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Mycotic keratitis: epidemiology, diagnosis and management

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Abstract

Mycotic keratitis (an infection of the cornea) is an important ocular infection, especially in young male outdoor workers. There are two frequent presentations: keratitis due to filamentous fungi (*Fusarium*, *Aspergillus*, phaeohyphomycetes and *Scedosporium apiospermum* are frequent causes) and keratitis due to yeast-like fungi (*Candida albicans* and other *Candida* species). In the former, trauma is usually the sole predisposing factor, although previous use of corticosteroids and contact lens wear are gaining importance as risk factors; in the latter, there is usually some systemic or local (ocular) defect. The clinical presentation and clinical features may suggest a diagnosis of mycotic keratitis; increasingly, *in vivo* (non-invasive) imaging techniques (confocal microscopy and anterior segment optical coherence tomography) are also being used for diagnosis. However, microbiological investigations, particularly direct microscopic examination and culture of corneal scrape or biopsy material, still form the cornerstone of diagnosis. In recent years, the PCR has gained prominence as a diagnostic aid for mycotic keratitis, being used to complement microbiological methods; more importantly, this molecular method permits rapid specific identification of the aetiological agent. Although various antifungal compounds have been used for therapy, management of this condition (particularly if deep lesions occur) continues to be problematic; topical natamycin and, increasingly, voriconazole (given by various routes) are key therapeutic agents. Therapeutic surgery, such as therapeutic penetrating keratoplasty, is needed when medical therapy fails. Increased awareness of the importance of this condition is likely to spur future research initiatives.

Keywords: Azoles, confocal microscopy, culture, direct microscopy, epidemiology, fungal keratitis, mycotic keratitis, polymerase chain reaction, voriconazole

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Mycotic keratitis (International Nomenclature of Diseases disease number 2100) is a general term for a mycosis of the cornea, and can be caused by a wide variety of fungi [1]. This condition is usually manifested by severe inflammation, the formation of a corneal ulcer, and hypopyon, with the presence of fungal hyphae within the corneal stroma. Synonyms include 'keratomycosis' and 'oculomycosis' (in part), but 'mycotic keratitis' is recommended in preference to 'keratomycosis' so as to have similar names for the diseases caused by fungi, bacteria and viruses [1]. If the fungal species causing the infection is identified, a term such as '*Fusarium* keratitis'(or, more specifically, 'keratitis due to *Fusarium solani*') is recommended [1].

Epidemiology

Epidemiology refers to the study of the distribution and determinants of a disease in a given population in a given period of time. Whereas *prevalence* is the rate or frequency with which the disease is found in a group or population under study at a particular point in time, *incidence* is the frequency with which new cases of a disease arise over a defined period of time [2]. Going strictly by these definitions, there are no published reports on the prevalence of mycotic keratitis in the community, but there is one study, in the UK, that has reported on the incidence of mycotic

keratitis in a community (0.32 (95% CI 0.24–0.44) cases per million individuals per year) [3]. However, in less strict usage, modified for clinical series [2], it is possible to look at the prevalence of mycotic keratitis among individuals presenting with keratitis (corneal inflammation) to a hospital; this provides an estimate of the magnitude of the problem. In this respect, mycotic keratitis may account for more than 50% of all patients with culture-proven microbial keratitis [4,5], especially in tropical and subtropical environments. In terms of absolute numbers, this condition apparently occurs more frequently in developing countries (e.g. China and India) than in the developed world (e.g. the USA, Australia) A single institution in Hyderabad (India) reported that 1360 individuals with culture-proven mycotic keratitis were seen over a period of 10 years and 5 months [6], and another institution in northern China reported 654 patients with this condition over a 6-year period [4]. In contrast, mycotic keratitis was documented in just 56 eyes (56 patients) in Melbourne (Australia) and in 61 eyes (57 patients) in New York (USA) over 8-year and 16-year periods, respectively [7,8].

Although a high incidence of mycotic keratitis might be expected in countries with similar annual rainfall and temperature range, this is not always so and incidence also appears to depend on the extent of urbanization [9]. Mycotic keratitis associated with the wearing of contact lenses may also be on the rise [10] A statistically significant increase in the relative frequency of mycotic keratitis during the years 1997 to 2007 was noted in Egypt; this rise was found to correlate significantly with rises in minimum temperature and the maximum atmospheric humidity in the greater Cairo area over the same period [11]. A review of the data from studies on microbial keratitis conducted worldwide noted that whereas the highest proportion of bacterial corneal ulcers was reported from studies in North America, Australia, the Netherlands and Singapore, the highest proportion of fungal corneal ulcers was reported from studies in India and Nepal; interestingly, the Spearman correlation coefficient demonstrated a statistically significant inverse correlation between gross national income and percentage of fungal isolates in the studies [12]. A study in Brazil sought to predict the epidemiology of mycotic keratitis by monitoring the sales distribution of antifungal eye drops in Brazil; a linear regression model displayed a significant association between reduced relative humidity and sales of antifungal drugs, which was interpreted to mean a seasonal distribution of mycotic keratitis, with a higher incidence during the third quarter of the year (when the climate is drier and when agricultural activity is more intense in Brazil) [13].

Types and Aetiological Agents of Mycotic Keratitis

In terms of occurrence, risk factors and therapeutic approaches, two basic types of this condition are recognized, namely, keratitis due to filamentous fungi and keratitis due to yeast-like and related fungi (keratitis due to thermally dimorphic fungi has only rarely been reported). There appears to be a strong geographical influence on the occurrence of the different forms of mycotic keratitis. The proportion of corneal ulcers caused by filamentous fungi has shown a tendency to increase towards tropical latitudes, whereas in more temperate climates, fungal ulcers appear to be uncommon and to be more frequently associated with *Candida* species than filamentous fungi [14].

Keratitis due to filamentous fungi

Filamentous fungal keratitis usually occurs in healthy young males engaged in agricultural or other outdoor work; these fungi do not penetrate an intact epithelium and invasion is secondary to trauma. Trauma is the key predisposing factor, occurring in 40–60% of patients [5,6]; other reported risk factors include previous ocular surgery, ocular surface disease, previous use of corticosteroids (either topical or systemic) and contact lens use [10,15,16]. Interestingly, in one study on mycotic keratitis, response to antifungal therapy and or surgery was observed in none of six patients with previous ocular surgery, two of six patients with previous ocular trauma, two of six patients with ocular surface disease, all three patients with contact lens use and six of 16 patients with previous use of corticosteroids [16]. Traumatizing agents of plant or animal origin (even dust particles) either directly implant fungal conidia in the corneal stroma or abrade the epithelium, permitting fungal invasion [4–6,17,18].

