The Epidemiology of Microbial Keratitis in South Western Uganda

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Declaration

I, Simon Arunga, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

18/9/2019

Signature Date

Glossary

AAO	American Academy of Ophthalmology
AMG	Amniotic Membrane Graft
ARMD	Age Related Macular Degeneration
BCVA	Best Corrected Visual Acuity
BVDU	BromoVinylDeoxyUridine
CF	Conjunctival Flap
CFW	Calcofluor white
CoNS	Coagulase-negative *Staphylococci*
CXL	Corneal Cross Linking
DAG	Direct Acyclic Graphs
DM	Diabetes Mellitus
DNA	Deoxyribonucleic Acid
ECM	Extra Cellular Matrix
EQ-5D	European Quality of Life Questionnaire
Exo	Exotoxin
FK	Fungal Keratitis
FLV	Functional Low Vision
HC	Health Centre
HEDS	Herpetic Eye Disease Study
HIV	Human Immune Virus
HRQoL	Health Related Quality of Life
HSV	Herpes Simplex Keratitis
ICAMB	Intra Cameral Amphotericin B
IND-VFQ	Indian visual Function Questionnaire
IOP	Intra Ocular Pressure
ISV	Intra Stromal Voriconazole
IVCM	In Vivo Confocal Microscopy
KCMC	Kilimanjaro Christian Research Centre
KCRI	Kilimanjaro Christian Research Institute
KOH	Potassium Hydroxide
LK	Lamellar Keratoplasty
LMIC	Low and Middle Income Countries
LogMAR	Logarithm of the Minimum Angle of Resolution

LSHTM	London School of Hygiene and Tropical Medicine
MALDI-TOF	Matrix-Assisted Laser Desorption/Ionization Time Of Flight
MIC	Minimum Inhibitory Concentration
MK	Microbial Keratitis
MOH	Ministry of Health
MRC	Medical Research Council
MRRH	Mbarara Regional Referral Hospital
MUHREC	Mbarara University and Referral Hospital Eye Centre
MUST	Mbarara University of Science and Technology
MUTT	Mycotic Ulcer Treatment Trial
MVI	Moderate Visual Impairment
NEI-VFQ	National Eye Institute Visual Function Questionnaire
OA	Ophthalmic Assistant
OCO	Ophthalmic Clinical Officer
PCR	Polymerase Chain Reaction
PHMB	PolyHexaMethylene-Biguanide
PMCD	Pellucid Marginal Corneal Degeneration
PNFP	Private Not For Profit
PPP	Private for Profit
PUK	Peripheral Ulcerative Keratitis
PVL	Panton-Valentine leukocidin
QOL	Quality of Life
RAAB	Rapid Assesment of Avoidable Blindness
RCT	Randomised Controlled Trial
REC	Ruharo Eye Centre
RR	Risk Ratio
SCUT	Steroids for Corneal Ulcers Treatment
SIGN 50	Scottish Intercollegiate Guidelines Network
SMD	Standard Mean Difference
SSA	Sub Saharan Africa
SVI	Severe Visual Impairment
T3SS	Type III secretion system
TEM	Traditional Eye Medicine
TLR	Toll Like Receptor
TPK	Therapeutic Penetrating Keratoplasty

TT	Trachomatous Trichiasis
UK	United Kingdom
USA	United States of America
UVRI	Uganda Virus Research Centre
VAS	Visual Analogue Scale
VKC	Vernal Kerato Conjunctivitis
VRQoL	Vision Related Quality of Life
VZV	Varicella Zoster Virus
WHO	World Health Organisation
WHO/PBD VF 20	WHO Prevention of Blindness and Deafness 20-item Visual Functioning Questionnaire
WHOQOL-BREF	WHO generic Quality of Life tool
Zmp	Zinc Metallo Proteases

Abstract

Background: Microbial Keratitis (MK) is the leading cause of unilateral blindness after cataract in Tropical regions and is responsible for 2 million cases of blindness per year. In Sub-Saharan Africa, MK is a neglected problem, most ophthalmic centres do not have diagnostic services, patients present late, appropriate drugs are often not available, corneal transplant services are rarely available. Subsequently, outcomes are poor in this area. Currently there are very limited data to guide policy and practice.

Methods: In a main cohort design, individuals with MK presenting to the two referral eye hospitals in South Western Uganda were enrolled over a one-year period. Clinical history and presentation journey were recorded. Their eyes were carefully examined, and samples were collected for microbiology. Patients were tested for HIV and Diabetes. At three months, patients were followed up in their homes and at this point healthy community controls were enrolled to compare risk factors in a nested case-control study and assess the impact of the disease on the Quality of Life (QoL). A separate situation analysis survey of lower health centres was additionally conducted to understand the role of the health system in management of MK.

Results: Three hundred and thirteen individuals were enrolled. Median age was 47 years (ra nge 18-96) and 174 (56%) were male. Median presentation time to the eye hospital was 17 days from onset (IQR 8-32). Trauma was reported by 29%. Majority presented with severe in fections (median infiltrate size 5.2 mm); 47% were blind in the affected eye (vision <3/60), fu ngal cases were 62%. At 3-months, 30% of participants were blind in the affected eye, while 9% had lost their eye from the infection. Predictors of poor vision at 3-months were: baseline vision (aOR 2.98 [95%CI 2.12-4.19], p<0.0001), infiltrate size (aOR 1.19 [95%CI 1.03-1.3 6], p<0.020) and perforation at presentation (aOR 9.93 [95% CI 3.70-26.6], p<0.0001).

Traditional Eye Medicine (TEM) use was reported in 188/313. TEM users had a delayed presentation; median presenting time 18 days versus 14 days, p= 0.005; had larger ulcers 5.6 mm versus 4.3 mm p=0.0005; a worse presenting visual acuity median logarithm of the minimum angle of resolution (Log MAR) 1.5 versus 0.6, p=0.005; and, a worse visual acuity at 3 months median Log MAR 0.6 versus 0.2, p=0.010. In the qualitative analysis, reasons for TEM use included lack of confidence in conventional medicine, health system breakdown, poverty, fear of the eye hospital, cultural belief in TEM, influence from traditional healers, personal circumstances and ignorance.

In the case-control analysis, HIV OR 83.5 (95%CI 2.01-3456), p=0.020, Diabetes OR 9.38 (95% CI 1.48-59.3), p=0.017 and a farming occupation OR 2.60 (95%CI 1.21-5.57), p=0.014 were main risk factors of MK. In the Quality of Life (QoL) analysis, mean QoL scores of the cases were lower than controls across all domains. Determinants of QoL among the cases at 3-months included visual acuity at 3-months and history of eye loss.

Although most patients presented early to the primary health centres (median 2days IQR 0-5 days), there were severe weaknesses along the health system in identification and early referral of MK. Only 12% of the health workers could make a diagnosis of MK. None of the health facilities had a stock of the recommended first line treatment options for MK (ciprofloxacin and Natamycin eye drops).

Conclusion: This is the first large epidemiological cohort in SSA studying MK and provides a baseline understanding of the epidemiology, aetiology and outcomes, and what needs to be done to improve the situation and reduce the devastating visual outcomes currently experienced by many people

Format of the Thesis

The thesis for this PhD utilises the “research papers” format, recently introduced by the London School of Hygiene and Tropical Medicine. It therefore includes several papers which are either published, accepted or in submitable format for publication in peer-reviewed journals. The chapters listed in italics in the Contents are in this research/review paper format, and each chapter includes publication details in a cover sheet, including acknowledgement of the contributions of other people. The other chapters of the thesis are composed of “linking material” which includes information/data not covered in the research papers and helps to make the thesis a coherent body.

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Dedication

I dedicate this thesis to the Microbial Keratitis patients who offered themselves to participate i n the study for the betterment of mankind, and to my Bishop Ap Allan Bukuru Jonathan (late) who would have loved to read this book but is now with the Lord.

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Chapter 1. Background

Ronald a 25-year old young father with Fungal Keratitis

Overview

Microbial keratitis (MK), or infection of the cornea, can be caused by a range of pathogens. The causative organisms include bacteria, viruses, protozoa (e.g. acanthamoeba), and fungi (yeasts, moulds and microsporidia). It is characterised by an acute or sub-acute onset of pain, conjunctival hyperaemia and corneal ulceration with a stromal inflammatory cell infiltrate. MK frequently leads to sight-loss from dense corneal scarring, or even loss of the eye, especially when the infection is severe and/or appropriate treatment is delayed.[1] Blindness is defined as a presenting distance vision worse than 3/60; bilateral blindness is distance vision worse than 3/60 in the better eye.[2]

MK has been described as a "silent epidemic", which leads to substantial morbidity, related to blindness and other consequences such as pain and stigma.[3] It is the leading cause of unilateral bli ndness after cataract in tropical regions and is responsible for about 2 million cases of monocular b lindness per year in Africa and Asia.[4] The World Health Organization (WHO) estimated (2017) that 1.3 million individuals are bilaterally blind from corneal opacity globally (excluding trachoma a nd vitamin A deficiency), accounting for 3.2% of binocular blindness globally[5]. In Sub-Saharan Afric a (SSA), MK is an important cause of binocular blindness and is responsible for about 15% of monocular blindness in the Nigeria National Survey (personal communication).[6,7]

MK typically affects people in the most economically productive stage of life, median age ~40 years.[6,8] It reduces vision-related quality of life.[9,10] Treatment is costly and prolonged. Outcomes in Low and Middle-Income Countries (LMIC) are typically poor, with ~60% of eyes rendered blind.[8,11,12]

A good outcome depends on early appropriate treatment, correct identification of the causative organism, and careful follow-up.[8,9] In LMIC, MK presents major challenges. The outcomes in SSA are frequently poor.[10-12] Presentation is usually delayed and advanced infections have poor outcomes.[12] Patients may use TEM, which often contains plant matter or inappropriate "conventional" medication (such as a corticosteroid), exacerbating the problem.[12-14] Primary health-care staff have little training in recognising, treating and referring MK. It is frequently not possible to clinically distinguish bacterial and fungal MK. Microbiology services are usually unavailable. Fungal keratitis is particularly difficult to treat. Current topical anti-fungals are not consistently effective and infection can progress despite prompt treatment.[12,15-19] Anti-fungal drops are rarely available in SSA and often scarce elsewhere.[12] The eye may be lost through progressive deep corneal ulceration and perforation.[12,16]

Epidemiology of Microbial Keratitis in Africa and Elsewhere

Incidence

The Incidence of MK varies between high-income countries and Low- and Middle-Income Countries (LMIC). A recent review has described global incidence rates.[20] A summary of the global incidence rates is presented in Table 1. Overall, the incidence of MK is highest in Asian countries (except Hong Kong) and lowest in Europe and North America.[21-24]

There is only one older report of the incidence of MK in SSA from Malawi.[14] In this study, all patients with corneal lesions (in which fluorescein stain was visible with a torch) that presented to ophthalmic medical assistants at two district hospitals (Mulanje and Chikwawa) in Malawi were enrolled. For one district (Chikwawa) with a known population, the incidence of corneal disease per 10,000 population was calculated. From this district, most of the patients came from two main subdistricts (Kasisi and Katunga). Fifty-six patients came from Kasisi (total population 24,300, incidence 231/100,000 persons) while twenty-four patients came from Katunga (total population 13,850, incidence 173/100,000 persons). Pooling these cases gave an incidence of 210/100,000/year.[14] However, a more recent update on the burden of serious fungal infections in Malawi has given a much lower figure of (10.3/100,000) fungal cases per year based on the number of cases presenting to the main eye hospital department in Malawi.[25]

Table 1. Incidence of microbial keratitis in population-base studies

Country	Year	Estimate	Source
Africa			
Malawi (Kasisi, Katunga)	1994	210/100,000/year	Courtright[14]
Malawi	2018	10.3/100,000/year	Kalua[25]
Asia			
India (Madurai)	1997	113/100,000/year	Gonzales[26]
Nepal (Bhaktapur)	2001	799/100,000/year	Upadhyay[22]
Hongkong	2002	6.3/100,000/year	Lam[21]
Myanmar	2004	710/100,000/year	WHO / Country Report[27]
Bhutan	2004	339/100,000/year	WHO / Country Report[27]
North America and Europe			
USA	2010	27.6/100,000/year	Jeng[23]
UK	2012	40.3/100,000/year	Ibrahim[24]

Variation in Causative Organisms by Geographical Region

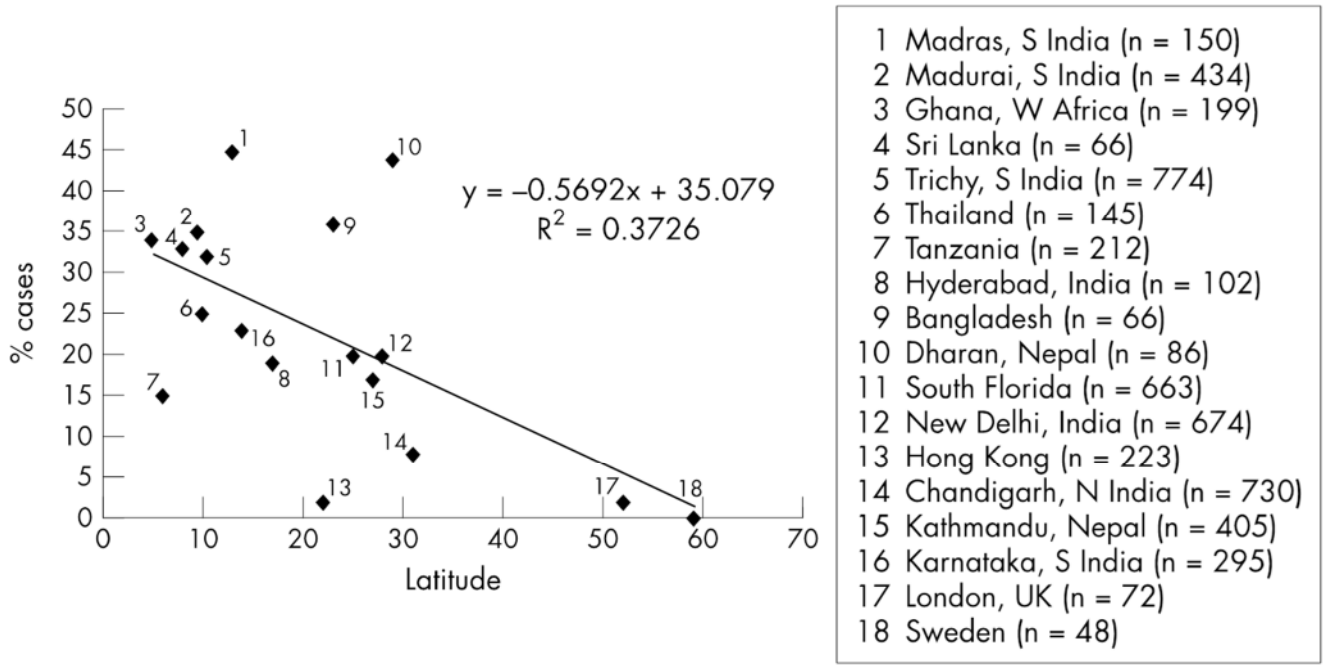
A wide range of microorganisms can infect the cornea: bacteria, fungi, viruses and protozoa. The pattern of causes seems to be more geographical although urbanisation and seasonal variation has also been reported to influence specific causes.[28] There are only a few, limited studies of the microbiology of MK in SSA. Table 2 shows the distribution of the causes of MK in SSA.

In global epidemiology of MK, three large reviews have looked at the distribution of organisms according to geographical region.[20,29,30] The first review in 2002 looked at the global proportions of fungal keratitis.[31] The authors mapped the proportion of fungal keratitis against latitude and demonstrated that the proportion of filamentous fungi as a cause of MK generally increases the lower the latitude, with the highest proportion being found around the equator (figure 2).[31-34] In tropical regions filamentous fungi cause about half of MK.[12,31,35] The second epidemiological review in 2011 described associations between a country's gross national income and types of causative organism.[29] The highest proportion of bacterial corneal ulcers was reported in studies from North America, Australia, Europe and Singapore. The highest proportions of fungal infections were found in studies from India and Nepal.[29] There was a significant correlation between a country's gross national income and type of infection (fungal or bacterial). The higher the income of a country, the higher the proportion of bacterial MK and vice versa.[29] A more recent review (2019) looked at the global incidence and proportions of MK based on large population studies and large case series.[20] The summary of the reports considered in these reviews have been updated and presented in Table 3.

Generally, in temperate climates most corneal infections are bacterial and are frequently related to contact lens use although reports of recent increases in fungal keratitis in the UK have been reported.[13] In the literature of large case series from North America, Europe and South America, the proportion of fungal keratitis was low ranging from 0-26%.[24,36-40] Conversely, fungal rates from African and Asian studies were high ranging at an average of 50%.[31,41-43]

Specifically, regardless of geographical location, Gram positive organisms (*Streptococcus pneumoniae, Staphylococcus aureus*) and Gram-negative pathogens (*Pseudomonas aeruginosa*) are the most frequent bacterial causes while *Fusarium spp and Aspergillus spp* are the most common fungal causes.[31,44-48] However, in temperate climates, Candida spp have been commonly reported. [24,36-40]

Figure 1 Distribution of filamentous fungi by Latitude[31]



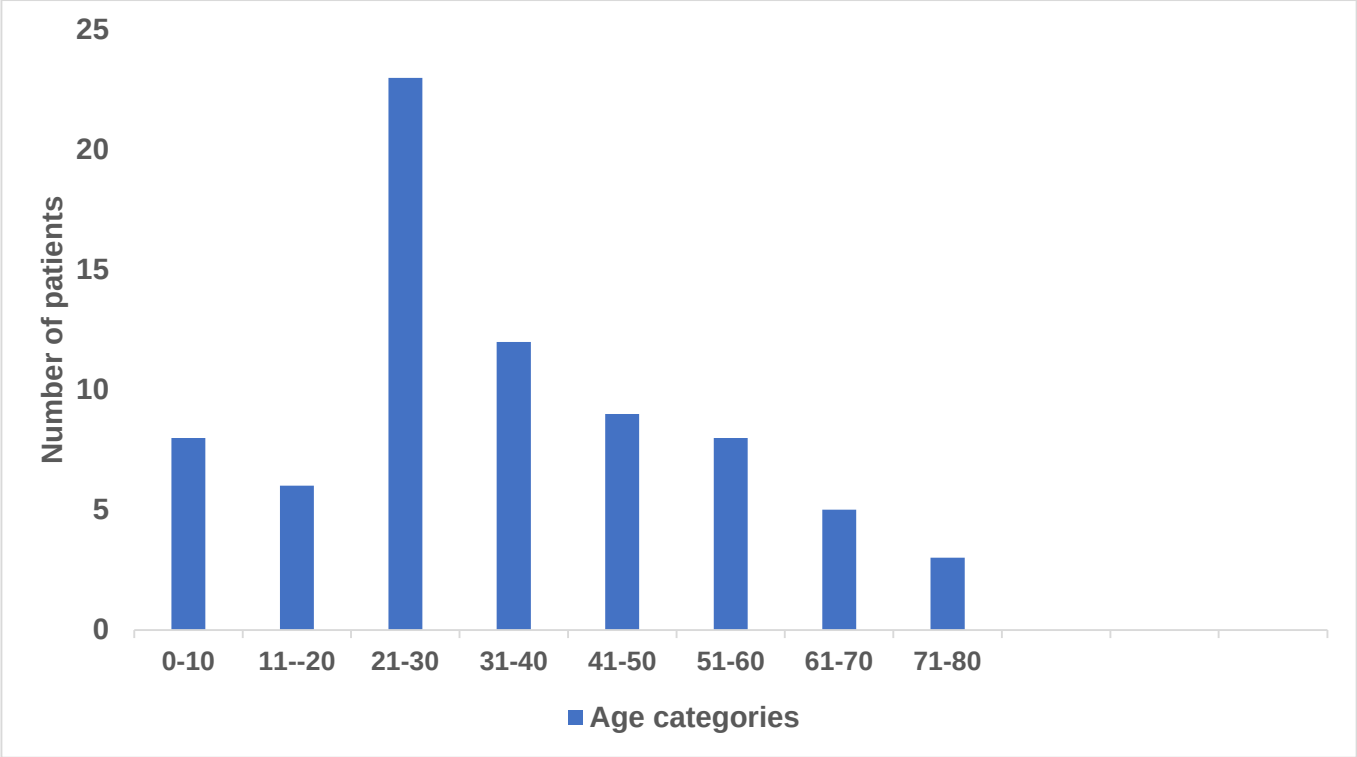
Risk factors

There are many potential risk factors that may predispose a person towards developing MK with some risk factors being more specific to settings (region, income status and organism) and some being ubiquitous. Table 4 shows some of the summary results on studies from SSA that have reported data on risk factors of MK, and some select global studies.

Age

Although age may not be an independent risk factor for MK, infectious keratitis is a more severe disease in elderly than in younger patients with more complications and a worse prognosis. Elderly patients have multiple and more diverse risk factors, making prevention difficult to manage.[49] Age influences other risk factors: for example, trauma is more common in the lower age groups versus ocular surface diseases which are more common in older folk.[49] However, the peak affected age group in many African studies is between 20-40.[50,51] Table 2 summarises the epidemiology of MK in SSA and shows that in almost all the studies the peak/median age of presentation was between 20-40 years. Only one report from Nigeria indicated a dual peak in the age group of 21-30 years and 51-60 years.[52] In our pilot work before this project, we noted a similar picture in our setting as shown in Figure 3, the peak age group for MK was between 21-30 years.

Figure 2 Age distribution among patients presenting to Ruharo Eye Centre with MK, a pilot study (n=75)



Sex

Although sex is not an independent risk factor for MK, almost all papers from SSA have reported a male predominance among MK patients ranging from 54-87%.[12,48,50,51,53,54] A similar pattern has been shown in Asian studies with an average of 60-70% of all the MK patients being male.[41,43,45,55] In our settings, this difference might be due to occupational differences where males may be at a higher risk of trauma compared to females.).[56-58] However, there does not seem to be a difference in the male and female proportions among studies from Europe and the USA with some reporting a lower proportion of males compared to females.[23,59-61] This pattern has also been reported in other non-European but equally developed regions such as Hong Kong.[62] In this 10 year review study in a tertiary centre in Hong Kong where 347 scrapes were performed in the 10-year period, the proportion of males in this study was 43.4%.[62]

Trauma / Occupation

Trauma has been reported in almost all studies regardless of geographical region as a key predisposing factor for MK (Table 4). Risk factors such as trauma especially with vegetative matter have been associated with fungal keratitis compared to a pre-existing ocular disease for bacterial keratitis.[56,57] Injury with mud strongly linked to Acanthamoeba keratitis.[57] In addition, agricultural work and foreign body in the eye have been implicated, these are fairly trauma related.[56,58,63]. In the reports from SSA, the rates of trauma range from 23-54%.[12,48,50,51,53,54]

Traditional Eye Medicine (TEM)

In Low and Middle-Income Countries (LMIC), use of Traditional Eye Medicine (TEM) for treatment of many eye conditions is a common practise.[64-66] A recent large population-based study in 25 randomly selected clusters of Rural Gurgaon, Haryana, India found that of the 2160 participants interviewed, 396 (18.2%) reported using ophthalmic medications without consulting an ophthalmologist, mainly for symptoms like watering (37.1%), redness (27.7%), itching (19.2%) and infection (13.6%).[66] Additionally, 25.7% (529) participants resorted to home remedies like 'kajal'(61.4%), honey (31.4%), ghee (11.7%) and rose water (9.1%).[66]

In SSA, one study from Malawi interviewed 800 adults in the study areas. Self-treatment was reported for the last episode of eye disease by 39.8% of the study population of which 72% had used TEM.[65] Another prospective case-controlled study where 150 pterygium patients and 150 controls participated found that 52.6% of the 150 cases and 40% of the 150 controls had used TEM (odds ratio (OR) 2.03; p=0.009.[64] Another study from Tanzania enrolled 257 consecutive patients with eye injury.[67] TEM was used by 49% of all patients; the main types of traditional medicines used were plant juices, milk mixed with black powder and pounded roots; the main route of application was instillation into the conjunctival sac.[67] In Uganda, small local study at Ruharo Eye Centre in Mbarara found that 60% of the people attending the outpatient clinic for various problems first used TEM, before coming to hospital (unpublished). The proportion of TEM among people presenting with MK in SSA has been reported varying from 4-35%.[12,44,52,68] There may be some underreporting due to “fear” among patients.

Since most of the TEM involves plant products such as fresh leaves, it could have a major role in the pathogenesis of fungal keratitis, which has been associated with injuries involving vegetative matter.[45,69] One earlier report from Tanzania reported TEM as an independent risk factor for TEM.[13] In this study, 103 patients presented with MK of which 26% admitted to having used TEM. Out of these, 58% had no other attributable risk factor for MK and were then considered as TEM induced MK.[13]

In addition, TEM has been found to lead to complications such as corneal scarring and delayed presentation of patients to hospital resulting in poor outcomes.[67,70] In one study from Malawi, 197/583 (33.8%) patients who presented with corneal disease reported using TEM during the current eye disease episode.[14] The patients who reported TEM use took longer (mean 50 7 (SD 35 3) days) to reach the district hospital than patients who did not report TEM use (mean 12-9 (SD 17-8) days) (p<0001).[14] In addition, patients who reported TEM use were more likely to have bilateral disease and poor vision on presentation.[14]

The pilot study in Uganda found that patients had used different vegetative concoctions, including cow dung derivatives. Most patients reported a firm belief in TEM and did not appreciate the potential danger. This is compounded by the traditional understanding that MK, which is known as "*akavurugye*" in the local Bantu language (literally translated as 'that which distorts the eye'), is only treatable by TEM. Patients presenting with severe MK reported, on being asked why they came late, that they first tried TEM for "*akavurugye*". This belief has been reported in other parts of SSA. In the earlier study from Malawi, One hundred and ninety-four (33.3%) patients had consulted a traditional healer at least once before presentation at the district hospital (113 as first source and 81 as second source).[14] Treatment received from the healers was perceived to be equally helpful (41.6%) as that received from health centres (42.8%). Of the 173 patients who attended a health centre first, 59 (34. 1%) went to a traditional healer for subsequent care.[14]

HIV

HIV is a common problem in many parts of SSA. In Uganda, the prevalence of HIV is at 6.3%.[71] Two studies have reported HIV as a potential risk factor for MK.[12,51] In the first study in 1999 enrolled 212 patients with MK that presented to Muhimbili Medical Centre, Dar es salaam, Tanzania.[51] As part of the work up, patients were tested for HIV as well as microbiological workup. There was a total of 86/212 (40%) HIV positive patients in this study population. Twenty-six of 32 (80%) patients with fungal keratitis were HIV positive; and 33% patients with non-fungal keratitis were HIV positive (P-value was < 0.001).[51] This was before the onset of Anti Retro Viral Therapy (ART) for HIV care. Later in 2003, a study in Kilimanjaro (about 462km from Dar es salaam) enrolled 170 patients with MK over a 27-month period. As part of the work up, HIV infection was diagnosed in 16% of individuals tested, which was approximately twice the prevalence found in the wider population in Tanzania at that time.[12] This study however did not specifically compare the HIV proportions in bacterial versus fungal MK.

HIV is uncommon in other regions outside of SSA. However, one large population study in the USA recorded incidence rates of ulcerative keratitis over a 12-month period.[23] Multivariate relative risk regression was conducted to evaluate potential risk factors for ulcerative keratitis. Within the target population of 1,093,210 patients, 302 developed ulcerative keratitis. The incidence of ulcerative keratitis was 27.6 per 100 000 person-years (95% confidence interval, 24.6-30.9).[61] Seven of 2,944 people known to be infected with HIV developed ulcerative keratitis giving an incidence of 238.1 per 100 000 person-years (95% confidence interval, 95.7-490.5). Compared to HIV negative individuals, the with an odds of developing ulcerative keratitis among the HIV positive patients was 9.31 (7.42-11.7; P < .001).[23]

DM

Diabetes Mellitus (DM) has been the most commonly reported systemic risk factor, especially following keratoplasty or corneal trauma.[45,72,73] Diabetes Mellitus is a growing public health concern in many parts of the world. The latest International Diabetes Foundation (IDF) Diabetes atlas reported 451 million adults with Diabetes globally in 2017[74]. The expected number will be 552 million by 2030 and 693 million in 2045 with the greatest increase in Low and Middle Income countries (LMICs) [74]. The prevalence of DM in Uganda is about 2% in the rural population and 4% in the urban population.[75] There is no report from SSA on DM as a risk factor for MK. However, a number of studies from India and Asia have reported DM as one of the risk factors for MK accounting for 3.2-7.6% of MK cases (table 4).[41,43,45,62]

Steroids

Topical steroids are used in ophthalmology to treat a number of conditions such as inflammatory conditions, uveitis, post-surgical care, allergic conditions and some forms of Peripheral Ulcerative Keratitis (PUK).[76] However, they can impair the immune host response of the cornea making it susceptible to infection.[77] In many parts of SSA, patients access these steroid eye drops as off over the counter medication. In one 10 year review from Nigeria, self-medication with topical steroids as a risk factor accounted for 5.7% of all the 82 MK cases.[44] In another report from Tanzania, use of steroids as a risk factor accounted for 17.1% of all the 170 MK cases.[12] In many other settings in SSA, the type of the drug used by the patients prior to presentation are difficult to ascertain especially if the patients do not present to hospital with the bottles/pack information of the medicines they have been using.

Ocular Surface Disease (OSD)

Pre-existing OSD includes several conditions such as scarring, dryness, eyelid problems such as blepharitis which compromise the integrity of the intact corneal epithelium.[62] This has been reported more in studies from Europe, USA and Hong Kong ranging from 17-29% (table 4).[61] Apart from a report from Ethiopia, a trachoma endemic region which reported a high proportion of blepharitis (29.2%) among the patients developing MK and an older report from Tanzania (another trachoma endemic area) which reported a 32% (previous corneal scar) proportion of OSD among the MK cases, other literature from SSA has not reported much lower rates of OSD compared to European studies.[68,78]

Contact Lens wear associated with MK affects more people in high-income countries as opposed to the use of traditional eye medicines, which is more of a problem in LMIC.[12,14,59,79,80] For example, in the recent large multicentre study from Asia-pacific, the overall proportion of people who developed MK due to contact lens wear was 10.7% across all the countries.[41] In the subgroup analysis by

country, contact lens wear was the greatest risk factor in Singapore (68.2%), Taiwan (43.3%), and Japan (25.6%) and lowest in India (0.8%) and China (0.4%).[41]

However, all previous African studies are limited by the absence of control subjects for comparison. One of the aims of this PhD, therefore, was to investigate the role of multiple risk factors (HIV infection, DM, farming) which are preventable or modifiable by comparing MK cases to disease free community controls, matched for age, sex and village in Uganda.

Table 2 Epidemiology of MK from studies in SSA

Author	Country	N	Males	Mean age	(%) culture positive	Fungal*	Bacterial*	Bacteria (%) ǂ	Fungi (%) ǂ
Mafwiri[50]	Tanzania	202	66.8%	-	76%	48%	52%	*Staphylococcus spp (37%), Streptococcus spp (23.5%) Escherichia Coli (7.4%) Pseudomonas aeruginosa (6.2%)*	*Candida spp (22%)*
Aliraki[81]	Uganda	78	66%	30	38%	12.5%	87.5%	*Staphylococcus aureus (7.5%), Streptococcus pneumoniae (7.5%) Pseudomonas aeruginosa (10%)*	*Dermatophyte (2.5%)*
Poole[68]	Tanzania	44	66%	44	55%	50%	50%	*Staphylococcus epidermidis (6.8%) Staphylococcus aureus (4.6%) Pseudomonas aeruginosa (13.6%)*	*Fusarium spp (20.5%) Aspergillus (2.3%)*
Carmicheal[54]	South Africa	283	87%	42	45%	5%	95%	*Streptococcus pneumoniae (37.7%), Pseudomonas aeruginosa (16.9%)*	-
Hagan[48]	Ghana	207	69%	36	50%	56%	44%	*Streptococcus spp (8.6%) Staphylococcus spp (5.4%), Pseudomonas aeruginosa (12.5%)*	*Fusarium spp (26.5%) Aspergillus (7.8%)*
Capriotti[82]	Sierra-Leone	73	-	-	95%	32%	68%	*Staphylococcus aureus (27.4%, Pseudomonas aeruginosa (39.7%)*	*Aspergilus spp (5.5%)*
Burton[12]	Tanzania	170	54%	46	50%	51%	49%	*Staphylococcus epidermidis (14.1%), Pseudomonas aeruginosa (5.3%)*	Fillamentary fungi *(24.6%), Candida spp (3.5%)*
Wani[47]	Zimbabwe	43	58%	33	26%	0%	100%	*Staphylococcus spp (54.5%), Escherichia Coli (18.5%), Pseudomonas aeruginosa (9.1%), Klebsiella spp (9.1%), Norcadia spp (9.1%)*	-
Ezegwui[52]	Nigeria	82	46%	-	-	-	-	-	-
Mselle[83]	Tanzania	212	59%	-	-	32%	68%	-	*Fusarium spp (75%),*

									Aspergillus spp (18.75%)
Oladigblou[44]	Nigeria	228	57%	-	58%	-	-	*Staphylococcus aureus, Streptococcus pneumoniae Pseudomonas spp*	-
Limaiem[84]	Tunisia	100	55%	-	42	21%	79%		-
Leck[31]	Ghana	290	-	-	-	73%	27%	*Staphylococcus spp (10%) Streptococcus spp (20%), Pseudomonnas spp (52.5%)*	*Fusarium spp (42.2%), Aspergillus (17.4%)*

*proportion out of the culture positive cases, ǂ most common bacterial, fungal organisms identified

Table 3 Global epidemiology of MK, updated literature.

Author	Country/year	N	(%) culture positive	Fungal*	Bacterial*	Bacteria (%) ǂ	Fungi (%) ǂ
Khor[41]	Asia-pacific/2018	6626	43.1%	32.7%	38%	*Pseudomonas aeruginosa (10.7%), Streptococcus pneumoniae (6.3%),*	*Fusarium spp (18.3%), Aspergillus spp (8.3%)*
Chidambaram[43]	India/2018	252	83%	77%	7%	*Streptococcus pneumoniae (47%), Pseudomonas aeruginosa (21%), Norcadia spp (16%)*	*Fusarium spp (39%), Aspergillus spp (18%)*
Khanal[85]	Nepal/2005	447	64%	47.8%	34%	*Staphylococcus aureus (56.7%), Streptococcus pneumoniae (20%), Pseudomonas aeruginosa (20%)*	*Aspergillus spp* (38.4%) *Fusarium spp* (22%). *Aureobasidium spp*. (12.3%)
Dunlop[86]	Bangladesh/1994	142	63%	35.9%	53.5%	*Pseudomonas spp (24%) Streptococcus pneumoniae (17%)*	*Aspergillus spp* (13%) *Fusarium spp* (7%) *Culvularium spp* (6%)
Panda[87]	India/2007	1000	56.8%	49.1%	37.5%	*Staphylococcus spp (27.4), Pseudomonas spp (12.1%)*	*Aspergillus spp* (20.4%)
Sharma[88]	India/2007	170	69.4%	13%	55.2%	*Staphylococcus epidermidis (38.3%), Streptococcus pneumoniae (22.3%), Pseudomonas spp (6.3%)*	*Fusarium spp (72.7%),*
Bharathi	India/2007	3183	71%	34.4%	32.77%	*Streptococcus pneumoniae (36%), Pseudomonas aeruginosa (20%)*	*Fusarium spp (41.92%), Aspergillus spp (25%)*
Basak[55]	India/2005	1198	68%	62.7%	22.7%	*Staphylococcus aureus (42.6%), Pseudomonas spp (21.1%)*	*Aspergillus spp* (59.9%) *Fusarium spp* (21.2%)
Sharma	India/2002	1092	35%	37.5%	62.5%	*Staphylococcus spp (23.7), Streptococcus spp (15.4%), Pseudomonas spp (6.9%)*	*Aspergillus spp* (11.1%)

Leck[31]	India/2002	800	69%	44%	29.3%	*Streptococcus spp (46.8%), Staphylococcus spp (24.7), Pseudomonas aeruginosa (14%)*	*Aspergillus spp* (39.9%) *Fusarium spp* (21.5%) *Culvularium spp* (9.6%)
Vajpayee[89]	India/2000	100	65%	20%	52%	*Staphylococcus spp (33.9%), Pseudomonas aeruginosa (15.4%)*	*Aspergillus spp* (9%)
Srinivasan[90]	India/1997	434	68%	46.8%	47.1%	*Streptococcus pneumoniae (44.3%), Pseudomonas spp (14.4%)*	*Fusarium spp* (47.1%) *Aspergillus spp* (16.1%)
Far East							
Lin[91]	China/2017	2973	46.1	44.6%	41.9%	*Staphylococcus epidermidis (31.9%) Pseudomonas aeruginosa (12.4%)*	*Fusarium spp. (29.3%) Aspergillus spp. (24.1%)*
Hsiao[92]	Taiwan/2016	2,012	49.3%	16%	81.1%	*Pseudomonas aeruginosa* (24.4%) *Staphylococcus spp* (16.6%)	-
Xie[42]	China/2006	1,056	75.6%	77.9%	16.2%	-	*Fusarium spp. (73.3%) Aspergillus spp. (12.1%)*
Europe							
Ting[37]	UK/2018	914	44.5%	4.2%	91.0%	CoNS *(28.5%) Staphylococcus aureus (14.9%) Streptococcus spp. (13.3%)*	Yeasts (50.0%) Filamentous fungi (50.0%)
Tan[38,93]	UK/2017	4,229	32.6%	7.1%	90.6%	CoNS *(26.9%) Staphylococcus aureus (16.7%) Streptococcus spp. (14.7%)*	Yeasts (53.2) *Fusarium spp.* (25.7%)
Ibrahim[24]	UK/2009	1,254	63.8%	0%	85.4%	*Staphylococcus epidermidis (31.7%) Pseudomonas aeruginosa (12.0%) Staphylococcus aureus (11.5%)*	-

North America							
Hernandez[39]	Mexico/2015	1,638	37.6%	11.7%	88.3%	*Staphylococcus epidermidis* (27.4%) *Pseudomonas aeruginosa* (12.1%) *Staphylococcus aureus* (9.0%)	*Fusarium spp* (50.0%) *Aspergillus spp* (19.4%) *Candida spp* (8.3%)
Lichtinger[40]	Canada/2012	1,701	57.4%	6.0%	91.8%	CoNS (36.5%) *Streptococcus spp* (17.4%) *Staphylococcus aureus* (17.2)	-
Alexandrakis[36]	USA/2000	2,920	50.3%	-	91.1%`	*Pseudomonas aeruginosa* (25.7%) *Staphylococcus aureus* (19.4%)	-
South America							
Cariello[94]	Brazil/2011	6,804	48.6%	11.0%	78.9%	CoNS (26.3%) *Staphylococcus aureus* (21.1%) *Pseudomonas aeruginosa* (11.8%)	*Fusarium spp* (51.9%) *Candida spp* (14.3%) *Aspergillus spp* (9.1%)
Laspina[95]	Paraguay/2004	660	79.4%	26.0%	51.0%	CoNS (25.1%) *Staphylococcus aureus* (23.7%) *Pseudomonas aeruginosa* (10.7%)	*Acremonium spp* (37.8%) *Fusarium spp* (19.6%) *Aspergillus spp* (17.7%)

*proportion out of the culture positive cases, ǂ most common bacterial, fungal organisms identified. CoNS=Coagulase Negative *Staphylococcus spp*

Table 4 Summary results of select papers on risk factors of MK

Author	Country	year	N	% fungal	% Bacterial	% Male	% Trauma	% Steroid	% TEM	% CL	% OSD	% POS	% HIV	% DM
Africa														
Gebremariam[78]	Ethiopia	2015	24	-	100	87.5	37.5	-	-	-	29.2	-	-	-
Mafwiri[50]	Tanzania	2013	202	37	40	66.8								
Burton[12]	Tanzania	2011	170	25	-	54	24	17.1	4	-	5.9	-	16	-
Oladigbolu[44]	Nigeria	2013	228			57	51.3	5.7	17.1	0.4	4	-	-	-
Ezegwui[52]	Nigeria	2010	82	-	-	46	52.4	-	19.5	-	-	-	-	-
Poole[68]	Tanzania	2002	44	27.3	27.3	66	38.7	-	9.1	-	32	-	-	-
Mselle	Tanzania	1999	212	15.1	32.5	59	23.1	-	-	-	-	-	40	-
Hagan[48]	Ghana	1995	207	30.9	16.4	69	39.2	-	-	-	-	-	-	-
Asia														
Khor[41]	Asia-pacific	2018	6563	32.	38.0	60.8	34.7	-	-	10.7	4.2	6.8	-	-
Chidambaram[43]	India	2018	252	77	7	64	72	11	19	-	-	-	-	7
Lap-Ki[62]	Hong Kong	2015	347	10	90	43.4	7.8	-	-	31.9	23.6	-	-	7
Nath[45]	India	2011	310	60.6	31.6	69.3	71.3	-	-	-	2.5	0.6	--	3.2
Xie[55]	China	2006	654	61.9	-	60.6	25.7	-	-	-	-	-	-	-
Basak[55]	India	2005	1198	62.7	22.7	70.6	82.9	19.3	-	0.3	10.1	0.6	-	7.6
Gopinathan	India	2002	1352	100	-	71.1	54.4							
Europe and USA														
Ong[96]	UK	2016	112	100	-	41.4	11.6	32.1	-	57.1	22.3	22.3	-	-
Keay	USA	2011	733	100	-		25			37	29	-	-	-
Jeng[23]	USA	2010	302	-	-	42.7	11	-	-	55	17.9		2.3	-
Saeed[60]	N.Ireland	2009	90	3	84.8	52.2	14.4	-	-	37.4	21.1	1.1	-	-

TEM=Traditional Eye Medicine CL=Contact Lens OSD=Ocular Surface Disease POS=Prior Ocular surgery HIV=Human Immune Virus DM=Diabetes Mellitus

Causative organisms

In SSA, the most common bacterial agents responsible for keratitis include *Staphylococcus aureus*, *Streptococcus pneumoniae and Pseudomonas aeruginosa* while the most frequent fungal causes are *Fusarium spp* and *Aspergillus spp* (Table 2-3). [31,44-48] Figure 4 shows the morphology of these organisms. In this section, we describe in brief the structure and virulence of these most common organisms.

Staphylococcus aureus

This gram-positive round shaped facultative anaerobe is part of the normal flora of the body and resides most frequently in the upper respiratory tract and on the skin.[97] It is a major pathogen of the eye able to infect the tear duct, eyelid, conjunctiva, cornea, anterior and posterior chambers, and the vitreous chamber and has potential to cause a loss in visual acuity or even blindness.[97] Although the ocular structures are inherently protected by a constitutive expression of antimicrobial factors and a protective host response to the organism, certain predisposing factors weaken this protection such as use of extended-wear contact lenses, the trauma caused by cataract surgery or intravitreal injection.[97]

Staphylococcus aureus produces numerous virulence factors which protect the organism from host defense and these include proteins among which are alpha-toxins, beta-toxins, gamma-toxins, leukocidins and protein A that mediate tissue damage and induce inflammatory response.[98] Strains associated with keratitis are different with each strain containing genetic loci that encode virulence factors. One such locus encodes Panton-Valentine leukocidin (PVL), a pore-forming toxin comprising of protein subunits which bind to neutrophils, monocytes, and macrophages, but not to lymphocytes resulting in in leukocyte cell death and the release of inflammatory cytokines.[99] Since there are several *Staphylococcus aureus* strains, Aminoglycosides, cephalosporins and synthetic Penicillins are used for treatment due to a broad spectrum of activity and they account for methicillin resistant and sensitive strains.[100]

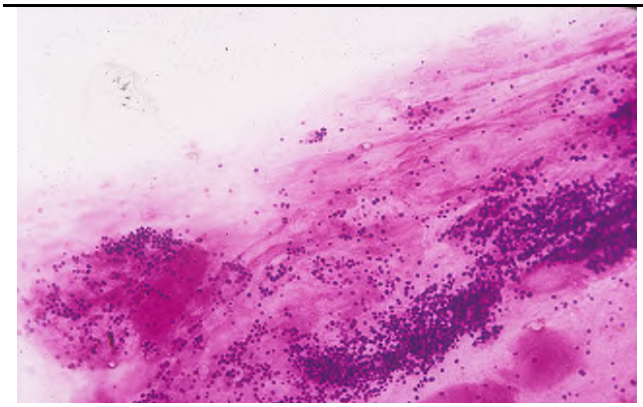
Streptococcus pneumoniae

This gram-positive alpha haemolytic facultative anaerobe causes several illnesses such as Pneumonia, Meningitis, Bacteremia, Otitis media, Sinusitis and Keratitis.[101] Surgery and trauma are predisposing factors of *Streptococcus pneumoniae* infection in Microbial Keratitis.[101]

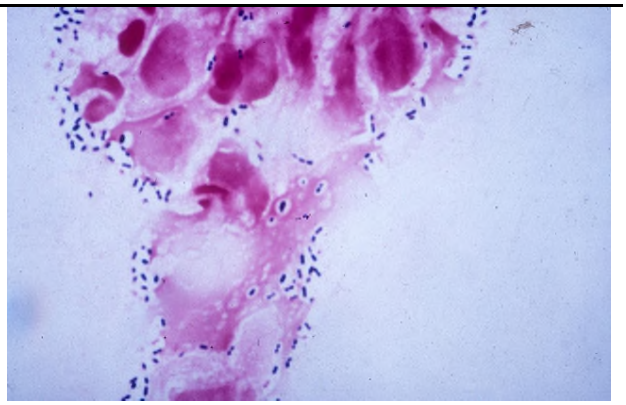
Streptococcus pneumoniae has several virulence factors including a polysaccharide capsule which reduces IgG and C reactive protein binding in turn aiding in the evasion of host complement system; pneumolysin which is a family of cytolysins and includes perfringolysin, streptolysin and listeriolysin that have a damaging effect; neuraminidases which enable this pathogen to cause disease; and,

three zinc metalloproteinases, IgA1 protease, ZmpB, and ZmpC for bacterial adherence to epithelial cells[102]

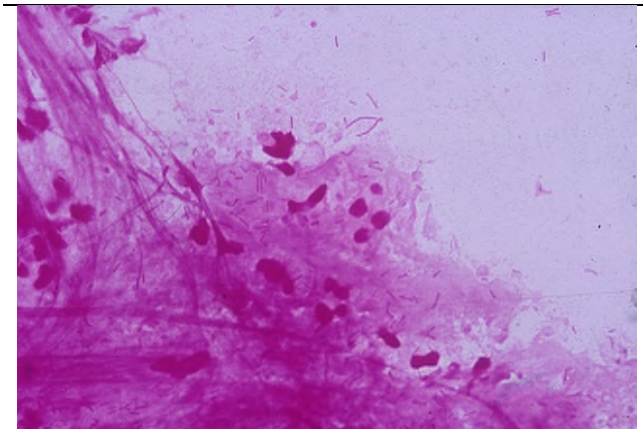
Figure 3: Morphology of the different causative organisms of MK



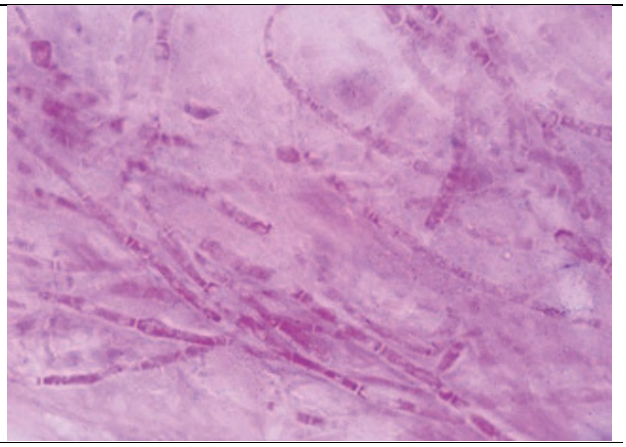
Corneal scrape stain showing Gram positive cocci. Photo courtesy of Dr Astrid Leck



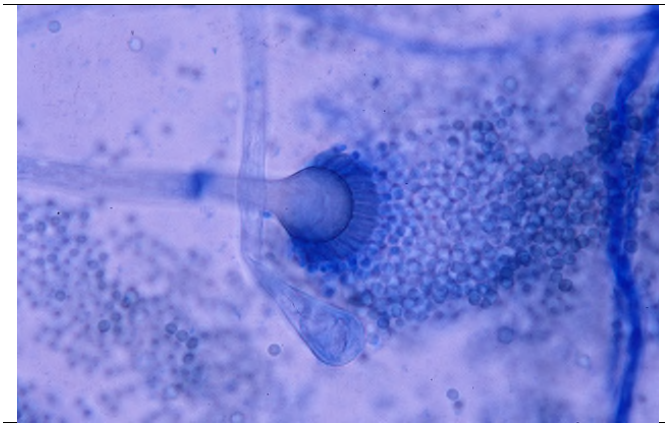
Corneal scrape stain showing Pneumococci. Photo courtesy of Dr Astrid Leck



Corneal scrape stain showing Gram negative bacilli. Photo courtesy of Dr Astrid Leck



Fungal Hyphae visible after gram stain of a corneal scrape. Photo courtesy of Dr Astrid Leck



Aspergillus spp stained on Lacto Phenol Cotton Blue stain. Photo courtesy of Dr Astrid Leck

Conidiophores and Conidia of *Fusarium spp.* Photo courtesy of Dr Astrid Leck

Streptococcus pneumoniae elicits a wide array of antimicrobial peptide expression in corneal epithelial cells. The corneal epithelium mediates innate immune responses by secreting cytokines,

chemokines, and antimicrobial peptides.[103] Treatment *Streptococcus pneumoniae* infection is with Aminoglycosides, cephalosporins and synthetic Penicillins.[100]

Pseudomonas aeruginosa

This is an encapsulated, gram-negative, rod-shaped bacterium that is commonly found in environment.[104] *Pseudomonas aeruginosa* keratitis is most commonly associated with contact lens use and can also occur following ocular trauma.[104]

Keratitis caused by *Pseudomonas aeruginosa* is associated with worse visual outcomes than that caused by other bacterial pathogens.[105] This is attributed to its virulence factors which are involved in acute infection: flagella, adhesins, toxin secretion, proteases, pili, lipopolysaccharides, proteases, Exotoxin A.[105] Type III secretion system (T3SS) is another virulence factor that transports toxins to host cell and involves four effector proteins namely ExoU, ExoS, ExoT, and ExoY.[106] These effector proteins determine type of T3SS exotoxin secreted, which categorizes *Pseudomonas aeruginosa* strains as either invasive or cytotoxic genotypes.[105,106] Invasive strains possess exoS gene and these are capable of invasion of corneal epithelium, cytotoxic strains with the exoU gene cause rapid necrotic death of host cells within 1 to 2 hours.[106] Cytotoxic strains are associated with contact lens wear while invasive strains are related to poor prognosis and increased resistance to fluoroquinolones.[107] These virulence factors interfere the innate response by influencing the cytokine profile and reducing neutrophil recruitment or activity hence promoting bacterial survival in the cornea.[108]

Treatment of Pseudomonas keratitis is either monotherapy with fluoroquinolones or fortified Aminoglycosides, cephalosporins and synthetic Penicillins.[100]

Aspergillus spp

The most commonly isolated species in Aspergillus Keratitis includes *Aspergillus fumigatus* and *Aspergillus flavus.*[109,110] It is common among healthy young males engaged in agricultural or other outdoor work.[109] These filamentary fungi do not penetrate the intact epithelium; infection occurs following traumatic inoculation of *Aspergillus* conidia into the cornea either through injury or corneal surgical procedures.[45,111]

Aspergillus conidia live ubiquitously in the air but do not cause inflammation and disease on inhalation except in immune suppressed individuals.[112] The pathogenesis of *Aspergillus* keratitis arises out of an interplay of various factors which enhance the virulence of the organism. In many models, the most studied species is *Aspergillus flavus*.

Firstly, Conidia are coated by a hydrophobic rodlet layer composed of regularly arranged RodA hydrophobins (RodA), which are covalently bound to cell wall polysaccharides by GPI anchor

proteins which helps them to evade the immune surveillance system.[113] In the absence of RodA, beta 1,3-glucan and alpha-mannan are exposed on the cell wall of Aspergillus which activates the C-type lectins Dectin-1 and Dectin-2 to mediate the host response.[114] Ability to evade early recognition by Dectin-1 and Dectin-2, enables conidia to germinate and form hyphae prior to immune recognition, which thereby enhances fungal survival during infection.[114]

Secondly, *Aspergillus fumigatus* conidia attenuates host proinflammatory responses through modulation of Toll-like receptor (TLR)2 and TLR4 signalling.[115] In a model to study modulation effects of *Aspergillus* cell wall polysaccharide constituents responsible for the modulation of host capability to mount a proinflammatory response, Beta-glucan specifically suppressed TLR4-induced response, while alpha-glucan inhibited IL-6 induced through TLR2- and TLR4-stimulation. Galactomannan diminished TLR4-mediated response, while its inhibitory effects on TLR2-signalling were limited. Chitin, on the other hand, did not have significant immunomodulatory capability.[115]

Thirdly, *Aspergillus fumigatus* can form a biofilm both in the natural and artificial environments.[116] Biofilms are a highly structured consortia of microorganisms that adhere to a substrate and are encased within an extracellular matrix (ECM) that is produced by the organisms themselves.[116] Biofilm formation around the fungal hyphal colony in the cornea can reduce the ability of the host inflammatory cells to physically reach the fungi.[116]

Fourthly, *Aspergils flavus* has been shown to produce aflatoxin B1 which occurs more in keratitis causing strains of Aspergillus than environmental strains.[117] In addition, keratitis causing *Aspergillus spp* strains produce proteases (ALP1) which have a role in corneal tissue destruction and can impair immune response.[118]

Fusarium spp.

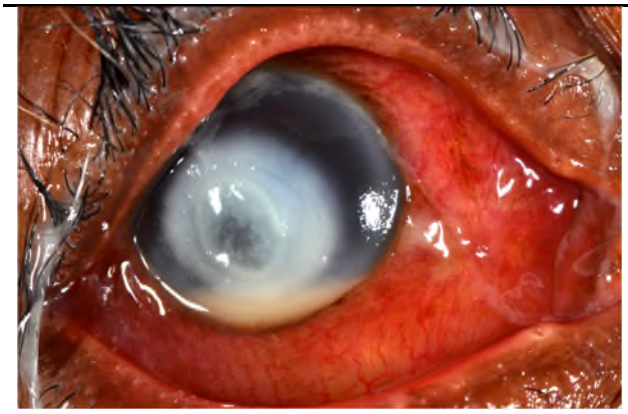
Fusarium species are common plant pathogens, particularly of cereal crops or saprophytes of plant debris and are found in soil.[31] The most implicated keratitis causing species in this genus are *Fusarium solani* and *Fusarium oxysporum*.[56] Risk factors for keratitis are usually trauma often involving plant material where the conidia are inoculated into the breeched cornea.[119]

The virulence of *Fusarium spp* is dependent on several factors. The PacC gene in *Fusarium oxysporum* was demonstrated in a model to facilitate quick invasion and penetration of through corneal anterior stroma.[120] This gene has a role in allowing the fungus to adhere to and grow successfully within corneal tissue.[120] In addition, *Fusarium sp*p have been shown to express extracellular proteases that are capable of degrading collagens in the host corneal tissue, thus enabling invasion into deeper stroma.[118] Like in *Aspergillus spp*, *Fusarium spp* form biofilms around the fungal mass in the cornea, especially in *Fusarium solani* keratitis which protects the fungi from

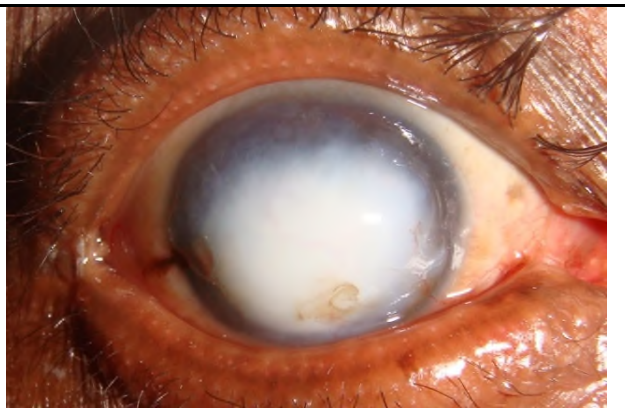
the host immune response and antifungal treatments.[121] Finally, Fusarium sp. are also able to generate myotoxins that could theoretically damage host cells.[122]

Making the diagnosis

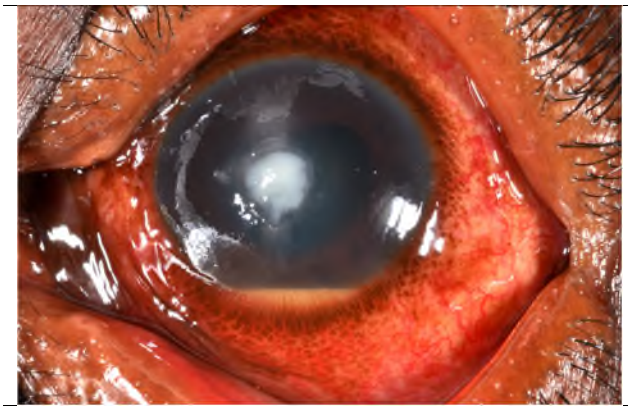
Figure 4: Some of the clinical features of MK



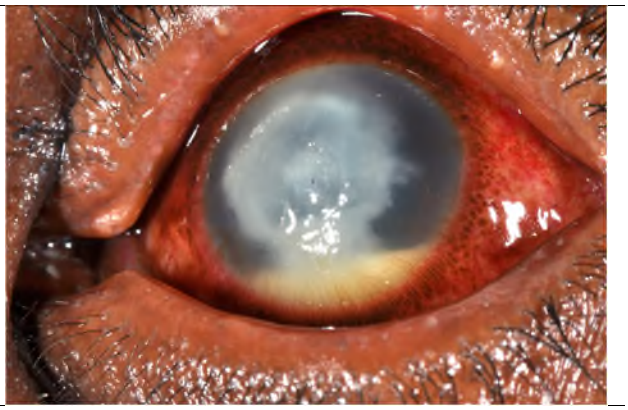
(A) Active microbial keratitis with signs of acute inflammation and corneal ulceration.



