Aspergillus tamarii keratitis in a contact lens wearer

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ABSTRACT

Keratitis produced by Aspergillus tamarii has been previously described associated to an ocular injury. We report a case in a contact lens wearer with a history of previous bilateral myopic LASIK ablation, bilateral intracorneal rings and vitrectomy and scleral buckling in his left eye. The fungus could be quickly identified combining phenotype, microscopy and mass spectrometry. Treatment with intravenous amphotericin, oral voriconazole, and topical amphotericin and natamycin and voriconazole was needed for corneal preservation.

1. Introduction

Fungal eye infections can be very severe and rapid diagnosis is crucial for a successful treatment. Mass spectrometry is being available increasingly in most clinical microbiology laboratories and can be very useful for a prompt diagnosis. A case of keratitis by A tamarii is presented where microscopy, culture and mass spectrometry were sequentially used for the management of the patient.

2. Case report

In August 2015 a patient attended our emergency department complaining of six days of blurred vision, pain and discharge in his left eye (day 0). The symptoms had started four days before, when he was on a tourist trip in Paris, where he was prescribed antibiotic eyedrops (ticarcillin, gentamicin and vancomycin every hour). His previous ophthalmic history included bilateral myopic LASIK ablation, bilateral intracorneal rings and vitrectomy and scleral buckling in his left eye. The fungus could be quickly identified combining phenotype, microscopy and mass spectrometry. Treatment with intravenous amphotericin, oral voriconazole, and topical amphotericin and natamycin and voriconazole was needed for corneal preservation.
misidentifying of *A. tamarii* and *A. nomius* as *A. flavus* when only phenotypic methods are used.

Identification of the microorganism of the specimen was based on the nucleotide sequences of its ITS region [3] and its conserved regions of the calmodulin "cmd" [4] and betatubulin "bt2" [5] genes. For that purpose, 100 ml buffered flasks containing 30 ml of Potato Dextrose Broth were inoculated with a single colony of the microorganism in duplicate and incubated for 48 h (30 °C, shaking) to obtain pure cultures of the specimen. Total DNA was extracted from each culture using the "UltraClean® Microbial DNA Isolation Kit" and DNA quality assessed by spectrophotometry. ITS, cmd and bt2 regions were amplified by PCR using AmpliTaq Gold® Master Mix and the primer pairs ITS1/ITS4 and ITS5/ITS4, cmd5/6 and bt2a/bt2b, respectively. Amplicons were verified by electrophoresis on 1.2% agarose gels (Fig. 4) and sequenced with the corresponding primers used for their amplification. Nucleotide sequences were analyzed by the Chromas Lite program and assembled with CAP3. No differences were found in the genetic sequences of either DNA sample (see Annex I and II). Finally, the nucleotide sequences of the ITS, cmd and bt2 regions were blasted against GenBank using the NCBI/BLAST tool (see Annex III and IV).

Identity percentages were greater than 99% with respect to different types of collection strains of *Aspergillus tamarii* (strain NRRL20818, ITS region, accession number, NR135325; strain NRRL 4966, calmodulin gene, accession number, EF661527; strain NRRL20818, betatubulin gene, accession number, AY017540).

The Etest method (bioMerieux) for molds was performed for determining the MICs to guide the antifungal treatment. Strips containing amphotericin B (0.002–32 µg/ml), fluconazole (0.016–256 µg/ml), posaconazole (0.002–32 µg/ml), and caspofungin (0.002–32 µg/ml), were used on RPMI agar (bioMerieux). After 48 h of incubation at 30 °C, the CMI was read as the drug concentration at which the inhibition zone intersected the Etest strip. *A. tamarii* showed the following CMIs:

- 0.25 µg/ml to amphotericin B,
- > 256 µg/ml to fluconazole,
- 0.5 µg/ml to caspofungin,
- 0.094 µg/ml to posaconazole.

