**The Global Incidence and Diagnosis of Fungal Keratitis**

**Authors:**

Lottie Brown1, Astrid K. Leck PhD2, Michael Gichangi MSc3, Matthew J. Burton FRCOphth2,4, David W. Denning FRCP 1,5,6\*,

**Affiliations:**

1. The University of Manchester, Oxford Road, Manchester, M13 9PL, UK

2. International Centre for Eye Health, London School of Hygiene & Tropical Medicine, London, UK 3. Ministry of Public Health and Sanitation, Kenya, Nairobi

4. Moorfields Eye Hospital NHS Trust, London, UK

5. National Aspergillosis Centre, Wythenshawe Hospital, Manchester University NHS Foundation Trust, Manchester Academic Health Science Centre, Southmoor Road, Manchester M23 9LT, UK.

6. Global Action Fund for Fungal Infections, Geneva, Switzerland

**\* Correspondence:** Professor David Denning, National Aspergillosis Centre, Education and Research centre, Wythenshawe Hospital, Southmoor Road, Manchester M23 9LT, UK; Tel +441612915353, FAX +441612915806 and ddenning@manchester.ac.uk or ddenning@GAFFI.org

**Keywords:** blindness, *Fusarium*, *Aspergillus*, *Candida*, microscopy, cornea

**Running title:** Global incidence of fungal keratitis

**Word count:**

**ABSTRACT**

Fungal keratitis is a severe corneal infection which often results in blindness and eye loss. The disease is most prevalent in tropical and subtropical climates, and sufferers are largely young agricultural workers of low socioeconomic status. Early diagnosis and treatment may preserve vision. Here we provide a narrative review of the fungal keratitis diagnostic literature and we estimated the global burden through a complete systematic literature review from 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 (range 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 (range 1,036,641 - 1,925,191). In three series, 8-11% of patients had to have the eye removed, an annual loss of 84,143 - 115,697 eyes. Fungal keratitis likely affects over a million people annually. An inexpensive simple diagnostic method and global availability of affordable treatment are needed.

***Funding:*** No external funding. MJB and AL are supported by The Wellcome Trust (207472/Z/17/Z).

**Key Points**

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.

Despite publication of >3,600 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 rises to over 1.4 million if culture negative cases are assumed to be fungal.

The highest rates and proportion of fungi as a cause of microbial keratitis occur in subtropical and tropical countries, in male agricultural workers.

Calcofluor white with florescence microscopy is superior in sensitivity to potassium hydroxide, Giemsa, lactophenol cottom blue and gram stain in the diagnosis of fungal keratitis.

Fungal culture is essential and >100 different fungal species have caused fungal keratitis.

Probably about 100,000 eyes are removed annually as a result of late diagnosis and poor therapeutic outcome.

**INTRODUCTION**

Fungal keratitis, also known as mycotic keratitis, keratomycosis or oculomycosis, is a severe sight threatening condition. It is a highly damaging corneal infection, often leading to permanent blindness and eye loss1,2. 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 climates3. Fungal keratitis tends to be a poorly treated condition with a very high morbidity1,2,4. 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 the majority of cases. Sufferers are frequently young, healthy agricultural or outdoor workers who are unfortunate enough to experience an injury from organic or vegetative matter such as during harvesting2. Traumatising agents from a variety of plant and animal sources have been recorded, even dust particles2,4. As males make up a greater proportion of agricultural and outdoor workers, they are more prone to the disease6. In one series nearly 4% of cases were found in children, although the vast majority of cases are seen in adults aged 20-50 years7. 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 immunosuppression such as in HIV/AIDS2,4,8. Traditional eye remedies, which are often plant-based and non-sterile, may also introduce infection9. Conversely, in temperate regions, ocular surface disease such as insufficient tear secretion and defective eye-lid closure can predispose to *Candida* and *Candida*-like keratitis. *Candida* infections may 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 the fungal keratitis. Contact lens wearers of low socioeconomic status are more at risk of developing the condition and this is attributed to a lack of education about hygienic eye care and sufficient cleaning solution6.

Fungal infections of the cornea are caused by over 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 the vast majority of infections in tropical and subtropical climates, with yeast more frequent in temperate climates. Corneal infections caused by filamentous fungi tend to have a worse prognosis than those caused by yeast species2.

