The global incidence and diagnosis of fungal keratitis

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Fungal keratitis is a severe corneal infection that often results in blindness and eye loss. The disease is most prevalent in tropical and subtropical climates, and infected individuals are frequently young agricultural workers of low socioeconomic status. Early diagnosis and treatment can preserve vision. Here, we discuss the fungal keratitis diagnostic literature and estimate the global burden through a complete systematic literature review from January, 1946 to July, 2019. An adapted GRADE score was used to evaluate incidence papers—116 studies provided the incidence of fungal keratitis as a proportion of microbial keratitis and 18 provided the incidence in a defined population. We calculated a minimum annual incidence estimate of 1 051 787 cases (736 251–1 367 323), with the highest rates in Asia and Africa. If all culture-negative cases are assumed to be fungal, the annual incidence would be 1 480 916 cases (1 036 641–1 925 191). In three case series, 8–11% of patients had to have the eye removed, which represents an annual loss of 84 143–115 697 eyes. As fungal keratitis probably affects over a million people annually, an inexpensive, simple diagnostic method and affordable treatment are needed in every country.

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Introduction

Fungal keratitis, also known as mycotic keratitis, keratomycosis, or oculomycosis, is a severe sight- threatening condition. This highly damaging corneal infection often leads to permanent blindness and eye loss.[1,2] The condition is most prevalent in tropical and subtropical locations and has been estimated to account for 20–60% of all culture-positive corneal infections in these climates.[3] Fungal keratitis tends to be a poorly treated condition with very high morbidity.[1,2,4] Corneal infections have been declared a silent epidemic,[5] yet the size of this epidemic has never been carefully estimated.

Fungal keratitis occurs secondary to often minor ocular trauma in most cases. Infected individuals are frequently young, healthy agricultural or outdoor workers who experience an injury from organic matter such as during harvesting.[2] Traumatising agents from a variety of plant and animal sources have been recorded, even dust particles.[2,4] As men make up a greater proportion of agricultural and outdoor workers, they are more prone to the disease than women.[6] In one case series, nearly 4% of cases were found in children,[6] although the vast majority of cases are seen in adults aged 20–50 years.[7] Other reported predisposing factors for filamentous fungal keratitis include previous ocular surgery, ocular surface disease, contact lens use, previous use of corticosteroids (topical or systemic), and immunosuppressive conditions such as HIV/AIDS.[2,4,8] Traditional eye remedies, which are often plant-based and non-sterile, can also introduce infection.[9] Conversely, in temperate regions, ocular surface disease such as insufficient tear secretion and defective eyelid closure can predispose to candida and candida-like keratitis. *Candida* spp infections might superimpose on pre-existing Herpes simplex keratitis or corneal defects from contact lens wearing. Unsafe hygiene practices such as overnight wear and ineffective cleaning have been associated with fungal keratitis. Contact lens wearers of low socioeconomic status are at increased risk of developing the condition, attributed to a inadequate education about hygienic eye care and insufficient cleaning solution use.[6]

Fungal infections of the cornea are caused by more than 100 different species, although over 95% are caused by the filamentous fungi *Fusarium* spp and *Aspergillus* spp and the yeast *Candida* spp. Filamentous fungi are responsible for most fungal infections in tropical and subtropical climates, with yeast being more frequent in temperate climates. Corneal infections caused by filamentous fungi tend to have a worse prognosis than those caused by yeast species.[2]

Fungal keratitis typically presents subacutely with eye pain, followed by blurred vision, redness, excessive tearing or discharge, and photophobia. It progresses to ulceration, opacification of the cornea and, more rarely, endophthalmitis.[10] Corneal perforations are common and five to six times more likely than in bacterial keratitis, and often result in the need for evisceration.[1,4] For the patient, the consequences range from visual impairment and blindness, to loss of the globe and disfiguration.[1]

The differential diagnosis includes fungal, bacterial, viral, amoebic, oomycete, or parasitic causes. Certain

