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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 corneal ectasia treated bilaterally with intracorneal rings and retinal detachment in his left eye treated with vitrectomy and scleral buckling (16, 8 and 6 years before, respectively). At the moment of the diagnosis, he was also using contact lenses in both eyes to correct his residual refractive error. The physical examination of his left eye showed a corneal abscess in the nasal region of 2,7 mm of biggest diameter with surrounding corneal oedema, normal intraocular pressure (IOP) and a best corrected visual acuity of 0.1 (decimal scale). A bacterial corneal infection was suspected and topical treatment was started with topical vancomycin (50 mg/ml) and ceftazidime (50 mg/ml) every hour, oral ciprofloxacin (500 mg twice a day) and topical 1% atropine twice a day. After twenty days of a favorable evolution, when topical antibiotics were tapered until one drop every 3 h, the clinical features worsened with an increase in epithelial defect, stromal thinning, corneal and conjunctival swelling and more pain (day +20).

Samples from the corneal ulcers were taken for microbiological studies. The Gram stain of a corneal scraping taken with a intramuscular (IM) needle showed numerous hyphae (Fig. 1) and treatment was initiated immediately with topical amphotericin B 0,5%, as it was the only antimycotic topical drug available in our center at that time. In the following 24 h grew a velvety hyaline fungus, which was initially identified by microscopy as belonging to the genus *Aspergillus* spp. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS, Bruker Biotyper system) [1] was performed on colonies pretreated with formic acid and identified the strain as *Aspergillus tamarii* (certainty grade of 1.628) (day +21).

The sample was inoculated onto Sabouraud glucose agar and incubated at 30 °C. In the following days the fungus grew rapidly covering all the plate. Colonies were olive green in the surface (Fig. 2) and white-pale yellow on the reverse side, which became darker with time. They appeared rough and powdery with a wavy edge. Blue lactophenol staining showed septate hyphae, with 5–7.5 μm diameter rough conidiophores which ended into a globose vesicle, 35–50 μm diameter. Uniseriate phialides were scattered across the surface of the vesicle (Fig. 3). Previous reports [2] have warned about the common

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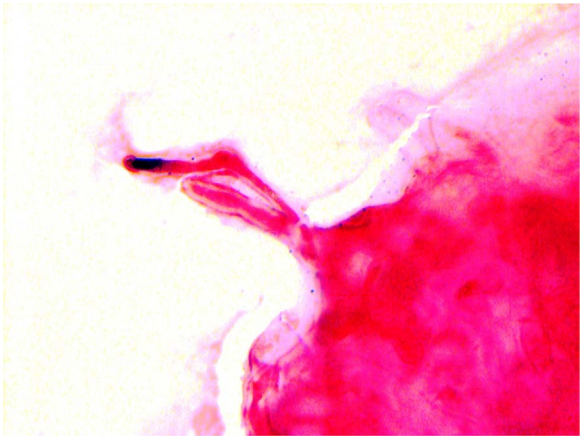

Fig. 1. Hyphae on Gram´ stain (× 1000 high power field, scale bar in microns).

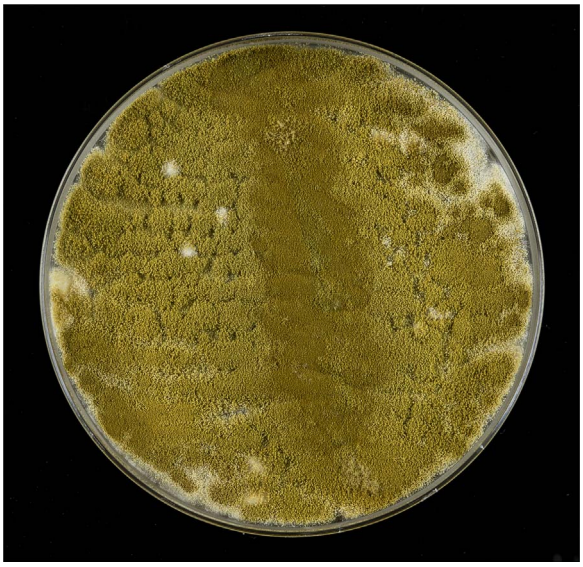

Fig. 2. Olive green velvety mold in Saboureaud´s medium.