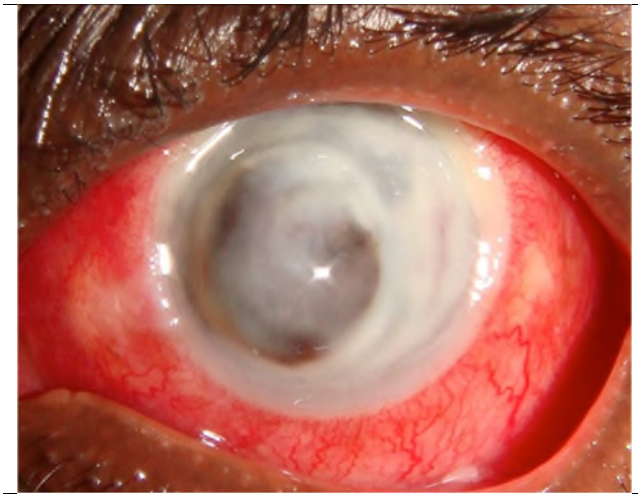
(B) Corneal Scar, the sequel of a resolved episode of microbial keratitis, no current signs of acute inflammation



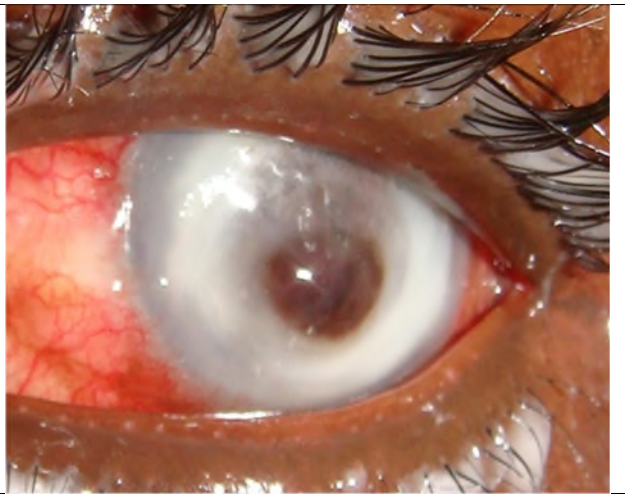
(C) Early filamentary fungal keratitis; admitted and started immediately on intensive topical natamycin treatment.



(D) The same case as (C) one week later, unresponsive to intense anti-fungal treatment, with progression of the infection



(E) Bacterial Keratitis, rapid severe damage



(F) Unknown cause, perforation with iris plugging

History

The history might contain certain clues that point to the most likely agent. In most of the forms of MK ocular symptoms include a degree of pain, redness, discharge, blurred vision and photophobia.[44] In some forms like Acanthamoeba, the pain is usually more marked than the clinical picture.[123]

The duration of symptoms are usually shorter in bacteria compared to other forms of MK. A study from India reviewed 345 patients with laboratory proven diagnoses (115 bacterial, 115 fungal, 115 Acanthomoeba).[124] In this report, differentiating features were more common for acanthamoeba keratitis than for bacterial or fungal keratitis. Compared to patients with bacterial or fungal keratitis, patients with acanthamoeba keratitis were more likely to be younger and to have a longer duration of symptoms.[124]

A history certain risk factors may be associated with as infection such as trauma, use of traditional eye medicine, immune suppression, swimming, contact lens wearing as Herpes Simplex Virus (HSV) keratitis, Varicella Zoster Virus (VZV) keratitis, previous bacterial keratitis, trauma, dry eye, and previous ocular surgery. These are discussed in the subsequent sections of this book.

A recent prospective study of 252 participants with MK from India reported data on factors on patient history associated with bacterial, fungal and Acanthomoeba keratitis at presentation.[43] A history of trauma was present in all forms of MK, although trauma with vegetative matter was more common in the bacterial keratitis (77%) group and least common in Acanthomoeba keratitis group (23%), p=0.033. In the same study, prior steroid use was more common in the Acanthomoeba group. There were 17 patients with DM of which 15 had fungal keratitis.[43]

Clinical diagnosis

The clinical features are usually dependant on the infectious agent and the presentation period.[125] However, there have not been many well designed studies to objectively analyse the correlation between clinical features to microbiological diagnosis. One large prospective study in India looked at whether the presence of characteristic clinical features can be used as a diagnostic aid for suppurative keratitis caused by filamentous fungi.[125] In this study, 360 patients presenting with suppurative keratitis in India underwent detailed clinical examination followed by microbiological investigation of corneal scrapes. A partial diagnostic score based upon the strength of the association, as estimated by the odds ratio, between reported clinical features and laboratory confirmed diagnoses was devised and subsequently tested using a case series from Ghana.[31]

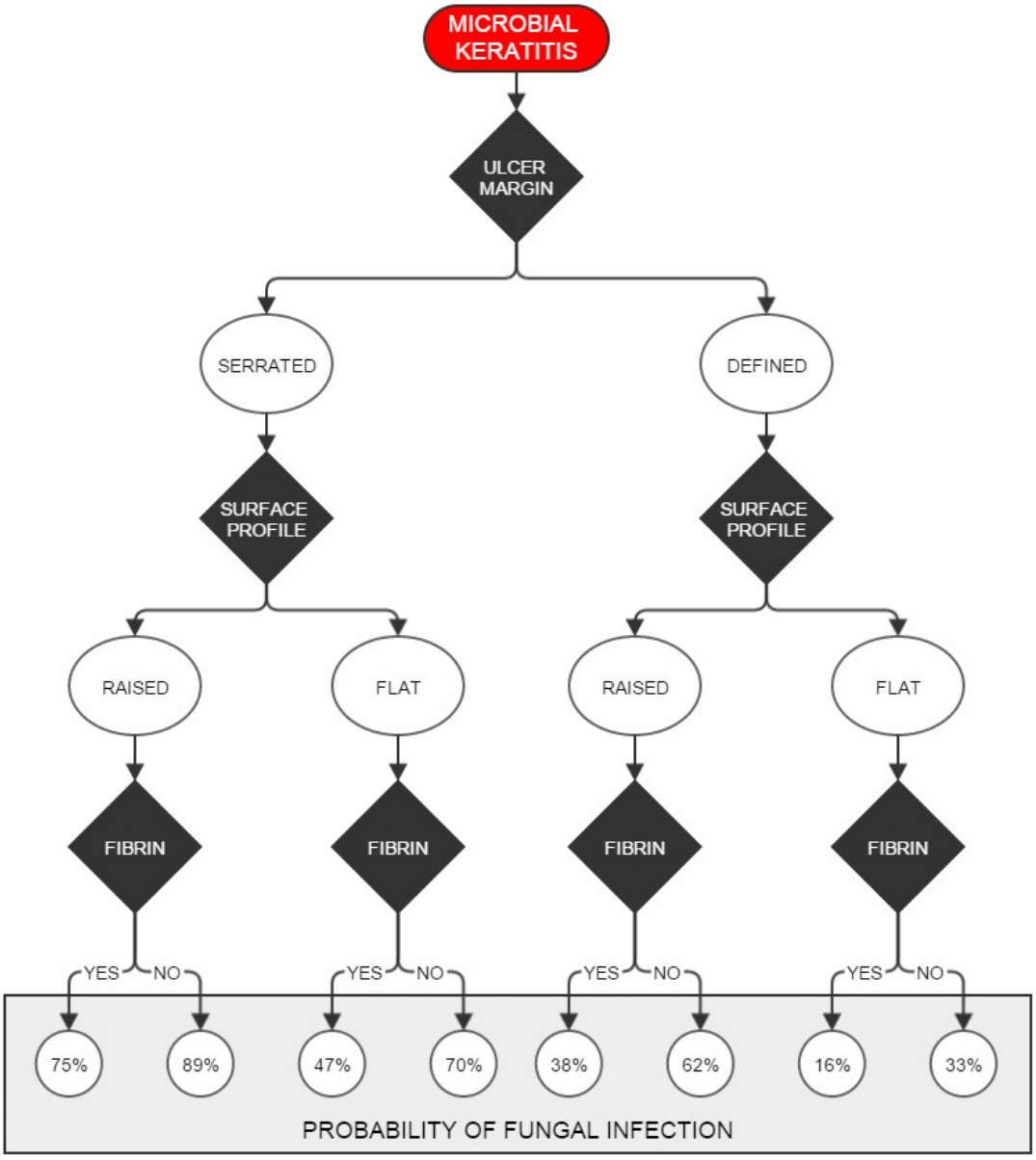
Tables 5 shows the main findings from this study that were associated with a confirmed laboratory diagnosis. On univariable logistic regression analysis, serrated margins, raised slough, dry texture, satellite lesions and coloration other than yellow occurred more frequently in cases of filamentous

fungal keratitis than bacterial keratitis ($p<0.05$). Hypopyon and fibrinous exudate were observed more frequently in bacterial keratitis ($p<0.05$).[125] When incorporated into a backwards stepwise logistic regression model, only serrated margins, raised slough, and colour were independently associated with fungal keratitis; these features were used in the scoring system. The probability of fungal infection if one clinical feature was present was 63%, increasing to 83% if all three features were present.[125] Another study by the Proctor and Aravind group randomised 80 corneal photographs of eyes with culture-proven bacterial keratitis (40 photographs) or smear-proven fungal keratitis (40 photographs) were randomly selected from 2 clinical trials (Steroids for Corneal Ulcer Treatment trial (SCUT) trial and Mycotic Ulcer Treatment Trial (MUTT)).[126] Fifteen cornea specialists from the Proctor Foundation and the Aravind Eye Care System assessed the photographs for prespecified clinical signs of keratitis, and they identified the most likely causative organism. The study found that using pre specified clinical signs of keratitis, clinicians were able to correctly distinguish bacterial from fungal aetiology 66% of the time ($p < 0.001$). The presence of an irregular/feathery border was associated with fungal keratitis, whereas a wreath infiltrate or an epithelial plaque was associated with bacterial keratitis.[126]

In a more recent prospective observational study of 252 adults presenting with severe microbial keratitis (MK), ulcer clinical features were recorded at presentation and compared to aetiologic agent in a regression model.[43] Fungal keratitis cases were 191 (75.7%), 18 had Acanthamoeba keratitis (7.1%), 19 had Bacterial keratitis (7.5%), 4 (1.6%) had mixed bacterial and fungal while 20 were microbiologically negative.[43] Logistic regression analysis of fungal ulcers versus all others showed that feathery margins were strongly associated with fungal ulcers, ring infiltrate associated with Acanthamoeba while Bacterial ulcers were more likely to have a hypopyon.[43]

Using clinical data correlated with microbiological diagnosis, a diagnostic algorithm was suggested by colleagues to aid in making a clinical diagnosis (Figure 6).[30] An example in this algorithm is that the probability of an ulcer being fungal keratitis is 89% if the ulcer margin is serrated, the surface profile raised and no fibrin. Conversely, if the ulcer margin is well defined, the surface profile flat and fibrin present, the probability of fungal keratitis in such an ulcer is only 16%.

Figure 5: Algorithm for clinical differentiating between fungal and bacterial keratitis in a tropical environment.[30]



Microbiology

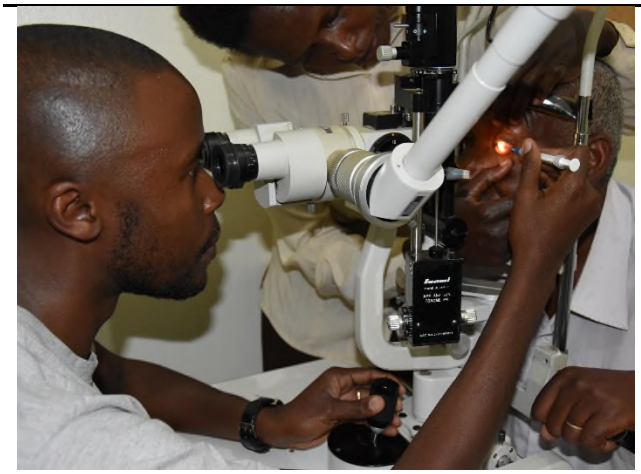
A major challenge in the management of MK is the identification of the causative organism. In many resource-limited settings, little is known about the possible infectious causes of MK. This is because routine microbiology investigations are not readily available outside the larger centres. It is very important to understand the "local" aetiology of MK to develop a rational, empirical protocol if there is limited access to microscopy and culture in a given region.[31]

Current techniques used to isolate the pathogen are direct staining of corneal smears or inoculation of media for microbiological culture. Gram stain, 10% potassium hydroxide (KOH), Calcofluor white and Giemsa stains are all used to visualise corneal pathogens as part of standard practice in many centres throughout the world. The techniques on how to take a corneal scrapping have been previously reported (Figure 7).[127] The recommendations from this guide mention that in a resource limited setting, the minimum resources needed to do a corneal scrape include 21-gauge needles or Kimura scalpel, 2 clean microscope slides, 1 blood agar plate, 1 Sabouraud/Potato Dextrose Agar plate, 1 brain heart infusion broth.[127]

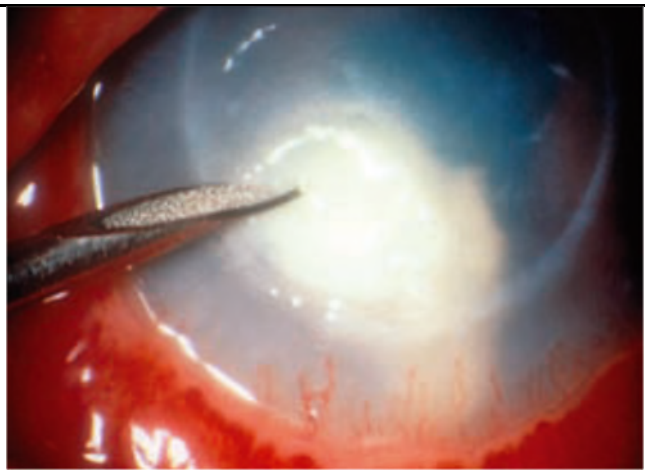
KOH staining can identify fungus in corneal ulcers with a sensitivity of 90-94% of those which are culture positive and has been considered in some studies to be a confirmed fungal diagnosis even in the absence of a positive culture result.[31,128,129] Overall, usually about half of the corneal scrapping samples will show a positive yield on microscopy (Tables 2 and 3).[12,31]

Although microbiological culture remains the gold standard in diagnosis of the causative organism in microbial keratitis, it is positive in only 50-65% of cases.[31,130,131] There are many reasons for this low culture positivity rate, which include only partially viable organisms in the corneal scrape due to previous antimicrobial therapy, and the small sample size of corneal scrape specimens.[132] Yield can be improved by withdrawing the use of antimicrobial agents for 24 hours prior to sampling (if possible), using liquid phase media which serves as a diluent that reduces the concentration of the drug below the minimum inhibitory concentration (MIC), using preservative free anaesthetic drops and deep tissue scrapping when fungal or amoebic infection is suspected.[127] In addition, after collecting the sample, it should be gently smeared on the surface of agar in C-streaks (taking care not to puncture the surface of the agar), the lid of the plate should be sellotaped and incubation should be immediately after.[127] The agar plates should have been taken out of the fridge and allowed to warm to room temperature not to shock the inoculated microorganisms.[127]

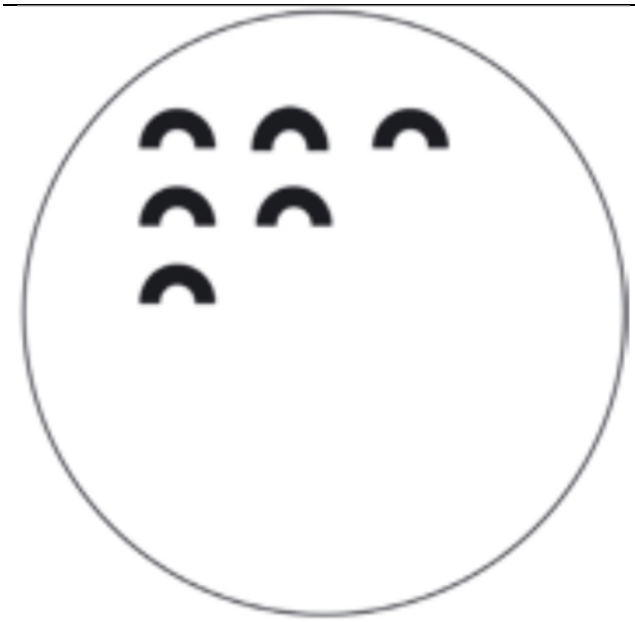
Figure 6: Corneal scraping and media inoculation



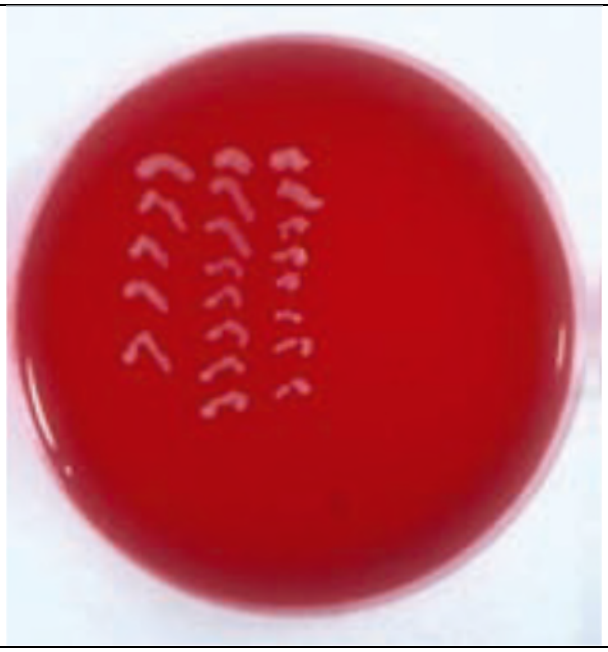
Corneal scrapping on a slit lamp while the assistant retracts the eyelid



Zoomed in image of taking a corneal scrape (Image courtesy of Prof John Dart)



C streaking on the surface of the slide (Image courtesy of Dr Astrid Leck)



Bacterial growth in a C streak pattern (Image courtesy of Dr Astrid Leck)

Polymerase chain reaction (PCR) is useful in such settings as it can detect the presence of nucleic acid from small samples and even non-viable organisms. It involves cyclical amplification of small amounts of nucleic acid and synthesis of new complementary strands until adequate amounts of nucleic acid material (billion copies) have reached detectable levels.[133] This usually takes about 30 cycles in most standard PCR machines.[133] One challenge with this method is that it is difficult to rule out contamination especially where organisms such as fungi are ubiquitously living in the environment. In such a scenario, quantitative PCR (real time PCR) allows for estimation of the amount of DNA in the initial sample.[134] In this technique, the amount of product formed is monitored

during the course of the reaction by monitoring the fluorescence of dyes or probes introduced into the reaction that is proportional to the amount of product formed, and the number of amplification cycles required to obtain a particular amount of DNA molecules is registered. Assuming a certain amplification efficiency, which typically is close to a doubling of the number of molecules per amplification cycle, it is possible to calculate the number of DNA molecules of the amplified sequence that were initially present in the sample.[134]

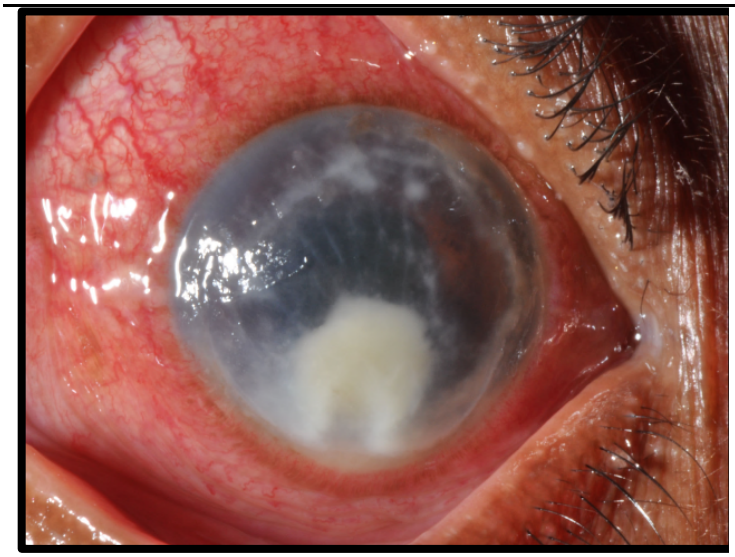
Previous studies employing this technique for microbial keratitis have found PCR to be effective in detecting organisms and often display higher overall pick up rates than culture or microscopy.[135] In this study, patients with eye findings suspected of fungal keratitis were enrolled for cornea sampling. Scrapings from the affected areas of the infected corneas were divided into two parts one for microbiology and the other for PCR.[135] Potassium hydroxide, Gram staining, culture and nested PCR results (either positive or negative) matched in 76.3, 42.1, 68.4 and 81.6%, respectively.[135] In another large prospective study from India, 108 consecutive corneal ulcers were cultured and analyzed by PCR using pan-bacterial and pan-fungal primers and compared to culture results.[130] Of the 108 samples, 56 were culture-positive, 25 for bacteria and 31 for fungi; 52 were culture-negative. After eliminating false-positive PCR products, 94 of 108 were positive by PCR, 37 for bacteria and 57 for fungi. Nineteen of 25 bacterial culture-positive samples were positive by PCR, and 29 of 31 samples culture-positive for fungi were positive by PCR equivalent to a sensitivity of 76% and 93.5%, respectively.[130]

There is an even newer technique called Matrix-Assisted Laser Desorption/Ionization Time Of Flight (MALDI-TOF) mass spectrometry.[136] According to the patent description, this method involves, providing a liquid biological sample containing any proteins and/or lipids and/or salts and/or polysaccharides and/or oligosaccharides and/or monosaccharides capable of forming complexes with said proteins and/or lipids and/or salts from an infected mammal. The sample is then treated with a biological liquid to extract said polysaccharides and/or oligosaccharides and/or monosaccharides. After extraction, the presence of these markers is then determined by MALDI-TOF mass spectrometry which confirms presence of a fungal infection.[136] This method is thought to be easier and cheaper to run than PCR.[137]

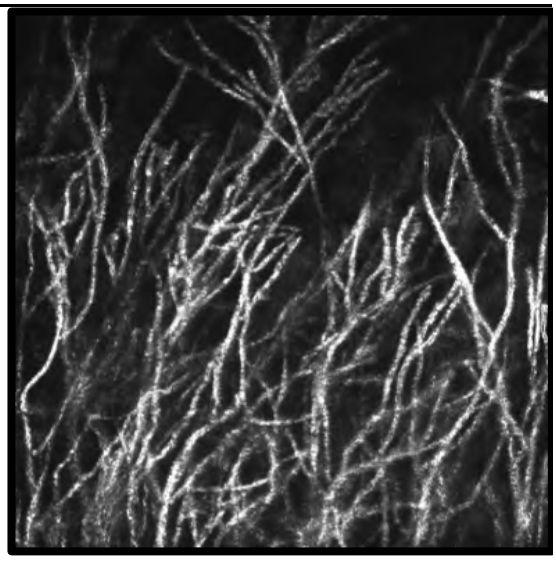
Although the use of this technique has not been widely reported in clinical studies, one report that compared MALDI-TOF with conventional morphology and PCR on 24 consecutive clinical isolates of Aspergillus collected during 2012-2014 in Turkey.[138] In this study, there was good agreement between the conventional morphology and PCR and MALDI-TOF methods.[138] Two other reports described use of MALDI-TOF to detect uncommon bacterial strains for microbial keratitis.[137,139] One was a case report of a 94-year woman with suppurative keratitis due to *Corynebacterium propinquum* and another was a case series of ocular infections caused by *Moraxella spp* in Japan.

In Vivo Confocal Microscopy (IVCM)

Figure 7: Images of IVCM



Corneal picture of a patient with filamentary keratitis, satellite lesions and a ring infiltrate. (photo Courtesy of Prof Matthew Burton)



IVCM scan of the same cornea showing extensive, branching fungal hyphae. Scale bar 100µm. (photo Courtesy of Prof Matthew Burton)

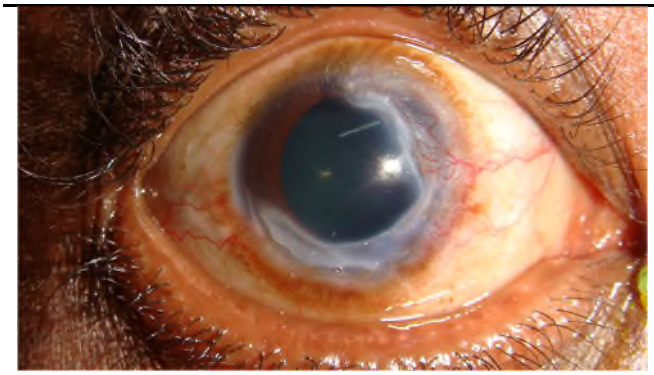
In vivo confocal microscopy (IVCM) is a non-invasive imaging technique that allows direct visualization of pathogens within the patient's cornea.[140] The 2 imaging modalities in current clinical use are the scanning slit IVCM (ConfoScan, Nidek Technologies, Fremont, CA) and the laser scanning IVCM (HRT3 with Rostock Corneal Module [RCM], Heidelberg Engineering, Heidelberg, Germany). The ConfoScan has a resolution of 1 micron laterally and up to 24 microns axially; the HRT3/RCM also has a lateral resolution of 1 micron but higher axial resolution of 7.6 microns.[141]

One recent large double masked prospective study from India tested the diagnostic accuracy of IVCM for moderate to severe microbial keratitis (MK).[141] In this study, The study enrolled 239 patients presenting to Aravind Eye Hospital, Madurai, India were consecutively enrolled. Following examination, the corneal ulcer was scanned by IVCM (HRT3/RCM, Heidelberg Engineering, Heidelberg, Germany). Images were graded for the presence or absence of fungal hyphae or Acanthamoeba cysts by the confocal microscopist who performed the scan (masked to microbial diagnosis) and 4 other experienced confocal graders (masked to clinical features and microbiology).[141] The regrading of the shuffled image set was performed by 3 graders, 3 weeks later. Corneal-scrape samples were collected for microscopy and culture. The main outcome measures

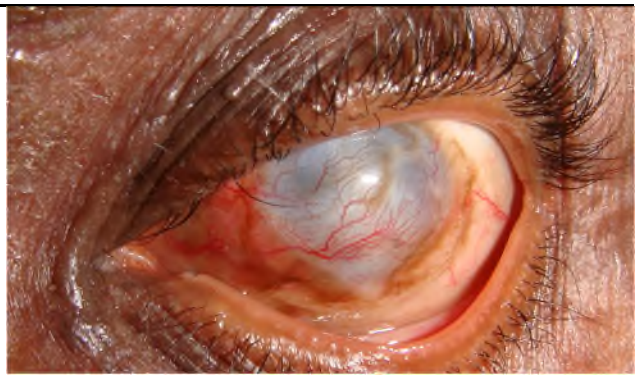
were sensitivity, specificity, and positive and negative predictive values of IVCM compared with those of a reference standard of positive culture or light microscopy. Fungal infection was detected in 176/239 (74%) and Acanthamoeba in 17/239 (7%) by microbiological methods. IVCM had an overall pooled (5 graders) sensitivity of 85.7% (95% confidence interval [CI]: 82.2%-88.6%) and pooled specificity of 81.4% (95% CI: 76.0%-85.9%) for fungal filament detection. For Acanthamoeba, the pooled sensitivity was 88.2% (95% CI: 76.2%-94.6%) and pooled specificity was 98.2% (95% CI: 94.9%-99.3%).[141] The authors of this study concluded that Laser scanning IVCM performed with experienced confocal graders has high sensitivity, specificity, and test reproducibility for detecting fungal filaments and Acanthamoeba cysts in moderate to large corneal ulcers in India. This imaging modality was particularly useful for detecting organisms in deep ulcers in which culture and light microscopy results were negative.[141]

Differential Diagnosis

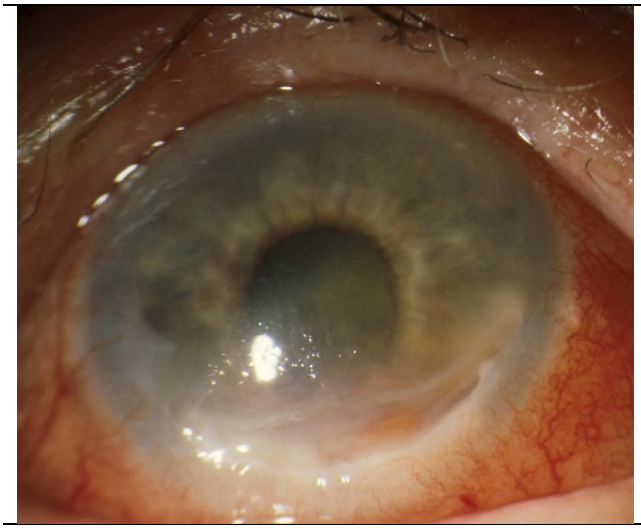
Figure 8: Differential diagnosis of microbial keratitis



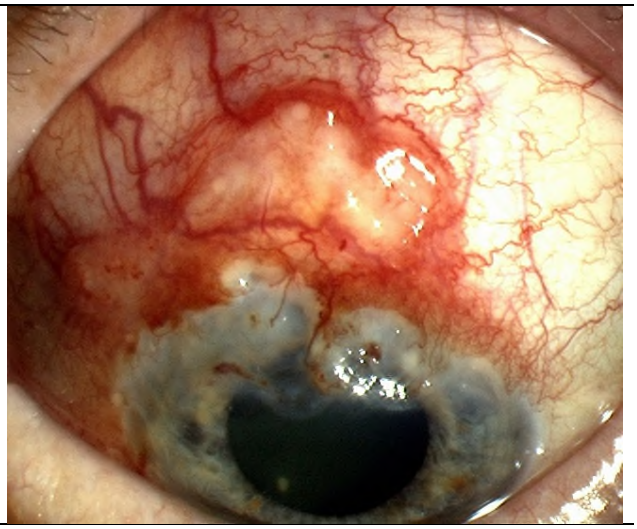
A case of Mooren's Ulcer with rapidly encircling limbal thinning. (photo Courtesy of Prof Matthew Burton)



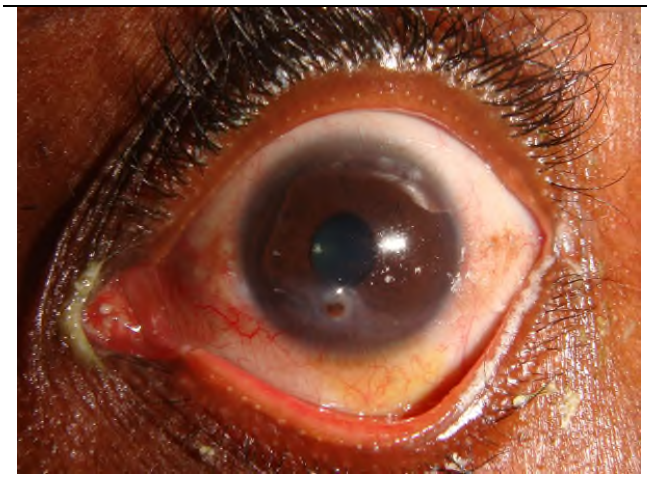
The same case of Mooren's after resolving of the inflammation with total corneal opacification. (photo Courtesy of Prof Matthew Burton)



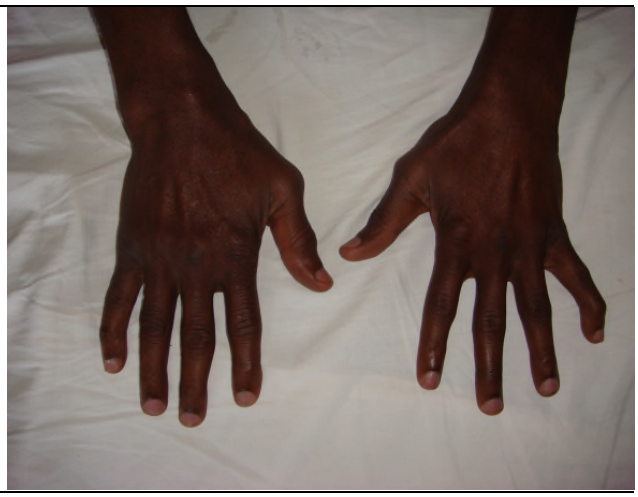
Rheumatoid Arthritis (photo Courtesy of Prof John Dart)



Wegner's Granulomatosis (photo Courtesy of Prof John Dart)



A patient with PUK and a sealed perforation. (photo Courtesy of Prof Matthew Burton)



The hands of the same patients showing peripheral joint distortions for Rheumatoid Arthritis. (photo Courtesy of Prof Matthew Burton)

The differential diagnosis for microbial keratitis includes Peripheral Ulcerative Keratitis (PUK), Mooren's and non-infectious degenerative lesions (Figure 9).

PUK is a corneal inflammatory disease syndrome that has multiple associations. It usually presents as juxta-limbal peripheral thinning/ulcer, there can be stromal infiltration and an epithelial defect ± perforations. In SSA, the most common differential diagnosis is Mooren's ulcer.

Mooren's ulcer is described as a chronic and painful ulceration of the cornea that often starts in the periphery and may gradually progress centrally or circumferentially to eventually involve the entire cornea.[142] In one audit from Nigeria, the demographic and clinical characteristics of patients with Mooren's ulcer seen over a 10 year period were described.[143] Thirty-one eyes of 23 patients were enrolled. The mean age of participants was 31±16.6 years, males were 18/23 (78%). Eight (34.8%) patients had bilateral lesions. Most common presenting symptoms were redness and pain, with an average of 6.8 clock hour-limbal involvement. In this group, the presenting visual acuity was >6/18 in 29%, 6/18 to 3/60 in 58% and <3/60 in 13%.[143] Treatment options included topical and systemic steroids, systemic methotrexate or cyclophosphamide (8 patients who did not respond to steroids) and surgical treatment with conjunctival resection and cryotherapy in 14 (60.9%) patients (21 eyes).[143] Patients presenting with perforation also had scleral patch graft or direct repair. The ulcer healed with varying degrees of corneal opacity in 18 (85.7%) of the 21 eyes, while the remaining three eyes developed descemetocele, anterior staphyloma or corneal perforation.[143]

Our group recently described a series of Mooren's ulcer cases from Uganda.[144] In this 3 year audit, 52 patients (80% male) presented with Mooren's ulcer to our hospital, majority (90.4%) had unilateral disease. The median age was 24.5 IQR 16.5 years. At the last follow-up, 40.4% of the patients had a worse visual acuity, 20 patients (38.5%) had no change in visual acuity while 21.2% had an improvement in vision.[144]

In comparison to India, there have been two large case series from North and South India.[145] One large prospective study from North India, 76 eyes of 65 consecutive patients with PUK were evaluated over an 18 month period and followed for 3 years.[145] The mean age of the study participants was 45.5±17.9 years and 66% were male. Unilateral disease was present in 83% of patients (54/65). The most common aetiology was Mooren's ulcer (31.5% cases (24/76 eyes)) followed by infection (20%) and systemic collagen vascular disease (20%).

The other study from South India described the clinical characteristics at presentation of 166 patients (242 eyes) that presented to the cornea clinic at Aravind Eye Hospital Madurai, Tamil Nadu, India, over a 10-year period.[146] The median and mean ages at presentation were 65 and 61 years, respectively, with a range of 13–95 years. The proportion of males was 82% while bilateral ulcers

were present in 46% of the cases.[146] One eye was affected in 90 of 166 (54%) patients. Visual acuity in the affected eye at presentation was 6/12 or better in 34 of 242 (14%) eyes, between 6/12 and 3/60 in 168 (69%) eyes, and worse than 3/60 in 40 (17%) eyes.[146] Identified risk factors in the cohort included evidence of prior corneal surgery (22%), corneal trauma (17%) and corneal infection (2%).[146]

In comparison with the far east, one large study from China reported on clinical characteristics and treatment of 550 consecutive cases of Mooren's ulcer (715 eyes) over a period of 36 years. [147] The average age of onset was 48.4 years of age. The proportion of males was 56% and 165 (30%) cases had bilateral disease.[147]

PUK can be associated with several causes such as presenting sign of a systemic disease Collagen Vascular Disease. Studies from the UK reported on PUK with other differential diagnoses. One 3 year prospective study reported on 21 cases enrolled from 1995-1997.[148] The proportion of males in this study was 50%, mean age was 65-years and the most common systemic associations were Rheumatoid Arthritis (71%), Wegner's Granulomatosis (24%) and Microscopic polyarteritis (2%).[148] Another 10-year audit reviewed case notes of all the patients attending a specialist corneal immunosuppression clinic between June 2002 and July 2012. Of the 70 cases, 57% were female and the mean age was 65-years.[149] The most common associations were Rheumatoid Arthritis (66%), Wegner's Granulomatosis (3%) and Psoriasis (6%).[149]

In Rheumatoid Arthritis, PUK is a rare but very serious inflammatory condition: a warning sign of impending vasculitis which can led to mortality.[150] Treatment is with Immune suppressive therapy.[150] Wegner's granulomatosis, the ocular manifestations range from mild conjunctivitis and episcleritis to more severe inflammation with keratitis, scleritis, uveitis, and retinal vasculitis.[151] Ocular involvement will not respond to topical agents, but rather to systemic anti-inflammatory and immunosuppressive regimens.[151] Other differentials include ocular rosacea.[152] This is part of the general syndrome that includes facial and ocular manifestations. There is facial flushing, the appearance of telangiectatic vessels and persistent redness of the face, eruption of inflammatory papules and pustules on the central face, and hypertrophy of the sebaceous glands of the nose. Ocular changes are present in more than 50 percent of patients and range from mild dryness and irritation with blepharitis and conjunctivitis (common symptoms) to sight-threatening keratitis (rare).[153] Treatment is with artificial tears, eyelid hygiene, fucidic acid, and metronidazole gel applied to lid margins if there is associated blepharitis.[153] Keratitis can also be as a result of hypersensitivity to bacterial antigen most common being *Staphylococcus aureus* in cases of chronic blepharitis.[154] Treatment is with steroids and lid hygiene.[154]

Corneal degenerations with thinning can occur in other benign conditions such as Dellen, Terriens marginal degeneration and pellucid marginal degeneration.

Dellen are small, saucer-like excavations at the margin of the cornea.[155] They occur most often following processes which produce a paralimbal elevation such as a conjunctival growth and a post trabeculectomy bleb.[156] This elevation induces a localized break in the precorneal oily film layer of the tears which, in turn, causes a localized dehydration and thinning of the cornea. Treatment is directed towards rehydration of the cornea and reduction of the limbal elevation.[155]

Terrien's disease occurs in middle-aged patients and is characterised by an insidious thinning of the cornea near the limbus.[157] In most cases, this results in a peripheral ectasia associated with a severe degree of astigmatism.[157] Although thought to be non-inflammatory in nature, there have been reports to show that patients can get recurrent, disabling, episodic inflammation with severe pain and an associated episcleritis or superficial.[158] Management is symptomatic during episodes of inflammation and penetrating keratoplasty for the corneal ectasia.[157]

Pellucid Marginal Corneal Degeneration (PMCD) of the cornea is a bilateral, clear, inferior, peripheral corneal-thinning disorder.[159] Protrusion of the cornea occurs above a band of thinning, which is located 1 to 2 mm from the limbus and measures 1 to 2 mm in width.[159] In one large series from India, clinical presentation of 116 eyes of 58 patients with PMCD seen between 1990 and 2002 was described.[160] The diagnosis of PMCD was based on the presence of corneal thinning with ectasia of the normal cornea above or below the area of thinning with no evidence of scarring, vascularization, or lipid deposition and typical topographic features whenever topography was performed.[160] In this study, all cases were bilateral, 77.6% were male and the mean ager was 34.0±14.8 years.[160] In these series, PMCD was not strongly associated with Vernal Kerato Conjunctivitis (VKC).[160] However, the most common associations were Keratoconus (10% of the eyes) and Keratoglobus (13%). The most common site was inferior PMCD (85%). Severe astigmatism was present in 42.6% of the eyes and 6% had hydrops. Most patients were treated with spectacles or contact lens.[160] Surgery for PMCD lamellar keratoplasty and crescentic lamellar keratoplasty, if indicated usually results in significant residual astigmatism.

Table 5: Uni and multivariate analysis of clinical features occurring in fungal and bacterial Keratitis

Clinical feature	n	(% fungal)	n	(% bacterial)	OR	(CI)	p value	aOR	(CI)	P value
Serrated margins	180/228	79%	63/132	48%	4.09	(2.57-6.56)	<0.0001	3.45	(2.12-5.68)	<0.0001
Raised slough	135/228	59%	52/132	39%	2.23	(1.44-3.55)	<0.0001	2.32	(1.43-3.74)	<0.0001
Dry texture of slough	101/228	44%	37/132	28%	2.04	(1.29-3.26)	<0.0001			
Satellite lesions	51/222	23%	17/132	13%	1.95	(1.08-3.61)	0.04			
Hypopyon	105/219	48%	83/128	65%	0.5	(0.32-0.78)	<0.0001			
Fibrin	21/210	10%	28/125	22%	0.38	(0.20-0.70)	<0.0001	0.39	(0.20-0.77)	0.01
Colour (not yellow)	213/228	93%	106/132	80%	3.47	(1.77-6.98)	<0.0001	2.85	(1.34-6.06)	0.01

OR=univariable Odds Ratio, aOR=adjusted Odds Ration Sens=sensitivity, Spec=specificity, PPV=positive predictive value. Table reproduced with permission from a previous report by Leck et al.[30]

Management of Microbial Keratitis

Bacterial Keratitis

A recent review of treatment options for bacterial keratitis concluded that topical antibiotics remain the best treatment for bacterial keratitis.[161] In that review, all commonly prescribed topical antibiotics were found to be equally effective. A 2014 Cochrane review also found no comparative differences between fluoroquinolones, aminoglycosides and cephalosporines.[162] In this review, a total of 16 high quality RCTs were included in the analysis; the main trial results are summarized in Table 6. In this report, a metanalysis of topical fluoroquinolone compared with topical fortified aminoglycoside–cephalosporin with a pooled total of 672 individuals found no difference in chance of treatment success. However, the concentrations of some of the agents was variable. The evidence from these trials shows that fluoroquinolones can be given as monotherapy while aminoglycosides and cephalosporins should be as combination therapy.

Although there seems to be no difference in efficacy of the antibiotics, the treatment of bacterial keratitis should take into consideration the local sensitivity patterns. Some of the background work conducted by our group on the local sensitivity patterns on bacterial keratitis isolates found that there was good sensitivity to ciprofloxacin and ofloxacin, modest sensitivity to gentamycin but resistance to chloramphenicol and tetracycline (Table 7). In a study of antimicrobial susceptibility patterns of external ocular surface bacterial isolates among 131 per-operative routine cataract patients at Mulago National Hospital in Kampala, Uganda, eyelid margin and conjunctival swabs were collected and processed to identify bacterial isolates and their respective antimicrobial susceptibility patterns.[163] The most common organisms identified were Coagulase-negative *Staphylococci* (CoNS) (65.9%) and *Staphylococcus aureus* (21.0%). CoNS showed the highest resistance to tetracycline (58.2%) and erythromycin (38.5%) whereas in S. aureus the resistance to tetracycline and erythromycin were 55.2% and 31.0% respectively. Methicillin resistant CoNS (MRS) and Methicillin resistance *S. aureus* (MRSA) were 31.9% and 27.6% (8/29) respectively. There were low resistance rates for CoNS, *S. aureus* and other bacterial isolates to ciprofloxacin (11.1%-24.2%), gentamicin (5.6-31.0%), tobramycin (17.2% -25.3%) and vancomycin 0.0%).[163]

Recently, a review of antimicrobial resistance in East Africa was published.[164] Following an extensive literature search across PubMed database and African Journals Online archives for published reports on antimicrobial resistance, a total of 12 studies were included in this

analysis. A summary of the sensitivity data published in this report is presented in Tables 8 and 9. In this review, high levels of resistance to commonly used antibiotics were reported, including resistance to ampicillin and cotrimoxazole (50% – 100%), emerging resistance to gentamicin (20% – 47%) and relatively high levels of resistance to ceftriaxone (46% – 69%) among Gram-negative infections. Much of the resistance was reported to be in *Klebsiella* species and *Escherichia coli*. Among Gram-positive infections, extensive resistance was reported to ampicillin (100%), gentamicin and ceftriaxone (50% – 100%), with methicillin-resistant Staphylococcus aureus prevalence ranging from 2.6% – 4.0%. However, although it gives an indication of regional sensitivity patterns, its usefulness and applicability are limited for ocular infections. The method of sensitivity testing for the bacteria colonies reported was the Kirby-Bauer disc diffusion method.[165]This method tests a restricted concentration of the drug that can be used safely in the blood stream; however, the concentrations that can be achieved in the ocular structures with topical antibiotics are usually much higher. This means that an antibiotic reported as “resistant” might still be effective in treating ocular infections.

Despite a good choice of antibiotic cover, outcomes remain poor secondary to corneal melting, scarring and perforation. Adjuvant therapies aimed at reducing the immune response responsible for much of the morbidity associated with keratitis seem to have a role. The large, randomized controlled Steroids for Corneal Ulcers trial randomised 500 patients with culture positive bacterial keratitis to topical Moxifloxacin 0.5% and prednisolone sodium phosphate solution 1% (250 patients) in one arm and to topical moxifloxacin 0.5% and placebo (250 patients) in another arm. The primary outcome measure was best corrected visual acuity (BCVA) at 3 months. The study found that steroids provided no significant improvement overall in BCVA at 3-moths, scar size, time to re-epithelialization. However subgroup analyses by baseline BSCVA, ulcer location, and infiltrate depth showed a significant effect of corticosteroids.[166] In this trial, there was no evidence of potential harmful effects of corticosteroids in treating bacterial keratitis.