Key messages

- Microbial keratitis is an avoidable cause of usually unilateral blindness and sometimes eye loss; fungal (mycotic) keratitis is generally more difficult to diagnose and has a worse outcome than other types of microbial keratitis
- Despite publication of more than 3600 papers about microbial keratitis, the annual incidence of fungal keratitis has never been ascertained; a minimum of 1 million cases of fungal keratitis occur annually—this number rises to over 1·4 million if culture-negative cases are assumed to be fungal
- The highest annual incidence of fungal keratitis and the highest ratio of fungi versus bacteria as a cause of microbial keratitis occur in subtropical and tropical countries, predominantly in male agricultural workers
- Calcofluor-white with florescence microscopy is superior in sensitivity to potassium hydroxide, Giemsa, lactophenol cotton blue, and Gram stain in the diagnosis of fungal keratitis
- Fungal culture is essential for diagnosis, and more than 100 different fungal species are known to have caused fungal keratitis
- Probably about 100 000 eyes are removed annually because of late diagnosis and poor therapeutic outcome

clinical features are suggestive of a filamentous fungal infection: firm or dry elevated slough, an irregular or feathery stromal infiltrate edge, satellite infiltrates, an immune ring, and endothelial plaques.[2] A hypopyon (pus in the anterior chamber) might also be present. Because of the overlap in the clinical signs at presentation, it is often not possible to clinically distinguish fungal keratitis from other types of corneal infection.[6,11]

Corneal scrapings for direct microscopy and culture are required for definitive diagnosis, although other modalities might be helpful, including molecular methods and in-vivo confocal microscopy.[2,7] Sometimes a corneal biopsy is required. There is currently no point-of-care diagnostic test for fungal keratitis, which is major obstacle in improving health outcomes for the condition.

General trends and risk factors are widely reported, but there has been very little epidemiological research conducted in Africa, Asia, and central and South America to calculate its global incidence. The aim of this Review was to appraise the existing literature concerning the incidence of fungal keratitis, the optimal means of making the diagnosis, and to use the most reliable data to estimate the global burden of this condition.

Search strategies

We did a systematic literature review on the epidemiology of fungal keratitis from Jan 1, 1946, to July 26, 2019, using Embase, MEDLINE, PubMed, CINAHL, and Cochrane (search terms given in the appendix, p 1). Papers presenting incidence of fungal keratitis within a defined population were evaluated using an adapted GRADE score[12] on the basis of the following features: diagnostic accuracy, study size (using a cutoff of >30 cases), decade of study, with more recent studies scoring higher, and documentation of fungal keratitis as a proportion of microbial keratitis (appendix p 5).

See Online for appendix

Those with an adapted GRADE score of more than 2 were deemed acceptable and enabled a minimum estimation of the global burden of fungal keratitis. Regions of the world were assigned an estimated incidence rate based on data from a country within that region, or from a country that bore similarity to that region (ie, climate, socioeconomic status). Where more than one incidence rate was available for a country, we calculated a weighted mean from the studies that were less than 10 years old. In all case series there were cases of keratitis without a confirmed microbiological diagnosis, probably because of previous therapy and the insensitivity of both microscopy and culture. Therefore, the proportion of culture-negative and microscopy-negative cases was recorded from all included studies in each country and a weighted mean was taken. Studies from the past decade were given preference, but where there were none, the most recent recorded proportion was used. Mixed infections (both bacteria and fungi cultured or seen on smears) were counted as cases of fungal keratitis only, as bacterial co-infection might be secondary to fungal invasion. Rates per 100 000 people were derived using the UN World Population Prospects 2015 database,[13] between the ages of 20 and 70 years, as fungal keratitis predominantly affects this age group.[2,7]

Data were particularly few for sub-Saharan Africa, so we took the annual county reports of microbial keratitis from Kenya collected by the Ministry of Health over the years 2013–17 and adjusted for incomplete records. The assumption was made that 45% of the cases were fungal in origin, and absolute numbers of fungal keratitis cases and an incidence per 100 000 people.

To address diagnostic performance, we did multiple separate searches in MEDLINE of the diagnosis of fungal keratitis for any comparator data on any diagnostic modality—clinical, confocal microscopy, microscopy, histopathology, culture, and PCR. Only those papers in which fungal-specific staining methods and culture were done were included. These searches were expanded by seeking references used in papers that we identified.

Epidemiology

Our epidemiology searches identified 3668 records, of which 397 were selected for full-text assessment after title and abstract screening. Duplicates were then removed, leaving 241 unique full manuscripts to be assessed for eligibility. We excluded 59 full-text articles for the following reasons: 33 were not related to the epidemiology of fungal keratitis, 16 did not present their original data, and seven presented data on bacterial keratitis only. This selection left a total of 187 studies from 50 different countries for detailed analysis. A total of 118 studies provided the incidence of fungal keratitis as a proportion of microbial keratitis. Only 18 papers provided the incidence of fungal keratitis in a defined population and these were the key papers used for country, regional, and, ultimately, our global estimation of the annual incidence of fungal keratitis (appendix pp 6–8).

