




Filamentous Fungal Keratitis in Greece: A 16-Year Nationwide Multicenter Survey

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Abstract In a multicenter, prospective study of filamentous fungal keratitis in Greece, predisposing factors, etiology, treatment practices, and outcome, were determined. Corneal scrapings were collected from patients with clinical suspicion of fungal keratitis, and demographic and clinical data were recorded. Fungal identification was based on morphology, molecular methods, and matrix assisted laser desorption ionization time-of-flight mass-spectrometry. A total of 35 cases were identified in a 16-year study

period. Female to male ratio was 1:1.7 and median age 48 years. Corneal injury by plant material, and soft contact lens use were the main risk factors (42.8% and 31.4%, respectively). Trauma was the leading risk factor for men (68.1%), contact lens use (61.5%) for women. *Fusarium* species were isolated more frequently (n = 21, 61.8%). *F. solani* was mostly associated with trauma, *F. verticillioides* and *F. proliferatum* with soft contact lens use. Other fungi were: *Purpureocillium lilacinum* (14.7%), *Alternaria* (11.8%), *Aspergillus* (8.8%), and *Phoma foliaceiphila*, *Beauveria bassiana* and *Curvularia spicifera*, one

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case each. Amphotericin B and voriconazole MIC₅₀s against *Fusarium* were 2 mg/L and 4 mg/L respectively. Antifungal therapy consisted mainly of voriconazole locally or both locally and systemically, alone or in combination with liposomal AmB. Cure/improvement rate with antifungal therapy alone was 52%, keratoplasty was required in 40% of cases, and enucleation in 8%. In conclusion, filamentous fungal keratitis in Greece is rare, but with considerable morbidity. A large proportion of cases resulted in keratoplasty despite appropriate antifungal treatment.

Keywords Fungal keratitis · Filamentous fungi · *Fusarium* · *Purpureocillium lilacinum* · Epidemiology · Greece

Introduction

Fungal keratitis is a serious, difficult-to-treat infection of the cornea, usually resulting in poor visual outcome. Keratitis caused by filamentous fungi is considered an ophthalmic emergency often leading to eye loss, and a major global public health problem mainly in tropical and subtropical regions. High rainfall, longer rainy season and high humidity have been identified as favorable environmental conditions for fungal growth in those areas [1]. Moreover, people from these countries are often engaged in agriculture, or work outdoors. This makes them more vulnerable to corneal injury by plant material, causing a defect in the epithelial barrier and allowing fungi to gain access into the corneal stroma [1]. This defect may be also caused by contact lenses, a compromised ocular surface, the use of topical and systemic corticosteroids, or a previous ocular surgery [2]. Eradication of the fungi becomes extremely difficult after penetration into the

deeper structures of the eye [3]. Topical antifungals are not effective in these cases and surgical therapy, typically with therapeutic penetrating keratoplasty is recommended [4, 5]. Therefore, an early and accurate diagnosis is of great importance for a possible good outcome. Diagnosis is challenging due to overlapping signs and symptoms with other ocular infections, including bacterial, viral, and parasitic keratitis, although certain clinical characteristics may suggest fungal infection [6].

The proportion of fungal keratitis as a subset of microbial keratitis varies from 1 to 67% between countries. The largest proportions occur in areas with tropical or subtropical climate and the lowest in Europe [7]. The annual incidence has been estimated to be 1–1.4 million cases per year globally, with variations between countries [7]. There are also regional variations regarding involved fungi, risk factors of the affected patients and mode of transmission. Knowledge of the local epidemiology is helpful in decision making for prompt initiation of the appropriate treatment. Moreover, monitoring the local epidemiology may bring out new emerging pathogens or even outbreaks.

The purpose of this study was to provide an overview of the burden and epidemiology of fungal keratitis in Greece, focusing on filamentous fungi as causative microorganisms and their susceptibility to antifungals. The underlying patient risk factors, usual treatment practices and outcome, were also studied.

Materials and Methods

Participating Hospitals/Centers

During the years 2005–2021, a nationwide multicenter, prospective, observational study took place. The following centers participated with several cases: seven tertiary care academic or state hospitals (Attikon University Hospital, Laikon General Hospital of Athens, General Hospital of Athens “Georgios Gennimatas”, Evangelismos General Hospital of Athens, General Hospital of Nikaia, Piraeus, University Hospital of Patras, General Hospital of Nea Ionia), one private general hospital (Hygeia), one private ophthalmology hospital (Athens Eye Hospital), as well as the Department of Microbiology, Medical

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Patients

Patients with symptoms and signs of keratitis were examined in the Ophthalmology departments of the participating hospitals. Corneal scraping specimens from patients were collected at the hospital of admission and were inoculated on nutrient media as per standard of care. A direct Gram stain and potassium hydroxide (KOH) microscopy for the presence of fungal hyphae were also performed. Only adult patients with positive cultures were included in the study, whereas no case with a positive direct microscopy and a negative culture came to our notice. Demographic and clinical data, risk factors, antifungal treatment, and outcome of each case were recorded in a Case Report Form and sent to our department (4th Department of Internal Medicine, Medical School, National and Kapodistrian University of Athens, Attikon hospital) together with the isolated fungus, for further identification to species level and antifungal susceptibility testing. An informed consent of the patient was not required, as the patients received treatment as per standard of care. This was an observational study, approved by the ethics committee and Scientific Council of our hospital.

Methods

All samples were taken by the ophthalmologist at the examination site, using a surgical blade or a Kimura spatula, after local anesthesia. Very rarely a thioglycollate-moistened Dacron swab was used. Corneal scrapes were immediately inoculated onto the following media: 2 blood agar plates (BA), one for aerobic and one for anaerobic culture, chocolate agar (CHOC), Sabouraud dextrose agar (SDA) plates, non-nutritive agar (NNA), and thioglycollate broth. In addition, glass slides were smeared for Gram stain and KOH (10%) preparation, for light microscopy. The corneal scrapes were transferred onto the solid media using the “C” streak technique, with multiple C-shaped streaks on each medium. The scraping was repeated several times, and a fresh one was used for each medium. In order to determine the microbial cultures as significant, the following criteria were used: (i) growth of the same organism on two or more solid culture media at

the site of inoculation; (ii) semi-confluent growth at the site of inoculation or growth on one solid medium consistent with microscopy; or (iii) growth of the same organism on repeated scraping [6]. The SDA plates were incubated at 30 °C and 37 °C aerobically for 1–4 weeks.

Initially, all isolates were identified morphologically by standard mycology methods including evaluation of the macroscopic and microscopic characteristics of the colony. *Fusarium* isolates were further identified to species level by molecular methods and/or matrix assisted laser desorption ionization time-of-flight mass-spectrometry (MALDI-TOF MS).

Molecular Identification

Fusarium isolates were molecularly identified at the Università degli Studi di Milano, Milan, Italy, in the framework of the ECMM survey of fusariosis in Europe [8] by sequencing of the translation elongation factor 1- α gene (TEF1- α), and/or at the Westerdijk Fungal Biodiversity Institute (formerly CBS-KNAW Fungal Biodiversity Centre, Utrecht, the Netherlands) by multilocus sequence typing using the internal transcribed spacer region (ITS), the TEF1- α and the second largest subunit of RNA polymerase (rPB2), as previously described [9].

