American Journal of Ophthalmology Case Reports 36 (2024) 102176

Contents lists available at ScienceDirect

American Journal of Ophthalmology Case Reports

journal homepage: www.ajocasereports.com/

A rare case of keratitis due to co-infection with *Plectosphaerella cucumerina* and *Acanthamoeba* T5 genotype

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ARTICLE INFO

Keywords:
Keratitis
Acanthamoeba
Plectosphaerella
Co-infection
Genotype T5

ABSTRACT

Aims: This report summarizes clinical findings, diagnosis, management, and long term outcomes of a recalcitrant *Plectosphaerella* and *Acanthamoeba* keratitis.
Methods and materials: An otherwise healthy male patient presented with a corneal infiltrate with predominantly fungal characteristics, but atypical in terms of progression. The infiltrate remained undiagnosed even after several scrapings, and aqueous tap cultures. Since conventional therapy with antifungals and intrastromal voriconazole injections was ineffective, a therapeutic keratoplasty was done.
Results: The excised corneal button was cultured; molecular identification of the *Acanthamoeba* and fungus isolates revealed the rare T5 genotype and the even rarer *Plectosphaerella cucumerina*. Judicious use of antifungals and anti-amoebic drops in the post-operative period, combined with gradual introduction of steroids resulted in favourable visual outcome.
Conclusions: The T5 is the second most frequent environmental *Acanthamoeba*, but rarely isolated clinically. *Plectosphaerella* keratitis has been reported only 4 times in the literature. Neither of these have been previously reported from India.

1. Introduction

The genus *Plectosphaerella* is a well-known plant pathogen, but is a very rare cause of keratitis, with only four reported cases.[1–3] Genotyping of *Acanthamoeba* has revealed that T4 accounts for most of the cases of *Acanthamoeba* keratitis.[4] Although T5 is the second most common genotype found in the environment, it has only rarely been found to be pathogenic.[5] We report an unusual co-infection involving both *Plectosphaerella* and *Acanthamoeba* genotype T5.

2. Subject and methods

A 24-year-old male in apparently good health and a non-contact lens user, reported with a 2-day history of redness and photophobia in the right eye (RE); he was not a diabetic, and had no chronic illness or disease. His only relevant medical history was trauma to the eye with dust particles. He had used moxifloxacin eye drops, but noted no improvement.

At presentation, his uncorrected visual acuity was 20/60 RE and 20/40 in the left eye (LE). RE slit-lamp bio-microscopic examination showed minimal lid edema with conjunctival congestion and a paracentral

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https://doi.org/10.1016/j.ajoc.2024.102176
Received 18 June 2024; Received in revised form 20 August 2024; Accepted 13 September 2024
Available online 20 September 2024

epithelial defect 2.2mm in size just beneath the visual axis. The stroma was clear, the anterior chamber quiet, the pupil brisk and the lens clear. Slit-lamp examination of LE was within normal limits. The disc, the macula and vessels up to the 3rd crossing, were within normal limits on fundus examination with a 90D lens. The patient was treated with ciprofloxacin 0.3 % eye drops. He presented 5 days later with worsening symptoms and a significant drop in visual acuity to 20/250. Slit-lamp evaluation revealed a central stromal lesion 5mm × 5mm in size with minimal patchy infiltration and hyphate edges, and a thin central superficial plaque.

Corneal Scrapings were obtained and plated onto Blood Agar, Sabouraud's Dextrose Agar, and Non-Nutrient Agar. Smears were also prepared - the KOH mount was negative, but the Gram's stain revealed sparsely septate hyphae.

As the clinical presentation was atypical (the clinical diagnosis now being fungal keratitis or mixed infection), a confocal microscopy was done - which was suspicious for fungal filaments (Fig. 1). Therapy was initiated with natamycin 5 %, voriconazole 1 %, and homatropine 2 %. When reviewed 2 days later, vision had dropped to 2/60. The plaque-like area in the centre of the lesion was surrounded by some amount of scarring, suggestive of response to therapy, but the periphery of the lesion had dot-like extensions as well as hyphate edges. Once again corneal scrapings were repeated, and although the Gram stain revealed few hyphae, the cultures were negative.