Fungal keratitis typically presents sub-acutely 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, endophthalmitis10. Corneal perforations are common and 5-6 times more likely than in bacterial keratitis, and often result in the need for evisceration1,4. For the patient, the consequences range from visual impairment and blindness, to loss of the globe and disfiguration1.

The differential diagnosis includes fungal, bacterial, viral, amoebic or parasitic causes. Certain 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 plaques2. A hypopyon (pus in the anterior chamber) may 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 infection6,11.

Corneal scrapings for direct microscopy and culture are required for definitive diagnosis, although other modalities may assist including molecular methods and *in vivo* confocal microscopy2,7. Sometimes a corneal biopsy is required. There is currently no point of care diagnostic test for fungal keratitis and this remains a 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 to making the diagnosis and to use the most reliable data to estimate the global burden of this condition.

**Search strategies**

We conducted a systematic literature review on the epidemiology of fungal keratitis from January 1946 to 26th July 2019 using Embase, Medline, PubMed, CINAHL and Cochrane (search terms given in supplementary data) (Supplementary Table 1). Papers presenting incidence of fungal keratitis within a defined population were evaluated using an adapted GRADE score12 based on 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 (Supplementary Table 2).

Those with an adapted GRADE score of >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 (i.e. climate, socioeconomic state). 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 series there were cases of keratitis without a confirmed microbiological diagnosis, probably because of prior therapy and the insensitivity of both microscopy and culture. Therefore, the proportion of culture and microscopy negative cases were 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 may be secondary to fungal invasion. Rates per 100,000 population were derived using the UN World Population Prospects 2015 database, between the ages of 20 and 70 years, as fungal keratitis predominantly affects this age group2,7.

Data were particularly limited 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 2013-2017 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 a rate per 100,000 population.

To address diagnostic performance, we conducted multiple separate searches in Medline on 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 performed were included. These searches were expanded by seeking references used in papers 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, and this left 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 articles did not present their original data and 7 presented data on bacterial keratitis only. This left a total of 189 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 (Table 1).

We estimated that the global annual incidence of fungal keratitis is 1,051,787 and this is outlined, along with total regional burdens, in Table 2 and figure 1. The highest estimated incidence rates are in Asia and Africa, and the lowest in Europe (figure 1). We estimated the error rate to be +/-30% which gives a range of 736,251 to 1,367,323 cases per year. There known to be regional variations within countries with significant climatic differences – so fungal keratitis is much more common the South of the USA and China than the North, as examples. Only in China are the data robust enough to estimate this formally, and even there the gradations in climate make this division somewhat arbitrary.

The mean rate 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 these cases were all unconfirmed fungal keratitis, the global incidence rate estimate increases to 1,480,916 cases (range 1,036,641 to 1,925,191). This assumption relies on the known insensitivity of both direct microscopy and culture, but it is recognised that some bacterial keratitis will also be culture negative (although may 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 of this are shown in figure 2, and documented in Table 3. The proportions varied from 1.0% in Spain to 60.0% in Vietnam among 37 countries in which an estimate was available. In the instance of multiple studies, values from studies less than 10 years old were always used in preference to those more than 10 years old. In the instance of more than 1 study less than 10 years old, a weighted mean was calculated based on 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 logged GDP per capita, though the adjusted r2 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 GDP per capita and distance from the Equator. A scatterplot of these variables using distance as weighted marker revealed a lot of variability left unexplained. The most common causative fungal species was *Fusarium spp.*, followed by *Aspergillus spp.* and *Candida spp*. The most common regional aetiological species were not mapped because sensitivity of culture differs for each pathogen and laboratory.

For some countries multiple reports over time were available, Table 4. 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 same drawbacks. First, the majority of investigators used traditional microbiological techniques (culture, smear stains) to diagnose microbial keratitis. Even in the hands of experts, culture only has a sensitivity of 73% at best7 and so under-diagnosis is highly likely. Second, many sufferers of fungal keratitis in rural distant communities will never present to healthcare professionals due to cost of treatment, loss of earnings, lack of an escort and often long distance. Third, as most studies were conducted at tertiary healthcare facilities which are designed to accept referrals and more severe cases of disease, it is possible that fungal keratitis was over-represented, as fungal keratitis will not respond to the first line anti-bacterial treatment. A strength of our estimate is that the data for sub-Saharan Africa was based on real-world data from Kenya, supported by local experience in Malawi13.