Species of *Fusarium*, *Aspergillus*, *Curvularia* and other phaeohyphomycetes, *Scedosporium apiospermum* and *Paecilomyces* are the principal causes of filamentous fungal keratitis, but many other species have been implicated [18–21] (Table 1). Environmental factors (humidity, rainfall, wind) appear to have a bearing on the occurrence of filamentous fungal keratitis and may also determine seasonal variations in the frequency of isolation of fungi and the fungal species isolated [14]. Along the Gulf of Mexico, keratitis due to *Curvularia* spp. appeared to occur more frequently during the hotter, moister, summer months, possibly because of an increase in airborne *Curvularia* spores during these months [22].

Although *Fusarium* species have been cultured from soft contact lenses during use [23], it was still a surprise when, from mid-2005 to around July 2006, a multi-country outbreak of contact lens-associated keratitis due to *Fusarium* species

TABLE 1. Reported aetiological agents in mycotic keratitis[a]

Genus	Species
1. Hyaline filamentous fungi	
Acremonium	*A. atrogriseum*, *A. curvum*, *A. kiliense*, *A. potronii*, *A. recifei*[b], *Acremonium* species[b]
Arthrographis	*A. kalrae*
Aspergillus	*A. clavatus*, *A. fischerianus*, *A. flavipes*, *A. flavus*, *A glaucus*, *A. fumigatus*, *A. janus*[b], *A. niger*, *A. terreus*, *A. nidulans*[b], *A. oryzae*, *A. wentii*[b]
Beauveria	*B bassiana*
Cephaliophora	*C. irregularis*
Chrysonilia	*C. sitophila*[b] (formerly *Neurospora sitophila*)
Chrysosporium	*C. parvum*[b]
Cylindrocarpon	*C. lichenicola* (*C. tonkinense*)
Diplosporium	*Diplosporium* species[b]
Engyodontium	*E. alba* (formerly *Beauveria alba*)
Epidermophyton	*Epidermophyton* species[b]
Fusarium	*F. aquaeductum*, *F. dimerum*, *F. oxysporum*, *F. solani*, *F. verticilloides* (*F. moniliforme*), *F. nivale*[b], *F. subglutinans*, *F. ventricosum*
Glenospora	*G. graphii*[b]
Metarhizium	*M. anisopliae*
Microsporum	*Microsporum* species[b], *M. canis*
Myrathecum	*Myrathecum* species[b]
Ovadendron	*O. sulphureo-ochraceum*
Paecilomyces	*P. farcinosus*, *P. lilacinus*, *P. variotii*
Penicillium	*P. citrinum*, *P. expansum*
Rhizoctonia	*Rhizoctonia* species
Sarcopodium	*S. oculorum*
Scedosporium	*S. apiospermum* (reported as *Pseudallescheria boydii*; previously *Allescheria boydii*, *Petriellidium boydii*, *Monosporium apiospermum*)
Scopulariopsis	*S. brevicaulis*
Tritirachium	*T. oryzae*
Ustilago	*Ustilago* species[b]
Verticillium	*V. searrae*[b], *Verticillium* species
2. Phaeohyphomycetes	
Alternaria	*A. alternata*, *A. infectoria*[b], *Alternaria* spp.[b]
Aureobasidium	*A. pullulans*[b]
Bipolaris	*B. hawaiiensis*, *B. spicifera* (formerly *Drechslera*)
Cladosporium	*C. cladosporioides*[b]
Curvularia	*C. brachyspora*, *C. geniculata*, *C. lunata*, *C. pallescens*, *C. senegalensis*, *C. verruculosa*[b]
Dichotomophthoropsis	*D. nymphearum*, *D. portulacae*
Doratomyces	*D. stemonitis*
Exophiala	*E. jeanselmei* var. *dermatitidis*, *E. jeanselmei* var. *jeanselmei*
Exserohilum	*E. rostratum*, *E. longirostratum*
Fonsecaea	*F. pedrosoi*
Lecytophora	*L. mutabilis*[b]
Phaeoisaria	*P. clematitidis*
Phaeotrichoconis	*P. crotalariae*
Phialophora	*P. bubakii*, *P. verrucosa*
Tetraploa	*T. aristata*
3. Phaeoid Sphaerosidales	
Colletotrichum	*C. capsici*[b], *C. coccodes*[b], *C. dematium*[b], *C. graminicola*, *C. gloenosporioides*, *Colletotrichum* state of *Glomerulla cingulata*
Lasiodiplodia	*L. theobromae*
Microsphaeropsis	*M. olivacea*[b]
Phoma	*P. oculo-hominis*[b], *Phoma* species
Sphaeropsis	*S. subglobosa*
4. Yeast and yeast-like fungi	
Candida	*C. albicans*, *C. famata*, *C. glabrata*[b], *C. guilliermondii*, *C. krusei*, *C. parapsilosis*, *C. tropicalis*[b]
Cryptococcus	*C. laurentii*, *C. neoformans*[b]
Geotrichum	*G. candidum*[b]
Malassezia	*M. furfur*[b]
Rhodotorula	*R. glutinis*[b],*R. rubra*[b], *Rhodotorula* species
Rhodosporidum	*R. toruloides*[b]
5. Dimorphic fungi	
Blastomyces	*B. dermatitidis*
Coccidioides	*C. immitis*
Paracoccidioides	*P. brasiliensis*
Sporothrix	*S. schenckii*
6. Other fungi	
Absidia	*A. corymbifera*
Chlamydoabsidia	*C. padenii*
Pythium	*P. insidiosum*
Ulocladium	*U. atrum*
Scytalidium	*Scytalidium* sp.
Blastoschizomyces	*B. capitatus*
7. Newly reported agents	
Aspergillus viridinutans [19]	
Candida fermentati [20]	
Thelavia subthermophilia [21]	

[a]Modified from ref [18].
[b]Key to Uncertain: 1 = not listed in MedLine; 2 = deemed questionable by McGinnis [69]; 3 = details of identification inadequate; 4 = morphology in tissue not consistent with the fungus isolated. *Paecilomyces variotii* + = initially identified as *Paecilomyces viridis*.

occurred because, until that time, filamentous fungi had been infrequently linked to contact lens-associated keratitis. Epidemiological and microbiological studies implicated the use of a specific brand of contact lens multipurpose solution in many patients affected by the outbreak. The high polymer content of the solution, as well as non-compliance by the patients, was hypothesized to have facilitated contamination of the solution by *Fusarium* strains derived from the local environments of the patients. [24].

