed are tentacle like infiltrate (nearly 50%), hypopyon (40%), endothelial

exudates (30%), dot like infiltrate (15-65%), superficial plaque (15%), total corneal ulcer (10%), peripheral furrowing (10%) and perforated corneal ulcer (5%) [8,22] (Figure 1).



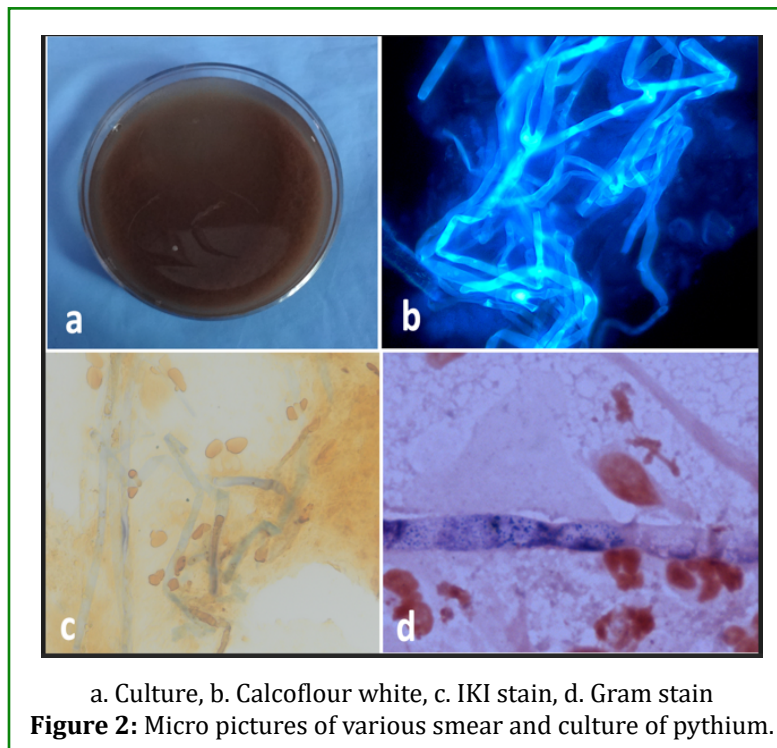
**Figure 1:** Clinical features of pythium keratitis.

## Diagnosis

History taking and slit-lamp examination are first line for diagnosis. A high clinical suspicion with protracted treatment to which patient doesn't respond properly may give a hint of pythium keratitis diagnosis [29].

Microbiological examination helps to establish a definitive diagnosis. Corneal scrapping is subjected to 10% KOH wet mount, fungal culture, PCR and DNA sequencing. 10% KOH shows long sparsely septate hyaline hyphae of *Pythium*

*insidiosum* with presence of numerous vesicles within the hyphae with ribbon like folding. Gram stain image shows the thick cell wall, a few septate and mass of vesicles inside. Potassium iodide-sulfuric acid (KI-H<sub>2</sub>SO<sub>4</sub>) staining of the slides of the specimens shows 100 % sensitivity and specificity [30]. Culture of *P. insidiosum* at 37°C grown on 5% sheep blood agar with flat, feathery edged, partially submerged, colourless or light brown colonies with filiform margins on blood agar were grown from corneal scrapings or buttons [8] (Figure 2).



a. Culture, b. Calcofluor white, c. IKI stain, d. Gram stain  
**Figure 2:** Micro pictures of various smear and culture of pythium.

