Fungal Keratitis Caused by *Laetisaria Arvalis*

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Abstract

A 53-year-old female presented with keratitis (right eye) after fall of insect 10 days back. The ulcer worsened in spite of aggressive treatment with topical natamycin (5%) and amphotericin-B (0.15%) eye drops and finally perforated. Iris tissue sealed the perforation site, and ulcer healed with formation of adherent leucoma. This case represents first reported case of keratitis caused by *Laetisaria arvalis* and stresses on aggressive course of keratitis caused by this fungus. Importance of DNA sequencing in identification of unidentified fungal species is also highlighted.

Keywords: DNA sequencing, fungal keratitis, *Laetisaria arvalis*

**INTRODUCTION**

*Laetisaria* is a new genus described in the family *Corticiaceae*. *Laetisaria arvalis* is a soil-inhabiting basidiomycete. The generic placement of *L. arvalis* is still somewhat unclear as it possesses characters intermediate between *Phanerochaete* and *Laetisaria*. The species was thought to be reminiscent of a species of *Phanerochaete*, but such characteristics as the production of sclerotia, probasidial-type cells from which the basidia arise, size of the basidia and basidiospores and habitat and mycoparasitic capability are unusual for *Phanerochaete* species. Thus, *L. arvalis* is now considered in *Laetisaria* because of the absence of clamps in the basidiocarp proper, large basidia and basidiospores, reduced probasidial cell, parasitic capability and production of differentiated vegetative structures.[¹]

*L. arvalis* has potential importance as a biological control agent which has been extensively studied. It has been indicated that it has a role as biological control agent against *Thanatephorus cucumeris*,[¹] *Pythium ultimum*,[²] *Rhizoctonia solani* on cucumber (*Cucumis sativus L.*), snap bean and dry edible bean (*Phaseolus vulgaris L.*) and sugar beet.[³,⁴] *L. arvalis* has been shown to form a fungicidal metabolite 8-hydroxylinoleic acid, which can have a role in fungicidal properties of the fungus.[⁵]

To the best of our knowledge, *L. arvalis* has not been associated with human infections. Ocular trauma with vegetative matter can predispose to keratitis by the fungus which might have been undiagnosed earlier by conventional phenotypic methods of identification. This is the first reported case of fungal keratitis caused by *L. arvalis*.

**CASE REPORT**

A 53-year-old female presented with pain, redness in her right eye since 10 days following fall of insect in the eye. She is not a known diabetic or hypertensive. On examination, her visual acuity in the right eye was hand movements only, conjunctiva had diffuse circumciliary congestion and cornea had an epithelial defect of 2.4 mm × 2.4 mm with surrounding stromal infiltrate measuring 4 mm × 3 mm and an associated hypopyon measuring 1 mm. As per our routine clinical protocol, a flame-sterilised Kimura spatula was used to obtain the scrape from the leading edge and base of the corneal ulcer. Sample was smeared and evaluated using Gram’s stain and 10% KOH mount and inoculated in sheep’s blood agar and potato dextrose agar. Fungal smears were considered positive when fungal elements were seen under low power magnification and reduced light. Fungal cultures were considered positive with growth on any two media or moderate-to-heavy growth on one medium. Fungal filaments were noticed on smear evaluation (Gram stain and KOH mount), following which the patient was...
started on topical natamycin (5%) eye drops. The patient was followed daily for a month, but the ulcer did not respond to monotherapy, following which 0.15% amphotericin-B eye drops was added. Perforation was noted 18 days following addition of amphotericin-B eye drops, for which cyanoacrylate glue and bandage contact lens were applied. Ulcer healed with formation of adherent leucoma with visual recovery up to 1/60 which improved further to 6/18 after a cataract surgery done 6 months later [Figure 1a and b]. Wooly colonies of the fungus covered the Petri dish containing blood agar and potato dextrose agar in 3 days, but the fungus could not be identified using conventional phenotypic methods due to lack of sporulation even after 4 weeks of inoculation. This prompted the use of polymerase chain reaction (PCR) and DNA sequencing.