MALDI-TOF MS

For the MALDI-TOF MS analysis two platforms were used: the Bruker Biotyper (microflex LT/SH, Bruker Daltonik GmbH, Leipzig, Germany) and the Autof ms1000 (Autobio Diagnostics, Zhengzhou, China). Procedures were performed according to the manufacturers’ instructions. In brief, each *Fusarium* isolate was inoculated on SDA plate (Oxoid, UK) and incubated at 35 °C for 3–5 days, until well-isolated colonies were clearly visible and could be harvested.

The samples were treated for protein extraction before their transfer to the target plate, either by a rapid formic acid on-target extraction approach or, in case of “no identification result”, an in-tube protein extraction approach with ethanol, formic acid, and acetonitrile. Cutoff scores for reliable species/genus identification were 9.000-10.000/6.000-8.999 for Autof ms1000 (Library database version 1.1.11), and 2.00-3.00/1.70-1.99 for Bruker Biotyper (BDAL Filamentous Fungi/577 MSPs). Analysis of each strain

was performed in duplicate, and the results with a higher range/score were used for final identification [10–12].

Antifungal Susceptibility Testing (AST)

For the AST the EUCAST E.Def 9.3.2 broth microdilution reference method for conidia forming molds was applied [13]. For amphotericin B (AmB) and the azoles, the minimum inhibitory concentration (MIC) was determined, whereas for the echinocandins (anidulafungin, micafungin and caspofungin) the minimum effective concentration (MEC) was evaluated microscopically after 48 h of incubation, as described [13].

Results

Demographic, clinical and laboratory data are presented in Table 1.

Demographic Data

Twenty-eight patients were treated for confirmed filamentous fungal keratitis during the 16-year study period and reported to our survey. In addition, seven incidents were published as case reports during the same period [14–20]. As our study did not preclude publication of isolated cases, authors were free to publish their cases independently of the registry. These cases occurred at the same time-period, and we were aware of some of them as they happened. Therefore, we considered that they would not introduce any bias and were included in our prospective study. One more published case involving fungi complicating acanthamoeba keratitis [21] was not included in the study due to lack of mycological data. Twenty-two (62.8%) patients were men, with female-to-male ratio 1:1.7. The median age at the time of diagnosis was 48 years.

Clinical Presentation

The patients usually sought medical advice 2–8 days after trauma or initial symptoms. Signs upon presentation varied, including corneal infiltration and ulcer of at least 4 mm diameter with or without satellite lesions, diffuse keratitis with stromal edema, hypopyon, and progressive corneal thinning. The patients complained of blurred vision, pain, tearing and photophobia. Other predisposing factors were bullous keratopathy (two cases), herpes infection, irradiation-related local immunosuppression, use of topical dexamethasone, while no data were reported for 2 cases (5.7%).

Risk Factors

Corneal accidental injury and the use of soft contact lenses were the main risk factors [15 cases (42.8%), and 11 cases (31.4%), respectively]. Different risk factors were observed between men and women: for men the leading risk factor was trauma (15/22 cases, 68.1%). They reported accidental injury with a piece of wood or other plant material (a tree branch, onion leaf, or olive fruit have been reported) while working outdoors or during agricultural activities, or accidentally rubbing the eye with soil. In six cases the patients were farmers or agricultural workers, four of which were immigrants from Asiatic countries. Contact lens use and previous ocular surgery followed [three, (13.6%), and two (9%) cases, respectively]. Of note, in one of the cases keratitis was diagnosed after keratoplasty which was performed after accidental injury (case 21, Table 1). No underlying risk factors were reported for the remaining two cases. In contrast, the predominant risk factor for women was contact lens use (8/13 cases, 61.5%), followed by previous ocular surgery or other underlying ocular condition (Table 1). No trauma case was reported among female patients.

Fungal Pathogens

Fusarium spp. was the predominant cause of fungal keratitis (n = 21, 60%) followed by *Purpureocillium lilacinum* (n = 5, 14.3%), *Alternaria* spp. (n = 4, 11.4%), *Aspergillus* spp. (n = 3, 8.6%), and *Phoma foliaceiphila*, *Beauveria bassiana* and *Curvularia spicifera*, one case each. In one case an *Aspergillus*

Table 1 Reported filamentous fungal keratitis cases in Greece, 2005–2021

Case	Organism	ID method	Date	Age	Gender	Risk factor	Antifungal therapy	Outcome (cure or improvement Y/N)	Cure after keratoplasty
1	<i>Fusarium oxysporum</i>	Molecular	2005	NA	M	Soft lens	NA	NA	
2	<i>Fusarium solani</i>	Molecular	2008	NA	M	Trauma	NA	NA	
3	<i>Alternaria</i> sp.	Morphology	2009	74	M	Trauma	VOR eye drops + VOR p.o	Y	
4	<i>Purpureocillium lilacinum</i>	Morphology	2009	41	F	Soft lens	VOR eye drops + VOR p.o	Y	
5	<i>Fusarium</i> sp.	Morphology	2009	46	F	Post operative ^a	nystatin eye drops + VOR systemic	N; enucleation	
6	<i>Purpureocillium lilacinum</i>	Morphology	2009	NA	M	Trauma	NA	NA	
7	<i>Fusarium verticillioides</i>	Molecular	2010	30	F	Soft lens	VOR eye drops + VOR i.v	Y	
8	<i>Fusarium</i> sp.	Morphology	2010	NA	F	Soft lens	NA	NA	
9	<i>Fusarium verticillioides</i>	Molecular	2010	29	F	Soft lens	VOR eye drops + VOR i.v	N; keratoplasty	Relapse, lost to follow up
10	<i>Fusarium verticillioides</i>	Molecular/ MALDI-TOF	2011	NA	M	Soft lens	NA	Y	
11	<i>Fusarium proliferatum</i>	Molecular/ MALDI-TOF	2012	53	F	Soft lens	AmB eye drops + AmB i.v	Y	
12	<i>Fusarium verticillioides</i>	molecular/ MALDI-TOF	2012	69	M	Trauma	Natamycin eye drops	Y	
13	<i>Fusarium solani</i>	Molecular/ MALDI-TOF	2012	42	M	Trauma	NA	N; need for keratoplasty, lost to follow up	
14	<i>Fusarium faiciforme</i>	Molecular/ MALDI-TOF	2013	77	M	Trauma	VOR eye drops + VOR i.v., AmB eyedrops + AmB i.v	Y	
15	<i>Fusarium oxysporum</i>	Molecular/ MALDI-TOF	2013	78	F	bullous keratopathy	VOR eye drops/4 weeks	Y	
16	<i>Fusarium proliferatum</i>	Molecular/ MALDI-TOF	2013	22	M	Soft lens	no, suspected <i>Acanthamoeba</i> infection	Referred, lost to follow up	