**Diagnosis**

Timely diagnosis of fungal keratitis can prevent irreversible corneal destruction and drastically improve the chances of complete recovery14. Diagnosis of this disorder starts with a strong clinical suspicion10. 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 condition14. It is important to note 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 may be made on this basis, although this is unreliable2.

**Diagnosis based on clinical presentation**

Detailed clinical examination can aid diagnosis prior to 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 et al2, 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 (Figure 4). A diagnostic algorithm was devised comprising three of the clinical signs which were independently associated with fungal and bacterial keratitis. Colour was considered to be too subjective for inclusion for this dataset, however, pigmented ulceration is a characteristic feature of fungal keratitis caused by some of the dematiaceous moulds (Figure 4B)15. 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 chamber11.

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 the minority of cases16. 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 performed when possible14.

*Acanthamoeba* keratitis is difficult to distinguish from fungal keratitis on clinical characteristics alone, though a high index of suspicion is indicated if uveitis, ring infiltrate, endothelial plaque and corneal thinning are observed15. Contact lens wear and associated poor hygiene combined with exposure to amoeba-containing water sources are the most common predisposing risk factors for infection18. Microsporidial keratitis in non-immunocompromised patients has a distinctive multi-focal punctate appearance19, which is quite distinctive and unlike fungal, bacterial or *Acanthamoeba* keratitis. In immunocompromised patients, microsporidial keratitis is proportionally more common and can cause deep corneal ulceration. Pythium keratitis mimics fungal keratitis in its appearances, with feathery margins, but also distinctive dot-like infiltrates adjacent to the ulcer20.

***Iv vivo* confocal microscopy**

In vivo confocal microscopy (IVCM) examination of the cornea is a non-invasive technique which enables real-time identification of the causative agent in microbial keratitis, specifically filamentous fungal elements (Figure 5) and *Acanthamoeba* cysts. IVCM provides a magnification of around x500, which enables a lateral resolution of 1 μm. One can examine all corneal layers and their micro-anatomic structures (cells, nuclei and nerves), even in those affected by oedema, inflammatory infiltrates and fibrosis21,22.

Several studies have prospectively examined the diagnostic accuracy of IVCM for identifying fungal keratitis21,22-25. These have found the sensitivity to range between 85% to 94% and the specificity to range between 71% and 92%. This technique requires skill in both the acquisition and interpretation of images23,25. There has been some interest in whether or not IVCM can be used to distinguish between the principle types of causative fungi (*Aspergillous* and *Fusarium*) on the basis of their different branching angles26,27. 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 the large majority of cases. It also allows a diagnosis of infection which is focused in the deep layers of the cornea, which may not be readily accessible to sampling for microbiological analysis23. However, the current high cost and limited availability of this technology may also deter ophthalmologists from supporting its utility in LMIC21. Indeed, the technology is usually available at a few centres within 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 visualised22,28. Although non-invasive, a confocal microscope is a contact diagnostic tool and thus may cause ocular discomfort in a sensitive eye, which will likely increase eye movements and could blur the images. In order to obtain high quality images, a high degree of patient cooperation is necessary22. 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 instillation of local preservative-free anaesthetic and prior to the application of fluorescein. A variety of solid and liquid media are inoculated and multiple slides prepared for microscopy11,29. 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 specimen29. As fungi generally penetrate deep into the cornea, the yield of fungi obtained using swabs is usually inadequate to confirm a diagnosis30. Although the action of scraping debrides necrotic tissue, one study advised against excessive scraping due to the risk of scarring and subsequent deterioration in visual acuity31.

**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 (CFW) preparation and either potassium hydroxide (KOH) or lactophenol cotton blue (LPCB) stained preparations. More specialised stains (Giemsa, periodic acid Schiff, Gomori methenamine silver stain) can also be used14. Well trained microscopists find CFW with florescence microscopy superior in sensitivity to KOH, in the diagnosis of fungal keratitis, itself superior to LPCB32. Fluorescence microscopy was the most sensitive technique in a recent comparison with Giemsa staining, but less specific33. 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 stain33,34. Calcofluor white is said to be a mainstay of diagnosis, and when combined with KOH stains, sensitivity has been shown to rise to 98.3%30. There is evidence that alternative stains like methylene blue may also be used for rapid diagnosis but CFW is superior35. 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 may be the only means of staining available29,36.

