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Case of Keratitis Caused by *Aspergillus tamarii*[▽]

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We report a case of *Aspergillus tamarii* keratitis. Ocular injury was known to be a predisposing factor. Topical natamycin and econazole treatment and subsequent systemic ketoconazole treatment proved effective. The isolate was identified by morphological characteristics and sequence analysis as *A. tamarii*, a member of *Aspergillus* section *Flavi* not hitherto reported from keratomycosis.

CASE REPORT

A 32-year-old female from Coimbatore was presented to the Aravind Eye Hospital, Coimbatore, South India, on December 27, 2005, with complaints of pain, redness, and defective vision of a 4-day duration in the left eye. She indicated that she had suffered an ocular injury caused by an iron piece while hammering 4 days earlier. On examination, her uncorrected visual acuities in the right and left eyes were 6/9 (partial) and 1/2/60, respectively. An anterior segment examination of the left eye showed lid edema and conjunctival congestion. The cornea showed a central 3-by-3-mm ulcer with an anterior midstromal infiltrate with feathery edges and surrounding edema. The anterior chamber showed a moderate number of cells (2+ grade). The lens was clear. The anterior segment of the right eye and the posterior segments of both eyes were within normal limits.

With due aseptic precautions, the ulcer was scraped and two smears were made on glass slides for a 10% KOH wet mount and Gram staining. The microscopic examination of the KOH wet mount and Gram staining showed fungal filaments. Material from scraping was also directly inoculated onto potato dextrose agar and incubated at 25°C. Based on the colony appearance, the fungus was identified as an *Aspergillus* sp. Topical antifungal therapy was started with 5% natamycin suspension and 2% econazole drops every half hour, along with 1% homatropine three times a day.

When reviewed after 3 days, the patient's uncorrected visual acuity in the left eye had improved to 6/36, but the corneal midstromal infiltrate was still active. The anterior chamber showed a hypopyon of 0.5 mm. The patient was admitted as an inpatient and advised to continue the same medications along with 200 mg oral ketoconazole and 0.2% subconjunctival fluconazole based on the results of our previous study (6). The patient showed improvement during the next 3 weeks; the infiltrate reduced gradually, and the anterior segment inflammations subsided. On the last review, the anterior segment of the left eye showed a central nebular scar, with the best corrected visual acuity having improved to 6/12. The patient was advised to use glasses and to report for review after 6 months.

The clinical isolate was further examined at the CBS Fungal Biodiversity Centre and at the University of Szeged for species assignment and antifungal susceptibility tests.

Mycological study and diagnosis. The fungus was subcultured on malt extract agar plates and identified as *Aspergillus tamarii* Kita based on the colony morphology and microscopic features of the isolate (Fig. 1 and 2). Colonies on malt extract agar at room temperature attained diameters of 6.0 to 7.0 cm in 10 days, producing abundant conidial heads in dull yellowish green shades becoming metallic bronze at maturity (24). The conidiophore stipe was hyaline and rough walled; the conidial heads were radiate; the vesicles were globose to subglobose, 25 to 50 μm in diameter. The phialides were borne directly on the vesicle or on metulae (mostly on large heads). The conidia were globose to subglobose, 5 to 6.5 μm in diameter, and brownish yellow. However, in contrast with those of typical wild *A. tamarii* isolates (Fig. 2A), some conidia of this isolate were not ornamented with tubercules and warts but were smooth walled and hyaline (Fig. 2B). The isolate grew well at 37°C but was unable to grow at 42°C on malt extract agar medium.

For 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 a Masterpure yeast DNA purification kit (Epicenter Biotechnologies, Madison, WI) according to the manufacturer's instructions. The internal transcribed spacer (ITS) region of the rRNA gene complex, incorporating ITS 1, the 5.8S rRNA gene, and ITS 2, was amplified using primers ITS1 and ITS4 (26). A segment of the calmodulin gene was amplified using 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 (9). DNA sequences were determined using a BigDye Terminator v3.1 cycle sequencing kit