Table 1 continued

Case	Organism	ID method	Date	Age	Gender M/F	Risk factor	Antifungal therapy	Outcome (cure or improvement Y/N)	Cure after keratoplasty
17	<i>Alternaria alternata</i> [16]	Morphology	2013	66	F	post operative ^b	VOR eye drops + AmB eye drops + VOR i.v	N, keratoplasty	Y
18	<i>Fusarium solani</i>	Molecular/ MALDI- TOF	2013	34	M	Trauma	VOR eye drops + NAT eye drops + AmB inj	N, keratoplasty	Y
19	<i>Alternaria chlamydospora</i>	Morphology	2013	50	M	Trauma	VOR i.v + VOR eye drops, VOR p.o	Y	
20	<i>Fusarium proliferatum</i>	Molecular/ MALDI- TOF	2014	NA	M	Trauma	NA	NA	
21	<i>Purpureocillium lilacinum</i>	Morphology	2014	49	M	Trauma	VOR eye drops	N, keratoplasty	2nd graft/Y
22	<i>Fusarium</i> sp + <i>Aspergillus flavus</i>	Morphology	2014	NA	M	Post operative	VOR eye drops	N, keratoplasty	NA
23	<i>Fusarium solani</i>	MALDI-TOF	2015	NA	M	Trauma	NA	NA	
24	<i>Fusarium</i> sp.	Morphology	2015	46	F	Soft lens	VOR eye drops + AmB eye drops	Y	
25	<i>Aspergillus flavus</i>	Morphology	2015	30	F	Soft lens	VOR i.v + VOR eye drops, VOR p.o	Y	
26	<i>Aspergillus nidulans</i>	Morphology	2015	60	M	Trauma	VOR i.v + VOR eye drops, VOR p.o	Y	
27	<i>Alternaria alternata</i>	Morphology	2016	NA	M	NA	NA	NA	
28	<i>Fusarium solani</i>	MALDI-TOF	2016	NA	F	Herpes infection	NA	N, enucleation	
29	<i>Purpureocillium lilacinum</i>	Morphology	2016	NA	M	NA	NA	NA	
30	<i>Fusarium</i> sp.	Morphology	2018	NA	M	Trauma	NA	Y	
31	<i>Phoma foliaceiphila</i> [17]	Molecular	2019	47	F	soft lens	AmB eye drops + AmB inj + VOR systemic	N, keratoplasty	2nd graft, PP vitrectomy
32	<i>Purpureocillium lilacinum</i> [18]	Morphology	2019	62	F	Post operative ^c	VOR eye drops + VOR p.o. + AmB p.o	N, keratoplasty	Y
33	<i>Fusarium dephinoides</i> (<i>F. dimerum</i> SC)	MALDI-TOF	2020	NA	M	Trauma	NA	NA	
34	<i>Beauveria bassiana</i> [19]	Molecular	2021	84	M	Post operative ^d	NAT eye drops + POS p.o., post- operatively	N, keratoplasty	Y

Table 1 continued

Case	Organism	ID method	Date	Age	Gender M/F	Risk factor	Antifungal therapy	Outcome (cure or improvement Y/N)	Cure after keratoplasty
35	<i>Curvularia spicifera</i> [20]	MALDI-TOF	2021	29	M	Trauma	VOR eye drops + fluconazole p.o	N, keratoplasty	Y

NA no data available, VOR voriconazole, AmB Amphotericin B, NAT natamycin, POS posaconazole, PP pars plana

^aPterygium excision

^bKeratoplasty due to bullous pseudophakic keratopathy

^cIrradiation-related local immunosuppression after primary penetrating keratoplasty, conjunctival melanoma 10 y earlier, professional exposure to plant material

^dPrimary penetrating keratoplasty for advanced keratoconus 10 years earlier, diabetes mellitus II

and a *Fusarium* strain were isolated simultaneously. Among *Fusarium* spp., isolates belonging to the *F. solani* species complex (SC) (including five *F. solani* sensu stricto and one *F. falciforme*) predominated (6/21, 28.5%), followed by *F. fujikuroi* SC isolates: *F. verticillioides* (4/21, 19%) and *F. proliferatum* (3/21, 14.3%). *F. oxysporum* SC was isolated from two cases (9.5%) and *F. delphinooides* (*F. dimerum* SC) from one. Five (23.8%) *Fusarium* strains were unspiciated as they were no longer available (Table 1). *Fusarium* infection was mostly associated with the use of soft contact lens (8 out of 11 contact lens wearers, 72.7%). Three *F. verticillioides* (30%), two *F. proliferatum* (20%), one *F. oxysporum* (10%) and two unspiciated strains were involved. Other risk factors for *Fusarium* keratitis were trauma (9 out of 15 trauma cases, 60%), bullous keratopathy, and herpes infection.

Fusarium solani was isolated only from trauma (5/15, 33.3%) and from a case with underlying herpes infection. *F. verticillioides* and *F. proliferatum* were isolated from soft lens users (three and two cases, respectively), followed by trauma (one case each), while risk factors for two *F. oxysporum* infections were soft lens use and bullous keratopathy.

Purpureocillium lilacinum was the cause of five keratitis cases. Trauma was the risk factor in two of them, soft contact lens in one, and irradiation-related local immunosuppression after primary penetrating keratoplasty in one patient with history of conjunctival melanoma 10 years earlier, but with professional exposure to plant material. There were no available data for the fifth case.

Aspergillus flavus was identified in two and *A. nidulans* in one of three cases of *Aspergillus* keratitis. No risk factors were found in two cases, whereas in the third case, *A. flavus* was isolated with *Fusarium* sp. from a patient after unspecified eye surgery.

Alternaria was identified in four cases: two *A. alternata*; one *A. chlamydospora*; one *Alternaria* sp. One case occurred after injury with a tree branch, another complicated bullous keratopathy, while there are no data for the third and fourth cases.

Antifungal Susceptibility

AmB and voriconazole MIC_{50s} against *Fusarium* strains were 2 mg/L and 4 mg/L, respectively. Geometric means were 1.2 mg/L and 2.2 mg/L, respectively. No clinical break points exist currently for

Table 2 Antifungal susceptibility profile of isolated fungi

Case	Organism	Antifungal MICs (mg/L)						
		AmB	VOR	POS	ITZ	CAS*	AND*	MCF*
7	<i>Fusarium verticillioides</i>	0.25	1	–	0.50	> 16	> 16	> 16
10	<i>Fusarium verticillioides</i>	2	4	–	0.25	> 16	> 16	> 16
12	<i>Fusarium verticillioides</i>	2	2	–	1	> 16	> 16	> 16
11	<i>Fusarium proliferatum</i>	2	4	–	4	> 16	> 16	> 16
16	<i>Fusarium proliferatum</i>	2	4	–	4	> 16	> 16	> 16
2	<i>Fusarium solani</i>	0.25	16	16	4	> 16	> 16	> 16
13**	<i>Fusarium solani</i>	2	4	–	4	> 16	> 16	> 16
18**	<i>Fusarium solani</i>	2	4	–	4	> 16	> 16	> 16
14	<i>Fusarium falciforme</i>	2	4	–	4	> 16	> 16	> 16
15	<i>Fusarium oxysporum</i>	2	2	–	2	> 16	> 16	> 16
22**	<i>Fusarium</i> spp.	1	0.13	0.13	0.25		0.02	0.02
24	<i>Fusarium</i> spp.	0.5	0.02	> 16	> 16	> 16	> 16	> 16
30	<i>Fusarium</i> spp.	1	> 16	> 16	–	> 16	> 16	> 16
4	<i>Purpureocillium lilacinum</i>	1	1	0.50	2	> 16	> 16	> 16
6	<i>Purpureocillium lilacinum</i>	4	0.03	–	0.50	> 16	> 16	> 16
21**	<i>Purpureocillium lilacinum</i>	0.50	0.06	0.25	0.25	> 16	> 16	> 16
25	<i>Aspergillus flavus</i>	0.12	0.06	0.50	0.50	1	1	1
26	<i>Aspergillus nidulans</i>	0.25	0.03	0.12	0.12	0.50	0.50	0.50
17**	<i>Alternaria alternata</i>	0.06	0.13	0.06	0.02	0.13	–	–
27	<i>Alternaria alternata</i>	0.25	0.13	0.13	0.25	–	–	–
19	<i>Alternaria chlamyospora</i>	1	0.50	0.25	–	–	–	–
3	<i>Alternaria</i> spp.	0.13	0.01	–	0.03	–	–	–
34**	<i>Bauveria basiana</i>	2	4	0.25	0.25	–	0.25	0.13
35**	<i>Curvularia spicifera</i>	1.5	0.02	–	–	32	–	–

