Mycotic Keratitis Caused by *Fusarium solani sensu stricto* (FSSC5): A Case Series

Hazal Boral · Anne van Diepeningen · Elif Erdem · Meltem Yağmur · G. Sybren de Hoog · Macit Ilkit · Jacques F. Meis · Abdullah M. S. Al-Hatmi

Received: 21 January 2018 / Accepted: 11 June 2018 / Published online: 21 June 2018

© Springer Nature B.V. 2018

**Abstract** Owing to a lack of appropriate diagnostic and therapeutic approaches for mycotic keratitis, approximately one million cases of preventable corneal blindness are reported each year. The number of keratitis cases due to infection with *Fusarium* is increasing significantly worldwide, many of which are not treated adequately and in a timely manner due to frequent misdiagnosis. In the current report, we describe three cases of keratitis caused by *Fusarium solani sensu stricto* (FSSC5) from Turkey and The Netherlands, following ocular trauma. The etiological agent of keratitis, FSSC5, identified by sequencing of the partial *tef1*-α gene, exhibited low minimum inhibitory concentrations (MICs) of 1 μg/mL for amphotericin B and high MICs above the published epidemiological cutoff values for voriconazole (8 μg/mL). Patients were successfully treated with topical amphotericin B and voriconazole with complete recovery.

Handling Editor: Philip Aloysius Thomas.

H. Boral · M. Ilkit (✉)
Division of Mycology, Department of Microbiology, Faculty of Medicine, University of Çukurova, 01330 Adana, Turkey
e-mail: macitilkit@gmail.com

A. van Diepeningen
BU Biointeractions and Plant Health, Wageningen University and Research, Wageningen, The Netherlands

E. Erdem · M. Yağmur
Department of Ophthalmology, Faculty of Medicine, University of Çukurova, Adana, Turkey

G. S. de Hoog · A. M. S. Al-Hatmi
Westerdijk Fungal Biodiversity Institute, Royal Dutch Academy of Arts and Sciences, Utrecht, The Netherlands

G. S. de Hoog · J. F. Meis · A. M. S. Al-Hatmi
Centre of Expertise in Mycology Radboud University Medical Centre/Canisius Wilhelmina Hospital, Nijmegen, The Netherlands
Keywords  Amphotericin B · Chlorhexidine gluconate · Keratomycosis · Soft contact lenses · Voriconazole

Introduction

Mycotic keratitis is classified as an ophthalmological emergency. The incidence of this disorder has increased over the last 30 years. Some of the possible causes of keratitis due to *Fusarium* species are the frequent use of topical corticosteroids, overuse of antibacterial agents, and the use of contact lenses [1, 2]. *Fusarium* keratitis is one of the most common fungal infections of the cornea. This infection may have severe complications, especially in individuals who wear contact lenses or work in outdoor fields [3].

Early-stage *Fusarium* keratitis remains a diagnostic and therapeutic challenge for ophthalmologists. Nearly 50% of all fusariosis cases are attributed to members of the *Fusarium solani* species complex (FSSC). The most commonly reported species within the FSSC include *F. falciforme* (FSSC3–4), *F. keratooplasticum* (FSSC2), *F. lichenicola* (FSSC16), and *F. petroliphilum* (FSSC1). *Fusarium solani* sensu stricto (FSSC5) and *F. metavorans* (FSSC6) [4] are rarely reported, along with several currently unnamed phylogenetic species.

Species-level identification of *Fusarium* strains that cause keratitis is important, since antifungal susceptibilities may vary between different species of the genus [5]. Confocal microscopy and anterior segment optical coherence tomography are used to determine the clinical features of the infected eye and make an initial diagnosis of mycotic keratitis [6]. However, standardization of culture, microscopy, and molecular identification is required for proper detection and identification of the pathogen [1]. Laboratory approaches begin with a Gram stain of the corneal scraping material, followed by a wet preparation of the sample using potassium hydroxide (KOH), lactophenol cotton blue, Giemsa, or an optical brightener, and culture from scrapings or biopsies [2]. Subsequent identification is done by PCR and sequencing, for which identification of partial *tef1*-α is recommended, in the case of *Fusarium* [7]. Furthermore, matrix assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) has emerged as a promising tool for the identification and diagnosis of yeast and mold infections, including *Fusarium* [8].