We estimated that the global annual incidence of fungal keratitis is 1051787 cases and we outline this incidence along with total regional burdens in the table, and in figure 1. The highest estimated incidences are in Asia and Africa, and the lowest in Europe (figure 1). We estimated the error rate to be plus or minus 30%, which gives a range of 736 251–1 367 323 cases per year. Regional variations are known within countries with major climatic differences—so fungal keratitis is much more common in the south of the USA and China than the north, as examples. Only in China are the data robust enough to estimate these variations formally, and even there the gradations in climate make this division somewhat arbitrary.

The mean proportion of culture negative microbial keratitis in our included studies was 40·8% (14 024/34 257). The range was 5% (Sierra Leone) to 74·4% (Thailand). If we assume that these cases were all unconfirmed fungal keratitis, the global annual incidence estimate increases to 1 480 916 cases (1 036 641–1 925 191). This assumption relies

	Annual incidence	Annual incidence per 100 000 people	Extrapolated from
Africa total	75 196	13·5	..
Eastern Africa	23 241	13·3	Kenya (unpublished)
Middle Africa	8625	13·3	Kenya (unpublished)
Northern Africa	17 556	14	Zaki and Denning (2017)[14]
Southern Africa	5096	14	Zaki and Denning (2017)[14]
Western Africa	20 678	13·3	Kenya (unpublished)
Asia total	939 895	33·9	..
China (north, west, Hong Kong)	3686	1·3	Zhu et al (2013)[15]
China (south, east, central)	107 124	15·2	Lin et al (2017)[16]
Eastern Asia (excluding China)	2061	1·3	Zhu et al (2013)[15]
Central Asia	530	1·3	Zhu et al (2013)[15]
Southern Asia	768 325	73	Khwakhali and Denning (2015)[17]
Southeastern Asia	57 990	15	Imwidthaya (1995)[18] and Chayakulkeeree and Denning (2017)[19]
Western Asia	179	0·12	Hilmioglu-Polat (2018)[20]
Europe total	99	0·02	..
Eastern Europe	41	0·02	Ong (2016)[21]
Northern Europe	13	0·02	Ong (2016)[21]
Southern Europe	20	0·02	Ong (2016)[21]
Western Europe	25	0·02	Ong (2016)[21]
North America and Oceania total	17 427	..	..
Northern America	15 660	6·8	Jeng (2010)[22]
Oceania	1767	14·5	Thew (2008)[23]
Latin America total	19 170	5	..
Caribbean	1305	5	Alvarez-Moreno et al (2018)[24]
South America	12 895	5	Alvarez-Moreno et al (2018)[24]
Central America	4970	5	Alvarez-Moreno et al (2018)[24]
World total	1 051 787	23·6	..

Table: **Estimated annual incidence of fungal keratitis by region (as defined by the UN) and source**

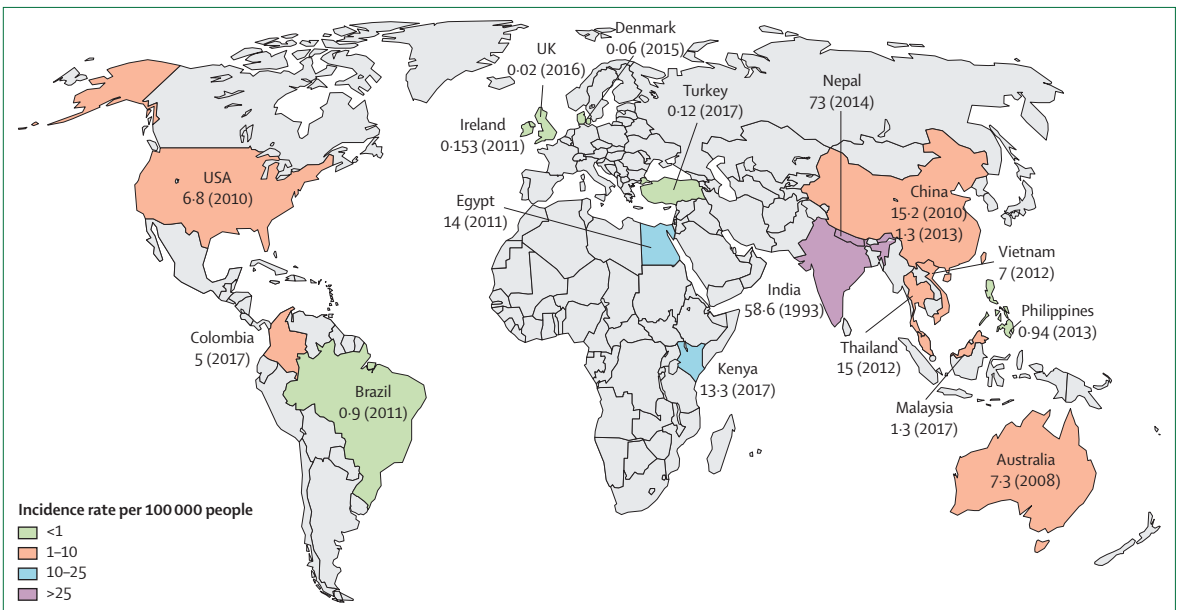

Figure 1: **Estimated annual burden of fungal keratitis by continent and regions within those continents**

