Pythium keratitis: Clinical profile, laboratory diagnosis, treatment, and histopathology features post-treatment at a tertiary eye care center in Eastern India

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Purpose: The aim of this work was to study demography, clinical profile, laboratory diagnosis, and management of *Pythium* keratitis at a tertiary eye care center in Eastern India. **Methods:** Eighteen patients with culture-positive *Pythium* keratitis managed at our center between January 2016 and December 2018 were included in this retrospective study. Clinical features, laboratory investigations, treatment, and outcomes were analysed. **Results:** *Pythium* keratitis commonly affects middle-aged males with low socioeconomic profile and history of trauma. Samples stained with Gomori methenamine silver showed 93.8% positivity and Iodine-potassium iodide-sulfuric acid showed 100% positivity. Periodic acid-Schiff's showed negative staining in 62.5% and weak in 37.5%. Kirby-Bauer disc diffusion method showed zone of inhibition as 30.25 ± 4.61 mm for Linezolid and 23.56 ± 6.86 mm for Azithromycin. 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. Patients were divided into early and late presenters. Late presenters had more complications and worse final visual outcome. **Conclusion:** *Pythium* keratitis can be differentiated from fungal keratitis by its characteristic appearance on slit-lamp examination, smear, culture, and histopathology. Early presentation, detection, and treatment with antibacterial drugs like linezolid and azithromycin results in a better prognosis. Early full-thickness corneal transplant should be considered for *Pythium* keratitis not responding to treatment.

Key words: Fungus, keratitis, Pythium

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Pythiosis, caused primarily by *Pythium insidiosum*, is an emerging and life-threatening infectious disease in humans and animals.[1] Apart from *P. insidiosum*, other oomycetes like *Lagenidium* spp. and *P. aphanidermatum* have also been rarely recognized as human pathogens.[2-4] It can manifest as cutaneous/subcutaneous, ocular, vascular or as disseminated forms of infection.[5] Unlike fungus, its cell wall contains cellulose and β-glucans but lacks chitin. Absence of ergosterol in the cytoplasmic membrane renders most of the antifungal medications ineffective, which acts primarily by inhibiting ergosterol synthesis.[6] The clinical and microbiological resemblance with fungus, absence of a standardized treatment protocol, relatively uncommon incidence, and aggressive nature of the disease are the challenges in the management of ocular pythiosis. In India, few studies, mostly from Southern India, have been published on *P. insidiosum* keratitis.[7-15] We aim to review the predisposing factors, clinical profile, microbiology, histopathology, treatment, and outcomes of the first reported *Pythium* keratitis cases series from Eastern India.

Methods

We retrospectively reviewed the clinical and laboratory charts of all patients who were culture positive for *Pythium* in corneal scraping or buttons, at our tertiary eye care center. The study period was of 36 months, between January 2016 and December2018; adhering to the tenets of the Declaration of Helsinki. Appropriate approval was obtained from the Institutional Review Board of our institute.

Corneal scrapings were done with a Bard-Parker knife 15 No. blade under topical anesthesia and aseptic conditions. For patients presenting with large corneal perforation or extensive infection, samples were collected from the half corneal button, AC (anterior chamber) following TPK (therapeutic penetrating keratoplasty), or eviscerated eye sample.

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Smears were evaluated by direct microscopy using gram stain and calcofluor white with 10% potassium hydroxide (CFW + KOH). Iodine-potassium iodide-sulfuric acid (IKI-H_2SO_4) staining was performed whenever there was a clinical or microbiological suspicion for *Pythium*, as per published protocols.[16] Any significant growth of pathogens on various inoculated solid (chocolate agar/blood agar and Sabouraud dextrose agar/potato dextrose agar) and liquid (Brain heart infusion broth, Robertson's cooked meat media and thioglycolate broth) media were examined regularly for 2 weeks. Significant growth of *Pythium* on any media was further confirmed by the induction of zoospores as per published protocols.[7] Antimicrobial susceptibility against specific panels of drugs (linezolid, minocycline, doxycycline hydrochloride, tigecycline, clarithromycin, azithromycin, tetracycline, mupirocin, chloramphenicol) for *Pythium* was also done using Kirby-Bauer disc diffusion method.[10]

Histopathology evaluation with special stains was performed for cases that underwent TPK or eviscerated eye samples using; GMS (Gomori methenamine silver), PAS (periodic acid-Schiff's) and IKI-H_2SO_4 (potassium iodide sulfuric acid) stains.

Results

Among 1251 patients who were diagnosed to have microbial keratitis in our institute during the study period, corneal scrape or corneal button samples of 18 patients (1.4%) were found to be culture positive for *Pythium*.

Demography and history

The mean age of presentation was 45.50 ± 15.35 years, with male preponderance (83.3%). Most of the patients belonged to low socioeconomic status which was defined by those patients who could not afford to pay for the services at our institute (88. 8%). All patients had unilateral involvement [Table 1].