## Laboratory Diagnosis

Identification with zoospore induction requires expertise and is a time-consuming process. *P. insidiosum* cultures with zoospore induction by water culture containing grass leaves and nested PCR were used to provide definite diagnoses of *P. insidiosum* infection. So Polymerase chain reaction (PCR) with DNA sequencing is very useful diagnostic method showing vesicles with zoospores which develops after incubation just before zoospore release [14,25-29,31]. In vivo confocal microscopy (IVCM) is noninvasive and provides real-time measurement of the entire corneal thickness [32]. Study shows repeated scanning of the centre and edges of the corneal infiltration yields approximately 350 images per scan showing pathogenic hyphae with the presence of hyperreflective, double-walled filaments varying in size [25]. Further molecular -based diagnosis of *Pythium* is employed for species identification and study phylogenetic relationship. Proteomic analysis (MALDI-TOF MS) is a powerful tool for identification of *p. insidiosum* [33].

Histopathological examination is with GMS, PAS and H & E stains [27,34] Novel recombinant election (EL1025) is a newly developed immunohistological assay target elictions present only in oomycete reports to be highly specific. Molecular analysis- (Genotyping&phylogenetic analysis study of corneal ulcer). Based on COX II gene, *P. insidiosum* is categorised into three clades, CLADE I (American stains), CLADE II (Asian, Australian) & CLADE III (Thailand strain). Molecular tools based on PCR and gene blast technique are main methods for identification of the pathogen. ITS1, ITS2 and 5.8 S r RNA gene are main target gene amplified and sequenced by PCR. Exo -1, 3, beta- glucanase was promoted as a novel target for *P. insidiosum* detection [35].

Loop mediated isothermal amplification (LAMP) for identification of *P. insidiosum*- it uses a set of four primers which anneal to six distinct location on the target rDNA sequence and the SYBR green illumination, the detection of colour change (from orange to green) will interpret a LAMP result [36]. LAMP result though not more specific than multiplex PCR but has shorter identification assay compared to M PCR.

## Treatment

There are various treatment options for *Pythium* keratitis. Weak or lack of in vitro activity of several individual antifungal agents (terbinafine, caspofungin, miconazole, ketoconazole, fluconazole) against *P. insidiosum* has been shown in the past [32]. Inhibitory action of macrolides and tetracycline antibiotics against *P. insidiosum* is also noted [31,37-40]. Treatment can be medical and surgical intervention. Since aggressive medical intervention often fails to cure the infection, prompt surgical intervention is key

to control the infection.

According to Hasika et al, medical management included topical natamycin , combination of natamycin and voriconazole eyedrops, itraconazole 1% eyedrops; out of which few patients responded to itraconazole therapy with complete resolution of ulcer [7]. Other successful medical management includes antibiotic combination of topical linezolid(0.2%) hourly and 1% azithromycin eye ointment twice daily with oral azithromycin 500mg once daily for 2 weeks and minocycline eye ointment 4 times per day, oral linezolid 1200mg twice daily and topical chloramphenicol eyedrops hourly combination [24,27,42,43]. Triple drug regimen with topical (0.2%) and oral linezolid and topical 1% azithromycin eye ointment has showed good outcome [20]. Oral linezolid was chosen based on the result of in vitro susceptibility of the *Pythium* culture isolates with largest zone of inhibition, low minimum inhibitory concentration value and high concentrations achieved in aqueous and vitreous [44]. Cryotherapy i.e. Nitrous oxide cryotherapy (boiling point -88.5°C) using a retinal cryoprobe with a tip diameter of 2 mm was used for 7-8 s to create an iceball followed by a slow thaw. In eyes with contiguous sclera infiltration, in addition to cryotherapy, the surface was swabbed with 99.9% absolute alcohol over and beyond the area of cryotherapy application extending posteriorly until the edge of the infiltrated sclera [44-45]. TPK (therapeutic keratoplasty) was performed in patients who either had a perforated corneal ulcer at presentation itself or with nonresponsive to treatment [5,46]. Recurrence was noted more in patients who underwent TPK alone compared with TPK with cryotherapy and alcohol swab. In patients not responding to any treatment with further aggressiveness of disease can go for evisceration or enucleation.