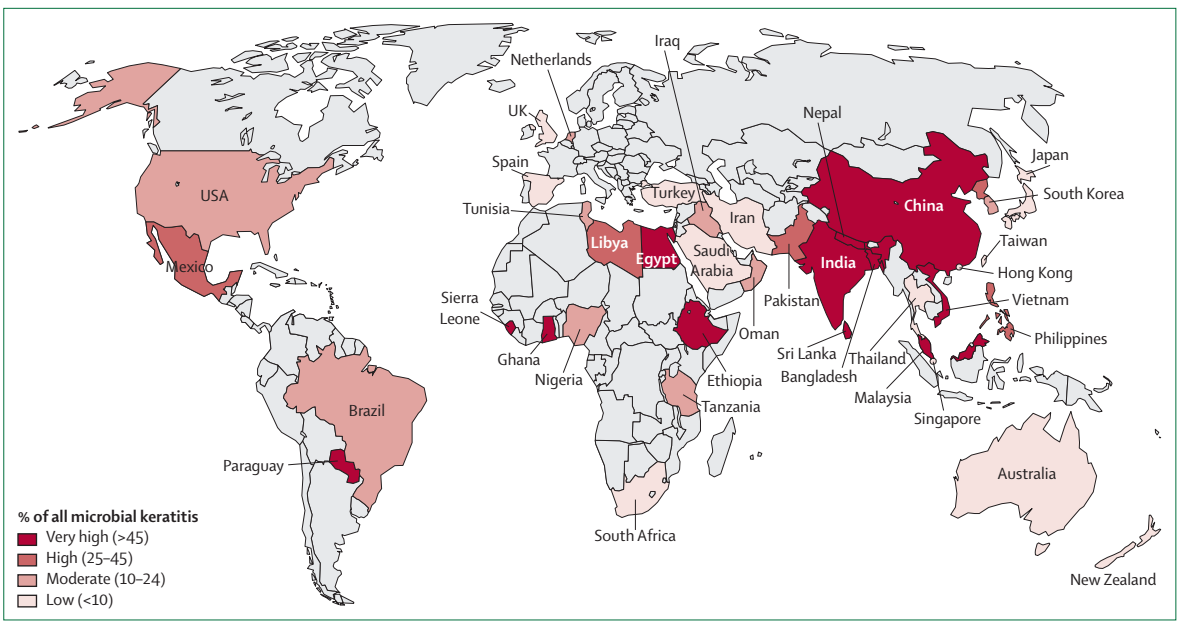

Figure 2: **Proportion (%) of cases of microbial keratitis shown to be caused by fungi**

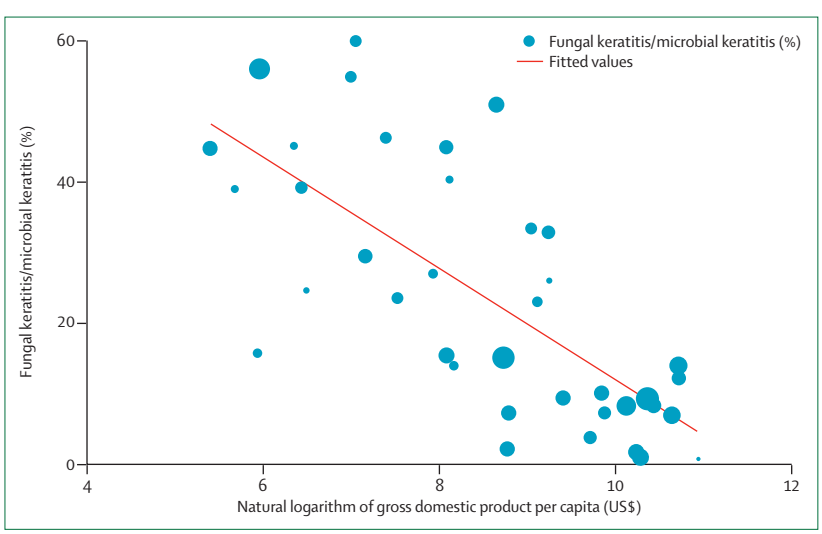

Figure 3: **Proportion of patients with fungal keratitis (of all those with microbial keratitis) and transformed values (log_e) of both gross domestic product per capita and distance from the equator (p<0·001)**

on the known insensitivity of both direct microscopy and culture, but it is recognised that some bacterial keratitis will also be culture-negative (although it might be Gram-stain-positive).