Table 6 Major Randomised Controlled Trials on topical antibiotic therapy against bacterial keratitis

Author	Year	Country	N	Arms	Drugs	Primary outcomes	Finding
Sampaio[167]	1994	Spain	30	2	• Ciprofloxacin 0.3% • Gentamycin 1.4% and Cefazolin 5%	Healing of the ulcer	No difference across the 2 arms
Obrien[168]	1995		248	2	• Ofloxacin 0.3% • Tobramycin 1.5% and Cafazolin 10%	Time to healing of the ulcer	No difference across the 2 arms
Hyndiuk[169]	1996	India	176	2	• Ciprofloxacin 0.3% (82) • Tobramycin 1.3% and Cefazolin 5% (94)	Healing of the ulcer	Overall healing 87% No difference across the 2 arms
Pavesio[170]	1996	UK	122	2	• Ofloxacin 0.3% (• Gentamycin 1.5% and cefuroxime 5%	Healing of the ulcer	No difference across the 2 arms
Panda[171]	1999	India	30	2	• Ofloxacin 0.3% (15) • Tobramycin 1.5% and Cafazolin 10% (15)	Healing of the ulcer	Overall healing 90% No difference across the 2 arms
Kosrirukvongs[172]	2000	Thailand	41	2	• Ciprofloxacin 0.3% (17) • Gentamycin 1.4% and Cafazolin 5% (24)	Healing of the ulcer	Overall healing 66% No difference across the 2 arms
Zhang[173]	2000	China	132	2	• Ofloxacin 0.3% • Levofloxacin 0.3%	Healing of the ulcer	No difference across the 2 arms
Prajna[174]	2001	India	217	2	• Ofloxacin 0.3% (112) • Ciprofloxacin 0.3% (105)	Healing of the ulcer	Overall healing 81% No difference across the 2 arms
Erjongmanee[175]	2004	Thailand	40	2	• Lomefloxacin 0.3% (20) • Gentamycin 1.5% and cefuroxime 5% (20)	Time to healing of the ulcer	No difference across the 2 arms
Booranapong[176]	2004	Thailand	41	2	• Lomefloxacin 0.3% (23) • Ciprofloxacin 0.3% (18)	Healing of the ulcer	No difference across the 2 arms
Parmar[177]	2006	India	104	2	• Gatifloxacin 0.3% (50) • Ciprofloxacin 0.3% (54)	Healing of the ulcer	Gatifloxacin 39/50 eyes (95.1%) vs Ciprofloxacin 38 (80.9%). p=0.042
Constantinou[178]	2007	Australia	229	3	• Moxifloxacin 1.0% (77) • Ofloxacin 0.3% (74) • Tobramycin 1.33% and Cafazolin 5% (78)	Healing of the ulcer	Overall healing 94% No difference across the 3 arms

Dehghani[179]	2009	Iran	89	2	• Gentamycin and Cafazolin (41) • Vancomycin and Ceftazidime (48)	Time to healing	Gentamycin group 17.7 ± 4.3 days Vs Vancomycin group 13.8 ± 2.6 days, p=0.04
Shah[180]	2010	India	61	3	• Tobramycin 1.3% and Cafazolin 5% (20) • Gatifloxacin 0.3% (21) • Moxifloxacin 0.5% (20)	Healing of the ulcer	Overall healing 93% No difference across the 3 arms
Kasetsuwan[181]	2011	Thailand	71	2	• Levofloxacin 0.5% (34) • Amikacin 5% and Cefazolin 5% (37)	Healing of the ulcer	Overall healing 86% No difference across the 3 arms
Sharma[182]	2012	India	224	2	• Tobramycin 1.3% and Cafazolin 5% (114) • Moxifloxacin 0.5% (110)	Healing of the ulcer	Overall healing 80% No difference across the 2 arms

Table 7 Showing culture and sensitivity of a pilot study in Mbarara, N=15

Drug	Sensitivity	*S. aureus*		*S. pneumonae*		Other strep		*P. aeruginosa*		Coliforms		Total	
		N=4	*(%)*	N=3	(%)	N=1	*(%)*	N=4	(%)	N=3	(%)	N=15	(%)
Chloramphenicol	S	1	(25)	1	(33.3)	1	(100)	0	(0)	2	(66.7)	5	(33.3)
	I	3	(75)	0	(0)	0	(0)	1	(25)	0	(0)	4	(26.7)
	R	0	(0)	2	(66.7)	0	(0)	3	(75)	1	(33.3)	6	(40)
Ciprofloxacin	S	2	(50)	1	(33.3)	1	(100)	4	(100)	2	(66.7)	10	(66.7)
	I	1	(25)	2	(66.7)	0	(0)	0	(0)	1	(33.3)	4	(26.7)
	R	1	(25)	0	(0)	0	(0)	0	(0)	0	(0)	1	(6.6)
Gentamycin	S	1	(33.3)	0	(0)	0	(0)	2	(50)	1	(33.3)	4	(26.7)
	I	3	(66.7)	2	(66.7)	1	(100)	1	(25)	1	(33.3)	8	(53.3)
	R	0	(0)	1	(33.3)	0	(0)	1	(25)	1	(33.3)	3	(20)
Tetracycline	S	2	(50)	1	(33.3)	1	(100)	1	(25)	1	(33.3)	6	(40)
	I	2	(50)	1	(33.3)	0	(0)	0	(0)	0	(0)	3	(20)
	R	0	(0)	1	(33.3)	0	(0)	3	(75)	2	(66.7)	6	(40)
Ofloxacin	S	4	(100)	3	(100)	1	(100)	4	(100)	2	(66.7)	14	(93)
	I	0	(0)	0	(0)	0	(0)	0	(0)	1	(33.3)	1	(6.7)
	R	0	(0)	0	(0)	0	(0)	0	(0)	0	(0)	0	(0)

S=sensitive, I=Intermediate, R=resistant

Table 8: Antibiotic resistance patterns among patients with bloodstream infections; percentage resistant by Kirby Bauer disk diffusion method

Bacteria	No of species	AMP	SXT	CHLO	PEN	CRO	GENT	Author	Country
Gram-positive									
Staphylococcus aureus	20	35	77	37	-	-	12	Kitara[183,184]	Uganda
	69	88	-	73	-	-	4	Mugalu[185]	Uganda
	17	47	58	23	-	-	29	Dagnew[186]	Ethiopia
	30	100	-	-	-	-	-	Blomberg[187]	Tanzania
Enterococcus faecalis	9†	-	-	62	83	-	44	Blomberg[187]	Tanzania
	6‡	-	-	67	25	-	33	Blomberg[187]	Tanzania
Enterococcus faecium	12†	75	63	-	90	-	23	Blomberg[187]	Tanzania
	9‡	89	100	-	100	-	77	Blomberg[187]	Tanzania
Streptococcus pneumoniae	11	27	100	27	-	-	-	Bachou[184]	Uganda
Streptococcus agalactiae	7	14	-	14	-	-	57	Mugalu[185]	Uganda
Gram-negative									
Escherichia coli	6	83	100	50	-	-	-	Bachou[184]	Uganda
	17	-	-	-	-	-	29	Mugalu[185]	Uganda
	5	100	40	20	40	-	-	Dagnew[186]	Ethiopia
	24†	96	-	-	-	22	29	Blomberg[187]	Tanzania
	13‡	85	-	-	-	4	46	Blomberg[187]	Tanzania
Klebsiella spp	8	75	50	62	-	62	37	Dagnew[186]	Ethiopia
	19†	100	-	-	-	26	47	Blomberg[187]	Tanzania
	34‡	100	-	-	-	15	47	Blomberg[187]	Tanzania
Pseudomonas aeruginosa	4	100	75	25	-	25	-	Dagnew[186]	Ethiopia
	7†	100	-	-	-	80	24	Blomberg[187]	Tanzania
	6‡	100	-	-	-	100	27	Blomberg[187]	Tanzania
Salmonella spp	5	80	80	-	-	-	20	Bachou[184]	Uganda
	3	-	-	-	-	-	33	Mugalu[185]	Uganda
Haemophilus spp	2	100	100	50	-	-	-	Bachou[184]	Uganda

AMP, Ampicillin; CRO, Cefuroxime; CHLO, Chloramphenicol; GENT, Gentamicin; PEN, Penicillin; SXT, Trimethoprim-sulfamethoxazole.
†, Hospital-acquired; ‡, Community acquired. Table reproduced with permission from a review paper by Ampaire et al.[164]

Table 9 Antibiotic resistance patterns among patients with bloodstream infections; percentage resistant by Kirby Bauer disk diffusion method

	No. of species	AMP	SXT	TTC	CHLO	ERY	GEN	AMX	CEFT	CIPRO	DOXY	F	KAN	CL	Author	Country
Gram-positive																
Coagulase-negative *Staphylococci*	6	50	66	50	16	-	16	33	-	50	-	16	33	-	Demilie[188]	Ethiopia
	9	100	11	55	55	55	44	-	44	44	33	-	-	-	Kitara[183]	Uganda
Staphylococcus aureus	3	66	33	66	33	-	-	-	-	33	-	33	-	-	Demilie[188]	Ethiopia
	66	25	50	54.7	65.6	92.2	100	-	-	98.4	-	-	-	-	Kitara[183]	Uganda
	64	100	89	42	15	46	18	-	-	-	-	-	-	-	Seni[189]	Uganda
	300	-	62	-	-	47	-	-	-	-	-	-	-	36	Mwambi[190]	Uganda
Streptococcus agalactiae	2	50	50	50	50	-	50	50	-	50	-	-	50	-	Seni[189]	Uganda
Enterococcus spp	23	30	-	74	30	65	21	-	-	60	-	-	-	-	Seni[189]	Uganda
Gram-negative																
Escherichia coli	16	81	56	43	56	-	31	75	-	18	-	-	63	-	Demilie[188]	Ethiopia
	9	78	67	66	-	-	-	90	55	44	66	22	44	-	Mulu[191]	Ethiopia
	6	67	67	100	17	-	67	50	50	50	-	-	-	-	Wondimeneh[192]	Ethiopia
	72	100	81	72	41	-	54	-	77	72	-	-	-	-	Seni[189]	Uganda
Klebsiella pneumoniae	39	100	92	76	71	-	76	-	92	66	-	-	-	-	Seni[189]	Uganda
Pseudomonas aeruginosa	2	100	100	-	100	-	100	100	100	100	50	50	50	-	Mulu[191]	Ethiopia
	2	100	50	50	100	-	50	50	-	-	-	-	50	-	Demilie[188]	Ethiopia
	5	100	60	20	80	-	40	100	100	40	80	100	60	-	Mulu[191]	Ethiopia
	12	-	100	100	-	-	16	-	16	-	-	-	-	-	Seni[189]	Uganda

	No. of species	AMP	SXT	TTC	CHLO	ERY	GEN	AMX	CEFT	CIPRO	DOXY	F	KAN	CL	Author	Country
Proteus mirabilis	5	100	60	20	80	-	40	100	100	40	80	100	60	-	Mulu[191]	Ethiopia
	2	50	50	-	50	-	-	100	-	-	-	100	-	-	Mulatu[193]	Ethiopia
Citrobacter freundi	13	69	54	62	77	-	62	69	46	54	-	-	-	-	Wondimeneh[192]	Ethiopia
	2	100	100	-	100	-	-	100	-	100	-	-	-	-	Mulatu[193]	Ethiopia
Salmonella																
Serogroup A	1	-	-	-	-	-	-	-	100	-	-	-	-	-	Mulatu[193]	Ethiopia
Serogroup B	3	-	-	-	-	-	-	-	100	-	-	-	-	-	Mulatu[193]	Ethiopia
Shigella spp	11	63	-	54	9	90	27	100	55	-	-	-	-	-	Mulatu[193]	Ethiopia
Campylobacter spp	20	30	20	15	-	55	70	80	-	-	-	-	-	-	Mulatu[193]	Ethiopia
Acinetobacter spp	52	-	98	65	-	-	88	-	-	77	-	-	-	-	Seni[189]	Uganda
Neisseria gonorrhoeae	123	-	-	-	-	-	-	-	-	81	-	-	-	-	Vandepitte[194]	Uganda

AMP, Ampicillin; AMX, Amoxicillin; CEFT, Ceftriaxone; CHLO, Chloramphenicol; CIPRO, Ciprofloxacin; CL, Clindamycin; DOXY, Doxycycline; ERY, Erythromycin; F, Nitrofurantoin; GENT, Gentamicin; KAN, Kanamycin; SXT, Trimethoprim-sulfamethoxazole; TTC, Tetracycline. Table reproduced with permission from a review paper by Ampaire et al.[164]

Viral Keratitis

Viral Keratitis is relatively uncommon in SSA. In many parts of SSA, topical acyclovir is the first line for Herpes Simplex Virus (HSV) Keratitis. According to the American Academy of Ophthalmology (AAO) treatment guidelines for HSV keratitis (2014), there are 2 topical agents (ganciclovir and trifluridine) and 3 systemic agents (acyclovir, famciclovir and valacyclovir) that are used in the treatment of HSV keratitis in the United States.[77]

Several trials have compared topical ganciclovir or trifluridine to acyclovir and found that all these agents are comparable in efficacy.[77,195-197] In the trials that compared trifluridine to acyclovir, one multicentre double-blind trial of 59 herpes keratitis patients. Acyclovir 3% ophthalmic ointment was compared with trifluorothymidine 2 percent ointment. Ninety percent of acyclovir-treated patients and 75 percent of trifluorothymidine-treated patients had healed within 14 days. There was no significant difference in the rate of healing between the two treatment groups.[195] In another double blind randomized study of 50 patients with epithelial HSV keratitis, 25 patients received 3% acyclovir ophthalmic ointment and other 25 patients 2% trifluorothymidine ophthalmic ointment. The mean duration of treatment in the 2 study groups before healing of the epithelial ulceration was obtained was 6.7 days and 5.9 days, respectively (no statistically significant difference) and there were no clinically significant adverse effects recorded in the 2 arms.[196] Another randomized double-blinded clinical trial compared four antiviral agents--1% idoxuridine ointment (group 1), 2% trifluorothymidine ointment (group 2), 3% acyclovir ointment (group 3) and 1% bromovinyldeoxyuridine (BVDU) ointment (group 4)-for uncomplicated and treatment naive cases of in HSV keratitis. Cure rates of 60%, 90%, 90% and 95% were obtained in groups 1, 2, 3 and 4 respectively. The average healing time was 13.4, 8.9, 8.5 and 7.5 days respectively. Side effects (follicular conjunctivitis, epithelial keratopathy and stinging) were more frequent in group 1 than in the other groups.[197]

In comparison of topical ganciclovir to acyclovir, one multicentre randomized clinical trial involving 67 patients compared 2 strengths of ganciclovir gel (0.05 and 0.15%) were compared with 3% acyclovir ointment in the treatment of superficial HSV keratitis.[198] The results showed no statistically significant difference between the treatment groups, although local tolerance was found to be superior with the gel formulation of ganciclovir with fewer complaints of discomfort (stinging, burning) or blurred vision after application of the drug.[198] Another randomised trial compared topical ganciclovir 0.15% gel and acyclovir 3% ointment in the treatment of HSV dendritic keratitis. Patients were assigned randomly to one of the two treatment groups for the purpose of the trial. They were then examined on days 2, 7, 10, and

14 to assess the rate of healing of the dendritic ulceration. There was no statistically significant difference detected in the rate of healing between the two treatment groups over the course of the trial.[199] Evidence from these trials show that these agents (acyclovir, ganciclovir and trifluridine) may all be used as first line agents depending on local availability and tolerance. In addition to these topical agents, oral acyclovir and other oral anti-viral agents such as Valacyclovir and Valganciclovir are used in managing other non HSV keratitis.[200,201]

Depending on the site of active inflammation, adjunctive topical or oral steroids can be added in stromal type HSV keratitis. The Herpetic Eye Disease Study (HEDS) enrolled 106 participants with stromal keratitis (57 treated with topical trifluridine and topical prednisolone phosphate and 49 with topical trifluridine and placebo). In this study, time to resolution of stromal keratitis and uveitis was significantly shorter in the steroid group compared with the placebo group.[202]

In addition, the HEDS also compared oral acyclovir in treating stromal keratitis caused by herpes simplex virus (HSV) in patients receiving concomitant topical corticosteroids and trifluridine. Patients were randomized to receive a 10-week course of either oral acyclovir (400 mg 5 times daily, n = 51) or placebo (n = 53). All patients also received a standard regimen of topical prednisolone phosphate and trifluridine. There was no statistically or clinically significant beneficial effect of oral acyclovir in treating HSV stromal keratitis in patients receiving concomitant topical corticosteroids and trifluridine with regard to time to treatment failure, proportion of patients who failed treatment, proportion of patients whose keratitis resolved, time to resolution, or 6-month best-corrected visual acuity.[203]

One peculiar problem with HSV is the recurrent nature of the stromal keratitis which can result into significant visual impairment and blindness through corneal scarring and astigmatism. According to American Academy guidelines, evidence from 3 clinical trials suggests that long term low dose antiviral agents (oral acyclovir 400mg BD or oral valacyclovir 500mg OD) are the only proven agents to prevent incidence of recurrent HSV stromal keratitis. However, this effect is more useful among people with previous episodes of stromal keratitis and does not continue after cessation of the medication.[204-206] In the first 2 studies by the HEDS, 703 immunocompetent patients who had had ocular HSV disease within the preceding year were randomised to receive 400 mg of acyclovir or placebo orally twice daily. The study outcomes were the rates of development of ocular or nonocular HSV disease during a 12-month treatment period and a 6-month observation period.[204,205] The cumulative probability of a recurrence of any type of ocular HSV disease during the 12-month treatment period was 19 percent in the acyclovir group and 32 percent in the placebo group (P<0.001). Among the 337

patients with a history of stromal keratitis, the most common serious form of ocular HSV disease, the cumulative probability of recurrent stromal keratitis was 14 percent in the acyclovir group and 28 percent in the placebo group (P=0.005). The cumulative probability of a recurrence of nonocular (primarily orofacial) HSV disease was also lower in the acyclovir group than in the placebo group (19 percent vs. 36 percent, P<0.001). There was no rebound in the rate of HSV disease in the six months after treatment with acyclovir was stopped.[205] Further analysis of these results showed that the magnitude of absolute benefit was greatest among patients with the highest number of prior episodes of ocular HSV disease. The benefit in preventing stromal keratitis was seen solely among patients with a history of stromal keratitis.[204] In another smaller trial involving 52 immunocompetent patients with a history of recurrent ocular HSV disease, 26 were randomized to the valacyclovir group (one 500 mg tablet daily), and 26 patients were randomized to the acyclovir group (one 400 mg tablet twice daily). The recurrence rate of ocular HSV disease during 12 months of treatment and drug-related side effects were monitored.[206] Recurrence of any type of ocular HSV disease during the 12-month treatment period was 23.1% in the valacyclovir group, compared with 23.1% in the acyclovir group. No difference between the two groups was observed regarding the nature, frequency, or severity of adverse events. The most frequent adverse events were nausea and headache. The conclusion from this trial was that One-year suppression therapy with oral valacyclovir (500 mg tablet daily) was shown to be as effective and as well tolerated as acyclovir (400 mg tablet twice daily) in reducing the rate of recurrent ocular HSV disease.[206]

Acanthamoeba Keratitis

Acanthamoeba is a difficult form of MK to treat because of the nature of the parasite which may occur in cystic (highly resistant to therapy) and trophozoite forms (more readily responsive).[207] Acanthamoeba is not a known common cause of MK in SSA: only one case has been reported.[53] Where it is prevalent, current treatment options for Acanthamoeba Keratitis include Diamidines and Biguanides.[208] Diamidines such as propamidine-isethionate (Brolene), hexamidine-diisethionate (Hexacyl), and dibromopropamidine (Golden Eye) are used in 0.1% concentration while Biguanides, such as polyhexamethylene-biguanide (polyhexanid) (PHMB Lavasept), and chlorhexidine (Curasept) are applied in 0.02% concentration.[208]

These topical antimicrobials are administered every hour day and night immediately after corneal debridement or for the first several days of therapy. Depending on clinical response, they are then continued hourly (while the patient is awake) for 3 days depending on clinical

response. The frequency is then reduced to every 3-hourly. Two weeks may be required before a response is observed, and the total duration of therapy is a minimum of 3–4 weeks. Moreover, when therapy is discontinued, close observation of the patient is suggested in order to avoid recurrent infection.[207]

A more recent review of management of Acanthamoeba keratitis recommends PHMB or chlorhexidine as the most effective drugs for treatment of infection.[207] They have been reported to be effective against both cysts and trophozoites.[209] Some patients have been successfully treated using an antiseptic as monotherapy: one double-masked, randomize trial compared the therapeutic outcomes of PHMB and chlorhexidine for Acanthamoeba keratitis.[210] Fifty-six eyes of 55 patients with Acanthamoeba keratitis were randomized to receiving PHMB 0.02% or chlorhexidine 0.02%.[210] The primary outcome measure in this trial was treatment failure defined as failure to induce a favorable clinical response within two weeks.[210] Outcomes were similar in the two arms, 18 (78%) PHMB patients were treatment successes compared with 24 (85.7%) chlorhexidine patients (P = .71). Five eyes worsened while receiving PHMB vs four eyes worsening while receiving chlorhexidine.[210] In non-responding cases,

Polymicrobial Infections

This is one of the causes of worsening infection if not detected and managed. Generally, the proportion of patients with polymicrobial infections has been reported to range between 1-6%. In an earlier study from Ghana in 1995, culture results of 199 patients with MK were reported.[48] In that study, the proportion of mixed infections (bacterial/fungal) was 5.5%. This included a mix of Gram +ve and -ve bacteria only (1.5%), Gram +ve bacterial and fungus only (2%), Gram -ve bacteria and fungus only (1%), Gram +ve. Gram -ve and 1 fungus (0.5%) and Gram +ve, Gram -ve and 2 fungus (0.5%).[48] Seven years later, in 2002, another study from Ghana reported a much less proportion of mixed infections (1.4%) among 290 patients with MK.[31] This study also reported data on 800 patients with MK from India: the proportion of mixed infections was 5.5%. A more recent study of 252 patients with MK from India reported mixed infections 2%.[43] In this study, all the mixed infections were Gram +ve bacteria and fungi.[43]

Treatment of polymicrobial infections requires good laboratory setup to be able to do initial and subsequent corneal scrapping analysis. In many reports, the preferred approach is to rule out mixed infections and proceed with mono microbial therapy.[211-214] However, use of prophylactic antibiotic cover has been reported in treatment of fungal keratitis. In a trial by

Arora et al from India in 2011 that tested topical Natamycin 5% versus Voriconazole 1% for treatment of fungal keratitis, patients also received topical ofloxacin 0.3% as part of treatment.[215] In another recent large series investigating the role of intracameral amphotericin B for treatment of recalcitrant fungal keratitis, patients received prophylactic topical moxifloxacin 0.5% three times a day as part of their management protocol.[216]

Outcomes of MK in Sub Saharan Africa

There is limited data on outcomes of MK in SSA. In the few papers that have reported on outcome, visual acuity, healing with corneal scarring and evisceration rates have been mentioned as outcome measures.[52,217,218] In one study from Nigeria reviewed medical records of 82 patients with corneal ulcers and compared the pre and post treatment visual acuity. The follow-up duration was not indicated.[52] The proportion of people with poor vision (VA <6/60) was 67.7% while 9.2% were eviscerated. The risk factors of a poor outcome in this study were not analyzed. Another study from Nigeria prospectively enrolled 54 patients with corneal ulcers and followed them up for at least 6 weeks after the start of their treatment. In this study, 93.5% of the patients healed with corneal scarring. The risk factors for poor outcome were centrally located lesion, a large lesion (greater than 4mm) and lesions affecting deeper layer of the corneal.[218] One study from East Africa reported outcome data among patients with MK in Tanzania.[12] In this study, a review of medical records of 170 individuals with microbial keratitis was conducted. At discharge (period not stipulated), 81% had poor vision (VA<6/60) while 8% had undergone evisceration. The risk factors to poor vision in this study were reported as a large infiltrate at presentation (>5mm), delayed presentation (>5 days) and corneal perforation.

In comparing SSA to Asia, one recent large multicenter study by the Asia Cornea Society Infectious Keratitis Study group presented data of 6626 eyes of 6563 individuals recruited in a large multicenter study from thirteen study centers and 30 sub-centers across India, Singapore, China, Philippines, South Korea, Japan, Thailand and Hongkon.[41] In this study, patients with MK were consecutively enrolled over a 12-18 months period where a performed standardized data collection protocol was used. Treatment of the infectious keratitis was decided by the managing ophthalmologist. Subjects were observed for up to 6 months. Main outcome measures were final visual acuity and the need for surgery during infection. Cornea transplantation was performed in 628 eyes to manage ongoing infection, but 289 grafts (46%) had failed by the end of the study. Moderate visual impairment (Snellen vision less than 20/60) was documented in 3478 eyes (53.6%).

Until this project, we had no prospective medium or longer-term data on the outcome of treatment for MK in Uganda. Mostly this was due to poor follow up. However, our indication was that the outcomes were poor. For example, when we looked at the evisceration rate among 50 patients with MK who had been admitted from April to August 2014 at Ruharo Eye Centre (REC), one of the main eye hospitals in South Western Uganda, 28% had been eviscerated. This was a relatively high rate compared to 9.2% reported in a study in Nigeria and 8% in a Tanzania study [12,52].

Impact of Microbial Keratitis on Quality of Life

Quality of Life (QoL) is a very important component of management of any disease and treatments should be targeted to improving QoL.[219] There is a need to better understand how MK and its outcomes affect people, to develop improved ways of management, counselling and support. One way of doing this is to use validated vision and health related QoL tools, which collect data in a quantitative way to draw out differences between cases and controls. The most commonly used tools in eye care are the WHO generic Quality of Life tool (WHOQOL-BREF), the European Quality of Life Questionnaire (EQ-5D) and WHO Prevention of Blindness and Deafness 20-item Visual Functioning Questionnaire (WHO/PBD VF 20).[220-222]

VRQoL: The WHO/PBD VF20 tool measures vision related quality of life, which was developed in India.[220] It assesses the impact of visual impairment in several domains including mental wellbeing, dependency and social functioning. The WHO/PBD-VF20 consists of 20 questions divided into four sub-scales: "General Vision" subscale (1 question); "Visual Symptoms" subscale (3 questions); "General Functioning" subscale (12 questions); and "Psychosocial" subscale (4 questions). It begins by asking the patient *"Overall, how would you rate your eyesight using both eyes?"*; and uses a five-point scale answer option such as *"very good", "good", "moderate", "bad", "very bad"*. Each subsequent question also has a 5-point response option: one indicates the highest and five the lowest score.[220]

HRQoL: The WHOQOL-BREF (WHOQOL Group, 1998) is a summarised version of the WHO generic QoL tool that was designed in 1991 (WHOQoL-100).[223,224] It has good applicability in low and middle-income countries (LMIC) as it was developed simultaneously from concept across 18 countries in Africa, Asia and Latin America.[221] It measures 4 domains of health: Physical Health, Psychological Health, Social Relationships, and Environment. It asks respondents 26 questions. These include the frequency they have experienced issues and/or were able to do things (e.g. feel safe, able to concentrate, enjoy life) in the past 4 weeks and how satisfied they are with certain aspects of their lives (e.g. sleep, capacity for work).[221,224]

EQ-5D: The European Quality of Life Questionnaire This scale was designed by the European quality of life (EuroQol) group to be brief, simple and practical for use in surveys alongside disease-specific measures.[222] The EQ-5D includes two components. The first consists of five descriptive dimensions: mobility, self-care, usual activity, pain/discomfort and anxiety/depression, each with three response options: no problem, some problem or extreme problem. The second is a Visual Analogue Scale (VAS), with scores ranging from 0 (''worst

imaginable health state'') to 100 (''best imaginable health state''). Respondents are asked to indicate on the scale where they rate their ''own health state today''.[222]

These tools have been used in a number of other vision related studies such as cataract, trichiasis and even MK to show a difference in QoL.[225,226] The first eye disease related QoL study in East Africa was conducted in Kenya in 2007 where the WHO/PBD VF20 and some questions from the European Quality of Life Questionnaire (EQ-5D) were used to describe the relationship between cataract visual impairment and vision and generic health-related quality of life, in people >50 years of age in Nakuru district, Kenya.[225] In this study, 196 patients with visual impairment from cataract and 128 population-based controls without visual impairment from cataract were identified through a district-wide survey and additional cases were identified through case finding. The modified WHO/PBD VF20 demonstrated good psychometric properties and was found to be a valid and reliable scale to assess vision-related quality of life associated with cataract visual impairment in this Kenyan population.[225] The second study was from Ethiopia in 2015 where the two tools WHOQOL-BREF WHO/PBDVF20 were used to compare the mean QoL scores between 1000 adult trichiasis cases and 200 trichiasis-free controls.[227] In this study, trichiasis cases had substantially lower Vision and Health related QoL than controls.[227]

Although the visual function tools were designed for use on binocular vision, they have been demonstrated to be effective in detecting differences in monocular visual impairment in the MUTT1 trial in India and in another case series in China.[10,228]

In the MUTT1 trial, The Indian visual function questionnaire (IND-VFQ) was administered to MUTT I study participants at 3 months to determine the risk factors for a low vision related functioning.[229] The IND-VFQ is a modified WHO/PBD VF 20 tool that is reduced to a 45 questions of four subscales (mobility subscale, 6 questions; activity limitation subscale, 10 questions; psychosocial impact subscale, 5 questions; visual function subscale, 7 questions .[230] In this study involving 292 patients who completed the IND-VFQ at 3 months, baseline visual acuity, need for a therapeutic penetrating keratoplasty and being unemployed were strong predictors of a low vision related QoL at 3 months.[229] The main conclusion of this study by the authors was that monocular vision loss from corneal opacity due to fungal keratitis reduced vision-related quality of life and that given the relatively high worldwide burden of corneal opacity, improving treatment outcomes of corneal infections should be a public health priority.[229] In addition, this trial also compared the 3-months QoL scores among the study participants in the different treatment groups (Natamycin versus Voriconazole).[231] In this study, the participants who had received Natamycin had a significantly better QoL score than those

who had received Voriconazole, and this difference was even more apparent among people where the causative agent was *Fusarium spp*.[231]

The Case series from China used the 25-item National Eye Institute Visual Function Questionnaire (NEI VFQ-25) to determine the vision related QoL in 65 patients with infectious keratitis.[10] The 51-list-item NEI VFQ is a vision-targeted survey that assesses the influence of visual impairment on HRQOL and was specifically developed to target 5 common chronic conditions (age-related cataracts, age-related macular degeneration, diabetic retinopathy, primary open-angle glaucoma, cytomegalovirus retinitis) and low vision from any cause.[232] The 51-list-item NEI VFQ includes a multi-list-item scales to rate overall health (2 list-items), overall vision (2 list-items), difficulty with near vision (7 list-items), difficulty with distance vision (7 list-items), limitations in social functioning due to vision (4 list-items), role limitations due to vision (5 list-items), dependency on others due to vision(5 list-items), mental health symptoms due to vision (8 list-items), future expectations for vision (3 list-items), driving difficulties (4 list-items), and pain and discomfort around the eyes (2 list-items).[232] In addition, it has single list-items to assess peripheral and color vision. Each subscale is scored so that 0 represents the lowest and 100 the best possible score.[232] In this study, there was a strong correlation between the QoL score and the Best Corrected Visual Acuity (BCVA) of the worse-seeing eye, duration of the disease, history of operation, and gender.[10]

Specific problems of MK in SSA

The problems of MK in SSA can be summarised in one sentence. **MK is a neglected disease**. There has been little investment in terms of research and improving care and outcomes.

Neglected problem with limited literature

Because of the limited research, there are many knowledge gaps in the epidemiology, risk factors and outcomes specific to the local context. A literature search on MK in SSA returned only a handful of papers most of which were of modest quality. Part of the aim of this research project was to make a scientific contribution by conducting well designed and powered studies.

Poor outcomes

Although there were only 2 studies that mentioned outcomes of MK in SSA, it is common knowledge among the ophthalmologists that outcomes for MK are grim.[12,52] The predictors for these have been reported to be large ulcers, delayed presentation and perforation. We have no medium or longer-term data on the outcome of treatment after discharge in Uganda. Mostly this has been due to poor follow up. However, our small audit of 50 patients admitted at Ruharo Eye Centre with MK from January to August 2014 indicated that 28% had to be eviscerated.

Limited resources

SSA is generally a limited resource setting, this includes the human resources, diagnostics and medicines.[233,234] There is limited access to appropriate treatment and care: people have to travel long distances to the capital to access an ophthalmologist. In addition, there is limited availability of effective medicines such as Natamycin which is currently not available in Uganda and many places in SSA yet the best current evidence indicates that topical natamycin is the treatment of choice for filamentous fungal keratitis.[213] This drug was recently added to the WHO Essential Medicines List, which will hopefully result in greater availability. Even when available, challenges of cost will still be a barrier to access as many people may not be able to afford it.

Weak health systems

Because of weak health systems, there is poor triage and referral of patients which leads to late presentation and poor outcomes.[12] For example, the Uganda health is a tier-based system with the lowest point of care being at the village level. However, physically, a Health Centre (HC) II is the lowest unit and is located at a parish level. These units have different staffing and capacity in terms of service provision. Patients are referred along the tier system depending on the complexity of their condition. Our experience is that there are many factors

along this system that further contribute to the delayed presentation of MK patients to the eye hospital. In an earlier retrospective study in Tanzania, a risk factor for corneal perforation among patients with MK was having visited a HC[12,235] Although the authors argued that the association may be that the lower-level HC were more likely to refer the 'bad' cases, it is also plausible that some of these lower-level HC are inherently deficient in capacity to diagnose and offer appropriate treatment to patients with MK. Lower HC play a role in delayed referral and presentation of patients to eye hospitals, and could therefore be a key rate limiting step in the presentation journey of patients with MK.

No corneal tissue

Therapeutic keratoplasty is a good option to salvage the eye and control infection.[236,237] Later, an optical keratoplasty can be done to improve visual outcomes.[237] Although there is a rising number of corneal specialists in SSA, keratoplasty for corneal blindness (not even MK) is not routinely performed due to lack of tissue. There is only one known corneal bank in Ethiopia but there is no current legal framework available to export tissue to other countries in SSA.[238]

Role of TEM and self-medication

Many people in SSA use TEM. Our experience is that by the time patients with MK come to hospital, about half have used some form of TEM. Many people probably choose to try TEM for several days before attending hospital as it can be easily obtained within or close to home. Its use appears to contribute to poor outcomes, substantially adding to the risk of poorer presenting vision. In Uganda, TEM is usually made from plant products. This is concerning, as such substances may be toxic or harbour infectious agents, such as fungal spores.[13,14]

Those who do not use TEM will use an off the counter eye drop without any expert advice. In Uganda, it is possible to obtain a range of medicines from pharmacies without a prescription. Our experience is that many eye-drop preparations sold over the counter in our region contain a corticosteroid component. This risks exacerbating fungal keratitis, compounding poor outcomes.[239,240]

Impact on QoL

Although data is not available, our impression is that treatment of corneal infections is very challenging for patients. Firstly, most patients come when they have tried all sorts of medications, which leave them financially, physically and mentally fatigued. On admission, they may spend many days or even weeks in hospital. They are the ones "in the corner". They are usually seen last on a ward round, because of appropriate concerns about transmission

of infection; which frequently means they are only reviewed by one of the more junior clinicians and our observation is that they are not actively or optimally managed.

Most people with MK are adults who have financial responsibilities to earn the income to support their families. Loss of income and inability to provide for family members is a major concern. Some of the female patients we interact with are worried about being abandoned for a second wife by their husbands, or not being able to plant crop for the season because they are sick or not being able to get married because of their looks. These and many other examples we learn about daily show that MK has far reaching short and long term physical and psychosocial ramifications. This is contrary to what has been the general assumption that since MK tends to be a monocular condition, the person still has the “other eye” to live and work with and therefore, leads a “normal” quality of life.

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Chapter 2. Management of Fungal Keratitis: a systematic review and metanalysis

A patient recieves treatment at Mbarara University and Referral Hospital Eye Centre (MURHEC

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SECTION A – Student Details

Student ID Number	LSH1511754	**Title**	Dr
First Name(s)	Simon		
Surname/Family Name	Arunga		
Thesis Title	Epidemiology of Microbial Keratitis in South Western Uganda		
Primary Supervisor	Prof Matthew Burton		

If the Research Paper has previously been published please complete Section B, if not please move to Section C.

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Where is the work intended to be published?	Survey of Ophthalmology
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SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I searched the databases and extracted the data; conducted the analysis with guidance from, Victor Hu, David Macleod and M.J Burton. I have drafted the manuscript with consideration of comments from all the co-authors pending additonal comments from Prof Dart and NV Prajna

SECTION E

Student Signature	AS
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MANAGEMENT OF FUNGAL KERATITIS: A SYSTEMATIC REVIEW

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ABSTRACT

Purpose: To review published data on the treatment of fungal keratitis and make evidence-based recommendations.

Methods: A literature search was performed using the search terms Fungal Corneal Ulcer, Fungal Keratitis, Fungal Corneal abscess, Fungal Infective Keratitis, Fungal Corneal abscess, Fungal corneal abscess, Mycotic keratitis and Mycotic corneal ulcer. Only Randomised Controlled Trials (RCTs) were considered for the topical and oral treatment groups. The search was updated on 26/06/2019.

Results: A total of 14,396 results were returned of which 55 papers were ultimately included. Treatments for fungal keratitis included medical, injections, surgery, corneal cross linking and argon laser. A meta-analysis of two trials comparing topical chlorohexidine to natamycin, found a non-significant trend favouring chlorhexidine for cure / healing at 21 days (RR 0.72, 95% CI 0.46-1.12, RR> 1 favours natamycin). A meta-analysis of four trials comparing topical natamycin to voriconazole, found natamycin had favourable vision outcomes at 3 months (Standardised Mean Difference (SMD) in Log MAR units 0.34, 95% CI 0.17-0.50, SMD>0 favours natamycin). A meta-analysis of two trials of intrastromal voriconazole found this was associated with increased risk of perforation and a worse vision at 3 months, compared to topical treatment alone (Standardised Mean Difference (SMD) in Log MAR units 0.56, 95% CI 0.16-0.96, SMD>0 favours topical treatment alone). Five studies on intra-cameral amphotericin B (ICAMB) reported favourable outcomes in patients with severe, deep infiltrates. The surgical interventions reported several different outcome measures: anatomical integrity (64-90%), graft clarity (26-94%), recurrence of infection (0-47%). One RCT reported increased perforation following CXL (p=0.02). Another RCT reported better outcomes in people with moderate fungal keratitis. Two RCTs for Argon laser reported better outcomes in recalcitrant fungal Keratitis.

Conclusions: There was strong evidence favouring natamycin as the treatment of choice for filamentary fungi. Intrastromal voriconazole did not have favourable outcomes. Use of ICAMB showed benefit especially for deep lesions. Evidence for surgical techniques was inconclusive, however, there is a role of all these procedures in salvaging eyes and controlling infection. Evidence around the use of CXL and Argon laser is currently inconclusive, more studies are needed to define their potential indication.

INTRODUCTION

Microbial Keratitis (MK), or corneal infection, is a major ophthalmic public health problem, particularly in low and middle-income countries (LMIC). Corneal scarring, which is frequently caused by MK, accounts for an estimated 3.2% (1.3 million people) of binocular blindness globally and ~10% in Sub-Saharan Africa (SSA).[1] In the Nigerian National Survey corneal scarring caused 15% of monocular blindness.[2] Older estimates suggest ~2 million people in LMIC develop monocular blindness from MK annually, however, the true figure is probably somewhat higher.[3] MK has been described as a "silent epidemic" leading to significant morbidity and sight loss.[4]

Fungal Keratitis (FK) accounts for around 50% of MK occurring in tropical regions, becoming increasingly more common closer to the Equator.[5] It is usually caused by filamentous organisms with *Fusarium spp.* and *Aspergillus spp.* the most frequent causes.[6] In temperate regions, fungal corneal infections are much less frequent, accounting for less than 5% of all MK.[7,8] Historically, yeast infections were more frequent in temperate areas, however, recent reports suggest a shift towards increasing numbers of filamentous infections.[9-11] The predisposing factors for fungal keratitis are varied and include: trauma (particularly related to agricultural work), contact lens wear, chronic ocular surface disease, topical steroid use, corneal surgery, traditional eye medicine and immunosuppression.[10,12,13]

Treating fungal keratitis is challenging as cases tend to be severe, particularly with late presentation in LMIC, and are often associated with poor outcomes.[14,15] The armamentarium for treating fungal keratitis treatment is relatively limited, and drug availability very variable. Treatment options are broadly subdivided into medical, laser and surgical. Medical options include topical antifungal agents, systemic antifungal agents and injections which can be subconjunctival, intrastromal or intracameral. Surgical options for fungal keratitis include therapeutic penetrating keratoplasty (TPK), partial keratectomy, debridement, conjunctival flaps, amniotic membrane graft and corneal collagen crosslinking (CXL).

Many studies have reported outcomes of specific treatments, although these are often simple case series of an individual treatment. There remains considerable uncertainty regarding the best management for fungal keratitis, and outcomes are often poor. Here we review the published evidence on the treatment of fungal keratitis, and propose a treatment approach to assist ophthalmologists in making decisions.

METHODS

Treatments for fungal keratitis were grouped as follows: Medical treatment (topical and oral treatment), injections, surgical treatment (penetrating keratoplasty, lamellar keratoplasty, conjunctival flaps and amniotic membrane grafts), and, other treatment (corneal cross linking and argon laser).

Search strategy

We searched Embase and extended across Medline, Global health, Pubmed, Clinical Trials.Gov, Ethos, IndMed and google scholar. Authors approved the search strategy. Only Randomised Controlled Trials (RCTs) were considered for the topical and oral treatment groups. We allowed greater inclusion flexibility for other forms of treatment, because there were few if any RCTs for those treatment groups. We last searched electronic databases on 26/06/2019. The following key words were used for each individual treatment method:

Topical and oral treatment; ((Fungal Corneal Ulcer OR Fungal Keratitis OR Fungal Corneal abscess OR Fungal Infective Keratitis OR Fungal Corneal abscess OR fungal corneal abscess OR Mycotic keratitis OR Mycotic corneal ulcer) AND (Management OR Treatment OR fluconazole OR natamycin OR econazole OR chlorohexidine OR clotrimazole OR voriconazole OR itraconazole OR amphotericin B OR fluconazole) AND (Randomised Controlled Trial OR RCT OR Trial OR Clinical Trial OR intervention study OR intervention trial)). TW.

Fluconazole injection; ((Fungal Corneal Ulcer OR Fungal Keratitis OR Fungal Corneal abscess OR Fungal Infective Keratitis OR Fungal Corneal abscess OR fungal corneal abscess OR Mycotic keratitis OR Mycotic corneal ulcer) AND (Management OR Treatment OR fluconazole, Subconjunctival, Intrastromal, Intracameral)).TW.

Amphotericin B injection; ((Fungal Corneal Ulcer OR Fungal Keratitis OR Fungal Corneal abscess OR Fungal Infective Keratitis OR Fungal Corneal abscess OR fungal corneal abscess OR Mycotic keratitis OR Mycotic corneal ulcer) AND (Management OR Treatment OR amphotericin B OR Subconjunctival Intrastromal OR Intracameral)).TW.

Voriconazole injection; ((Fungal Corneal Ulcer OR Fungal Keratitis OR Fungal Corneal abscess OR Fungal Infective Keratitis OR Fungal Corneal abscess OR fungal corneal abscess OR Mycotic keratitis OR Mycotic corneal ulcer) AND (Management OR Treatment OR voriconazole, Subconjunctival, Intrastromal, Intracameral)).TW.

Collagen cross linking; ((Fungal Corneal Ulcer OR Fungal Keratitis OR Fungal Corneal abscess OR Fungal Infective Keratitis OR Fungal Corneal abscess OR fungal corneal abscess OR Mycotic keratitis OR Mycotic corneal ulcer) AND (Collagen Cross linking OR CCL OR CXL OR X-Linking OR Corneal Cross linking OR Collagen Corneal cross linking OR riboflavin OR Ultraviolet-A OR PACK-CXL)). TW.

Surgery; ((Fungal Corneal Ulcer OR Fungal Keratitis OR Fungal Corneal abscess OR Fungal Infective Keratitis OR Fungal Corneal abscess OR fungal corneal abscess OR Mycotic keratitis OR Mycotic corneal ulcer) AND (surgery OR surgical OR corneal allograft OR corneal transplant OR Keratoplasty OR Therapeutic Keratoplasty OR TPK OR PK OR Conjunctival flap OR Flap OR Gunderson flap OR debridement OR gluing OR Keratectomy OR amniotic membrane graft)).TW.

Argon laser; ((Fungal Corneal Ulcer OR Fungal Keratitis OR Fungal Corneal abscess OR Fungal Infective Keratitis OR Fungal Corneal abscess OR fungal corneal abscess OR Mycotic keratitis OR Mycotic corneal ulcer) AND (argon laser OR laser)).TW.

A separate PubMed search was made specifically for topical treatment for candida keratitis using the search terms **Candida Keratitis.**

Inclusion criteria:

- Participants: Patients of any age with fungal keratitis
- Interventions: Topical antifungal treatment, antifungal injections, systemic antifungal treatment, surgical treatment (conjunctival flap, amniotic membrane flap, lamellar keratoplasty, therapeutic penetrating keratoplasty), corneal cross linking, or argon laser treatment (any of these)
- Comparisons: Topical antifungal treatments, Topical vs injections, or Topical vs surgery (any of these)
- Outcomes: Best Corrected Visual Acuity (BCVA), improvement in vision, complication rate, recurrence rate, time to heal, or residual scar size (any of these)
- Type of study: Randomised controlled trials, case series with at least 10 cases and published audits. For topical and oral treatments, we only included randomised clinical trials.
- Language: English
- Publication status: Published

Study selection:

One author assessed all the studies identified in the search for eligibility. Initial selection was made by reviewing title and abstract; full texts were retrieved for studies considered to possibly meet inclusion criteria. The full text was reviewed and assessed against the inclusion criteria. Studies

meeting inclusion criteria were included in the review. These were independently verified by a second author.

Data collection:

We extracted the following data: Author and year of publication, study design, participants, inclusion criteria, fungal diagnosis, severity, fungal species, intervention (drug or surgery), indication for surgery, follow-up period, main outcome variables (BCVA, time to heal, complications, recurrence, scar size)

Risk of bias:

The risk of bias in clinical trials was assessed using the Cochrane risk of bias tool.[16] Parameters assessed included: random sequence generation, allocation concealment, masking of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting. In addition, all studies were allocated a general score of strength of evidence using the Scottish Intercollegiate Guidelines Network (SIGN 50) criteria as shown in Table 1.[17]

RESULTS:

1) TOPICAL TREATMENT

The search returned 426 articles on topical and oral anti-fungal treatment. Among these were 8 randomised controlled trials comparing topical antifungal agents. The key features of the study designs and the main findings from these trials are presented in detail in Table 2. All the trials were conducted in South Asian countries (India, Bangladesh and Nepal), where there are enough FK cases for clinical trials. Many cases enrolled in these trials had filamentous fungi (*Fusarium spp.* and *Aspergillus spp.* being most frequent), rather than yeasts. All trials included topical natamycin as one of the treatments being compared. Where the Risk Ratio (RR) is presented in Table 2, natamycin is considered the reference treatment. The methodologies were varied in terms of treatment used, follow-up schedules and outcome measures, which limits potential meta-analysis. The studies are considered in chronological order.

Two trials (*Rahman et al 1997, 1998*) compared topical natamycin and chlorhexidine.[18,19] These were relatively small, with a combined size of 130 participants. In the first, three different concentrations of chlorhexidine (0.05%, 0.1%, 0.2%) were compared to natamycin 5%.[20] There were trends towards more favourable responses by five days and “cure” at 21 days with increasing chlorhexidine concentration. The authors concluded that a chlorhexidine concentration of 0.2% was required. In the second trial, chlorhexidine 0.2% was compared to natamycin 2.5% (half the standard concentration).[21] Chlorhexidine 0.2% was associated with more favourable responses at 5 days (RR of a bad outcome 0.23, 95%CI 0.08 – 0.63, p=0.004) and no significant difference in healed ulcers at 21 days (RR of a bad outcome 0.78, 95%CI 0.54 – 1.14, p=0.200). In a meta-analysis of these two studies, combining the chlorhexidine and natamycin groups with different concentrations, there was a non-significant trend favouring chlorhexidine over natamycin for cure / healing at 21 days (Figure 2).

One trial (*Prajna et al 2003*) compared topical natamycin 5% to econazole 2%, in 116 participants.[22] The primary outcome measure was healed or healing ulcers at the final visit. No difference was found between the two arms (RR 1.04, 95% CI 0.64-1.72, p=0.86).

Four studies have compared topical natamycin 5% and voriconazole 1%. The first trial (*Prajna et al 2010*) used a factorial design with 120 patients randomised to natamycin or voriconazole, with or without repeated corneal scrapping.[23] The primary outcome was best spectacle-corrected visual acuity (BSCVA) at 3 months: there was a non-significant trend in favour of voriconazole (0.98 logMAR better, 95%CI: -0.28 to 0.83, p=0.29). In the second trial (*Arora et al 2011*) 30 patients were randomised to natamycin or voriconazole.[24] There was a non-significant trend in favour of natamycin. In the third trial (*Prajna et al 2013*), 323 patients were randomised to natamycin or voriconazole. This

found strong evidence for the superiority of natamycin for the primary outcome of BSCVA at 3 months. The fourth trial (*Sharma et al 2015*) randomised 119 patients to natamycin or voriconazole. The overall final visual acuity was better in the natamycin group.[25] Meta-analysis of the 3-month, final visual acuity for these four trials indicates that natamycin is associated with a more favourable visual acuity outcome than voriconazole (Figure 3).

Three of the studies reported perforation rates.[26-28] Overall, there was evidence that this was lower in natamycin treated patients (Figure 4). Two trials reported a subgroup analysis comparing fungal keratitis due to *Fusarium spp* compared to non-*Fusarium spp* (*Aspergillus spp* and others).[25,28] In the larger trial (*Prajna et al 2013*), the *Fusarium spp* results were better with natamycin for BSCVA at 3 months, perforation rates, infection resolution at 6 days and the scar size at 3 months.[28] Sharma et al also reported that the final visual acuity was better in patients with *Fusarium* keratitis but not with *Aspergillus* keratitis.[25]

Topical Treatment for *Candida spp*.

The search returned 479 papers were returned from which 30 were selected for abstract review, most were dropped for being case reports and finally 5 articles were considered for full text review. [29-33] These included 1 case series and 4 audits. Overall, the most commonly reported drugs were topical Amphotericin B, topical fluconazole and oral azoles.[33] An earlier case series had reported 6 eyes with Candida Keratitis which all improved on topical fluconazole.[31] In one audit from Japan, 10 patients with Candida Keratitis were initially treated with fluconazole (topical and oral), then miconazole or natamycin eyedrops if they showed no improvement. Of the 10, 7 responded well and 3 with recalcitrant *Candida parapsilosis* had to be managed with 0.1% Mucafungin.[30] In another audit of 29 patients, topical amphotericin B (17 eyes), natamycin (4 eyes), and miconazole (2 eyes) ± oral ketoconazole or itraconazole were used to successfully manage the cases.[33] In a more recent audit of 128 patients with fungal keratitis all the 16 patients with Candida Keratitis were treated with topical amphotericin B (0.15%). In addition, those with deep infiltrate, increase in hypopyon, or size of infiltrate received additional intracameral injection of amphotericin B (10 mg in 0.1 mL). All patients received oral itraconazole as adjunctive systemic therapy.[32] Good outcomes were noted in 14/16 with only 2 patients needing TPK.[32] In an earlier audit, 4 cases with had been successfully treated with topical antifungal therapy (amphotericin B 0.7%, 5-fluorocytosine 1%, one drop every hour) and oral itraconazole (400 mg daily).[29]

2) ORAL TREATMENT

Oral treatments are sometimes used in addition to topical therapy for cases with deep invasive disease (Table 3). There is limited trial data to guide practice in this area. We identified three randomised trials for oral treatments. In the first trial (*Agrawal et al 2001*) 54 patients with fungal keratitis, who were all treated with topical itraconazole, were randomised to receive oral itraconazole

or no oral treatment (no placebo).[34] Oral itraconazole was not found to provide additional benefit of healing by 6 weeks (RR 1.0, 95%CI 0.37 – 2.71).

In a second trial (*Prajna et al 2016*) 240 patients with particularly severe fungal keratitis were all treated with topical voriconazole ± topical natamycin.[35] They were randomised to oral voriconazole or placebo. There was no additional benefit from oral voriconazole for the primary outcome measure of corneal perforation (RR 1.2, 95%CI 0.78 – 1.80).

In the third trial (Sharma et al 2017), 50 patients with severe fungal keratitis were randomised to receive oral voriconazole or oral ketoconazole as adjunct treatment to topical natamycin.[36] Patients who received oral voriconazole had better BSCVA at 3 months (primary outcome) compared to the ones who received oral ketoconazole (mean Log MAR 1.3 ± 0.07 Vs 1.5 ± 0.07, p=0.02). In this trial, 3/25 patients in the voriconazole group and 5/25 in the ketoconazole group developed corneal perforation (RR 0.6 95% CI 0.2-2.2, p=0.45)

3) INJECTED TREATMENT

Several types of ocular injection have been reported: subconjunctival, intrastromal and intracameral injections (Table 4). Indications for this treatment tend to be severe and/or unresponsive fungal keratitis. The reported injected agents include fluconazole, voriconazole and amphotericin B.

Fluconazole Injection

The search for fluconazole injections returned a total of 2550 articles. Following title and abstract review, we identified 12 papers for full article review. We eliminated seven articles that did not meet the inclusion criteria. Five articles were included: 1 RCT, 3 case series and 1 retrospective audit. The route of injection was subconjunctival in four studies and intrastromal in one report. These studies are summarised in detail in Table 4.

Subconjunctival Fluconazole: In the RCT from Egypt (*Mahdy et al 2010*), 48 patients were randomised into two arms: (1) topical amphotericin B 0.05% eye drops and 1mL of sub-conjunctival fluconazole 2mg/mL, injected daily for 10 days and then every 48 hours for a further 10 injections; (2) topical amphotericin B 0.05% eye drops.[37] The main outcome measures were the proportion healed at 3 months ("healing rate") and the time to being healed. In the combination treatment group, the 3-month healing rate was 83% and the mean time to healing was 31 days. However, this trial had several methodological limitations with respect to randomization and masking. In the topical only group the healing rate was 67% and time to healing was 37 days ($p < 0.05$).

In the three prospective case series (*Yilmaz et al 2005, Dev et al 2006, Mahdy et al 2010*), subconjunctival fluconazole was found to have no local toxicity.[38-40] Outcomes in terms of healing,

improvement in visual acuity and ulcer/infiltrate resolution, were variable (Table 4). Healing or resolution was variable and was reported in 54-100% of the patients. Outcomes were not separately analysed by species for significance.[38,41]

Amphotericin B injection

The search returned 3071 articles for amphotericin B injection. After title and abstract review, we identified 23 papers for full review. We eliminated 17 studies which did not meet the inclusion criteria, leaving five articles (Table 5): one RCT, two non-randomised trials, two case series that reported intracameral use of amphotericin B; one audit reported use of intrastromal amphotericin B.

Intracameral Amphotericin B (ICAMB): One RCT (*Sharma et al 2015*) of 45 patients with fungal keratitis examined the effect of intracameral amphotericin B, in addition to topical and systemic treatments; there was no additional benefit in terms of healing or BCVA.[42]

In the first non-randomised prospective *study* (*Sharma et al 2015*) 104 patients were allocated to groups depending on how they responded to conventional topical antifungal treatment by day 7.[43] Those who seemed to be responding were maintained on the same treatment, while those who showed no response received additional intracameral amphotericin injection. The two groups were compared for healing time, time of hypopyon resolution, complication rate and improvement in final BCVA. The group which received the additional amphotericin injection had a non-significant trend to slightly better outcomes in all parameters compared to the group that did not. Another more recent non-randomised study (*Gupta et al 2019*) enrolled patients with recalcitrant fungal keratitis and allocated one group to early ICAMB (at 2 weeks) intervention and another to late ICAMB (at 4 weeks). Patients in the early ICAMB intervention group had a quicker healing time compared to the ones in the late intervention group.[44]

Among the case series, one report (*Yoon et al 2007*) audited the outcomes of two groups, one was treated with conventional antifungal drops and intracameral amphotericin B while the other was treated with only conventional antifungal treatment. Both groups were compared for final visual acuity, time to healing, hypopyon resolution, time for epithelial defect resolution and overall treatment success. Although overall treatment success was comparable in the two groups, the group that received intracameral amphotericin B had a better final vision and shorter healing times.[45] The second prospective series (*Yilmaz et al 2007*) of 14 eyes of 12 patients that had not responded to initial topical and systemic therapy, were treated with repeated doses of ICAMB. Healing was reported in 12 out of 14 eyes, cataract was also reported as a complication in this series.[46]

There were mild differences in the doses used in these studies (5-10 µg/0.1ml given up to 3 times 1-10 days apart). Favourable outcomes for ICAMB were reported in other studies, however, these

had a low level of evidence. Overall, literature on the use of intracameral amphotericin is inconclusive, some have reported favourable outcomes and others not. However, there appear to be specific situations where there may be advantages, such as unresponsive deep filamentary fungal keratitis with hypopyon. The literature on this was too scanty and the level of evidence too weak to draw any firm conclusions. A fully powered RCT for ICAMB for deep, recalcitrant intraocular fungal infections is needed.

Intrastromal Amphotericin B: An audit from Egypt (*Nada et al 2017*) reviewed 68 cases of unilateral fungal keratitis.[47] Forty-one cases that had not responded to the initial topical antifungal therapy were treated with a single intrastromal injection of amphotericin B + topical fluconazole 2%. These were then compared to 27 cases treated with only topical amphotericin B 0.3% monotherapy. Healing was reported to be faster in the amphotericin injection group. However, the overall evidence level was quite weak.

Voriconazole Injection

The search returned a total of 3911 articles for voriconazole injection. After title and abstract review, we identified 34 papers for full article review. We eliminated 27 papers for not meeting the inclusion criteria. The 7 studies that we included were: 2 RCT and 5 case series. Use of voriconazole was intrastromal in 5 studies (2 RCTs and 3 case series); intracameral in one case series; and intracameral combined with intrastromal in one case series. Details of these studies are in Table 6.

Intrastromal Voriconazole: The one RCT (*Sharma et al 2013*) randomised 40 patients into two groups.[48] One group received topical Voriconazole 1% and Natamycin 5% while the other group received intrastromal Voriconazole and topical Natamycin 5%. For all outcome measures of treatment success, BCVA at 3 months, time to heal, complications and scar size, the group that received topical Voriconazole had a better outcome than the group that received intrastromal Voriconazole.[49] In a more recent RCT (*Narayana et al*) 70 patients were randomised to two groups comparing topical Natamycin and Intrastromal Voriconazole (ISV) *vs* topical Natamycin alone. For all the outcome measures of microbiological cure, BSCVA, scar size, perforation rate, there was no evidence of benefit in adding ISV injections to topical natamycin in the primary treatment of moderate to severe filamentous fungal ulcers. Meta-analysis of these 2 trials shows that ISV is associated with more perforation rates and a worse vision at 3 months than topical treatment alone (figure 5 and 6). However, in the three case series (*Sharma et al 2011, Kalaiselvi et al 2015, Nagar et al 2015*), intrastromal voriconazole was found to be effective in treating deep seated fungal infiltrates/stromal abscesses unresponsive to 5% topical natamycin and 1% voriconazole, and oral itraconazole.[48,50-52]

Intracameral Voriconazole: Intracameral voriconazole injection have been reported in a few case series.[53,54] In a study from China (*Shen et al 2010*), 10 patients with fungal keratitis progressing to

endophthalmitis were given treated with intracameral voriconazole once daily until resolution of the endothelial infiltrate.[53] The injections varied from 1-8 (median 5). Patients were followed up for 4 months. The infiltrates resolved in 6 out of 10 patients and there was no reported intra or postoperative complications.

Intrastromal and intracameral Voriconazole: This combination has been reported in a study from India (*Killani et al 2015*); 30 patients with proven fungal corneal ulcers with deep stromal infiltrates and endothelial plaque not responding to routine antifungal drugs (5% natamycin, 1% voriconazole, oral itraconazole) were given 1-2 intrastromal and intracameral voriconazole injection 48 hours apart in addition to the topical treatment. Infection resolved in 25 patients, without significant complications.

4. SURGICAL TREATMENT

Medical treatment alone is sometimes insufficient to successfully manage severe cases of fungal keratitis. Surgical interventions may be required, including: therapeutic penetrating keratoplasty, lamellar keratoplasty, keratectomy, amniotic membrane graft, and conjunctival flaps. The main aims are to eliminate the infection and maintain the integrity of the globe. Indications usually include progressive or unresponsive keratitis, perforations, impending perforations, posterior corneal involvement, and persistent epithelial defects.

The search returned 3387 articles on the surgical treatment of fungal keratitis. After reviewing titles and abstracts, we identified 201 papers for full review. We eliminated 181 papers which did not meet the inclusion criteria. The surgical interventions described in the 20 studies included were: Therapeutic Penetrating Keratoplasty (11 studies), Lamellar Keratoplasty (5 studies), conjunctival flap (2 studies) and amniotic membrane graft (2 studies). Details of these studies are in Table 7.

Therapeutic Penetrating Keratoplasty (TPK): Eleven audit studies looked at the outcomes of fungal keratitis in patients who had undergone TPK (Table 7).[55-65] These studies were mostly from Asian countries. Follow-up ranged from 2 weeks to 5 years. The indications for TPK included perforations, impending perforations, non-healing ulcers despite medical treatment, infiltrates progressing to involve deep corneal layers or limbus. Figure 6 shows a summary of the indications.

The studies reported several different outcome measures: anatomical integrity, graft clarity, “cure”, recurrence of infection, vision and complications.

- Anatomical integrity was reported in 5 studies and ranged from 64-92%.[55-58,61]
- Graft clarity was reported in 7 studies as 26-94%.[55,56,58,59,61,63,65]
- Recurrence rate was reported in 9 studies from 0-47%.[55,56,58-63,65]
- Improvement in vision was reported in 3 studies.[56,57,60] One study in India (*Sharma et al 2014*)

reported it as 5.66% (6/106 patients with a BCVA>6/60).[57] However, it was reported as high as 88% in two other studies (*Palakash et al 2015, Xie et al*).[56,60] It was not clear whether the improvement was after the primary TPK or a secondary optical keratoplasty. Vision data in these studies was not disaggregated by the main vision categories.

- Complications reported included glaucoma 2-64% and cataract 5-20%.[55,56,60,62,63]

Lamellar Keratoplasty (LK): Five studies (3 retrospective audits and 2 case series), all from China, examined the outcomes of fungal keratitis in patients who had undergone LK (Table 7).[66-70] Follow-up ranged from 1-20 months. The main indication for LK was non-healing ulcers despite medical treatment. However, only infiltrates limited to anterior and middle cornea, where vision less than 6/60 were considered. Some of the main outcome measures reported were:

- Graft clarity was reported as 100% by Gao et al and at 92% by Xie et al. [70] [66]
- Cure of disease was reported study at 92% and 93% by xie et al in 2 separate audits. [70] [69]
- Recurrence rate was reported at 7% and 8% in 2 audits [66,69]. However, it was reported at 0% in a case series of 47 fungal keratitis patients in China by Zhang et al where porcine corneas were used. [68]
- Improvement in vision was reported at 72% using porcine corneas [66] while Gao et al reported it at 100% in a smaller series of 14 patients. [68]
- Complications: corneal neo-vascularisation was reported in 15%. [68]

Amniotic Membrane Graft (AMG): We found one 8-year audit of 23 cases from China (*Chen et al 2006*) meeting our inclusion criteria.[71] Indications for AMG included: corneal perforation (35%), descemetocele (35%), and deep ulcer (95% stromal loss with poor reepithelialisation, 30%). In this audit, immediate improvement in visual acuity was reported at 61%. The main complications reported were; secondary glaucoma (17%) and graft failure (13%). In a separate head to head RCT from Egypt (Abdulhalim et al 2015) comparing AMG with conjunctival flap (CF) for keratitis, AMG and CF were all comparable for re-epithelialisation time, persistence of infection, improvement in visual acuity and complications.[72]

Conjunctival flaps: These are frequently used for microbial keratitis which is failing to respond to treatment in a resource limited setting. The introduction of a vascular bed over the ulcer is thought to help control infection and promote healing. Literature on this method is generally scanty. In a recent series from China (Zhong et al 2018), 17 patients with recalcitrant fungal keratitis underwent conjunctival flap. The globe was preserved in 15/17(88%) and none developed raised Intra Ocular Pressure (IOP).[73]

5. COLLAGEN CROSS LINKING

The search returned 886 articles related to corneal cross linking (CXL). After title and abstract review,

we identified 45 articles for full paper review. We eliminated 42 papers for not meeting the inclusion criteria. The 3 selected studies were exclusively fungal keratitis cases, one was a randomised trial, one a retrospective comparative study and the third a case series. Details of these studies are in Table 8.