Immunotherapy- PIA vaccine shifts immune response from T-helper 2 to T- helper 1 for cytotoxic killing of hyphae [46] can be used as a compassionate therapy [5,9] prevents post keratoplasty recurrence in corneal grafts. Study from Thailand reported 43-47% patient needed evisceration/enucleation to be cured of disease even after 3doses of PIA. One milliliter of a 2 mg/mL PIA (a crude antigen) solution, prepared according to the protocol by Mendoza L, et al. [46,47] was injected subcutaneously in laboratory-proven cases, six booster doses can be given at 0.5, 1, 1.5, 3, 6, and 12 months. According to Vilavun et al 17 cases were treated out of which PIAI was continued for a year as an adjunct therapy in few cases but result was not so inconclusive to give a comment [48].

Role of topical ethanol- Shweta et al studied the efficacy and safety profile of topical ethanol of concentration 20%, 40%, 60%, 80%, 99.9% in *pythium insidiosum* and found inhibition of organism in 20% and no growth in rest

concentration in microbiological tests applied in 2 cases one case with recurrent pythium keratitis even after keratoplasty,

one large infiltrate before keratoplasty, both cases showed no further growth of organism [49] (Table 2).

Author, Journal, Type Of Study	Duration of Study	Sample Size	Clinical Features	Diagnosis	Treatment Given	Result
Bagga B, et al, BJO 2018 (Retrospective study) [27]	2014-2016	116	Dot like infiltrates and Tentacle and reticulate pattern	Through gram stain, KOH and culture sensitivity	In 2014-15 topical 5% natamycin given hourly +- topical voriconazole drops, in 2016- topical antibiotics like 0.2% linezolid drops hourly and azithromycin eye ointment twice a day and oral azithromycin 500mg once a day for 2 weeks	Rate of TPK was higher in patients on antifungal therapy compared to antibacterial therapy
Agarwal S, et al, BrJO 2018 (retrospective study) [44]	2014-2017	46	Infiltrate with superficial radiating reticular pattern with or without hypopyon.	PCR DNA sequencing along with stain and culture	Medical management (MM) (topical azithromycin and linezolid with oral azithromycin at presentation), surgery (S) (therapeutic penetrating keratoplasty, TPK), surgical adjunct (SA) (cryotherapy ± alcohol with TPK) and medical adjunct (MA) (MM after TPK).	In this study, MM/MA measures showed no benefit with recurrence or worsening of infection requiring resurgery, adjunctive procedures during TPK appear to have additional benefit with low risk of recurrence

<p>Hasika R, et al, IJO 2019( Retrospective study) [8]</p>	<p>Jan 2016- Nov 2017</p>	<p>71</p>	<p>Tentacle like infiltrate, Dot like infiltrate, Peripheral furrowing, Perforated corneal ulcer, Total corneal ulcer.</p>	<p>Grams stain,10% KOH, culture on blood agar and potato dextrose agar. It was further confirmed both by PCRbased DNA sequencing targeting internal transcribed spacer (ITS) region and with zoospores formation identifying the organism as <i>P. insidiosum</i> in all the isolates.</p>	<p>A combination of 5% natamycin and 1% voriconazole was given to 42% patients, and natamycin alone was given to 39.4% patients. 1% itraconazole eye drops alone was initiated in 7 (10%) patients .Therapeutic keratoplasty (TPK) was performed in 48 (67.6%) patients</p>	<p>None of the primary grafts remained clear after a period of 1 month. Twentysix eyes (54.2%) had graft reinfection and all these eyes either developed anterior staphyloma (4) or were eviscerated (3) and 13 eyes became phthisical. The remaining 22 patients who had TPK resulted in failed graft.</p>
<p>Sane SS, et al, Cornea 2020 (Retrospective study) [26]</p>	<p>Jan 2016- Aug 2019</p>	<p>21</p>	<p>Central and paracentral infiltrate with tentacle like infiltrate, dot like infiltrates with or without hypopyon</p>	<p>Gram stain, KOH-CFW stain, iodine potassium iodide-sulfuric acid (IKI-H<sub>2</sub>SO<sub>4</sub>) stain, culture, histopathological examination in 1 patient</p>	<p>Patients were prescribed 0.2% linezolid eye drops, 1% azithromycin eye drops, and linezolid 600 mg twice a day. Topical medications were used every hour for the first 2 weeks and then reduced to 2 hours based on the response. Oral linezolid was prescribed for 1 month. Four cases (4/19) underwent therapeutic keratoplasty,</p>	<p>On triple drug regimen, 14/17 cases (82.35%) resolved.</p>