Keratitis due to yeast-like and related fungi

In keratitis due to *Candida albicans* and related fungi, one or more ocular (e.g. insufficient tear secretion, defective eyelid closure) or systemic (e.g. diabetes mellitus, immunosuppression) conditions predispose to the infection [25]. This form of mycotic keratitis may also supervene on a pre-existing epithelial defect due to herpes keratitis or due to abrasions caused by contaminated contact lenses [25].

Diagnosis of Mycotic Keratitis

If the diagnosis of mycotic keratitis is made within a short time, it improves the chances of a complete recovery. Obtaining a detailed clinical history should be followed by a meticulous search for ocular or systemic defects that may have predisposed to the keratitis, because these need to be corrected to ensure that the condition does not recur. Symptoms resemble those reported in other forms of keratitis but, possibly, are more prolonged in duration (5–10 days).

Diagnosis based on clinical presentation

Filamentous fungal keratitis may involve any area of the cornea and usually exhibits the following features: firm (sometimes dry) elevated slough; 'hyphate' lines extending beyond the ulcer edge into the normal cornea; multifocal granular (or feathery) grey-white 'satellite' stromal infiltrates (Fig. 1); 'immune ring'; minimal cellular infiltration in the adjacent stroma; mild iritis [18,26,27]. An elevated firm slough and hyphate margins are found in more than 50% of culture-proven cases [18]. Although every case of filamentous fungal keratitis may exhibit some of these basic features, there may be variations, depending on the aetiological agent. Chronic, severe filamentous fungal keratitis may resemble bacterial suppuration and involve the entire cornea. Keratitis due to yeast-like fungi (e.g. *C. albicans*) and related fungi usually resembles bacterial keratitis, with an overlying epithelial defect, a more discrete infiltrate and slow progression [25].

In a logistic regression model, serrated margins, raised slough and colour other than yellow were found to be

independently associated with mycotic keratitis; the probability of fungal infection, 63% if one clinical feature occurred, increased to 83% if all three features occurred [28]. In a recent study [29], clinicians were able to correctly differentiate a bacterial aetiology from a fungal aetiology 66% of the time, but the Gram stain, genus and species were accurately predicted less frequently (46%, 25% and 10% of the time, respectively). The presence of an irregular/feathery border was associated with mycotic keratitis, whereas a wreath infiltrate or an epithelial plaque was associated with bacterial keratitis [29]. Hence, although certain clinical signs of infectious keratitis may be associated with a bacterial or fungal aetiology, appropriate microbiological tests should be performed at presentation wherever possible [28–30].

In vivo diagnosis of mycotic keratitis

Non-invasive techniques are being increasingly used for 'real-time' detection of the aetiological agent in patients presenting with suspected microbial keratitis. Non-invasive methods of diagnosis include confocal microscopy [31–34], and anterior segment optical coherence tomography [33,35].

The confocal microscope allows *in vivo* examination of the cornea. First-generation confocal microscopes have given way to more advanced configurations, such as the advanced tandem scanning confocal microscope and the Heidelberg Retina Tomograph II-Rostock Cornea Module (HRTII-RCM). Recently, HRTII-RCM *in vivo* confocal microscopy aided the diagnosis of a fungal aetiology in a patient with keratitis due to *Colletotrichum gloeosporioides*, with many septate, hyphae-like interlocking and branching white lines being visible in the area of the infiltrate [31]; confocal microscopy has been used similarly in a patient with *Cylindrocarpon lichenicola* keratitis [32]. The HRTII-RCM was also recently used to demonstrate sub-basal corneal nerve alterations (reduced total corneal nerve lengths and counts, and number of main nerve trunks and nerve branching) in patients with acute *Acanthamoeba* keratitis and mycotic keratitis (compared with normal controls and patients with herpetic keratitis [34]. Spectral domain anterior segment optical coherence tomography of 20 eyes (20 patients) with proven fungal or bacterial keratitis (including 12 eyes with culture-proven *Aspergillus* spp. keratitis) revealed that mycotic keratitis presented in two unique patterns, namely, early localized and diffuse necrotic stromal cystic spaces [35].

In addition to diagnosis, *in vivo* confocal microscopy and anterior segment optical coherence tomography may also be used to monitor the response of mycotic keratitis to treatment. After 1 month of antifungal therapy to a patient with keratitis due to *Alternaria alternata* [33], confocal microscopy demonstrated a significant reduction in inflammatory cells and the presence of hyper-reflective scar-like tissue and absence of branching hyphal infiltrates in the affected cornea; optical coherence tomography also documented the healing process and the complete recovery of the central and peripheral stromal thickness of the affected cornea [33].

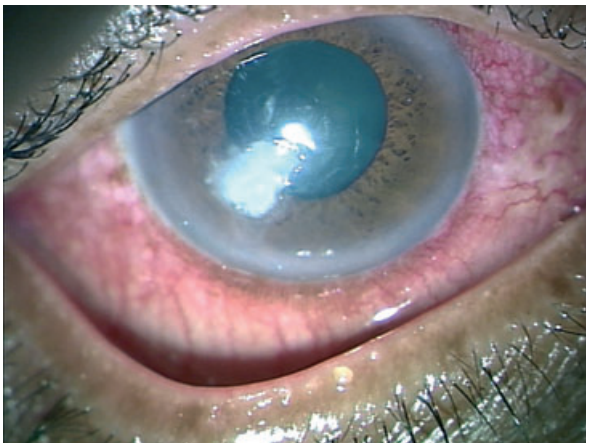

FIG. 1. Keratitis due to *Fusarium solani;* the irregular edges of the corneal lesion are prominent.