Predisposing risk factors such as fall of foreign body, ocular trauma, vegetative injury was identified in 66.67% of the patients of which injury with agricultural material, dust, and soil were predominant (9/18). The duration between the onset of symptoms to presentation in our hospital ranged between 5 and 90 days (24.72 ± 20.77 days).

At presentation 12/18 patients were on topical antifungals of which eight were in combination with antibiotics. Four patients were using steroids.

Clinical features

Visual acuity worse than 20/200 was seen in 17/18 patients. On slit-lamp examination, 10 (55.56%) patients had corneal epithelial defect ≤ 64 mm^2. Six patients with epithelial defect >64 mm^2 had either near-total (16.7%) or total corneal epithelial defect with infiltrates (16. 7%). In two patients (11.11%) size of the epithelial defect could not be ascertained [Table 2].

Among patients with corneal infiltrate of ≤8 mm x 8 mm on initial presentation workable visual acuity of ≥20/200 was obtained in 5/10 patients (50%) of which vision of pt. no. 13 decreased later due to post TPK glaucoma. In this group, eye of 1 patient went into phthisis while 1 required evisceration. Among patients with corneal infiltrate of >8 x 8 mm or with perforated corneal ulcer with size of infiltrate >8 mm x 8 mm, 2/8 (25%) underwent evisceration, 1/8 (12.5%) went into phthisis while 5/8 (62.5%) had visual acuity ≤20/200.

Apart from findings noted by the physicians in the first visit, we also did a retrospective photographic review of the patients included in the study. In six patients (33.33%) with total corneal ulcer [Fig. 1a], no characteristic features could be determined. Of the rest, features such as 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 [Fig. 1c] (n = 4, 22.22%), descemetocele (n = 2, 11.11%) and ring infiltrate [Fig. 1d] (n = 5, 27.78%) could be identified. Reticular dot-like infiltrates and tentacle-like extensions [Fig. 1b and d] were seen in 8 (44.44%) patients each. Hypopyon [Fig. 1b and d] was ≥2 mm was present in 8 patients (44.44%) while <2 mm was present in 3 patients (16.67%). Anterior chamber contents could not be visualized due to ≥ near-total corneal infiltrate [Fig. 1a] in 3 patients (16.67%).

At presentation, clinical diagnosis of fungal keratitis was made in 13/18 patients (72.22%). Four (pt. no. 4,6,7,14) (22.22%) were suspected to have *Pythium* and one as *Acanthamoeba* keratitis (pt. no. 3) (5.56%).

Microbiology features

Based on initial smear examination, 3 patients (pt. no. 12,14,17) with typical findings i.e., broad, sparsely septate or aseptate, ribbon-like hyaline filaments were suspected to have *Pythium* [Fig. 1e]. Of these 2 patients (pt. no. 14,17) were confirmed for *Pythium* on IKI-H_2SO_4 staining. Fungus was suspected in 7 patients. In 5 patients (pt. no. 4,7,8,9,18) smear findings were inconclusive and a dilemma between *Pythium* or fungus could not be ascertained. On IKI-H_2SO_4 staining patient no. 18 was confirmed to be *Pythium*. Three samples didn't reveal any organism.

Pythium colonies on culture plates like blood agar or chocolate agar showed typical characteristic features like flat, carpet-like, feathery and, colorless colonies which could not be scraped off easily. Eleven patients grew *Pythium* on culture from corneal scrape samples and the mean time of growth being 4.72 ± 2.19 days.

Following TPK, half corneal button culture showed *Pythium* growth in 10 patients out of 15 (66.67%), while eviscerated corneal button sample of 1/2 patients also showed growth of *Pythium*. The mean duration of growth from post TPK samples was 3.82 ± 3.60 days. Of these, 6 patients (pt. no. 6,7,13,15,16,17) weren't identified as *Pythium* keratitis from the initial corneal scraping results. Patients whose corneal button samples did not show growth of *Pythium* grew the same in the initial corneal scrape samples except for one patient (pt no. 4) whose AC exudate sample post initial TPK showed growth of *Pythium* on culture.

IKI staining results on scrapes suspicious for Pythium

Corneal scrape samples of three patients (pt. no. 14,17,18) were re-evaluated with IKI-H_2SO_4 stain and were found to be positive.