Stained corneal material may contain artefacts which can result in the reporting of false positives; conversely, less common fungal species may not be detected by the aforementioned stains. Finally, although it may be possible to differentiate between yeast and filamentous fungi, and in some cases, dematiaceous fungi which appear pigmented, it is not possible to differentiate between genera and species of fungi based on microscopic examination of the corneal smear preparation alone14. For this reason, it is advised that both microscopy and culture are performed whenever possible.

**Culture**

Blood agar (BA), chocolate blood agar (CBA) and Sabouraud dextrose agar (SDA) are inoculated with corneal scrape material using “C”-shaped streaks, due to the very small size of the inoculum, and only colony growth within these parameters are regarded significant37. An Indian study compared growth time and cost of BA, CBA and SDA plates smeared with corneal scrapings: fungal species grew on 56% of BA 56%, 46% of CBA and on 43% of SDA. They deduced that BA and CBA are able to support the growth of some but not all fungi that cause infectious keratitis and are more cost-effective than SDA30. However, in resource-limited settings where fungal keratitis is prevalent, procuring animal blood to prepare blood agar is a challenge. The use of SDA (or potato dextrose agar (PDA) is still advised for optimal growth and reliable identification of filamentous fungi38. 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 prior treatment with antimicrobial agents37.

Despite corneal smears and culture being the current gold standard mode of diagnosis, culture is both 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 hours, 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 may be necessary to wait for 2 weeks before confirming no growth in culture30. Some samples have even taken up to 35 days to grow39. The significance of this is huge, given that a delay in diagnosis and treatment is an important factor contributing to poor prognosis1. Repeat cultures taken at 6 days of therapy were, if still positive, a marker of poor outcome40. 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 significant drawbacks to culture have led to the development of molecular tools as a diagnostic tool for fungal keratitis. The molecular tool of choice is Polymerase Chain Reaction (PCR) which only requires a small quantity of sample. PCR has been shown to have high sensitivity and specificity when compared to smear stains and culture30. In a 10 year retrospective non-randomized 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 CFW preparations identified 66.6% and culture identified just 59.3%39. Another study reported similar identification rates of 42.1%, 68.4% and 81.6% respectively41. The speed and accuracy of PCR have prompted certain researchers to advocate for its widespread use in the diagnosis of fungal keratitis. However, it is currently of limited use low resource settings, where the burden of disease is greatest30.

**Ocular outcomes**

Four series describe the ocular outcomes, aside from randomized clinical trials. In Pakistan4, investigators reported that 590 eyes (59%) had a final vision of <6/60 and eviscerations were necessary in 11% of cases. In East Africa1, 66% of eyes had a final vision of <6/60, 30% resulted in corneal perforations and 8% required eviscerations. Even in a high-resource setting such as Germany, a multi-year series (2000-2017)42 found that penetrating keratoplasty was performed in 57% of cases and enucleation was necessary in 9%. In the UK7, 20% of eyes were rendered blind, and 56% were left with good vision (6/5–6/12). Using these figures, we calculate that 94,753 to 115,810 eyes are surgically removed each year. In countries where eye care is sub-optimal, the loss of eyes will likely be greater. Using outcome data from the Pakistan study for low and middle-income countries, we predict that 610,821 eyes will go blind due to fungal keratitis each year.

**SUMMARY**

The annual global incidence of fungal keratitis has never before been estimated. There are few epidemiology 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 keratitis45. Fungal microscopy and culture both have low sensitivity for fungal keratitis, and so estimates based on these diagnostic modalities under-estimate incidence. Despite these limitations, we estimate that over a million eyes are affected each year from fungal keratitis and probably more like 1.4 million, assuming that culture negative cases are usually cases of fungal keratitis., in high incidence areas. Given that ~10% of eyes will perforate or need removal and that over 60% are left with mono-ocular blindness (even if treated), major improvements in eye healthcare are required to improve this dismal situation.

The majority of epidemiology 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 based on clinical evidence. Of the corneal specimens which were negative for both microscopy and culture, we suspect there were a significant proportion caused by fungi, but this is unknown. 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 microbiology service to interpret findings.