AmB amphotericin B, VOR voriconazole, POS posaconazole, ITZ itraconazole, CAS caspofungin, AND anidulafungin, MCF micafungin

*Minimum effective concentration (MEC)

**Keratoplasty

–Not done

Fusarium species. Based on clinical break points of *A. fumigatus* [22], we found that against five out of the 13 isolates tested (38.5%), the AmB MIC values were lower or equal to 1 mg/L. Similarly, the MICs of voriconazole and itraconazole were lower than 1 mg/L against 3/13 (23.1%), and 4/13 (30.8%) of isolates, respectively. The echinocandins exhibited no antifungal activity, except against one unspiciated *Fusarium* isolate (Table 2). Against *P. lilacinum* strains, MICs of AmB and voriconazole were low, while echinocandins showed no activity. Only in one of them AmB MIC was 4 mg/L. All antifungals used were active against two *Aspergillus* isolates.

Treatment

Data on the applied antifungal therapy were available for 22 patients. One of them received antifungals only after keratoplasty. The most used was voriconazole

(18 patients), locally or both locally and systemically, and in combination with other antifungals. Liposomal AmB followed, either topically or systemically (seven cases). In five cases liposomal AmB was used in combination with voriconazole or replaced it when in vitro susceptibility results were available (Table 1). In two cases liposomal AmB was used also for subconjunctival injections. The patients did not receive any antibiotic or antifungal treatment before specimen collection unless they had an underlying disease or preceding surgery. All patients received antibiotics simultaneously, as per standard of care.

Outcome was known for 25 of 35 cases (71.4%). Thirteen of 25 patients (52%) were cured with antifungal therapy alone (Table 1). In these cases, improvement was obvious after 1–2 days of treatment. MICs of AmB and voriconazole were low in seven out of 11 with known treatment (63.6%, Table 2). For another 10 patients (40%) penetrating keratoplasty

was required due to deterioration despite antifungal treatment. Data on drug therapy were available in nine of them, as shown in Table 1, and MICs in seven (Table 2). Interestingly, in four of them (57.1%) AmB and voriconazole MICs were low. Cure or improvement of visual acuity after keratoplasty was observed in five cases. Three needed a second graft, while no data were available for the remaining two. Two of the patients with a second keratoplasty improved and one was lost to follow up. Enucleation had to be performed in two out of the 25 patients (8%) with known outcome, including one published case [15]. Data on time elapsed between onset of symptoms and initiation of antifungal treatment was available for nine keratoplasty and one enucleation patients. The median time was 20 days (range 7 to > 30 days). For the cured with antifungal therapy alone, data were available only for three patients, with a median of 7 days (range 3–15 days).

Discussion

Fungal keratitis constitutes a particular burden in tropical developing countries, where it may comprise up to 67% of infectious keratitis cases [23, 24]. In tropical and subtropical climates filamentous fungi are the predominant etiological microorganisms while trauma is the main risk factor [7]. In contrast, limited cases of fungal keratitis are reported in developed countries, in Europe and USA, where it is considered to be a rare ocular infection often misdiagnosed, with use of contact lenses as the predominant risk factor [25–35].

Our data show that in Greece, trauma and soft contact lens use equally contributed to the disease burden. It was remarkable that trauma was associated only with men. Moreover, four of our 15 trauma patients were immigrants. This high representation can be easily explained, as immigration has grown in recent years in Greece and many young men are occupied in agricultural activities. Therefore, they are exposed to accidental injuries by plant material, or contact of the eyes with organic matter or contaminated soil.

Among women, the leading risk factor was contact lens wear, as in other European countries [27–29], followed by previous ocular surgery and bullous keratopathy.

Regarding fungi involved in keratitis, *Fusarium* and *Aspergillus* species are globally more commonly reported [5], followed by dematiaceous fungi of the genus *Curvularia* [4]. *Fusarium* was the predominant cause of keratitis in the present study. *Fusarium* species are ubiquitous in the environment, and they are also important phytopathogens affecting a wide variety of crops. In humans they cause a wide spectrum of infections, from superficial or locally invasive in immunocompetent, to disseminated life threatening infections in immunosuppressed patients. Keratitis is among the most prevalent infections in the immunocompetent, along with onychomycosis and skin infections [36].

Fusarium ocular infections are associated with high morbidity and poor clinical outcome [37]. Management is often difficult as *Fusarium* is usually resistant to antifungals currently available, including AmB, azoles, echinocandins and terbinafine [37]. Furthermore, in vitro studies have demonstrated that *F. solani* can form biofilms resulting in increased resistance to antifungal agents [38]. *Fusarium* keratitis is common in tropical and subtropical countries where it is mainly associated with trauma by plant material [39]. In recent years it also concerns developed countries with moderate climate, where it is associated with contact lens use as predominant risk factor [23]. An outbreak of contact lens-related *Fusarium* keratitis has taken place during the years 2005–2006 in Asia (Singapore and Hong Kong), North America and in a few European countries. It has been associated with the multipurpose disinfection solution ReNu® with MoistureLoc® (Bausch and Lomb, Rochester, NY) [40, 41] and it ended as soon as the solution was recalled and withdrawn from the market. The investigations that took place to find the source of the epidemic showed that a critical factor in this event was not any contamination of the solution (the strains isolated from the users were not related), but the formula of the solution itself, which exhibited reduced activity against *Fusarium* under certain conditions. More specifically, the antimicrobial alexidine, contained in the solution, appeared to lose its effect on *Fusarium* strains when its ReNu plastic bottle was heated [42, 43]. No cases using that specific solution were reported in Greece. Undoubtedly, poor hygiene practices by the contact lens wearers and no compliance with the manufacturer's instructions increases the risk for fungal infections. A Greek study by Mela EK et al.

2015 on contact lens storage cases of asymptomatic users, showed that isolation of fungi is possible even among users that reported compliance, therefore they are at risk for contamination [44]. Furthermore, disinfectant solutions containing hydrogen peroxide were found to be less effective than various alternative solutions in eliminating fungal species [44].