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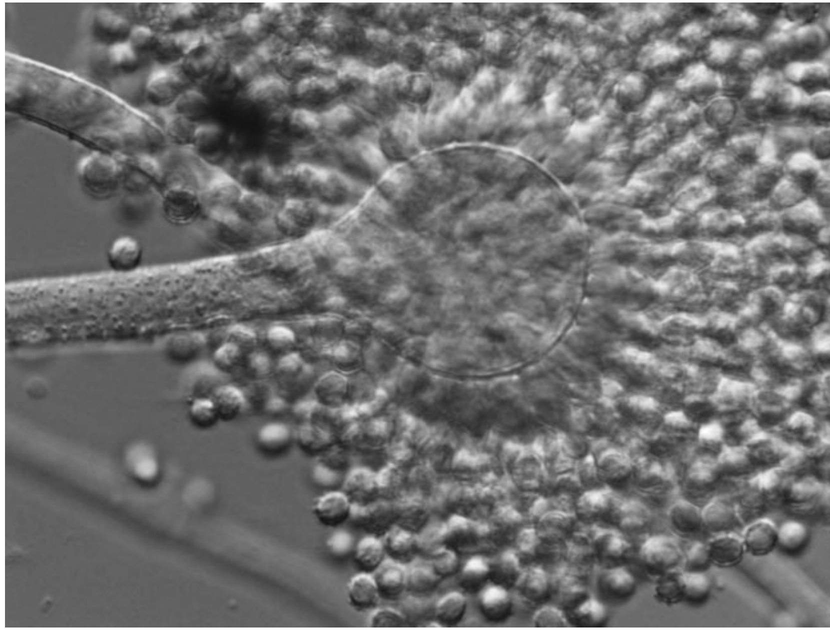

FIG. 1. Conidial head of *A. tamarii* 2342/05.

(Applied Biosystems Inc., Foster City, CA) and an ABI 3100 DNA sequencer. Both strands of each fragment were sequenced. The resulting sequences were deposited in the GenBank database. Sequence analysis was carried out by a BLASTN similarity search (2) at the website of the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/blast/).

Table 1 lists *A. tamarii* sequences with complete homology to those of the case isolate. The ITS, tubulin, and calmodulin sequences of the case isolate proved to be completely identical to the corresponding sequences of *A. tamarii* strain NRRL 25565 (13).

Living cultures were deposited at the Department of Microbiology, Aravind Eye Hospital and Postgraduate Institute of Ophthalmology, Coimbatore, India (strain number 2342/05), and at the Centraalbureau voor Schimmelcultures (CBS 121598).

Antifungal susceptibility testing. The Etest method (AB BIODISK, Solna, Sweden) for molds was applied for the determination of MICs of amphotericin B, fluconazole, itracona-

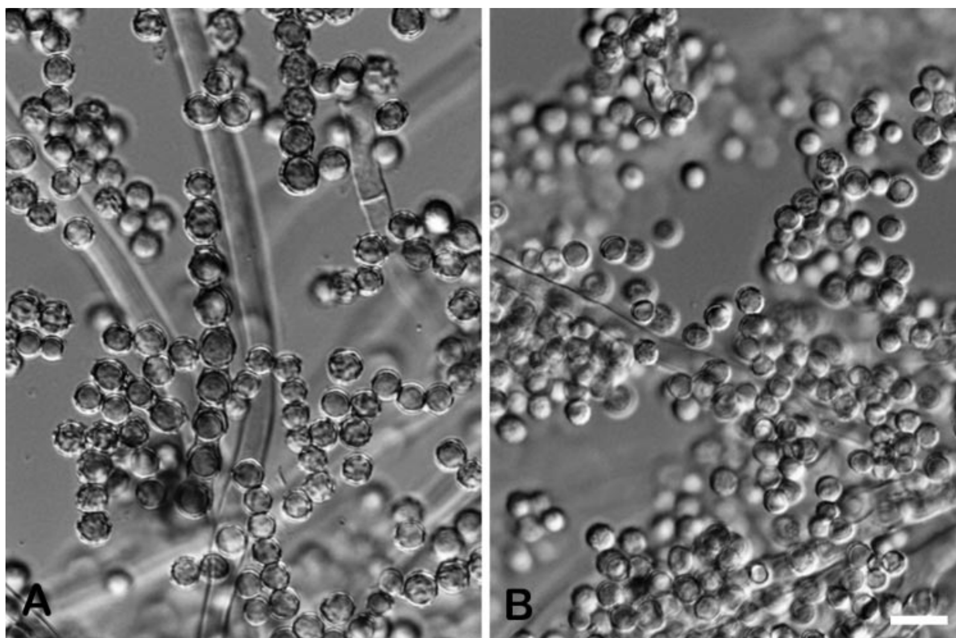

FIG. 2. Conidia of a typical *A. tamarii* strain (CBS 484.65) (A) and that of *A. tamarii* 2342/05 (B). The scale bar represents 10 μm.