Fungal keratitis is initially managed medically, but various surgical procedures may also be required. Medical therapy includes specific antifungal agents (topical or systemic), and non-specific, supportive methods (such as cycloplegics). Treatment responses to topical antifungal therapy are fairly reasonable, with 75% of corneas not severely affected and 60% of those severely affected being effectively managed by topical 5% natamycin, now listed by the WHO as an Essential Medicine (EML) (30). Penetrating keratoplasty, if a donor cornea is available, may 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 Tanzania1 reported a median delay of 14 days to presenting at the hospital, and this was extended to 21 days if another facility was visited first. A similar delay was recently reported from Uganda43. Inadequate or inappropriate initial treatment is common1. Often, patients present too late for treatment to preserve sight, as extensive and deep corneal lesions have already developed1,4. Furthermore, despite the addition of natamycin eye drops to the WHO EML14, the availability of antifungal eye drops is still limited ([www.gaffi.org/antifungal-drug-maps/](https://www.gaffi.org/antifungal-drug-maps/%22%20%5Ct%20%22_blank)). Diagnostic facilities such as skill to do a corneal scraping, fungal microscopy, fungal culture and colony identification are also limited in most areas. Guidelines for the management of fungal keratitis were last published in 2004 by the South East Asian Regional Office of WHO44.

Fungal keratitis likely affects over a million people annually and probably three quarters will lose their eye or their sight. It 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.

**ACKNOWLEDGMENTS**

We are indebted to John Belcher at Wythenshawe Hospital, Manchester for analysing the relationship between proportion of fungal keratitis and GDP. AKL and MJB are funded by The Wellcome Trust (207472/Z/17/Z).

**POTENTIAL CONFLICTS OF INTEREST**

The authors declare no conflict of interest.

**AUTHOR CONTRIBUTIONS**

LB undertook 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 its 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 and organised the statistical analyses and finalized the writing.

**Figure legends**

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) (FK/MK) and transformed values (log e) of both GDP per capita and distance from the Equator (p<0.001).

Figure 4: Two examples of severe fungal keratitis: A Fungal keratitis caused by *Fusarium spp* and B by *Curvularia lunata,* respectively.

Figure 5: *In vivo* confocal microscopy (IVCM) image of ulcer caused by *Fusarium* spp.

**References**

1. Burton MJ, Pithuwa J, Okello E, et al. Microbial keratitis in East Africa: why are the outcomes so poor? *Ophthal epidemiol* 2011; **18**:158-163.

2. 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;**19**:210-220.

3. Bongomin F, Gago S, Oladele RO. Denning DW. Global and Multi-National Prevalence of Fungal Diseases—Estimate Precision. *J Fungi* 2017;**3**:57.

4. Shah SIA, Shah SA, Rai P, Katpar NA, Abbasi SA, Soomro AA. Visual outcome in patients of keratomycosis, at a tertiary care centre in Larkana, Pakistan. *J Pak Med Assc* 2017;**67**:1035-1038.

5. Whitcher JP, Srinivasan M. Corneal ulceration in the developing world—a silent epidemic. *Br J Ophthalmol* 1997;**81**:622-623.

6. Deorukhkarl S, Katiyarl R, Sainil S. Epidemiological features and laboratory results of bacterial and fungal keratitis: five-year study at tertiary-care hospital in western Maharashtra, India. *Singapore Med J* 2012;**53**:264-267.

7. Ong HS, Fung SS, Macleod D, Dart JK, Tuft SJ, Burton MJ. 2016. Altered patterns of fungal keratitis at a London ophthalmic referral hospital: an eight-year retrospective observational study. *Am J Ophthalmol*2016;**168**:227-236.

8. Mselle J. Fungal keratitis as an indicator of HIV infection in Africa. *Trop Doctor* 1999;**29**:133-135.

9. Arunga S, Kintoki GM, Mwesigye J, Ayebazibwe B, Onyango J, Bazira J, Newton R, Gichuhi S, Leck A, Macleod D, Hu VH. Epidemiology of Microbial Keratitis in Uganda: A Cohort Study. *Ophthal Epidemiol*. 2020;**27**(2):121-31.

