

Not Fungal After All: The Diagnostic Puzzle of Pythium Keratitis – First Case Report from Pakistan



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ABSTRACT

Pythium keratitis, caused by the oomycete *Pythium insidiosum*, is a rare and emerging cause of vision-threatening corneal ulcers. Clinically, it mimics fungal keratitis but is unresponsive to conventional antifungal therapy. Microbiologically confirmed cases have been rarely reported from Pakistan. A corneal scraping from a patient under antifungal therapy for presumed fungal keratitis was submitted for microbiological evaluation. Inoculation onto blood agar and Mueller Hinton agar with 10% KOH and Lactophenol Cotton Blue (LCB) staining revealed white colonies. Microscopy demonstrated hyphae, sporangia, sporangiophores, and sporangiospores, confirming *Pythium insidiosum*. The corneal abscess leads to perforation for which emergency sclero-conjunctival flap was performed; however, this incidental finding highlights the importance of suspecting *Pythium* in refractory corneal ulcers. Ophthalmologists should consider corneal scrapings in non-responsive cases and recognize characteristic tentacle-shaped stromal infiltrates to guide early diagnosis and management.

Keywords: Keratitis, Corneal Ulcer, Oomycetes, Infectious, Fungal.

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INTRODUCTION

Infectious keratitis is one of the leading causes of corneal blindness worldwide. Among rare pathogens, *Pythium insidiosum*, an aquatic oomycete, has emerged as an important cause of keratitis in tropical and subtropical regions.^{1,2} Clinically, *Pythium* keratitis closely mimics fungal keratitis, presenting with stromal infiltrates, hypopyon, and ulceration.^{3,4} Its lack of ergosterol renders conventional antifungal therapy largely ineffective.⁵

Characteristic features such as tentacle-like stromal extensions, peripheral guttering, and rapid progression may help differentiate *Pythium* from true

fungi.¹ We present a case of a patient with *Pythium* keratitis undergoing antifungal therapy which emphasizes the diagnostic challenges associated with *pythium* keratitis.

Case Presentation

A 60-year-old patient presented with severe pain and redness of right eye from the past 15 days for which he was using over-the-counter medication. On examination central corneal ulcer with 6x6mm abscess, circum-lesional guttering and 1.5mm hypopyon was noted for which admission was advised and full anti-fungal therapy was started. No history of trauma, contact lens use, or aquatic exposure was documented. Despite treatment, the ulcer showed limited response, so the patient was selected for corneal scraping.

Informed consent was taken and the corneal scraping was inoculated onto blood agar in a C-shaped streak pattern without damaging the agar surface. A few drops of 10% KOH and Lactophenol Cotton Blue (LCB) were added. Plates were incubated at 25°C–

