**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 salvage 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

Pythiosis, caused primarily by *Pythium insidiosum*, is an emerging and life-threatening infectious disease in humans and animals. Apart from *P. insidiosum*, other oomycetes like *Lagenidium* spp. and *P. aphanidermatum* have also been rarely recognized as human pathogens. It can manifest as cutaneous/subcutaneous, ocular, vascular or as disseminated forms of infection. Unlike fungus, its cell wall contains 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. 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. 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 December 2018; 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$_2$SO$_4$) staining was performed whenever there was a clinical or microbiological suspicion for Pythium, as per published protocols. 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. 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. 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$_2$SO$_4$ (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 (11.1%) 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 marginals (n = 5, 27.78%), irregular ulcer marginals (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$_2$SO$_4$ 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$_2$SO$_4$ 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$_2$SO$_4$ 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,
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$_2$SO$_4$ 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.

| Variable                     | Number of cases n (%) | Variable                     | Number of cases n (%) |
|------------------------------|-----------------------|------------------------------|-----------------------|
| **Table 1: Demography, prior treatment, clinical features, microbiology, outcome** |                       |                              |                       |
| **Gender**                   |                       | **Clinical features**        |                       |
| 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**               |                       |                              |                       |
| Lower socioeconomics         | n=16                  |                              |                       |
| Farmer                       | n=7 (38.89%)          |                              |                       |
| 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
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) | 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) | 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+     | 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   | 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+     | 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    | 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     | 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) | 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    | 788             |
| 10     | 36/M/Driver/LE            | Insect fall | Vegetative matter injury | 45 days           | Corneal melt, perforation | Evisceration After TPK | Evisceration 35 (later evisceration done) | NA 11    | NA (required evisceration later) | No vision | 86                  |
| 11     | 36/M/Farmer/LE            | Vegetative matter injury | 20 days             | PL+PR acc         | Near total corneal infiltrate, hypopyon >2 mm | Evisceration After TPK | NA 11    | NA (required evisceration later) | No vision | Evisceration 607 |
| 12     | 74/M/Farmer/LE            | Vegetative matter injury | 15 days             | PL+PR inacc       | Total corneal infiltrate, thinning | Evisceration 35 (later evisceration done) | 26 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 → CF 2m (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) | Anatomical |
|--------|---------------------------|-------------|----------------------|-------------------|-------------------|------------------------------------|------------------------------------------|---------|-----------------|------------|
| 14     | 45/M/Labour/RE 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 LE 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 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 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 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
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

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**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
success (without ptosis 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 (22.2%) (pt. no. 4, 5, 7, 17) post TPK, of which one had graft failure post repeat TPK (pt. no. 4), two went into ptosis (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 22.2% (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.[7,10 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 socioeconomic status with agricultural background. The most common predisposing risk factors were agricultural 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.[19] 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 (22.2%) 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.[10] 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-H₂SO₄ 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-H₂SO₄ 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.[17] *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-H₂SO₄ stain, which correlated well with the growth of *Pythium* on
Aseptate to sparsely septate Pythium. Most common ones: β
Better on SDA, PDA
Linezolid
Age/Gender
Better on BA, CA
Differences
Broad, ribbon-like folds,
Ring infiltrates
Cellulose in cell wall
Satellite lesions
Feathery margin
Natamycin
IKI-H
GMS, H&E
in vitro
Young/Male
Septate
PAS
[10]
Agricultural
Septate

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). [23]
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.[19]
Recurrence has been widely reported. In our study, relatively
less recurrence was attributed to early keratoplasty with a wide

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

| Similarities | Differences |
|--------------|-------------|
| **History**  | **Pythium** |
| Age/Gender  | Young/Male  |
| Injury      | Agricultural |
| **Clinical feature** | **Fungus** | **Pythium** |
| Irregular margin | Feathery margin | Tentacle like extensions, Reticular dot-like infiltrates |
| Dry looking, whitish Convex hypopyon Chronic | Satellite lesions | Peripheral thinning (gutter) |
| **Microbiology** | **Shape** | **Growth** |
| Shape | Thin | Better on SDA, PDA |
| Stain | GMS, H&E KOH-CFW | PAS Septate |
| Gram stain | PAS Septate | Better on BA, CA |
| **Growth Identification** | Better on SDA, PDA | 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 |
| **Medical management** | Mydriatic | β-glucans in cell wall |
| | Natamycin | Linezolid | Azithromycin |

GMS=Gomori Methenamine Silver stain, H and E=Hematoxylin and Eosin stain, KOH-CFW=Potassium hydroxide-calcofluor white, IKI-H2SO4=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
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.[13] Studies have also shown beneficial role
of prophylactic intraoperative cryotherapy and absolute
alcohol.[6] 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 anti fungal 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.

**References**

1. Krajaejun T, Sathapatayavongs B, Pracharktam R, Nitiyanant P,
Leelachaikul P, Wanachiwanawin W, et al. Clinical and
epidemiological analyses of human pythiosis in Thailand. Clin
Infect Dis 2006;43:569-76.

2. Calvano TP, Blatz PJ, Vento TJ, Wickes BL, Sutton DA, Thompson EH,
et al. *Pythium* aphanidermatum infection following combat trauma.
J Clin Microbiol 2011;49:3710-3.