Antimicrobial sensitivity

Mean zone of inhibition for different antibiotics were; 30.25 ± 4.61, 29.50 ± 8.37, 28.88 ± 6.51, 26.63 ± 8.94, 25.13 ± 8.64, 23.56 ± 6.86, 23.38 ± 5.55, 22.13 ± 8.31 and 20 ± 8.52 mm for Linezolid, Doxycycline, Minocycline, Tigecycline,

Table 1: Demography, prior treatment, clinical features, microbiology, outcome

Variable	Number of cases *n* (%)	Variable	Number of cases *n* (%)
Gender		**Clinical features**	*n* >18
M	*n*=15 (83.33%)	Feathery margins	*n*=5 (27.78%)
F	*n*=3 (16.67%)	Irregular/blurred ulcer margins	*n*=1 (5.56%)
Age		Peripheral corneal thinning	*n*=5 (27.78%)
1-20	*n*=0 (0%)	Reticular dot like infiltrates	*n*=8 (44.44%)
21-40	*n*=8 (44.44%)	Tentacle like extensions	*n*=8 (44.44%)
41-60	*n*=7 (38.89%)	Ring infiltrate	*n*=5 (27.78%)
>60	*n*=3 (16.67%)	Corneal perforation	*n*=4 (22.22%)
Occupation		Corneal melt	*n*=2 (11.11%)
Lower socioeconomics	*n*=16	Inconclusive	
Farmer	*n*=7 (38.89%)		*n*=6 (33.33%)
Laborer	*n*=3 (16.67%)	Descemetocele	*n*=2 (11.11%)
Fisherman	*n*=1 (5.56%)	Hypopyon<2 mm	*n*=3 (16.67%)
Driver	*n*=1 (5.56%)	Hypopyon ≥ 2 mm	*n*=8 (44.44%)
Nil	*n*=4 (22.22%)		
Middle class	*n*=2	**Initial microbiological diagnosis of *Pythium* based on**	*n*=18
Businessman	*n*=1 (5.56%)		
Private job	*n*=1 (5.56%)	Smear	*n*=3 (16.67%)
Risk factors		Culture	*n*=7 (38.89%)
Agricultural injury	*n*=5 (27.78%)	Repeat smear	*n*=0
Soil and Dust	*n*=4 (22.22%)	Repeat culture	*n*=1 (5.56%)
Insect	*n*=2 (11.11%)	Growth from CB post -TPK	*n*=5 (27.78%)
Cement	*n*=1 (5.56%)	Growth from CB post- evisceration	*n*=1 (5.56%)
Nil	*n*=6 (33.33%)	Histopathology from CB	*n*=1 (5.56%)
Previous treatment			
Only antifungals	*n*=4 (22.22%)	**Outcome analysis**	
Only antibacterial	*n*=1 (5.56%)	**Early Presenters (Within 15 days)**	*n*=9
Antifungals and antibacterial	*n*=8 (44.44%)	Clear graft / Minimal graft edema	*n*=2 (22.22%)
Antiviral and antibacterial	*n*=1 (5.56%)	Severe graft edema/failed graft	*n*=5 (55.56%)
Steroids and antibacterial	*n*=3 (16.67%)	Graft Infiltration	*n*=0 (0.00%)
Antiviral, steroids and antibacterial	*n*=1 (5.56%)	Phthisis / evisceration	*n*=2 (22.22%)
Initial smears suggestive of		**Late Presenters (More than 15 days)**	*n*=9
Pythium	*n*=3 (16.67%)	Clear graft / Minimal graft edema	*n*=2 (22.22%)
Pythium / Fungus	*n*=5 (27.78%)	Severe graft edema / failed graft	*n*=3 (33.33%)
Fungus	*n*=7 (38.89%)	Graft Infiltration	*n*=1 (11.11%)
Nil	*n*=3 (16.67%)	Phthisis / evisceration	*n*=3 (33.33%)

M - Male, F - Female, CB - Corneal button, TPK - Therapeutic penetrating keratoplasty

Clarithromycin, Azithromycin, Tetracycline, Mupirocin, and Chloramphenicol respectively [Table 3].

Histopathological features

Half corneal button post TPKs and eviscerated eye samples were subjected to histopathological examination with special stains. Out of 16 corneal buttons studied, PAS stain showed negative staining in 10/16 samples (62.5%), while 6 samples (37.5%) showed weak staining in few filamentous structures. GMS stain was positive in all except one (93.8%) [Fig. 1f]. IKI- H_2SO_4 staining was done for 15 samples and all stained the filaments as bluish black structures (100%) [Fig. 1g]. Out of 15 patients who underwent TPK, anti-*Pythium* therapy (APT) was initiated for 5 patients prior to TPK. However, 3/5 (pt. no. 7,12,17) had received therapy only for 5-7 days and necessitated TPK. Corneal button of these cases showed full thickness stromal destruction, necrosis, thinning with infiltration of predominantly polymorphonuclear leukocytes and admixed eosinophils. Thick anterior chamber exudates were seen in 2/3 cases. Filaments of *Pythium* were noted involving full thickness of stroma (3/3) and even in AC in 2/3 cases. Two of five cases underwent TPK after 61 and 73 days of APT. The patient (pt. no. 8) that received APT for 61 days also showed full thickness stromal involvement by *Pythium* filaments, however the patient (pt. no. 9) that received APT for 73 days displayed posterior stromal necro inflammatory tissue with numerous posterior stromal histiocytes and multinucleated giant cells

