We report the first known case of fungal keratitis caused by *Aspergillus niusius*. Ocular injury was known as a predisposing factor. The patient was treated with natamycin and econazole eye drops, itraconazole eye ointment, and oral ketoconazole. A therapeutic penetrating keratoplasty was performed 16 days after presentation. A sequence-based approach was used to assign the isolate to a species.

**CASE REPORT**

A 64-year-old woman with no significant ophthalmic or systemic history presented with a history of pain, redness, and defective vision of the right eye of 4 days' duration following a minor trauma sustained by mud splashing into the eye. She had been treated elsewhere earlier, and her medications consisted of the use of tobramycin, phenylephrine, natamycin, and moxifloxacin eye drops for 1 day.

On examination, her uncorrected visual acuity was less than 20/800 (<1/60) in the right eye. Slit lamp examination of the right eye was significant for an area of infiltration (6.2 to 7.5 mm) involving the central cornea and extending toward the limbus temporally and superiorly. The infiltrate involved all the layers of the stroma, and there was a dense endothelial plaque. A hypopyon of 1 mm was present. Scrapings obtained from the corneal infiltrate were stained (Gram's stain and 10% KOH) and plated on 5% sheep blood agar, chocolate agar, and potato dextrose agar. Both the Gram stain and the KOH mounts were positive for fungal filaments, and the cultures subsequently grew a fungus which was initially identified as a member of *Aspergillus* section *Flavi*.

Given the severity of the infection, the patient was admitted to the hospital, and intensive topical antifungal therapy was initiated. Natamycin (5%) and econazole (2%) eye drops were started on a half-hourly basis, while itraconazole (1%) eye ointment was applied three times a day. This was supplemented with cycloplegics (homatropine) and medication for the relief of pain. Oral antifungal medication in the form of tablets (200 mg ketoconazole twice a day) was also given.

In spite of intensive therapy, the infiltrate continued to progress with thinning and melting of the cornea. Doxycycline tablets were administered to decrease the collagenolytic activity, and amphotericin B (50 µg/ml) eye drops were also added to the medical regimen, but the infiltrate continued to progress to involve the entire cornea with descemetocele formation and finally perforation. A therapeutic penetrating keratoplasty was performed 16 days after presentation. Scleral extension was noted intraoperatively at the superior limbus. The corneal button removed at the time of surgery was also positive for a heavy growth of *Aspergillus*. Postoperatively the same medical regimen was continued in a tapering fashion, and the patient was discharged on the 10th postoperative day. After an interval of 35 days postoperatively, she once again presented with an area of infiltration at 11 o'clock of the superior sclera; this was treated successfully medically, and the infiltrate healed in a week. She was asked to continue natamycin and econazole (2%) four times a day along with itraconazole (1%) eye ointment three times and homatropine (2%) eye drops twice a day.

The patient was lost to follow-up until 40 days later, when she presented with pain and redness. Examination was remarkable for the presence of a tender nodule involving an area of the sclera from 7 o'clock to 9 o'clock. The patient was once again placed on intensive antifungal therapy with amphotericin B (50 µg/ml) eye drops and econazole (2%) eye drops being administered half-hourly, along with itraconazole (1%) eye ointment three times a day and ketoconazole (200 mg) twice daily. She was once again lost to follow-up and presented intermittently over the next 5 months with scleral involvement, either active scleritis or involvement with ocular congestion. Throughout the follow-up period, her vision in the right eye continued to be perception of light. The graft continued to be edematous.

Her compliance continued to be poor. At each presentation, she was placed on topical and systemic antifungals as well as topical and systemic nonsteroid anti-inflammatory drugs. Therapy always provided symptomatic relief as well as resolution of signs of scleritis. She was last seen 9 months after the initial presentation. Her vision in the right eye was positive for perception of light. An area of scleral thinning extending from 7 to...
12 o’clock was noted along with an area of persistent inflammation in the superonasal quadrant.