3. Farmer AR, Murray CK, Driscoll IR, Wickes BL, Wiederhold N,
Sutton DA, et al. Combat-related pythium aphanidermatum
invasive wound infection: Case report and discussion of utility of
molecular diagnostics. J Clin Microbiol 2015;53:1968-75.

4. Reinprayoon U, Permpalung N, Kasetsuwank P, Plongla R,
Mendoza L, Chindamporn A. *Lagenidium* sp. ocular infection
mimicking ocular pythiosis. J Clin Microbiol 2013;51:2778-80.

5. Hilton RE, Tepedino K, Glenn CJ, Merkel KL. Swump cancer: A case
of human pythiosis and review of the literature. Br J Dermatol
2016;175:394-7.

6. Mendoza L, Newton JC. Immunology and immunotherapy
of the infections caused by *Pythium* insidiosum. Med Mycol
2005;43:477-86.

7. Sharma S, Balne PK, Motukupally SR, Das S, Garg P, Sahu SK,
et al. *Pythium* insidiosum keratitis: Clinical profile and role of
DNA sequencing and zoospor formation in diagnosis. Cornea
2015;34:438-42.

8. Agarwal S, Iyer G, Srinivasan B, Agarwal M, Panchalam Sampath
Kumar S, Therese LK. Clinical profile of pythium keratitis:
Perioperative measures to reduce risk of recurrence. Br J
Ophthalmol 2018;102:153-7.

9. Agarwal S, Iyer G, Srinivasan B, Benurwar S, Agarwal M,
Narayanan N, et al. Clinical profile, risk factors and outcome
of medical, surgical and adjunct interventions in patients with
*Pythium* insidiosum keratitis. Br J Ophthalmol 2019;103:296-300.

10. Bagga B, Sharma S, Madhuri Guda SJ, Nagpal R, Joseph J,
Manjulatha K, et al. Leap forward in the treatment of *Pythium*
insidiosum keratitis. Br J Ophthalmol 2018;102:1629-33.

11. Hasika R, Lalitha P, Radhakrishnan N, Rameshkumar G, Prajna NV,
Srinivasan M. *Pythium* keratitis in South India: Incidence, clinical
profile, management, and treatment recommendation. Indian J
Ophthalmol 2019;67:42-7.

12. Kalra P, Bagga B, Garg P. *Pythium* insidiosum keratitis:
Histopathology and rapid novel diagnostic staining technique.
Cornea 2018;37:e14.

13. Raghavan A, Bellamkonda P, Mendoza L, Rammohan R. *Pythium*
insidiosum and Acanthamoeba keratitis in a contact lens user. BMJ
Case Rep 2018;11:bcr2018226386. doi: 10.1136/bcr-2018-226386.

14. Ramappa M, Nagpal R, Sharma S, Chaurasia S. Successful medical
management of presumptive pythium insidiosum keratitis.
Cornea 2017;36:511-4.

15. Thomas PA. Current perspectives on ophthalmic mycoses. Clin
Microbiol Rev 2003;16:730-97.

16. Mittal R, Jena SK, Desai A, Agarwal S. *Pythium* insidiosum keratitis:
Histopathology and rapid novel diagnostic staining technique.
Cornea 2017;36:1124-32.

17. Chitasombat MN, Jongkhajornpong P, Lekhanont K, Krajaejun T.
Recent update in diagnosis and treatment of human pythiosis.
PeerJ 2020;8:e8555.

18. Thanathanee O, Enketchakul O, Rangsir R, Warasawapati S,
Samerpitak K, Suwan-apichon O. Outbreak of *Pythium* keratitis
during rainy season: A case series. Cornea 2013;32:199-204.

19. Vanittanakom N, Supabandhu J, Khamwan C, Praparattanapan J,
Thirach S, Prasertwitayakij N, et al. Identification of emerging
human-pathogenic *Pythium* insidiosum by serological and
molecular assay-based methods. J Clin Microbiol 2004;42:3970-4.

20. Chaiprasert A, Samerpitak K, Wanachiwanawin W, Thasnakorn P.
Induction of zoospore formation in Thai isolates of *Pythium*
insidiosum. Mycoses 1990;33:317-23.

21. Loreto ES, Tondolo JS, Piloto MB, Alves SH, Santurio JM. New
insights into the *in vitro* susceptibility of *Pythium* insidiosum.
Antimicrob Agents Chemother 2014;58:7534-7.

22. Cavalleiro AS, Maboni G, de Azevedo MI, Argenta JS, Pereira DJ,
Spader TB, et al. *In Vitro* activity of terbinafine combined with
caspofungin and azoles against *Pythium* insidiosum. Antimicrob
Agents Chemother 2009;53:2136-8.

23. Leisersuthirat T, Sangcakul A, Lohnoo T, Yingyong W, Rujirawat T,
Krajaejun T. Evolution of the sterol biosynthetic pathway of
*Pythium* insidiosum and related oomycetes contributes to
antifungal drug resistance. Antimicrob Agents Chemother
2017;61:e02352-16. doi: 10.1128/AAC.02352-16.

24. Rath A, Chakraborti A, Agarwal T, Pushker N, Patil M, Kamble H,
et al. *Pythium* Keratitis Leading to Fatal Cavernous Sinus
Thrombophlebitis. Cornea 2018;37:519-22.