Over the next few weeks, the lesion had a waxing and waning course with some areas appearing to scar, and other areas with active infiltration. A third set of scrapings done at 2 weeks was once again negative. Twenty days post-presentation (Fig. 2), an Aqueous (Anterior Chamber) tap was performed, and intrastromal and intracameral voriconazole was administered. Cultures of the Aqueous tap on Blood Agar, NNA and SDA were negative. Two more injections of intrastromal voriconazole were given on the 25th and 29th days. At 43 days only the plaque on the surface remained, while the stromal component appeared to be healing well. Since plaque-like lesions are common in *Aspergillus* infections, Amphotericin-B 0.15 % was added along with 1.0 % itraconazole eye ointment, and the natamycin was withheld. Once again there appeared to be a transient improvement. Chlorhexidine 0.04 % was also administered since *Acanthamoeba* was in the differential diagnosis. At 2 months the vision was hand movements, the infiltrate now involved the entire thickness of the stroma measuring 4mm × 4 mm (Fig. 3). There was a central epithelial defect and a hypopyon of 1mm. Given the recalcitrant nature of the infection, a therapeutic keratoplasty was carried out 63 days after presentation and the excised corneal button was submitted for cultures. Post-operatively both Amphotericin-B and voriconazole were continued at 8 times/day and itraconazole ointment thrice daily. Six days after keratoplasty, fungal colonies were identified on the SDA plate and a day later *Acanthamoeba* was identified on the NNA plate. Poly hexamethylene biguanide (PHMB) 0.04 % was added and 10 days after keratoplasty oral prednisolone 50mg per day was initiated. Thirteen days after keratoplasty the graft was clear with no signs of recurrence and 1 % prednisolone ophthalmic suspension (predforte) 3 times a day was started. The PHMB, voriconazole, and Amphotericin-B were continued at 8 times a day.

One month post-keratoplasty topical medicines were tapered to 6 times a day, predforte increased to 4 times and oral steroids discontinued. Over the ensuing few months antifungals and PHMB were slowly tapered and the medications discontinued one-by-one. Amphotericin-B was discontinued 2 months after keratoplasty, PHMB at 3 months, and voriconazole at 4 months. Tacrolimus eye ointment (0.03 % w/v) was added at 2 months and the prednisolone gradually tapered to once a day by the 9th month.

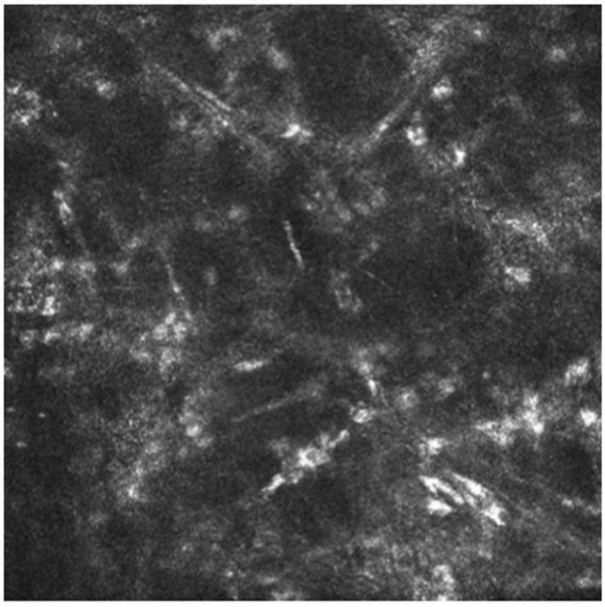

Fig. 1. Confocal microscopy.

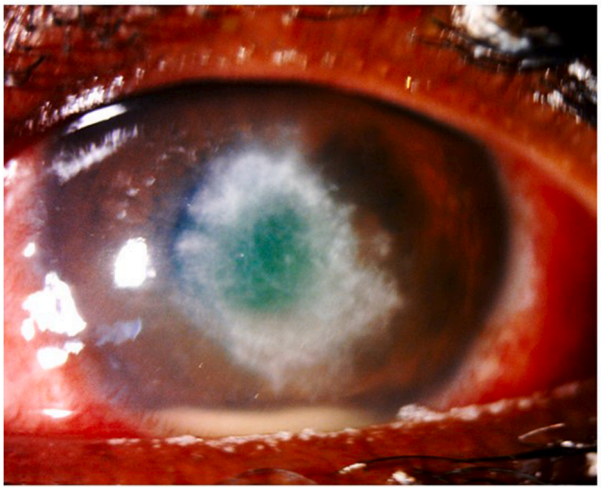

Fig. 2. Clinical picture 20 days post-presentation.