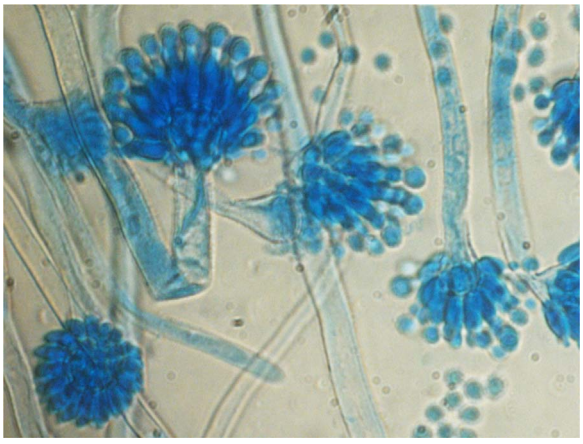

Fig. 3. Conidiophores with uniseriate phialides across the vesicles (× 400).

misidentifying of *A tamarii* and *A nomius* as *A flavus* when only phenotipic 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 buffled flasks containing 30 ml of Potato Dextrose

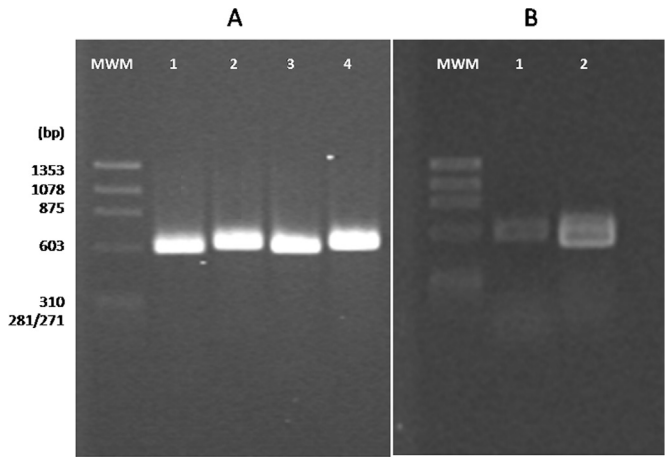

Fig. 4. Agarose gel electrophoresis of amplicons obtained by PCR amplification of total DNA from pure cultures of the specimen. MWM: Φ174 *Hae*III. Template DNA sample 1 (lanes 1 and 2) and sample 2 (lanes 3 and 4). **A**: Primer pair ITS 1/4 (lanes 1 and 3) or ITS 5/1 (lanes 2 and 4) using the following PCR program: 95 °C for 2 min followed by 34 cycles of 95 °C for 30 s, 55 °C for 30 s, 72 °C for 1 min, and a final extension at 72 °C for 10 min **B**: Template DNA sample 1 with primer pair cmd5/6 (lane 1) or bt2a/b (lane 2) using the following PCR program: 95 °C for 2 min followed by 32 cycles of 94 °C for 1 min, 58 °C for 1 min, 72 °C for 1 min, and a final extension at 72 °C for 6 min.

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® 360 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, calmoludin 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 caspofungine (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 caspofungine and 0.094 μg/ml to posaconazole.

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 micafungin 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/kd/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

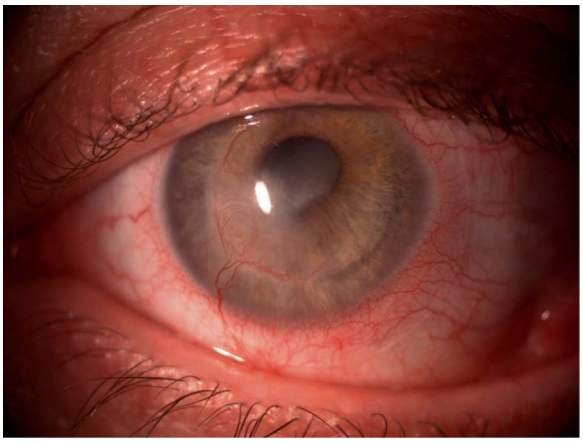

Fig. 5. Extensive corneal scar with corneal vessels, posterior synechiae and cataract after 4 months of treatment with topical and systemic antifungals.

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 doxicicline (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 his first visit (day +170), the patient shows no epithelial defect, hypopyon, hyperemia, 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 Brasil 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 scleral buckling) and was a contact lens wearer. The identification of *Aspergillus tamarii* 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 tamarii 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 fumigaclavines produced by this species [13]. Other clinical reports of infections caused by *A tamarii* are one case of mycotic keratitis [13] and single cases of eyelid, nasosinusal, 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.