Table 2: Demography, clinical features, treatment, outcome of all patients

Pt no.	Age/Gender/ Occupation/ Eye	Risk factor	Duration of symptoms	VA At presentation	Clinical features	Duration of medical treatment (days)		Duration of Surgical intervention (days)		Outcome		Follow up (days)
						Prior to TPK	Post TPK	TA	TPK	VA	Anatomical	
*1	29/F/Nil/LE	Nil	45 days	PL + PR acc	Perforated corneal ulcer, hypopyon-1mm	After TPK	8	0	8	20/400→PL + PR acc (Graft infiltrate Post-PK)	Secondary graft infiltration and melt	116
2	37/M/Farmer/ RE	Vegetative matter injury	10 days	PL + PR acc	Near total corneal infiltrate, feathery margins, descemetocele	After TPK	20	NA	16	HM+	Failed graft (secondary fungal infection)	602
3	32/M/ Fisherman/LE	Nil	5 days	20/125p	5.5 x 6 mm, reticular dots, feathery margin, tentacles, hypopyon-0.5 mm, ring infiltrate	After TPK	60	NA	17	20/50p	Good vision, Mild graft edema	800
4	29/M/Labour/ LE	Nil	15 days	HM+	7.5 x 7.5 mm, ring infiltrate, reticular dots, tentacles, feathery margin hypopyo-1 mm	After TPK	58	NA	10	HM+	Failed graft	741
5	64/M/Labour/ LE	Vegetative matter injury	30 days	PL+PR acc	Total corneal infiltrate	After TPK	67	NA	1	PL+PR inacc	Phthisis	686
6	23/F/Nil/RE	Agricultural tool injury	22 days	PL+PR acc	Near total corneal infiltrate, reticular dots, tentacles, thinning, hypopyon >2 mm	After TPK	137	NA	5	CF 1m	Graft edema, VH, RD	144
7	71/M/Farmer/ LE	Soil	12 days	PL+PR acc	6 x 8 mm epithelial defect with infiltrate, thinning	After TA (7 days)	103	0	16	NPL	Phthisis	333
*8	60/M/Farmer/ RE	Nil	15 days	HM+	7 x 5 mm epithelial defect, ring infiltrate, reticular dots, thinning	After TA (61 days)	44	0	71	20/400→HM+ (Glaucoma post TPK)	Failed graft	502
9	41/M/ Businessman/ RE	Nil	8 days	HM+	5.5 x 5.8 mm epithelial defect, hypopyon-2 mm, ring infiltrate, feathery margin, tentacles, reticular dots	23 days before TA, 50 days after TA (total 73 days)	122	26	79	CF CF	Failed graft (Glaucoma post TPK)	788
10	36/M/Driver/LE	Insect fall	45 days	PL+PR acc	Corneal melt, perforation	Evisceration	Evisceration	NA	NA	No vision	Evisceration	86
11	36/M/Farmer/ LE	Vegetative matter injury	20 days	PL+PR inacc	Near total corneal infiltrate, hypopyon >2 mm	After TPK	35 (later evisceration done)	11	5 (required evisceration later)	No vision	Evisceration	607
12	74/M/Farmer/ LE	Vegetative matter injury	15 days	PL+PR inacc	Total corneal infiltrate, thinning	5	26	NA	6	HM+	Failed graft	33
*13	52/M/Farmer/ LE	Insect hair	45 days	HM+	3 x 6 mm epithelial defect, hypopyon >2 mm, tentacle, reticular dots	After TPK	88	NA	14	20/40→CF2m (Glaucoma post TPK)	Graft edema, Glaucoma post TPK	768

Table 2: Contd...

Pt no.	Age/Gender/ Occupation/ Eye	Risk factor	Duration of symptoms	VA At presentation	Clinical features	Duration of medical treatment (days)		Duration of Surgical intervention (days)		Outcome		Follow up (days)
						Prior to TPK	Post TPK	TA	TPK	VA	Anatomical	
14	45/M/Labour/ RE	Cement	6 days	CFCF	4 x 6 mm epithelial defect with infiltrate, tentacles, hypopyon-2 mm, reticular dots, ring infiltrate	50	NA	NA	NA	20/80p	Good vision, Mild graft edema	212
15	30/F/Nil/LE	Nil	90 days	PL+PR inacc	5 x 5 mm epithelial defect with infiltrate, irregular margin, perforation, tentacles	After TPK	25	NA	1	20/160	Good vision, Mild graft edema	668
16	56/M/Nil	Mud	25 days	PL	Total corneal infiltrate, corneal melt, perforation, hypopyon >2 mm	After TPK	28	NA	1	CFCF	Failed graft	56
17	54/M/Farmer	Mud	22 days	HM+	8 x 8 mm epithelial defect with infiltrate, descemetocele, hypopyon >2 mm	5	22	NA	5	20/60	Good vision, Clear graft	376
18	50/M/Private job	Dust	15 days	CF 2m	4.5 x 5.5 epithelial defect with infiltrate, feathery margin, tentacles, reticular dots, thinning, hypopyon-2 mm	9	NA	NA	NA	No vision	Evisceration	390