F. solani keratitis is a severe disease requiring a long time of therapy. Moreover, it has been demonstrated that it has higher pathogenic potential and is associated with untreated infections with subsequent keratoplasty more often than non-*solani* *Fusarium* species [45]. Our study focused particularly on *Fusarium* species epidemiology. Of the ten patients requiring emergency keratoplasty despite antifungal therapy, four suffered from *Fusarium* keratitis, two of which with *F. solani*. Furthermore, in both cases resulting in enucleation, *Fusarium* strains were involved, one *F. solani* and one non-specified [15]. Although *F. solani* SC is considered to be the most prevalent species worldwide, in our case series it was outnumbered by other species, among which *F. fujikuroi* SC was isolated more frequently. This is in line with the findings of the ECMM study on invasive infections due to *Fusarium* species in Europe [8], in which *F. fujikuroi* SC (formerly *Giberella fujikuroi*) was the most frequent etiology of proven or probable disseminated infections, with most cases reported from Southern European countries. Therefore, there may be an association with the geographical distribution of species. *F. solani* SC was mainly the cause of localized infections in the ECMM study [8]. An interesting finding in the present study was that *F. solani* was isolated only from trauma patients and a patient with underlying herpes infection, but the statistical significance of this difference could not be assessed, as the number of patients was very small. If confirmed in larger studies, probably different ecological distribution of species, selective growth in soft lens storage solutions, or differences in pathogenesis might explain this finding. This merits further investigation, as *F. solani* and *F. verticillioides* (also known as *F. moniliforme*) have usually different antifungal susceptibility profiles, *F. solani* being more susceptible to AmB and more resistant to azoles [8]. In line with the literature [46], *Fusarium* keratitis isolates in the present study were associated with higher antifungal MICs, but no interspecies differences were noted.

Echinocandins had no effect, as *Fusarium* is intrinsically resistant to them [46].

Two more cases requiring keratoplasty had infection caused by *P. lilacinum*. *P. lilacinum* (formerly *Paecilomyces lilacinus*) is a saprophytic fungus found in soil, air, wood and plants, and a rare cause of severe keratitis, usually after trauma. It has also been found as a contaminant of antiseptic skin lotions and solutions used as sterilizers for artificial lenses [47]. Other risk factors are chronic keratopathy, previous ocular surgery, corneal trauma or contact lens wear [48]. Cases without prior ocular trauma but associated with immunosuppression, use of systemic immunosuppressants and topical corticosteroids have been described as well [49]. In our study trauma was the predominant risk factor. *P. lilacinum* is associated with high antifungal MICs, causing a difficult to treat ocular infection. Voriconazole has been found to be the most effective, but further studies are required for the establishment of the optimal treatment [50, 51].

A. flavus has been identified in two of the three cases of *Aspergillus* keratitis in the present study. This species has been shown to be the leading cause of ocular infections among other *Aspergillus* species in several published studies [52–54]. Following *A. fumigatus*, *A. flavus* is the leading cause of invasive and allergic aspergillosis, particularly in climates with high temperature and humidity, where it appears to be the predominant agent of sinusitis, keratitis, and cutaneous infections [55]. All of them are difficult to treat infections due to the significant resistance of *A. flavus* to azoles [55, 56]. In one of our cases the isolate appeared to be wild-type according to EUCAST [22] and was successfully treated with voriconazole. The second case had a double infection with *Fusarium* sp. and resulted in keratoplasty.

In the present study four patients suffered from *Alternaria* keratitis. In two patients *A. alternata*, a saprophytic dematiaceous mold was identified. The most common human infections due to *Alternaria* species include cutaneous and subcutaneous infections, oculomycosis (keratitis, endophthalmitis), onychomycosis and sinusitis [57]. Trauma, previous ocular surgery, and pre-existing corneal diseases are more commonly associated with *Alternaria* keratitis [57, 58]. However, cases of soft contact lens related *Alternaria* keratitis have also been reported [59, 60]. In our study, ocular trauma with a tree branch in one case and bullous keratopathy in another were reported

as predisposing risk factors. According to literature, *Alternaria* has variable responses to topical and systemic antifungals. AmB has shown variable in vitro activity, while triazoles including itraconazole, voriconazole and posaconazole, have exhibited low MICs [57], as in our cases. Terbinafine and caspofungin have been found to be in vitro active against *Alternaria* [57].

Curvularia spicifera (formerly *Bipolaris spicifera*) was isolated from one patient in our study. *Bipolaris* is a large genus of dematiaceous hyphomycetes, including several significant plant pathogens with worldwide distribution. The species considered to be pathogenic in humans are *B. spicifera*, *B. hawaiiensis*, *B. australiensis* and, to a lesser extent, *B. papendorffii* [61]. After the most recent taxonomic revisions based on molecular phylogenetic studies, the *Bipolaris* human pathogens have now been shifted to the genus *Curvularia* [62]. They can infect both immunocompetent and immunosuppressed patients, mainly in tropical and subtropical areas. The most common infections are allergic sinusitis, keratitis, endophthalmitis, onychomycosis, peritoneal dialysis-associated peritonitis, lung, and skin infections [62]. Keratitis caused by *C. spicifera* has been rarely reported [20].

The present study revealed also new opportunistic fungi as causes of keratitis in Greece. *Phoma* spp. are known to be phytopathogens that are widely distributed in the environment mainly in aquatic systems and soil [17]. *Beauveria bassiana* is also an opportunistic fungus ubiquitous in soil and is widely used as biopesticide due to its entomopathogenic properties [19]. Both can become pathogens in patients who are at risk due to immunosuppression.

Therapy of fungal keratitis consists of topical antifungal medication with or without surgical interventions. Many of the antifungals differ in their corneal penetration activity and effectiveness [7]. Natamycin (5%) is the treatment of choice for filamentous fungi, but topical liposomal AmB (L-AmB) (0.3–0.5%) and voriconazole (1%) are suggested as alternatives [63]. Topical voriconazole is particularly useful in fungal keratitis not responding to natamycin [23]. In our study the most used was voriconazole, locally or both locally and systemically, and in combination with other antifungals. In two cases intraocular injection of L-AmB was administered. Due to the low number of patients, different

etiologies, and poor information of the severity of infection upon seeking medical advice, no conclusions can be made on the efficacy of treatment by antifungals alone.

By poor response to antifungals, surgery must take place to prevent perforation. Periodic debridement is useful to remove necrotic tissues from the cornea and achieve better penetration of antifungals. Patch graft and transplant can be used as final attempt to restore the cornea and normal vision [23]. In our study, keratoplasty was required for a considerable number of patients, and in two cases enucleation was performed. Recently published results from the German Fungal Keratitis Registry were similar, with 54.6% of patients requiring keratoplasty and 8.6% enucleation [23].

According to our data, it is understood that despite the improvement of diagnosis and treatment of fungal keratitis, its prognosis is still poor. It remains a severe ocular infection with unpredictable outcome, resistance to antifungals in most cases, and necessity of long healing time.