The Broth microdilution method (BMD) was carried out in the Spanish National Center of Microbiology according to methodology developed by the Antifungal Subcommittee of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [6]. The MIC values obtained by BMD were as follows:

- 0.25 µg/ml to amphotericin B,
- 0.5 µg/ml to voriconazole,
- 0.06 µg/ml to posaconazole,
- 0.5 µg/ml to caspofungin,
- 0.12 µg/ml to miconafungin and
- 0.5 µg/ml to anidulafungin.

Due to the progressive worsening of the corneal ulcer, the patient was admitted to hospital and started treatment with topical voriconazole 1% and intravenous liposomal amphotericin B (2 mg/kg/day), once the initial E-test susceptibility tests were known. After 10 days of IV treatment, the intracorneal rings in his left eye were removed due to progressive corneal melting and the development of hypopyon in
order to improve corneal trophism and healing (day +30). At the end of the surgical procedure of rings extraction, amphotericin B was applied intrastromal and in the corneal tunnels. Five days later (day +35), the corneal melting halted its progression and intravenous treatment was ended and the patient was discharged for daily outpatient follow up with topical natamycin 5% every two hours, topical voriconazole 1% every two hours, topical atropine 1% every 12 h, oral voriconazole (200 mg/12 h), oral dexamethasone (100 mg/24 h), and Vitamin C (1 tablet/24 h). One week later (day +42), topical preservative-free dexamethasone 1 mg/ml every 72 h was added due to excessive anterior segment inflammatory reaction. Five months after its first visit (day +170), the patient shows no epithelial defect, hypopyon, hypermia, nor corneal swelling. However, he presented an extensive corneal scar, with corneal vessels, posterior synechiae and cataract (Fig. 5). The patient received at this time still with topical voriconazole and natamycin every 6 h, topical dexamethasone every 12 h and oral voriconazole (200 mg/12 h). A very slow tapering process of topical medication was needed in our case, as a first attempt of rapid withdrawal of topical treatment lead us to clinical worsening and the need to re-start high dose topical antifungal treatment.

3. Discussion

Fungal keratitis can account for up 50% of all culture proven microbial keratitis [7,8] and those produced by filamentous fungi have been described frequently in rural areas of India, China and Brazil and associated to eye trauma [9–12]. However, in countries like USA, the use of contact lens was the most common predisposing factor (37% of the patients), well ahead of ocular trauma (25%), as a large retrospective multicenter study (n = 733) has shown [8]. In a 3 year long survey performed in India with 3183 corneal ulcers, 30% were mycotic and Fusarium sp and Aspergillus sp caused up to 70% of all these fungal infections [12]. Non filamentous fungi like yeasts were more common in patients with ocular surface disease [8]. Our patient presented clear predisposing factors as previous ocular surgery (LASIK myopic ablation, intracorneal rings implantation, vitrectomy and sceleral buckling) and was a contact lens wearer. The identification of Aspergillus tamarit in this corneal abscess was possible just 24 h after culturing the specimens by the use of mass spectrometry technology [2] prompting the use of topical voriconazole, an antifungal previously reported as active for this species [13]. Although topical natamycin has been found more effective than voriconazole in the treatment of fungal ulcers [14], was not available at this moment in our center and could not be initially used. Due to the severe condition of the patient, IV amphotericin treatment was initiated, because our strain was sensitive according to the results of the E-test, following the results of a recent publication [15], where a comparison between the standard broth dilution method (BMD) [6] and the Etest showed >90% of agreement, as we could corroborate later with our own strain.

Although more than 70 species of Aspergillus have been described as pathogenic to the cornea [16], this is only the second case of ocular infection by A tamarit described so far. We can only speculate that the protacted and torpid evolution of the patient could be due to some of the toxic secondary metabolites as cyclopiazonic and fumigacavines produced by this species [13]. Other clinical reports of infections caused by A tamarit are one case of mycotic keratitis [13] and single cases of eyelid, nasosinusial, onychomycosis and cutaneous aspergillosis [17–20]. As this case shows, the combination of microscopy, culture and rapid mass spectrometry can provide also an adequate and rapid microbiological diagnosis in exceptionally rare fungal infections.

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Conflict of interest

There are no conflicts of interest.

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