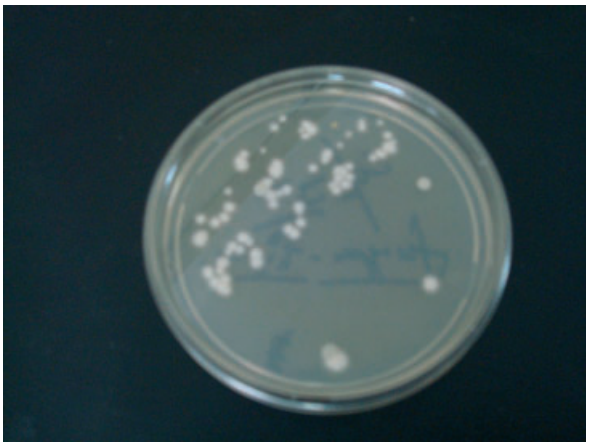

FIG. 2. Growth of a filamentous fungus on the 'C'-streaks of corneal scrape material made on a culture plate of Sabouraud glucose–neopeptone agar (after 48 h incubation at 30°C).

The use of confocal microscopy as a diagnostic aid in microbial keratitis was recently evaluated, with conflicting viewpoints. Using a positive tissue diagnosis as the reference standard, the authors of one study did not recommend stand-alone use of confocal microscopy for the diagnosis of microbial keratitis [36]. Conversely, when conventional microbiology findings were used as the gold standard, the authors of another study concluded that confocal microscopy provides accurate and reliable diagnosis in mycotic keratitis, particularly when the corneal infiltrate is deep-seated or patients are on treatment or when microbial keratitis develops after intracorneal

TABLE 2. Techniques for detection of fungi by direct microscopy

Method	Advantages	Disadvantages
1. Potassium-hydroxide (KOH) mount	a. Single-step, Inexpensive simple method. b. 86% positivity in a series of *Fusarium* keratitis c. Oil immersion magnification not required	a. Artefacts are common b. Corneal cells do not swell to produce transparent preparations
2. Gram-staining	a. Stains *Candida* blastospores and pseudohyphae b. Stains hyphae of fungi c. Bacteria stained and differentiated d. Preparation can be restained with methenamine silver e. Takes 5 min to perform	a. May stain fungal hyphal cytoplasm irregularly or not at all in some cases b. Stains fibrous protein, causing opacity c. False-positive artefacts common d. Crystal violet precipitation may obscure detail and cause confusion
3. Giemsa staining	a. Differential staining of tissue and cellular elements b. Stains yeast cells and hyphae c. Stains chlamydiae, viral inclusions and protozoa	a. Disadvantages similar to Gram stain b. Tissue cells stain, forming opaque area where smear is thick c. False-positive artefacts common d. Buffer and working solutions need careful preparation e. Staining time of 60 min
4. Lactophenol cotton blue staining. Sensitivity: 78%	a. Stain easily available with shelf-life of 1 year b. Rapid, simple, inexpensive one-step method c. Important ocular fungi can be seen and identified d. Wet mount is a semi-permanent preparation that can be kept for years e. *Acanthamoeba* can be detected	a. No digestion of tissue b. Unusual fungi may escape detection c. Contrast between fungi and background material may sometimes be insufficient
5. Methenamine silver staining (modified)	a. Stains fungal cell wall clearly; no interference f rom background b. Positives more frequent and reliable than in methods 1–6 c. Negatives more reliable	a. Excessive deposit of silver may obscure cell wall and septa b. Stains cellular debris and melanin c. Gelatin-coated slides needed d. Controls needed; reagents and procedure need standardization e. Takes 60 min to perform
6. Calcofluor white (CFW) Fluorescent dye with high affinity for polysaccharides, such as fungal cell walls. This dye is used to detect fungi in corneal scrapings and in aqueous and vitreous samples .	a. Excellent sensitivity and good specificity b. Detects yeast blastoconidia and hyphae of filamentous fungi c. Material from KOH mount can be used for subsequent CFW staining	a. Not all fungi are adequately stained b. Ultraviolet microscope needed c. Corneal collagen stained, which may cause confusion with the hyphae of filamentous fungi d. Reagents and procedure need standardization and expertise

Modified from Ref [18]

implants [37]. These imaging facilities are valuable in regions where cost is no constraint to the investigation of infectious keratitis.

***In vitro* diagnosis using conventional microbiological methods**

Wherever possible, microbiological investigations should be performed in patients presenting with suspected microbial keratitis [14,38]. Material is collected using a corneal spatula or blade, which is used to scrape the base and edges of the ulcerated part of the cornea several times. Material collected in one scraping is used to inoculate culture plates and material collected in an additional scraping is used to prepare smears or mounts for direct microscopic examination (the spatula can be flamed after use and cooled before using again; a set of blades can be used for one patient). Corneal biopsy may have to be performed where scrapings yield negative results; aqueous humour may also have to be obtained from the anterior chamber. Corneal material is usually inoculated on culture plates in the form of multiple 'C's (Fig. 2); only growth on the 'C'-streaks is deemed significant [38]. Two agar media that should be used are blood agar (incubated at 37°C) and Sabouraud glucose–neopeptone agar (incubated at 22–25°C); additional media can be used if warranted [14,38]. In addition to the solid media, it may be useful to use a liquid medium, such as brain–heart infusion broth, containing an antibacterial drug to suppress bacterial growth; however, not all investigators agree on this point [14,39]. An incubation temperature of 30°C and the use of liquid-shake cultures may also help in isolation of ocular fungi. Fungal growth usually occurs within 3–4 days (Fig. 2) but culture media may require incubation for up to 4–6 weeks. Growth in culture is deemed significant if the same growth is obtained (i) on more than one occasion, (ii) on the 'C' streaks on more than one culture medium, or (iii) on one solid or in one liquid medium with direct microscopy of corneal material revealing the presence of fungal hyphae or yeast cells [14].