Here, we report three cases of mycotic keratitis, caused by the recently described species *F. solani* sensu stricto (FSSC5) [9] that were diagnosed and treated in the early stages of the disease.

Case 1

A 15-year-old male was referred to the Cornea Service Section at the Department of Ophthalmology, Faculty of Medicine, Çukurova University (Adana, Turkey), with complaints of keratitis in the left eye that did not respond to topical antibiotic (moxifloxacin) treatment. The patient had a history of ocular trauma, due to the splashing of soil into both eyes, 10 days prior to presentation. He had no previous history of ocular or systemic disorders. The presenting symptoms were blurred vision, redness, burning, and pain in the left eye. A slit-lamp examination revealed a diffuse conjunctival hyperemia and a paracentral dense corneal infiltration on the left eye (Fig. 1a). The best-corrected visual acuity was 1.0 in the right eye and 0.6 in the left eye. No abnormalities were observed upon fundus examination of either eye. The patient was hospitalized with a preliminary diagnosis of mycotic keratitis, caused by a history of trauma with organic matter and a feathery margin around the infiltrate that was unresponsive to topical antibiotic medications. Fortified voriconazole (10 mg/mL) was administered topically every hour. The clinical symptoms improved rapidly within 3 days. The conjunctival hyperemia disappeared, the size of the infiltration reduced, the transparency of the perilesional cornea increased, and the visual acuity improved to 0.9 in the affected eye (Fig. 1b). The best-corrected visual acuity was 1.0 in the right eye and 0.6 in the left eye. No abnormalities were observed upon fundus examination of either eye. The patient was hospitalized with a preliminary diagnosis of mycotic keratitis, caused by a history of trauma with organic matter and a feathery margin around the infiltrate that was unresponsive to topical antibiotic medications. Fortified voriconazole (10 mg/mL) was administered topically every hour. The clinical symptoms improved rapidly within 3 days. The conjunctival hyperemia disappeared, the size of the infiltration reduced, the transparency of the perilesional cornea increased, and the visual acuity improved to 0.9 in the affected eye (Fig. 1b). Cytological examination using PAS 3 days after presentation revealed the presence of fungal hyphae (Fig. 1c). Cultures proved that the infection was due to a member of the FSSC.

At the end of the second week, voriconazole dosing frequency was reduced to six times per day and treatment was discontinued within 2 months. The patient showed complete resolution of the disease 20 days after presentation. The last visit was in the eighth month of follow-up examination; the visual acuity was 1.0, and a superficial semi-translucent scar was apparent at the location of the lesion, out of the visual axis (Fig. 1d).
Case 2

A 61-year-old Dutch female, who wore soft contact lenses, with complaints of pain in the left eye 5 days after bathing in a city swimming pool and 4 days after working in her garden, was examined by the ophthalmologist. There was no recollection of any trauma. Previously, a general physician had prescribed topical tobradex (a combination of 1 mg/mL dexamethasone and 3 mg/mL tobramycin) eight times a day but with no improvement after 5 days. Slit-lamp examination showed an ulcer (1.5 × 1.7 mm) with satellite lesions. A hypopyon was not present. Cultures identified mycotic keratitis caused by FSSC. Treatment was started with amphotericin B (2.5 mg/mL), combined with chlorhexidine gluconate (0.2%), every 30 min for the first 24 h, every hour for the second 24 h, and then the dosage was tapered to eight times a day for another 18 days. The patient showed complete resolution of the disease 20 days after presentation. At the last visit in the fifth month of follow-up examination, a translucent scar (2.3 × 1.8 mm) without infiltrates was still apparent.