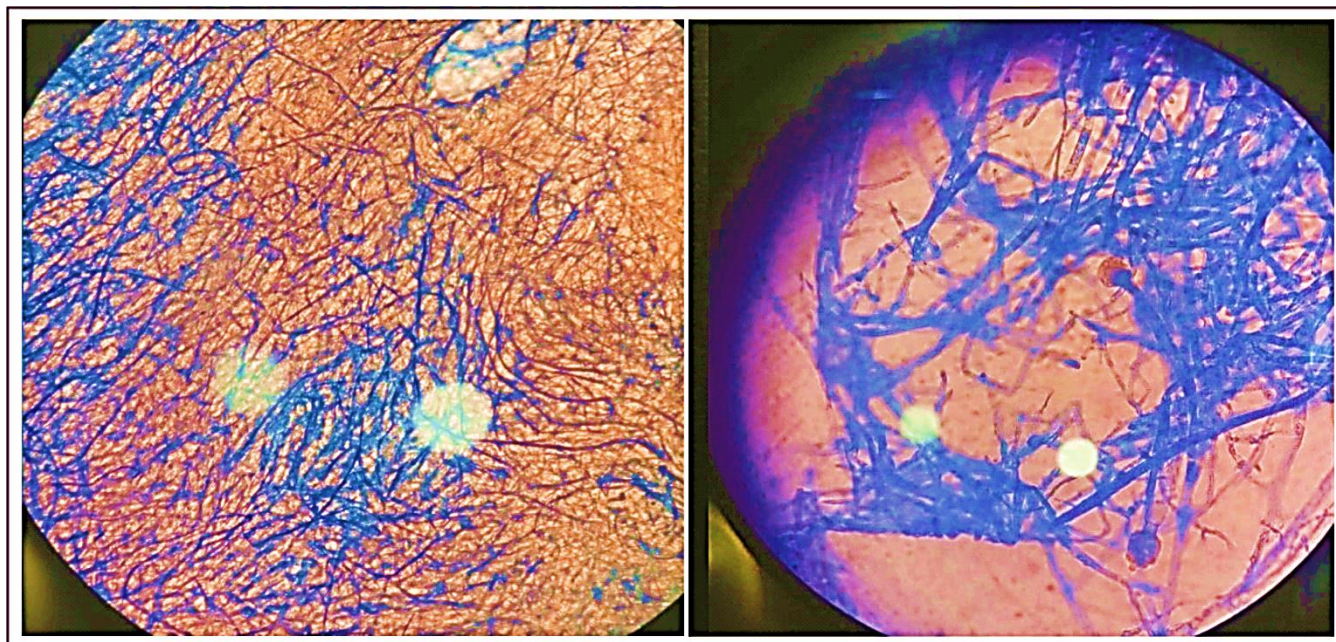


Figure 1: Left shows Lactophenol cotton blue–stained wet mount from culture isolate. Right image shows broad, sparsely septate, ribbon-like hyphae with irregular branching are visible, consistent with the morphology of *Pythium* species.

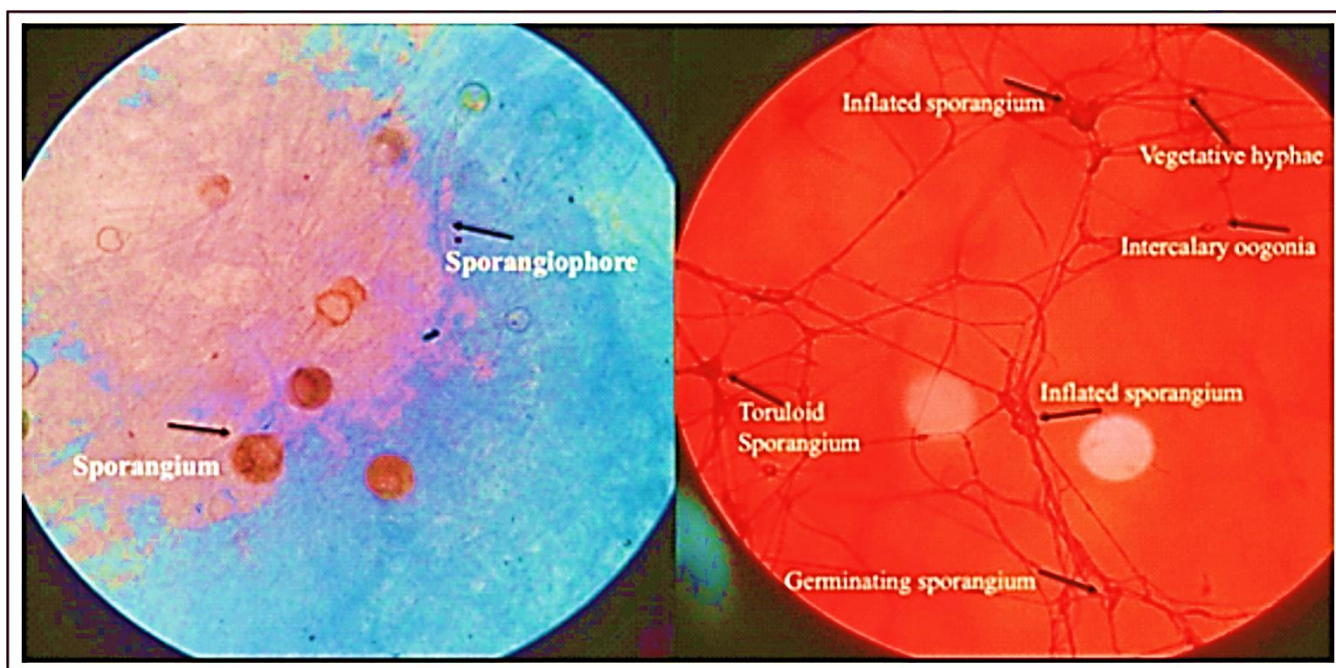


Figure 2: Microscopic view demonstrating characteristic sporangia and sporangiophores of *Pythium insidiosum*. Round to oval sporangia with smooth walls and emerging sporangiophores are present, representing key reproductive structures that differentiate *Pythium* from filamentous fungi. Intercalary oogonia can also be identified which differentiates *Pythium* from filamentous fungi.