*Initial good VA post TPK but later on worsened owing to either secondary glaucoma post TPK or secondary graft infection, Pt no.=Patient number, F- Female, M - Male, RE - Right eye, LE - Left eye, VA - Visual Acuity, PL - Perception of light, PR - Perception of rays, Acc - Accurate, Inacc- Inaccurate, HM - Hand movements, CF - Counting Finger, CFCF - CF close to face, NPL- no perception of light, 1mm- 1 millimeter, 2mm- 2 millimeter, TPK - Therapeutic penetrating keratoplasty, TA - Tissue adhesive, NA- Not applicable, VH - Vitreous hemorrhage, RD- Retinal detachment

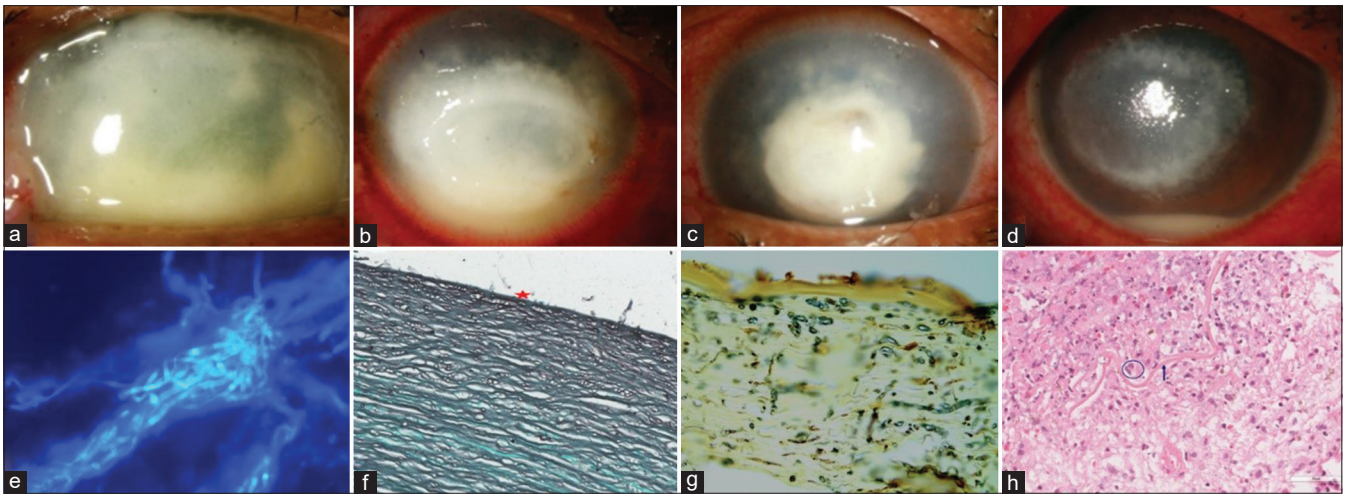

Figure 1: (a-h): Slit-lamp picture of total and near-total corneal infiltrate (a and b); corneal perforation (c); ring infiltrate (d); Direct microscopy of corneal scrapings revealing broad, aseptate, hyaline filaments with ribbon-like folds in KOH + CFW, 40x (e); Photomicrograph of corneal tissue with full thickness involvement by filamentous structures extending from Bowman's membrane (Asterix marked), GMS (30X) (f;) Bluish black filaments, IKI-H2SO4 stain (40X) (g); Perforated cornea with diffuse stromal necrosis and fragmented Descemet membrane (arrow marked). Posterior stromal histiocytes and multinucleated giant cell noted (circle), PAS (40X) (h)

Table 3: Sensitivity of *Pythium* to different antibiotics

Name of antibiotics in descending order of efficacy	Zone of inhibition (mean±SD) (13) (*n*=16)	Zone of inhibition (range) (13) (*n*=16)
Linezolid (*n*=16)	30.25±4.61	18-40
Minocycline (*n*=16)	29.88±6.51	18-40
Doxycycline hydrochloride (*n*=16)	29.50±8.37	20-40
Tigecycline (*n*=16)	26.63±8.94	12-40
Clarithromycin (*n*=16)	25.13±8.64	06-44
Azithromycin (*n*=16)	23.56±6.86	10-34
Tetracycline (*n*=16)	23.38±5.55	18-40
Mupirocin (*n*=16)	22.13±8.31	06-40
Chloramphenicol (*n*=16)	20.00±8.52	06-40

SD - Standard deviation

and did not demonstrate any definite organisms on routine and special stains [Fig. 1h]. Upon correlating, we found that culture of corneal button of this patient (pt. no. 9) did not show growth of Pythium whereas growth was seen in rest of the 4 corneal button samples (pt. no. 7,8,12,17).