Our study is the first to present collective data on filamentous fungal keratitis in Greece, there are however certain limitations. As fungal keratitis is not a notifiable disease, cases might be underreported. Though one of the participating hospitals is a significant ophthalmology referral center (General Hospital of Athens "Georgios Gennimatas"), serving patients with more complex medical conditions throughout Greece, this remains a point of concern. Furthermore, we did not have reported cases diagnosed as fungal keratitis based solely on the light microscopy results (culture-negative). Light microscopy is less sensitive than culture, however, a diagnosis can still be established if fungal hyphae can be demonstrated on Gram stain or KOH preparations [64, 65]. Taking into account the number of collected cases only, a tentative estimate of annual incidence of fungal keratitis would result in 0.1–0.5 per million population/year (based on the 2011 population-housing census ~ 11 million). This is in line with incidence reported in other European countries [30, 32–34]. Another limitation of the study is that several data and final outcome are missing in a number of cases. As our main contacts for obtaining data were the Microbiology departments of the hospitals, we did not have always full access to the records of the patients, whereas some isolates were no longer available for further analysis. We were able to

show however, that keratoplasty was unavoidable for a large proportion of patients, despite appropriate antifungal therapy. It appears that many patients waited too long before seeking medical help or were treated for bacterial keratitis at least for a week prior to the diagnosis of fungal keratitis. Therefore, awareness for the risks for a fungal infection should be increased, and samples for direct microscopy and culture should be taken timely.

In summary, our study shows that filamentous fungal keratitis in Greece is rare, but with considerable morbidity. Both trauma and soft contact lens use were the most usual risk factors, the first concerning men and the latter mainly women. *Fusarium* species were the organisms isolated more frequently, while cases with more unusual fungi were also revealed. Despite appropriate antifungal treatment, a large proportion of cases resulted in keratoplasty as a last attempt to restore vision.

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Author Contributions AM collected data, performed diagnostic tests, and wrote the first draft. MD-A and GP conceived, designed, and supervised the study. MS provided data and performed diagnostic tests. MD-A analyzed data and edited the manuscript. JM, MS, MC, IC, AA, EP, GV and GP, reviewed and commented on previous versions of the manuscript. All authors provided data and approved the final manuscript.

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Declarations

Conflict of interest The authors have no conflicts of interest to declare.

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References

- Kibret T, Bitew A. Fungal keratitis in patients with corneal ulcer attending Minilik II Memorial Hospital, Addis Ababa. *Ethiop BMC Ophthalmol*. 2016. <https://doi.org/10.1186/s12886-016-0330-1>.
- Knutsson KA, Iovieno A, Matuska S, Fontana L, Rama P. Topical corticosteroids and fungal keratitis: a review of the literature and case series. *J Clin Med*. 2021. <https://doi.org/10.3390/jcm10061178>.
- Alfonso EC, Forster RK, Garg P. Fungal infections. In: Foster CS, Azar DT, Dohlman CH, editors. *Smolin and Thoft's the cornea scientific foundations & clinical practice*, vol. 4. Baltimore: Lippincott Williams & Wilkins; 2005. p. 405–16.
- Hoffman JJ, Burton MJ, Leck A. Mycotic keratitis—a global threat from the filamentous fungi. *J Fungi (Basel)*. 2021. <https://doi.org/10.3390/jof7040273>.
- Manikandan P, Abdel-Hadi A, Randhir Babu Singh Y, Revathi R, Anita R, Banawas S, et al. Fungal keratitis epidemiology, rapid detection, and antifungal susceptibilities of *Fusarium* and *Aspergillus* isolates from corneal scrapings. *Biomed Res Int*. 2019. <https://doi.org/10.1155/2019/6395840>.
- Thomas PA, Leck AK, Myatt M. Characteristic clinical features as an aid to the diagnosis of suppurative keratitis caused by filamentous fungi. *Br J Ophthalmol*. 2005. <https://doi.org/10.1136/bjo.2005.076315>.
- Brown L, Leck AK, Gichangi M, Burton MJ, Denning DW. The global incidence and diagnosis of fungal keratitis. *Lancet Infect Dis*. 2021. [https://doi.org/10.1016/S1473-3099\(20\)30448-5](https://doi.org/10.1016/S1473-3099(20)30448-5).
- Tortorano AM, Prigitano A, Esposito MC, Arsic Arsenijevic V, Kolarovic J, Ivanovic D, et al. ECMM working group. European Confederation of medical mycology (ECMM) epidemiological survey on invasive infections due to *Fusarium* species in Europe. *Eur J Clin Microbiol Infect Dis*. 2014. <https://doi.org/10.1007/s10096-014-2111-1>.
- Al-Hatmi AMS, Ahmed SA, van Diepeningen AD, Drogari-Apiranthitou M, Verweij PE, Meis JF, de Hoog GS. *Fusarium metavorans* sp. nov.: the frequent opportunist “FSSC6.” *Med Mycol*. 2018. <https://doi.org/10.1093/mmy/myx107>.
- Cassagne C, Normand AC, L'Ollivier C, Ranque S, Piarrou R. Performance of MALDI-TOF MS platforms for fungal identification. *Mycoses*. 2016. <https://doi.org/10.1111/myc.12506>.
- Peng Y, Zhang Q, Xu C, Shi W. MALDI-TOF MS for the rapid identification and drug susceptibility testing of filamentous fungi. *Exp Ther Med*. 2019. <https://doi.org/10.3892/etm.2019.8118>.
- Gómez-Velásquez JC, Loaiza-Díaz N, Norela Hernández G, Lima N, Mesa-Arango AC. Development and validation of an in-house library for filamentous fungi identification by MALDI-TOF MS in a clinical laboratory in Medellín (Colombia). *Microorganisms*. 2020. <https://doi.org/10.3390/microorganisms8091362>.
- Eucast Definitive Document E.Def 9.1: method for the determination of broth dilution minimum inhibitory concentrations of antifungal agents for conidia forming moulds.