There were many more studies published describing the proportion of fungal keratitis as a subset of microbial keratitis. The proportion of keratitis cases attributed to fungi and the global discrepancies in prevalence are shown in figure 2 and documented in the appendix (pp 6–8). The proportions varied from 1·0% in Spain to 60·0% in Vietnam among 37 countries for which an estimate was available. In the instance of multiple studies, values from studies younger than 10 years were always used in preference to those older than 10 years. In the instance of more than one study younger than 10 years, a weighted mean was calculated on the basis of study size. Countries with proportions of fungal keratitis above 25% tended to be near the equator, but not universally so. A significant negative slope (p<0·001) was found between the proportion of patients with microbial keratitis who had proven fungal keratitis and a logged gross domestic product per capita, although the adjusted R^2 value was limited at 34% (figure 3).

Multivariable regression was used to assess the relationship between the proportion of patients with fungal keratitis (of all those with microbial keratitis) and transformed values (log_e) of both gross domestic product per capita and distance from the equator. A scatterplot of these variables using distance as a weighted marker revealed a lot of unexplained variability. The most common causative fungal species was *Fusarium* spp, followed by *Aspergillus* spp and *Candida* spp. Although marked regional variations in fungal aetiology were noted in the literature, these apparent variations were not mapped because sensitivity of culture varies for each pathogen and by laboratory.

For some countries multiple reports over time were available (appendix p 9). There is some evidence of an increasing trend in the proportion of all microbial keratitis being diagnosed as fungal. We considered formal statistical analysis of these data, but the time blocks are too disparate to allow pooling.

Each paper was analysed to assess its reliability and limitations, and most were found to have some drawbacks. First, most investigators used traditional microbiological techniques (culture, smear stains) to diagnose microbial

keratitis. Even when done by experts, culture only has a sensitivity of 73% at best[7] and so under-diagnosis is highly likely. Second, many people with fungal keratitis in rural distant communities will never present to health-care professionals because of cost of treatment, loss of earnings, lack of an escort, and often long distance. Third, as most studies were done at tertiary health-care facilities, which are designed to accept referrals and more severe cases of disease, fungal keratitis was possibly over-represented, as fungal keratitis will not respond to first-line anti-bacterial treatment. A strength of our estimate is that the data for sub-Saharan Africa were based on real-world data from Kenya, supported by local experience in Malawi.[25]

Diagnosis

Timely diagnosis of fungal keratitis can prevent irreversible corneal destruction and drastically improve the chances of complete recovery.[26] Diagnosis of this disorder starts with a strong clinical suspicion.[10] On presentation of a patient with suspected mycotic keratitis, a thorough history must be obtained, with a particular focus on symptoms, preceding events, and risk factors. A meticulous search for local ocular or systemic defects should follow and these should be managed to prevent recurrence of the condition.[26] It is important to note that the symptoms reported (blurred vision, eye pain, excessive tearing, etc) are not unique to fungal keratitis, but are seen in various forms of infectious keratitis. However, the duration of symptoms in fungal infection is typically more prolonged (5–10 days) and some distinction might be made on this basis, although such distinction is unreliable.[2]

Diagnosis based on clinical presentation

Detailed clinical examination can aid diagnosis before microbiological testing or in its absence. In low-resource settings, many ophthalmologists do not have access to specialised diagnostic facilities (microbiological or otherwise) and so must base their diagnosis solely on clinical presentation with a slit lamp and treat their patients empirically. For these ophthalmologists, the main challenge is differentiating between bacterial and fungal infections. A study by Thomas and colleagues,[2] using a logistic regression model, found that the following features were independently associated with fungal keratitis: serrated margins, raised slough, and colour (other than yellow) and that the presence of fibrin in the anterior chamber was independently associated with bacterial infection (appendix p 3). A diagnostic algorithm was devised comprising three of the clinical signs that were independently associated with fungal and bacterial keratitis. Colour was too subjective for inclusion in this dataset; however, pigmented ulceration is a characteristic feature of fungal keratitis caused by some of the dematiaceous moulds (appendix p 3).[27] Using this algorithm, it is possible to obtain a probability score of 89% likelihood that the infection is fungal if serrated, feathery infiltrate margins, and raised slough (surface profile) are present, and fibrin is absent from the anterior chamber.[11]