Treatment details

Medical management

Based on smear findings, in three patients (pt. no. 12,14,17) APT was initiated with topical or systemic linezolid (0.2% eyedrop, 600 mg tablet) and/or azithromycin (1% eye ointment, 500 mg tablet). In another 4 patients (pt. no. 7,8,9,18), we were able to start APT based on growth on culture. The mean duration of treatment with linezolid and/or azithromycin prior to Keratoplasty was 30 ± 30.08 days and median was 9 days.

Antifungals, such as topical natamycin (1% eye drop) and/or systemic ketoconazole (200 mg tablet) was started in the remaining 11/18 patients.

Later post TPK, all the patients were started on topical/systemic linezolid and/or azithromycin; except for one patient who underwent evisceration (pt. no. 10) on the fourth day of presentation.

The mean total duration of treatment with APT was 61.65 ± 51.01 days (8-195 days) and median was 50 days.

Surgical management

Tissue adhesive was needed in 5 patients with significant thinning after a mean time of 7.4 ± 11.44 days of medical management. TPK was needed in 15/18 (83.33%) patients. The mean time of interval between presentation to TPK was 17 ± 24.23 days. Of these patients 4/15 needed a repeat TPK due to graft failure in 2 (pt. no. 13,15), residual infection/recurrence in 1 (pt. no. 4) and residual infection/recurrence along with graft failure in 1 (pt. no 17). Pt. no. 15 again required keratoplasty due to graft failure. Evisceration was required for 1/15 patient post TPK. Overall, three patients underwent evisceration (16.7%).

Outcome analysis

The average duration of follow up was 439.33 ± 278.69 days (range, 33-800 days).

Overall globe salvation (eyes not requiring evisceration) was possible in 15 patients (83.3%), while anatomical

success (without pthisis or evisceration) was attained in 13 patients (72.2%). Of these, workable visual acuity ≥ 20/200, was seen in 7 patients (38.9%) at 2 months postoperative period. Later, however, 3 of these worsened owing to either secondary glaucoma or secondary graft infection (* in Table 2). Graft failure was identified in 6/15 patients (40%).

Residual infection was seen in 4 patients (22.22%) (pt. no. 4,5,7,17) post TPK, of which one had graft failure post repeat TPK (pt. no. 4), two went into phthisis (pt. no. 5,7), while good vision (20/60) was achieved in the fourth patient (pt. no. 5,7) with initial scleral patch graft followed by repeat TPK.

In 5 patients APT (pt. no. 7,8,9,12,17) was started prior to TPK and in 1 patient before evisceration (pt. no. 18) while 1 patient was given only medical management (pt. no. 14). APT was started after 8 days in 2 patients (pt. no. 7,8), 3 days in 1 patient (pt. no. 9), 1 day in 1 patient (pt. no.12), 9 days in 1 patient (pt. no 18) and zero days in 2 patients (pt. no. 14,17). In spite of early medical management in these patient's workable visual acuity was obtained in 2 patients only (pt. no. 14,17). In rest of the patients APT was started after TPK or not at all in 1 patient who underwent evisceration (pt. no. 10).

The patients were divided into two groups: early presenters (presenting within 15 days) and late presenters (after 15 days). There was near-total corneal involvement in 22.22% (2/9) of early presenters as compared to 66.67% (6/9) among late presenters. Evisceration was required in 2 of the late presenters. One patient in the early presenter group underwent evisceration. This patient refused for TPK, 4 months prior to the evisceration. Final visual acuity of ≤ hand movement (HM) was seen in 44% of late presenters and 22.2% early presenters.

Discussion

Pythium keratitis has been increasingly reported in Southern India.[7-15] Hasika *et al.* have reported that the reason for this disparity is not clear.[11] We assume that it is present in other geographical regions too but it is underdiagnosed as well as underreported. In our study, we report 18 patients with culture-positive *Pythium* keratitis, the dilemma of its diagnosis, and treatment outcome from a tertiary eye care center in Eastern India.