2008. https://www.eucast.org/astoffungi/previous_versions_of_documents/. Accessed 25 Feb 2022.
14. Vagiakou E, Orfanidou M, Mitsakakis D, Zourla K, Petrou E, Goula A. Fungal keratitis caused by *Alternaria* spp following injury. *Acta Microbiol Hell*. 2008;53:44–8.
 15. Peponis V, Rosenberg P, Chalkiadakis SE, Insler M, Amariotakis A. Fungal scleral keratitis and endophthalmitis following pterygium excision. *Eur J Ophthalmol*. 2009. <https://doi.org/10.1177/112067210901900326>.
 16. Konidaris V, Mersinoglou A, Vyzantiadis TA, Papadopoulou D, Boboridis KG, Ekonomidis P. Corneal transplant infection due to *alternaria alternata*: a case report. *Case Rep Ophthalmol Med*. 2013. <https://doi.org/10.1155/2013/589620>.
 17. Adamopoulou A, Sakellaris D, Koronis S, Balidis M, Zachariadis Z, Tranos P, Kozeis N, Gatziofias Z, Anogeianakis G, Mikropoulos DG, Konstas AG. Rare persistent corneal infection by *phoma* sp.—a case report. *Ophthalmol Ther*. 2019. <https://doi.org/10.1007/s40123-019-0163-0>.
 18. Mikropoulos DG, Kymionis GD, Voulgari N, Kaisari E, Nikolakopoulos KA, Katsanos A, Konstas AG. Intraoperative photoactivated chromophore for infectious keratitis—corneal cross-linking (PACK-CXL) during penetrating keratoplasty for the management of fungal keratitis in an immunocompromised patient. *Ophthalmol Ther*. 2019. <https://doi.org/10.1007/s40123-019-0196-4>.
 19. Atzamoglou S, Siopi M, Meletiadis J, Markopoulos I, Kontomichos L, Batsos G, Paroikakis E, Peponis V. A corneal perforation related to *Beauveria bassiana* and post-penetrating keratoplasty management discussion. *Cureus*. 2021. <https://doi.org/10.7759/cureus.15724>.
 20. Petrou E, Orfanidou M, Georgios S, Dimtsas GS, Moutzouri-Sidiri M, Voulgaridi I, Vyzantiadis TA, Vagiakou E, Droutsas K. A case of keratomycosis caused by *Curvularia spicifera* (previously *Bipolaris spicifera*) and review of the literature. *Acta Microbiol Hell*. 2022; 67(1): 59
 21. Kymionis GD, Kouroupaki AI, Liakopoulos DA, Arandjelovic IR, Tsoulras KI. Multiorganism, drug-resistant keratitis treated by corneal crosslinking. *Eur J Ophthalmol*. 2016. <https://doi.org/10.5301/ejo.5000755>.
 22. Clinical breakpoints for fungi (*Candida* and *Aspergillus* species). In: The European committee on antimicrobial susceptibility testing—EUCAST. 2019. <https://www.eucast.org/astoffungi/clinicalbreakpointsforantifungals/>. Accessed 25 Feb 2022.
 23. Acharya Y, Acharya B, Karki P. Fungal keratitis: study of increasing trend and common determinants. *Nepal J Epidemiol*. 2017. <https://doi.org/10.3126/nje.v7i2.17975>.
 24. Chitamparam S, Lim TH, Tai E, Ibrahim M. Mycotic keratitis in a tertiary hospital in Northeastern Malaysia. *Turk J Ophthalmol*. 2020. <https://doi.org/10.4274/tjo.galenos.2020.57609>.
 25. Ong HS, Fung SSM, Macleod D, Dart JKG, Tuft SJ, Burton MJ. Altered patterns of fungal keratitis at a London ophthalmic referral hospital an eight-year retrospective observational study. *Am J Ophthalmol*. 2016;168:227–36. <https://doi.org/10.1016/j.ajo.2016.05.021>.
 26. Rosa RH Jr, Miller D, Alfonso EC. The changing spectrum of fungal keratitis in South Florida. *Ophthalmology*. 1994. [https://doi.org/10.1016/s0161-6420\(94\)31225-5](https://doi.org/10.1016/s0161-6420(94)31225-5).
 27. Mosquera Gordillo MA, Barón Cano N, Garralda Luquin A, López Gutiérrez C, Mengual Verdú E, Trujillo Cabrera G, et al. Keratitis secondary to *Fusarium* spp. in Spain 2012–2014. *Arch Soc Esp Oftalmol (Engl Ed)*. 2018. <https://doi.org/10.1016/j.oftal.2017.08.005>.
 28. Olivier R, Knoeri J, Leveziel L, Negrier P, Georgeon C, Kopal A, et al. Update on fungal keratitis in France: a case-control study. *Acta Ophthalmol*. 2021. <https://doi.org/10.1111/aos.14910>.
 29. Roth M, Holtmann C, Daas L, Kakkassery V, Kurzai O, Geerling G, et al. Results from the German fungal keratitis registry: significant differences between cases with and without a history of contact lens use. *Cornea*. 2021. <https://doi.org/10.1097/ICO.0000000000002705>.
 30. Oliveira Dos Santos C, Kolwijck E, van Rooij J, Stoutenbeek R, Visser N, Cheng YY, et al. Epidemiology and clinical management of *Fusarium* keratitis in the Netherlands 2005–2016. *Front Cell Infect Microbiol*. 2020. <https://doi.org/10.3389/fcimb.2020.00133>.
 31. Nowik KE, Wylęgała A, Nowik K, Wylęgała E. A single-centre retrospective observational study of fungal keratitis in Poland with a review of findings in Europe. *Ann Agric Environ Med*. 2020. <https://doi.org/10.26444/aaem/109414>.
 32. Tuft SJ, Tullo AB. Fungal keratitis in the United Kingdom 2003–2005. *Eye (Lond)*. 2009. <https://doi.org/10.1038/eye.2008.298>.
 33. Farrell S, McElnea E, Moran S, Knowles S, Murphy CC. Fungal keratitis in the Republic of Ireland. *Eye (Lond)*. 2017. <https://doi.org/10.1038/eye.2017.82>.
 34. Nielsen SE, Nielsen E, Julian HO, Lindegaard J, Højgaard K, Ivarsen A, Hjortdal J, Heegaard S. Incidence and clinical characteristics of fungal keratitis in a Danish population from 2000 to 2013. *Acta Ophthalmol*. 2015. <https://doi.org/10.1111/aos.12440>.
 35. Bograd A, Seiler T, Droz S, Zimmerli S, Früh B, Tappeiner C. Bacterial and fungal keratitis: a retrospective analysis at a university hospital in Switzerland. *Klin Monbl Augenheilkd*. 2019. <https://doi.org/10.1055/a-0774-7756>.
 36. Van Diepeningen AD, Feng P, Ahmed S, Sudhadham M, Bunyaratavej S, de Hoog GS. Spectrum of *Fusarium* infections in tropical dermatology evidenced by multilocus sequencing typing diagnostics. *Mycoses*. 2015. <https://doi.org/10.1111/myc.12273>.
 37. Sara S, Sharpe K, Morris S. Multidrug-resistant *Fusarium* keratitis: diagnosis and treatment considerations. *BMJ Case Rep*. 2016. <https://doi.org/10.1136/bcr-2016-215401>.
 38. Sav H, Rafati H, Öz Y, Dalyan-Cilo B, Ener B, Mohammadi F, Ilkit M, van Diepeningen AD, Seyedmousavi S. Biofilm formation and resistance to fungicides in clinically relevant members of the fungal genus *Fusarium*. *J Fungi (Basel)*. 2018. <https://doi.org/10.3390/jof4010016>.
 39. Chen CA, Hsu SL, Hsiao CH, Ma DH, Sun CC, Yu HJ, Fang PC, Kuo MT. Comparison of fungal and bacterial keratitis between tropical and subtropical Taiwan: a prospective