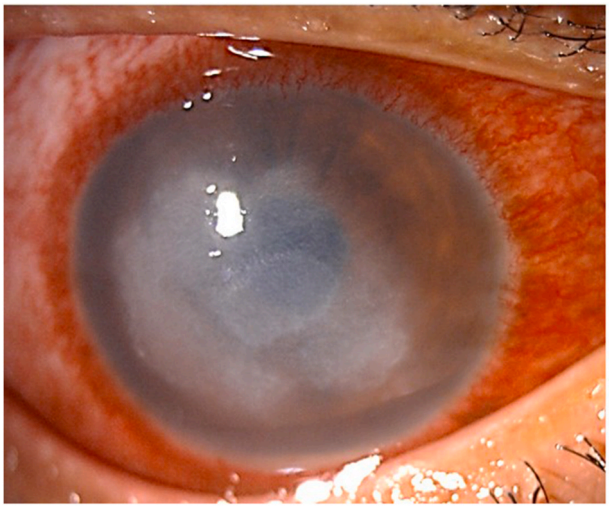

Fig. 3. Clinical picture 2-months post-presentation.

3. Results

Over the next year the patient continued to do well, and at 2 years post-surgery, vision was BCVA of 20/30 with a correction of - 2.25 to - 3.00 at 110.

The filamentous colony which had appeared after 6 days of incubation on SDA, closely resembled *Fusarium*, and gradually turned pinkish after 2 weeks. Microscopic examination of colony stained with lactophenol cotton blue (LPCB) showed no sporulation. After a month, the colony became dull salmon-pink, flat and smooth, and then gradually radiated out concentrically as dull dirty-grey from the centre to the periphery (Fig. 4). LPCB mounts showed hyaline uni-septate or sparsely septate slightly curved short conidia (Fig. 5). Since this colony and the spores did not look like *Fusarium* or a similar fungus, the isolate was sent to the WHO Collaborating Centre for Research on Fungi of Medical Importance, Chandigarh 160 012, India, for identification. The isolate failed to sporulate and thus DNA sequencing was performed, which showed 99.8 % sequence homology with *Plectosphaerella cucumerina*.

The *Acanthamoeba* isolate was sent to the University Institute of Tropical Diseases and Public Health, University of La Laguna, Canary Islands, Spain, where sequencing confirmed the isolate as a T5 genotype.

4. Discussion

The genus *Plectosphaerella* comprises both plant pathogens and soil-borne species.[6] *Plectosphaerella cucumerina* causes blight or spot diseases in vegetables and flowers.[7] Though cosmopolitan, it is widespread specifically in tropical and subtropical regions.[8] Of the five known species of *Plectosphaerella* (*Plectosphaerella cucumerina*, *Pa. citrullae*, *Pa. pauciseptata*, *Pa. plurivora,* and *Pa. ramiseptata*), only *Pa. cucumerina* causes keratitis.[2,3] To date, there are only four reported cases of *Plectosphaerella* keratitis, the causative organism being identified only via molecular methods or matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS).[1–3]

The case described by Kamada et al. was initially treated with topical miconazole, topical fluconazole, pimaricin ointment, and intravenous miconazole. These were subsequently replaced with topical voriconazole, intravenous voriconazole, and oral itraconazole. It took 3 months of topical and systemic therapy to achieve resolution.[1] The case described by Shen et al. (identified by MALDI-TOF analysis) resolved in a month with topical 5 % pimaricin and oral voriconazole. The 3rd case,

Fig. 4. Colony on Sabouraud Dextrose Agar after 1 month of culture.

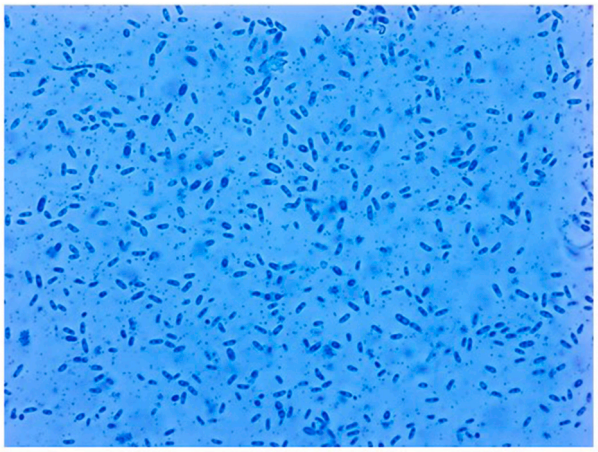

Fig. 5. Lacto-Phenol Cotton Blue Mount from colony.