DNA from the clinical fungal isolates was extracted by phenol-chloroform method with minor modification in the protocol. PCR targeting the internal transcribed spacer (ITS) region 1 and 4 was carried out in 50 μl reactions, containing 1 μl of 200 mM dNTPs, 5 μl of reaction buffer (Tris with Mgcl2), 10 μM forward primer (ITS1: 5' TCCGTAGGTGAACCTGCGG 3') and reverse primer (ITS4: 5' TCTTCCGCTTATTGATATG 3'), 1.2 U/μl of Taq polymerase and 2 μl of genomic DNA (500 ng/μl), and amplification was carried out in Thermal Cycler (BioRad-PTC 200 USA). The amplification of ITS region yielded 595 bp product [Figure 2], following which DNA sequencing of ITS amplicons was carried out after purification of amplified products using commercially available kit (Bio Basic Inc., Bangalore Genei, India). Cyclic PCR was carried out using Big Dye Terminator Ver 3.1 (Applied Biosystems). Sequencing reaction was carried out using Genetic Analyser 3130 (ABI). The sequences obtained then were used to perform nucleotide–nucleotide searches using the BLASTn database at the National Centre for Biotechnology Information website (http://www.ncbi.nlm.nih.gov/BLAST/). BLAST outputs were sorted based on maximum identity, and identifications were made when BLAST searches yielded 98% coverage and for closely related species >90% of query coverage.

We conducted a thorough MEDLINE search with predefined keywords to retrieve articles. To the best of our knowledge, L. arvalis as a corneal pathogen has not been reported previously. We deposited the sequence in the GenBank (GenBank Accession No: JF909549).

**DISCUSSION**

Laetisaria grows rapidly on malt-extract agar covering the Petri dish in 2–3 days. At first, wooly radiating aerial fibrils form, after 2–4 weeks they become more appressed, with scattered radiating aerial cordons sometimes possessing basidia, fruiting patches developing later between cordons.[1] Long time taken for sporulation and difficulty in recognising spores as the fungi are not expected in ocular samples can delay identification and hence delay the treatment. Molecular diagnostic techniques play a major role in identifying these rare causative fungal organisms which are generally grouped as ‘unidentified fungi’. The results of DNA sequencing are available within hours, hastening appropriate management.

There is no known case of human infection by L. arvalis reported in literature and the case presents first reported case of keratitis by L. arvalis. Injury with vegetative matter or soil infected with the fungus may cause keratitis with L. arvalis. In this case, the patient gave a history of injury due to fall of insect which could have been carrier of the fungus or might have caused corneal damage predisposing infection with opportunistic fungi. Ulcer showed an aggressive course and eventually perforated. Many novel fungi such as *Pythium insidiosum* and *Lophotrichus* have been reported to cause aggressive keratitis. Such novel causative fungi can cause epidemics of keratitis.[8] Sensitivity pattern of such causative fungi is unknown, unlike *Fusarium* and *Aspergillus* species.[10] This highlights the urgent need to be prepared for outbreaks of cases of keratitis by rare fungi. There is a need to identify

![Figure 1](image1.png)

Figure 1: (a) Slit lamp photograph at 3 weeks showing circumciliary congestion and active keratitis with 1 mm hypopyon in the anterior chamber (b) Slit lamp photograph at 3 months, showing residual scar with no circumciliary congestion

![Figure 2](image2.png)

Figure 2: Polymerase chain reaction: amplified product of unidentified fungal isolate. Lane 1–7: Amplified fungal DNA, NC: Negative control, M: Marker (100 bp), Lane 7: 595 bp
as many fungi as possible which can cause keratitis and study their epidemiology, clinical course and response to treatment. This will help in taking appropriate control measures and formulating a definitive treatment protocol for various fungi involved in fungal keratitis. The presented case highlights the role of molecular diagnosis in identifying the fungi-causing keratitis which remains unidentified by conventional phenotypic methods. DNA sequencing identifies organisms up to the species level within hours. This case report also stresses the aggressive course of keratitis caused by L. arvalis, unresponsive to topical antifungals.

Declaration of patient consent
The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest
There are no conflicts of interest.

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