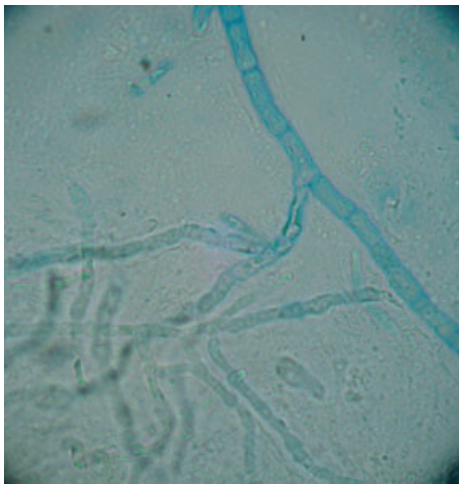

FIG. 3. Wet film of corneal scrape material stained with lactophenol cotton blue, demonstrating the presence of septate hyaline fungal hyphae (× 400 magnification).

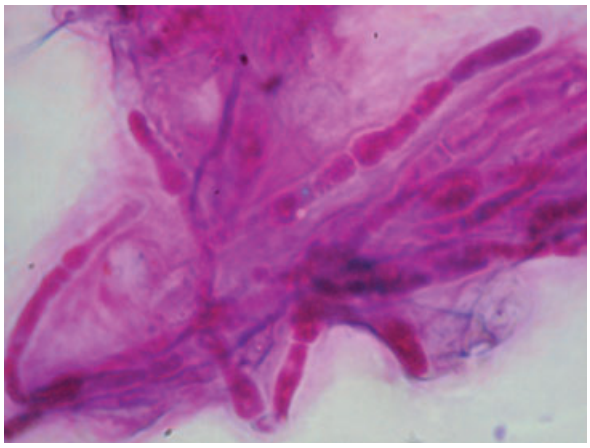

FIG. 4. Smear of corneal scrape material stained by the Gram method, demonstrating the presence of septate hyaline fungal hyphae (× 1000 magnification).

Direct microscopic examination of corneal scrapings permits a rapid presumptive diagnosis of mycotic keratitis; the techniques used have several advantages and disadvantages [6,38–41] (Table 2). A suggested set of smears for direct microscopic detection of fungal structures in corneal material would be: a wet preparation (potassium hydroxide or lactophenol cotton blue) (Fig. 3); a Gram-stained smear (Fig. 4); a smear for staining by special fungal stains (Giemsa, periodic acid Schiff, Gomori methenamine silver stain, calcofluor white). The corneal material should be spread out as thinly as possible on the slides so as to facilitate visualization of the fungal hyphae or yeast cells (Fig. 3,4).

In vitro diagnosis using molecular tools

Conventional microbiological methods used for diagnosis of mycotic keratitis suffer from inherent drawbacks [38,39]. If fungal hyphae or yeast cells are detected by direct microscopic examination of corneal material, a rapid presumptive diagnosis of mycotic keratitis can be made. However, an inexperienced observer may not be able to detect these fungal structures; more importantly, it is rarely possible to accurately identify the fungal genus and species involved [39]. Culture of corneal material overcomes this limitation of direct microscopic examination; however, a positive result (growth in culture or specific identification of the fungus isolated) usually requires a minimum of 48–72 h, and some expertise is required for precise identification of the fungal species isolated [39]. These limitations have led to the evaluation of molecular methods, specifically PCR, as a diagnostic tool for mycotic keratitis. A recent paper [42] elegantly summarizes the key features of the more than 25 reports in the literature (from 1996 to 2011) that evaluated PCR as a diagnostic tool for mycotic keratitis.

PCR is an ideal diagnostic method for mycotic keratitis because only a small quantity of sample (corneal scrape or corneal biopsy material) is required to perform the test. While the cutinase gene was the target in the first report in 1996, nearly all the other studies targeted the fungal ribosomal DNA regions, such as 18S rRNA, 28S rRNA and internal transcribed spacer regions. Many of the reported studies have sought to detect fungal DNA in the corneal sample by PCR-based amplification using universal (panfungal) primers or more specific primers, followed by identification of the fungus by sequencing of the amplified fungal DNA; other studies have reported molecular identification of fungi isolated in culture from corneal scrapings [42]. The biggest advantage reported is the speed at which a diagnosis of mycotic keratitis can be made [43,44] and, perhaps more importantly, accurate species identification can be achieved; this has led certain investigators to advocate the use of PCR as the reference standard for diagnosis of mycotic keratitis [42]. On the other hand, a careful evaluation of previous studies reveals good agreement between the results obtained using conventional tests and those obtained using PCR [44]. Moreover, one set of investigators [45] cautioned against the routine everyday use of the technique because non-pathogenic microorganisms could conceivably be amplified, therein confusing the diagnosis. PCR cannot be used to monitor the response of a patient with mycotic keratitis to antifungal therapy because it is not possible to differentiate viable from non-viable fungi. Performing the PCR for the diagnosis of mycotic keratitis may be more expensive than using conventional microbiological methods. Hence, PCR may possibly be reserved for diagnosis of mycotic keratitis in patients in whom conventional tests do not yield positive results. Probably, in the context of mycotic keratitis, the most important use of the PCR is in permitting the rapid identification of the infecting fungal strain, because routine morphological identification may not suffice to identify certain species of *Fusarium* and of species previously unreported as causes of mycotic keratitis [19–21,39].

Management

Keratitis due to filamentous fungi continues to be difficult to treat despite the use of topical and systemic antifungal agents and adjuvant surgery, such as corneal transplantation. A recent Cochrane Database systematic review of medical interventions for mycotic keratitis (an update of a review in 2005) analysed nine randomised controlled trials involving 568 participants who were randomized to various comparisons (including 1% topical itraconazole versus 1% topical itraconazole and oral

itraconazole, voriconazole 1% versus natamycin 5%). It was concluded that, based on the available literature, there is no evidence to suggest that any particular drug, or combination of drugs, is more effective than any other in the management of mycotic keratitis; the trials included in this review were found to be of variable quality and were generally underpowered [46]

Mycotic keratitis is managed by medical or surgical means. Medical therapy consists of non-specific measures and the use of specific antifungal agents. Cycloplegics are used to relieve the iridocyclitis that usually accompanies mycotic keratitis; broad-spectrum antibacterial agents may be needed to combat secondary bacterial infection.