In another study, clinicians were able to accurately differentiate between a fungal and bacterial keratitis in 66% of cases. However, for the fungal infections, the Gram stain, genus, and species were only accurately predicted in few cases.[28] Furthermore, chronic filamentous fungal keratitis involves the entire cornea, resembling bacterial suppuration and so the two are easily confused. Unfortunately, as clinical features are not specific to types of microbial keratitis, the sensitivity of clinical diagnosis is low and appropriate in-vivo or in-vitro testing should always be done when possible.[26]

Acanthamoeba keratitis is difficult to distinguish from fungal keratitis on clinical characteristics alone, although a high index of suspicion is indicated if uveitis, ring infiltrate, endothelial plaque, and corneal thinning are observed.[29] Contact lens wear and associated poor hygiene combined with exposure to amoeba-containing water sources are the most common predisposing risk factors for infection.[30] Microsporidial keratitis in non-immunocompromised patients has a distinctive multifocal punctate appearance,[31] unlike fungal, bacterial, or *Acanthamoeba* keratitis. In immunocompromised patients, microsporidial keratitis is proportionally more common and can cause deep corneal ulceration. Pythium (oomycete) keratitis mimics fungal keratitis in its appearances, with feathery margins, but also distinctive dot-like infiltrates adjacent to the ulcer.[32]

In-vivo confocal microscopy

In-vivo confocal microscopy (IVCM) examination of the cornea is a non-invasive technique that enables real-time identification of the causative pathogen in microbial keratitis, specifically filamentous fungal elements (appendix p 4) and *Acanthamoeba* cysts. IVCM provides a magnification of around ×500, which enables a lateral resolution of 1 µm. With this technique, all corneal layers and their micro-anatomic structures (cells, nuclei, and nerves) can be examined, even in those affected by oedema, inflammatory infiltrates, and fibrosis.[33,34]

Several studies have prospectively examined the diagnostic accuracy of IVCM for identifying fungal keratitis.[33,34–37] They have found the sensitivity to range between 85% and 94% and the specificity to range between 71% and 92%. This technique requires skill in both the acquisition and interpretation of images.[35,37] There has been some interest in whether or not IVCM can be used to distinguish between the principal types of causative fungi (*Aspergillous* spp and *Fusarium* spp) on the basis of their different branching angles.[38,39] However, no convincing difference between them has been found.

The great value of IVCM is that it provides the clinician with a real-time diagnosis of fungal infection if this is present in most cases. The technique also allows a diagnosis of infection that is focused in the deep layers of

the cornea, which might not be readily accessible to sampling for microbiological analysis.[35] However, the current high cost and low availability of this technology might also deter ophthalmologists from supporting its utility in low-income and middle-income countries.[33] Indeed, the technology is usually available at a few centres in high-income countries. It is also important to note that although the value of confocal microscopy has been demonstrated for fungal and *Acanthamoeba* keratitis, the resolution of this imaging technique limits its use in confirming bacterial infection, as the organisms are too small to be visualised.[34,40] Although non-invasive, a confocal microscope is a contact diagnostic tool and thus might cause ocular discomfort in a sensitive eye, which will probably increase eye movements and could blur the images. To obtain high-quality images, a high degree of patient cooperation is necessary.[34] The future role of confocal microscopy in low-resource settings, where it could potentially provide the greatest benefit, remains uncertain given the high costs and need for specialist training.

Sample collection

Samples for microbiology are collected using a sterile Kimura spatula, surgical blade, or hypodermic needle (21 or 23 gauge) from the base and edges of corneal ulcers, following the local instillation of preservative-free anaesthetic and before the application of fluorescein. A variety of solid and liquid media are inoculated, and multiple slides prepared for microscopy.[11,41] Slides and culture media are inoculated in the clinic. The reason for this multiplicity, a practice unique to ocular microbiology, is the need to detect different types of causative organism. Care must be taken to spread out the corneal material into a thin layer on the slides, so that the observer is able to visualise the specimen well. Caution is also required when inoculating solid culture media to avoid puncturing the surface of the agar with the sharp instruments used to collect the specimen.[41] As fungi generally penetrate deep into the cornea, the yield of fungi obtained using swabs is usually inadequate to confirm a diagnosis.[42] Although the action of scraping debrides necrotic tissue, one study advised against excessive scraping because of the risk of scarring and subsequent deterioration in visual acuity.[43]

Microscopy

Direct microscopy of corneal smears allows the clinician to rapidly differentiate between a fungal infection and other types of microbial keratitis and is considered the gold standard for diagnosis of fungal infection—if fungal hyphae are visualised in a corneal specimen, the clinician can be confident to commence antifungal therapy. Furthermore, some stains allow detection and differentiation between bacterial and amoebic infection simultaneously.