10. FlorCruz NV, Evans JR. Medical interventions for fungal keratitis. *Cochrane Database Syst Rev*. 2015(4)

11. Leck AK, Thomas PA, Hagan M, et al. Aetiology of suppurative corneal ulcers in Ghana and south India, and epidemiology of fungal keratitis. *Br J Ophthalmol* 2002; **86**:1211- 1215.

12. Guyatt GH, Oxman AD, Kunz R, Vist GE, Falck-Ytter Y, Schünemann HJ. What is “quality of evidence” and why is it important to clinicians? *Br Med J* 2008;**336**:995-998.

13. Kalua K, Zimba, B, Denning DW. Estimated Burden of Serious Fungal Infections in Malawi. *J Fungi* 2018; **4**(2):61.

14. Thomas PA, Kaliamurthy J. Mycotic keratitis: epidemiology, diagnosis and management. *Clin Microbiol Infect*. 2013;**19**(3):210-20.

15. Kumar A, Khurana A, Sharma M, Chauhan L. Causative fungi and treatment outcome of dematiaceous fungal keratitis in North India. *Indian J Ophthalmol*. 2019;**67**(7):1048.

16. Dahlgren MA, Lingappan A, Wilhelmus KR. The clinical diagnosis of microbial keratitis. *Am J Ophthalmol*. 2007;**143**(6):940-4.

17. Lee MH, Abell RG, Mitra B, Ferdinands M, Vajpayee RB. Risk factors, demographics and clinical profile of Acanthamoeba keratitis in Melbourne: an 18-year retrospective study. *Br J Ophthalmol*. 2018;**102**(5):687-91.

18. Maycock NJ, Jayaswal R. Update on Acanthamoeba keratitis: diagnosis, treatment, and outcomes. *Cornea*. 2016;**35**(5):713-20.

19. Khurana S, Agrawal SK, Megha K, Dwivedi S, Jain N, Gupta A. Demographic and clinical profile of microspodial keratitis in North India: an underreported entity. *J Parasit Dis*. 2019;**43**(4):601-6.

20. Mittal R, Jena SK, Desai A, Agarwal S. Pythium insidiosum keratitis: histopathology and rapid novel diagnostic staining technique. *Cornea*. 2017;**36**(9):1124-32.

21. Vaddavalli PK, Garg P, Sharma S, Sangwan VS, Rao GN, Thomas R. Role of confocal microscopy in the diagnosis of fungal and acanthamoeba keratitis. *Ophthalmology*. 2011;**118**(1):29-35.

22. Labbé A, Khammari C, Dupas B, Gabison E, Brasnu E, Labetoulle M, Baudouin C. Contribution of in vivo confocal microscopy to the diagnosis and management of infectious keratitis. *Ocul Surf*. 2009;**7**(1):41-52.

23. Chidambaram JD, Prajna NV, Larke NL, et al. Prospective Study of the Diagnostic Accuracy of the In Vivo Laser Scanning Confocal Microscope for Severe Microbial Keratitis. *Ophthalmology*. 2016;**123**(11):2285‐2293.

24. Kanavi MR, Javadi M, Yazdani S, et al. Sensitivity and specificity of confocal scan in the diagnosis of infectious keratitis. *Cornea* 2007;**26**:782–6.

25. Kheirkhah A, Syed ZA, Satitpitakul V, et al. Sensitivity and Specificity of Laser-Scanning In Vivo Confocal Microscopy for Filamentous Fungal Keratitis: Role of Observer Experience. *Am J Ophthalmol*. 2017;**179**:81‐89.

26. Chidambaram JD, Prajna NV, Larke N, et al. In vivo confocal microscopy appearance of *Fusarium* and *Aspergillus* species in fungal keratitis. *Br J Ophthalmol*. 2017;**101**(8):1119‐1123.

27. Tabatabaei SA, Soleimani M, Tabatabaei SM, Beheshtnejad AH, Valipour N, Mahmoudi S. The use of in vivo confocal microscopy to track treatment success in fungal keratitis and to differentiate between Fusarium and Aspergillus keratitis. *Int Ophthalmol*. 2020;40(2):483‐491. doi:10.1007/s10792-019-01209-2

28. Mirdehghan A, Rezaei Kanavi M, Javadi MA, Nazari R. Sensitivity and Specificity of Confocal Scan in the Diagnosis of Fungal and Acanthamoeba Keratitis. *Bina J Ophthalmol*. 2007;**12**(2):203-10.