Case 3

A 58-year-old Dutch female, who wore soft contact lenses, with a painful red left eye was examined by the ophthalmologist. She had no recollection of any trauma. Examination revealed several superficial infiltrates. She was initially treated with tobradex and ofloxacin (3 mg/mL) eight times a day. After 3 days, a member of the FSSC was detected in the cultures, after which treatment was changed to amphotericin B (2.5 mg/mL) and voriconazole (10 mg/mL). Recovery was slow, and after 2 weeks of treatment, a penetrating cornealplasty was necessary. Amphotericin B and voriconazole drop therapy were continued for another 2 weeks. After 3 months, her visual acuity was 1.0.
Laboratory Investigations

Corneal scrapings from the ulcers of all the patients were collected by an experienced ophthalmologist under aseptic precautions. The samples were immediately sent to the microbiology laboratory. Samples were inoculated as multiple “C” streaks on blood agar (Biomark, Pune, India) and brain heart infusion agar (Merck, Darmstadt, Germany) plates and incubated at 37 °C. They were also inoculated on sabouraud glucose agar (SGA; Merck) and potato dextrose agar (PDA; Merck) plates and incubated at 28 °C. Cultures were checked daily and were considered positive when the growth of the same organism was demonstrated on two or more solid media and/or whether there was confluent growth at the site of inoculation in at least one solid medium. SGA plates were positive for the presence of a filamentous fungus after 5 days of incubation. Further, fungal growth was observed on SGA and PDA plates as woolly, cream-colored aerial mycelia, and colonies with a cream-colored reverse.

Preliminary identification at the genus or species complex level was performed by analysis of the macro- and micromorphology on 2% malt extract agar (MEA; Sigma-Aldrich, St.Louis, MO, USA), incubated at 24 °C. Further identification of the strains was undertaken (at the Westerdijk Fungal Biodiversity Centre, Utrecht, The Netherlands) by partial sequencing of the elongation factor 1 alpha (tef1-α) gene, according to the methods described by Al-Hatmi et al. [10]. Results of a BLAST search against the sequences in GenBank revealed 100% identity with the Fusarium sp. FSSC_5bb strain GJS 09-1470 (Accession No. KT313615.1) for the three strains studied (CBS 138564, CWZ 10070479, and CWZ 608056699). The strains were consequently identified as F. solani sensu stricto (Table 1).

Antifungal susceptibility testing (AFST) was performed using the Clinical and Laboratory Standards Institute (CLSI) broth microdilution methods, as described in the CLSI document M38-A2 [11]. The following drugs were used: Amphotericin B (Bristol-Myers Squibb, Woerden, The Netherlands), fluconazole (Pfizer, Groton, CT, USA), itraconazole (Janssen Research Foundation, Tilburg, The Netherlands), voriconazole (Pfizer), posaconazole (Merck, Whitehouse Station, NJ, USA), isavuconazole (Basilea Pharmaceutica, Basel, Switzerland), micafungin (Astellas Pharma Inc., Ibaraki, Japan), and anidulafungin (Pfizer). Three reference strains (Paecilomyces

<table>
<thead>
<tr>
<th>Case</th>
<th>Origin</th>
<th>Age/sex</th>
<th>Application date</th>
<th>Predisposing factor</th>
<th>Duration of symptoms (days)</th>
<th>Affected eye</th>
<th>Ulcer diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Turkey</td>
<td>15/M</td>
<td>2014/February</td>
<td>Ocular trauma</td>
<td>10</td>
<td>Left</td>
<td>2.0 × 3.0</td>
</tr>
<tr>
<td>2</td>
<td>The Netherlands</td>
<td>61/F</td>
<td>2015/May</td>
<td>Soft contact lens usage</td>
<td>9</td>
<td>–</td>
<td>1.5 × 1.7</td>
</tr>
<tr>
<td>3</td>
<td>The Netherlands</td>
<td>58/F</td>
<td>2016/August</td>
<td>Soft contact lens usage</td>
<td>3</td>
<td>Left</td>
<td>–</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Case</th>
<th>Origin</th>
<th>Prior treatment</th>
<th>Visual acuity initial/final</th>
<th>Laboratory diagnosis</th>
<th>Treatment</th>
<th>Response to treatment</th>
<th>GenBank accession no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Turkey</td>
<td>Topical moxifloxacin</td>
<td>0.6/0.9</td>
<td>Fungal culture, PAS staining, and partial sequencing of the tef1-α gene</td>
<td>Voriconazole</td>
<td>Resolved</td>
<td>KT272100</td>
</tr>
<tr>
<td>2</td>
<td>The Netherlands</td>
<td>Topical tobradex</td>
<td>–</td>
<td>Fungal culture and partial sequencing of the tef1-α gene</td>
<td>AmB combined with chlorhexidine gluconate</td>
<td>Resolved with a translucent scar</td>
<td>MH15524</td>
</tr>
<tr>
<td>3</td>
<td>The Netherlands</td>
<td>Tobradex and ofloxacin</td>
<td>–/-1.0</td>
<td>Fungal culture and partial sequencing of the tef1-α gene</td>
<td>AmB and voriconazole</td>
<td>Resolved after penetrating corneaplasty</td>
<td>MH15525</td>
</tr>
</tbody>
</table>
In the three cases described here, both the classical and recent types of ocular *Fusarium* infection were recorded, i.e., soil association (Case 1) and probably the use of contact lenses (Cases 2 and 3). Owing to the rapid growth of *Fusarium* strains, antifungal treatment was initiated directly after laboratory confirmation of a fungal pathogen, with preliminary identification as *Fusarium* sp. A positive clinical response was observed within the first couple of days; the visual acuity improved in all cases, and no recurrence of the infection occurred during the eight months of follow-up examination. In vitro susceptibility results showed that variable MICs were obtained following treatment with amphotericin B and voriconazole (Table 1).