TABLE 1. GenBank sequences with 100% similarity to the ITS (EF525554), β-tubulin (EF525555), and calmodulin (EF525556) sequences of strain 2342/05

Locus	GenBank accession no.	Species	Strain designation	Reference
ITS	AB008420	*A. tamarii*	JCM2259	19
ITS	AF004932	*A. tamarii*	NRRL 26066	21
ITS	AF272579	*A. tamarii*	NRRL 26594	13
ITS	AF272576	*A. tamarii*	NRRL 25565	13
ITS	AF004929	*A. tamarii*	NRRL 20818	13
ITS	D84358	*A. tamarii*	A0754	16
ITS	AY373870	*A. tamarii*	SRRC 1088	11
ITS	AY213635	*A. tamarii*	UWFP534	22
β-Tubulin	AF255074	*A. tamarii*	NRRL 26594	13
β-Tubulin	AF255071	*A. tamarii*	NRRL 25565	13
β-Tubulin	AF255069	*A. tamarii*	NRRL 20818	13
Calmodulin	AF255033	*A. tamarii*	NRRL 25565	13
Calmodulin	AF255032	*A. tamarii*	NRRL 26594	13

zole, ketoconazole, and voriconazole according to the manufacturer's instructions on RPMI 1640 agar (15 g in 1,000 ml) supplemented with 20 g glucose per 1,000 ml medium (1). The Etest drug concentrations ranged from 0.016 to 256 μg/ml for fluconazole and from 0.002 to 32 μg/ml for itraconazole, ketoconazole, voriconazole, and amphotericin B. The MIC was read as the drug concentration at which the elliptical inhibition zone intersected the scale of the Etest strip. The MICs of natamycin and econazole were determined by the broth microdilution method according to NCCLS M38-A in RPMI broth (18). The MICs for natamycin, amphotericin B, fluconazole, itraconazole, ketoconazole, voriconazole, and econazole proved to be >1,024, 0.125, >256, 0.064, 0.25, 0.125, and 0.064 μg/ml, respectively.

Discussion. Corneal infections of fungal etiology are very common and represent 30% to 40% of all cases of culture-positive infectious keratitis. Combating fungal keratitis is of special importance in India, which harbors the largest agrarian population at risk for developing blindness due to the limited availability of antifungal drugs and the lack of response during therapy. Certain *Aspergillus* species, mainly *Aspergillus flavus* (23, 25), *Aspergillus terreus* (25), *Aspergillus fumigatus* (23, 25), and *Aspergillus niger* (4), have long been regarded as important pathogens in eye infections, especially keratitis. Other members of the genus less frequently occurring in keratitis include *Aspergillus glaucus* and *Aspergillus ochraceus*. Most of the *Aspergillus* strains isolated from keratomycosis patients are being identified and reported at the genus level only (10). Their molecular identification at the species level would be of great importance, as the pathogenic potential may vary between different species of the genus.

A. tamarii is a member of *Aspergillus* section *Flavi* (8). This species is widely used in the food industry for the production of soy sauce (known as red Awamori koji) (14) and in the fermentation industry for the production of various enzymes, including amylases, proteases, and xylanolytic enzymes (3, 7, 17). Although *A. tamarii* is able to produce several toxic secondary metabolites, including cyclopiazonic acid and fumigaclavines (24), it has rarely been encountered as a human pathogen. The only known cases are an eyelid infection (5), invasive nasosinusal aspergillosis in an immunocompetent patient (20), and onychomycosis in a 3-year-old boy (15). To our knowledge, the present case of fungal keratitis is the first report on an ocular infection caused by *A. tamarii* and the fourth known case worldwide involving this unusual opportunistic human pathogen.

Nucleotide sequence accession numbers. The GenBank accession numbers for the sequences of the case isolate are EF525554 (ITS), EF525555 (β-tubulin), and EF525556 (calmodulin).

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