29. Leck A. Taking a corneal scrape and making a diagnosis. *CEHJ*. 2015;**28**(89):8.

30. Ansari Z, Miller D, Galor A. Current thoughts in fungal keratitis: diagnosis and treatment. *Curr Fung Infect Rep*. 2013;**7**(3):209-18.

31. Prajna NV, Mascarenhas J, Krishnan T, Reddy PR, Prajna L, Srinivasan M, Vaitilingam CM, Hong KC, Lee SM, McLeod SD, Zegans ME. Comparison of natamycin and voriconazole for the treatment of fungal keratitis. *Arch Ophthalmol*. 2010;**128**(6):672-8.

32. Sharma S, Silverberg M, Mehta P, Gopinathan U, Agrawal V, Naduvilath TJ. Early diagnosis of mycotic keratitis: predictive value of potassium hydroxide preparation. *Indian J Ophthalmol*. 1998;**46**(1):31.

33. Zhang W, Yang H, Jiang L, Han L, Wang L. Use of potassium hydroxide, Giemsa and calcofluor white staining techniques in the microscopic evaluation of corneal scrapings for diagnosis of fungal keratitis. *J Int Med Res*. 2010;**38**(6):1961-7.

34. McLeod SD, Kolahdouz-Isfahani A, Rostamian K, Flowers CW, Lee PP, McDonnell PJ. The role of smears, cultures, and antibiotic sensitivity testing in the management of suspected infectious keratitis. *Ophthalmology*. 1996;**103**(1):23-8.

35. Moemen D, Bedir T, Awad EA, Ellayeh A. Fungal keratitis: Rapid diagnosis using methylene blue stain. *EJBAS*. 2015;**2**(4):289-94.

36. Bharathi MJ, Ramakrishnan R, Meenakshi R, Mittal S, Shivakumar C, Srinivasan M. Microbiological diagnosis of infective keratitis: comparative evaluation of direct microscopy and culture results. *Br J Ophthalmol*. 2006;**90**(10):1271-6.

37. Leck A. Taking a corneal scrape and making a diagnosis. *CEHJ/ICEH*. 2009;**22**(71):42-3.

38. Reddy AK, Brahmaiah U, Narayen N, Reddy RK, Reddy RK, Chitta M, Prasad S, Swarup R, Mohiuddin SM, Reddy M, Aasuri MK. Is blood agar an alternative to sabouraud dextrose agar for the isolation of fungi in patients with mycotic keratitis. *Int Ophthalmol*. 2013;**33**(3):251-4.

39. Ferrer C, Alió JL. Evaluation of molecular diagnosis in fungal keratitis. Ten years of experience. *J Ophthalmic Inflamm Infect*. 2011;**1**(1):15-22.

40. Ray KJ, Lalitha P, Prajna NV, Rajaraman R, Krishnan T, Srinivasan M, Ryg P, McLeod S, Acharya NR, Lietman TM, Rose-Nussbaumer J. The utility of repeat culture in fungal corneal ulcer management: a secondary analysis of the MUTT-I randomized clinical trial. *Am J Ophthalmol*. 2017;**178**:157-62.

41. Badiee P, Nejabat M, Alborzi A, Keshavarz F, Shakiba E. Comparative study of Gram stain, potassium hydroxide smear, culture and nested PCR in the diagnosis of fungal keratitis. *Ophthalmic Res*. 2010;**44**(4):251-6.

42. Roth M, Daas L, Renner-Wilde A, Cvetkova-Fischer N, Saeger M, Herwig-Carl M, Matthaei M, Fekete A, Kakkassery V, Walther G, von Lilienfeld-Toal M. The German keratomycosis registry: Initial results of a multicenter survey. *Ophthalmologe*. 2019;**116**(10):957.

43. Arunga S, Kintoki GM, Gichuhi S, Onyango J, Newton R, Leck A, Macleod D, Hu VH, Burton MJ. Delay Along the Care Seeking Journey of Patients with Microbial Keratitis in Uganda. *Ophthalmic Epidemiol.* 2019;**26**(5):311-20.

44. World Health Organization. Guidelines for the management of corneal ulcer at primary, secondary and tertiary care health facilities in the South-East Asia region. WHO Regional Office for South-East Asia; 2004.