In the RCT (*Udaraju et al 2015*), patients with severe fungal keratitis not responding to topical antifungal therapy after 2 weeks of treatment were randomised to either receive CXL treatment in addition to the topical treatment or continue with the antifungal topical treatments only.[74] The main outcome was treatment failure at 6 weeks, defined as a composite score of perforation and/or increase in the infiltrate by 2mm or more; other secondary outcomes included uncorrected vision at 6 weeks. This study was prematurely stopped after only 13 patients had been enrolled as there was already a significantly higher rate of perforation among the CXL group compared to the non CXL group (p=0.02). This trial only enrolled patients with severe, extensive, and deep ulcers.

In a more recent trial (Wei et al 2019) 41 patients with fungal ulcerative keratitis were randomised to CXL combined with antifungal medications (CXL-M) or antifungal medications alone (M).[75] Patients were followed up for 6 months. In the cured patients the area of corneal ulcers, the duration of ulcer healing, the time to non-observed fungal hyphae by In Vivo Confocal Microscopy (IVCM), the number of antifungal medications, the frequency of administered medications, and the maximum ulcer depth decreased significantly after CXL compared with the M group.

Vajpayee et al conducted a retrospective audit of 41 eyes with moderate fungal keratitis, 21 whom had received CXL on admission and continued with 5% Natamycin and 20 who only received 5% Natamycin. CXL was performed on the day of presentation using a standard surgical technique. Medical management was continued after the CXL. Main outcome measures were resolution of infection, BCVA at 3 months, rate of corneal perforation and size of residual scar. There was no difference in all the outcome variables between the two groups, CXL was not particularly associated with any adverse events.[76]

One case series of 8 patients with culture proven fungal keratitis not responding or worsening on topical antifungal treatment were treated with CXL in addition to 5% natamycin. The main outcome measure was resolution of the infiltrate. After the CXL treatment, in all patients, re-epithelialisation occurred within 3 to 8 days. Hypopyon had resolved in 8—11 days, vision improved in 6 patients, remained unchanged in 1 and worsened in 1. None of the patients required a corneal transplant. The times of review were not specified in this study.[77]

Another recent small series treated 13 fungal Keratitis patients who were unresponsive to topical voriconazole with CXL.[78] Seven patients (54%) were healed with topical voriconazole and CXL

adjuvant treatment while the remaining six patients did not respond to CXL treatment. Those who responded had small and superficial mycotic ulcers.

6. ARGON LASER FOR FUNGAL KERATITIS TREATMENT

The search returned a total of 165 articles on laser treatment. After title and abstract review, we identified 11 articles for full paper review. We eliminated 9 papers for not meeting the inclusion criteria. Two RCTs: Argon laser *Vs* intrastromal voriconazole, and argon laser *Vs* Amniotic Membrane Graft (AMG) were included. Details of these papers are in Table 9.[79]

***Argon laser Vs intrastromal voriconazole*:** In this RCT (Khater et al, 2016), 40 patients with culture proven mycotic keratitis not improving on topical treatment of 0.15% amphotericin, or 5% natamycin, or 1% voriconazole, or 1% itraconazole or 0.2% fluconazole were randomised into two groups to receive Argon laser or intrastromal voriconazole.[80] Argon laser was applied as follows: After application of surface anaesthesia and a drop of fluorescein sodium 0.25%. Argon laser therapy was done using argon green wavelength (Carl Zeiss LSL 532s AG; Meditec, Inc.). A spot size of 500 m, pulse duration of 0.2s, and power of 900 mW were used. Number of shots varied from one case to another depending on the size of ulcer. The bed and edge of the ulcer were targeted during argon laser therapy with laser shots. Argon laser was found to have a significantly quicker healing time of 2-4 weeks and fewer patients needing AMG. More patients in the voriconazole group had improvement in vision but this was not significant. This RCT had a relatively weak design in randomisation, masking, allocation concealment and reporting.[80]

Argon laser and AMG Vs AMG: In this RCT (Kharter et al,2016) 40 patients with culture proven mycotic keratitis not improving on topical treatment with 0.15% amphotericin, or 5% natamycin, or 1% voriconazole, or 1% itraconazole or 0.2% fluconazole were randomised into two groups to receive AMG or Argon laser. Argon laser therapy was done using a similar machine and protocol as in the previous study. AMG was done in all cases of the study, after argon laser therapy in the laser treatment group and after tissue debridement in the AMG group. The multilayer amniotic membrane was sutured to the cornea using nylon 10/0 suture (Ethilon, Ethicon Inc.) under general anaesthesia (inlay technique). The amniotic membrane was sewn in with the epithelium/basement membrane side facing outwards so that neighbouring epithelial cells of the recipient would migrate over the amniotic membrane, allowing ulcer healing. Topical drops were continued after either intervention. Argon laser was found to be comparable to AMG in improving vision. However, patients in the argon laser arm had a significantly faster healing time compared to those in the AMG arm. [79]

Figure 1: Flow diagram of the search results

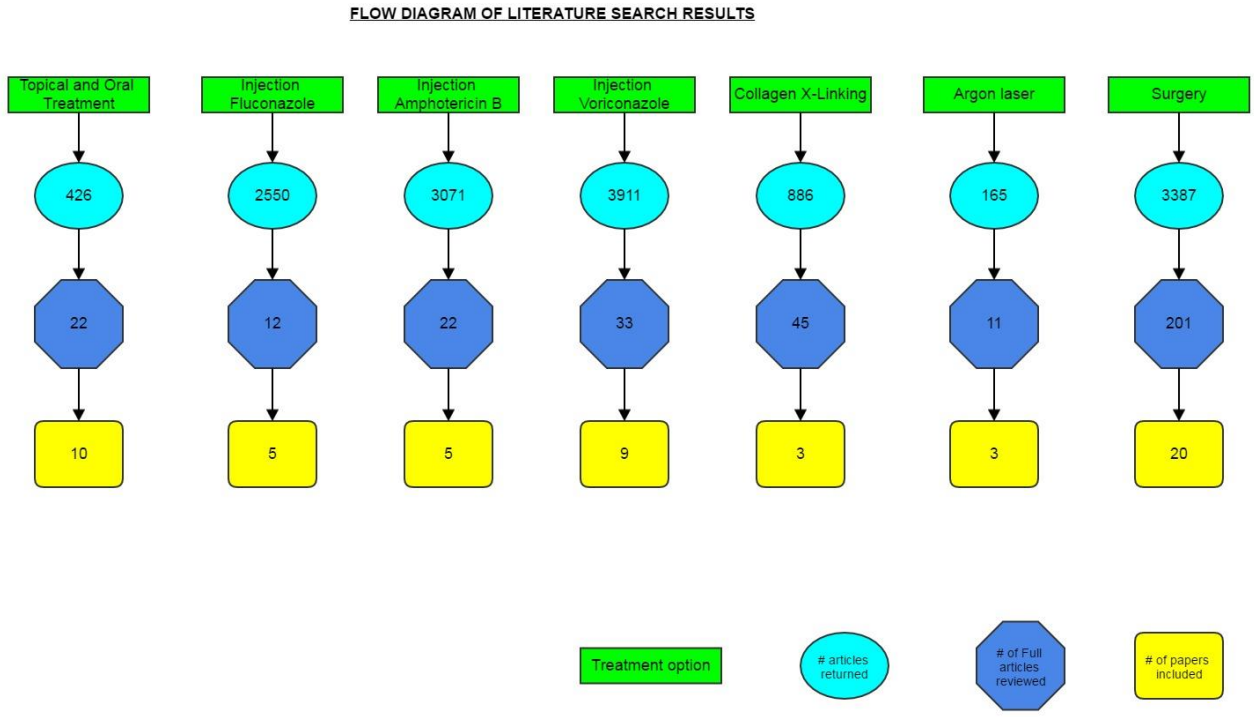


Figure 2: Metanalysis of a healed outcome by 21 days in randomised trials of Natamycin Vs Chlorohexidine: RR > 1 favours Natamycin

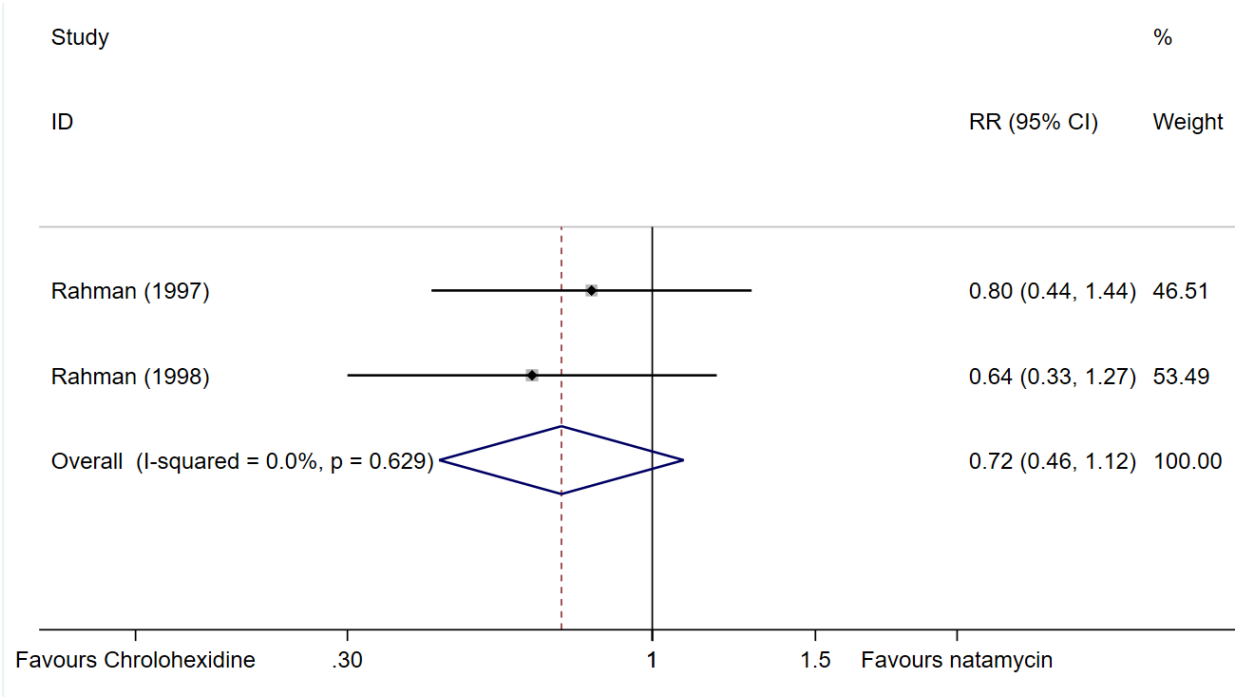
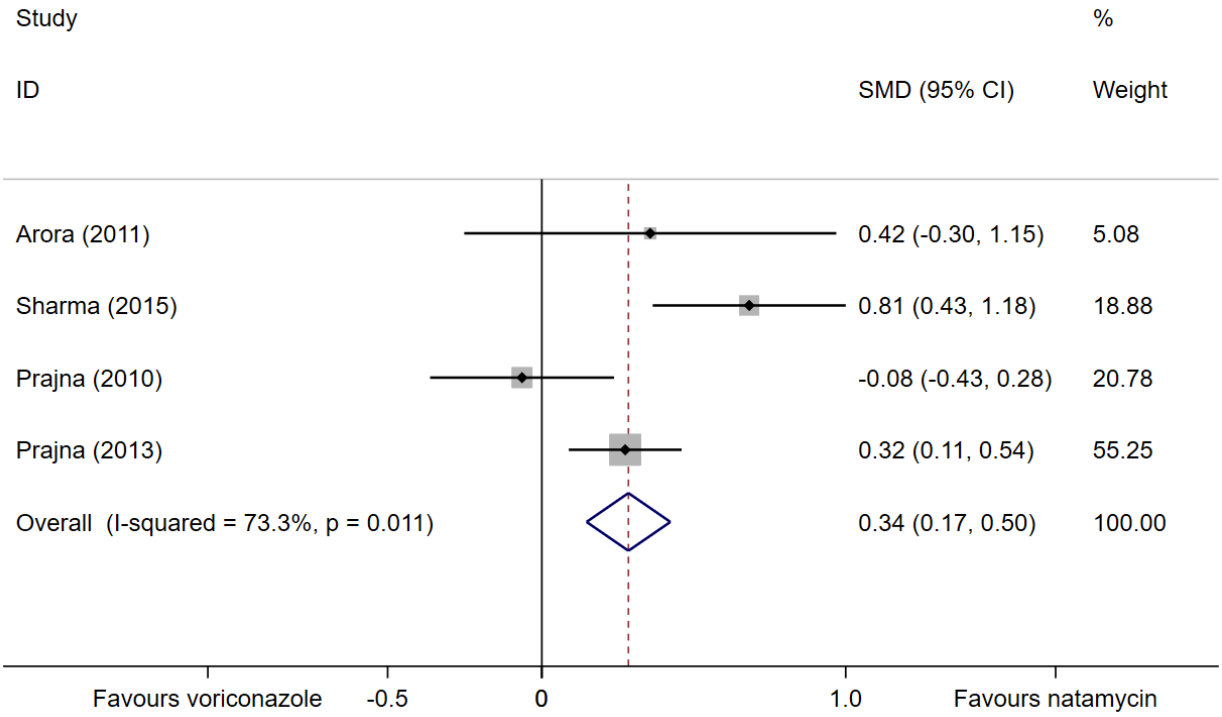


Figure 3: Metanalysis of mean difference in final best corrected visual acuity of randomised trials comparing Natamycin to Voriconazole*: A difference of >0 favours Natamycin*



SMD: Standardised Mean Difference in Log MAR units.

Figure 4: Metanalysis *perforation rates in randomised trials comparing* Natamycin *to* Voriconazole: RR > 1 favours Natamycin

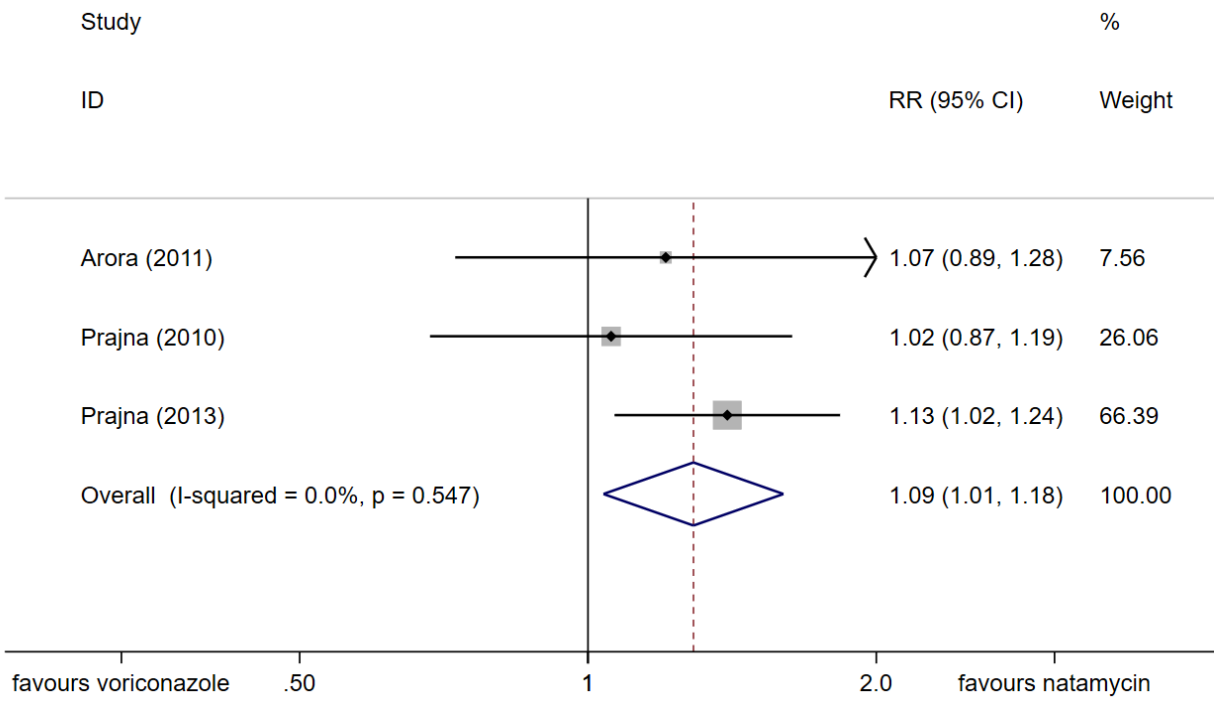


Figure 5: Metanalysis perforation rates in randomised trials comparing topical Natamycin ±Voriconazole to Intrastromal Voriconazole: RR > 1 favours Topical treatment alone

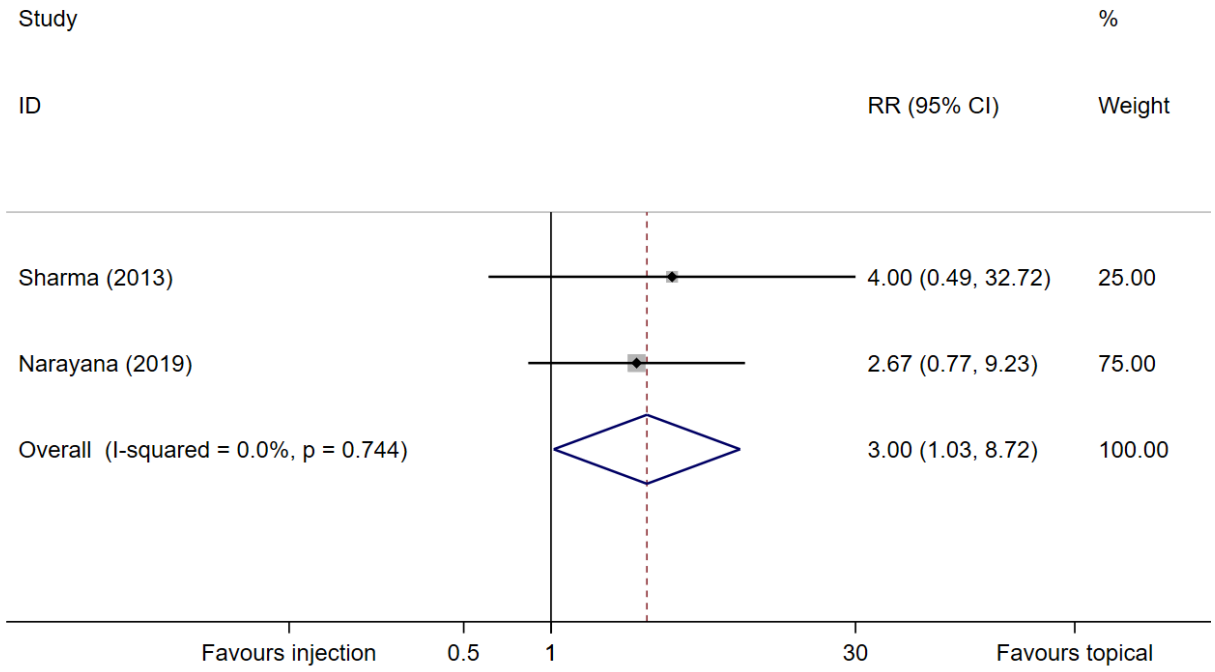
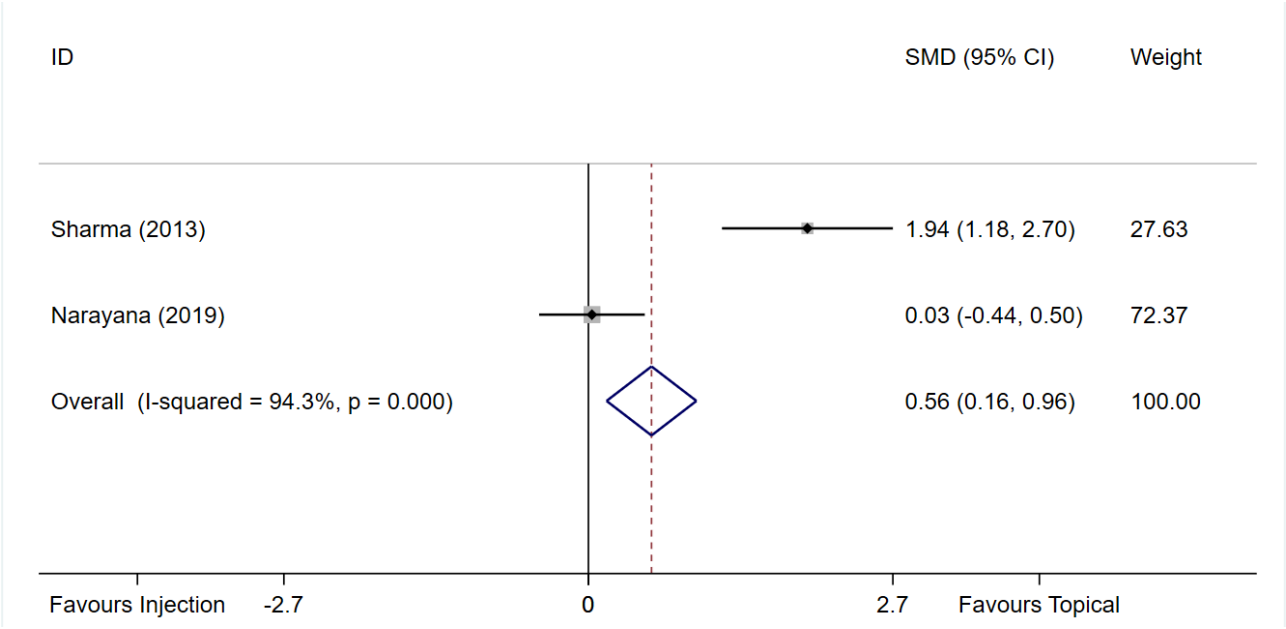


Figure 6: Metanalysis of mean difference in final best corrected visual acuity of randomised trials comparing Intrastromal Voriconazole to topical Natamycin ± topical Voriconazole.



SMD: Standardised Mean Difference in Log MAR units.

Figure 7: Indications of Therapeutic Penetrating Keratoplasty

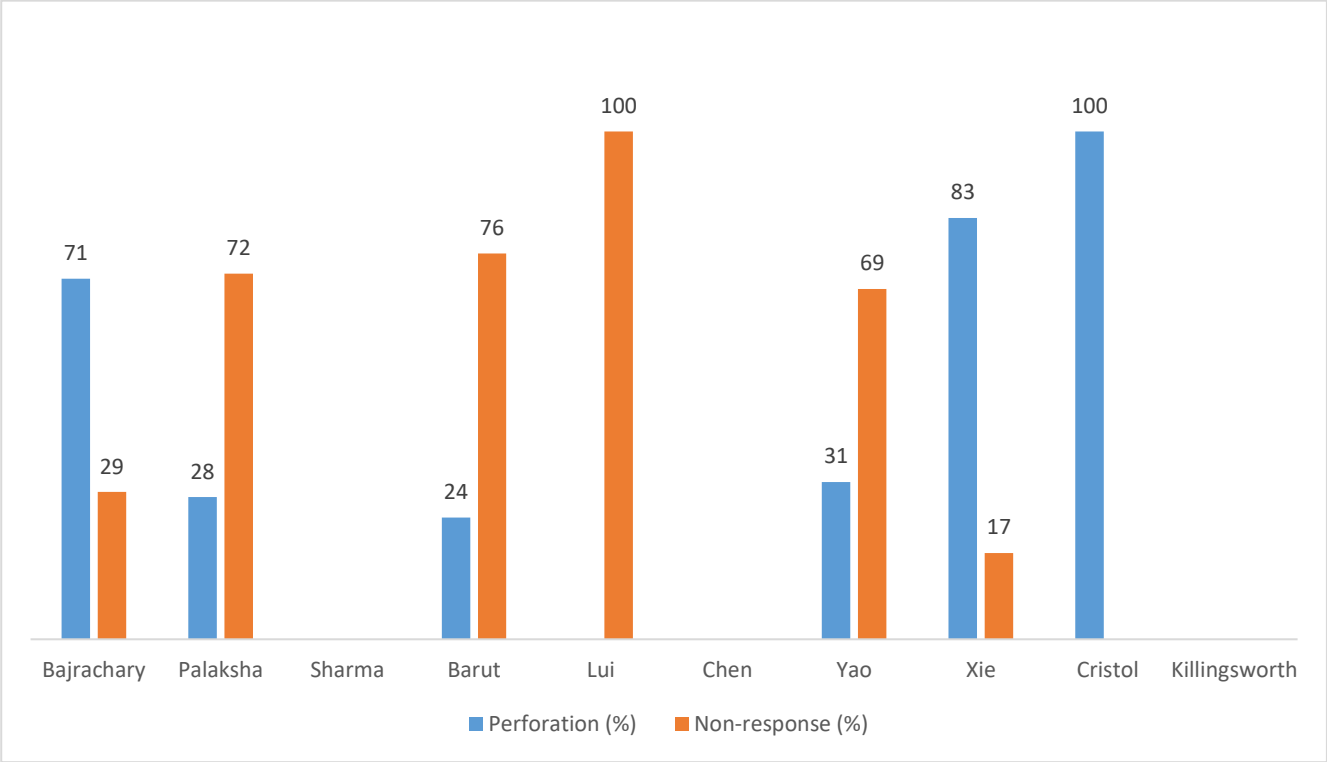


Table 1: Evidence grading using the SIGN 50 criteria.[17]

Evidence Grade	Description
1++	High-quality meta-analyses, systematic reviews of RCTs, or RCTs with a very low risk of bias
1+	Well-conducted meta-analyses, systematic reviews of RCTs, or RCTs with a low risk of bias
1-	Meta-analyses, systematic reviews of RCTs, or RCTs with a high risk of bias
2++	High-quality systematic reviews of case–control or cohort studies High-quality case–control or cohort studies with a very low risk of confounding, bias or chance and a high probability that the relationship is causal
2+	Well-conducted case–control or cohort studies with a low risk of confounding, bias or chance and a moderate probability that the relationship is causal
2-	Case–control or cohort studies with a high risk of confounding, bias, or chance and a significant risk that the relationship is not causal
3	Non-analytic studies (for example, case reports, case series)
4	Expert opinion, formal consensus

Table 2: Topical Treatment

STUDY	DESIGN	RESULTS	COMMENTS
NATAMYCIN VS CHLORHEXIDINE			
Rahman et al 1997 India [18]	RCT with 4 parallel arms: • g-Natamycin 5% • g-chlorhexidine 0.05% • g-chlorhexidine 0.1% • g-chlorhexidine 0.2% Drop Frequency • Day 1: Half hourly for the 1st 3 hours, hourly rest of the day • Day 2: 2 hourly during day • Day 5 onwards: 3 hourly Inclusion: • suppurative corneal ulcers • fungal elements on microscopy Exclusion: • Patients with only one eye • Patients with diabetes mellitus • Patients with mixed infections • Unwilling to come for follow up • Less than 1 year of age • Perforated • Lived far away Outcome Measures (primary not designated): • Favourable response at 5 days • Healed ulcer by 21 days • Toxicity	N=60 • 16 patients in g-Natamycin 5% arm • 17 patients in g-chlorhexidine 0.05% arm • 17 patients in g-chlorhexidine 0.1% arm • 8 patients in g-chlorhexidine 0.2% arm Fungal species: 22 *Fusarium*, 10 *Aspergillus*, 3 *Curvularia*, 6 other, 19 unidentified. 1) Favourable response at 5 days: • g-Natamycin 5% - 7 / 16 (43.8%) • g-chlorhexidine 0.05% - 8 / 17 (47.1%) • g-chlorhexidine 0.1% - 10 / 17 (58.5%) • g-chlorhexidine 0.2% - 6 / 8 (75.0%) 2) Healed ulcer by 21 days: • g-Natamycin 5% - 7 / 14 (50.0%) • g-chlorhexidine 0.05% - 7 / 12 (58.3%) • g-chlorhexidine 0.1% - 8 / 14 (57.1%) • g-chlorhexidine 0.2% - 5 / 6 (83.3%) If the data from all three chlorhexidine arms are combined the following results are obtained (RR>1 favours Natamycin): • Favourable response at 5 days; RR 0.76, 95%CI: 0.44-1.33; p=0.3) • Healed ulcer at 21 days; RR 0.73, 95%CI: 0.36-1.45, p=0.4 • Toxicity: no toxic effects observed.	Pilot trial to compare three concentrations of chlorhexidine. The sample size is small and insufficient for a comparison with natamycin 5%. Masking examiner to natamycin is difficult as it can leave a white precipitate on ocular surface. Masking may not have been possible for treatment failures after 5 days. Data on 12 severe cases is excluded from the analysis, because none were cured in 21 days. Level of evidence: 1- .
Rahman et al 1998 Bangladesh [19]	RCT with 2 parallel arms: • g-Natamycin 2.5% • g-chlorhexidine 0.2% Drop Frequency • Day 1: Half hourly for the 1st 3 hours, hourly for 2 days • Day 3-7: 2 hourly • Day 7-21: 3 hourly	N=71 • 36 patients in g-Natamycin arm • 35 patients in g-Chlorhexidine arm Fungal species: 22 *Fusarium*, 22 *Aspergillus*, 5 *Curvularia*, 10 other, 19 unidentified. Primary outcome: (RR>1 favours Natamycin): • Healed ulcer at 21 days: RR 0.78, 95%CI: 0.54-1.14, p=0.2	This study used natamycin of half the usual (2.5% instead of 5%). Masking nurses and ophthalmologists was not possible due to appearance differences of the formulations Level of evidence: 1-

	Inclusion: • suppurative corneal ulcers • fungal elements on microscopy Exclusion: • Patients with only one eye • Patients with diabetes mellitus • Patients with mixed infections • Unwilling to come for follow up • Less than 1 year of age • Perforated Outcome Measures (primary not designated): • Favourable response at 5 days • Healed ulcer by 21 days (Primary) • Toxicity	• g-chlorohexidine 0.2%-14/32 (43.8%) • g-Natamycin 2.5%-9/32 (28.1%) Secondary outcomes: • Favourable response at 5 days: RR 0.24, 95%CI: 0.09 – 0.63, p=0.0039 • g-Chlorohexidine 0.2%-31/35 (88.6%) • g-Natamycin 2.5% 18/35 (51.4%) • Toxicity: 1 patient receiving chlorhexidine developed temporary punctate epitheliopathy	
NATAMYCIN VS ECONAZOLE			
Prajna et al 2003 India [22]	RCT with 2 parallel arms: (1) g-Natamycin 5% (2) g-econazole 2% Drop frequency • Day 1-7: Hourly 7am-9pm • Day 7 onwards: 2 hourly 7am-9pm Inclusion: • suppurative corneal ulcers • fungal elements on microscopy • Ulcer size 2 – 60 mm^2 Exclusion: • Unwilling to participate Outcome Measures (primary not designated) • Healed ulcer • Time to heal	N=116 • 53 patients in g-Natamycin arm • 59 patients in g-Econazole arm Fungal species: 64 *Fusarium*, 30 *Aspergillus*, 6 *Curvularia*, 12 other, 4 unidentified. Outcomes: Healed ulcer by four weeks (RR>1 favours Natamycin): • Natamycin 36 / 55 (65.5%) • Econazole 39 / 61 (63.4%) • RR 1.04, 95% CI 0.64-1.72, p=0.86. Time to heal: • No difference: log rank 0.52, p=0.47	Randomisation not clear Masking examiner to NATA is problematic as can leave a white precipitate on ocular surface. Patients were followed up for 4 weeks Level of evidence: 1-
NATAMYCIN VS VORICONAZOLE			
Prajna et al 2010 India [23]	RCT with a 4-arm factorial design: • g-Natamycin 5% with scraping • g-Natamycin 5% no scraping • g-Voriconazole 1% with scraping • g-Voriconazole no scraping	N=120 • 60 patients in each arm Fungal species: 44 *Fusarium*, 19 *Aspergillus*, 39 other, 8 unidentified	Double masking done by using identical opaque bottles and having a study nurse wipe the natamycin residue before examination primary and secondary outcomes assessed.

	Scrapping was done at baseline for all ulcers and then repeated in the scraping arms at one and two weeks. Drop frequency • Day 1-7: Hourly while awake • Day 7-21: 2 hourly while awake Inclusion: • Corneal ulcer • fungal elements on microscopy Exclusion: • Mixed infections • Younger than 16 years • Bilateral disease • Live >200km away • Pregnant • VA <6/60 in fellow eye • Allergy to antifungal medication • Previous corneal transplant • Epithelial defect < 0.5mm Follow-up was 3 months Outcome Measures: • BSCVA at 3 months (Primary) • Scar size • Perforations • Time to re-epithelialize	Primary outcome: • After adjusting for scraping, voriconazole treatment was associated with a non-significant trend towards a slightly better logMAR visual acuity at 3 months: 0.98 logMAR better, 95%CI: -0.28 to 0.83, p=0.29. • Eyes that had repeated scraping showed a non-significant trend towards having a worse logMAR visual acuity at 3 months: 0.71 worse, 95%CI: -0.007 to 0.35, p=0.06 Secondary outcomes: • Scar size; voriconazole treatment was associated with 0.17 mm larger infiltrate / scar size diameter (95% CI, 0.20 mm smaller to 0.53 mm larger; p=0.37) • Time to re-epithelialization; no difference in the time to re-epithelialization between the NATA and VOR groups. Voriconazole associated with a hazard ratio of −0.05 (95% CI, −0.13 to 0.14; =.61)	Level of evidence: 1-
Arora et al 2011 India [24]	RCT with 2 parallel arms: • g-Natamycin 5% • g-Voriconazole 1% Drop frequency: • Day 1-14: Hourly • Day 14: Titrated as per response Inclusion: • Positive fungal KOH scraping Exclusion • Prior use of antifungal drugs • History of herpetic keratitis	N=30 • 15 patients in each arm Fungal species: 3 *Fusarium*, 12 *Aspergillus*, 9 *Curvularia*, 1 other, 5 not identified Primary Outcome: • Non-significant difference in the time to resolution, which was shorter in the NATA Vs the VOR groups: 24 Vs 27 days (p>0.05) Secondary outcome (s): RR>1 favours Natamycin • Healing rate NATA 15/15, VOR 14/15.	Sample size small and insufficient for a comparison Randomisation by lottery Double masking by using identical opaque bottles and having a nurse wipe any white residue from the patient's eye before examination Level of evidence: 1+

	• Previous corneal scars • Impending perforation • NPL Follow-up 2 months or until complete resolution Outcome measure: • Time to healing of ulcer (Primary) • Best Corrected Visual Acuity (BCVA) at 3/12 • Proportion of healed cases • Scar size	• Scar size: Not significant • Final BCVA: NATA: 1.368 +/-0.887 Vs VOR 1.775 +/-1.036 (P = 0.227). • RR 3, 95% CI 0.13-68.2, p=0.49	
Prajna et al 2013 India [81]	RCT with 2 parallel arms: • g-Natamycin 5% • g-Voriconazole 1% Drops frequency • Hourly until re-epithelialisation. • Then QID for at least 3 further weeks. Inclusion • Corneal ulcer • fungal elements on microscopy • Visual acuity: 6/12 to 6/120 Exclusion • Mixed infections • Younger than 16 years • Bilateral disease • Unwilling follow-up • Pregnant • VA <6/60 in fellow eye • Allergy to antifungal medication • Previous corneal transplant • Epithelial defect < 0.5mm Follow-up was 3 months Outcome Measures • BCVA at 3/12 (Primary) • Corneal perforation • TPK	N=323 • 162 patients in each arm Fungus speciated in 255 cases: 128 *Fusarium*, 54 *Aspergillus*, 20 *Curvularia*, 56 other, 68 not identified Primary Outcome: • logMAR BSCVA at 3 months: VOR 1.8 lines poorer, p=0.006 Secondary Outcomes: • logMAR BSCVA at 3 weeks: VOR 1.1 lines poorer, p=0.03 • Fusarium Cases Only: logMAR BSCVA at 3 months: VOR 4.1 lines poorer, p<0.001 • Day 6 re-culture positive: NATA 15% Vs VOR 48%, p<0.001. • Re-epithelialization: faster in Fusarium cases with NATA: hazard ration 1.89, p=0.005 • Scar size: Fusarium cases had significantly smaller scars at 3 months if treated with NATA, -1.02mm, 95%CI: -1.46 to -0.58. • Perforations and /or Therapeutic Penetrating Keratoplasty: 18 NATA Vs 34 VOR. All OR 0.42, 95%CI: 0.22 – 0.80, p=0.009. Fusarium only: OR 0.06, 95%CI: 0.01 – 0.28, p<0.001.	The arms were well balanced in terms of the demographic, baseline clinical signs and organisms cultured. Randomisation code autogenerated Double masking done by using identical opaque bottles and having a study nurse wipe the natamycin residue before examination Primary and secondary outcomes reported Level of evidence: 1+
Sharma et al 2015	RCT with 2 parallel arms: • g-Natamycin 5%	N=118. • 60 patients in g-Natamycin arm	The arms were well balanced in terms of the demographic, baseline clinical signs and organisms

India [25]	• g-Voriconazole 1% Drops frequency • Day 1-3: Hourly day and night • Day 4: Hourly during day, 3 hourly at night • Day 5: 2 Hourly while awake until cured Inclusion: • above 18 years old • symptoms < 14 days • epithelial defect (2-6mm) + stromal infiltrate +/- hypopyon • corneal scrapping +ve Exclusion: • allergy to Natamycin/ Voriconazole • Scleral involvement • Posterior 1/3 corneal involvement • Perforation/ impending perforation • Mixed infection • Comorbidities Outcome Measures • Healing • Final VA at last follow-up (Primary) • VA on day 7	• 58 patients in g-Voriconazole arm Fungal species: 29 *Fusarium*, 15 *Aspergillus*, 46 other , 28 no growth. Primary outcome (s): RR>1 favours Natamycin • % of patients with healed or resolving ulcer; NATA (50/56) Vs VOR (34/51) P<0.005 • Final VA at last follow-up; NATA LogMar 0.6 (CI 0.4-0.8) Vs VOR LogMar 1.1 (CI 0.9-1.2) P=0.01 • RR 3.1, 95% CI 1.3-7.2, p=0.009 Secondary outcome • Visual acuity on day 7; VA in NATA marginally better than VOR (p=0.04)	cultured. Randomisation code autogenerated Double masking done by using identical opaque bottles and having a study nurse wipe the natamycin residue before examination Primary and secondary outcomes reported Shorter incubation time for fungus- 2 weeks instead of 3 It was not clear when the final review date was. Level of evidence: 1+

Table 3: Oral Treatment

STUDY	DESIGN / INTERVENTION	RESULTS	COMMENT
TOPICAL ITRACONAZOLE VS TOPICAL ITRACONAZOLE + ORAL ITRACONAZOLE			
Agrawal et al 2001 India [34]	RCT Arms: • g-itraconazole 1% • g-itraconazole 1% + itraconazole 100mg, Drug frequency: • Hourly drops • 100mg PO BD*3/52 Inclusion: • Suspected fungal keratitis Exclusion: • Unclear Follow-up 6 weeks Outcome measures: (primary not designated) • Therapeutic effect • Healing of the ulcer by 6 weeks	N=54 • 27 patients in each arm Fungal species: 4 *Fusarium*, 28 *Aspergillus*, 6 *Penicillium*, 2 *Candida*, 4 Other fungi, 10 No growth Outcome measure: • Healing of ulcer by 6 weeks. No added benefit found of using an oral anti-fungal in addition to topical treatment. RR 1.0, 95%CI: 0.37 – 2.71 • Fusarium was unresponsive to itraconazole	Method of randomisation unclear Inclusion and exclusion not clear Not placebo-controlled Concerns over blinding as treatment different Topical treatment was continued for 6 weeks after the resolution of keratitis Level of evidence: 1-
Oral Voriconazole + g-Voriconazole or g-natamycin Vs Placebo + g-Voriconazole or g-natamycin			
Prajna et al 2016 India / Nepal [35]	RCT Arms • Tablets Voriconazole 400mg BD then 200mg BD + g-Natamycin or g-Voriconazole • Placebo+ g-Natamycin or g-Voriconazole Drug frequency: • Oral Voriconazole; 400mg BD *24hrs then 200mg BD* 20/7 • Drops; week 1-Houlry while patient awake; week 2-3-2 hourly while patient awake Inclusion	N=240 • 119 patients in the oral voriconazole arm • 121 patients in the placebo group Fungal species: 72 *Fusarium*, 63 *Aspergillus*, 8 *Culvuralia*, 4 *Bipolaris*, 12 Unidentified hyaline, 18 unidentified dematiaceous, 16 Other spp, 44 No growth Primary outcome • There was no difference in rate of perforation or need for TPK: HR 0.82 (CI 0.57 to 1.18) P=0.29, HR<1 favours oral Voriconazole Secondary outcome (s) • BCVA at 3 weeks and 3 months: no difference in the two groups	Randomisation Masking by having identical placebo and Voriconazole tablets Primary and secondary outcomes reported No benefit seen in adding oral voriconazole to topical antifungal treatment for severe fungal keratitis. Level of evidence: 1+

	• Confirmed fungal keratitis (KOH/culture) • Severe keratitis VA 3/60 or worse Exclusion • Mixed infections • Younger than 16 years • Bilateral disease • Pregnant • Liver disease • Allergy to antifungal medication • VA <6/60 in the fellow eye Follow-up was for 3 months Outcome measures • Perforation rate/need for TPK at 3/12 (primary) • (BSCVA) at 3/52 & 3/12 • Infiltrate and/or scar size at 3/52 & 3/12 • Time to re-epithelialization • Microbiologic cure at6days (±1day) • Complications such as endophthalmitis and evisceration.	• Infiltrate/scar size at 3 weeks and 3 months: no difference in the two groups • Time to re-epithelialization: no difference in the two groups • Cure rate at 6 days: no difference in the two groups • Rate of adverse events: statistically significant increase among the oral voriconazole Vs placebo group (58/120 Vs 28/120; P = .001)	
Oral Voriconazole Vs Oral Ketoconazole			
Sharma et al 2017 India[36]	RCT Arms • Tablets Voriconazole (VCZ) 200mg BD + g-Natamycin 5% • Tablets Ketoconazole (KCZ) 200mg BD+ g-Natamycin or g-Voriconazole Drug frequency: • Oral Voriconazole or Ketoconazole 200mg BD (duration not specified) • Drops; g-Natamycin 5% every 1 hour, for the first 48 hours, every 2 hours during waking hours until epithelial healing, and then every 4 hours for 3 weeks. Inclusion	N=50 • 25 patients in the oral voriconazole arm • 25 patients in the placebo group Fungal species: 8 *Fusarium*, 6 *Aspergillus*, 1 *Culvuralia*, 1 *Alternaria*, 1 Helminthosporium spp, 33 No growth Primary outcome • BSCVA at 3 months; oral VCZ mean Log MAR 1.3 ± 0.07 oral KCZ mean log MAR 1.5 ± 0.07, p=0.02 Secondary outcome (s) • Re-epithelialisation time VCZ 41± 11 Vs KCZ 43± 12 days, p=0.71. • Final scar size VCZ 4.47 6± 0.11 Vs KCZ 4.79 6 ± 0.11mm, p=0.03 •	Randomisation by computer Double masking, all tablets were wrapped in uniformly coloured paper Primary and secondary outcomes reported Oral VCZ more effective than oral KCZ in cases of severe fungal keratitis, Level of evidence: 1+

	• Confirmed fungal keratitis (KOH/culture) • Maximum diameter >5mm • Involving >4mm of the centre of the cornea • >50% stromal diameter Exclusion • Mixed infections • Evidence of herpetic Keratitis (history/examination) • Perforation/impending • Younger than 18 years • Bilateral disease • Pregnant/Lactating • Unwilling for follow-up • Previous antifungal treatment • Diabetes Follow-up was for 3 months Outcome measures • BSCVA at 3 months (primary) • Percentage of healed cases • Scar size		

Table 4: Results of studies on Fluconazole injection for treatment of fungal keratitis

STUDY	DESIGN	RESULTS	COMMENT
Sub conjunctival Fluconazole RCT			
Mahdy et al 2010 Egypt [37]	RCT Arms. • g-Amphotericin B and sub conjunctival Fluconazole • g-Amphotericin B alone. Drug frequency: • Fluconazole was injected daily for 10 injections and after every 48 hours for another 10 injections • Drops 2 hourly * 21/7 Inclusion: • Clinical presentation of Fungal Keratitis Exclusion: • Not clear Follow-up: 3 months Outcome measures: (primary not designated) • Healing time • Healing rate at 3/12	N=48 • 24 patients in g-Amphotericin B and sub conjunctival Fluconazole arm • 24 patients in g-Amphotericin B alone arm Fungal species: 0 *Fusarium*, 20 *Aspergillus*, 14 *Candida*, 4 *Penicillium*, 10 No growth Outcomes: • Healing time: Fluconazole group 31 days Vs Amphotericin B alone 37 days ($p<0.05$). Fluconazole had a faster healing time • Healing rate at 3/12. Fluconazole 20/24 (83%), Amphotericin B only 16/24 (67%) ($p<0.05$) • RR 1.2, 95% CI 0.9-1.7, p=0.2. RR> 1 favours Fluconazole	Methodologically this is a weak study. Inclusion and exclusion not clear Method of randomisation unclear. May not have been completely random allocation – as the two groups appear matched for fungal species. Observers were not masked to allocation. Randomisation and blinding protocol unclear Outcome measures not clear Level of evidence: 1-
Case series			
Yilmaz et al 2005 Turkey [38]	Case series • Sub conjunctival fluconazole. Drug frequency • Sub conjunctival fluconazole 2% up to 1ml BD, for at least 5/7. If necessary, therapy was continued OD for a maximum of 14/7 after 5/7 of injections. Inclusion: • Severe microscopy confirmed fungal keratitis, not responding to initial treatment (Topical fluconazole 2% hourly, Intravenous fluconazole and oral itraconazole) at 10/7. Exclusion: • Negative culture result Outcomes: (primary not designated)	N=13 Fungal species: 1 *Fusarium*, 1 *Aspergillus*, 4 *Candida*, 7 No growth Outcome (s): • Healed 13/13 • Time to heal, 6 healed in 5/7, 4 in 10/7 and 3 in 14/7 • Improvement in Visual acuity (11/13), • Hypopyon clearance time (mean 5.06 days SD 2.06 days) • Corneal ulcer resolution (mean 28.40 days SD 11.66 days) • Recurrence rate (3/13) • Complications (0%)	This series included only severe fungal keratitis cases Sub conjunctival fluconazole given as second line treatment Dose of fluconazole was not the same for all patients Follow-up time for the patients not clear Small series Level of evidence: 3

	• Healed • Healing time • Improvement in VA • Hypopyon clearance time • Corneal ulcer resolution • Recurrence rate • complications		
Dev et al 2006 India [40]	Case series • Sub conjunctival Fluconazole Drug frequency • Fluconazole Injection given for 5-40 days Inclusion: • Culture proven fungal keratitis • Not responding to topical (5% natamycin, 2% Econazole, 1% clotrimazole, 1% Itraconazole) and Oral Ketoconazole 200mg BD Exclusion: • Not clear Outcomes (primary not designated) • Eradication of the infection • Toxicity	N=33 Fungal species: 22 *Fusarium*, 10 *Aspergillus*, *Curvularia* Outcomes: • Eradication of infection 18/33 (54.05%) • Toxicity 0/33 •	Letter to the Editor All fungal species in this series were filamentous Level of evidence: 3
Mahdy et al 2010 Egypt [41]	Case series. • Topical Amphotericin B and sub conjunctival Fluconazole Drug frequency • 0.5-mL sub-conjunctival injection of fluconazole 2 mg/mL was injected daily for 10 injections and then every 48 hours for another 10 injections • Drops 2 hourly * 21/7 Inclusion: • Clinical fungal keratitis (elevated slough, feathery edges, gutter formation), • Not responding to topical treatment after 7 days Exclusion: • No clear	N= 12 Fungal species: 0 *Fusarium*, 2 *Aspergillus*, 5 *Candida*, 1 *Penicillium*, 4 No growth • Healing at 21 days; 9/12 (75%) • Complications; conjunctival haemorrhage 3/12 (25%)	Case series was mainly to assess safety and efficacy of a combination therapy of topical amphotericin B and sub conjunctival fluconazole for the treatment of fungal keratitis Pre-trial pilot Exclusion criteria not clear Included clinical fungal cases Level of evidence: 3

	Follow-up 3 months Outcome measures (primary not designated) •		

Table 5: Results of studies on Amphotericin B injection for treatment of fungal keratitis

STUDY	DESIGN	RESULTS	COMMENT
Intracameral Amphotericin B RCT			
Sharma 2015 India [42]	RCT 3 Arms • Topical 5% Natamycin and 0.15% topical amphotericin B + oral ketoconazole • Topical 5% Natamycin and 0.15% topical amphotericin B + Intracameral amphotericin B (ICAMB)+oral Ketoconazole • Topical 5% Natamycin and 0.15% topical amphotericin B + Intracameral amphotericin B (ICAMB)+oral Ketoconazole+ drainage of hypopyon Drug frequency • g-Natamycin 5% hourly during day and 2 hourly at night • g-Amphotericin B 0.15% 2 hourly • ICAMB 0.5µg in 0.1ml 5% dextrose repeated 72hours if no improvement to a maximum of 3 injections. • PO ketoconazole BD Inclusion: • Smear positive fungal keratitis • Involvement of anterior one-third or more with concomitant hypopyon. Exclusion: • One-eyed patients • children <12 years of age • Cases with keratitis limited to anterior third of the stroma, • corneal thinning (<300 mm) on anterior segment OCT • concurrent sclera involvement were excluded. Follow-up time not clear Outcomes (Primary not designated) • Treatment success • Time to disappearance of hypopyon • Time to healing • Final BCVA • Cataract • Hyphema	N=45 • 15 patients in each arm Fungal species: 2 *Fusarium*, 7 *Aspergillus*, 30 No growth Outcomes: • Treatment success rate (resolution of stromal infiltrates, healing of epithelial defect and disappearance of endothelial plaque). Topical treatment alone (11/15) Vs ICAMB (9/15), Vs ICAMB + draining of hypopyon (11/15) P=0.66 Secondary Outcomes: • Tine to disappearance of hypopyon P=0.7 • Time to heal P=0.18 • Final visual acuity P=0.8 • Presence of complications, Cataract P=0.1, Hyphema P= 0.5 Occurrence of cataract as a complication was more commonly seen in ICAMB and hypopyon wash +ICAMB groups compared to topical treatment group	Sample might have been small for the comparisons A big proportion of the fungal cases were not culture positive Randomisation done using tables It was not feasible to mask Primary and secondary outcomes not designated 3 arms might be difficult to compare Level of evidence: 1-
Sharma 2015 India [43]	Non-randomised clinical trial, 2 arms. All patients were started on topical natamycin or fluconazole. They were evaluated at one week	N=104, • 49 patients in the good response arm (topical treatment alone)	Non-randomization, selective bias per response. Group allocation was per response or no response to topical treatment at 7/7

	and additional intra cameral AMB was injected if there had been no response by one week. • Arm I showed **good response** at one week to topical 5% Natamycin or 0.3% fluconazole or topical itraconazole and tablets fluconazole • Arm II showed **No response** at one week to topical 5% Natamycin or 0.3% fluconazole or topical itraconazole and tablets fluconazole Drug frequency • Drops- Hourly during day and 2 hourly at night • ICAMB 5-10µg in 1ml of 5% dextrose repeated 3-6 days depending on the response Inclusion: • smear-positive deep fungal keratitis; • corneal ulcers presenting with history of trauma from organic matter; • ulcers not responding to topical and systemic antifungal • medications for 7 days (with history of trauma from organic matter) • abscess at presentation (with history of trauma from organic matter Exclusion: (not clear) Follow-up for 4 weeks Outcomes • Time for epithelialization/decrease in infiltrates and ulcer resolution (Primary) • Improvement in BCVA • Time for hypopyon disappearance • Scars • Complications	• 55 patients in the No response arm (ICAMB) Fungal species: Not reported Primary outcome: (no significant difference) • Time for epithelialization with a decrease in infiltrates and ulcer resolution. Topical treatment 13.08 ± 4.33 days Vs ICAMB arm 12.37 ± 5.50 days (p value not given but calculated as p=0.15) Secondary outcomes: • Improvement in best-corrected visual acuity (BCVA). Topical treatment 0.55 ± 0.30 logMAR Vs ICAMB arm 1.40 ± 0.2 logMAR. (Calculated p<0.01) • Mean time for hypopyon disappearance: Topical treatment 17.12 ± 8.7 days Vs ICAMB 13.4 ± 8.0 days (calculated p<0.01) • More scars in Topical treatment Vs ICAMB (P<0.05) • Less complications in ICAMB Vs Topical treatment (p<0.05)	No masking Primary and secondary outcomes reported Level of evidence: 1-
Gupta 2019 India[44]	Non-randomised clinical trial 2 arms of cases that were not responding to standard treatment. All patients received Intracameral Injection of Liposomal Amphotericin B (ICAMB) 10 micrograms/0.1 mL + g-Natamycin 5% + Oral Itraconazole 200mg BD. However, the timing of the ICAMB injection was different between the arms. • Arm I **early** at 2 weeks • Arm II **late** at 4 weeks Drug frequency	N=50, • 25 patients in the **early** arm (Group I) • 25 patients in the **late** arm (Group II) Fungal species: 4 *Fusarium*, 11 *Aspergillus*, 1 *Curvularia*, 1 *Bipolaris,* 32 No growth Outcome: • The mean healing time in Group I was 17.5±	Non-randomization No masking Primary and secondary outcomes reported Level of evidence: 1-

	• 0.1ml of 10µg of ICAMB repeated every 48-72 hours until a maximum cumulative dose of 50µg or resolution of infiltrates • g-Natamycin 5% hourly for 2 weeks • Oral Itraconazole 200mg BD • g-Moxiflocxacin tds • g-Atropine 1% tds Inclusion: • Confirmed fungal keratitis (Microscopy/culture) • >18 years old • infiltrates more than 5 mm in size and involving more than 2/3rd of the corneal thickness • ulcers not responding to conventional antifungal for 2 weeks Exclusion: • Not willing for follow-up • Perforation/impending • Scleral involvement • Endophthalmitis • Mixed infection • Antifungal allergy • Immune compromised • Renal disease • Liver disease Follow-up: 3 months Outcomes (Primary not designated) • Treatment success defined as resolution of the corneal infiltrate with scarring, disappearance of the corneal endothelial plaque and hypopyon, and healing of the epithelial defect • Final visual acuity • Corneal opacity Complications • Cataract, hyphema, increased inflammation, pain	3.64 days and 32.2± 8.89 days in Group II (p<0.001) • Maculo-leucomatous corneal opacity in 12 (48%) eyes in Group I versus 4 (16%) eyes in Group II (p=0.03) • leucomatous corneal opacity in 13 (52%) eyes in Group I versus 16 (64%) eyes in Group II (p=0.56) • Adherent leucoma in none of the eyes in Group A versus 5 (20%) eyes in Group II (p=0.05). • None of the eyes in Group I required additional surgical intervention while 10 eyes in Group II developed corneal perforation, thus requiring surgical intervention (p=0.006).	
Intracameral Amphotericin B Case series			
Yoon et al 2007 China [45]	Audit • Conventional topical antifungal + Intra Cameral Amphotericin B (ICAMB) • Conventional topical antifungal alone (0.15% amphotericin B and 1% fluconazole and PO fluconazole 200 mg/d.	N=32 • 14 ICAMB • 17 topical alone	Non randomised-topical antifungal group was treated July-Dec 2005, ICAMB Jan-June 2006 Primary and secondary outcomes not designated