**Mycological study and diagnosis.** The case isolate was subcultured on Czapek Dox yeast extract and malt extract agar plates for morphological identification. The fungus was identified as *Aspergillus* species from section *Flavi* based on colony morphology and microscopic features of the isolate (Fig. 1). Colony surface on Czapek Dox yeast extract was velvety to floccose, consisting of white or light orange-brown vegetative mycelium and sparse to moderately abundant conidial structures. Colony reverse was light yellow or orange-brown. Sclerotia could not be observed. Conidiphores were variable in length (600 to 1,500 μm), uncolored, and echinulate (Fig. 1A). Conidial heads were yellowish green, biseriate, and radiate, often splitting into several columns. Vesicles were globose to subglobose, 45 to 65 μm in diameter. Conidia were globose to subglobose, echinulate, and 4 to 6 μm in diameter (Fig. 1B). Living cultures were deposited at the Department of Microbiology, Aravind Eye Hospital and Postgraduate Institute of Ophthalmology, Coimbatore, India (strain number: 823/07), and at the Centraalbureau voor Schimmelcultures (CBS 123901).

For the purposes of molecular identification, mycelia grown in liquid YPG medium (0.5% Bacto yeast extract, 0.5% Bacto peptone, 1% glucose) for 1 day were subjected to DNA isolation by the Masterpure yeast DNA purification kit (Epicentre Biotechnologies, Madison, WI) according to the manufacturer’s instructions. The internal transcribed spacer (ITS) region of the rRNA gene complex, incorporating ITS1, the 5.8S rRNA gene, and ITS2, was amplified using primers ITS1 and ITS4 (31). Part of the calmodulin gene was amplified using the primers cmd5 and cmd6 as described by Hong et al. (12), while a segment of the β-tubulin gene was amplified using primers bT2a and bT2b (10). DNA sequences were determined using a BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems Inc., Foster City, CA) and an ABI 3100 DNA sequencer. Sequence analysis was carried out by BLASTN similarity search at the website of the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/BLAST) (2). Molecular identification based on sequence analysis of ITS, β-tubulin, and calmodulin sequences revealed that the isolate belongs to the species *Aspergillus nomius*.

**Antifungal susceptibility testing.** The Etest method (AB Biodisk, Solna, Sweden) for molds was used to determine the MICs of the isolate to amphotericin B, fluconazole, itraconazole, ketoconazole, voriconazole, and caspofungin. In accordance with the manufacturer’s instructions, RPMI 1640 agar (15 g in 1,000 ml) supplemented with 20 g glucose per 1,000 ml medium was used in the tests (1). The MICs of natamycin (Natamet; 5% suspension; Sun Pharmaceutical Ind. Ltd., Halol, India), econazole (Aurozole; 2% suspension; Aurolab, Madurai, India), and clotrimazole (Auroclot; 1% suspension; Aurolab, Madurai, India) were determined by the broth microdilution technique NCCLS M38-A (22). Both the Etest and microdilution plates were incubated at 30°C for 72 h. *Candida parapsilosis* ATCC 22019 was used as the quality control for econazole, clotrimazole, ketoconazole, and amphotericin B during the susceptibility tests. Results obtained for these strains were in accordance with the quality control ranges published previously for these isolates (11, 24). Results of antifun-
The fact that the patient missed scheduled postoperative follow-up visits possibly contributed to the complications. It is uncertain whether the repeated episodes of scleral involvement were due to the spread of infection from the involved areas as noted during surgery or to recurrence of infection due to poor compliance. However, since this is the first such reported case of *A. nomius* infection, all possibilities remain hypothetical.

To our knowledge, the presented case of fungal keratitis is the first report on an ocular infection caused by *A. nomius* and, furthermore, the first known case of human disease with the involvement of this species from *Aspergillus section Flavi*.

**Nucleotide sequence accession numbers.** The GenBank accession numbers for the sequences of the case isolate are GQ221261 (ITS), GQ221262 (β-tubulin), and GQ221263 (calmodulin).

This work was supported by the Indian National Science Academy and the Hungarian Academy of Sciences within the frames of the Indo-Hungarian bilateral exchange program No.1A/INSA-HAS Project/2007 and by the intergovernmental project DST-TIF OMBP-00285/2008. L.K. is a grantee of the János Bolyai Research Scholarship.

**REFERENCES**