The following stains for microscopic evaluation are recommended: Gram stain, calcofluor-white preparation, and either potassium hydroxide or lactophenol cotton blue stained preparations. More specialised stains (Giemsa, periodic acid Schiff, Gomori methenamine silver stain) can also be used.[26] Well trained microscopists find calcofluor-white with florescence microscopy superior in sensitivity to potassium hydroxide, in the diagnosis of fungal keratitis, itself superior to lactophenol cotton blue.[44] Fluorescence microscopy was the most sensitive technique in a 2010 comparison with Giemsa staining, but less specific.[45] Generally, this diagnostic modality is inexpensive, relatively simple, and yields results rapidly, which renders it suitable in low-resource settings. Furthermore, the sensitivity for detecting fungal keratitis has been reported to be 61–94% using potassium hydroxide, 85% using lactophenol blue, but just 36–50% using a traditional Gram stain.[45,46] Calcofluor-white is said to be a mainstay of diagnosis, and when combined with potassium hydroxide stains, sensitivity has been shown to rise to 98·3%.[42] There is evidence that alternative stains like methylene blue might also be used for rapid diagnosis but calcofluor-white is superior.[47] The value of the Gram stain to visualise fungal hyphae in direct microscopy of corneal scrape preparations should not be underestimated, especially in settings where this might be the only means of staining available.[41,48]

Stained corneal material might contain artefacts, which can result in the reporting of false positives; conversely, less common fungal species might not be detected by the aforementioned stains. Finally, although it might be possible to differentiate between yeast and filamentous fungi, and in some cases, dematiaceous fungi that appear pigmented, it is not possible to differentiate between genera and species of fungi on the basis of microscopic examination of the corneal smear preparation alone.[26] For this reason, it is advised that both microscopy and culture are done whenever possible.

Culture

Blood agar, chocolate agar, and Sabouraud dextrose agar are inoculated with corneal scrape material using C-shaped streaks, because of the very small size of the inoculum, and only colony growth within these parameters are regarded significant.[49] An Indian study[42] compared growth, time, and cost of these three types of agar plates smeared with corneal scrapings: fungal species grew on 56% of blood agar, 46% on chocolate blood, and on 43% of Sabouraud dextrose agar. The authors deduced that blood agar and chocolate agar are able to support the growth of some, but not all, fungi that cause infectious keratitis and are more cost-effective than Sabouraud dextrose agar.[42] However, in resource-limited settings where fungal keratitis is prevalent, procuring animal blood to prepare blood agar is a challenge. The use of Sabouraud dextrose agar (or potato dextrose agar) is still advised for optimal growth and reliable identification of filamentous fungi.[50] Use of liquid phase media, for example brain–heart infusion and

thioglycolate broths, is common practice to enhance recovery of microorganisms, diluting out the effect of previous treatment with antimicrobial drugs.[49]

Despite corneal smears and culture being the current gold standard mode of diagnosis, culture is insensitive and the wide variety of fungal pathogens implicated (>100 species) means that considerable mycological skill and knowledge are required for prompt identification in positive cases and to rule out contaminants. Fungal growth generally requires 48–72 h, and so diagnosis based only on culture is often delayed and microscopy is always advised. Some of the less common species take longer to grow and it might be necessary to wait for 2 weeks before confirming the absence of growth in culture.[42] Some samples have even taken up to 35 days to grow.[51] The effect of this difference on the patient is huge, given that a delay in diagnosis and treatment is a factor contributing to poor prognosis.[1] Repeat cultures taken at 6 days of therapy were, if still positive, a marker of poor outcome.[52] It is sometimes necessary to try to make the microbiological diagnosis of a corneal infection from corneal biopsy tissue when all other approaches have not yielded a result.

PCR

The considerable drawbacks to culture have led to the development of molecular tools as diagnostics for fungal keratitis. The molecular tool of choice is PCR, which only requires a small quantity of sample. PCR has been shown to have high sensitivity and specificity when compared with smear stains and culture.[42] In a 10-year retrospective non-randomised trial, samples from 20 patients with proven fungal keratitis were used to evaluate the sensitivity of microscopy, culture, and PCR. PCR positively identified the causative fungal species in 92·6% of cases, Gram stain and calcofluor-white preparations identified 66·6%, and culture identified just 59·3% of cases.[51] Another study reported similar identification rates of 81·6%, 42·1%, and 68·4% respectively.[53] The speed and accuracy of PCR have prompted certain researchers to advocate for its widespread use in the diagnosis of fungal keratitis. However, the technique is currently of limited use in low-resource settings, where the burden of disease is greatest.[42]