	Drug frequency • 10 µg of amphotericin B in 0.1 mL into the anterior chamber single dose Inclusion • Fungal keratitis • Minimum follow-up of 3/12 Exclusion: • Not clear Outcomes (primary not designated) • Time to heal of epithelial defect • Time to disappearance of hypopyon • Final VA • Time to final improvement • Final outcome • Side effects	Fungal species: 10 *Fusarium*, 7 *Aspergillus*, 3 *Alternaria*, 1 *Curvularia*, 6 *Candida*, 4 Not identified Outcomes: • Mean final visual acuity (log MAR) ICAMB 1.6 ± 1.1 Vs Topical alone 1.3 ± 1.4 (P = 0.24) • Time to hypopyon disappearance, Group ICAMB 9.4+/-9.4 Vs Topical alone 26.7± 21.3 (P=0.03) • Time to epithelial defect closure, ICAMB 19.8± 10.4 Vs Topical alone 32.6 +/-22.8 (P = 0.08) • Time to final improvement, ICAMB 26.6 ± 9.2 Vs Topical alone 52.8 ± 38.2 days (P = 0.04) • Treatment success, ICAMB 92.9% Vs Topical alone 82.4% (P = 0.38) ICAMB had a significantly better time to final improvement and faster hypopyon clearance compared to topical alone	Study not powered to test differences Level of evidence: 2-
Yilmaz 2007 Turkey [82]	Case series • Intracameral Amphotericin B Drug frequency • ICAMB 0.5µg in 0.1ml 5% dextrose repeated PRN 1-10 days apart Inclusion • Fungal keratitis not responding to 0.3% topical and intravenous fluconazole and oral itraconazole for 14/7 Exclusion: Not clear Follow-up: 6 months Outcomes (Primary not designated) • Treatment success defined as resolution of the corneal infiltrate, disappearance of the endothelial plaque, and healing of the epithelial defect • Complications	N=14 eyes of 12 patients Fungal species: 2 *Fusarium*, 4 *Aspergillus*, 2 *Candida*, 7 No growth Outcomes • Treatment success; 12/14 • Complications rate; 4/14 (cataract)	This study had a small sample size Included only severe cases Level of evidence: 3
Intracameral & Intrastromal Amphotericin B Case series			
Hu 2016 China [83]	Case series • Intracameral Amphotericin B	N=9	Small sample size

	Drug frequency • ICAMB 50µg/0.1ml + Intrastromal 25µg/0.1ml Injections were repeated PRN until resolution. • Intrastromal injections were repeated after more than 5/7, and intracameral after more than 3/7 Inclusion • Fungal keratitis not responding to 0.5% topical fluconazole combined with 5% natamycin or 0.25 topical amphotericin B and oral itraconazole for 7/7 • Fungal keratitis presenting with serious corneal damage and intraocular extension Exclusion • Cases that had some involvement of adjacent sclera, frank corneal perforation, shallow anterior chamber, and presence of intravitreal fungal mass by B- ultrasound scanning were excluded Follow-up 2-4 months Outcomes (primary not designated) • Treatment success defined as resolution of the corneal infiltrate, disappearance of the anterior chamber inflammation, and healing of the epithelial defect • Complications rate • Recurrence rate	Fungal species: 3 *Fusarium*, 1 *Aspergillus*, 1 *Alternaria*, 1 Unidentified, 2 No growth Outcomes: • Treatment success. 9/9 • Complications rate: Bleeding 2/9, uveitis 9/9, secondary glaucoma 6/9, cataract 9/9 • Recurrence rate: 0/9	Mixed routes for amphotericin injection (intrastromal and intracameral) Difficult to conclude on which route had the most effect A significant number of complications were reported in this study Level of evidence: 3
Intrastromal Amphotericin B			
Nada et al 2017 Egypt [47]	2-year Audit (2015-2016) • Group A: Intrastromal Amphotericin +g-Fluconazole 2%. 47 cases resistant to topical treatment (g-Natamycin 2.5% (12), g-Amphotericin B 0.3 mg/mL (11), g-Itraconazole 2% (13), and g-fluconazole 2% (5) • Group B: treated with topical amphotericin B (Fungizone 50 mg vial) 0.3 mg/mL in 5% dextrose five times daily, in addition + regular debridement of the ulcer every 48 hours to facilitate penetration of the drug. Drug frequency: • Intrastromal Amphotericin B 2–3 mid stromal injection sites around the ulcer with 0.1–0.15 mL containing 2–3 µg of amphotericin B (single dose)	N=68 • 41 patients received Intrastromal Amphotericin B (Group A) • 27 patients received g-Amphotericin B (Group B) Fungal species: 11 *Fusarium*, 17 *Aspergillus*, 31 *Candida*, 5 *Alternaria*, 4 *Penicillium* Outcome (s) RR > 1 favours intrastromal Amphotericin • Complete healing: Group A 34/41 (82.9%), Group B 16/27 (59.3%). RR 1.4 95% CI 0.7-3.1, p=0.3	No randomisation was done Patients were allocated depending on their response rate Level of evidence: 2-

	• Topical drops; five times daily Inclusion • Culture positive fungal keratitis Exclusion not clear Follow-up 2 months Outcomes (primary not designated) • Complete healing • Mean duration of healing • Burning sensation	• Mean duration of healing: Group A 24± 6.42 days, Group B 39.66± 13.6 days, (calculated p<0.01) • Burning sensation: Group A 4/41 (9.8%), Group B 11/27 (40.7%). RR 5.6 95% 2.0-15.4 p<0.01 Intrastromal Amphotericin B had a significantly better healing rate, faster healing time and less burning effect than topical amphotericin B)	

Table 6: Results of studies on Voriconazole injection for treatment of fungal keratitis

STUDY	DESIGN	RESULTS	COMMENT
Intrastromal Voriconazole RCT			
Sharma 2013 India [48]	RCT 2 Arms • **Arm I**: g- Voriconazole 1% + g-Natamycin 5% • **Arm II**: Intrastromal Voriconazole injections + g-Natamycin 5% Drug frequency • Drops 2 hourly while awake for 72 hours then 4 hourly • Five injection spots of intrastromal voriconazole (50µg/0.1 ml). At least 3 injections were given 72 hours apart Inclusion: • Smear- or culture-proven fungal ulcers + • Larger than 2mm + • Involving up to 2/3 of the stromal thickness + • Not showing any signs of clinical improvement after 2 weeks of topical natamycin therapy. (Increase in size of epithelial defect, a decrease of less than 20% of stromal infiltrate or scar complex, or increasing hypopyon) Exclusion: • Mixed infection on smear or culture analysis • Evidence of herpetic keratitis in history or upon examination • Impending perforation • Bilateral ulcers • Those with vision less than 6/60 in the fellow eye • Patients younger than 18 years Follow-up 3 months Outcomes • BSCVA at 3 months (primary) • Time to healing • Size of scar • Perforation rate	N=40 • 20 patients in each arm Fungal species: 7 *Fusarium*, 12 *Aspergillus*, 5 *Curvularia*, 1 *Alternaria*, 15 No growth Primary outcome: • Best spectacle-corrected visual acuity (BSCVA) at 3 months. It was significantly better in the topical arm: Mean BSCVA g-VOR 1.295 ± 0.5 log MAR Vs intrastromal VOR 1.692 ± 0.29 log MAR (p=0.008) Secondary Outcomes: • Time to healing. g-VOR 28.9 ± 19.1 days Vs intrastromal VOR 36.1 ± 20.0 days (p=0.38) • Size of the scar. g-VOR 4.36 ± 1.38 mm Vs intrastromal VOR 5.3 ± 1.4 mm (p=0.06) • Perforation rate. g-VOR (1/20) Vs intrastromal VOR (4/20) (p=0.22). RR >1 favours g-VOR. RR=4, 95% CI 0.4-32.7, p=0.2	Randomisation done by variable block Masking was impossible Primary and secondary outcomes clearly reported Sample size may not have been adequate for some of the secondary outcomes Level of evidence: 1+
Narayana 2019 India[84]	RCT 2 Arms • **Arm I**: Intrastromal Voriconazole (ISV) + g-Natamycin 5% • **Arm II**: g-Natamycin 5% alone	N=70 • 35 patients in each arm	Randomisation done by 1:1 fashion Outcome masking possible for examining physicians, microbiologist, optometrist at 3months,

	Drug frequency • 3-5 injection spots of intrastromal voriconazole (50µg/0.1 ml) repeated on day 3 and 5 • Frequency of g-Natamycin not mentioned Inclusion: • Smear- or culture-proven fungal ulcers + • Visual acuity worse than 20/70 (Log MAR 0.54) Exclusion: • Mixed infection • Impending or perforation • Limbal involvement • NPL in affected eye • Those with vision less than 20/200 (6/60) in the fellow eye • Patients younger than 18 years or older than 70 years • Patients who were cognitively impaired • Patients unable to complete follow-up Follow-up 3 months Outcomes • Microbiological cure at 3 days (primary) • BSCVA at 3 weeks & 3months • Size of infiltrate/scar at 3 weeks & 3months • Perforation rate/need for TPK • Microbiological cure at 7 days	Fungal species: 19 *Fusarium*, 17 *Aspergillus*, 4 *Curvularia*, 15 *Others*, 13 No growth Primary outcome: • Microbiological cure at 3 days. Culture negative results 21/35 (60%) in g-Natamyicn + ISV Vs 23/35 (68%) in g-Natamycin only group. aOR of a culture positive result in the ISV arm Vs g-Natamycin only arm was 1.82 (95% CI 0.65-5.83; p=0.26) Secondary Outcomes: • BSCVA at 3 weeks. Those randomized to ISV injection showed 1.6 log MAR (approximately 1.5 Snellen lines) worse visual acuity at 3 weeks after controlling for baseline visual acuity (95% CI, -1.2 to 4.4 log MAR; p=0.25) • Size of the infiltrate/scar was 0.69 mm larger among those who were randomized to ISV injection after controlling for baseline measurements (95% CI, 0.04-1.33 mm; p=0.04). • Perforation rate. ISV (8/35) Vs intrastromal VOR (3/35) aHR=2.85 (95% CI 0.76-10.75, p=0.12) • Microbiological cure at 7 days; aOR of a culture positive result in the ISV arm Vs g-Natamycin only arm was 1.98 (95% CI 0.69-5.91; p=0.20)	Primary and secondary outcomes clearly reported Sample size may not have been adequate for some of the secondary outcomes Level of evidence: 1+
Intrastromal Voriconazole Case series			
Sharma 2011 India [85]	Case series • Intrastromal Voriconazole (50µg/0.1ml) Drug frequency • Week 1-2: g-Natamycin 5% 2 hourly, if no improvement, • Week 2-4: g-Natamycin 5% + g-Voriconazole 1% 2 hourly + PO Voriconazole 200mg BD, if no improvement, • Week 4: Five injection spots of intrastromal voriconazole (50µg/0.1 ml) repeated 72hours if no improvement. In addition topical and PO treatment	N= 12 Fungal species: 3 *Fusarium*, 8 *Aspergillus*, 1 *Curvularia* Outcomes • Healing at 3 months (10/12) • Improvement in BCVA at 3 months (10/12)	Included only fungal cases resistant to g-Natamycin and Voriconazole + PO Voriconazole Level of evidence: 3

	Inclusion • Proven fungal keratitis non-responsive on 4 weeks of topical Natamycin (5%) and 2 weeks of topical Voriconazole (1%) and oral Voriconazole (200 mg BD) Exclusion • Involvement of adjacent sclera • Impending or frank corneal perforation • Presence of descemetocele • Concomitant endophthalmitis Follow-up: Follow-up 3 months Outcomes • Healing at 3 months • Improvement in BCVA at 3 months	Intrastromal Voriconazole had good response to fungal cases resistant to g-Natamycin, g-Voriconazole and oral Voriconazole.	
Kalaiselvi 2015 India [51]	Case series • Intrastromal Voriconazole (50 µg/0.1 mL) Drugs frequency: • Week 1-2: g-Natamycin 5% 2 hourly, if no improvement, • Week 2-4: g-Natamycin 5% + g-Voriconazole 1% 2 hourly + PO Voriconazole 200mg BD, if no improvement, • Week 4: Five injection spots of intrastromal voriconazole (50µg/0.1 ml), repeated within 1 week if no imprvement Inclusion • Proven fungal keratitis non-responsive on 4 weeks of topical Natamycin (5%) and 2 weeks of topical Voriconazole (1%) Exclusion • Mixed corneal infections • Perforated corneal ulcers or those with impending perforation • Presence of descemetocele • Involvement of the adjacent sclera • Ulcers with clinical features of non-infective and autoimmune conditions • Fungal ulcer associated with endophthalmitis • Patients <16 years and beyond 80 years • Patients with one eye. Follow-up: 3 months	N=25 Fungal species: 13 *Fusarium* , 4 *Aspergillus*, 1 *Curvularia*, 2 *Exserohilum*, 2 Unidentified hyaline fungus, 3 Unidentified Outcomes • Healing at 3 months (18/25) • Improvement in BCVA (16/25) Intrastromal Voriconazole had good response to fungal cases resistant to g-Natamycin, g-Voriconazole	Included only fungal cases resistant to g-Natamycin and Voriconazole + PO Voriconazole Level of evidence: 3

	Outcomes (primary not designated) • Treatment success-healing with scar, improvement in vision • Treatment failure-non healing, progression, perforation		
Nagar 2015 India [52]	Case series • Intrastromal Voriconazole (50 µg/0.1 mL) Drug frequency • Week 1-2: g-natamycin 5% 2 hourly and PO itraconazole 100mg BD • Week 2: Five injection spots of intrastromal voriconazole (50µg/0.1 ml) as one time dose Inclusion • Fungal keratitis involving deep corneal stroma not responding to 5% topical natamycin and oral itraconazole Exclusion • Perforated corneal ulcer • Anaesthetic cornea • Lagophthalmos Follow up: variable Outcomes (primary not designated) • Improvement in visual acuity at 4 months (20/30) • Resolution of the infection (26/30) • Healing time, Mean 5 weeks +/-1 week	N=30 Fungal species: 7 *Fusarium*, 7 *Aspergillus*, 2 *Candida* 14 No growth Outcomes • Improvement in visual acuity at 4 months (20/30) • Resolution of the infection (26/30) • Healing time, Mean 5 weeks ± 1 week	Not sure if this was peer reviewed Level of evidence: 3
Intracameral Voriconazole			
Shen 2010 China [53]	Case series • Intracameral Voriconazole 100µg/0.1ml Drug frequency • Intracameral Voriconazole 100µg/0.1ml OD until resolution (1-8 injections) Inclusion • Proven fungal keratitis progressing to endophthalmitis on 5% topical Natamycin, 0.15% topical Amphotericin B, 1% Voriconazole, 200mg Itraconazole	N=10 Fungal species: 6 *Fusarium*, 2 *Aspergillus*, 1 *Alternaria*, 1 *Acremonium* Outcomes • Resolution (6/10)	Included patients with severe fungal keratitis with AC spread Level of evidence: 3

	Exclusion • Not clear Follow-up 4 months Outcomes • Resolution • TPK Injection given OD until resolution of the AC fungal infiltrate		
Intracameral + Intrastromal Voriconazole			
Killani 2015 India [86]	Case series • Intracameral and intrastromal voriconazole (50 µg/0.1 mL) Drug frequency • Week 1-3, g-Natamycin 5% & g-Voriconazole 1% 2 hourly, + PO itraconazole 100mg BD • Week 2-3 if no improvement, ring intrastromal voriconazole 50µg/0.1ml and intracameral 50µg/0.1ml. A second injection was given 48 hours later if no clinical response. Not more than 2 injections of intracameral and intrastromal Voriconazole were given. Inclusion • Proven fungal corneal ulcers with deep stromal infiltrates and endothelial plaque not responding to routine antifungal drugs (5% Natamycin, 1% Voriconazole, oral Itraconazole) Exclusion • Perforated ulcers • Children < 10 Follow-up not given Outcomes (primary not designated) • Time of healing after the injection • Clinical outcome-scar formation, perforation • Improvement in visual acuity	N=30 Fungal species: 15 *Fusarium*, 12 *Aspergillus*, 3 *Candida* Primary Outcome • Resolution of infection (25/30) • Perforation (5/30) • Time of healing 4-12 weeks •	This study has combined interventions, intrastromal and AC voriconazole Level of evidence: 3

Table 7: Results of studies on surgical options for fungal keratitis

STUDY	DESIGN	RESULTS	COMMENT
TPK-AUDIT			
Bajracharya 2015 Nepal [55]	5-year audit study, 2006-2010 Inclusion: • All infectious MK that underwent TPK Exclusion: • Patients with <2 months follow-up Patients who had a second TPK, only their first was considered for outcome assessment Follow-up: variable (2months minimum) Outcomes (primary not designated) • Anatomical stability • Recurrence • Graft Clarity • Development of glaucoma	N=180, Culture proven Fungal (n)=49 Fungal species: 13 *Fusarium*, 23 *Aspergillus*, 5 *Cladosporium*, 8 *Unidentified* Indications: • Perforation (128/180) • Impending • Non-healing Outcome (s): • Anatomic stability (34/44) • Recurrence (13/49) • Graft clarity (9/34) • Development of glaucoma (22/34)	All aetiologies of MK were included but was disaggregated by aetiology. We restricted outcome data to fungal proven cases 5 fungal keratitis patients were not analysed for anatomical success because they had < 2 months' follow-up. Level of evidence: 3
Palaksha 2015 India [56]	2-year audit study, 2012-2014 Inclusion: • Non-healing fungal keratitis Exclusion: • Posterior segment disease • Glaucoma Follow-up 6 months Outcomes (primary not designated) • Anatomical integrity • Recurrence • Visual acuity improvement • Graft clarity • Complications rate	N=25 Fungual species: Culture not done, cases were identified by KOH Indication: • Perforation (7/25) • Non-healing (14/25) Outcome (s): • Anatomical integrity (23/25) • Recurrence (7/25) • Visual acuity improvement (22/25) • Graft clarity (9/25) • Complication (epithelial defect 10/25, glaucoma 7/25, cataract 2/25, scleral abscess 2/25)	Level of evidence: 3
Sharma 2014 India [57]	10-year audit 1999-2009 Inclusion • All patients > 14 years who underwent TPK • Patients with at least 1 year follow-up	N= 506, fungal cases n=106 Fungal species: 42 *Aspergillus*, 64 other fungal species	All aetiologies of MK were included but was disaggregated by aetiology. Different fungal species not mentioned

	Exclusion • Patients with follow-up < 1 year • Patients < 14 years Follow-up mean 26.7 months Outcomes: (primary not designated) • Anatomical success • Visual success • complications	Indication: • Infiltrates progressing to deeper stroma/limbus or sclera despite appropriate treatment • cases of impending perforation/perforation. Outcome (s): • Anatomical success; 94/106 • Visual success (VA>6/60), 6/106	We restricted outcome data only to fungal cases. Data on complications was not disaggregated Proportions of indication not mentioned Level of evidence: 3
Barut 2014 Turkey [58]	7-year audit study, 2006-2013 Inclusion: • All culture proven fungal keratitis that underwent TPK Exclusion: • Not mentioned Mean follow-up was 14 months Outcomes (primary not designated) • Anatomical stability • Recurrence rate • Graft clarity • Evisceration • Phtisis Bulbi	N=17 Fungal species: 6 *Fusarium*, 4 *Aspergillus*, 4 *Acremonium*, 1 *Candida*, 1 *Penicillium*, 1 *Colletotrichum* Indications: • Corneal perforation (4/17) • Severe disease (13/17) Outcome (s) • Anatomical stability (11/17) • Recurrence rate (8/17) • Graft clarity (5/17) • Evisceration (4/17) • Pthisis Bulbi (2/17)	Level of evidence: 3
Lui 2013 China [59]	2-year audit study, 2007-2009 Inclusion: • All culture proven fungal MK who underwent PKP Exclusion: • Not mentioned • Mean follow-up 28 months Outcomes (primary not designated) • Graft clarity • Recurrence	N= 19 Fungal species: 4 *Fusarium*, 14 *Aspergillus*, 1 *Candida* Indications • Ulceration deteriorated or did not improve after intensified antifungal Outcome (s) • Graft clarity (18/19) • Recurrence (1/19)	Minimal trephination technique used Level of evidence: 3
Xie 2007 China [60]	5-year audit study, 1999-2004 Inclusion • Fungal keratitis that underwent TPK	N= 52 Fungal species: 35 *Fusarium*, 3 *Aspergillus*, 8 other filamentous, 2 *Candida,* 4 No growth	Level of evidence: 3

	Exclusion • Not mentioned Follow-up: 2 weeks-24 months (mean 12 months Outcomes (primary not designated) • Complications: graft rejection, recurrence, cataract, glaucoma, graft ulcer • Improvement in visual acuity	Indications: • Not mentioned Outcome (s) • Complications; immune graft rejection (20/52), fungal recurrence (8/52), Cataract (10/52), glaucoma (7/52), graft ulcer (3/52) • Improvement in visual acuity; 46/52	
Chen 2003 Taiwan [61]	14-year audit study, 1987-2001 Inclusion: • All patients who underwent TPK Exclusion: • Herpetic • Re-grafts for surgically uncontrolled microbial keratitis within 3 months after first therapeutic PKP were excluded • Patients with <1month's follow-up Follow-up variable (minimum 1 month) Outcomes (primary not designated) • Graft clarity at 1 month and 1 year post op • Cure rate • Anatomical success rate • Recurrence rate	N= 108, Culture proven fungal (n)=52 Fungal species: 13 *Fusarium* , 21 *Aspergillus*, 5 *Candida*, 4 *Acremonium*, 4 *Paecilomyces*, 3 *Cephalosporum*, 2 *Mycelium*, Indications • Perforation or impending perforation • Unresponsiveness and progression of • the infection after extensive medical treatment • Impending scleral involvement of the infection Outcome (s) • Graft clarity rate at 1 year postoperative (20/39) • Cure (36/52) • Anatomical success rate (44/52) • Recurrence (20/52)	All aetiologies of MK were included but was disaggregated by aetiology. We restricted outcome data only to fungal cases Patients who had a second TPK, only their first was considered for outcome assessment Specific proportions of the indications were not given Level of evidence: 3
Yao 2003 China [62]	6-year audit study, 1995-2001 Inclusion: • Post TPK with cryo-preserved donor cornea • Culture proven fungal keratitis Exclusion: • <6/12 follow-up Follow-up: 7-37 months Outcomes (primary not designated) • Control of infection • Glaucoma	N=45 Fungal species: 14 *Fusarium*, 12 *Aspergillus*, 7 *Verticillium*, 5 *Microsporum*, 7 Others Indication (s) • AC collapse by fibrinoid membrane formation post hypopyon resorption (29/45) • Perforation (14/45) • Extensive suppuration (1/45) • Large infiltrate >8mm (3/45) Outcome (s) • Infection control (39/45)	No explanation on why all the 39/45 did not receive optical PKP Some of the eyes had more than 1 indication for TPK Level of evidence: 3

	• Enucleation • Optical PKP	• Glaucoma (4/45) • Enucleation (2/45) • Optical PKP (23/45)	
Xie 2001 China [63]	3-year audit study, 1996-1999 Inclusion: • All fungal keratitis who underwent TPK Exclusion: • Not clear Follow-up 12-24 months Outcomes (Primary not desgniated) • Graft clarity • Recurrence • Failure • Enucleation • Cataract • Glaucoma	N=108 Fungal species: 63 *Fusarium*, 14 *Aspergillus*, 9 *Candida*, 4 *Penicillium*, 7 Unidentified, 11 No growth Indication: • Ulcer < 6 mm & not cured on intensive medical treatment • Ulcer was 6–8mm & the infection continued to progress during 72 hours of intensive medical treatment • Ulcer > 8 mm or corneal perforation/impending perforation Outcome (s) • Graft clarity (86/108) • Recurrence (8/108) • Failed grafts (15/108) • Enucleation (4/108) • Cataract (5/108) • Glaucoma (2/108)	Specific proportions of the indications were not given Level of evidence: 3
Cristol 1996 USA [64]	8-year audit study, 1979-1987 Inclusion • All patients who underwent PKP in the study period Exclusion • Non-MK patients Follow-up: mean was 13.6 months (3 weeks-5 years) Outcomes (primary not designated) • Final visual acuity • Graft survival time • Failure rate	N=21, fungal cases n=10 Fungal species: 9 *Fusarium*, 1 *Aspergillus* Indications • Perforation (21) • Impending perforation (5) Outcome (s) • VA on the last visit; 3/60-NPL • Time to graft failure: Median was 4 weeks • Graft failure rate (9/10)	Bacterial and fungal aetiologies of MK were included but results were disaggregated by aetiology. We restricted outcome data only to fungal cases Indications were more than N because some patients had more than 1 graft Level of evidence: 3
Killingsworth 1993 USA [65]	9-year audit study, 1980-1989 Inclusion	N= 70 fungal cases n=15	All aetiologies of MK were included but results were disaggregated by aetiology.

	• All cases who underwent PK Exclusion • Patients whose follow-up was <1month Follow-up: mean 9.2 months Outcomes (primary not designated) • Graft clarity • Cure of the disease	Fungal cases: 6 *Fusarium*, 3 *Candida*, 6 Other filamentous fungi Indications: • Advanced infections not responding to medical treatment (15/15) Outcome (s) • Graft clarity (9/15) • Cure of the disease (15/15)	We restricted outcome data only to fungal cases Level of evidence: 3
THERAPEUTIC KERATOPLASTY (TPK) VS LAMELLAR KERATOPLASTY (LKP)			
Singh 1972 India [67]	5-year audit study, 1964-1969 • Group I: 10 patients TPK • Group II: 7 patients Lamellar KP (LKP) Inclusion: • Not clear Exclusion: • Not clear Follow-up not clear Outcomes (primary not designated) • Improvement in VA • Recurrence of infection • Graft clarity	N=17, 10 patients underwent TPK 7 patients underwent LKP Fungal species: 2 *Fusarium*, 7 *Aspergillus*, 5 *Candida*, 2 *Penicillium* Indications • Massive hypopyon • Perforation Outcomes: RR > 1 favours TPK • Improvement in VA (TPK: 3/10 Vs LKP 2/7), RR 1, 95% CI 0.5-1.8, p=0.9 • Recurrence of Infection (TPK: 4/10 Vs LKP 6/7) RR 2.1 95% CI 0.9-4.8, p=0.06 • Graft clarity (TPK: 3/10 Vs LKP: 1/7), RR 1.2 95% CI 0.7-2.0, p=0.4 • TPK has significantly less recurrence of infection compared to LKP. TPK also had better outcomes on improvement in VA and graft clarity although not significant	This was a non-randomised study that compared two keratoplasty options Specific proportions of the indications were not given Level of evidence: 2-
LAMELLAR KERATOPLASTY (LK) FOR FUNGAL KERATITIS			
Xie 2008 China [70]	7-year audit study, 1998-2005 Inclusion • Patients with fungal keratitis who underwent LK Exclusion	N=218 Fungal species: 142 *Fusarium*, 26 *Aspergillus*, 9 *Alternaria*, 8 *Candida*, 5 *Penicillium*, 28 Unidentified, 23 No growth	A large number of patients were reviewed in this audit Level of evidence: 3

	• Not clear Follow-up: 20 months Outcomes • Successful treatment (clear graft, no recurrence, improved vision)	Indication • Uncontrolled infection with intensive antifungal therapy but did not penetrate into the anterior chamber (218/218) Outcome (s) • Successful treatment (201/218)	
Xie 2007 China [87]	6-year audit study, 2000-2006 Inclusion • Culture +ve fungal infection • Post Lamellar keratoplasty (LK) Exclusion • Mixed infection Follow-up: 1 month Outcome • Recurrence rate	N=174 Fungal species: 148 *Fusarium*, 11 *Aspergillus*, 8 *Alternaria*, 4 *Penicillium*, 3 *Candida* Indication (s) • Only the upper or middle corneal stroma was infected, • Not improving after 1 week of antifungal medications • Uncorrected VA less than 20/200 Outcome • Recurrence rate (15/174)	Level of evidence: 3
Xie 2001 China [69]	1-year audit 1998-1999 Inclusion • Post LK fungal keratitis Exclusion • Infection already spread into the AC Follow-up: 8-18 months Outcome (Primary not designated) • Curing fungal keratitis • Surgical complication rate	N=55 Fungal species: 33 *Fusarium*, 6 *Aspergillus*, 3 *Candida*, 1 *Penicillium,* 9 No growth Indication (s) • Fungal keratitis not controlled in 7 days on antifungal medication (55/55) Outcomes • Curing fungal keratitis (51/55) • Surgical complication rate (0/55)	46/55 were culture positive for fungus. Other diagnosis was made clinically and with confocal microscopy Level of evidence: 3
LKP CASE SERIES			
Gao 2013 China [66]	Case series Inclusion • Deep infectious purulent Keratitis • Infiltrate >4/5 of corneal thickness	N= 17, fungal cases n=14 Fungal species: Indications	Cases of fungal and bacterial keratitis were included but results were disaggregated DALK assisted by big bubble technique was used

	Exclusion • Not mentioned Follow-up: 9 months Outcomes (primary not designated) • Perioperative complications • Recurrence • Graft status transparency • Visual recovery	Outcome (s) • Perioperative complications • Recurrence (1/14) • Graft status transparent (14/14) • Visual recovery (14/14)	We restricted outcome data only to fungal cases Level of evidence: 3
LAMELLAR KERATOPLASTY WITH PORCINE CORNEA			
Zhang 2015 China [68]	Case series study: TPK using acellular porcine corneas Inclusion: • Proven fungal keratitis • Not responding to antifungal medications • At risk of perforation Exclusion: • Not indicated Follow-up: 6 months Outcomes • Improvement of BCVA by 2 lines • Recurrence • Severe neo-vascularisation	N=47 Fungal species: Not reported Indications • Unresponsive to antifungal medication • At risk of perforation Outcome (s) • Improvement of BCVA by 2 lines (34/47) • Recurrence (0/47) • Severe neo-vascularisation (7/47)	Diagnosis was by microscopy and confocal. No culture results were reported Level of evidence: 3
AMNIOTIC MEMBRANE GRAFT (AMG)			
Chen 2006 China [71]	8-year audit study, 1994-2002 Inclusion: • Culture confirmed fungal keratitis who received AMG Exclusion: • Not mentioned Follow-up was 6-65 months Outcomes • Immediate improvement in visual acuity • Epithelial healing rate in days • Treatment failure necessitating therapeutic penetrating keratoplasty (TPK);	N=23 Fungal species: 10 *Fusarium*, 3 *Aspergillus*, 4 *Candida*, 6 other Indications: • Corneal perforation (8/23) • Descemetocele (8/23) • Deep ulcer (95% stromal loss with poor reepithelialisation) (7/23) Outcome (s): • Immediate improvement in visual acuity (14/23) • Epithelial healing rate in days (6-26 days) • Treatment failure necessitating therapeutic	Individuals who had concomitant bacterial infections were treated and included Final visual acuity improved in 17/23 cases. However, other surgeries were preformed to improve vision in 3 cases All cases who developed glaucoma had corneal perforation with AC collapse on presentation Double layered AMG was done with people who had collapsed AC, the rest had single layer AMG Level of evidence: 3

	• The persistence of infection • Subsequent surgeries necessary for visual recovery • Other complications such as secondary glaucoma., graft failure	penetrating keratoplasty (TPK); (3/23) • The persistence of infection (2/23) • Subsequent surgeries necessary for visual recovery (11/23) • Other complications such as secondary glaucoma. (4/23), graft failure (3/23)	
AMNIOTIC MEMBRANE GRAFT (AMG) VS BIPEDICLE CONJUNCTIVAL FLAP (CF)			
Abdulahimm 2015 Egypt [72]	RCT arms • AMG • CF Inclusion • Culture positive non-viral cases Exclusion • Not clear Follow-up 6 months Outcome • Primary: location, size and depth of the lesion, epithelialisation time and persistence of infection. • Secondary outcome measures included visual acuity and other complications	N=40 • 20 patients had AMG, 13 were fungal cases 13 • 20 patients had CF, 12 were fungal cases Fungal species: 9 *Fusarium*, 5 *Aspergillus*, 6 *Candida* (6), 5 Other filamentous Indications: • >50% stromal loss with poor re-epithelialisation) • Descemetocele or corneal perforation • Medical treatment failure (no improvement after 2 weeks from intensive medical therapy). Outcome (s) specific outcome data was not disaggregated Primary • Epithelialisation time; No difference • Persistence of infection; No difference Secondary • Visual acuity; No difference • Complication; No difference	Cases of fungal and bacterial keratitis were included but some of the results were disaggregated Computer generated randomisation was done Masking was not done Primary and secondary outcomes clearly stated Specific proportions of the indications were not given Level of evidence: 1-
CONJUNCTIVAL FLAP			
Zhong 2018 China[73]	Case series • Full Thickness Conjunctival flap Covering Surgery (FCCS) Inclusion • Culture positive FK • Poor response to topical therapy at 1 month (g-Natamycin, g-Fluconazole, occ-Fluconazole, PO Voriconazole 300mg BD) • Increasingly large lesion an increasingly large lesion	N=17 Fungal species: 6 *Fusarium*, 4 *Aspergillus*, 1 *Curvularia*, 4 Other filamentous, 2 No growth Indications: • Poor response to medical treatment	Prospective design used Small numbers Moderate follow-up period Evidence level: 3

	involving the entire cornea • Gradual thinning of the cornea with no observable perforation by fluorescein staining • No endophthalmitis according to B-scan ultrasounds Exclusion • Corneal perforation was present • Endophthalmitis detected by B-scan ultrasound • Hypopyon present in the eye • Good response with antifungal medication Follow-up 12 months Outcome (Primary not desginated) • BCVA • Raised IOP • Eye loss	7 patients received sclerokeratoplasty 3 months after FCCS 8 patients received conservative medication Outcome (s) specific outcome data was not disaggregated • Raised IOP 0/15 • Eye loss 2/17	

Table 8: Results of studies on corneal cross linking for treatment of fungal keratitis

STUDY	DESIGN	RESULTS	COMMENT
CXL RCT			
Uddaraju 2015 India [74]	RCT 2 arms • CXL + topical antifungal • Topical antifungal Procedure • Dresden protocol followed. • Aseptic precautions followed • Topical anaesthesia with 0.5% proparacaine (Aurocaine; Aurolab, Madurai, India). • For the first 30 minutes the cornea was soaked with 0.1% riboflavin drops (riboflavin dextran solution; Intacs XL, Dorset, UK) every 2 minutes. Over the next 30 minutes the cornea was exposed to ultraviolet-A radiation of 370 nm with 3 mW/cm2 (IROC-UVX, Zurich,Switzerland) along with continued instillation of riboflavin drops. • Topical antifungal therapy was restarted 1 hour after the procedure. Inclusion: • Culture positive fungal keratitis +, • Not improved after 2 weeks of medical treatment + • Infiltrate 5mm or more in diameter + • Infiltrate involving posterior 2/3 of the cornea Exclusion: • Not clear Outcomes • Treatment failure at 6 weeks (primary) • Perforation rate (primary) • Uncorrected VA (secondary) Follow-up: 6 weeks	N=13, • 6 patients received CXL + topical antifungal • 7 patients received topical only Fungal species: 3 *Fusarium*, 4 *Aspergillus* 4 Unidentified hyaline, 2 Unidentified dematiaceous Primary outcome: (RR> favours CXL) • Treatment failure at 6 weeks (perforation and/or increase in infiltrate by more than 2mm). CXL 5 Vs Topical 4 (p=0.56). RR 0.6 95% CI 0.3-1.4, p=0.3 • Perforation rate CXL 4 Vs Topical 0 (p=0.02) RR 0.1 95% CI 0.0-1.5, p=0.09 Secondary Outcomes: • Uncorrected VA at 6 weeks CXL HM (6/60-LP) Vs Topical 2/60 (6/12-HM) (p=.08)	Computer generated randomisation was done Allocation concealment done The trial was stopped prematurely due to the perforation rate in the CXL group. Primary and secondary outcomes were reported This study included only deep severe unresponsive cases Level of evidence: 1+

Wei 2019 China[75]	RCT 2 arms • CXL combined with antifungal medications (CXL-M) group • Antifungal medication alone (M) group Procedure • Aseptic technique • A topical anesthetic (oxybuprocaine hydrochloride • 0.4%, Santen Pharmaceutical Co., Ltd.) was instilled three times • The epithelium and the necrotic tissue of the ulcer were removed with a hockey knife; • Riboflavin drops (Medio-Cross riboflavin/dextran solution, 0.1%) were instilled on the ulcer of corneal every 3 min for 30 min. • The cornea was irradiated for 30 min using a Phoenix UV-A system (Peschke Meditrade GmbH, Huenenberg, Switzerland) at 365 nm with an irradiance of 3 mW/cm2 and a dose of 5.4 J/cm2. During the period of UV-A exposure, riboflavin was dropped onto the cornea every 1.5 min. • Topical antifungal (g-Natamycin + g-Voriconazole) therapy was restarted 2 hours after the procedure. Inclusion: • Culture/IVCM positive fungal keratitis Exclusion: • Perforated corneal ulcer • Corneal melting or perforation • Endophthalmitis • Collagen vascular disease • Corneal descemetocele • Immune diseases • Diabetes • Pregnancy Outcomes (Primary not designated) • Visual acuity • Area of the ulcer • Duration of the ulcer • Time to non-observed fungal hyphae by IVCM • The number of antifungal medications, • The frequency of medication administration • Hypopyon	N=41 • 21 CXL-M • 20 M Fungal species: 9 *Fusarium*, 17 *Aspergillus* 14 No growth Time to healing • CXL-M group (1.30 ± 0.93 months) Vs M group (2.21 ± 1.35 months; P = 0.036). Depth of Ulcers • CXL-M group (37.62 ± 4.79 μm) Vs M group (68.57 ± 5.52 μm, P = 0.049) Corneal thicknesses of ulcers after healing • No difference	Randomisation process not clear Masking not done Primary and secondary outcomes not designated This study had many outcomes and a small sample Level of evidence: 1-

	• The maximum depth of the ulcer • Corneal thickness and epithelial thickness of the ulcer after healing Follow-up: 6 months		
CXL case series			
Li 2013 China [88]	Case series • CXL + topical antifungal Procedure • Under topical anaesthesia, the epithelium surrounding the infiltrate was removed. Riboflavin (Medio-Cross riboflavin/dextran solution, 0.1%) was administered topically for 30 min at intervals of 2 min. The cornea was illuminated for 30 min using a UV light lamp (UV-X 1000 system, IROC Innocross AG Co, Switzerland; wavelength 365 nm, irradiance 3 mW/cm2, total dose 5.4 J/cm2). Riboflavin administration was continued during UV illumination at the same intervals. • Topical 5% natamycin was continued after CXL treatment. Inclusion: • Microbiologically proven fungal keratitis + • No response to topical treatment OR • Exacerbation of infection Exclusion: • Not clear Follow-up daily until resolution (variable) Outcomes (primary not designated)	N=8 Fungal species: 6 *Fusarium*, 2 *Aspergillus* Outcomes • Time to epithelial healing, 3-8 days in all patients • Time to complete healing, 3-11 days in all patients • Improvement in Visual acuity, CXL visual acuity improved in 6 cases, remained unchanged in 1 case, and deteriorated in 1 case • Need for keratoplasty, 0/8	A decision was made to include this study although it had less than 10 participants. Level of evidence: 3

	• Time to epithelial healing • Time to complete healing • Improvement in VA • Need for TPK		
CXL Retrospective Audit			
Vajpayee 2015 India [89]	Comparative audit-2 arms • CXL + topical antifungal • Topical antifungal Procedure: • Topical anaesthesia with 0.5% proparacaine hydrochloride (Paracaine; Sunways, Mumbai, India) was used before the surgery. The corneal epithelium was debrided over the area of infiltrate. One drop of isotonic riboflavin phosphate 0.1% (10 mg of riboflavin-5-phosphate in 10 mLof dextran-T-50020%solution,Medio-cross GmbH, Neudorf, Germany) was applied every 3 min for 30 min before irradiation and every 3 min during irradiation. Ultraviolet A (UVA) radiation (365 nm with the desired irradiance of 3 mW/cm2; UV-X, IROC, Zurich, Switzerland) was applied at 5 cm from the cornea for 30 min. Inclusion • Moderate fungal keratitis: largest diameter < 6mm + • Involving <60% of corneal thickness Exclusion • Diabetes • Immunosuppression • Collagen vascular disorders • Impending perforation • Endothelial plaque • Hypopyon Follow-up 3months Outcomes • Resolution of infection (primary) • Healing time • Final BCVA	N=41, • 20 patients received CXL & topical treatment • 21 patients received topical treatment only Fungal species: 5 *Fusarium*, 10 *Aspergillus*, 1 *Curvularia*, 25 No growth Primary outcome: RR > 1 favours CXL • Resolution of infection CXL 18/20 Vs topical 18/21, RR 1.4 95% CI 0.4-7.6, p=0.7 Secondary outcomes: • Healing time CXL 30.85+/- 26.6 days Vs topical 31.28+/-19.97 days (p=0.94) • Final BCVA at 3 months CXL 1.13+/-0.55 Vs topical 1.25+/-0.46 (p=0.46)	No randomisation This study included moderate cases Level of evidence: 2-

Erdem 2018 Turkey[78]	Audit Procedure • Dresden protocol • gutt-Voriconazole Inclusion • Fungal Keratitis in one eye • Poor response to conventional therapy/progression of infection Exclusion • Follow-up • 3-months Outcomes • Cure rate	N=13 Fungal species: 5 *Fusarium*, 3 *Aspergillus*, 6 No growth Primary outcomes • 7/13 (54%) cured • 6 of the 7 cured cases had superficial infection	Small audit Cases had mixed infection which was initially controlled Level of evidence: 3

Table 9: Results of studies on Argon laser for treatment of fungal keratitis

STUDY	DESIGN	RESULTS	COMMENT
RCT: ARGON Vs INTRASTROMAL VORICONAZOLE			
Khater 2016 Egypt [80]	RCT 2 arms • Argon laser • Intrastromal Voriconazole Procedure: • A spot size of 500 um, pulse duration of 0.2 seconds, and power of 900-1400 mW were used. Argon laser therapy was done using argon green wavelength (Carl Zeiss LSL 532s AG; Meditec, Inc). A spot size of 500 um, pulse duration of 0.2 seconds, and power of 900-1400 mW were used. The number of shots varied from one case to another, depending on the size of the ulcer where we targeted its bed and edge during argon laser therapy with laser shots Inclusion: • Fungal keratitis not responding to topical treatment of 0.15% Amphotericin, or 5% Natamycin, or 1% Voriconazole, or 1% Itraconazole or 0.2% Fluconazole at day 7 Exclusion: • Improvement on medical treatment Follow-up 3 months Outcomes (primary not designated) • Healing time • Need for AMG • Improvement in vision	N=40, • 20 patients received Argon laser, • 20 patients received Intrastromal Voriconazole Fungal species: Not mentioned Outcome (s): RR > 1 favours Argon laser • Healing time: Argon group 2-4 weeks Vs VOR group 2-6 weeks P=0.001 • Need for AMG: Argon group 2/20 Vs VOR group 4/20, P=0.047, RR 2 95% CI 0.4-9.7, p=0.3 • Improvement in vision: Argon 11/20 Vs VOR group 13/20, P=0.011, RR 0.8 95%CI 0.3-1.7, p=0.6 Argon laser had a faster healing time, fewer cases needed AMG. Intrastromal Voriconazole had better improvement in vision	28 cases were pure fungal, 12 were mixed fungal and bacterial. Inclusion and exclusion not clear Randomisation not clear Masking not possible Primary and secondary outcomes not clearly stated Level of evidence: 1-
RCT: ARGON VS AMNIOTIC MEMBRANE GRAFT (AMG)			
Khater Egypt 2016 [79]	RCT 2 arms • Argon laser • AMG Inclusion • Fungal keratitis not responding to topical treatment of 0.15% Amphotericin, or 5% Natamycin, or 1% Voriconazole, or 1% Itraconazole or 0.2% Fluconazole at day 7	N= 40, • 20 patients received Argon laser • 20 patients received AMG Fungal species: Not mentioned Outcome (s): RR > 1 favours Argon • Healing time: Argon 2-3 weeks Vs AMG 3-5 weeks P=0.001	28 cases were pure fungal, 12 were mixed fungal and bacterial. Inclusion and exclusion not clear Randomisation not clear Masking not possible Primary and secondary outcomes not clearly stated

	Exclusion • Improvement on medical treatment Follow-up 3 months Outcomes • Healing time • Improvement in vision	• Improvement in vision: Argon 8/20 Vs AMG 6/20 P=0.138, RR 1.2 95% CI 0.7-1.8, p=0.5 Argon laser had a significant faster healing time compared to AMG	Level of evidence: 1-

DISCUSSION AND RECOMMENDATION:

This review examined the evidence for the main reported options for fungal keratitis. Overall, the strength of the evidence was low in a significant proportion of studies: many of the studies were observational and few clinical trials had a low risk of bias.

Medical interventions for fungal keratitis

Topical treatments: While the evidence for topical antifungal treatment has been previously reported, including a Cochrane review in 2015, this current review also included a recent RCT by Sharma *et al* comparing 5% natamycin and 1% Voriconazole eye drops.[90] [25] We conducted metanalyses of natamycin vs chlorohexidine (2 trials) and natamycin Vs voriconazole (4 trials). The natamycin Vs chlorohexidine metanalysis had a non-significant trend for healing by day 21 in favour of chlorohexidine.[20,21] However, the concentrations natamycin were 2.5% in one trial and 5% in the other.[21] natamycin Vs voriconazole metanalysis compared two outcome measures: Best Corrected Visual Acuity at 3 months in 4 RCTs and perforation rates in 3 RCTs.[25-28] natamycin had a significant trend for less perforations compared to Voriconazole. Unlike in the Cochrane metanalysis, where natamycin had a non-significant trend towards better BCVA at 3 months (p=0.20), our metanalysis that included the recent trial (Sharma et al, 2015) showed a significant trend (p<0.01) for better 3 months BCVA with natamycin figure 3). Evidence from the other 2 trials that compared topical natamycin Vs econazole and natamycin Vs itraconazole was inconclusive.[91,92] In all these trials, majority of the fungal species were filamentary.

In view of the results from our review, our current recommendation is that filamentary fungal keratitis should be initially treated with topical natamycin 5%, rather than topical voriconazole. The caveat to this is that there is limited data on the variation between geographical regions in the sensitivity of fungi to the various alternative drugs. This is the best available evidence to guide our practice. More work is needed on use of chlorohexidine for fungal Keratitis. This is ongoing in Nepal and East Africa studies. Although it is widely used, particularly for candida, amphotericin has received little attention in formal studies and no RCTs

Oral treatments: Evidence from the three trials that compared adding an oral antifungal (oral itraconazole or oral voriconazole) to topical treatment did not show any benefit.[34,35] However, a smaller trial that compared oral voriconazole Vs oral ketoconazole showed a benefit in terms of BSCVA at 3 months in the oral voriconazole arm.[36]

The evidence for oral treatment is still too scanty to utilise in making recommendations. In addition, patients on these oral treatments required frequent liver function tests which might not be ideal in resource limited settings, they should be avoided.

Injection Fluconazole: Although the use of subconjunctival fluconazole was found to be safe, the outcomes were variable and evidence too weak to draw a conclusion.[38-40] The only RCT included in this review although showed some benefit of using injection fluconazole as an adjuvant to topical amphotericin B compared to topical amphotericin B monotherapy, it had several methodological limitations with respect to randomization and masking.[37]

The evidence for injection fluconazole is still scanty to utilise in making recommendations.

Injection Amphotericin B: This was described as an intracameral injection (ICAMB), intrastromal injection and mixed (intracameral and intrastromal). The most commonly described was ICAMB given in dosages ranging from 0.5-50 µg/0.1ml given 1 up to 3 times 1-10 days apart. [42,46,82,83] Our review reported data on 2 trials which used ICAMB. We were unable to summarise these two trials in a pooled measure because they were methodologically different. One RCT from India compared ICAMB across 3 arms did not report any difference in the main outcome measures. However, the numbers in each arm were small (n=15).[42] Another RCT from China reported favourable outcomes for ICAMB Vs normal saline placebo by: time of hypopyon resolution and healing.[46]

Favourable outcomes for ICAMB were also reported in other studies.[42,44] However, these had a low level of evidence. For instance, a non-randomised trial from India compared ICAMB in patients who had not responded at 7 days to antifungal treatment (topical Natamycin or topical fluconazole or topical itraconazole and tablets fluconazole) to those who responded. Non responders were given ICAMB while the responders were not given ICAMB.[43] ICAMB had significantly favourable outcomes on improvement in BCVA, time of hypopyon disappearance, less scars and less complications. Patients in the ICAMB arm could have responded better just because they had “more” treatment. Another more recent non-randomised study among patients with recalcitrant fungal keratitis allocated (one group to early ICAMB at 2 weeks and another to late ICAMB at 4 weeks) found that patients in the early ICAMB intervention group had a quicker healing time compared to the ones in the late intervention group.[44] Again this may not necessarily be because of the drug but the timing.

Other encouraging results were reported one audit from China that compared ICAMB Vs topical antifungal alone and in another small series which reported good outcomes of use of ICAMB in patients with recalcitrant fungal keratitis.[45,82] Majority of fungal species in all these studies were filamentary fungi.

Overall, literature on the use of ICAMB is inconclusive. However, there seems to be context specific advantages of using ICAMB such as unresponsive deep filamentary fungal keratitis with hypopyon. A large RCT to determine the role of ICAMB in treatment of deep fungal keratitis may be warranted.

Other routes of administration reported for Amphotericin B were intrastromal and a mixed intrastromal and ICAMB.[47,83] Intrastromal injection dose ranged from 2-25µg/0.1ml. A more recent audit in Egypt reported a significantly faster healing time and less burning sensation in the intrastromal Amphotericin B injection group. Majority of the fungal species in this study were yeasts at 45%.[47] A separate series reported on combined ICAMB and intrastromal Amphotericin B in recalcitrant cases. [83] Treatment success was reported at 100% but also complications such as uveitis at 100%.[83]

The evidence for intrastromal Amphotericin B is still scanty to utilise in making recommendations.

Injection Voriconazole: Use was described as intrastromal (IVS), intracameral and mixed routes. Majority of the papers reported intrastromal use. In many studies, it was given as 5 injection spots of intrastromal voriconazole (50µg/0.1 ml) at least 3 injections given 72 hours apart.[48,51,52,85] In two RCTs from India, Intrastromal Voriconazole injections + topical treatment was compared to topical treatment (Natamycin 5% ± Voriconazole 1%) as adjuvant therapy or first line treatment in fungal keratitis patients, there was no evidence of benefit for IVS in healing time, scar size, vision at 3 months.[48,84] Pooled data from the two trials showed strong evidence of increased perforation and a worse vision at 3 months among the IVS arms.

Some series documented use of ISV in patients with recalcitrant keratitis.[51,52,85] Healing at 3-4 months ranged from 76-87% and improvement in vision ranged from 64-83%. In all these studies, all fungal species were filamentary. The conclusion of the authors that ISV had good response to filamentary fungal cases resistant to topical Natamycin and Voriconazole.

Metanalysis of the two RCTs indicates that IVS has no additional benefit in treatment of fungal Keratitis and is associated with worse outcomes and increased perforation rates. Our recommendation is that IVS should be avoided.

Surgical Interventions for fungal keratitis

TPK: The major challenge in reviewing TPK data was that most studies reported TPK outcomes for mixed aetiologies. Two previous review articles have looked at the role of TPK in management of all cause MK in general.[93,94] In addition, most of the reports were audits with an average SIGN evidence level of 3 (weak evidence).

We restricted our outcome reporting to only cases of fungal aetiology. Majority of the fungal species were filamentary and the most common indications for TPK in these cases were perforation/ impending perforation and non-healing ulcers/infiltrates. These audits had variable follow-up (1

month to years) and variable outcomes: Based on pooled data, anatomical stability was at 84%, recurrence 20%, graft clarity 60%, cure rate 80%.[55,56,58-61,63] Glaucoma was the most reported complication at 15%.[55,56,60,62,63]

Post-operative care especially use of steroids was not systematically described in almost all these audits.

Overall, TPK has an important role in management of severe MK by being able to provide eye salvage and infection control options. More efforts are needed to optimise outcomes.

Lamellar Keratoplasty (LK). Most of the studies which reported LK were audits and case series.[66,68-70,87] Majority of the fungal species were filamentary and the most common indications were under controlled infection but not very deep/extending into the anterior chamber. Follow up ranged from 1 month to 20 months. Generally favourable outcomes were reported in many studies: successful treatment/ cure/ non recurrence was reported in 5 studies at 471/498 combined patients (95%: ranging from 91-100%).[66,68-70,87] One study retrospectively analysed a small set of patients who had had LK compared to those who had received TPK.[67] TPK had a non-significant trend to improvement in VA, graft clarity and less recurrence.

Although promising, indications for LK among patients with fungal keratitis need to be carefully considered since most of the fungal infections tend to be deep.

Amniotic Membrane Graft (AMG): Literature on AMGs was scanty to draw any conclusions, we found only 3 papers in our inclusion that systematically documented use of AMGs in fungal keratitis.[71,72,95] One audit in China recorded 23 culture proven fungal keratitis patients who had undergone AMG, the distribution of indications; perforation (35%), descemetocele (35%) and poor re-epithelialisation (30%) were similar to TPK.[71] In this audit, there was a high recovery rate in vision and control of infection. Many of the patients with high vision recovery had presented with perforation and flat anterior chambers. Like TPK, glaucoma was the most commonly reported complication at 18%.

The evidence for Amniotic Membrane Graft is still scanty to utilise in making recommendations.

Conjunctival Flap (CF): Although conjunctival flap is frequently used in most parts in SSA, literature on outcomes was scanty. We only found one recent series from China that fit into the inclusion criteria. The globe was preserved in majority of cases and none developed raised Intra Ocular Pressure (IOP).[73]

The evidence for Conjunctival Flap is still scanty to utilise in making recommendations. However, Due to lack of corneal tissue in many Low- and Middle-Income Countries, Conjunctival Flap has a role in salvaging eyes with severe infection.

AMG Vs Conjunctival Flap: On which of the two is better, one RCT compared outcomes of AMG Vs bipedicle conjunctival flap with 20 patients in each arm. Although this trial included some bacterial keratitis cases, it was included because majority of the cases (63%) in each arm were fungal keratitis.[72] In this trial, primary outcome measures were: epithelialisation time and persistence of infection. Secondary outcome measures included visual acuity and other complications. There were no differences in primary or secondary outcomes. The sample size was small.

The evidence for Amniotic Membrane Graft Vs Conjunctival Flap is still scanty to utilise in making recommendations.

Corneal Cross Linking (CXL): Our search included 3 papers that used fairly similar CXL protocols: 2RCTs, 2 series and 1 audit.[74,75,78,88,89] The trial in India compared use of CXL+ topical antifungal treatment (CXL-M) Vs topical antifungal treatment only (M) in severe recalcitrant fungal keratitis patients.[74] The trial was prematurely stopped because of futility: there were more patients in the CXL arm that perforated than in the topical arm. In a more recent trial (Wei et al 2019), Forty-one patients with fungal ulcerative keratitis were randomised to (CXL-M)or (M).[75] Patients were followed up for 6 months. In the cured patients, the area of corneal ulcers, the duration of ulcer healing, the time to non-observed fungal hyphae by In Vivo Confocal Microscopy (IVCM), the number of antifungal medications, the frequency of administered medications, and the maximum ulcer depth decreased significantly after CXL compared with the M group. The severity grades of the patients in this trial were not reported.

A separate case series in China that used CXL-M did not report any perforation incident.[88] Again, the severity grade of the cases in this series was not reported. Another audit that compared two groups: CXL-M and M only did not report any significant differences in the outcomes of resolution of infection, healing time and final BCVA.[74] This audit excluded severe cases and did not report perforation rates. Another recent small series treated 13 fungal Keratitis patients who were unresponsive to topical Voriconazole with CXL.[78] Seven patients (54%) were healed with topical voriconazole and CXL adjuvant treatment while the remaining six patients did not respond to CXL treatment. Those who responded had small and superficial mycotic ulcers.