- cohort study. *Ann Clin Microbiol Antimicrob*. 2020. <https://doi.org/10.1186/s12941-020-00353-3>.
40. Khor WB, Aung T, Saw SM, Wong TY, Tambyah PA, Tan AL, et al. An outbreak of *Fusarium* keratitis associated with contact lens wear in Singapore. *JAMA*. 2006. <https://doi.org/10.1001/jama.295.24.2867>.
 41. Chang DC, Grant GB, O'Donnell K, Wannemuehler KA, Noble-Wang J, Rao CY, Fusarium Keratitis Investigation Team, et al. Multistate outbreak of fusarium keratitis associated with use of a contact lens solution. *JAMA*. 2006. <https://doi.org/10.1001/jama.296.8.953>.
 42. Epstein AB. In the aftermath of the *Fusarium* keratitis outbreak: what have we learned? *Clin Ophthalmol*. 2007;1(4):355–66.
 43. Bullock JD, Warwar RE, Elder BL, Khamis HJ. Microbiological investigations of ReNu plastic bottles and the 2004 to 2006 ReNu with moistureloc-related worldwide *Fusarium* keratitis event. *Eye Contact Lens*. 2016. <https://doi.org/10.1097/ICL.0000000000000175>.
 44. Mela EK, Anastassiou ED, Gartaganis SP, Christofidou M. Fungal isolation from disinfectant solutions of contact lens storage cases among asymptomatic users. *Eye Contact Lens*. 2015. <https://doi.org/10.1097/ICL.0000000000000069>.
 45. Krysik K, Wroblewska-Czajka E, Lyssek-Boron A, Wylegala EA, Dobrowolski D. Total penetrating keratoplasty: indications, therapeutic approach, and long-term follow-up. *J Ophthalmol*. 2018. <https://doi.org/10.1155/2018/9580292>.
 46. Al-Hatmi AMS, Meis JF, de Hoog GS. *Fusarium*: molecular diversity and intrinsic drug resistance. *PLoS Pathog*. 2016. <https://doi.org/10.1371/journal.ppat.1005464>.
 47. Luangsa-Ard J, Houbraken J, van Doorn T, Hong SB, Borman AM, Hywel-Jones NL, Samson RA. *Purpureocillium*, a new genus for the medically important *Paecilomyces lilacinus*. *FEMS Microbiol Lett*. 2011. <https://doi.org/10.1111/j.1574-6968.2011.02322.x>.
 48. Almeida Oliveira M, Carmo A, Rosa A, Murta J. Posaconazole in the treatment of refractory *Purpureocillium lilacinum* (former *Paecilomyces lilacinus*) keratitis: the salvation when nothing works. *BMJ Case Rep*. 2019. <https://doi.org/10.1136/bcr-2018-228645>.
 49. Turner LD, Conrad D. Retrospective case-series of *Paecilomyces lilacinus* ocular mycoses in Queensland Australia. *BMC Res Notes*. 2015. <https://doi.org/10.1186/s13104-015-1591-0>.
 50. Sponsel W, Chen N, Dang D, Paris G, Graybill J, Najvar LK, et al. Topical voriconazole as a novel treatment for fungal keratitis. *Antimicrob Agents Chemother*. 2006. <https://doi.org/10.1128/AAC.50.1.262-268.2006>.
 51. Todokoro D, Yamada N, Fukuchi M, Kishi S. Topical voriconazole therapy of *Purpureocillium lilacinum* keratitis that occurred in disposable soft contact lens wearers. *Int Ophthalmol*. 2014. <https://doi.org/10.1007/s10792-014-9965-1>.
 52. Al-Hatmi AMS, Castro MA, de Hoog GS, Badali H, Alvarado VF, Verweij PE, Meis JF, Zago VV. Epidemiology of *Aspergillus* species causing keratitis in Mexico. *Mycoses*. 2019. <https://doi.org/10.1111/myc.12855>.
 53. Khor WB, Prajna VN, Garg P, Mehta JS, Xie L, Liu Z, ACSIKS Group, et al. The Asia cornea society infectious keratitis study: a prospective multicenter study of infectious keratitis in Asia. *Am J Ophthalmol*. 2018. <https://doi.org/10.1016/j.ajo.2018.07.040>.
 54. Manikandan P, Varga J, Kocsubé S, Anita R, Revathi R, Németh TM, Narendran V, Vágvölgyi C, Panneer Selvam K, Shobana CS, Babu Singh YR, Kredics L. Epidemiology of *Aspergillus* keratitis at a tertiary care eye hospital in South India and antifungal susceptibilities of the causative agents. *Mycoses*. 2013. <https://doi.org/10.1111/j.1439-0507.2012.02194.x>.
 55. Rudramurthy SM, Paul RA, Chakrabarti A, Mouton JW, Meis JF. Invasive aspergillosis by *Aspergillus flavus*: epidemiology, diagnosis, antifungal resistance, and management. *J Fungi (Basel)*. 2019. <https://doi.org/10.3390/jof5030055>.
 56. Zaini F, Lotfali E, Fattahi A, Siddig E, Farahyar S, Kouhsari E, Saffari M. Voriconazole resistance genes in *Aspergillus flavus* clinical isolates. *J Mycol Med*. 2020. <https://doi.org/10.1016/j.mycmed.2020.100953>.
 57. Hsiao CH, Yeh LK, Chen HC, Lin HC, Chen PY, Ma DH, Tan HY. Clinical characteristics of alternaria keratitis. *J Ophthalmol*. 2014. <https://doi.org/10.1155/2014/536985>.
 58. Monno R, Alessio G, Guerriero S, Caggiano G, Capolongo C, De Vito D, Sanguinetti M. *Alternaria* is an infrequent cause of keratitis: a case report and review of the literature. *Eye Contact Lens*. 2015. <https://doi.org/10.1097/ICL.0000000000000012>.
 59. Yildiz EH, Ailani H, Hammersmith KM, Eagle RC Jr, Rapuano CJ, Cohen EJ. *Alternaria* and *Paecilomyces* keratitis associated with soft contact lens wear. *Cornea*. 2010. <https://doi.org/10.1097/ICO.0b013e3181ba0cde>.
 60. Ursea R, Tavares LA, Feng MT, McColgin AZ, Snyder RW, Wolk DM. Non-traumatic *Alternaria* keratomycosis in a rigid gas-permeable contact lens patient. *Br J Ophthalmol*. 2010. <https://doi.org/10.1136/bjo.2009.164632>.
 61. da Cunha KC, Sutton DA, Fothergill AW, Cano J, Gené J, Madrid H, De Hoog S, Crous PW, Guarro J. Diversity of *Bipolaris* species in clinical samples in the United States and their antifungal susceptibility profiles. *J Clin Microbiol*. 2012. <https://doi.org/10.1128/JCM.01965-12>.
 62. Manamgoda DS, Rossman AY, Castlebury LA, Crous PW, Madrid H, Chukeatirote E, et al. The genus *Bipolaris*. *Stud Mycol*. 2014. <https://doi.org/10.1016/j.simyco.2014.10.002>.
 63. Thiel MA, Zinkernagel AS, Burhenne J, Kaufmann C, Haefeli WE. Voriconazole concentration in human aqueous humor and plasma during topical or combined topical and systemic administration for fungal keratitis. *Antimicrob Agents Chemother*. 2007. <https://doi.org/10.1128/AAC.00762-06>.
 64. Chidambaram JD, Venkatesh Prajna N, Srikanthi P, Lanjewar S, Shah M, Elakkiya S, Lalitha P, Burton MJ. Epidemiology, risk factors, and clinical outcomes in severe microbial keratitis in South India. *Ophthalmic Epidemiol*. 2018. <https://doi.org/10.1080/09286586.2018.1454964>.

65. Gopinathan U, Garg P, Fernandes M, Sharma S, Athmanathan S, Rao GN. The epidemiological features and laboratory results of fungal keratitis. *Cornea*. 2002. <https://doi.org/10.1097/00003226-200208000-00004>.

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