In vitro antifungal susceptibility testing

Determination of the pattern of susceptibility of a fungal isolate from keratitis to different antifungal agents by an agar dilution [47] or broth dilution [48] method may aid rational specific antifungal therapy, but most laboratories do not routinely perform such tests because the methods in use are diverse. Using an agar dilution method for antifungal susceptibility testing, pre-treatment *in vitro* susceptibility data were found to correctly predict clinical responses in 43% of clinical cases of *Aspergillus* keratitis and in 37% of cases of *Fusarium* keratitis [47]; however, oral itraconazole therapy was used to treat patients in this study. In a more recent study that determined the MICs of natamycin and voriconazole on isolates from fungal keratitis, a higher MIC was significantly associated with an increased likelihood of perforation; however, there was no significant association between MIC and 3-week or 3-month visual acuity or between MIC and 3-week or 3-month infiltrate/scar size [48]. The real value of *in vitro* antifungal susceptibility testing in mycotic keratitis lies, perhaps, not in predicting responses in individual cases, but in providing important baseline data on the spectrum of activity of antifungal compounds against ocular fungal isolates.

Medical therapy of fungal keratitis

To treat mycotic keratitis effectively, a drug must be non-irritating and non-toxic in the eye, must penetrate the eye well and have a high level of antifungal activity against at least one significant ocular pathogen. Antifungal agents that are useful in the treatment of mycotic keratitis include [16,17,38,49–53]:

- Topical natamycin (5%), econazole (1%), amphotericin B (0.15–0.3%), flucytosine (1%), clotrimazole (1%), miconazole (1%), ketoconazole (1–2%), itraconazole (1%), fluconazole (1%), voriconazole (1–2%) and caspofungin (0.5%);
- Subconjunctival miconazole (10 mg in 0.5 mL) and fluconazole (0.5–1.0 mL of a 2% solution);
- Intravenous amphotericin B and miconazole (600–1200 mg/day).
- Oral ketoconazole (200–600 mg/day); itraconazole (100–200 mg/day); fluconazole (50–200 mg/day) and voriconazole (400 mg/day);
- Intrastromal injection of voriconazole and amphotericin B (5 mg per 0.1 mL);
- Intracameral voriconazole (50 μg/0.1 mL);
- Intravitreal amphotericin B, fluconazole and voriconazole

More details are provided in Table 3. As all the available antifungal agents only inhibit growth of the fungus, and the host defence mechanisms must eradicate the organism, treatment is usually prolonged.

An antifungal agent chosen for therapy of mycotic keratitis should be easily available. Treatment can be commenced on the basis of direct microscopy findings alone if these are unequivocal and consistent with the clinical evaluation; otherwise, therapy should be withheld while awaiting the results of culture. Topical natamycin (5%) is usually chosen as initial therapy for superficial keratomycoses, regardless of whether septate hyphae or yeast cells are seen by direct microscopy; additional antifungal agents (e.g. amphotericin B, ketoconazole, itraconazole) are added for deep corneal infections. The initial antifungal agent may also be chosen depending on whether yeast cells or hyphae are seen by microscopy: if hyphae are definitely seen by microscopy, topical natamycin (5%) is the drug of choice (0.15% amphotericin B or, currently, 1% voriconazole [16,17] are alternatives); if yeasts or pseudohyphae are seen, topical 0.15% amphotericin B, 1% fluconazole or 1% voriconazole is preferred.

Once the organism has been identified by culture, the therapeutic regimen may be modified. Most recommendations in the literature concerning the choice of antifungal once the infecting fungus has been identified are probably based on personal experience or on the results of *in vitro* antifungal susceptibility testing. This led one investigator to review the therapy of keratitis caused by frequently encountered hyaline filamentous fungi, phaeohyphomycetes and yeast-like fungi based on reports in the published literature [38]. Some of the key observations made were:

- More than 70% of patients with superficial keratitis due to *Fusarium solani* and other *Fusarium* spp. apparently respond to medical therapy alone; although several antifungals have been found to be effective, administration of natamycin may prevent surgical intervention. However, almost 70% of patients with *Fusarium* keratitis with deep lesions do not respond to medical therapy alone, particularly if natamycin is not used, and some form of surgical intervention is necessary.