37°C for 24 hours.

White colonies appeared on blood agar. Microscopic examination of colonies stained with LCB (Lactophenol cotton blue) revealed broad hyphae,

sporangia and sporangiophores (Figure 1). Colonies appeared light greenish blue due to LCB staining, consistent with *Pythium insidiosum* morphology.^{1,3} Slide preparation involved transferring a small piece of

colony onto a glass slide under a coverslip. Microscopic examination of growth obtained on blood agar also demonstrated intercalary oogonia which is highly consistent with *Pythium* species differentiating it from other filamentous fungi (Figure 2).

The infection demonstrated an unusually aggressive course, progressing from keratitis to a corneal abscess despite intensive empiric antifungal therapy. This rapid deterioration ultimately resulted in corneal perforation, for which a sclero-conjunctival flap was performed to preserve globe integrity. Microbiological culture of the corneal scraping subsequently confirmed *Pythium insidiosum*, providing a clear explanation for the poor response to antifungal treatment and the fulminant clinical behavior.

DISCUSSION

Pythium keratitis is often misdiagnosed as fungal keratitis due to overlapping clinical features. Key clinical clues include tentacle-shaped stromal infiltrates, reticular dot-like patterns, and peripheral guttering.³ Non-responsive ulcers under antifungal therapy should prompt corneal scraping and culture. In this case, the diagnosis was incidental, highlighting the fact that routine fungal scrapings can reveal unexpected pathogens.

Pythium insidiosum is an oomycete with no ergosterol in its cell wall.² Culture on blood agar with LCB stain revealed broad hyphae, sporangia and sporangiophores which are characteristic morphologic and reproductive structures of *pythium*. Advanced diagnostics, including PCR or zoospore induction, may further confirm the organism.³

The cornerstone of modern medical management for *Pythium* keratitis involves using a combination of linezolid and azithromycin.^{3,6} This approach is necessitated by the fact that *Pythium insidiosum*, an oomycete, lacks ergosterol, rendering conventional antifungal medications ineffective.⁷ Linezolid and azithromycin function as broad-spectrum antibacterial agents that exhibit in-vitro activity against *P. insidiosum*.⁸ Their therapeutic effect stems from their ability to disrupt the organism's protein synthesis.

Linezolid inhibits the initiation of protein synthesis by binding to the 23S ribosomal RNA subunit within the organism's 50S ribosome.⁹ On the other hand Azithromycin, which is a macrolide antibiotic, binds to a different site on the 50S

ribosomal subunit, halting the elongation step of the protein synthesis process.^{7,9} When used in combination (typically topical 0.2% linezolid and 1% azithromycin), these drugs provide a synergistic effect, which has improved clinical outcomes and reduced the need for surgical intervention compared to previous antifungal-based regimens.⁷ Surgical intervention, particularly therapeutic keratoplasty, is often required in severe cases.⁴

This case represents a rare microbiologically confirmed case of *Pythium* keratitis in Pakistan. Ophthalmologists and microbiologists should maintain high suspicion for *Pythium* in refractory corneal ulcers, obtain scrapings for culture, and look for characteristic tentacle-like infiltrates clinically. Following are the key learning points derived from this case report.

1. Consider *Pythium* keratitis in non-responsive corneal ulcers.
2. Perform corneal scraping and culture, even if fungal keratitis is suspected.
3. Recognize tentacle-shaped infiltrates, peripheral guttering, and reticular patterns.
4. Early diagnosis facilitates targeted medical and surgical therapy.

The main limitation of this report is that corneal scraping was performed while the patient was already receiving empiric antifungal therapy. Nonetheless, culture still yielded *Pythium insidiosum*, reinforcing the value of microbiological evaluation even after antifungal exposure. The infection progressed rapidly to corneal abscess and perforation, limiting assessment of response to organism-specific therapy. Despite these constraints, the case highlights the need to consider *Pythium* in refractory keratitis and the importance of timely corneal scraping and culture.

CONCLUSION

Incidental identification of *Pythium insidiosum* in a corneal scraping in Pakistan highlights the importance of microbiological evaluation in refractory keratitis. Awareness of characteristic infiltrates and timely culture can guide early diagnosis and improve patient outcomes.

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