**Table 2** MIC and MEC (μg/mL) values determined for the three clinical isolates of *F. solani* sensu stricto (FSSC5)

<table>
<thead>
<tr>
<th>Case number</th>
<th>MIC (μg/mL)</th>
<th>MEC (μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AMB</td>
<td>FLC</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>&gt; 64</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>&gt; 64</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>&gt; 64</td>
</tr>
</tbody>
</table>


Discussion

In all three cases evaluated in the present study, mycotic keratitis was caused by *F. solani* sensu stricto, a lineage within the FSSC, known as FSSC5. The species was recently epitypified and re-described by Schoers et al. [9], based on a strain in the FSSC5 lineage. Consequently, this lineage can be referred to as *F. solani* sensu stricto. Typically, *Fusarium* keratitis is caused by trauma associated with soil or plant material [12, 13]; however, recently infection due to the inappropriate cleaning of contact lenses has been an emerging cause [14].

*Fusarium* is one of the few fungal genera whose members are able to infect both human and plant hosts [15, 16]. The infection is often favored by empirical and topical usage of antibiotics and steroids [17], based on a surmised bacterial infection. Therefore, poor visual outcome owing to delayed diagnosis allows for the development of large central ulcers and polymicrobial infection that may be aggravated by local and/or systemic host immunity [18–20]. In developed countries, the widespread use of contact lenses has become one of the most frequent causes of keratomycosis. Other risk factors include corneal abrasion, foreign body implantation, use of topical corticosteroids, and systemic diseases such as diabetes mellitus [21].

In the three cases described here, both the classical and recent types of ocular *Fusarium* infection were recorded, i.e., soil association (Case 1) and probably the use of contact lenses (Cases 2 and 3). Owing to the rapid growth of *Fusarium* strains, antifungal treatment was initiated directly after laboratory confirmation of a fungal pathogen, with preliminary identification as *Fusarium* sp. A positive clinical response was observed within the first couple of days; the visual acuity improved in all cases, and no recurrence of the infection occurred during the eight months of follow-up examination. In vitro susceptibility results showed that variable MICs were obtained following treatment with amphotericin B and voriconazole (Table 1).

**Conflict of interest** JFM received grants from Astellas, Basilea, and Merck. He has been a consultant for Astellas and Merck and received speaker’s fees from Gilead Sciences.

**Compliance with Ethical Standards**

| Conflicts of interest | JFM received grants from Astellas, Basilea, and Merck. He has been a consultant for Astellas and Merck and received speaker’s fees from Gilead Sciences. |

---

*variantii* ATCC 22319, *Candida krusei* ATCC 6258, and *Candida parapsilosis* ATCC 22019) were included for quality control. Table 2 summarizes the in vitro susceptibilities of the three isolates of *F. solani* to the aforementioned drugs, and the data are represented as MICs (minimum inhibitory concentrations)/MECs (minimum effective concentrations).

Merck, Pfizer, and United Medical. None of the other authors have competing interests.

References