TABLE 3. Antifungal drugs to treat mycotic keratitis

Drug, features and advantages (References)	Drawbacks (References)
1. Natamycin (Pimaricin)	a. Not commercially available as an ophthalmic preparation in many regions
a. Commercially available as topical 5% suspension for ophthalmic use in some countries, where it constitutes first-line therapy for mycotic keratitis	b. Effective only when applied topically
b. Ophthalmic preparation is well-tolerated, stable and can be sterilized by heat	c. Natamycin therapy may not be effective when keratitis is associated with deep stromal lesions
c. Relatively high levels reportedly achieved in cornea after topical application	d. Only about 2% of total drug in corneal tissue is bioavailable
2. Amphotericin B	a. Intravenous administration frequently associated with renal tubular damage, due to use of deoxycholate as vehicle
a. Good *in vitro* activity against *Aspergillus* spp. and *Candida* spp.; emergence of resistant mutants rare	b. Subconjunctival injection causes marked tissue necrosis at the site of injection
b. Can be administered by topical (0.15–0.30% solution), intracameral (7.5–30 μg/0.1 mL), intravenous (0.5–1 mg/kg BW/day) or intravitreal (1–5 μg/0.1 mL) routes	c. Topical application of concn > 5.0 mg/mL may cause ocular irritation (solutions of 1.5–3.0 mg/mL better tolerated)
c. Penetrates deep corneal stroma after topical application; bioavailability sufficient for susceptible fungi	d. Not commercially available as topical ophthalmic preparation; needs to be reconstituted from powder or intravenous preparation
Exerts direct fungicidal effect and exhibits immunoadjuvant properties	e. Poor intraocular penetration after intravenous administration
3. Miconazole	a. Use of intravenous preparation occasionally associated with toxicity due to the vehicle used
Reported routes of administration in mycotic keratitis: topical (1%), subconjunctival (10 mg/0.5 mL), intravenous (600–1,200 mg/day); topical and subconjunctival administration generally well-tolerated	b. Undetectable concentration of drug in rabbit corneas and vitreous after intravenous administration
	c. Generally considered useful in *Scedosporium apiospermum* ocular infections, but treatment failures have occurred
4. Ketoconazole	a. Oral doses >400 mg/day may cause transient rise in concn of serum transaminases
a. Given by oral (200–400 mg/day) or topical (1–2% suspension) routes in ophthalmic mycoses	b. Acid pH required for absorption
Well-absorbed and good tissue distribution after oral administration. Peak serum concn of 2–3 μg/mL 2–3 hours after 200 mg oral dose	c. Prolonged administration of high doses may cause impotence, gynaecomastia or alopecia or papilloedema. No commercially available solution of ketoconazole for topical or subconjunctival administration in ophthalmic mycoses
5. Itraconazole	a. Commercially available capsule (100 mg) should be taken with meal; difficult to give in infants and children
a. Synthetic dioxolane triazole	b. May be poorly absorbed after oral administration in certain groups of patients. Caution needed in patients with previous hepatic disease
b. Given by oral (200–400 mg/day) or topical (1% suspension) routes in ophthalmic mycoses. Oral solution and intravenous formulation recently developed; no reports of use in ophthalmic mycoses	c. Absorption after oral dosing affected by antacids and H_2 receptor antagonists; may interact with other drugs
c. Peak serum concn 0.3 μg/mL after single oral dose of 200 mg; increased to 3.5 μg/mL after 200 mg/day orally for 14 days	d. Poor penetration into rabbit ocular tissue, compared with fluconazole and ketoconazole, after oral dosing
	e. Intravitreal injection (>10 μg) causes focal retinal necrosis in rabbits
	f. No commercially available solution of itraconazole for topical or subconjunctival administration
6. Fluconazole	a. May interact with cisapride, oral antidiabetic drugs and phenytoin after oral administration
a. Synthetic bistriazole	b. Less active against *Candida glabrata* and *Candida krusei* than against *C. albicans*
Soluble in water, hence excreted through kidney; 10–20% protein bound in serum; long half-life	c. May not be effective in treatment of filamentous fungal keratitis
b. Given by oral (50–100 mg/day), topical (0.2 to 2% solution) or intravenous routes	
c. High bioavailability, low toxicity, good stability	
d. Commercially available for oral and intravenous use	
7. Voriconazole (Azole)	Voriconazole monotherapy may sometimes not effect cure; caspofungin may need to be added
a. Potent activity against a broad spectrum of yeasts and moulds	
b. Oral (200 mg twice daily), topical (1%), intravenous and intravitreal (100 μg/0.1 mL) routes of administration have all been described	
c. Achieves 53% and 38%, respectively, of plasma levels in aqueous and vitreous following oral administration	
d. Has been used successfully to treat keratitis	

Modified from ref [38]

- More than 80% of patients with keratitis due to *Aspergillus flavus*, *Aspergillus fumigatus* and other *Aspergillus* spp. respond to medical therapy alone with a variety of topical or systemic antifungals; however, in the presence of deep corneal lesions, almost 60% of patients do not respond to medical therapy alone, particularly if natamycin is not used, and surgical intervention is needed.
- Medical therapy of keratitis due to *Candida* spp. generally has a favourable prognosis, particularly when topical amphotericin B 0.15% is used alone or in combination with systemic azoles, and the presence of deep lesions is not a major hurdle.
- Most patients with keratitis due to *Curvularia* spp. can be treated with antifungals alone, particularly when natamycin is used; however, surgery may be required when deep lesions are present. Keratitis due to phaeohyphomycetes other than *Curvularia* spp. appears to respond to primary therapy with topical natamycin, oral and or topical ketoconazole, oral ketoconazole and topical miconazole, topical amphotericin B alone or oral itraconazole alone. However, therapy of keratitis due to *Lasiodiplodia theobromae* is often difficult to treat.
- Miconazole appears to be important in the treatment of keratitis due to *Scedosporium apiospermum*; its relative efficacy in comparison to natamycin is difficult to evaluate.

For topical therapy, most workers advise hourly application around the clock for several days and the dosage is then gradually reduced.

TABLE 4. Recent reports on voriconazole (VZ) for the treatment of mycotic keratitis