The evidence shows that although CXL might be dangerous for deep seated corneal infiltrates in causing perforation, it may not be dangerous in superficial infiltrates. The evidence on the beneficial effects of CXL on superficial infiltrates not responding to topical antifungal medicine is still inconclusive and more studies are warranted

Argon laser: One interesting finding in our review was reports on the use of argon laser in treatment of keratitis. Although not well understood, it is thought that the thermal energy is fungicidal and

mimics epithelial debridement in facilitating penetration of topical medication.[96] Although literature on Argon laser was insufficient, the two RCTs considered in our review showed some minimal evidence of the role of Argon laser in fungal keratitis not responding to medical treatment. One RCT compared Argon laser Vs intrastromal Voriconazole for treatment of fungal keratitis not responding to topical treatment at day 7.[80] Argon laser had a significantly faster healing time and fewer cases needed AMG. However, the Intrastromal Voriconazole arm had better improvement in vision. Another RCT compared Argon laser Vs AMG for the treatment of fungal keratitis not responding to topical treatment at day 7.[79] Argon laser had a significant faster healing time compared to AMG. However, these 2 RCTs had significant methodological weaknesses.

The evidence for Argon laser is still scanty to utilise in making recommendations. More studies

CONCLUSION

Our review systematically looked at all the evidence on treatment options for fungal keratitis. For some options such as topical Natamycin, it is becoming apparent that it is the first line treatment of choice for filamentary fungi. However, although there are trends, evidence on other options is still inconclusive. No apparent benefit has been documented for oral treatments (Voriconazole and Itraconazole). Among the injections, there is modest evidence against use of intrastromal Voriconazole and modest evidence for the use of Intra cameral Amphotericin B (ICAMB) especially for deep seated/anterior segment fungal Keratitis. A large RCT would be useful. Evidence for surgical techniques (TPK, LK, AMG, CF) was inconclusive, however, there is a role of all these procedures in salvaging the eye and controlling infection. There is need to optimise results. CXL is gaining ground especially in superficial keratitis that are not responding to medical treatment, however, there is strong evidence that it might be dangerous for deep seated corneal infiltrates in causing perforation. Argon laser may have a role but more studies are needed to generate more evidence.

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Chapter 3. Research setting

Uganda: An introduction into the research setting

Geography of Uganda

Figure 1 Political map of Africa showing the different countries in the continent, including Uganda

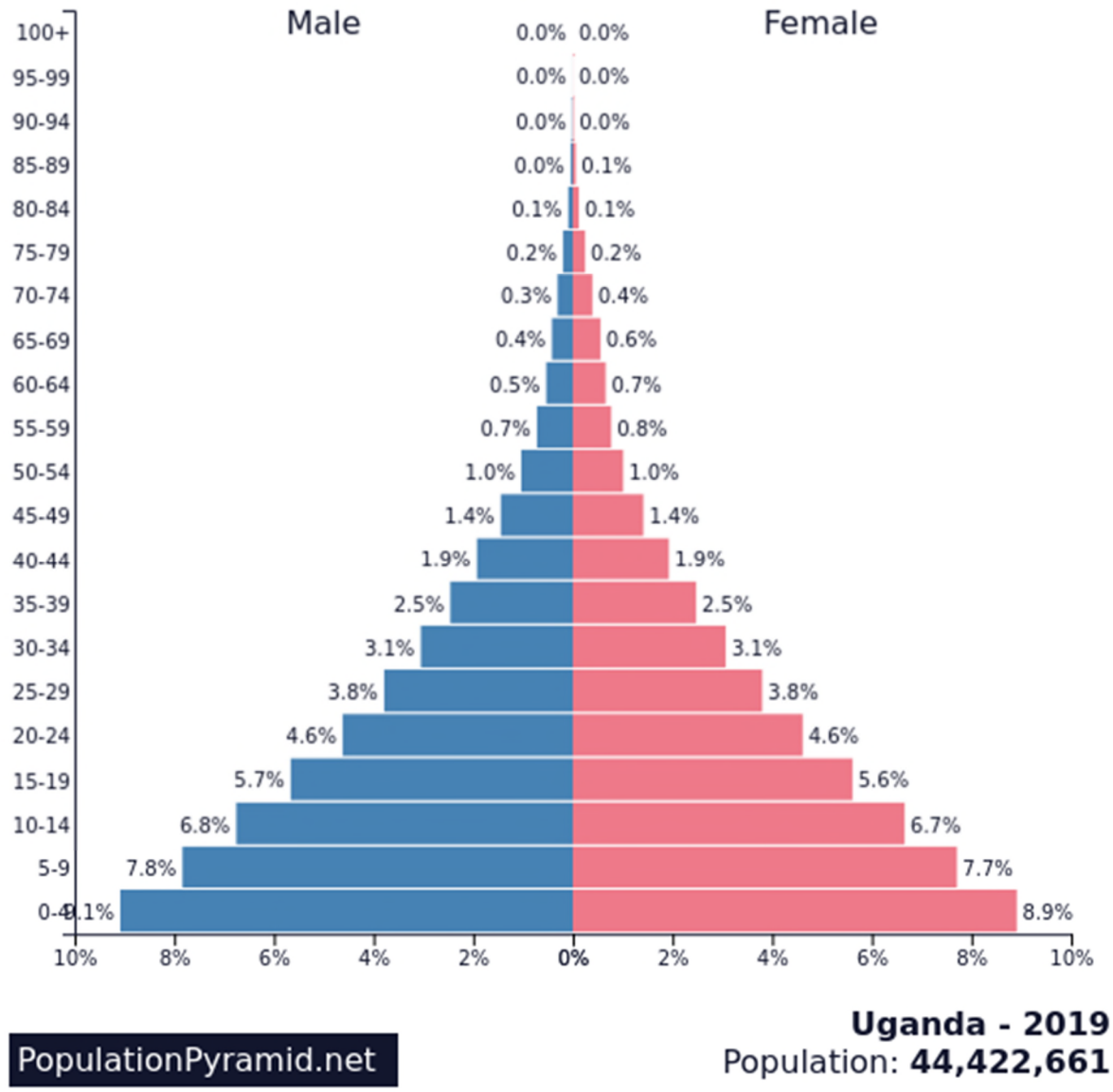
Figure 2 Political Map of Uganda

Uganda is a land locked country located in East Africa. It is bordered by five countries, Kenya in the east, South Sudan in the north, Tanzania in the south, Rwanda in the southwest and Democratic Republic of Congo in the west. Uganda is located on the Equator and thus has a tropical climate. The wet months are March to May and September to November. The country mainly experiences two dry seasons (December to February and June to August).[1]

Demography of Uganda

According to the National Census of 2014, the total population of Uganda was 34.6 million people. This represented an increase of 10.4 million from the census in 2002 with an annual growth rate averaging of 3%.[2] The population was 49.3% male and 50.7% female. Using this growth rate, the estimated population for Uganda as of June 2019 is 44.4 million.

Figure 3: The population pyramid. The majority of the population is youthful.



Uganda Health system

Table 1 gives a summary of the health system in Uganda; it is a tier-based system divided into 7 levels with the lowest point of care being at the village level. However, physically, a HC II is the lowest unit and is located at a parish level. These units have different staffing and capacity in terms of service provision. Patients are referred along the tier system depending on the complexity of their condition. Special Clinics are facilities which provide specialised services only such as HIV treatment services.

Table 1: Structure of the Uganda health system

Level	Status	Population	Staffing	Services Provided
One	Health Centre 1 (HCI) Village Level	1,000	Health Volunteer	Community Based preventive health services
Two	Health Centre II (HCII) Parish level	5,000	Enrolled nurse	Outpatient curative health services and outreach care
Three	Health Centre III (HCIII) sub-county level	20,000	Registered nurse, midwife, Clinical Officer	Outpatient curative health services, maternity, in patients and laboratory services
Four	Health Centre IV (HC IV) County level	100,000	Medical Officer plus HC III cadre	HC III plus emergency surgery, blood transfusion
Five	District hospital	500,000	Registrars plus HC IV cadre	HC IV plus in service training, consultation, research, community based organisms
National	Regional Referral Hospital	2,000,000	Consultant specialists plus District hospital cadre	District hospital plus specialised services (REC and MURHEC are at this level)
	National Referral Hospital	25,000,000	Consultant specialists plus Regional referral Hospital cadre	Regional referral plus Comprehensive sub-specialist care

According to the National Health Facility Master list of 2017 (source, Ministry of Health Uganda), there are a total of 6,404 health facilities in Uganda. Most health facilities (48%) are public (exclusively government owned and aided), 15.0% (947) are Private and Not-For-Profit (PNFP) while the remaining 37.0% (2,373) are Private-For-Profit (PFP) facilities. The Government and PNFPs are mostly higher levels of health facilities while the Private-For-Profit facilities are largely lower level facilities (HC IIs and clinics).

The South Western Region where this study was based has 786 health facilities (2 Blood Collection and Distribution Points, 49 Clinics, 492 HC IIs, 174 HC IIIs, 41 HC IVs, 20 Hospitals, 2 Regional Referral Hospitals and 5 Special Clinics).

Eye care services are integrated within the general health service system. However, eye care providers (ophthalmologists, Ophthalmic Clinical Officers [OCOs], Ophthalmic Assistants [OAs] and refractionists) are few and mainly deployed at the regional referral hospital. Uganda still has a huge Human Resources for Eye Health gap. [3,4] According to the Uganda Medical and Dental Practitioners' Council specialist register of 2019 (https://www.umdpc.com/registers.php), there are 51 Ophthalmologists in Uganda for a population of 44 million people.[2] Out of these, 33 (63%) practice in the capital city Kampala, 7 (14%) in the second city Mbarara where referral eye hospitals for South western Uganda are located. Patients that need specialized eye care attention are referred to one of the 15 regional hospitals with ophthalmic services. Although ophthalmic outreach programme is organized by regional ophthalmic centres, they don't reach the rural community adequately.

According to several Rapid Assessment of Avoidable Blindness studies (RAABs) done in Uganda, the general population prevalence of blindness ranges from 1.5%-3.9% in people over 50 years.[5] Table 2 shows summary findings from the three RAABs conducted in different parts of Uganda. The causes of blindness have been reported to be Cataract, Glaucoma and Corneal Opacities (Table 3). In these RAABs, the non-Trachomatous Corneal opacities were responsible for 3.2-11.1% of all cause blindness. Among children aged below 16 years, two studies have been done in Uganda; one study was done in Eastern Uganda (prevalence 0.07%) and another in western Uganda (prevalence 0.02%).[6,7] The main causes of blindness in children were found to be cornea and lens pathologies.

Table 2: Extrapolated population estimates of prevalence of Blindness in Uganda

District/Region	**Mubende (Western)**			**Karamoja (Northern)**			**Hoima (Central)**		
	n	(%)	(95%CI)	n	(%)	(95%CI)	n	(%)	(95%CI)
Blindness	-	1.9	(1.5-2.4)	1075	3.1	(2.3-3.9)	858	2.0	(1.5-2.4)
SVI	-	1.1	(0.7-1.4)	498	1.5	(0.8-2.1)	778	1.8	(1.3-2.2)
MVI	-	5.5	(4.8-6.2)	2369	6.9	(5.6-8.2)	2309	5.2	(4.5-6.0)
FLV	-	-	-	878	2.6	(1.6-3.5)	679	1.5	(1.1-2.0)

Table extracted from the RAAB reports with permission from the Principal Investigator. SVI "Severe Visual Impairment" MVI "Moderate Visual Impairment" FLV "Functional Low Vision". Mubende report did not show the actual numbers but reported extrapolated percentages

Table 3: Causes of Blindness in Uganda

District	Mubende		Karamoja		Hoima	
	N=3729		**N=3727**		**N=3862**	
	n	(%)	n	(%)	n	(%)
Cataract untreated	36	57.5%	95	43.8%	31	49.20%
Cataract surgical complications	1	1.6%	4	1.80%	3	4.80%
Trachomatous corneal opacity	1	1.6%	57	26.3%	3	4.80%
Non-Trachomatous corneal opacity	7	11.1%	20	9.20%	2	3.20%
Phthisis		-	2	0.90%	3	4.80%
Onchocerciasis		-	0	0.00%	0	0.00%
Glaucoma	5	7.9%	18	8.30%	4	6.30%
Diabetic retinopathy		-	0	0.00%	0	0.00%
ARMD	1	1.6%	2	0.90%	1	1.60%

Table extracted from the RAAB reports with permission from the Principal Investigator. Mubende RAAB report had missing information

Research Partners

Study sites

Patients were recruited from two sites:

- Mbarara University and Regional referral Eye hospital (MURHEC)
- Ruharo Eye Centre (REC).

MURHEC is a tertiary eye hospital of Mbarara University of Science and Technology (MUST) Department of Ophthalmology. It is a government owned hospital providing free service and treats about 10,000 patients/year. MURHEC has a staffing of 4 consultant ophthalmologists, 22 ophthalmology residents, 3 ophthalmic clinical officers, 1 optometrist, 7 nurses. The hospital has a daily outpatient consultation schedule that includes a side microbiology laboratory, imaging facilities (fundus camera, Optical Coherence Tomography, ultrasound, visual fields) laser (YAG capsulotomy, Argon laser for retina, Argon laser trabeculoplasty), an admission ward and two theatres.

REC is a church based fee-paying tertiary eye hospital that has been in existence since the 1960s. REC sees about 25,000 patients/year. REC has a staffing of 2 consultant ophthalmologists, 10 ophthalmic clinical officers, 1 optometrist, 25 nurses. The hospital has a daily outpatient consultation schedule and a high-volume cataract surgery service. The ophthalmology residents from MURHEC do some of their rotations at REC.

Both hospitals are in Mbarara Municipality, South-western Uganda approximately four hours' drive from the capital Kampala. The two units are about 5km apart and work closely together. They receive patients mainly from south-western Uganda, parts of north western Tanzania, Rwanda and eastern Congo.

Laboratories

I. Mbarara University of Science and Technology, department of Microbiology-Ocular microbiology (Microscopy, culture and sensitivity)
II. Kilimanjaro Christian Medical College Laboratory-Pan fungal PCR DNA sequencing

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Chapter 4. Overview of the project design

Ruharo Eye Centre, Uganda. One of the project participant recruitment sites

Rationale for the study

Microbial keratitis is a common and serious eye problem that leads to loss of sight and a high morbidity for affected individuals. Currently, there is very little information on MK in Sub-Saharan Africa; it has been a neglected area of research.

The overall purpose of this research programme was to make a major contribution to the development of strategies to reduce blindness from microbial keratitis (MK) in East Africa. This would be achieved by carrying out detailed studies of the epidemiology, aetiology, microbiology and clinical presentation of microbial keratitis cases presenting to Mbarara University and Referral Hospital Eye Centre (MURHEC) and Ruharo Eye Centre (REC), south western Uganda.

The things learned through this project will help us to improve the management and outcomes for people with MK in Uganda and the wider East African region in several ways:

1. Identification of predisposing factors to corneal infections will help us to develop prevention strategies.

2. Understanding the barriers to timely presentation of patients with MK in Uganda will help us to design well-informed public health interventions to encourage early presentation.

3. A better understanding of the capacity of lower health centres in managing MK will enable us to design a training module for mid-cadre health workers, and also make recommendation to the Ugandan MOH stocking of medication in lower health centres.

4. Knowledge of the causative organisms and local antibiotic sensitivity patterns will help us make rational decisions on the choice of empirical treatment and to recommend to the MOH the choice of drugs.

5. The evaluation of specific clinical signs in relation to the underlying causative agent will help us develop a protocol for clinicians in settings where microbiological support is not available.

6. An understanding of the use and impact of TEM will help us to better engage with the wider community about the potential risks and develop health education messages to discourage its use (if it turns out to be detrimental).

7. A detailed understanding of the determinants of a poor outcome from MK will help clinicians to plan management accordingly such as whether to admit or not, and what the likely outcome of the treatment will be.

Theory of Change

We used a theory of change conceptual thinking process of the issues around MK that lead to poor outcomes. We used this model to narrow down to specific research aims. This was based on extensive literature which has been discussed in the background chapter, review of the local data in Uganda, knowledge of the disease process as well as discussion with fellow clinicians involved in managing eye conditions.

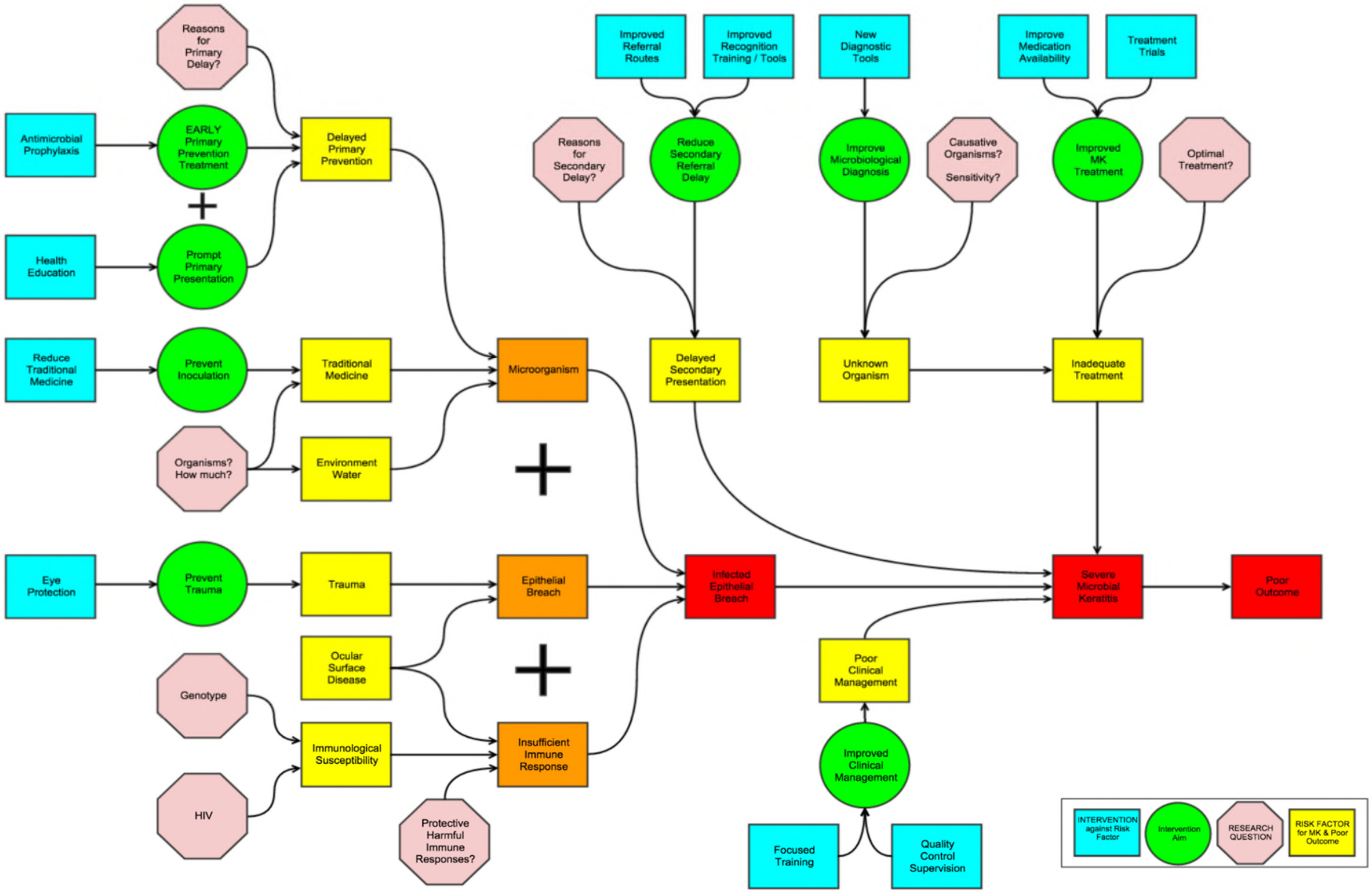
Our overall vision was to explore how to prevent vision impairment and vision loss due to MK. We explored reasons that contribute to poor outcomes among patients with MK. Figure 2 shows a logical cascade of events along this pathway. For one to get a severe MK infection, first there must be an epithelial breach, secondly, this becomes an infected epithelial breach which if not treated results in severe MK and ultimately poor outcome resulting in vision loss.

Broadly, there are “risk factors” that make one susceptible to developing MK. These could include, Traditional Eye Medicine use, Trauma, existing Ocular surface disease, environment contextual factors such as poverty, farming occupation, Immunological susceptibility (diabetes and HIV). [1-6]

Next are factors which exacerbate the disease such as delay starting treatment, lack of microbiological support in identifying the causative organisms and poor clinical management. [7-10] There is overlap and some of the pre-existing factors also contribute not only in susceptibility but in worsening the condition. Efforts to prevent blindness from MK can address to primary prevention against corneal epithelial breach (for example prevention of ocular trauma), secondary prevention against this breach becoming infected (antibiotic prophylaxis, avoiding TEM use) and once it gets infected, prompt treatment with effective antimicrobial agents.

Although this was logically clear, there were underlying questions along this though process that needed to be answered. This formed our basis for the aims and objectives mentioned below.

Figure 1 Theory of change model showing factors that lead to poor outcomes for Microbial Keratitis



Research Aims

1. To describe the epidemiology of MK; patient presentation, causative organisms and their anti-microbial sensitivity; outcome and its determinants among MK cases in south western Uganda.
2. To investigate risk factors for MK in Uganda including HIV infection, Diabetes Mellitus, Traditional Eye Medicine and Occupation.
3. To understand the patient perspective on the effect of MK on quality of life
4. To investigate the role of the health system particularly lower health centres in care of MK, and the barriers that need to be overcome.
5. To explore the contribution of Traditional Eye Medicine (TEM) to MK in Uganda

Table 1 Specific research objectives

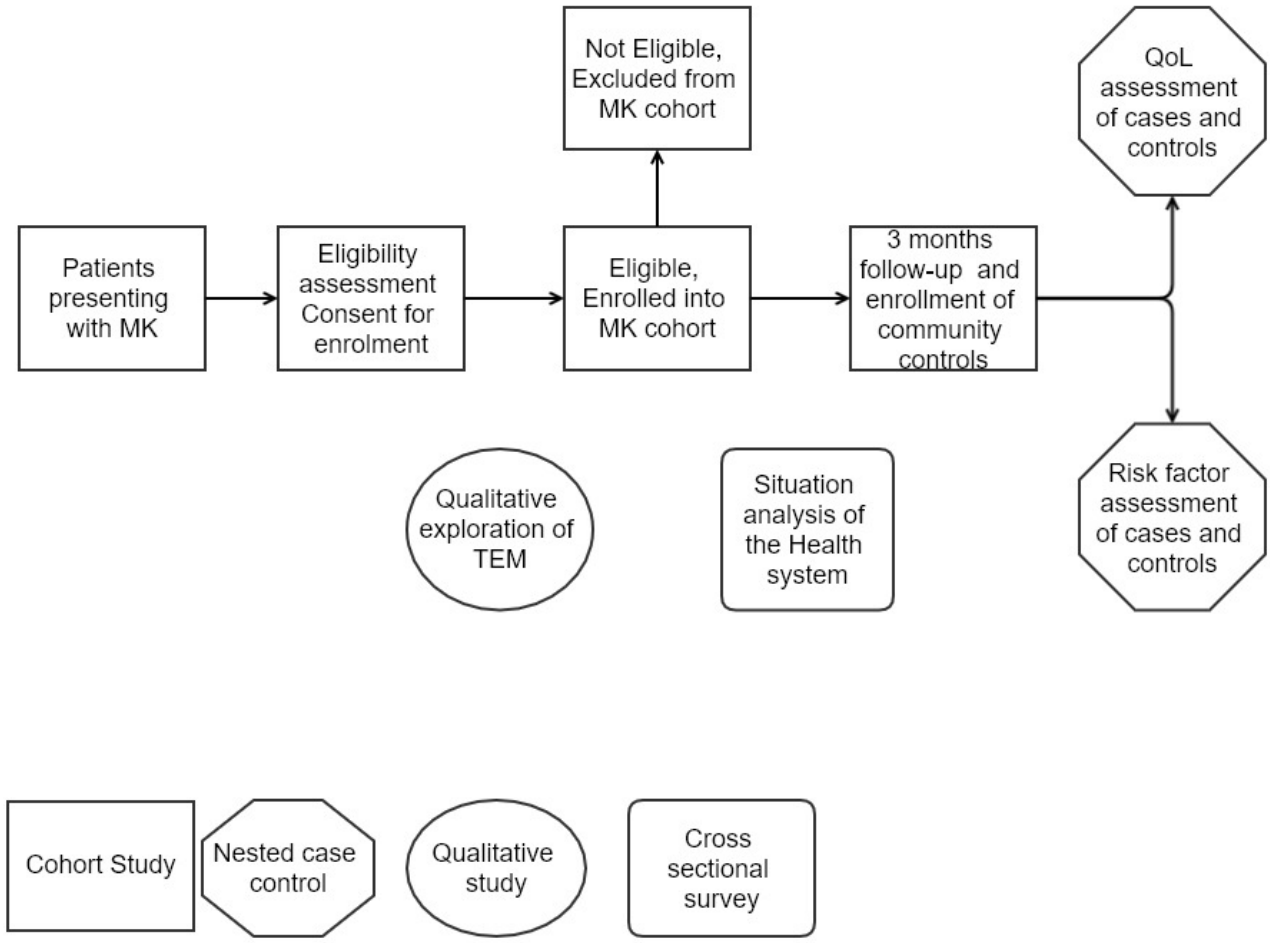
Study	Specific objectives	Design
Epidemiology of MK in Uganda	1. To describe the phenotypic presentation of MK patients in Uganda. 2. To describe the microbiological spectrum of MK in Uganda. 3. To describe the 3 months outcomes among MK patients in Uganda. 4. To determine the factors associated with poor outcomes among MK patients in Uganda	Cohort
Risk factors of MK in Uganda	1. To determine if HIV infection, Diabetes Mellitus, Traditional Eye Medicine use and farming occupation are risk factors for MK in Uganda?	Nested matched case-control
Effect of MK on Quality of Life (QoL)	1. To compare QoL amongst MK patients before and after treatment (at 3 months). 2. To compare QoL at 3 months among MK patients with individually matched healthy individuals without MK.	Cross sectional comparative study
Role of the healthy system in management of MK in Uganda	1. To determine the knowledge of mid-level health workers in managing MK. 2. To determine the capacity of different tiers in the health system in managing MK. 3. To determine the level of training given to mid-level health workers in eye health. 4. To describe the presentation pathways of MK patients through the different tiers of the health system in Uganda.	Cross sectional survey

Role of Traditional Eye Medicine (TEM)	1. To determine the proportion of people with MK who use TEM. 2. To determine the effect of TEM on the presentation and outcomes of MK in Uganda. 3. To determine factors associated with TEM use in Uganda 4. To explore reasons why people, use TEM for treatment of MK in Uganda.	Mixed methods

Methods Overview:

The overall design was a ***prospective cohort study*** of individuals presenting with MK to investigate their pattern of presentation, clinical features, microbiology and outcomes. We then conducted a ***nested case-control study***, recruiting community controls matched to a subset of the MK cases, to investigate the impact of MK on Quality of Life and identify addressable risk factors for this disease. In parallel, we explored in ***a qualitative study*** through one-to-one interviews and small group discussions reasons of use of TEM. A separate ***situation analysis survey*** was conducted to understand how patients with MK are currently cared for within the formal Health System in Uganda. Figure 3 shows the general schematic of how these studies were conducted.

Figure 2 Flow of studies



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Chapter 5. Factors Associated with Poor Presenting Vision Among Patients with Microbial Keratitis in Uganda

A study patient undergoes a smart phone-based peek visual acuity examination at Ruharo Eye Centre

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RESEARCH PAPER COVER SHEET

Please note that a cover sheet must be completed for each research paper included within a thesis.

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Thesis Title	Epidemiology of Microbial Keratitis in South Western Uganda		
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For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I designed the study, collected the data, conducted the analysis with guidance from David Macleod, Victor Hu, and M J Burton, prepared and submitted the final manuscript to MEAJO in consideration of comments from all co-authors.

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Type: Original article

Title: Factors Associated With Poor Presenting Vision Among Patients With Microbial Keratitis In Uganda

Running Title:Poor presentation of Microbial Keratitis in Uganda

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Consent for publication

Not applicable for this audit. All data were anonymized after extraction from clinical notes.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

SA, GA, DM, VHH and MJB conceived the design. SA, GA, JO, BA collected the data. SA, GA, DM, VHH, MJB analysed the data. All authors reviewed all the versions and have approved the final manuscript.

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Abstract

Purpose: To determine factors associated with poor presenting vision among patients with microbial keratitis in Uganda.

Methods: Retrospective audit of patients presenting with microbial keratitis at the two main eye units in Southern Uganda in 2015. We collected information on time to presentation, treatment history, use of traditional eye medicine, trauma and presenting final visual acuity. We analysed factors associated with a poor presenting vision in a regression model.

Results: There were 273 cases during 2015. The median presentation time was 7 days from onset (IQR 2-21, total range 0-366 days). Trauma was reported in 59/88 (67%) patients and 69/162 (43%) reported using traditional eye medicine. Visual acuity was reported in only 216/273 cases at presention. Visual acuity at presentation of less than 6/60 (severe visual impairment) was strongly associated with the use of traditional eye medicine (OR 5.13, 95%CI 2.17–12.1, p=0.001).

Conclusion: This audit highlighted the role of use of traditional eye medicine in causing poor presentation among patients with microbial keratitis in Uganda.

Keywords

Microbial Keratitis, Bacterial keratitis, Fungal keratitis, Keratitis, Traditional Eye Medicine, Uganda

Text

Introduction: Microbial keratitis (MK) can be caused by a range of pathogens including bacteria, viruses, protozoa and fungi. MK frequently leads to sight-loss from dense corneal scarring, or even loss of the eye, especially when the infection is severe and/or appropriate treatment is delayed.[1] MK has been described as a "silent epidemic", which leads to substantial morbidity, related to blindness and other consequences such as pain and stigma.[2] It is the leading cause of unilateral blindness after cataract in Tropical regions and is responsible for about 2 million cases of monocular blindness per year.[3]

A good outcome depends on early appropriate treatment, correct identification of the causative organism, and careful follow-up.[4, 5] In Low and Middle-Income Countries (LMIC), MK presents major challenges: Majority of patients present with advanced disease when little can be done. Ultimately, outcomes tend to be poor.[6, 7] Outcome data from SSA have reported overall cure rates with and without scarring of about 50% and the majority of patients end up with vision of less than 6/60.[6, 8-13]

The purpose of our audit was to determine factors associated with poor presentation among patients with MK in rural South-Western Region of Uganda.

Methods

We conducted a retrospective audit of all patients with MK that presented to Ruharo Eye Centre (REC) and Mbarara University and Referral Hospital Eye Centre (MURHEC) during the

whole of 2015. MURHEC is a government owned tertiary eye unit established in 2013. It provides mostly free services and sees about 6,000 - 10,000 patients/year. REC is a church-based fee-paying tertiary eye hospital founded in the 1960s. It sees about 20,000 - 25,000 patients/year. Both hospitals are located in Mbarara Municipality, South-Western Region, Uganda, approximately four hours drive from Kampala. The two units are about 5km apart and work closely together.

This study adhered to the tenets of Declaration of Helsinki. It was approved by the London School of Hygiene & Tropical Medicine Ethics Committee (Ref:10647). It was approved as an audit study by both MURHEC and REC. All data were anonymized after extraction from clinical notes.

We included all patients who were recorded to have a clinical diagnosis of either clinically diagnosed fungal or bacterial keratitis presenting between 1st January and 31st December 2015. Patients with other forms of keratitis were excluded. We reviewed and extracted information from the case records. This included patient demographics, history, recorded risk factors, presenting visual acuity, treatment and outcome.

Data were analysed in STATA v14. The main study variables were presentation time, use of traditional eye medicine, use of “other eye medicine”, history of trauma, presenting vision, follow-up rate, final visual acuity and loss of the eye. Visual acuity was categorised according to the WHO classification system.[14] Presentation time was classified as early (1-3 days) or late (4 days and above).[15]. For the purposes of this analysis we categorised poor presenting vision to be worse than 6/60.[6] Logistic regression analysis was used to identify factors associated with poor presenting vision.

Results

We enrolled 273 patient records with clinically diagnosed bacterial or fungal keratitis. Figure one shows the enrolment process.

Of the 273 individuals with bacterial or fungal keratitis, 178 (65%) were male (Table 1). Their median age was 36 years (IQR 19–55 years, Total Range 1–104 years). The time between the onset of symptoms and presentation was skewed: median 7 days, IQR 0–21 days, total range 0–366 days. Seventy three patients (30%) presented early (≤3 days) and 166 patients (70%) presented late (≥ 4 days). Patients had to travel considerable distances to reach the eye units: median 80 km, IQR 45-99 km, total range 1-378 km. There was no microbiology data recorded. At presentation, visual acuity was documented in 220 out of 273 patients (81%) of which 80/220 had a vision worse than 6/60.

We analysed the factors associated with a poor presenting vision (<6/60). The univariable and multivariable logistic regression models for these outcomes are presented in a table 3. Poor vision at presentation (<6/60) was associated with increasing distance from home to hospital (OR 1.02, 95%CI 1.01-1.03, p=0.002) and TEM use (OR 5.13, 95% CI 2.17-12.1, p=0.001).

Discussion

This audit highlights factors associated with a poor presenting vision. Multiple factors were hypothesised to contribute to poor outcomes. These included large distances from the eye hospital, delayed presentation, trauma and Traditional Eye Medicine (TEM).

In this audit, almost half of the patients with recorded information on TEM use reported having used TEM and this was strongly associated with worse presenting vision, even after controlling for delay in presentation. Many people probably choose to try TEM for several days before attending hospital as it can be easily obtained within or close to home. Its use appears to contribute to poor outcomes, substantially adding to the risk of poorer presenting vision. In Uganda, TEM is usually made from plant products. This is concerning, as such substances may be toxic or harbour infectious agents, such as fungal spores.[16, 17]

A large distance to the eye hospital was strongly associated with poor presenting vision. The units included in this audit constitute the referral centres for the whole region and many of the

patients came from substantial distances to seek treatment. While the evidence from our data was limited, distance is probably an important factor in the presentation, course and outcome of MK in our setting.

This retrospective audit had several limitations. Visual acuity was not recorded consistently for all patients at presentation and follow-up. Presenting vision was available in about 81% of the patients. From a clinical management point of view this is an important audit learning point. We have already introduced new procedures that ensure the consistent recording of vision data for all patients. It is possible that this might have introduced some systematic bias, with people with poorer vision being less likely to have this documented than those with better vision.

Loss to follow-up is generally a significant challenge in this region and makes it difficult to evaluate outcomes. Follow-up data was largely missing and so the analysis was based on presenting vision as a proxy of outcome.[18]

During 2015, samples were not sent for microbiological investigations, therefore, diagnosis and treatment choices were based purely on clinical evaluation. In the absence of a microbiological diagnosis, diagnostic uncertainty remains high, likely resulting in failure to treat appropriately.[19, 20] Following this audit, we have started a routine ocular microbiology service for all patients with MK.

Conclusion

This audit reflects the factors associated with a poor presentation among patients with MK. Delayed presentation, traditional eye medicine use, lack of laboratory support are all factors which need to be addressed in the effort to reduce avoidable blindness. Good quality data collection and research into strategies to manage MK are clearly needed.

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Figure 1 Patient chart evaluation for enrolment

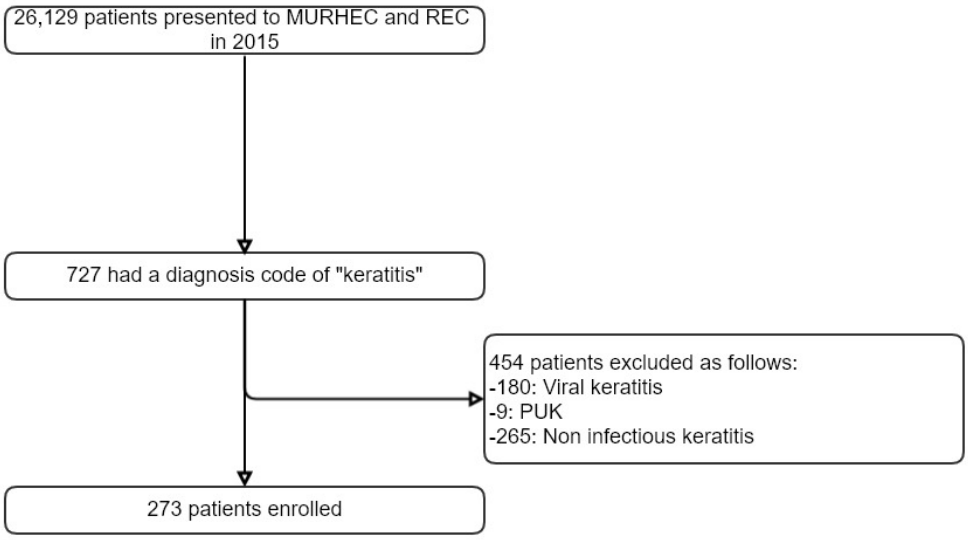

Table 1: Baseline characteristics of all 273 individuals with microbial keratitis.

Variable	**Median**	**IQR**	**(Total Range)**
Age (years)	36	19-55	(1-104)
Distance from Eye Hospital (km)	80	45-99	(1-378)
Time to presentation (days)	7	0-21	(0-295)
Variable	**N**	**Count**	**(%)**
Sex	273		
• Male		178	(65%)
• Female		95	(35%)
Trauma*	88	59	(67%)
Prior Treatment ł	154	147	(96%)
Traditional Eye Medicine use ǂ	162	69	(43%)
Hypopyon	271	42	(15%)
Visual acuity at presentation §	216		
6/5-6/18		107	(48%)
6/24-6/60		33	(15%)
5/60-3/60		8	(4%)
2/60-1/60		13	(6%)
0.5/60-PL		48	(22%)
NPL		11	(5%)

*Data not recorded in all clinical notes, denominator (N) indicates the number of records where reference to this variable was made. Out of the 273 patients, 88 had data on whether there was trauma or not (59/88, 67%, positive).

ł 147/154 (95%) patients reported prior use of some other eye medicine other than TEM, but the specific type was not recorded. ǂ 162 patients had data on whether they had used Traditional Eye Medicine (TEM) or not (69/162, 43%, positive). The different types of traditional medicine were not recorded in the charts. § only 216 out of 273 patients had recorded presenting vision. PL = Perception of Light; NPL = No Perception of Light

Table 2: Logistic regression for factors associated with a poor presenting vision among patients with Microbial Keratitis

Variable	Univariate analysis			Multivariate analysis		
	Crude OR	(95% CI)	p-value	Adjusted OR	(95% CI)	p-value
Age	1.02	(1.01-1.03)	0.016			
Sex (being female)	1.19	(0.66-2.13)	0.564			
Distance (for every 1Km increase)	1.02	(1.01-1.03)	0.001	1.02	(1.01-1.03)	0.002
TEM use	4.66	(2.18-9.95)	0.001	5.13	(2.17-12.1)	0.001
				5.19	(2.24-12.0)	0.001 ł
Trauma	1.28	(0.44-3.75)	0.645			
Delayed presentation	1.49	(0.75-2.92)	0.247			

In this model, there was a lot of missing data in the patient charts that not all the patients with reported baseline vision could be used for the analysis. The final model had 120 observations. ł TEM adjusted for delayed presentation

Chapter 6. Epidemiology of Microbial Keratitis in Uganda

A patient undergoes review at Mbarara University and Referral Hospital Eye Centre (MURHEC)

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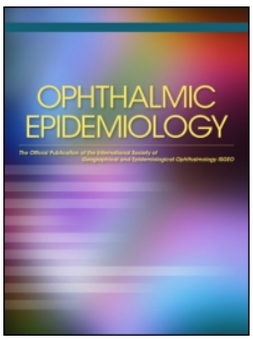

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Epidemiology of Microbial Keratitis in Uganda: A Cohort Study

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ABSTRACT

Purpose: To describe the epidemiology of Microbial Keratitis (MK) in Uganda.

Methods: We prospectively recruited patients presenting with MK at two main eye units in Southern Uganda between December 2016 and March 2018. We collected information on clinical history and presentation, microbiology and 3-month outcomes. Poor vision was defined as vision < 6/60).

Results: 313 individuals were enrolled. Median age was 47 years (range 18–96) and 174 (56%) were male. Median presentation time was 17 days from onset (IQR 8–32). Trauma was reported by 29% and use of Traditional Eye Medicine by 60%. Majority presented with severe infections (median infiltrate size 5.2 mm); 47% were blind in the affected eye (vision < 3/60). Microbiology was available from 270 cases: 62% were fungal, 7% mixed (bacterial and fungal), 7% bacterial and 24% no organism detected. At 3 months, 30% of the participants were blind in the affected eye, while 9% had lost their eye from the infection. Delayed presentation (overall $p = .007$) and prior use of Traditional Eye Medicine (aOR 1.58 [95% CI 1.04–2.42], $p = .033$) were responsible for poor presentation. Predictors of poor vision at 3 months were: baseline vision (aOR 2.98 [95%CI 2.12–4.19], $p < .0001$), infiltrate size (aOR 1.19 [95%CI 1.03–1.36], $p < .020$) and perforation at presentation (aOR 9.93 [95% CI 3.70–26.6], $p < .0001$).

Conclusion: The most important outcome predictor was the state of the eye at presentation, facilitated by prior use of Traditional Eye Medicine and delayed presentation. In order to improve outcomes, we need effective early interventions.

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KEYWORDS
Microbial keratitis; bacterial keratitis; fungal keratitis; keratitis; blindness; Uganda

Background

Microbial keratitis (MK) can be caused by a range of pathogens including, bacteria, viruses, protozoa, and fungi. It is characterized by acute or sub-acute onset of pain, conjunctival hyperemia, and corneal ulceration with a stromal inflammatory cell infiltrate.[1]

MK has been described as a "silent epidemic", which leads to substantial morbidity, related to blindness, pain, and stigma.[2] It is the leading cause of unilateral blindness after cataract in Tropical regions estimated at 2 million cases of monocular blindness per year.[3] In 2017, 1.3 million individuals were bilaterally blind from corneal opacity globally (excluding trachoma and vitamin A deficiency), accounting for 3.2% of the binocular blindness.[4] In Sub-Saharan Africa (SSA), MK is an important cause of binocular blindness and is responsible for about 15% of the monocular blindness (Nigeria National Survey).[5,6] The only report of the incidence in SSA is from Malawi in 1994, which suggested a rate of around 180/100,000/year.[7] Rates in high-income settings are lower at 5–10/100,000.[8–10]

MK frequently leads to sight-loss from dense corneal scarring, or even loss of the eye, especially when the infection is severe and/or appropriate treatment is delayed. A good outcome depends on early appropriate treatment, supported by correct identification of the causative organism, and careful follow-up.[11,12] In low and middle-income countries (LMIC), these resources are not readily available and outcomes tend to be poor.[13]

Literature on MK in SSA is extremely sparse, only one audit from an LMIC setting (Tanzania) has previously reported outcomes of MK at discharge in SSA.[13] Here, in this large prospective cohort study

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from South-Western Uganda, we describe patient presentation, causative organisms, 3-month outcomes, and investigate their determinants.

Methods

Ethical statement

This study followed the tenets of the Declaration of Helsinki. It was approved by the London School of Hygiene & Tropical Medicine Ethics Committee (Ref 10647), Mbarara University Research Ethics Committee (Ref 10/04-16) and Uganda National Council for Science and Technology (Ref HS-2303). Written informed consent in the local language was obtained before enrolment. If the patient was unable to read, the information was read to them, and they were asked to indicate their consent by application of their thumbprint, which was independently witnessed.

Study design and setting

In this cohort, we prospectively enrolled patients with MK that presented to Ruharo Eye Centre (REC) and Mbarara University and Referral Hospital Eye Centre (MURHEC) from December 2016 to March 2018. MURHEC is a government-owned tertiary eye unit established in 2013. It provides mostly free services and sees about 6,000–10,000 patients/year. REC is a church-run fee-paying tertiary eye hospital founded in the 1960s. It sees about 20,000–25,000 patients/year. Both hospitals are located in Mbarara Municipality, South-Western Region, Uganda. In order to investigate the seasonal variation in the presentation of MK, we aimed to recruit all MK cases presenting during at least one year.[13]

Study participants

MK was defined as loss of corneal epithelium (of at least 1-mm diameter) with underlying stromal infiltrate, associated with any or all signs of inflammation (conjunctival hyperemia, anterior chamber inflammatory cells, ± hypopyon).[14] We also included patients presenting with a deep corneal abscess (of at least 1 mm), defined as having all the features of MK, but without an epithelial defect. We excluded those not willing to participate, those not willing to return for follow-up, pregnant women, lactating mothers and those aged below 18 years.

Assessment

We documented basic demographic information and their ophthalmic history. This included the circumstances in which their eye became infected, predisposing factors, treatment received, and their "health care journey" before reaching the eye hospital. Presenting Log MAR (Logarithm of Minimum Angle of Resolution) visual acuity at 2 m in a dark room was measured using Peek Acuity software.[15] Participants were examined with a slit lamp to assess the anterior segment using a structured protocol, including eyelid assessment, corneal ulcer features, anterior chamber (flare, cells, hypopyon shape, and size) and perforation status. Infiltrate size was determined from the greatest diameter of the infiltrate (major axis) and the widest perpendicular diameter (minor axis).[14] The final infiltrate size was then derived as the geometric mean of these two diameters.[14] The same was repeated after fluorescein staining of the ulcer to determine epithelial defect sizes. High-resolution digital photographs with and without fluorescein staining were taken with a Nikon SLR 7200 digital camera with Macro lens.

Corneal scrape specimens were collected from the ulcer at a slit lamp or an operating microscope, using 21G needles after application of a proxymetacaine (minims) anesthetic eye drops. Samples underwent processing for the Gram stain, Potassium Hydroxide [KOH] stain, Calcofluor White [CFW] stain and direct inoculation on culture media (Sheep's Blood Agar [BA], Chocolate Agar [HBA], Potato Dextrose Agar [PDA] and Brain Heart Infusion broth [BHI]). Two sterile corneal swab samples were taken for pan fungal gene sequencing. The number of corneal samples was dependent on how much material could be safely scraped from the cornea. The order was samples for microscopy, agar, broth, and finally corneal swabs.

In addition, a random blood sugar test and HIV counseling and testing were offered, as per the Uganda Ministry of Health HIV testing protocol. For those who were confirmed as HIV positive, a CD4 test was performed to determine the level of immune suppression and they were referred to the HIV care center, which is on the hospital site.

Microscopy, culture, and antimicrobial sensitivity work were done at the Mbarara University Department of Microbiology. The technician underwent initial training in ocular microbiology at the Aravind Eye Hospital System, department of ocular Microbiology in Madurai, India and had a site supervision visit by a mycologist from the London School of Hygiene & Tropical Medicine. Immediate CFW staining was also done in the side lab at MURHEC on a fluorescein microscope (Zeiss Primostar ILED) by the attending ophthalmologist. Agar plates and broths were incubated and read daily at 35–37°C for bacteria for up to 7 days and at 25°C for up to 21 days for fungi. Organism identification and sensitivity testing (MIC/zone of inhibition) were performed using standard

microbiological techniques. We followed a previously described approach for reporting positive microbiology results.[16] Briefly, bacteria were identified using routine biochemical identification tests. Identification of fungi was according to the macroscopic appearance of cultures on potato dextrose and microscopic appearance of conidia and spore-bearing structures. Positive culture was growth at the site of inoculation or growth on one solid medium consistent with microscopy; or semiconfluent growth at the site of inoculation on one solid medium (if bacteria); or growth of the same organism on repeated scraping. If, by microscopy, hyphae were observed in corneal tissue, but failed to grow in culture, the causative organism was reported as fungal.

Treatment and follow-up

Patients were treated empirically at presentation and the treatment choice was reviewed when the microbiology results became available. Patients with fungal keratitis were treated with Natamycin 5% eyedrops (Zonat Sunways India), those with bacterial keratitis were treated with Ofloxacin 0.3% eyedrops (Biomedica Remedies-India). Patients with fungal infection were treated hourly day and night for the first 3 days and then hourly while the patient was awake for 2 weeks. This was changed to 2-hourly for another 2 weeks and then tapered to 4 times a day until healed. For bacterial infections, patients were treated hourly day and night for the first 3 days and then reduced to 6 times a day for a further week. All patients with fungal MK were also given Ofloxacin 0.3% eye-drops four times a day as prophylaxis until all epithelial defects were healed. In addition, those in pain were treated with Atropine 1% eye-drops (locally formulated) and oral Paracetamol tablets. Raised intraocular pressure was treated with Timolol 0.5% eye-drops (locally formulated). Those with presumed viral keratitis were treated with Acyclovir 3% eye ointment (CIPLA India) five times a day for 3 weeks. Most patients were admitted during the first week.

After the initial assessment patients were seen on day 2, day 7, day 21, and day 90 (3 months). Additional assessments were conducted as clinically indicated. The main outcome measures were final best-corrected vision at 3 months, blindness (<3/60 in the affected eye) at 3 months, and loss of the eye at 3 months. Scar density was also graded as "no scar" (clear cornea), "mild scar" (anterior chamber structures clearly visible through the scar), "moderate scar" (anterior chamber structures vaguely visible through the scar) and "dense scar" (anterior chamber structures completely obscured by the scar).

Analysis

Data were analyzed in STATA v14. To describe the presentation of MK, summary frequency tables of demographics, presentation time, clinical history and clinical features were generated. Presentation time was classified as prompt (0–3 days), early (4–7 days), intermediate (8–14 days), late (15–30 days) and very late (more than 30 days).[17] In addition, a summary tally of patients that presented by month across one year (2017) was generated to describe the presentation pattern. This was compared to local rainfall, humidity and temperature patterns. Local weather data were obtained from the weather and climate repository.[18] For presentation purposes, Log MAR visual acuity measurements were converted to the Snellen scale and categorized according to the WHO classification system.[19]

We used two different analytical approaches. We first took a causal modeling approach to explore the association of six risk factors of interest with visual acuity at presentation. These six factors were (Traditional Eye Medicine) TEM use, history of trauma, delayed presentation, distance from hospital, distance from nearest health center (HC), and organism type. In order to inform our modeling choices, we first drew Direct Acyclic Graphs (DAGs), using www.daggity.net v2.3 software, to identify relevant variables to adjust for in the multivariable logistic regression model.[20] A DAG is a representation of the hypothesized order of events from the exposure to the outcome. It allows the researcher to logically map out relationships between different variables and identify those to adjust for to determine the overall effect of the exposure on the outcome. A change in point estimate criteria was used to assess for confounding and multicollinearity. Each main exposure was separately adjusted for confounding factors and final adjusted odds ratios (aOR) recorded.

The second modeling approach was to build a predictive model for visual acuity outcomes at 3 months, using baseline clinical features. Patients without 3-month data were excluded from the analysis. Ordinal logistic regression analysis of the WHO Snellen visual acuity categories was used to identify factors associated with visual acuity at 3 months. Univariable regression was performed to generate

crude odds ratios (cOR). Variables with a *p*-value less than 0.1 were initially included in the multivariable model. A backward stepwise approach was then used until only the variables with a *p*-value of less than 0.05 were retained. Adjusted ORs were reported for the final model.

Results

Participants

Patient enrolment is illustrated in Figure 1. The baseline characteristics of the patients are shown in Table 1. Median age was 47 years (IQR 35–60, total range 18–96 years), and the majority (56%) were male. Over a quarter had never had any formal education. Most (70%) were married and most (70%) were the heads of households. Median distance from home to the eye hospital was 79 km (IQR 52–128, total range 0.2–378 km). Median distance from home to their nearest HC was 3 km (IQR 1–4, total range 0–45 km). The main occupation was farming (70%). The baseline characteristics of the patients who were lost to follow-up and those who completed 3 months were similar (Supplementary Table 1).

Presentation pattern

Figure 2 illustrates the number presenting per month throughout 2017, compared to rainfall, temperature and humidity patterns. Patients presented throughout the year, with peaks in May to July and October to November, which corresponded with the harvest seasons. April and November had the greatest rainfall. Temperature and humidity were constant throughout the year.

Table 1. Demographic characteristics of the study participants.

Variable	n/313	(%)
Age (median = 47, IQR 35–60) in years		
< 30 years	54	(17%)
30–40 years	63	(20%)
40–50 years	59	(19%)
50–60 years	66	(21%)
> 60 years	71	(23%)
Gender		
Female	139	(44%)
Male	174	(56%)
Occupation		
Farmer	220	(70%)
Non-farmer	93	(30%)
Education		
None	84	(27%)
Primary level	162	(52%)
Secondary level	45	(14%)
Tertiary level	22	(7%)
Marital status		
Unmarried[a]	95	(30%)
Married	218	(70%)
Economic status[b]		
Lower	85	(28%)
Middle	189	(63%)
Upper	26	(9%)
Being head of household		
Yes	212	(68%)
No	101	(32%)
Distance from the eye hospital (median = 79 km IQR 52–128)		
0–50 km	77	(25%)
50–100 km	111	(35%)
100–150 km	75	(24%)
>150 km	50	(16%)
Nearest health center (Median 3 km, IQR 1–4 km)[c]		
Clinic	10	(3%)
HC II	103	(33%)
HC III	96	(31%)
HC IV	43	(14%)
Hospital	32	(10%)
Don't know	29	(9%)

[a]Unmarried included single divorced and widowed.
[b]Economic status was self-reported where participants compared themselves with their neighborhood as "poor", "neither poor nor rich" or "rich", n was 300 with 13 non-reported values.
[c]The nearest health center was the health center that the patients considered nearest to them regardless of the level of that health center

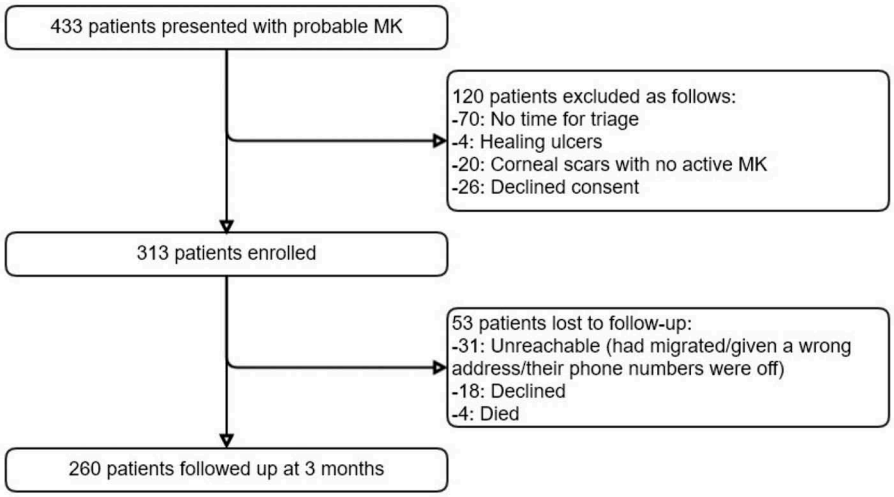

Figure 1. Flow diagram of participants who were enrolled in the cohort study.

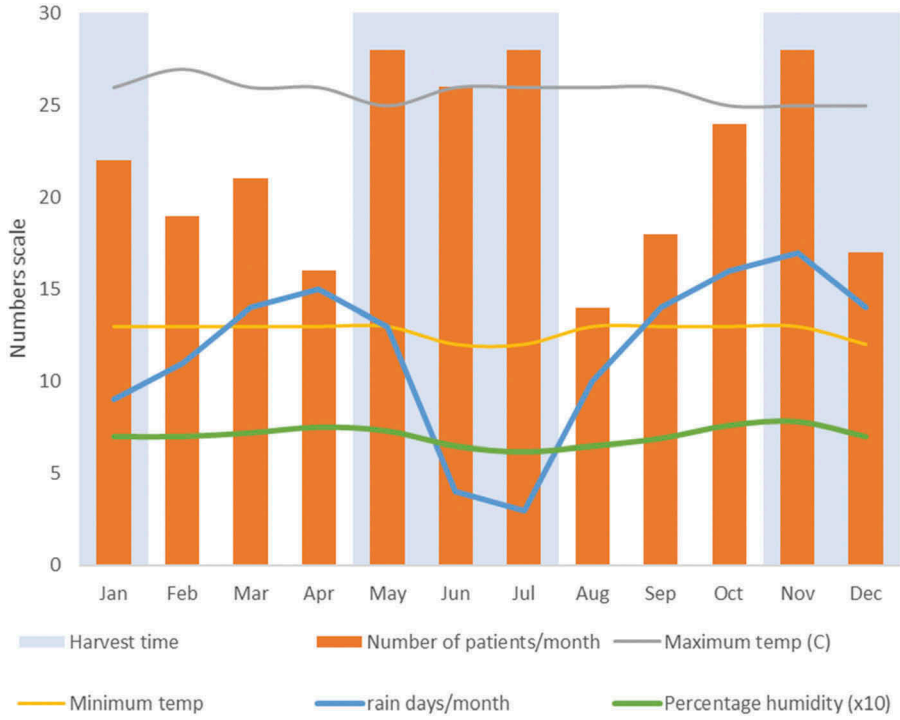

Figure 2. Presentation of patients with MK, by month in 2017 (n = 261). Monthly average minimum and maximum temperatures, average humidity and the number of days with rain are overlaid. Humidity was in percentage but was scaled to tens (divided by 10) to fit on the plot scale.