References

[1] R. Patel, Matrix-assisted laser desorption ionization–time of flight mass spectrometry in clinical microbiology, Clin. Infect. Dis. 57 (2013) 564–572.
[2] E.W. Tam, J.H. Chen, E.C. Lau, A.H. Ngan, K.S. Fung, K. Lee, et al., Misidentification of Aspergillus nomius and Aspergillus tamarii as Aspergillus flavus: characterization by internal transcribed spacer, β-tubulin, and calmodulin gene sequencing, metabolic fingerprinting, and matrix-assisted laser desorption ionization–time of flight mass spectrometry, J. Clin. Microbiol. 52 (2014) 1153–1160.
[3] T.J. White, T. Bruns, S. Lee, J. Taylor, Amplification and direct sequencing of fungal ribosomal RNA Genes for phylogenetics, in: M.A. Innis, D.H. Gelfand, J.J. Sninsky, T.J. White (Eds.), PCR Protocols : A guide to Methods and Applications, 322 Academic Press, San Diego, U.S.A, 1990.
[4] S. Hong, H. Cho, H. Shin, J.C. Frisvad, R.A. Samson, Novel Neosartorya species isolated from soil in Korea, Int. J. Syst. Evol. Microbiol. 56 (2006) 477–486.
[5] N.L. Glass, G.C. Donaldson, Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes, Appl. Environ. Microbiol. 61 (1995) 1323–1330.
[6] Subcommittee on Antifungal Susceptibility Testing (AFST) of the ESCMID European Committee for Antimicrobial Susceptibility Testing (EUCAST. EUCAST Technical Note on the Method for the Determination of Broth Dilution Minimum Inhibitory Concentrations of Antifungal Agents for Conidia–forming Moulds, Clinical Microbiology and Infection, 14, 2008, pp. 982–984.
[7] P.A. Thomas, J. Kaliamurthy, Mycotic keratitis: epidemiology, diagnosis and management, Clin. Microbiol. Infect. 19 (2013) 210–220.
[8] L.J. Keay, E.W. Gower, A. Iovieno, R.A. Oechsler, E.C. Alfonso, A. Matoba, et al., Clinical and microbiological characteristics of fungal keratitis in the United States, 2001–2007: a multicenter study, Ophthalmology 118 (2011) 920–926.
[9] L. Xie, W. Zhong, W. Shi, S. Sun, Spectrum of fungal keratitis in north China, Ophthalmology 113 (2006) 1943–1948.
[10] R. Nath, S. Baruah, L. Saikia, B. Devi, A.K. Borthakur, J. Mahanta, Mycotic corneal ulcers in upper Assam, Indian J. Ophthalmol. 59 (2011) 367.
[11] A. Chowdhary, K. Singh, Spectrum of fungal keratitis in North India, Cornea 24 (2005) 8–15.
[12] M.J. Bharathi, R. Ramakrishnan, R. Meenakshi, S. Mittal, C. Shivakumar, M. Srinivasan, Microbiological diagnosis of infective keratitis: comparative evaluation of direct microscopy and culture results, Br. J. Ophthalmol. 90 (2006) 1271–1276.
[13] L. Kredics, J. Varga, S. Kocsub, I. Dczi, R.A. Samson, R. Rajaraman, et al., Case of keratitis caused by Aspergillus tamarii, J. Clin. Microbiol. 45 (2007) 3464–3467.
[14] N.V. FlorCruz, J.R. Evans, Medical Interventions for Fungal Keratitis, The Cochrane Library, 2015.
[15] F. Lamoth, B.D. Alexander, Comparing etest and broth microdilution for antifungal susceptibility testing of the most-relevant pathogenic molds, J. Clin. Microbiol. 53 (2015) 3176–3181.
[16] L. Kredics, V. Narendran, C.S. Shobana, C. Vgvlgyi, P. Manikandan, Filamentous fungal infections of the cornea: a global overview of epidemiology and drug sensitivity, Mycoses 58 (2015) 243–260.
[17] S. Sharma, B.M. Yenigalla, S.K. Naidu, P. Pidakala, Primary cutaneous aspergillosis due to Aspergillus tamarii in an immunocompetent host, BMJ Case Rep. 2013 (2013) 010128.

[18] G. Paludetti, M. Rosignoli, E. Ferri, M.R. Cesari, G. Morace, M. Fantoni, et al., Invasive nasosinusal aspergillosis in an immunocompetent patient, Acta otorhinolaryngologica Italica: organo ufficiale della Societ italiana di otorinolaringologia e chirurgia cervico-facciale 12 (1992) 581–591.

[19] L. Kristensen, J. Stenderup, A. Otkjaer, Onychomycosis due to Aspergillus tamarii in a 3-year-old boy, Acta Derm. Venereol. 85 (2005) 261–262.

[20] R. Degos, G. Segretain, G. Badillet, A. Maisler, Aspergillosis of the eyelid, Bull. Soc. Fr. Dermatol. Syphiligr. 77 (1970) 732–734.