reported by Kiriyama et al., recovered after sustained (4-month) topical and oral treatment with voriconazole. Only the third scraping of the eye turned out to be fungus culture-positive – ultimately identified via PCR amplification and BLAST sequencing. In their 5-year retrospective study, Russello et al. re-identified one of their eight *Fusarium* spp as *Plectosporium tabacinum* via molecular analysis. No information is available on management or treatment of patient(s).[2,3]

Anifungal susceptibility *testing by* 2 independent workers demonstrated sensitivity to voriconazole (MIC_{50} 1ug/mL and MIC_{80} 0.125μg/mL), itraconazole (MIC_{80} 0.25μg/mL), or miconazole (MIC_{50} 1ug/mL), as well as to micafungin (MIC_{50} 2ug/mL, and MIC_{80} 0.25μg/mL), or pimaricin (MIC 2ug/mL).[3]

All the three successfully managed cases had been treated with voriconazole - either systemic, topical or both. Ours was a co-infection with *Acanthamoeba* and did not resolve with topical therapy. Though topical and intrastromal voriconazole was administered from the onset of the infection, the lesion failed to resolve. It is possible that we may have had a more favourable outcome with oral voriconazole, especially given the reported favourable outcomes in chronic *Acanthamoeba* keratitis.

The most common genotype reported in *Acanthamoeba* keratitis is the T4 (86 %) followed by T3 and T11. Together these three (the Group III) constitute 60 % of environmental isolates.[4] Although the genotype T5 represents more than one-quarter of all environmental isolates, the first reported case of keratitis was only in 2006.[5]

Isolates from only nine cases of keratitis have been identified as *Acanthamoeba* T5 genotype.[4,9] The first report was in 2006 by Spanakos, followed by Ledee in 2009. Iovieno in 2010 described a drug-resistant T5 isolate from a keratitis patient (who ultimately required an enucleation), and concluded that atypical *Acanthamoeba* genotypes, such as the T5, could be associated with poor prognosis and resistance to therapy. van Zyl isolated a T5 from the keratoplasty button of a patient with a rapidly progressive and severe episode of *Acanthamoeba* keratitis. Rahman et al., and Risler et al., reported one case each of T5 genotype in 2013. In 2018 Taher et al. from Egypt detected three T5 genotypes out of 32 keratitis isolates.[4,9]

Over the last couple of decades, a number of reports have described *Acanthamoeba* keratitis as co-infections, covering a wide range of pathogen diversity, from filamentous fungi such as *Fusarium*, *Aspergillus*, and the dematiaceous fungi, to several of the most common gram negative and gram positive bacteria, as well as *Pythium*. Clinical presentations in co-infections are often variable, and many tend to resemble fungal or bacterial ulcers, and establishing a co-infection frequently requires multiple scrapings and cultures.[10]

5. Conclusion

After conducting a literature review as of date, utilizing PubMed, and Google Scholar, we did not find any prior reports of keratitis co-infection with *Plectosphaerella* and *Acanthamoeba* T5. The possibility of unusual pathogens and or mixed infections should be considered in recalcitrant cases. Molecular diagnostic techniques will facilitate early diagnosis and appropriate treatment especially when conventional methods of identification fail.

Note

After conducting a literature review as of date, utilizing PubMed, and Google Scholar, we did not find any prior reports of keratitis co-infection with *Plectosphaerella* and *Acanthamoeba* T5.

Patient consent

Consent to publish this case report has been obtained from the patient in writing.

Funding

No funding or grant support.

Authorship

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

CRediT authorship contribution statement

Anita Raghavan: Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Conceptualization. **Jacob Lorenzo-Morales:** Writing – original draft, Investigation, Formal analysis. **Maitrayee Kaman Chabra:** Writing – original draft, Investigation. **Shivaprakash Rudramurthy:** Writing – original draft, Investigation, Formal analysis. **Narendran Venkatapathy:** Supervision, Resources. **Siddharth Narendran:** Writing – original draft, Investigation. **Ram Rammohan:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

None.

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