Presenting history

The median time from onset of symptoms to presentation time at the eye unit was 17 days (IQR 8–32, total range 0–370 days), Table 2. Only 7% of the participants presented "promptly" (within 3 days). Only 29% of the participants reported a history of trauma, and most (74%) of these were classified as organic in nature. Many patients (60%) reported use of TEM.

Table 2. Clinical history.

Variable	n/313	(%)
Presenting time (median = 17 days, IQR 8–32)[a]		
Prompt 0–3 days	23	(7%)
Early 4–7 days	46	(15%)
Intermediate 8–14 days	72	(23%)
Late 15–30 days	79	(26%)
Very late >30 days	90	(29%)
Most important symptom (self-reported)		
Pain	144	(46%)
Reduced vision	137	(44%)
Other	32	(10%)
History of trauma		
Yes	91	(29%)
No	220	(71%)
Used traditional eye medicine		
Yes	188	(60%)
No	125	(40%)
Used other treatment[b]		
Yes	275	(88%)
No	38	(12%)
Diabetic (n = 280)[c]	22	(8%)
HIV positive (n = 284)[c]	37	(13%)

[a]n was 310. For 3 patients the date of onset could not be well ascertained. Some patients had used other forms of eye drops prior to presentation and there was some overlap among those who used TEM and other eye drops.
[b]It was not possible to ascertain the forms of other treatment used.
[c]Some patients declined to be tested for HIV and diabetes

Clinical features and microbiology

Table 3 shows the clinical features at presentation, including detailed characteristics of the ulcers and microbiology results. Specimen for microbiology was collected in 270 patients. Due to limited amounts of sample material, it was not possible to perform all tests on all those sampled. Almost half of the participants (47%) had a visual acuity of less than 3/60 (blind) in the affected eye at presentation. Microbiology results were available in 270/313 (86.3%) participants. Corneal scrapping was not performed on 43 participants who either did not consent, had deep-seated infiltrates, or small infiltrates (less than 0.5 mm). Overall, most infections were fungal (62%), 7% were bacterial and 7% were mixed (fungal and bacterial). Fifty-seven (20%) of the corneal scrapping samples were negative on both microscopy and culture.

Outcomes

Table 4 shows the outcomes of the 260 participants seen at the 3-month follow-up. At 3 months, the visual acuity was better than baseline vision. Median final visual acuity (Log MAR) was 0.4 (IQR 0–1.5) compared to a baseline

Table 3. Clinical features and diagnosis at presentation (n = 313).

Variable	Median	(IQR [Total Range])
Infiltrate size (mm)[a]	5.2	(3.3–7.7 [0.5–13])
Epithelial defect size (mm)[a]	3.9	(2.4–6.5 [0–14])
Variable	n/313	(%)
Snellen Visual Acuity in affected eye (n = 312)		
6/5–6/18	102	(33%)
6/24–6/60	42	(12%)
5/60–3/60	24	(8%)
2/60–1/60	33	(11%)
Counting fingers-light perception	103	(33%)
No light perception	9	(3%)
Snellen visual acuity in non-affected eye (n = 312)		
6/5–6/18	278	(89%)
6/24–6/60	16	(5%)
5/60–3/60	2	(1%)
2/60–1/60	4	(1.2%)
Counting fingers-light perception	6	(2%)
No light perception	6	(1.8%)
Slough (n = 312)[b]		
No slough	62	(20%)
Flat	124	(40%)
Raised	126	(40%)
Infiltrate edge (n = 293)		
Defined	35	(12%)
Serrated	258	(82%)
Not visible	20	(6%)
Satellite lesions present (n = 304)		
Yes	178	(57%)
No	126	(40%)
Infiltrate colour (n = 288)		
White	148	(47%)
Cream	106	(34%)
Other colour	34	(11%)
Hypopyon (median height 1.3mm IQR 0.9–2.9, n = 301)		
Yes	94	(30%)
No	217	(69%)
Site of ulcer (n = 310)[c]		
Peripheral	27	(9%)
Paracentral	64	(21%)
Central	219	(70%)
Perforation status		
Not perforated	237	(76%)
Impending	31	(10%)
Perforated	48	(12%)
Perforated & sealed	7	(2%)
Overall Laboratory diagnosis (n = 270)[d]		
Unknown	65	(21%)
Bacterial	20	(6%)
Fungal	168	(54%)
Mixed (bacteria/fungal)	17	(5%)

Where n < 313 was due to some missing data: percentages calculated for 313 and rounded off to the nearest whole number.

[a]These were calculated as the geometrical means using the MUTT protocol. The upper limits exceeded normal corneal diameter for some lesions, which extended up to the sclera.

[b]Raised slough was when the corneal infiltrate profile was raised, flat slough was when the profile was flat while no slough is when there was no debris noted.

[c]Site of ulcer was peripheral when the ulcer was marginal, paracentral was when the ulcer was not marginal but not within 4 mm of the center of the cornea, central was when the ulcer was within the central 4 mm of the cornea.

Impending perforation is when the clinicians felt the ulcer would perforate in the next 48 h.

[d]Specimen for microbiology was collected in 270 patients. Due to limited amounts of sample material, it was not possible to perform all tests on all those sampled. The order of material collection was 3 slide smears (gram, KOH, CFW), 3 agar inoculations (blood, chocolate, PDA) and 1 broth (BHI) depending on available material.

Table 4. Outcomes at 3 months.

Variable	n/260	(%)
Visual acuity in the affected eye (Snellen)		
6/5–6/18	138	(53%)
6/24–6/60	37	(14%)
5/60–3/60	7	(3%)
2/60–1/60	14	(5%)
Counting fingers-light perception	31	(12%)
No light perception	33	(13%)
Visual acuity in the non-affected eye		
6/5–6/18	229	(90%)
6/24–6/60	11	(4%)
5/60–3/60	2	(1%)
2/60–1/60	0	(0%)
Counting fingers-light perception	6	(2%)
No light perception	7	(3%)
Outcome		
Healed no scar	34	(12%)
Healed mild scar	83	(30%)
Healed moderate scar	65	(24%)
Healed dense scar	46	(17%)
Eviscerated	24	(9%)
Not healed	20	(7%)
Staphyloma	4	(1%)

median of 1.3 (IQR 0.3–2.5). Visual acuity at 3 months improved in 139 participants, worsened in 66 participants and remained unchanged in 56 (sign rank test $p < .0001$). Visual acuity was categorized according to the WHO classification system and poor outcome was considered as vision < 6/60.[19] Thirty percent of the participants were blind in the affected eye (vision less than 3/60) and 9% had lost their eye to infection due to evisceration following endophthalmitis.

Causal modeling for poor presentation

Figure 3 shows the overall model for several variables of interest that we considered in the causal analysis for poor presenting vision. The results are summarized in Table 5 and their corresponding outputs from the DAGitty software in Supplementary Figures 1–5. Those who reported TEM were estimated to have overall 1.6 times the odds of being in a poorer vision category compared to those who did not use TEM (aOR 1.62 [95%CI 1.04–2.54], $p = .033$). It was considered plausible that some of this effect was mediated through delayed presentation and/or organism type, and after adjusting for these factors as well, the aOR was 1.47 [95%CI 0.91–2.38], $p = .11$. There was some evidence ($p = .033$) of an association between the category of presentation time and presenting vision, with the lowest odds of poorer vision being in those that present earliest and increasing odds as delay increases. No evidence ($p = .609$) was found of an association between trauma and presenting visual acuity, but strong evidence was found of an association between presenting visual acuity and both distance from the eye hospital ($p < .001$) and distance from the nearest HC ($p = .007$).

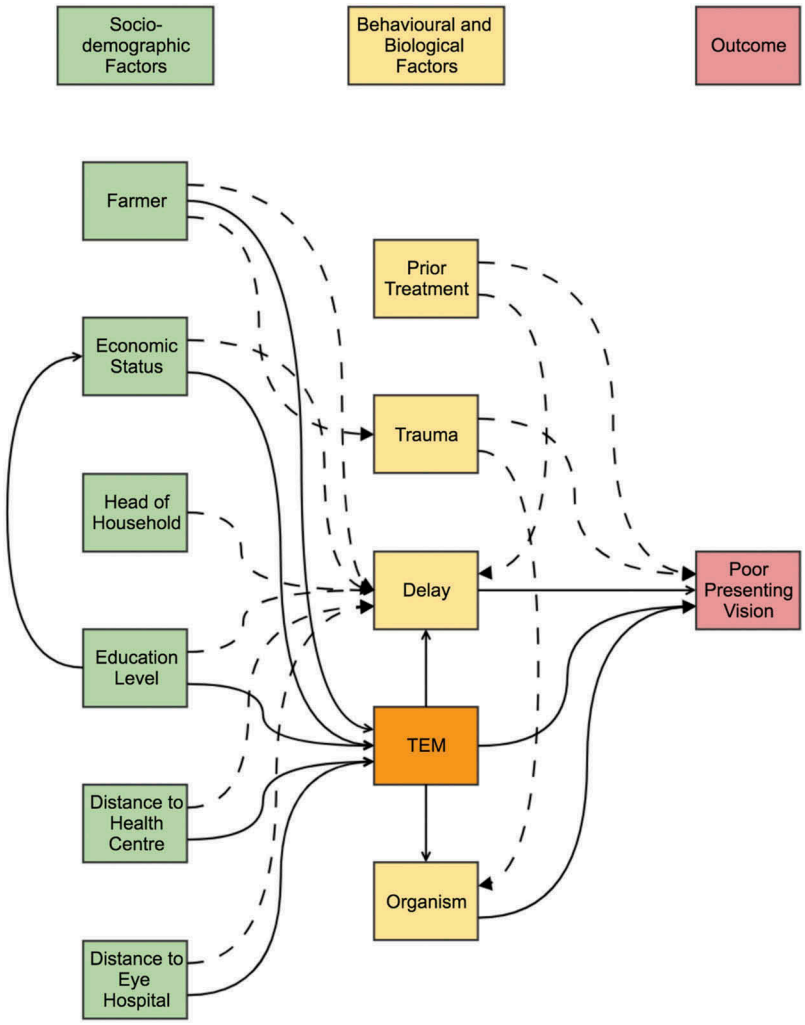

Figure 3. A DAG framework showing the causal pathways for poor presenting vision. This diagram is adjusted to illustrate the role of TEM. The solid lines indicate hypothesized direct relationships and the dashed lines indicate hypothesized indirect relationships.

Interestingly, even after adjusting for delayed presentation there remained strong evidence of an association ($p < .0001$ and $p = .009$).

Predictors of outcome

In the final multivariable model, worse visual acuity outcome at 3 months was associated with baseline vision, size of the infiltrate and perforation status at presentation Table 6.

Discussion

This study describes the clinical history, signs, microbiological etiology, causes, and outcomes of MK in Uganda. Most patients presented with poor vision. At 3 months, 30% had monocular blindness in the affected eye and 1 in 10 lost their eye to infection.

Delayed presentation was common. Very few (7%) presented within 3 days of symptom onset and this had a direct impact on outcomes, as previously reported.[13] In this study, delayed presentation after adjusting for being a farmer, distance, economic status, education status, trauma, TEM and previous use of other treatment was associated with poor presenting vision. Earlier studies indicate that prompt prophylactic antibiotic can prevent simple corneal abrasions developing into MK, leading to much better outcomes.[17,21,22] Most late presenters had advanced ulcers, where treatment could do little. We know from prior literature that once an ulcer is advanced, treatment does relatively little to change its course.[23] From previous studies, it is recommended that treatment of MK should be started as early as possible to achieve optimal outcomes.[17]

Another important cause of poor vision at presentation was Traditional Eye Medicine use. In this study, 60% of the patients reported TEM use. TEM increased the

Table 5. Causal modeling for poor presenting vision (n = 313).

	Univariable Analysis			Multivariable Analysis			Multivariable analysis for direct effect		
Variable	Crude OR[a]	(95% CI)	p-value	Adj. OR	(95% CI)	p-value	OR	(95% CI)	p-value
Model 1: Used Traditional Eye Medicine (TEM) as the main exposure of interest[b]									
Used traditional eye medicine (TEM)	1.78	(1.17–2.70)	0.007	1.62	(1.04–2.54)	0.033	1.47	0.91–2.38	0.11
Model 2: Delayed presentation as the main exposure of interest[c]									
Prompt 0–3 days	1		0.0004	1		0.033			
Early 4–7 days	2.78	(1.07–7.20)		1.94	(0.70–5.39)				
Intermediate 8–14 days	4.45	(1.84–10.7)		3.02	(1.17–7.79)				
Late 15–30 days	5.58	(2.33–13.3)		3.57	(1.40–9.07)				
Very late > 30 days	2.63	(1.11–6.25)		1.87	(0.74–4.72)				
Model 3: Trauma as the main exposure of interest[d]									
Positive history of trauma	0.94	(0.60–1.48)	0.810	1.13	(0.70–1.83)	0.609			
Model 4: Distance from the eye hospital in km as the main exposure of interest[e]									
0–50 km	1		< 0.0001	1		< 0.0001	1		< 0.0001
50–100 km	1.27	(0.73–2.21)		1.27	(0.73–2.21)		1.26	(0.71–2.21)	
100–150 km	2.90	(1.60–5.23)		2.90	(1.60–5.23)		2.63	(1.45–4.80)	
> 150 km	5.60	(2.87–10.9)		5.60	(2.87–10.9)		5.06	(2.58–9.92)	
Model 5: Distance from nearest health center (for every km increase)[e]									
Distance from nearest health center (for every km increase)	1.22	(1.05–1.41)	0.007	1.22	(1.05–1.41)	0.007	1.21	(1.05–1.41)	0.09
Model 6: Type of organism[f]									
No organism detected	1		0.105	1		0.101			
Bacteria	1.25	(0.50–3.15)		1.43	(0.55–3.66)				
Fungal	1.80	(1.05–3.07)		1.82	(1.06–3.13)				
Mixed	2.49	(0.93–6.62)		2.71	(1.07–2.79)				

[a]All crude estimates were adjusted for age and sex. [b]Use of TEM was adjusted for age, sex, being a farmer, economic status, education level, distance from the eye hospital, and distance from the nearest health center (n = 298). After adjusting for delay and organism type, the effect of TEM was OR 1.47 95% CI 0.91–2.38, $p = 0.11$. [c]Delayed presentation was adjusted for age, sex, being a farmer, distance, economic status, education level, TEM, trauma, and previous use of prior treatment before presentation (n = 295). [d]History of trauma was a priori based on literature from previous studies. It was adjusted for age, sex, being a farmer, TEM, distance, and prior treatment (n = 306). [e]Long distance from the eye hospital and long distance from the nearest health center were only adjusted for age and sex (n = 309). Their crude and adjusted point estimates are the same. However, the direct effect of distance to eye hospital and distance to nearest health center after adjusting for delay was still highly significant, $p < 0.0001$ and = 0.009. [f]Type of organism was a forced priori and was adjusted for trauma and use of TEM (n = 267).

Table 6. Factors at presentation predictive of a poor final visual acuity (WHO snellen ordinal scale) at 3 months (n = 260).

	Univariate Analysis			Multivariable Analysis		
Variable	Crude OR[a]	(95% CI)	p-value	Adjusted OR[b]	(95% CI)	p-value
Baseline visual acuity (for every line decrease in vision)	4.78	(3.59–6.35)	< 0.0001	2.98	(2.12–4.19)	< 0.0001
Presence of slough						
None	1		0.007			
Flat	1.91	(0.95–3.83)				
Raised	2.95	(1.46–5.95)				
Infiltrate edge being serrated	0.84	(0.58–1.24)	0.393			
Satellite lesions being present	0.64	(0.40–1.03)	0.068	0.51	(0.28–0.90)	0.021
Infiltrate color						
White	1		< 0.0001			
Cream	2.70	(1.56–4.63)				
Colored	6.37	(3.10–13.2)				
Hypopyon present	2.16	(1.38–3.55)	0.002			
Infiltrate size (for every 1 mm increase)	1.60	(1.44–1.79)	< 0.0001	1.19	(1.03–1.36)	0.020
Perforation status at presentation						
Not perforated	1		< 0.0001	1		< 0.0001
Impending perforation	11.9	(5.27–26.9)		2.86	(1.11–7.37)	
Perforated and sealed	5.60	(1.44–21.8)		1.57	(0.31–7.76)	
Perforated	41.0	(17.3–97)		9.93	(3.70–26.6)	
HIV status being positive	0.85	(0.39–1.85)	0.683			
Diabetes status being positive	0.81	(0.34–1.92)	0.630			
Microbiology						
No organism detected	1		0.063			
Bacteria	1.48	(0.53–4.14)				
Fungal	2.25	(1.19–4.26)				
Mixed	2.80	(0.86–9.01)				

[a]All crude estimates were adjusted for age and sex. [b]Final predictive model adjusted for age and sex.

odds of poor presentation by 60% after adjusting for age, sex, being a farmer, economic status, education level, and distance. In our model, some of the effects of TEM seemed to be mediated through delay and organism type. But after adjusting for these, there was still an estimated 40% increase in odds of poor presentation,

although the evidence for this association was weak. Many people probably try TEM before attending hospital, as it can be easily obtained within or close to home. In Uganda, TEM is usually made from plant products. This is concerning, as such substances may be toxic or harbor infectious agents, such as fungal spores.[7,24] Importantly, our patients were open in admitting use of TEM, a widely acceptable practice for treating MK.

Distance was an important cause of poor presenting vision. This included distance to the eye hospital and distance to the nearest HC. This highlighted a major underlying problem of access to health services: the further the HC, the lower the chances of promptly starting appropriate treatment. In our model, even after adjusting for delay, distance was still highly associated with poor presenting vision meaning that there were still other unexplained factors in this relationship.

As reported previously, severity of infection at presentation (vision, perforation status, and infiltrate size) was the strongest predictor of outcome.[23,25,26] Poor vision at presentation (WHO Snellen categories) was strongly associated with a worse visual outcome. Vision is an easily measurable and reliable prognostic measure that can support lower and mid-level cadres to make the right clinical decisions. A perforated eye at presentation had 10 times greater odds while an eye with an impending perforation had 3 times greater odds of a worse visual outcome compared to a non-perforated eye. This was not surprising because keratoplasty services are currently not available in Uganda. People who presented with threatened or full perforation underwent conjunctival flap or evisceration surgery depending on the extent of the perforation.

Most of our patients presented with large infiltrate and epithelial defect sizes. Such median sizes would be considered severe ulcers in a high-income setting. The epithelial defect size was not included in the analysis because it was highly correlated to the infiltrate size. A large infiltrate size was associated with increased odds of a worse final visual outcome.[25,26]

Most of the affected patients were aged between 31 and 60 years, which are the prime years for economic productivity.[13] About 70% of the affected people were heads of households and sole breadwinners in their home. Prolonged morbidity due to MK meant that they could not provide for their dependents. In an ongoing study, we have been exploring how MK affects the quality of life and household incomes (unpublished). The prevalence of HIV among our cohort was almost double the national prevalence and diabetes was 4 times the reported prevalence.[27,28] A high prevalence of HIV has been previously reported in people with MK.[13,29] HIV and diabetes predispose to MK through immune suppression: we conducted a nested case-control to test for risk factors of MK including HIV and diabetes which have been reported separately.

Understanding the seasonal pattern of presentation is important to prepare a surveillance mechanism and for hospitals to have expectant management. We found that the presentation of MK tended to follow rainfall patterns linked to agricultural activity. This was not surprising since the majority (70%) of patients were farmers. There was little variation in humidity and temperature throughout the year. This region of Uganda has two planting and harvesting seasons, one in each half of the year following rains. Harvesting time is May–July and November–January. These were the periods when we recorded increased numbers of presentations. Farming (especially harvesting) has been linked to ocular trauma which predisposes to MK.[30] These corresponded with peak presentation to hospital. April has modest farming activity, as people are waiting for the harvesting season and it usually corresponds to Easter holiday. August usually has almost no farming activity since it comes at the end of the harvesting season before the rains come again in September. December had fewer patients presenting, possibly due to the Christmas season.

It remains unclear if this seasonal variation was related to trauma, as there were no clear seasonal differences in the pattern of presentation among patients who reported trauma and those who did not. We were surprised that relatively few patients (29%) reported trauma, although this is consistent with other studies from sub-Saharan Africa (SSA). In an older study from Ghana, 39% of the MK cases reported some form of eye injury prior to onset.[31] In two separate studies from Tanzania, 24% and 39% of the cases were associated with trauma.[13,32] These levels are somewhat lower than those from South Asia, where around 75% are associated with an injury.[31,33–36] The reason for this difference is not apparent.

Ocular microbiology is not performed in many settings in SSA. As part of this study, we undertook to build the capacity of the hospital to provide this service. The overall microbiology yield was 80%. This was a composite of all the microscopy and culture results. Overall culture positive results were 55% similar to the expected yield reported in literature.[16,31]

Strengths/limitations

This is the first large prospective cohort study in SSA to describe outcomes of MK. Most of the reports have described etiology and presentation.[16,29,31,32] Only one audit had attempted to describe outcomes.[13] The large number of patients gave sufficient power to analyze

several factors associated with the main outcome measures. It was not possible to follow-up all the patients, with around 20% not having 3-month outcome data; however, no systematic differences were found between those with and without final follow-up data.

Conclusion

This study provides an understanding of MK epidemiology in Uganda. Majority of patients presented late after having traveled large distances to seek specialist care. Most patients presented with severe ulcers. The outcomes for many were poor, although around half had some improvement of vision with treatment. Predictive factors for these poor outcomes were the state of the eye at presentation. There is need to work on early interventions to prevent patients reaching such a stage where little can be done.

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Conflict of interest

None of the authors have any proprietary interests or conflicts of interest related to this submission.

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Submission statement

This submission has not been published anywhere previously and that it is not simultaneously being considered for any other publication.

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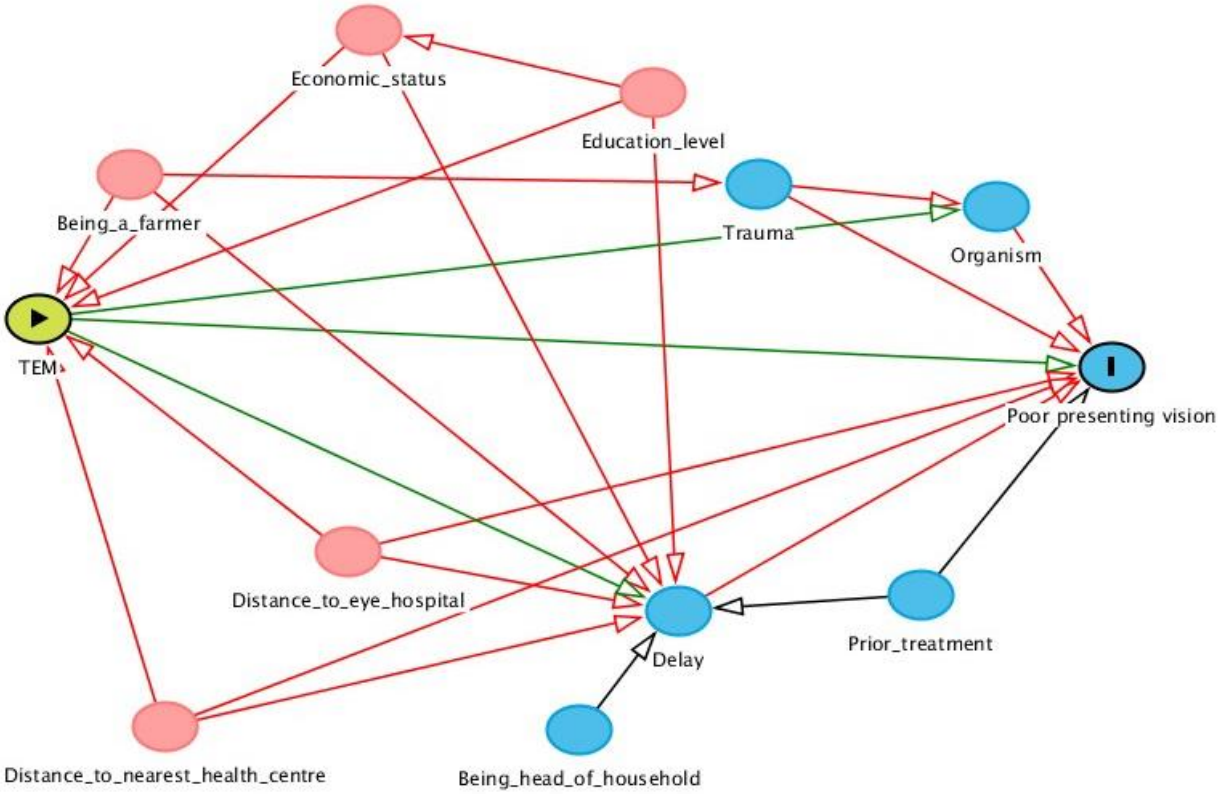
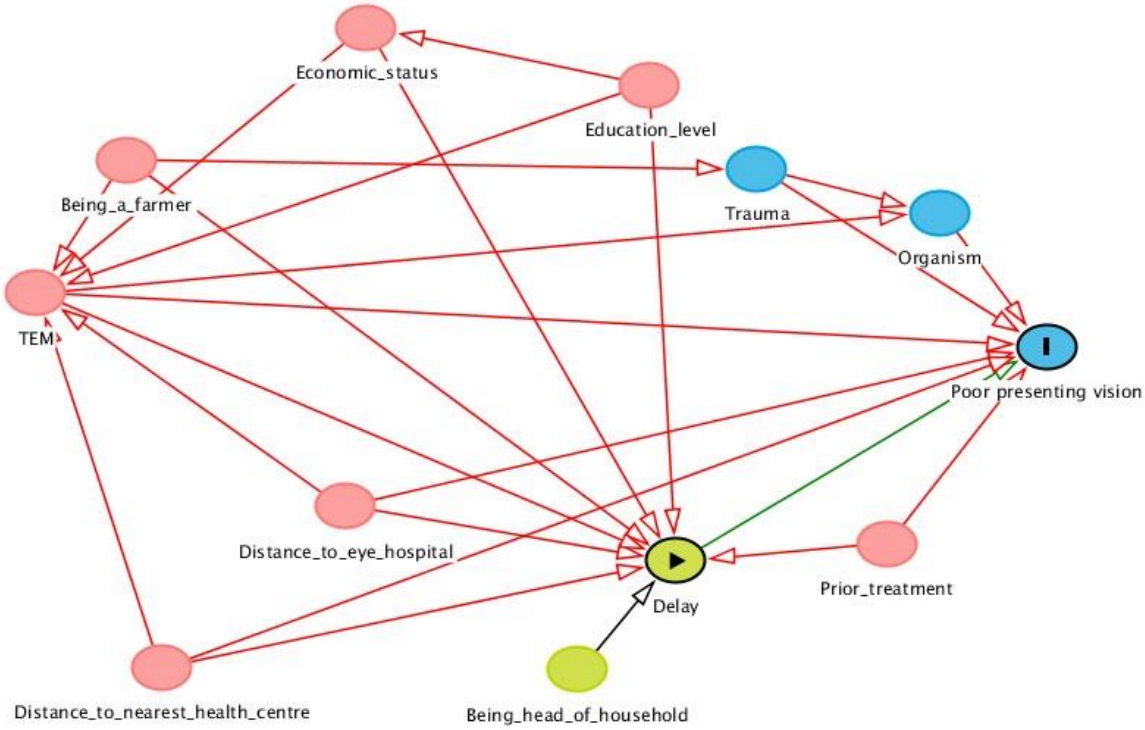


Supplimentary Figure 1: DAG for a causal effect of TEM on presenting vision

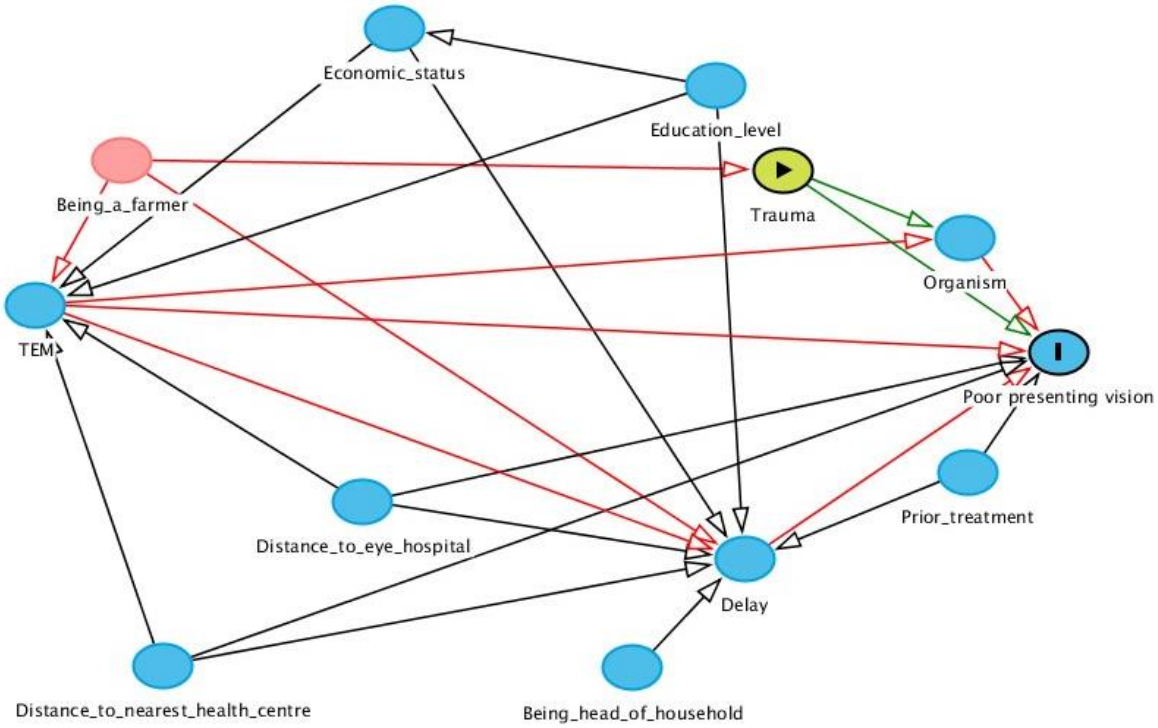
In this DAG, it was necessary to adjust for being a farmer, distance from the eye hospital and distance to the nearest health centre, economic status and education level to be able to correctly estimate the overall effect of using TEM on poor presenting vision. We also separately adjusted for delay and organism type to estimate a direct effect of TEM on poor presenting vision.

KEY: In the DAGiity software, Green with a black arrow represents exposure of interest, Green without an arrow represents ancestor of the exposure, Pink is ancestor of the exposure and outcome, Blue is ancestor of the outcome.



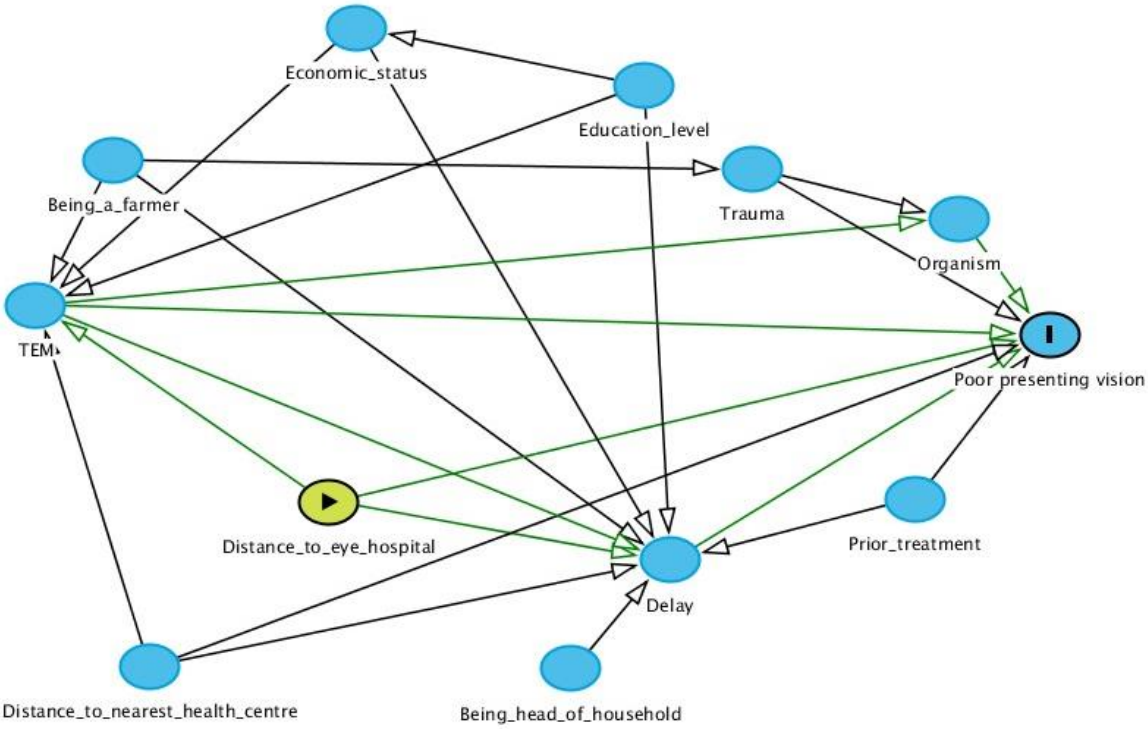
Supplimentary Figure 2: DAG for a causal effect of delayed presentation on presenting vision

In this DAG, it was necessary to adjust for being a farmer, distance, Economic status, Education status, trauma, TEM and previous use of other treatment to estimate the overall causal effect of delayed presentation on presenting vision



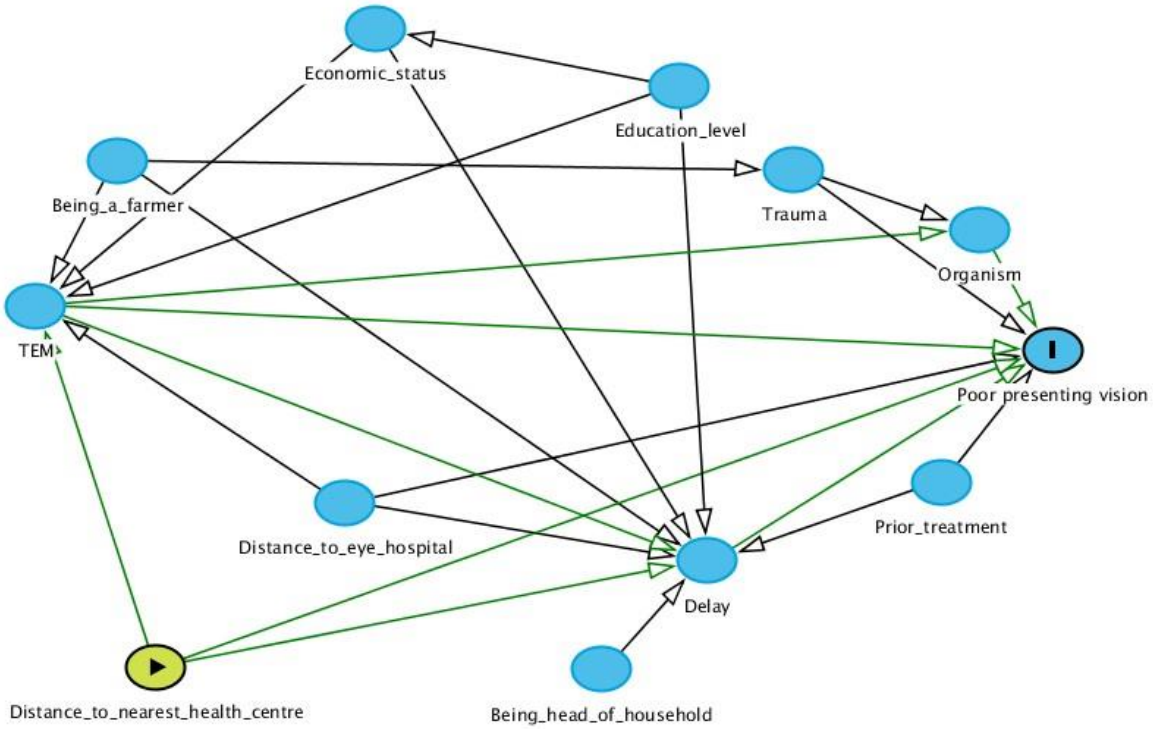
Supplimentary Figure 3: DAG for a causal effect of Trauma on presenting vision

In this DAG, it was necessary to adjust for being a farmer, delay, distance, TEM and prior treatment to estimate the overall effect of Trauma on presenting vision



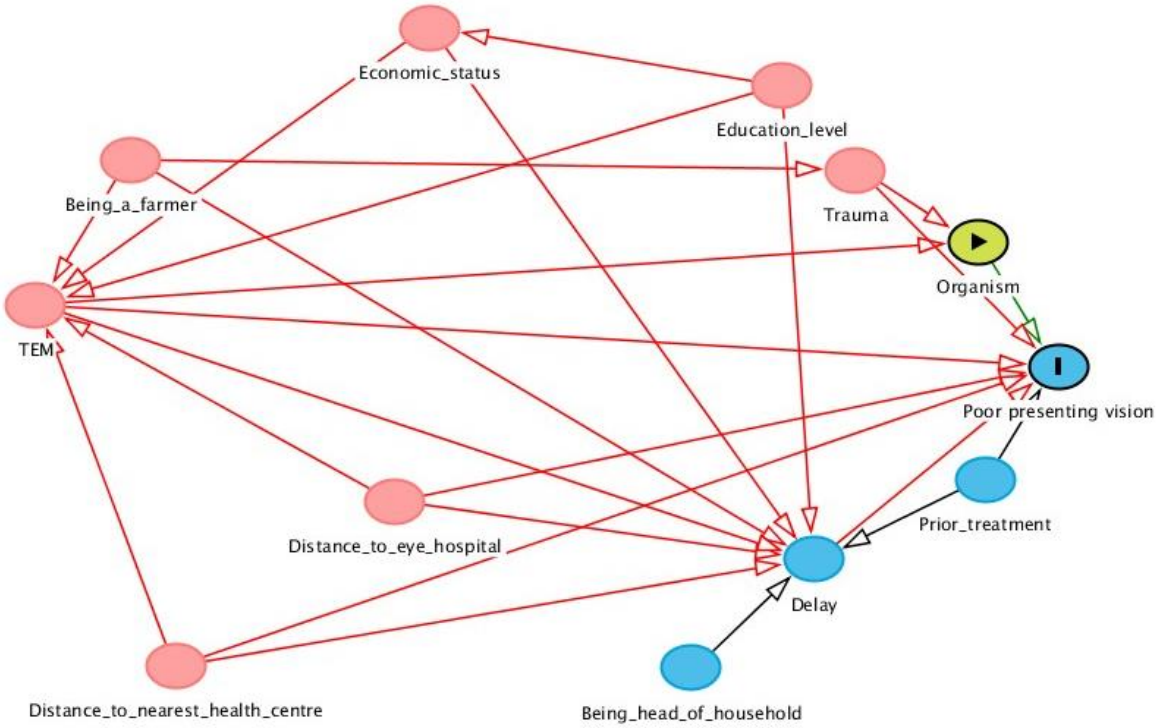
Supplimentary Figure 4: DAG for a causal effect of distance from the eye hospital on presenting vision

In this DAG, it was not necessary to adjust for anything apart from age and sex to estimate the overall causal effect of distance from the eye hospital on presenting vision. However, we separately for delay to estimate a direct effect of distance to the eye hospital on presenting vision.



Supplimentary Figure 5: DAG for a causal effect of distance from the nearest health centre on presenting vision

In this DAG, it was not necessary to adjust for anything apart from age and sex to estimate the overall causal effect of distance from the nearest health centre on presenting vision. However, we separately adjusted for delay to estimate a direct effect of distance to nearest Health Centre on presenting vision.



Supplimentary Figure 6: DAG for a causal effect of organism type on presenting vision

In this DAG, it was necessary to adjust for Trauma and use of TEM to estimate the overall causal effect of organism type on presenting vision

Microbiology results from patients with microbial Keratitis in Uganda (n=313)

Variable	Category	Count	(%)
Gram microscopy	Unknown	141	57%
	Bacteria	33	13%
	Fungal	73	30%
	Mixed	0	0
KOH	Unknown	158	67%
	Fungal	77	33%
Calcofluor white/KOH stain	Unknown	79	33%
	Fungal	163	67%
BHI microscopy	Unknown	126	57%
	Bacteria	22	10%
	Fungal	72	33%
BHI culture	Unknown	120	55%
	Bacteria	23	11%
	Fungal	74	34%
Blood agar culture	Unknown	109	51.5%
	Bacteria	21	10%
	Fungal	80	38%
	Mixed	1	0.5%
Chocolate agar culture	Unknown	101	52%
	Bacteria	20	10%
	Fungal	75	38%
Potato dextrose agar culture	Unknown	133	60%
	Fungal	90	40
Overall Laboratory diagnosis (n=257, 74 were not tested)	Unknown	56	22%
	Bacterial	21	8%
	Fungal	162	63%
	Mixed (Bacteria/Fungal)	18	7%
Cultured organisms	*Staph Aureus (2 mixed)*	8	3%
	Strep Pneumonae	8	3%
	Pseudomonas	6	2.5%
	Klebsiella	4	2%
	Norcadia	1	0.5%
	Fusarium (2mixed)	45	19%
	Aspegillus	18	8%
	Acremonium	13	6%
	Bipolaris	6	2.5%
	Scedospovium	1	0.5%
	Candida	3	1.5%
	Lasiodiplodia	2	1%
	Unidentified fungi	9	3.5%
	No growth	110	47%
Yield rates	Gram	106/247	43%
	KOH	77/235	33%
	CFW/KOH	163/242	67%
	BHI Gram	94/220	43%
	BHI culture	97/217	45%
	Blood agar	102/211	48%
	Chocolate agar	95/196	48%
	Potato dextrose agar	92/223	41%
	Composite	201/257	78%
Bacterial sensitivity	Ciprofloxacin	22/23	96%

	Ofloxacin	14/14	100%
	Levofloxacin	23/23	100%
	Amikacin	5/5	100%
	Ceftriaxone	23/23	100%
	Gentamycin	20/22	91%
	Chloramphenicol	0/23	0%
	Tetracycline	0/23	0%

This table shows the preliminary microbiology results and bacterial sensitivity results of the 313 patients with MK who were enrolled into the study. Of these, 74 microbiology specimens were not collected from 74 patients due to various reasons (small ulcers, deep ulcers, uncooperative patients). Corneal swabs were collected and shipped for pan fungal sequencing at a regional reference laboratory in Tanzania. A final microbiology profile will be published once these results are verified.

Chapter 7. Risk factors of Microbial Keratitis in Uganda

A traditional healer presents some of the common herbs used in the community for treating common eye conditions. Photo taken by Terry Cooper

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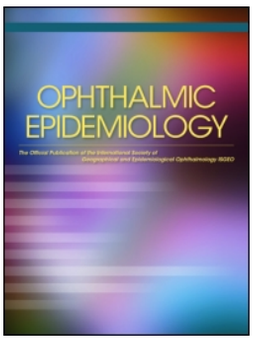

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Risk Factors of Microbial Keratitis in Uganda: A Case Control Study

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ABSTRACT

Purpose: Microbial keratitis (MK), is a frequent cause of sight loss worldwide, particularly in low and middle-income countries. This study aimed to investigate the risk factors of MK in Uganda.

Methods: Using a nested case control, we recruited healthy community controls for patients presenting with MK at the two main eye units in Southern Uganda between December 2016 and March 2018. Controls were individually matched for age, gender and village of the cases on a 1:1 ratio. We collected information on demographics, occupation, HIV and Diabetes Mellitus status. In STATA version 14.1, multivariable conditional logistic regression was used to generate odds ratios for risk factors of MK and a likelihood ratio test used to assess statistical significance of associations.

Results: Two hundred and fifteen case-control pairs were enrolled. The HIV positive patients among the cases was 9% versus 1% among the controls, $p = .0003$. Diabetes 7% among the cases versus 1.4% among the controls, $p = .012$. Eye trauma was 29% versus 0% among the cases and controls. In the multivariable model adjusted for age, sex and village, HIV (OR 83.5, 95%CI 2.01–3456, $p = .020$), Diabetes (OR 9.38, 95% CI 1.48–59.3, $p = .017$) and a farming occupation (OR 2.60, 95%CI 1.21–5.57, $p = .014$) were associated with MK. Compared to a low socio-economic status, a middle status was less likely to be associated with MK (OR 0.29, 95%CI 0.09–0.89, $p < .0001$).

Conclusion: MK was associated with HIV, Diabetes, being poor and farming as the main occupation. More studies are needed to explore how these factors predispose to MK.

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Background

Microbial keratitis (MK), or infection of the cornea, can be caused by a range of pathogens. The causative organisms include bacteria, viruses, protozoa (e.g. acanthamoeba), and fungi (yeasts, moulds and microsporidia). It is characterised by acute or sub-acute onset of pain, conjunctival hyperemia and corneal ulceration with a stromal inflammatory cell infiltrate. MK frequently leads to sight-loss from dense corneal scarring, or even loss of the eye, especially when the infection is severe and/or appropriate treatment is delayed.[1]

MK in low and middle-income countries (LMIC) has been described as a "silent epidemic", which leads to substantial morbidity, related to blindness and other consequences such as pain and stigma.[2] It is the leading cause of unilateral blindness after cataract in Tropical regions and is responsible for about 2 million cases of monocular blindness per year.[3] The World Health Organization (WHO) estimated (2017) that 1.3 million individuals are bilaterally blind from corneal opacity globally (excluding trachoma and vitamin A deficiency), accounting for 3.2% of binocular blindness.[4] In Sub-Saharan Africa (SSA), MK is an important cause of binocular blindness and is responsible for about 15% of monocular blindness (Nigeria National Survey).[5,6] The incidence of MK in South India was estimated at 113/100,000/year and in Nepal 799/100,000/year.[7,8] There is only one older report of the incidence of MK in SSA from Malawi, which suggested a rate of around 180/100,000/year.[9] Rates in high-income settings are lower.[10]

There are many potential risk factors that may predispose a person to developing MK with some risk factors being more specific to settings (region, income status and organism) and some being ubiquitous. Risk factors such as trauma especially with vegetative matter have been associated with fungal keratitis compared to

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a pre-existing ocular disease for bacterial keratitis.[11,12] Injury with mud is strongly linked to Acanthamoeba keratitis.[12] In addition, agricultural work and foreign body in the eye have been implicated.[11,13,14] Risk factors that are more setting specific include the use of contact lenses, which affects more people in high-income countries as opposed to the use of traditional eye medicines (TEM), which is more of a problem in Low and Middle-Income Countries (LMIC).[9,15–18] Other identified risk factors include age (trauma being common in the lower age groups versus ocular surface diseases in older folk), gender (males engaging more in outdoor activities than females) and poverty (MK mostly is more prevalent in among the poor).[11–13] Diabetes Mellitus (DM) has been the most commonly reported systemic risk factor, especially following keratoplasty or corneal trauma.[19–21]

In the few studies from SSA on the risk factors for MK, suggested factors include trauma and use of TEM.[22–24] The other risk factors reported in the literature are steroid use, severe staphylococcal eyelid infections and the HIV positive cases.[17,22–25] However, all previous studies from Africa, had a limited extrapolation of outcome due to the lack of controls.

The aim of this study, therefore, was to investigate the role of multiple risk factors (HIV infection, DM, farming) which are preventable or modifiable by comparing MK cases to disease free community controls, matched for age, sex and village in Uganda.

Methods

Ethical statement

This study followed the tenets of the Declaration of Helsinki. It was approved by the London School of Hygiene & Tropical Medicine Ethics Committee (Ref 10647), Mbarara University Research Ethics Committee (Ref 10/04-16) and Uganda National Council for Science and Technology (Ref HS-2303). Written informed consent in Runyankore, the local language, was obtained before enrolment. If the patient was unable to read, the information was read to them, and they were asked to consent by application of the thumbprint which was independently witnessed.

Study design and setting

A pair-matched case control study was used with a 1:1 case-to-control ratio. The cases were recruited during the main cohort study that prospectively enrolled patients with MK that presented to Ruharo Eye Centre (REC) and Mbarara University and Referral Hospital Eye Centre (MURHEC) from December 2016 to March 2018. MURHEC is a government owned tertiary eye unit established in 2013. It provides mostly free services and attends to about 6,000–10,000 patients/year. REC is a church-based fee-paying tertiary eye hospital founded in the 1960s. Attendance is about 20,000–25,000 patients/year. Both hospitals are in Mbarara Municipality, South-Western Region, Uganda, approximately four hours' drive far from Kampala. The two units are about 5km apart and work closely. Controls were enrolled in communities where the cases came from.

Study participants

For the purpose of this study microbial keratitis was defined as the loss of corneal epithelium (of at least 1mm diameter) with underlying stromal infiltrate, associated with any or all signs of inflammation (conjunctival injection, anterior chamber inflammatory cells, ± hypopyon).[26] Controls were healthy individuals (without any current eye complaint) matched for gender and address. For cases and controls, we excluded those not willing to participate, those not willing to return for follow-up, pregnant women, lactating mothers and those aged below 18 years.

Sample size

The prevalence of HIV in the general population in Uganda is 6%. A sample of 200 case-control pairs would have 80% power and 95% confidence to detect an odds Ratio of three between cases and controls. From Tanzanian data we expected that perhaps more than 10% of MK cases in our cohort would have HIV infection.[17,27]

Assessment

Cases

We documented baseline demographic information and ophthalmic history including how the eye became infected, predisposing factors such as trauma, prior use of Traditional Eye Medicine (TEM), treatment received, and their "health care seeking journey" before reaching to the eye hospital. In summary, cases underwent a detailed anterior and posterior segment examination on a slit lamp. Corneal scrapes were collected for microscopy, culture and sensitivity and molecular diagnosis. HIV counselling and testing were offered, as per the Uganda Ministry of Health HIV testing protocol where three rapid tests (Determine HIV-1/2/O [Abbott Laboratories, Abbott Park, IL],

HIV 1/2 Stat-Pak Ultra-Fast [Chembio Diagnostic Systems, Medford, NY] and Uni-Gold Recombinant HIV-1/2 [Trinity Biotech, Bray, Ireland]) were used to screen participants.[28] For those who were confirmed as HIV positive, a CD4 test was performed for level of immune suppression. They were referred to the HIV care centre, which is on the hospital site. If a patient refused the HIV test, they were still enrolled for the main cohort but were censored for the nested study. A peripheral prick for blood sugar was taken and WHO guidelines were used to make a diagnosis of Diabetes (random glucose >11.1mmol/L or fasting glucose of >7.0mmol/L).[29] Cases were treated empirically at presentation; the treatment choice was reviewed after microbiology results according to the hospital protocol. The study follow-up assessment was on day 2, day 7, day 21 and at day 90 to determine their outcome. Additional assessments were conducted as clinically indicated.

Controls

At 3 months, the cases were followed-up in their homes for a final assessment at which point healthy community controls were enrolled. Enrolment followed a similar method as previously used in Ethiopia.[30] The research team visited the villages (100–200 households), the local village head was asked to write down all the eligible controls in that village. They were people of the same gender and in a similar age bracket (decade) as the case. One person was randomly selected from this list using a lottery method, explained to the details of the study and invited to participate if eligible. If a selected control refused or was ineligible, another was randomly selected by lottery. Demographic data was collected as well as a detailed history of exposure to trauma, TEM use, DM and HIV status. A random blood sugar and HIV counselling and testing were offered, as per the Uganda Ministry of Health HIV testing protocol. Home testing for HIV is widely practised in Uganda. For those who were confirmed as HIV positive, a CD4 test was performed for the level of immune suppression. They were referred to the nearest HIV centre for appropriate care. Cases and controls were asked to self-report their wealth status compared to their neighbours using a scale of 1 "very poor" 2 "poor" 3 "neither poor nor rich" 4 "rich" 5 "very rich".[31]

Analysis

Data were analysed in STATA v14. All cases and controls were individually matched by age, gender and village. However, we noticed in the analysis that all the pairs had not been correctly matched on age because the village heads had subjectively guessed the ages of the controls. We thus adjusted for age throughout the analysis. We compared the proportions of potential risk factor exposures among cases and control and performed a Mcnemar's chi^2 test (binary exposures) and a univariable conditional logistic regression (categorical exposures) for significance of the differences. The main exposures of interest were HIV positive patients, DM patients, farmers and participants with a positive history of trauma and or TEM use.

Multivariable conditional logistic regression analysis was used to estimate odds ratios (ORs) and 95% CI's of risk factors of MK. The likelihood ratio test was used to assess statistical significance of associations. Variables with a p-value less than 0.1 were introduced in the multivariable model. For variables with a high collinearity, the variable of most interest was included in the model. A backward stepwise approach was then used until only the variables with a p-value of less than 0.05 were retained.

Because it was not possible to enrol controls for all the cases in the cohort, a separate analysis was performed to compare the baseline characteristics of the cases who had controls and those who did not to look for any systematic bias. A Pearson Chi test (categorical variables) or a Wilcoxon rank sum test (continuous variables) was used to test for significance of the differences.

Results

A total of 215 controls were enrolled out of 260 eligible cases who had 3-months outcome data. It was not possible to enrol controls for 45 cases because of several reasons. These included: not at home at the agreed time of the home visit (11), wrong home address (4), died (1), uncooperative village members (20), case address too far (9). The cases without controls were dropped from the matched risk factor analysis. We compared the baseline characteristics and exposure proportions between the cases for whom we were able to enrol controls versus the patients without controls (Table 1). Overall, these two groups were comparable across most of the characteristics. However, there was a significant difference in the proportion of HIV (22%) among the cases without controls versus the cases with controls (8%), (Chi-square test 10.7, p = .001, df 1). The overall prevalence of HIV was 12% and DM was 7%. Out of all the 37 HIV positive cases, 14 (38%) were newly diagnosed after presenting with MK. They were unaware of their current HIV status or had previously tested negative. The median CD4 count was 358 cells/μL (IQR 267–533, total range 154–1,053). Out of

Table 1. Comparison of people who were enrolled into the nested case-control and those who were not (n = 313).

	Enrolled into the case-control (n = 215)			Not enrolled (n = 98) ‡			
Variable	Median	(IQR)	(Total range)	Median	(IQR)	(Total range)	P value
Age	50	(37–60)	(18–96)	42	(33–59)	(18–87)	.040
Distance	78	(53–120)	(1.5–286)	85	(48–183)	(0.2–378)	.171
Household population	7	(5–8)	(1–28)	6	(3–8)	(1–18)	.030
Distance to nearest Health Centre in KM	3	(1–4)	(0–45)	2	(1–4)	(0–35)	.215
Variable	Category	count	(%)	count	(%)		P value
Gender	Female	101	(47)	38	(39)		.176
	Male	114	(53)	60	(61)		
Occupation	Farmer	157	(73)	63	(64)		.117
	Non-farmer	58	(27)	35	(36)		
Marital status	Not married*	61	(28)	34	(35)		.259
	Married	154	(72)	64	(65)		
Education status	None	60	(28)	24	(25)		.896
	Primary	110	(51)	52	(53)		
	Secondary	31	(14)	14	(14)		
	Tertiary	14	(7)	8	(8)		
Being head of household	Yes	146	(68)	66	(67)		.922
	No	69	(32)	32	(33)		
Being HIV positive (overall 12%) ł	Yes	18	(8%)	19	(22%)		.001
	No	197	(92%)	67	(78%)		
Being a Diabetic patient (overall 7%) ł	Yes	14	(7%)	8	(9%)		.385
	No	201	(93%)	77	(91%)		

*Not married refers to single, separated, divorced or widowed. ł missing results for HIV and diabetes, it was not possible to test everyone for HIV and Diabetes. ‡ These 98 include the 53 that were lost to follow up and the 45 cases with follow-up data at 3 months but to whom controls could not be enrolled.