Ocular outcomes

Four case series describe the ocular outcomes, aside from randomised clinical trials. In Pakistan,[4] investigators reported that 59% of eyes had a final vision worse than 6/60 and eviscerations were necessary in 11% of cases. In east Africa,[1] 66% of eyes had a final vision worse than 6/60, 30% resulted in corneal perforations, and 8% required eviscerations. Even in a high-resource setting such as Germany, a multi-year series (2000–17)[54] found that penetrating keratoplasty was done in 57% of cases and enucleation was necessary in 9%. In the UK,[7] 20% of eyes were rendered blind, and 56% were left with good vision (6/5–6/12). Using these figures, we calculate that 94753–115810 eyes are surgically removed each year. In countries where eye care is suboptimal, the loss of eyes will probably be greater. Using outcome data from the Pakistan study[4] for low-income and middle-income countries, we predict that 610821 eyes will go blind because of fungal keratitis each year.

Conclusions

The annual global incidence of fungal keratitis has never been estimated. There are few epidemiological data from Africa, Asia, and Latin America on which to base country incidence. Variations within countries are also likely, partly because of climate, but also occupational risk factors, as seen in bacterial keratitis.[55] Fungal microscopy and culture both have low sensitivity for fungal keratitis, and so estimates based on these diagnostic modalities underestimate incidence. Despite these limitations, we estimate that over a million eyes are affected each year from fungal keratitis—probably 1·4 million—assuming that in high-incidence areas culture-negative cases are usually cases of fungal keratitis. Given that approximately 10% of eyes will perforate or need removal and that over 60% of patients are left with mono-ocular blindness (even if treated), major improvements in eye health-care are required to improve this dismal situation.

Most epidemiological papers that we analysed used both microscopy and culture to diagnose microbial keratitis. In each study, a proportion of cases of clinically suspected infectious keratitis were not identified using these methods. However, they were treated empirically on the basis of clinical evidence. Of the corneal specimens that were negative for both microscopy and culture, we suspect a considerable proportion to have been caused by fungi, but this suspicion cannot be known with current methodology in most settings. Achieving optimal results is influenced by many factors but begins with obtaining a high-quality specimen from the clinic and relies on both a trained and dedicated microbiological service to interpret findings.

Fungal keratitis is initially managed medically, but various surgical procedures might also be required. Medical therapy includes specific antifungal drugs (topical or systemic), and non-specific, supportive methods (such as cycloplegics). Treatment responses to topical antifungal therapy are reasonable, with 75% of corneas not severely affected and 60% of those severely affected being effectively managed by topical 5% natamycin, now listed by WHO as an essential medicine.[56] Penetrating keratoplasty, if a donor cornea is available, might be necessary to treat an infection refractory to medical therapy or to rehabilitate vision when the infection has resolved. In intractable cases, with perforation of the eye and unavailability of donor corneas, evisceration is required.[2]

Late diagnosis contributes to a worse outcome. A study from Tanzania[1] reported a median delay of 14 days to presenting at the hospital, and this delay was extended to 21 days if another facility was visited first. A similar delay

For more on **availability of antifungal eye drops** see www.gaffi.org/antifungal-drug-maps

was reported from Uganda.[57] Inadequate or inappropriate initial treatment is common.[1] Often, patients present too late for treatment to preserve sight, as extensive and deep corneal lesions have already developed.[1,4] Furthermore, despite the addition of natamycin eye drops to the WHO essential medicines list,[26] the availability of antifungal eye drops is still poor. Diagnostic facilities and skill to do corneal scraping, fungal microscopy, fungal culture, and colony identification are also scarce in most areas. Guidelines for the management of fungal keratitis were last published in 2004 by the WHO Regional Office for South-East Asia.[58]

Fungal keratitis is likely to affect over a million people annually, of whom probably three quarters will lose an eye or sight. The condition is debilitating and refractory in its advanced stages, but usually treatable with early diagnosis and generic antifungal therapy. A point-of-care diagnostic method and global availability of affordable treatment are needed.

Contributors

LB did the epidemiology literature search and paper review, wrote much of the diagnostic text, managed the data analysis, and wrote the first draft of the paper. AKL reviewed the diagnostic literature and contributed much of this text. MG provided primary data from Kenya, assisted in its analysis, and made comments on the paper. MJB reviewed all the pertinent data to check their internal validity and commented on the text and figures. DWD conceived the project and the study design, reviewed many of the key papers, provided multiple drafts of the paper, organised the statistical analyses, and finalised the writing.

Declaration of interests

We declare no competing interests.

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