Our series shows a male preponderance and higher prevalence among people from lower socio-economic status with agricultural background. The most common predisposing risk factors were agriculture associated injuries and foreign body entry into eyes like dust and soil. Similar risk factors were identified from studies reported from Southern India.[10,11] Other risk factors described in the literature include contact lens wear, exposure to dust or foreign body, or direct contact with water from lake, river lagoon, swamp or swimming pool.[17] However, Agarwal *et al.* found it more common in software professionals and housewives with no exposure to vegetative matter or water.[8]

Prior to presentation, two-thirds of the patients in our series were using a topical antifungal of which few were in combination with antibiotics. This suggests that there is still a strong suspicion of fungus on clinical presentation to the primary physician. This is further augmented by the fact that 13/18 patients were clinically suspected to be of fungal keratitis by different physicians of our center.

The typical reticular dot-like pattern of sub epithelial and superficial stromal infiltrates at the ulcer border, described by Thanathanee *et al.*[18] to be characteristic of *Pythium* keratitis, were present in 44.44% of our patients. Features like sub-epithelial ring infiltration (27.78%), tentacle-like extensions (44.44%), peripheral thinning (27.78%), and hypopyon (61.11%) could also be identified. Bagga *et al.* reported dot-like infiltrates in 16.3% patients, tentacles in 6.1%, reticular pattern in 1%, hyphate edges in 5.1%, plaque-like lesions in 2%, ring infiltrate in 2%, endoexudates in 10.2% and hypopyon in 54% patients. In this series, 4 (22.22%) patients were clinically suspected to have *Pythium* keratitis based on these clinical features and all of them came out to be culture positive for *Pythium.* This strongly demonstrates that knowledge of characteristic clinical features and a high index of suspicion is required for clinical diagnosis of this entity. The most common organism which closely resembles *Pythium* keratitis is fungal keratitis which is also common in tropical countries like India.[16] The similarities and differences between both the microorganisms have been enumerated in Table 4.

On smears with KOH, only 3/18 could be diagnosed as *Pythium* species and started on APT treatment right away. Our co-authors have demonstrated that IKI-H_2SO_4 can be a fast, highly sensitive, specific and inexpensive stain for identification of filaments of *Pythium* and to differentiate them from fungal filaments as it doesn't stain fungi.[19] So henceforth this can be used in differentiating *Pythium* from fungus, in the initial identification on smears, where there is a dilemma in identification and differentiation from fungal filaments. On retrospective analysis, we conclude that when filamentous fungus is seen in KOH-CFW smears, IKI-H_2SO_4 staining of all corneal scrape samples could have helped to increase the detection rate of *Pythium*. In our case series, we probably misinterpreted 10/18 patients on initial examination on smears.

The mean duration from culture plating to the identification of *Pythium* was 5.09 days suggesting that demonstration of zoospores would be a simple, cost-effective, technically less-demanding methods. It takes 24–48 hours after growth on blood/chocolate agar at 37°C, which is similar to the time taken for DNA sequencing.[7,16,20] Characteristic features like flat, feathery-edged, partially submerged, colorless or light brown colonies with filiform margins, which are difficult to scrape off can be seen on culture.[7] *P. insidiosum* is the only widely recognized causative agent in humans, particularly for ocular *pythiosis*. However, PCR and DNA sequencing can further help in confirmation of the species, particularly in non-responsive and clinically suspicious cases, though it was not done for any of our patients.

Antibiotics sensitivity by Kirby–Bauer Agar disc diffusion method showed that linezolid had the greatest diameter of zone of inhibition in disc diffusion assay followed by doxycycline. Though zone of inhibition of azithromycin was not among the highest, we used it along with linezolid as it has already been reported to be effective,[14] easily available and known to be safer than other drugs.[10]

Histopathological evaluation of half corneal button samples showed negative or weak patchy staining with PAS and positive staining with GMS. All samples (half corneal button as well as corneal scrapes) showed positive staining with IKI-H_2SO_4 stain, which correlated well with the growth of *Pythium* on

Table 4: Similarities and differences in clinical, microbiological, anatomical features between fungus and *Pythium*

		Similarities	Differences	
			Fungus	Pythium
History	Age/Gender	Young/Male		
	Injury	Agricultural		
Clinical feature		Irregular margin	Feathery margin	Tentacle like extensions, Reticular dot-like infiltrates
		Dry looking, whitish	Satellite lesions	Peripheral thinning (gutter)
		Convex hypopyon		Ring infiltrates
		Chronic		
Microbiology	Shape		Thin	Broad, ribbon-like folds, rounded ends, fragile
	Stain	GMS, H&E	PAS	IKI-H_2SO_4
		KOH-CFW	Septate	Aseptate to sparsely septate
		Gram stain	Septate	Aseptate to sparsely septate
	Growth		Better on SDA, PDA	Better on BA, CA
	Identification		Most common ones: Aspergillus fumigatus- white colonies → later velvet green, Aspergillus niger- black colonies, Fusarium- white colonies → later buff colored with reverse pigmentation, Candida- smooth creamy white colonies	Carpet like flat growth on BA & CA that cannot be scraped off easily, Zoospore formation, DNA sequencing
Cell wall			Ergosterol in cytoplasmic membrane	Cellulose in cell wall
			Chitin in cell wall	β-glucans in cell wall
Medical management		Mydriatic	Natamycin Voriconazole	Linezolid Azithromycin