Puangsricharern V, et al, Clinical Ophthalmology 2021 (retrospective study) [48]	2006-2019.	25 (26 eyes)	multiple tentacle-like infiltrations, dot-like lesions, and reticular infiltrations.	Diagnosis of Pythium keratitis was confirmed by either a positive microbial culture or a positive polymerase chain reaction (PCR) from a corneal scraping or corneal button part after biopsy or TPK.	Topical natamycin or amphotericin B given initially to all suspected patients, subsequently one milliliter of a 2 mg/mL PIA (a crude antigen) solution, prepared was injected subcutaneously in laboratory-proven cases. Subsequently, six booster doses were scheduled at 0.5, 1, 1.5, 3, 6, and 12 months. For some cases, treatment with PIAI was continued for a year as an adjunct therapy.	The infection progressed in 24 eyes (92.3%) despite drug treatment. Twenty-one eyes underwent TPK, and three eyes had to be eviscerated because of uncontrollable disease.
Vishwakarma P, et al. IJO 2021 (Retrospective study) [45]	Jan 2016 and Dec 2018	18	Stromal infiltrates with feathery margins (n = 5, 27.78%), irregular ulcer margins (n = 1, 5.56%), peripheral corneal thinning [Fig. 1b] (27.78%), corneal melt (n = 2, 11.11%), corneal perforation descemetocele and ring infiltrate, Reticular dotlike infiltrates and tentaclelike extensions, Hypopyon	Direct microscopy using gram stain and CFW + KOH, IKI H <sub>2</sub> SO <sub>4</sub> staining was performed whenever there was a clinical or microbiological suspicion for Pythium, Culture and zoospore induction, Antimicrobial susceptibility against specific panels of drugs using KirbyBauer disc diffusion method and histopathological examination of corneal button	Medical management included topical/oral linezolid and azithromycin. Therapeutic penetrating keratoplasty (TPK) was done in 15 eyes (83.3%), repeat TPK in 4 eyes, and evisceration in 3 eyes (16.7%). One patient required only medical treatment.	Globe salvation was obtained in 15 (83.3%) eyes, and good visual outcome in 7 eyes (38.9%). There was graft failure in six eyes (40%) and two (11.1%) eyes went into phthisis.

**Table 2:** Various treatment and result of pythium keratitis.

## Follow-Up

Extensive infection leading to cavernous sinus thrombophlebitis has been reported in 1 case which led to a cerebrovascular attack and death of that patient [50].

## Conclusion

Pythium Keratitis seems to be increasing in recent times. They were underdiagnosed or misdiagnosed and there was

lack of proper knowledge and investigation to diagnose pythium keratitis due to which maximum cases went for TPK or in worse case, evisceration and enucleation. Old concept of giving anti-fungal agents is not effective against Pythium infections. However, with the recent advance and study suggesting better treatment with antibiotics and TPK combined with cryotherapy and alcohol swab can lead to more cases achieving better visual outcome. Still further extended long term studies may show more options or more details regarding treatment of this virulent organism. Response to therapy is extremely slow and prolonged. The role of early therapeutic PK with clear surgical margins is gold standard for a better visual outcome and globe salvage. Adjunct strategies include perioperative cryotherapy and absolute alcohol aid in preventing recurrence after surgery.

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