45. Shah A, Sachdev A, Coggon D, Hossain P. Geographic variations in microbial keratitis: an analysis of the peer-reviewed literature. Br J Ophthalmol. 2011;**95**(6):762-7.

**Table 1** Modified GRADE score for 18 papers that comprise the papers used for estimating annual incidence of fungal keratitis

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Country (year)** | **Diagnostic accuracy (/2)** | **Patient n >30 ((/1)** | **Up to date (/2)** | **%FK/MK (/1)** | **Overall score (/6)** | **Reference** |
| Malawi (2017) | 0 |  | 2 | 0 | 2 | 12 |
| Egypt (2012) | 1 | 1 | 1 | 1 | 4 | 13 |
| China (2013) | 1 |  | 2 | 1 | 4 | 14 |
| China (2010) | 1 | 1 | 1 | 1 | 4 | 15 |
| India (1993) | 1 | 1 | 0 | 1 | 3 | 16 |
| Nepal (2014) | 1 | 1 | 2 | 1 | 5 | 17 |
| Malaysia (2017) | 1 |  | 2 | 0 | 3 | 18 |
| Philippines (2013) | 1 |  | 2 | 1 | 4 | 19 |
| Thailand (2012) | 1 | 1 | 1 | 1 | 4 | 20 |
| Vietnam (2012) | 2 | 1 | 1 | 0 | 4 | 21 |
| Turkey (2017) | 1 | 1 | 2 | 1 | 5 | 22 |
| Denmark (2015) | 2 | 0 | 2 | 0 | 4 | 23 |
| Ireland (2011) | 2 | 1 | 1 | 1 | 5 | 24 |
| United Kingdom (2016) | 2 | 1 | 2 | 1 | 6 | 7 |
| Brazil (2011) | 1 | 1 | 1 | 0 | 3 | 25 |
| Columbia (2017) | 1 | 1 | 2 | 1 | 5 | 26 |
| USA (2010) | 1 | 0 | 1 | 1 | 3 | 27 |
| Australia (2008) | 1 | 1 | 1 | 1 | 4 | 28 |

**Table 2.** Estimated annual incidence of fungal keratitis by region (as defined by the United Nations) and source.

|  |  |  |  |
| --- | --- | --- | --- |
| **UN world regions** | **Annual Incidence**  | **Annual Incidence /100,000** | **Extrapolated from** |
| Eastern Africa  | 23,241 | 13.3 | Kenya (unpublished) |
| Middle Africa | 8625 | 13.3 | Kenya (unpublished) |
| Northern Africa  | 17,556 | 14 | Egypt (19) |
| Southern Africa  | 5096 | 14 | Egypt (19) |
| Western Africa  | 20,678 | 13.3 | Kenya (unpublished) |
| **Africa Total** | 75,196 | 13.5 |  |
|  |  |  |  |
| China (North, West, Hong Kong) | 3686 | 1.3 | China (20) |
| China (South, East, Central) | 107,124 | 15.2 | China (21) |
| Eastern Asia (exc China) | 2061 | 1.3 | China (20) |
| Central Asia  | 530 | 1.3 | China (20) |
| Southern Asia  | 768,325 | 73 | Nepal (22) |
| South-Eastern Asia  | 57,990 | 15 | Thailand (23,29) |
| Western Asia  | 179 | 0.12 | Turkey (24) |
| **Asia Total** | 939,895 | 33.9 |  |
|  |  |  |  |
| Eastern Europe | 41 | 0.02 | UK (25) |
| Northern Europe | 13 | 0.02 | UK (25) |
| Southern Europe | 20 | 0.02 | UK (25) |
| Western Europe | 25 | 0.02 | UK (25) |
| **Europe Total** | 99 | 0.02 |  |
|  |  |  |  |
| Northern America | 15,660 | 6.8 | USA (26) |
| Oceania | 1767 | 14.5 | Australia (27) |
| **North America and Oceania Total** | 17,427 |  |  |
|  |  |  |  |
| The Caribbean | 1305 | 5 | Columbia (28) |
| South America | 12,895 | 5 | Columbia (28) |
| Central America  | 4970 | 5 | Columbia (28) |
| **Latin America Total** | 19,170 | 5 |  |
|  |  |  |  |
| **World Total** | 1,051,787 | 23.6 |  |