GMS=Gomori Methenamine Silver stain, H and E=Hematoxylin and Eosin stain, KOH-CFW=Potassium hydroxide-calcofluor white, IKI-H_2SO_4=Iodine-potassium iodide-sulfuric acid stain, PAS=Periodic acid-Schiff's stain, BA=Blood agar, CA=Chocolate agar, SDA=Sabouraud dextrose agar, PDA=Potato dextrose agar

culture from samples of these patients, proving this stain to be 100% sensitive.

The major limitation encountered by the authors in the interpretation of *Pythium*, in laboratory, were patients with a low microbial load on sections (1-2 short filamentous structures), for which microbes might be absent on subsequent serial sections for special stains. The authors recommend that in a scenario of strong clinical suspicion of *Pythium*, special stains should be performed in the following order: IKI-H2SO4, GMS and PAS. Further negative staining on PAS stain should only call for a suspicion for *Pythium* and should not be interpreted as a confirmation for *Pythium* as fungal filaments might stain weak or negative for PAS in a case of treated fungal keratitis. Therefore, interpretation of a battery of stains is always recommended for increasing the sensitivity and specificity for identification of infections. De-staining of hematoxylin and eosin stained slide and re-staining it with IKI-H2SO4 stain can also be attempted successfully in patients with low microbial load.

Oral/topical linezolid and azithromycin are currently the treatment of choice for *Pythium* keratitis.[10] Majority of our patients needed surgical intervention. The reasons for this could be either late presentation or late diagnosis leading to a delay in starting anti-*Pythium* management. In our study, only one patient was successfully treated with medical management alone, with satisfactory visual outcome.

New antifungal agents with varied mechanisms of action, used in various combinations (terbinafine combined with either caspofungin, fluconazole, ketoconazole, itraconazole, voriconazole, miconazole or amphotericin B) have also been advocated with limited clinical improvement.[18,21-23] Loreto *et al.* determined the *in vitro* activity of several antimicrobial agents against *Pythium insidiosum* and found that azithromycin, clarithromycin, linezolid, mupirocin, doxycycline, minocycline, and tigecycline showed the largest zones of inhibition (disk diffusion) and the lowest BMD (broth microdilution) and E-test MICs (minimum inhibitory concentration).[21]

Complication like corneal perforation was seen only among the late presenters (beyond 2 weeks of symptoms). Late presenters also had a worse visual and anatomical outcome when compared with early presenters.

Although the mean duration between initial presentation and initiation of any surgical intervention was 14 days, with half of the patients operated on the next day, the final visual outcome was not satisfactory in most of the patients. Globe salvation and anatomical success was possible in 83.3% and 72.2% of the patients, respectively. In a study by Agarwal *et al.*, graft failure was reported in all patients with recurrence in 7 out of 10 patients (70%).[8] In our study, graft failure and recurrence/residual infection were less common.

In a study by Thanathanee *et al.*, *P. insidiosum* vaccine has been used in systemic, cutaneous and vascular *pythiosis*, to prevent systemic recurrences in thalassemia patients; and to prevent post keratoplasty recurrence in corneal grafts.[18] Recurrence has been widely reported. In our study, relatively less recurrence was attributed to early keratoplasty with a wide

surgical excision. Moreover, some of the patients were already on anti-*Pythium* therapy before therapeutic keratoplasty, also attributing to less recurrence. A recent study also recommends early TPK with clear surgical margins to be a gold standard in the management of *Pythium* keratitis in order to achieve globe salvage.[17] Studies have also shown beneficial role of prophylactic intraoperative cryotherapy and absolute alcohol.[8] Though we are still looking for better solutions but medical management with Linezolid and Azithromycin as well prophylactic intraoperative cryotherapy and absolute alcohol are some options in these challenging cases.

Conclusion

We conclude that *Pythium* keratitis is prevalent not only in Southern India but is also an emerging cause of fulminant microbial keratitis non-responding to antifungal treatment in Eastern India. This study emphasizes the importance of early detection and initiation of medical and surgical treatment for patients with keratitis mimicking *Pythium* keratitis. Awareness among the ophthalmologists and familiarity amongst the laboratory workers, about the risk factors; typical and atypical clinical presentations, is necessary in view of the high morbidity as well as the fatal nature of the disease in rare circumstances.[5,24] *Pythium* keratitis, pertaining to its effective medical and surgical therapy still stands out to be an unsolved puzzle requiring further studies.

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Conflicts of interest

There are no conflicts of interest.

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