Route of administration and number of patients [Reference]	Results	Microbiological data and comments
Topical 1% VZ vs topical 5% natamycin 120 patients (60 randomized to VZ, 60 randomized to natamycin) [49]	No significant differences in visual acuity, scar size and perforations between the two groups	*Fusarium* spp. isolated in 44 cases (21 received natamycin, 23 VZ), different *Aspergillus* spp in 19 (11 received natamycin, 8 VZ) and other filamentous fungi in 39 (21 received natamycin, 18 VZ); however, no information provided on the organism-wise response to either natamycin or VZ
Topical 1% VZ vs topical 5% natamycin 30 patients (15 randomized to VZ, 15 randomized to natamycin) [17]	Complete healing in all 15 natamycin-treated patients and 14 of 15 VZ-treated patients	*Aspergillus* spp. Isolated in 12 cases (6 received natamycin, 6 VZ), *Curvularia* spp. in 9 (4 received natamycin, 5 VZ), *Fusarium* sp. in 3 (1 received natamycin, 2 VZ) and *Aureobasidium* in, 1 (natamycin]; no growth in culture in 5 (3 received natamycin, 2 VZ); however, no information provided on the organism-wise response to either natamycin or VZ
Topical 1% VZ, oral VZ (400 mg), intracameral VZ and intrastromal VZ 26 patients (13 responded to medical therapy alone, 11 required additional surgery) [16]	13 cases healed a. 7 topical VZ only b. 1 topical VZ and 5% natamycin c. 5 initial topical natamycin or AB, then added topical VZ	a. *Fusarium* spp. isolated in 7 patients (3 responded), *Candida* spp. in 4 (2 responded), *Scedosporium apiospermum* in 3 (1 responded), *Aspergillus fumigatus* in 3 (2 responded), *Paecilomyces* spp. in 2 (neither responded) and *Bipolaris* spp. in 2 (both responded) b. Non-responders more likely to have peripheral infiltrates and hypopyon
a. VZ used systemically, topically and/or by intraocular injection b. 24 patients (15 keratitis, 9 endophthalmitis) Primary therapy in 8 Salvage therapy in 9 [56]	a. Overall response 67% (keratitis 73% and endophthalmitis 56%) b. Adjunctive surgery in 7 c. Response Primary therapy 63% Salvage therapy 69%	14 *Fusarium solani* infections, 9 (64%) responded; 8 *Fusarium* spp. infections, 8 (80%) responded 69% response when VZ used in combination with topical AB, caspofungin or natamycin) or systemic agents 64% response when VZ used alone
a. Intrastromal (50 μg in 0.1 mL) VZ b. Topical and systemic antifungal therapy 12 patients [57]	$\geq$ 1 intrastromal injection(s) of VZ at junction of clear cornea and infiltrates 5 quadrants to form a barrage around ulcer	Of 12 eyes (8 with *Aspergillus* infection, 3 with *Fusarium* infection and 1 with a *Curvularia* infection), 10 eyes healed with scar formation (mean resolution time was 39.75 $\pm$ 7.62 days) and showed improvement in visual acuity
Intracameral (50 μg/0.1 mL) and topical VZ 5 eyes (5 patients) with deep fungal keratitis with endoexudates ($\pm$ stromal infiltrates) [58]	In all eyes: a. size and density of endoexudates reduced b. complete resolution of infection c. marked improvement in visual acuity, within 3 weeks to 3 months	a. The aetiological fungus could not be isolated in 3 patients (diagnosis of fungal infection made by positive smear) b. *Aspergillus* spp. were isolated from the other two eyes
a. Topical and oral VZ or topical natamycin; 3 eyes (3 patients) all soft contact lens wearers [59] b. Topical 1% VZ; one eye [60] c. Topical 1% VZ and intracameral VZ; 2 eyes (2 patients) [61] d. Topical VZ and oral terbinafine; one eye [62]	a. Only one eye responded to medical therapy alone b. Healed within 1 month c. One eye healed with topical VZ alone after keratoplasty d. Healed	a. Keratitis due to *Paecilomyces* species other 2 needed other measures b. *Paecilomyces lilacinus* was isolated from the ulcer (there had been no response to topical natamycin or topical AB) c. *Paecilomyces lilacinus* isolated from both eyes; the second eye required VZ by multiple routes in addition to keratoplasty d. *Paecilomyces lilacinus* recovered in culture (there had been no response to initial topical natamycin and oral itraconazole)
a. VZ b. caspofungin c. natamycin d. fluconazole, Six eyes (6 patients); two were soft contact lens wearers [59,63,64]	a. Good response in five eyes b. Partial response in one eye	*Alternaria* spp. isolated in culture Two eyes responded well to topical and oral VZ or topical natamycin Three eyes responded well to topical fluconazole or combined VZ (intrastromal and topical) and caspofungin (intrastromal and topical)
Intracameral VZ 10 eyes (10 patients) with endophthalmitis due to keratitis [65]	VZ was injected intracamerally 1 to 8 times	Seven patients (6 with *Fusarium* and 1 with *Acremonium*) received 5 or more injections Three patients (2 with *Aspergillus* and 1 with *Alternaria*) received 4 or fewer injections

Voriconazole is a new generation triazole antifungal agent. Only marketed in systemic formulation and with broad-spectrum activity and high intraocular penetration, voriconazole has been reported to be useful in the treatment of mycotic keratitis. In 2008, a review of the results of over 40 clinical case reports of treatment with voriconazole led to the suggestion that voriconazole could be safely and effectively used against a broad range of fungal pathogens [54]. In 2010, the authors of another review concluded that topical voriconazole (usually prepared from the systemic preparation, and typically of 1% concentration) is well-tolerated by the eye and is stable; however, they believed that additional studies were needed to conclusively determine its efficacy as a first-line and stand-alone treatment, preparation of higher concentrations, and optimal dosing frequency in mycotic keratitis [55]. Table 4 summarizes the salient features of recent reports on the use of voriconazole in therapy of clinical mycotic keratitis [16,17,49,56–65]

Fungal keratitis usually responds slowly over a period of weeks to antifungal therapy. Clinical signs of improvement of a fungal corneal ulcer include a decrease in pain and in size of the infiltrate, disappearance of satellite lesions, rounding out of the feathery margins of the ulcer, and hyperplastic masses or fibrous sheets in the region of healing fungal lesions [27,66]. Signs of toxicity of the topical antifungal agent should also be looked for. Negative scrapings during treatment do not always indicate that the infecting fungus has been eradicated, as it may become deep-seated; hence therapy should be maintained for at least 6 weeks.

Although natamycin is widely used as first-line therapy for filamentous fungal keratitis, primary treatment failure has been reported in 31.3% of cases in a study of 115 patients [67]; large ulcer size, hypopyon and *Aspergillus* as the causative organism have been reported as predictors of poor outcome with topical 5% natamycin monotherapy. In another study that compared topical voriconazole with

topical natamycin as primary therapy for mycotic keratitis, rates of corneal perforations were 16.6% and 15% in the voriconazole and natamycin groups, respectively [49]. The authors did not find any significant differences in visual acuity and scar size between voriconazole-treated and natamycin-treated patients. A recent study sought to analyse the predictors of outcome in mycotic keratitis [68]. Older age and a larger infiltrate size at presentation significantly predicted a longer time to re-epithelialization and worsened 3-month visual acuity whereas a larger infiltrate size also significantly predicted a worsened 3-month infiltrate/scar size [68]. In addition, a larger epithelial defect size was a significant predictor of perforation.

Surgery for mycotic keratitis

Therapeutic surgery may be required for clinical cases of mycotic keratitis that respond poorly, or not at all, to medical therapy, or where perforation or descemetocele formation is imminent; however, every effort should be made to prolong medical therapy for the maximum duration possible, to render the infecting fungus non-viable before surgery and therein to improve the outcome. Surgery attempts to remove antigenic and infectious elements and also necrotic tissue and other debris, which may hinder complete healing of the lesion. Methods used include [27,38]:

- Debridement, tarsorraphy or superficial (lamellar) keratectomy (in combination with antifungal therapy) for small, superficial ulcers.
- Conjunctival flap or penetrating keratoplasty for severe keratitis that is unresponsive to medical therapy or where serious complications supervene.

Conclusions

Although mycotic keratitis is an important, sight-threatening problem, it has not always received the attention it deserves from medical personnel. A silver lining of the dark cloud that was the contact lens-associated outbreak of *Fusarium* spp. keratitis in 2005–06 is that it appears to have stimulated interest in the pathogenesis of this condition. Hopefully, this will lead to an increase in research initiatives on this complex problem.

Transparency Declaration

Neither of the authors has any proprietary interests or conflicts of interest related to this submission.

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