Table 2. A matched comparison of exposures among 215 case-control pairs. (gender and village and adjusted for age).

	Cases (215)		Controls (215)		P-value
Exposure	n	(%)	n	(%)	
Married	154	(72)	143	(67)	.215
Head of household	146	(68)	140	(65)	.441
Education status					
None	60	(28)	48	(22)	.148
Primary	110	(51)	114	(53)	
Secondary	31	(14)	32	(15)	
Tertiary	14	(7)	21	(10)	
Farming occupation (if yes)	157	(73)	168	(78)	.144
Trauma (if yes, n = 214)	63	(29)	0	(0)	<.0001
Traditional Eye Medicine (if yes)	133	(62)	1	(0.5)	<.0001
HIV (being positive) *	18	(9)	2	(1)	.0001
Diabetes Mellitus (being positive) ł	14	(7)	3	(1.4)	.012
Size of the household					
Small (1–4 people)	50	(23)	109	(51)	
Medium (5–10 people)	115	(54)	94	(44)	
Large (>11 people)	50	(23)	12	(5)	
Self-reported wealth status ‡					
Poor	36	(18)	20	(9)	.003
Middle	158	(74)	188	(89)	
Upper	21	(8)	6	(2)	
Type of water source					
Well	103	(50)	107	(52)	
Tap	85	(41)	74	(36)	
Other	17	(9)	25	(12)	
	median	(IQR)	median	(IQR)	
Distance to nearest Health centre	3	(1–4)	2	(1–3)	<.0001

*Twelve cases had missing HIV results, however, all the controls had HIV results reported. ł Nineteen Cases had missing Diabetes test results. self-reported wealth status was classified as poor (1" very poor" 2" poor"), middle (3 "neither poor nor rich") upper (4 "rich" 5 "very rich")

the 22 DM patients, 11 (50%) were diagnosed after presenting with MK.

Table 2 shows exposure comparison among the cases and controls matched for age, sex and village. The proportion of HIV positive patients among the cases was 9% versus 1% among the controls (p = .0003). DM was 7% among the cases versus 1.4% among the controls (p = .012). Sixty-one (29%) of the cases reported eye trauma before onset of symptoms, none of the controls reported any trauma in the previous 3 months. One hundred and twenty-eight (61%) of the cases reported having used TEM versus only one control who had recently used TEM. Cases more than controls had more people in the poor social economic bracket (p = .0001) and lived further from the nearest village health centre, median distance 3km (IQR 1–4, total

Table 3. A matched univariable and multivariable analysis of risk factors of Microbial Keratitis among 215 case-control pairs (matched for sex, village and adjusted for age).

	Univariate Analysis			Multivariable Analysis		
Variable	Crude OR	(95% CI)	p-value	Adjusted OR	(95% CI)	p-value
Farming occupation (if yes)	2.10	(1.12–3.92)	.021	2.60	(1.21–5.57)	.014
HIV (being positive)	18.3	(2.41–139)	.005	83.5	(2.01–3456)	.020
Diabetes Mellitus (being positive)	4.75	(1.29–17.6)	.019	9.38	(1.48–59.3)	.017
Size of the household*						
Small (1–4 people)	1	(reference)	<.0001			
Medium (5–10 people)	5.09	(2.89–8.94)				
Large (>11 people)	1.88	(0.69–5.12)				
Social economic status						
Poor	1	(reference)	<.0001	1	(reference)	<.0001
Middle or upper ǂ	0.21	(0.08–0.56)		0.29	(0.09–0.89)	
Upper	1.14	(0.23–5.58)		1.96	(0.34–10.9)	
Distance to the nearest Health Centre (increase/km)	1.32	(1.14–1.53)	<.0001	1.39	(1.14–1.67)	.001

*Family size was highly correlated with wealth status (p = 0.02) and was not included in the model. All analysis was adjusted for age

range 0–45) versus 2km (IQR 1–3, total range 1–15) among the controls (p < .0001).

Table 3 shows the univariable and multivariable analysis for risk factors of MK adjusted for age, sex and village. These were all adjusted for age, sex and village. In the final model, important risk factors were HIV (OR 83.5, 95%CI 2.01–3456, p = .020), DM (OR 9.38, 95% CI 1.48–59.3, p = .017), a farming occupation (OR 2.60, 95%CI 1.21–5.57, p = .014) and living far from a health facility (OR 1.39, 95%CI 1.14–1.67, p = .001) were strongly associated with MK. On the other hand, a middle compared to a low social economic status was less associated with MK (OR 0.29, 95%CI 0.09–0.89), p < .0001).

Discussion

This was the first case control study in SSA to investigate the risk factors of MK. We found that the significant risk factors were trauma, HIV, DM, farming, living far from a health facility and poverty.

The odds of being HIV-positive was higher among MK cases than in the controls, suggesting HIV is a risk factor for MK. HIV affects the immune system making its host susceptible to a range of opportunistic infections. Two previous studies, both from Tanzania, suggested a possible relationship between HIV and Keratitis.[17,25] In the first study in 1999, the proportion of HIV among MK cases was 40% with a statistically significant trend towards fungal Keratitis.[25] In the second study in 2003, the proportion of HIV among MK cases was 16%.[17] Even though anti-retroviral therapy (ART) is now widely available in most parts of SSA, the findings of our study confirmed that HIV is still an independent risk factor for MK. The proportion of HIV positive cases in our cohort was 12%, which was about double the national average of 6.3%.[27] In the group that was considered for the case control analysis, the proportion of HIV was much lower among the cases (9% as opposed to 12%) and controls (1% as opposed to 6% national average). We speculate that this might have been due to "healthy user bias" where people who thought they were HIV negative were more likely to consent as controls. Although HIV counselling and testing is widely practised in Uganda, almost 40% of the HIV positive patients in this study were identified after presenting with MK. They were unaware their HIV status or previously thought that they were negative.

We found that the possibility of MK is higher in DM. Although this had been suggested from other regions, there have not been any previous studies in SSA that described this association.[32,33] The proportion of DM among the MK cases was 7%, about thrice the national urban average and seven times the rural average.[34] DM also affects the immune system making the host susceptible to infection. Additionally, hyperglycaemia provides essential nutrients for the pathogens to thrive. This makes treatment more challenging as these patients tend to respond slowly. The prevalence of DM is on the rise globally due to lifestyle changes, In Uganda, there has been a three fold rise over the last decade.[34] We have since started offering routine HIV and Diabetes screening for all patients presenting with MK

A farming occupation was another identified risk factor; our hypothesis is that this was linked to trauma. However, trauma could not be tested in the model because of none of the controls reported trauma in the last 3 months. We found that even among the MK cases, trauma rates were lower than anticipated (29%). This is consistent with other studies from sub-Saharan Africa (SSA). In an older study from Ghana, 39% of MK cases reported some form of eye injury prior to onset.[22] In two separate studies from Tanzania 24% and 39% of cases were associated with trauma.[17,35] These levels appear to be lower than those reported from South Asia, where the proportion of MK

cases associated with an injury is typically around 75%.[11,36,37] The reason for this difference is not immediately apparent. Perhaps eye trauma was either not as common in SSA as South Asia or it was too subtle to be recalled. This might explain why there were even much less recall among the controls. As one intervention, farmers could be sensitized and encouraged to use eye protection while working.

Poverty and health are intricately related. It is linked to decisions and practises which predisposes individuals to disease, limit access to care and determine choices of treatment options (such as use of TEM). In our study, the odds of MK among individuals of a "low" economic status were about four times more than individuals over a "middle" economic status. According to the latest Uganda household survey, about 30% of the population is poor.[38] This translates into 12 million who are at an increased risk of MK. In this study, we noticed that cases were more likely to be poorer and live further from the nearest health centre.

Strengths and limitations

We were not able to enrol controls for all the MK cases in the cohort. However, the sample size was enough to detect important risk factors and enrolling all the controls may have provided minimal addition. Trauma could not be tested as risk factors because there was no reported episode among the controls. Although we were interested in TEM as a risk factor, it was not feasible to test this: we could not ascertain whether it had been applied before or after onset of MK. Before this study, HIV had been suggested as a potential risk factor. This study provided strong evidence of HIV as an independent risk factor for MK, and although the confidence interval is wide, the estimated effect is large. This was the first case control design to investigate risk factors of MK in SSA.

Conclusion

HIV, DM, a farming occupation and poverty were important risk factors for MK in Uganda. There is need for more work to be done to explore mechanisms of interaction and how these can inform prevention strategies against MK. Patients with MK should be offered HIV and diabetic screening.

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Conflict of interest

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Chapter 8. Delay along the care seeking journey of patients with microbial keratitis in Uganda

A typical road network in rural Uganda; photo taken during one of our outreach visits

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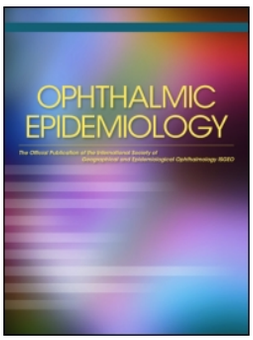

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ARTICLE

Delay Along the Care Seeking Journey of Patients with Microbial Keratitis in Uganda

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ABSTRACT

Purpose: To describe the care seeking journey and causes of delay among patients with Microbial Keratitis in Uganda.

Methods: A prospective cohort of patients presenting with microbial keratitis at the two main eye units in Southern Uganda (2016–2018). We collected information on demographics, home address, clinical history, and presentation pathway including, order of facilities where patients went to seek care, treatment advice, cost of care, and use of Traditional Eye Medicine. Presentation time was noted. We compared "direct" presenters versus "indirect" presenters and analysed predictors of delay.

Results: About 313 patients were enrolled. All were self-referred. Only 19% of the patients presented directly to the eye hospital. Majority (52%) visited one facility before presenting, 19% visited two facilities, 9% visited three facilities, and 2% visited four facilities. The cost of care increased with increase in the number of facilities visited. People in a large household, further distance from the eye hospital and those who used Traditional Eye Medicine were less likely to come directly to the eye hospital. Visiting another facility prior to the eye hospital and use of Traditional Eye Medicine aOR 1.58 (95%CI 1.03–2.43), p = .038 were associated with delayed presentation to the eye hospital.

Conclusion: This study provided information on patient journeys to seek care. Delay was largely attributable to having visited another health facility: a referral mechanism for microbial keratitis was non-existent. There is need to explore how these health system gaps can be strengthened.

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Microbial keratitis; bacterial keratitis; fungal keratitis; keratitis; blindness; Uganda

Introduction

Microbial keratitis (MK) can be caused by a range of pathogens, including bacteria, viruses, protozoa (e.g. acanthamoeba), and fungi (yeasts, moulds, and microsporidia). It is characterised by an acute or sub-acute onset of pain, conjunctival hyperaemia, and corneal ulceration with a stromal inflammatory cell infiltrate. MK frequently leads to sight-loss from dense corneal scarring, or even loss of the eye, especially when the infection is severe and/or appropriate treatment is delayed.[1] MK is important because it is a leading causes of uniocular blindness worldwide.[2,3]

In Sub Saharan Africa, the incidence of MK has been suggested to be around 180/100,000/year.[4] Bacterial (*staphylococcus, streptococcus* and *pseudomonas)* and fungal (*fusarium* and *aspergillus*) are the most common with an almost 50:50 proportion.[5–11]

In Low and Middle-Income Countries (LMIC), MK management is often more challenging because of late presentation, use of Traditional Eye Medicine (TEM), insufficient diagnostic support, lack of effective drugs and keratoplasty services.[11,12]

A critical step in effectively managing MK is ensuring that patients start appropriate treatment as early as possible. This is because once the infection is well established, there is little that can be done to change its course.[13] It is believed that many MK start following corneal abrasions. Studies in Burma and Bhutan showed that if people with a simple corneal abrasion applied antibacterial or antifungal medication within the first 24–48 hours, there was full recovery without any infectious sequalae.[14,15]

Delayed presentation of patients is a key determinant of outcomes.[12] Patients typically present at least two weeks after the onset of the first symptoms.[12] There are a number

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of factors that could contribute to this delay such as: distance from the hospital, transportation costs, poverty, self-medication, and tortuous referral pathways through the health system.[16–18] Prior visit to a non-specialist health facility has been implicated as a cause of delay in other eye conditions.[17,19]

In Uganda, the public health system has six levels, with the lowest point of care being at the village level (Village Health Committee).[20] However, physically, a Health Centre II (HC II) is the lowest unit and is located at a parish level, HC III at sub-county level, HC IV at county level, district hospital (HC V), and referral hospital (HC VI). These units have quite different staffing and capacity in terms of service provision. There are several different levels of private health care providers as well. Patients are referred up this tier system depending on the complexity of their condition.

Therefore, to investigate the role of the health system in providing care and onward referral of people with MK, here we describe the presentation pathway and factors associated with delayed presentation, among patients with microbial keratitis in Uganda.

Methods

Ethical statement

This study followed the tenets of the Declaration of Helsinki. It was approved by the London School of Hygiene & Tropical Medicine Ethics Committee (Ref 10647), Mbarara University Research Ethics Committee (Ref 10/04–16) and Uganda National Council for Science and Technology (Ref HS-2303). Written informed consent in "*Runyankore*" the local language was obtained before enrolment. If the participant was unable to read, the information was read to them by the research assistant. The participant was then asked to place a thumbprint on the consent form which was independently witnessed.

Study design and setting

This was part of a study where we prospectively enrolled patients with MK that presented to Ruharo Eye Centre (REC) and Mbarara University and Referral Hospital Eye Centre (MURHEC) from December 2016 to March 2018. MURHEC is a government owned tertiary eye unit established in 2013. It provides mostly free services and sees about 6,000–10,000 patients/year. REC is a church-based, fee-paying tertiary eye hospital founded in the 1960s. It sees about 20,000–25,000 patients/year. Both hospitals are in Mbarara Municipality, South-Western Region, Uganda, approximately 4 hours' drive from Kampala. The two units are about 5 km apart and work closely together.

Participants

All patients that were enrolled into the cohort study were included. In that cohort study, we aimed to recruit all MK cases presenting during a year in order to have a powerful sample set to answer detailed questions around the seasonal microbiological patterns. It was important to recruit for a full year as MK had been shown in other parts of the world to have seasonal variations in its' epidemiology.[21]

Study participants

The inclusion criteria for the bigger prospective study was the presence of acute MK at presentation to the hospital defined as EITHER (i) corneal epithelial ulceration (≥1 mm diameter) AND corneal stromal infiltrate AND evidence of acute ocular inflammation (e.g. Conjunctival injection/anterior chamber inflammatory cells/hypopyon); OR (ii) a corneal abscess (≥1 mm diameter) AND evidence of acute ocular inflammation. We excluded those not willing to participate, those not willing to return for follow-up, pregnant women, lactating mothers, those aged below 18 years.

Data collection procedures

Patients presenting with MK were introduced to the study and the informed consent processes followed. They were assigned a unique study number and their age, sex, occupation, and place of residence recorded. A history was taken of the circumstances in which their eye became infected, the predisposing factors (such as trauma and use of Traditional Eye Medicine [TEM]). A meticulous "journey" history was taken to document the date when they developed symptoms, where and when they sought treatment (name and level of the health centre), what medical advice and treatment was given (including whether they were referred to the eye hospital or not), how much each step cost them in Uganda shillings (transportation, consultation fees, medicines). The total amount of money recorded was for all the costs incurred before patients were enrolled into the study.

The place where they first received any form of treatment was denoted as "Facility 1", the second place visited (either as a result of formal referral or self-initiated referral) was denoted "Facility 2" and so on. GPS coordinates were generated for the patients' addresses (to the nearest village, parish, county school, or health centre depending on what was available on Google maps). Presenting Log MAR (Logarithm of Minimum Angle of Resolution) visual acuity at 2 m

in a dark room was measured using Peek Acuity software.[22] For visual acuities of counting fingers or less, Log MAR values were attributed as follows: counting fingers, 2.0; hand movements, 2.5; perception of light, 3.0; and no perception of light, 4.0.[23] The patients were then examined on a slit lamp and clinical signs carefully recorded. Infiltrate size was measured as the greatest diameter of the infiltrate (dimension 1) and the diameter of an imaginary line perpendicular to the widest axis (dimension 2). The final infiltrate size was then derived as the geometrical mean of the two diameters.[24] The same was repeated after fluorescein staining of the ulcer to measure the epithelial defect sizes. Corneal specimens were obtained for microbiological testing at Mbarara University Microbiology Department. Patients were treated as per the hospital treatment protocol and followed up periodically for up to 3 months to determine their outcome.

Analysis

Data were analysed in STATA v14. "direct" presenters were defined as participants whose first point of care was the eye hospital (MURHEC or REC). "Indirect" presenters are those who first went to other health centres before presenting to the eye hospital. Summary frequency tables of demographics and clinical presentation of "direct" versus "indirect" presenters were generated with appropriate statistical tests for each variable (Wilcoxon rank sum for the continuous variables and χ^2 test for the categorical variables). To determine where the participants came from, Google maps was used to pinpoint to the addresses of the participants. The presentation journey was described using interval times in days from home to Facility 1 or from Facility 1 to Facility 2 and so on (presented as median time in days with Inter Quartile Ranges [IQRs]). To describe the cost of care, the total patient expenditure at different facilities were summarised and cumulative expenditure derived depending on how many facilities an individual visited. Costs are presented as median expenditure in Uganda shillings with IQRs.

Presentation time was defined as the time in days it took a patient to come to the eye hospital after onset of symptoms. For analysis of delay, presentation time was divided into quartiles as "early" (0–7 days), "intermediate" (8–14 days), "late "(15–30 days), and "very late" (>30 days). Ordinal logistic regression was performed to determine the factors associated with these four quartiles of "delay", while logistic regression was performed to determine factors associated with direct presentation. Univariable regression was performed to generate crude Odds Ratios (OR). After assessing for collinearity, variables with a p value less than 0.1 were introduced in the multivariable model. A backward stepwise approach was then used, until only the variables with a p value <0.05 were retained. Adjusted OR were reported for the final model.

Results

Demographic features

During the study period, 313 patients were enrolled into this study. The baseline characteristics of direct versus direct presenters are shown in Table 1. Overall,

Table 1. Baseline characteristics of direct versus indirect presenters (n = 313).

	Direct presenters (n = 58)			Indirect presenters (n = 255)			
Variable	Median	(IQR)	(Total range)	Median	(IQR)	(Total range)	p value
Age	47	(35–60)	(18–96)	47	(35–60)	(18–87)	0.772
Distance to eye units	58	(16–85)	(0.2–244)	87	(57–131)	(2–378)	0.0001
Household population	5	(3–7)	(1–14)	7	(4–8)	(1–28)	0.006
Distance to nearest health centre in km*	2	(1–3)	(0–14)	3	(1–4)	(0–45)	0.174
Variable	Category	Count	(%)	Count	(%)		p value
Gender	Female	22	(38%)	117	(46%)		0.271
	Male	36	(62%)	138	(54%)		
Occupation	Farmer	34	(59%)	186	(73%)		0.031
	Nonfarmer	24	(41%)	69	(27%)		
Marital status	Unmarried ǂ	18	(31%)	77	(30%)		0.900
	Married	40	(69%)	178	(70%)		
Education status	None	15	(26%)	69	(27%)		0.407
	Primary	29	(50%)	133	(52%)		
	Secondary	7	(12%)	38	(15%)		
	Tertiary	7	(12%)	15	(6%)		
Being head of household	Yes	42	(72%)	170	(67%)		0.398
	No	16	(28%)	85	(33%)		
Needed an escort to hospital*	Yes	24	(41%)	49	(20)		<0.0001
	No	34	(59%)	202	(80)		

*Variables with some missing data: distance to nearest health centre was measured in km (n = 312, [direct 57]) needed an escort (n = 309, [direct 58]). ǂ Unmarried included single, divorced, and widowed,

the direct and indirect presenters were similar for many variables. However, the direct presenters lived closer to the eye hospital (median 58 km vs. 87 km; p = .0001), had fewer household members (median 5 people vs. 7 people; p = .006) and fewer were farmers (59% vs. 73%, p = .031).

Table 2 shows some select clinical history and signs of direct versus indirect presenters. Compared to indirect presenters, direct presenters had a shorter presentation time (median 8 days vs. 17 days; p < .0001), had slightly better presenting vision (median Log MAR 0.65 vs. 1.3; p = .075), a smaller infiltrate size (median 4.2 mm vs. 5.5 mm; p = .025) and a smaller epithelial defect (median 3.5 mm vs. 4.1 mm; p = .048). The proportion of people who had used TEM was higher among the indirect (63%) versus direct presenters (46%), p = .020. The direct and indirect presenters had similar proportions with a history of trauma, hypopyon, an opaque stromal opacity and perforation.

Factors associated with direct presentation

On univariable and multivariable analysis summarised in Table 3. People who lived far from the eye hospital (overall p = .003), those from large households OR 0.53 (95%CI 0.32–0.85), p = .0080 and those who had used TEM OR 0.48 (95% CI 0.25–0.90), p = .020 were less likely to be direct presenters.

Table 2. Clinical history and clinical signs of direct versus indirect presenters (n = 313).

	Direct presenters (n = 58)			Indirect presenters (n = 255)			
Variable	Median	(IQR)	(Total range)	Median	(IQR)	(Total range)	p value
Presentation time in days*	8	(2–18)	(0–116)	17	(8–32)	(0–370)	<0.0001
Presenting vision (Log MAR)	0.65	(0.1–2.5)	(0–4)	1.3	(0.3–2.5)	(0–4)	0.072
Infiltrate size in mm ł	4.2	(2.5–7.1)	(0.9–11)	5.5	(3.5–8)	(0.5–13)	0.025
Epithelial defect size in mm ł	3.5	(1.8–5.8)	(0–11)	4.1	(2.5–6.9)	(0–13)	0.048
Variable	Category	Count	(%)	Count	(%)		p value
History of trauma (overall 29%) ǂ	Yes	14	(25%)	77	(30)		0.388
	No	43	(75)	177	(70)		
Used traditional eye medicine (overall 61%)	Yes	27	(46)	161	(63)		0.020
	No	31	(53)	94	(37)		
Pain being the main complaint	Yes	26	(45%)	112	44		0.121
	No	32	55	143	56		
Opaque stromal opacity ǂ	Yes	25	(43)	107	(44)		0.918
	No	33	(57)	137	(56)		
Hypopyon ǂ	Yes	13	(22)	81	(32)		0.151
	No	45	(78)	172	(68)		
Perforated at admission	Yes	10	(17)	66	(26)		0.166
	No	48	(83)	189	(74)		

*Presentation time was measured as duration in days it took to come to the eye hospital after onset of symptoms. ł geometrical of the largest diameter and the diameter perpendicular to the largest diameter. ǂ variables that had less than 313 observations due to missing data (trauma n = 311 [direct57], opaque stromal opacity n = 302 [direct 58], hypopyon n = 311 [direct 58]).

Table 3. Univariable and multivariable logistic regression analysis of factors associated with direct presentation to the eye hospital (n = 309).

	Univariable analysis			Multivariable analysis		
Variable	cOR	(95% CI)	p value	aOR	(95% CI)	p value
Age in years	1.004	(0.987–1.022)	0.576			
Sex (being male)	1.38	(0.77–2.48)	0.273			
Marital status (being married)	0.96	(0.52–1.78)	0.900			
Occupation (being a farmer)	0.52	(0.29–0.94)	0.033			
Being head of household	1.31	(0.69–2.46)	0.399			
Number of people in household (increase/one person)	0.59	(0.38–0.90)	0.015	0.53	(0.32–0.85)	0.008
Distance to the eye hospital						
0–50 km	1		0.001			0.003
50–100 km	0.52	(0.26–1.01)		0.62	(0.30–1.27)	
100–150 km	0.16	(0.05–0.44)		0.16	(0.06–0.48)	
>150 km	0.42	(0.17–1.03)		0.52	(0.19–1.34)	
Distance from nearest health centre (increase per 1 km)	0.92	(0.822–1.029)	0.146			
Positive history of trauma	0.74	(0.38–1.44)	0.389			
Positive history of TEM Use	0.50	(0.28–0.90)	0.021	0.48	(0.25–0.90)	0.020
Education status						
None	1		0.462			
Primary	1.00	(0.50-1.99)				
Secondary	0.84	(0.31–2.25)				
Tertiary	2.14	(0.74–6.17)				

*patients with missing data were dropped from the model. OR less than 1 means they were less likely to come directly to the eye hospital

Care seeking pathway

Figure 1 shows where the patients came from in relation to the eye hospital (MURHEC or REC). Most came from the South Western region of Uganda and a handful from Northern Tanzania. Figure 2 shows the place where patients were first treated. Majority (46%) sought treatment at a nearby clinic/pharmacy/drug shop, 19% presented directly to the eye hospital, 15% were initially treated at home (either used TEM or an old eye drop) and 17% were treated at various levels of the health system (HC II, HC III, HC IV, and district hospital). Some patients (2%) did not know the type of facility where they first sought care and only 1% went to a traditional healer's shrine for treatment.

Figure 3 illustrates the pathway patients took to come to the eye hospital and the different times spent on each stage. Only 55 (20%) patients presented directly to the eye hospital, majority (134, 51%) visited one facility before presenting to the eye hospital, another 43 (19%) visited two facilities, 24 (9%) visited three facilities, and 5 (2%) visited four facilities. On average, patients took about a week to move from one facility to the next. The shortest response time was from onset of symptoms to Facility 1 and was even shorter among indirect presenters, median 2 days (IQR 0–5) versus direct presenters, median 8 (IQR 2–18), $p < .0001$. The longest interval time was from Facility 4 to the eye hospital, median 13 (IQR 10–33). The choice of the first facility did not affect overall presentation time. All the patients were self-referred.

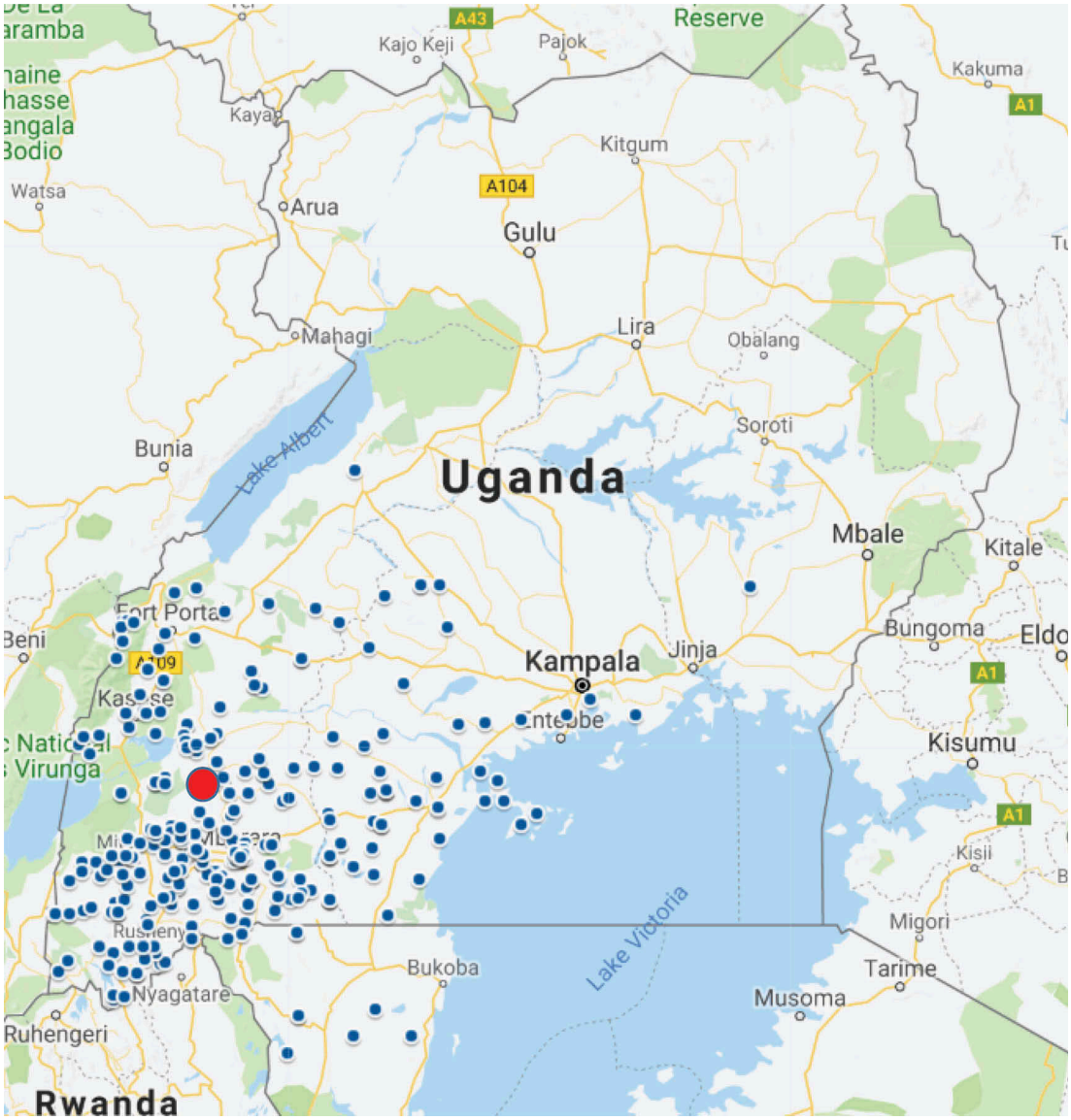

Figure 1. A map of Uganda showing patients homes.
Each point represents a patient. The red circle is the eye hospital where these patients presented.

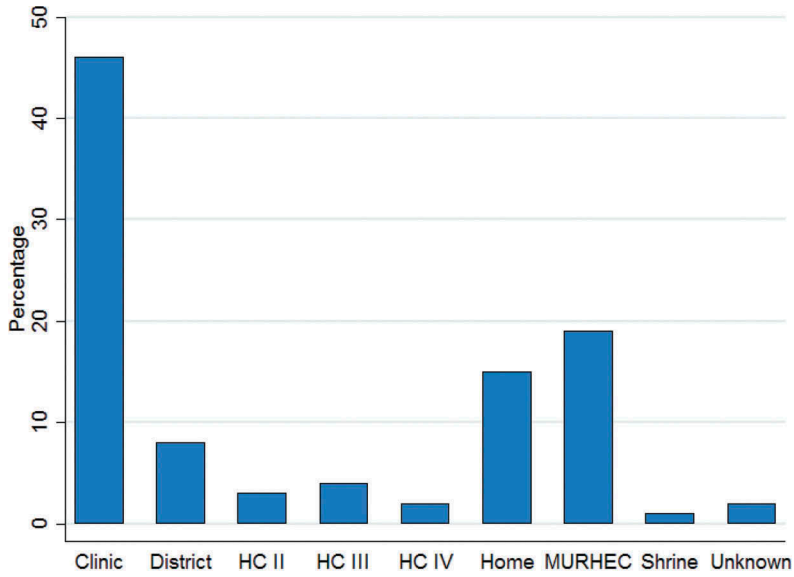

Figure 2. showing where patients first accessed treatment (n = 309).

Key: Clinic refers to clinic/pharmacy/drug shop, District is district hospital, MURHEC is the main eye hospital (Mbarara University and Referral Hospital Eye Centre and Ruharo Eye Centre).

We found in our study that most patients used TEM after having been to a health facility (secondary use). Out of the 188 who used TEM, only 51 used TEM as primary treatment (47 at home and 4 at the traditional healers' shrine). The rest (137/188) had secondary TEM application.

Cost of care

The cost of care in Uganda shillings (UGX) is presented in Table 4. The cost of care increased with increase in the number of facilities visited. There was evidence (Cuzick test for trend $p < .0001$), of an association between expenditure and number of facilities visited prior to presentation. The lowest spend was for direct presenters where the median expenditure was UGX 30,000 (IQR 7,000–63,000, total range 0–385,000) and the largest spend was among patients who had visited 4 facilities before presentation with a median expenditure of UGX 284,000 (IQR 118,000–-439,500, total range 96,000–864,000). Across the different expenditure lines, medicines were the most expensive followed by transportation, consultation fees were the least expensive.

Factors associated with delay

We tested for associations with delay in presenting to the eye hospital (Table 5). After adjusting for distance, visiting another facility prior to the eye hospital was strongly associated with delay but no obvious trend. Previous use of TEM was also found to be associated with delay OR 1.58 (IQR 1.03–2.43), $p = .038$

Discussion

This study aimed to describe the presentation journey and factors associated with delay. Factors associated with delay were having visited another health facility and prior use of Traditional Eye Medicine (TEM). This supported our hypothesis that an initial visit to a health facility introduced delay as had been reported previously for other eye conditions.[17,19,25] After onset of symptoms, the majority of patients quickly visited a health facility to seek treatment. This was an impressive median response time (within 48 hours). Although we did not explicitly ask their reasons for presenting early to these facilities, the painful nature of MK, proximity of the facilities and trauma (for those who had it) could have played a role. Perhaps, if appropriate treatment had been given or rapid referral made at this stage, the outcomes might have been better.[13,14]

At the first point of contact with the health system, there were three missed opportunities that we identified in our study, these were: to promptly initiate appropriate treatment; to triage and urgently refer; and health education advice against TEM use. We discuss these below.

Firstly, the health facility where most patients presented first were usually a nearby pharmacy/clinic. These are mostly private clinics that have sprouted up in many parts of Uganda. They are loosely regulated, manned by primary health workers and do not require a doctor's prescription to dispense treatment. Effective anti-microbial medication such as Natamycin and Ciprofloxacin eye drops are not available in such units. These could be potential stakeholders to target in promotion of triage and referral mechanisms for MK. We found that there was no referral

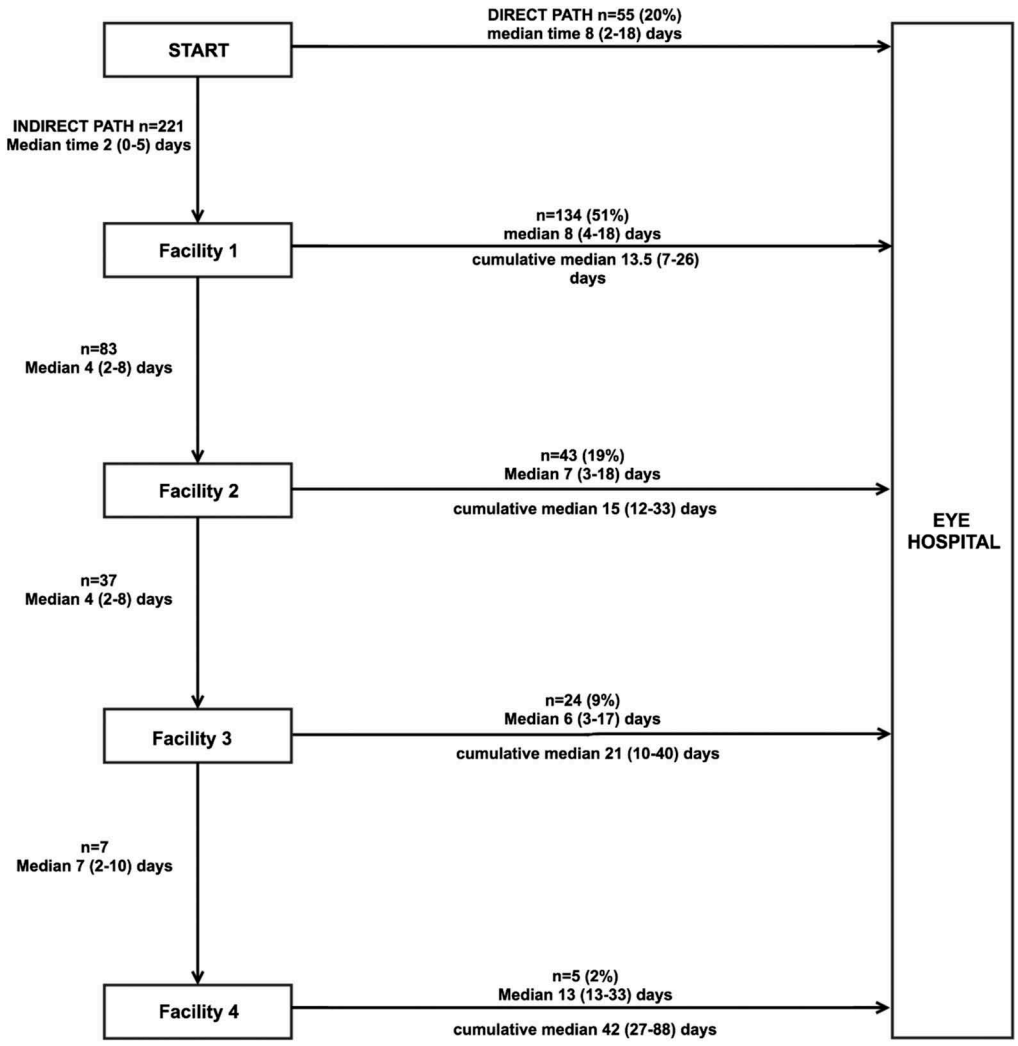

Figure 3. The care seeking journey of patients with microbial keratitis and the time taken at each step (n = 276).
In this analysis, only patients with complete data were included. START refers to when the symptoms started. Facility refers to a health centre or clinic/pharmacy and not necessarily the hierarchy of the health centres.

Table 4. Money spent by patients per number of facilities visited before coming to the eye hospital.

			Cost of care median (IQR) in Uganda Shillings*							
Facility	*n*	(%)	Transportation		Consultation		Medicine		Total expenditure	
0	58	(18.5%)	11,000	(4,000–20,000)	15,000	(0–15,000)	0	(0–27,000)	30,000	(7,000–63,000)
1	147	(52%)	19,500	(10,000–33,000)	15,000	(15,000–15,000)	19,800	(2,750–99,500)	52,000	(31,000–142,000)
2	58	(18.5%)	22,000	(15,000–37,000)	15,000	(0–15,000)	25,750	(6,000–80,000)	67,750	(34,250–142,500)
3	29	(9%)	30,000	(19,000–51,000)	15,000	(0-15,000)	28,500	(3,000–70,000)	78,250	(32,000–209,000)
4	6	(2%)	62,500	(33,000–143,000)	12,500	(10,000–30,000)	170,500	(78,000–343,500)	284,000	(118,000–439,500)
p value of test for trend										<0.0001

*All money is quoted in Uganda shillings. The US $ exchange rate was US $1: Uganda shillings 3,700 (2017). ł0-direct presenters who did not visit any other facility before coming to the eye hospital. Patients with incomplete data were not included in this analysis

mechanism for MK: all patients who came to the eye hospital were self-referred.

Secondly, all the patients who visited a health facility we given some treatment but none of the patients was ever referred for specialist care. Most of the health centres (II and III) are managed by mid-level cadres, who may not have the necessary skills and tools to appreciate the urgency and seriousness of MK. General eye health training has been previously reported to be limited among mid-level cadres in the region.[26] In addition, Uganda is still grappling with a major shortage of human resources for eye health. An eye specialist is found at some level six facilities and a mid-level ophthalmic cadre might be available in some level IV onwards.[27] We plan to conduct a study into factors around the health system that could be developed to strengthen treatment, triage and referral.

Table 5. Univariable and multivariable ordinal logistic regression analysis of factors associated with delay among patients with microbial keratitis (n = 309).

	Univariable analysis			Multivariable analysis		
Variable	cOR	(95% CI)	p value	aOR	(95% CI)	p value
Age in years	1.009	(0.994–1.019)	0.140			
Sex (being male)	1.06	(0.71–1.58)	0.792			
Marital status (being married)	0.86	(0.55–1.33)	0.316			
Occupation (being a farmer)	1.24	(0.80–1.93)	0.339			
Being head of household	0.83	(0.54–1.27)	0.394			
Number of people in household (increase/one person)	1.14	(0.85–1.51)	0.365			
Distance to the eye hospital (every 10km increase)	1.036	(1.003–1.)	0.034			
Distance from nearest health centre (increase per 1km)	1.01	(0.97–1.06)	0.501			
Positive history of trauma	0.96	(0.62–1.49)	0.860			
Positive history of TEM Use	1.73	(1.14–2.62)	0.010	1.58	(1.03–2.43)	0.038
Other facilities visited before eye hospital						
Nil (direct presenters)	1		0.0002	1		0.001
One facility	2.95	(1.63–5.38)		2.74	(1.53–4.92)	
Two facilities	3.62	(1.74–7.52)		2.58	(1.30–5.15)	
Three facilities	4.12	(1.82–9.34)		3.26	(1.42–7.45)	
Four facilities*	15.5	(2.65–90)		14.3	(2.45–83.7)	

*two patients had visited five facilities and one patient six facilities, these were dropped from the analysis

Thirdly, we found in our study that most patients used TEM after having been to a health facility (secondary use). This is worrying because these were patients who could have been sensitised against TEM use at the health facilities where they first presented. This was a missed opportunity that needs to be addressed.

Fifty-eight (19%) of the patients were direct presenters. As expected, people who had large households, those who lived far from the eye hospital and those who used TEM were less likely to present directly to the eye hospital. Understandably, use of TEM and having a large household were negative predictors for being a direct presenter. Most of the people who used TEM used it at home and this was marked as a treatment event in our study design. Many patients in our cohort were heads of households and the sole bread winners, they might have preferred to first seek treatment at a place near home.

The cost of care was variable depending on the number of facilities visited. Most of the money was spent on drugs, and transportation. The public health system in Uganda is largely free or highly subsided. Expenses are incurred on transportation and sometimes medicines when they are out of stock. For the case of MK, drugs such as Natamycin have only been erratically and expensively supplied by select private pharmacies and not available in the public health system. We anticipate this to change as Natamycin was recently added on the WHO essential medicines list.[28]

Strengths/limitations

This study was the first in SSA to systematically collect information on how MK patients seek care and what influences their pattern. It provides useful information on key health system gaps that need strengthening. Before this study, it had been thought that patients had poor health seeking behaviour, however, what we found was that majority of people presented to a health facility quite early after the onset of symptoms. Secondly, although TEM use was a known problem, this study showed that the bigger problem was secondary TEM use, that is patients who opted to use TEM even after they had been to a health facility.

Although we collected information on distance covered and treatment given at each level, it was difficult to analyse for these because most patients did not come to the eye hospital with their medicine and could not recall the names. There were many circular movements that made it complicated to analyse total distance covered by each patient. A qualitative approach in discussing with patients what informed their choice of self-referral or direct presentation would have strengthened the evidence in this study.

Conclusion

Delayed presentation to a specialist eye hospital is a problem in the care of MK, and that this appears to be largely attributable to slow referral through the health system. There are opportunities for health education, early referral, appropriate treatment and sensitization against TEM use that could be utilized to improve care of MK. More needs to be done to understand what goes on in the health system and how this can be strengthened.

Conflict of interest

None of the authors have any proprietary interests or conflicts of interest related to this submission.

Submission statement

This submission has not been published anywhere previously and that it is not simultaneously being considered for any other publication.

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Chapter 9. Role of Traditional Eye Medicine in treatment of MK in Uganda: A hospital and community based mixed methods study

A traditional healer squeezes sap from a leaf commonly used in treatment of Microbial Keratitis in Uganda. Photo taken by Terry Cooper

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Student ID Number	LSH1511754	**Title**	Dr
First Name(s)	Simon		
Surname/Family Name	Arunga		
Thesis Title	Epidemiology of Microbial Keratitis in South Western Uganda		
Primary Supervisor	Prof Matthew Burton		

If the Research Paper has previously been published please complete Section B, if not please move to Section C.

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SECTION E

Student Signature	AS
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Supervisor Signature	MJB
Date	20/9/19

RESEARCH ARTICLE

REVISED Traditional eye medicine use in microbial keratitis in Uganda: a mixed methods study [version 2; peer review: 2 approved]

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Abstract

Background: Traditional eye medicine (TEM) is frequently used to treat microbial keratitis (MK) in many parts of Africa. Few reports have suggested that this is associated with a worse outcome. We undertook this large prospective study to determine how TEM use impacts presentation and outcome of MK and to explore reasons why people use TEM for treatment in Uganda.

Methods: In a mixed method prospective cohort study, we enrolled patients presenting with MK at the two main eye units in Southern Uganda between December 2016 and March 2018 and collected information on history, TEM use, microbiology and 3-month outcomes. We conducted qualitative interviews with patients, carers traditional healers on reasons why people use TEM. Outcome measures included presenting vision and at 3-months, comparing TEM Users versus Non-Users. A thematic coding framework was deployed to explore reasons for use of TEM.

Results: Out of 313 participants enrolled, 188 reported TEM use. TEM Users had a delayed presentation; median presenting time 18 days versus 14 days, p= 0.005; had larger ulcers 5.6 mm versus 4.3 mm p=0.0005; a worse presenting visual acuity median logarithm of the minimum angle of resolution (Log MAR) 1.5 versus 0.6, p=0.005; and, a worse visual acuity at 3 months median Log MAR 0.6 versus 0.2, p=0.010. In a multivariable logistic regression model, distance from the eye hospital and delayed presentation were associated with TEM use. Reasons for TEM use

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	Invited Reviewers	
	1	**2**
REVISED **version 2** published 04 Oct 2019	✓ report	
version 1 published 06 Jun 2019	? report	✓ report

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2 **David Yorston**, NHS Greater Glasgow and Clyde, Glasgow, UK

Any reports and responses or comments on the article can be found at the end of the article.

included lack of confidence in conventional medicine, health system breakdown, poverty, fear of the eye hospital, cultural belief in TEM, influence from traditional healers, personal circumstances and ignorance.

Conclusion: TEM users had poorer clinical presentation and outcomes. Capacity building of the primary health centres to improve access to eye care and community behavioural change initiatives against TEM use should be encouraged.

Keywords
Microbial Keratitis, Traditional Eye Medicine, Traditional Healers, Blindness, Uganda

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REVISED **Amendments from Version 1**

In this revised version, we have addressed all the reviewer comments in a point by point format. The main differences are:

On lines 339–341, we have added a comment on why economic status was not significant in the multivariate analysis.

Data in Table 2 had been interchanged to show that TEM users had better presenting acuity than non TEM users, this has been corrected to show that TEM users had a worse vision.

On lines 364–365, we have acknowledged a limitation in not being able to enroll children and provided an explanation for this.

On Lines Line 48–55, we have provided a description on clinical examination and microbiological methods for the patients.

Typos in the abstract and in line 291 have been corrected.

Any further responses from the reviewers can be found at the end of the article

Introduction

Microbial keratitis (MK) frequently leads to sight-loss from dense corneal scarring, or even loss of the eye, especially when the infection is severe and/or appropriate treatment is delayed[1]. MK has been described as a "silent epidemic", which leads to substantial morbidity, related to blindness and other consequences such as pain and stigma[2]. It is the leading cause of unilateral blindness after cataract in tropical regions and is responsible for about 2 million cases of monocular blindness per year[3].

In Low and Middle-Income Countries (LMIC), use of Traditional Eye Medicine (TEM) for treatment of many eye conditions is a common practise[4–6]. In the few reported studies, TEM has been found to lead to complications such as corneal scarring and delayed presentation of patients to hospital resulting in poor outcomes[7,8].

Literature on TEM use for MK is scanty. However, among the three papers from Sub-Saharan Africa (SSA), TEM use among patients with MK was reported to be associated with a severe presentation. These studies did not report clinical outcomes[9–11]. In addition, since most of the TEM involves plant products such as fresh leaves, it could have a major role in the pathogenesis of fungal keratitis, which has been associated with injuries involving vegetative matter[12,13]. Our experience in Uganda is that TEM is widely used to treat a number of eye conditions including MK. However, the drivers of this practice are not well understood.

The aim of this study therefore was to determine how TEM use impacts presentation and outcome of MK and to explore reasons why people use TEM for treatment of MK in Uganda.

Methods

Ethical statement

This study adhered to the Declaration of Helsinki. It was approved by the London School of Hygiene & Tropical Medicine Ethics Committee (Ref 10647), Mbarara University Research Ethics Committee (Ref 10/04-16) and Uganda National Council for Science and Technology (Ref HS-2303). Written informed consent in Runyankore, the local language, was obtained before enrolment. If the patient was unable to read, the information was read to them, and they were asked to indicate their consent by application of their thumbprint. The collected source data is stored in a secure database at Mbarara University of Science and Technology. An anonymised digital version was also uploaded in a secure server. The data will be kept for 7 years according to institutional policy.

Participants

Due to the cultural complexity of TEM usage, we used a mixed methods approach. We prospectively enrolled patients with MK that consecutively presented to two tertiary eye hospitals in South-Western Uganda from December 2016 to March 2018. The case definition of MK was the presence of a corneal epithelial defect (of at least 1mm diameter) with an underlying stromal infiltrate, associated with signs of inflammation (conjunctival hyperaemia, anterior chamber inflammatory cells, +/- hypopyon). We excluded those not willing to participate, those not willing to return for follow-up, pregnant women, lactating mothers, those aged below 18 years.

Quantitative assessment

We documented basic demographic information and ophthalmic history using ophthalmic nurses as part of the routine hospital work up. This included treatment received including prior use of TEM. For those who reported use of TEM, a detailed structured history was taken on what they had applied, source of the medicines, cost, how it was prepared, duration of use and any complications experienced. A detailed description of the cases evaluation has been previously presented. In summary, after measurement of the presenting visual acuity (Logarithm of Minimum Angle of Resolution), cases underwent a detailed clinical examination on a slit lamp using a structured protocol, including eyelid assessment, corneal ulcer features, anterior chamber (flare, cells, hypopyon shape and size) and perforation status. Corneal scrapes were collected for microscopy, culture (blood agar, chocolate agar, potato dextrose agar) and molecular diagnosis. HIV, Diabetes counselling and testing were offered, as per the Uganda Ministry of Health HIV testing protocol. Cases were treated according to the hospital protocol, which usually involved a brief admission for the first few days. The study follow-up assessment schedule was days 2, 7, 21 and 90, to determine outcome. Patients were asked to return to the eye hospital for these reviews where their follow up data was collected as before. Additional assessments were conducted as clinically indicated. The primary outcome measure was final best corrected vision at 3 months. See extended data[14] for questionnaire used.

Qualitative assessment

All interviews and discussion groups were conducted by AA. They were audio recorded and summarised. Additional contextual information provided such as patient emotions, environment and any other aspect the interviewer found noteworthy.

Firstly, at presentation, patients who reported to have used TEM were asked if they would be willing to discuss their experiences. For such patients, an interviewer would return later that evening or the next day when the patient was more relaxed. Interviews were conducted in the local language by a social

scientist either at the hospital bedside (when quiet) or in the hospital compound depending on the patient's preference. The focus of the interview was to explore reasons why they had used TEM.

Secondly, we conducted informal group discussions (IGDs) with a sample of the MK patients involved in the study and relatives of people with MK on the practise and reasons why people use TEM. This was an opportunistic approach to allow flexible data collection. For example, a patient might present escorted by many family members and friends (common in this setting), such a group would then be invited to discuss issues around TEM. Such a naturally composed group was to result in a more relaxed discussion than a group of people who did not know each other who are brought together solely for the discussion.

Finally, we conducted in-depth interviews with traditional healers to learn about what they would usually do for people presenting with a problem like MK and why people go to them for treatment. Healers were identified from a traditional healers' registry at the local council headquarters. A random sample of 15 traditional healers were contacted through their coordinator. Those willing to share their knowledge and practise in treating eye problems particularly MK were visited and interviewed at their home or shrine.

For all the groups, topic guides were developed using available literature and experiences of the local ophthalmologists treating patients with MK (see extended data[14]). They included local understanding of MK, causes, treatment and experiences of using TEM. The guides were piloted among a few patients and modified accordingly. The final version was approved by all the authors who included senior social scientists (AA) and a professor (JS). In this report, our focus is on reasons why people use/do not use TEM. These were reviewed by one of the authors. They were then piloted among MK patients and revised accordingly. All interviews lasted about 30–45 minutes.

Analysis

Quantitative data were analysed using STATA v14. We compared demographic data, baseline clinical presentation and final vision outcomes at 3-months of patients who reported to have used TEM versus those who had not. Appropriate tests of significance (chi2 for categorical data and Wilcoxon rank sum for continuous data) were employed. Multivariable logistic regression analysis was used to identify factors associated with TEM use. Initially, univariable regression was performed to generate crude odds ratios (OR). Variables with a p-value less than 0.1 were introduced in the multivariable model. A back stepwise approach was then used, until only the variables with a p-value of less than 0.05 were retained. Adjusted OR were reported for the final model. Summary tables of proportions were constructed to describe the source, cost, complications and duration of use of TM.

For the qualitative data, all interviews were recorded with an audio recorder (Olympus WS-853 Digital Stereo Voice Recorder) and transcribed into summaries. These were independently reviewed several times by two of the authors (SA and JS). A coding framework was developed, and data were then manually coded. Emerging themes around reasons why people used/did not use TEM are presented. Specific conversation response clips from the respondents that supported the generated themes were extracted from the audio recordings and used as illustrative statements.

Results

We enrolled 313 people with MK, of whom 188 (60%) reported TEM use ("TEM Users") and 125 said they did not use TEM ("TEM Non-Users"). The demographic characteristics of both groups are shown in Table 1 (see underlying data[14]). There were some differences between TEM Users and Non-Users. TEM Users lived further from the eye unit, were more frequently farmers, were less likely to be married and had progressed less in formal education.

The clinical characteristics of both groups are shown in Table 2. There was evidence that the condition of TEM Users was worse than TEM Non-Users at presentation. The TEM Users presented later, had larger corneal ulcers (both infiltrate and epithelial defect), more frequent hypopyons and poorer vision.

We modelled factors associated with TEM use (Table 3). After adjusting for potential confounders, distance from the eye hospital and delayed presentation were associated with TEM use. Whereas, there was less TEM use among those who were married, had a history of trauma and a high education level.

At 3-months, 260 patients completed their follow-up. There was no systematic baseline difference between patients who were seen at 3-months and those that were not. The final LogMAR visual acuity was worse among TEM Users, median 0.6 (IQR 0-2.5), compared to TEM Non-Users, 0.2 (IQR 0-1.5), p=0.010.

Among the 188 patients who reported TEM use, 137 (73%) used TEM after they had been to a government health facility (secondary TEM use). TEM was mostly made from fresh leaves [154, (82%)]; the commonest preparation method was to freshly squeeze them [145, (77%)]. Most patients obtained TEM either from their home garden (40%) or from a neighbour (54%), only 5 patients (3%) obtained TEM from a traditional healer. TEM was generally free, 169 (90%) reported not to have spent any money to obtain it.

The qualitative study involved a total of 38 participants: 11 traditional healers, 21 MK patients who had used TEM and 6 MK patients who had not used TEM. The baseline characteristics of these individuals are presented in Table 4. Overall, it was a mix of male and female, young and old, not educated and highly educated. In addition, three informal group discussions (IGDs) were conducted, each with around 15 participants (these were naturally composed groups of patients who had used or not used TEM, relatives and friends).

The major factors coming out as the reasons for using TEM included lack of consumer confidence in conventional medicine, health system breakdown, poverty, fear, cultural belief in

Table 1. Baseline demographics characteristics of participants (n=313), comparing traditional eye medicine (TEM) users to non-users.

Variable		TEM Users (188)			TEM Non-Users (125)			
		Median	(IQR)	(Total range)	Median	(IQR)	(Total range)	P value
Age		48	(34–60)	(18–87)	45	(35–60)	(18–96)	0.651
Distance to eye hospital (km)		87	(59–132)	(1.5–378)	67	(42–121)	(0.2–316)	0.003
Distance to nearest Health Centre in (km)		3	(1–5)	(0–45)	2	(1–4)	(0–35)	0.528
		Count	**(%)**		**count**	**(%)**		**P value**
Gender	Male	101	(54)		73	(58)		0.415
Occupation	Farmer	140	(75)		80	(64)		0.047
	Non-farmer	48	(25)		45	(34)		
Education	None	59	(31)		25	(20)		0.016
	Primary Level	98	(52)		64	(51)		
	Secondary Level	23	(12)		22	(18)		
	Tertiary Level	8	(5)		14	(11)		
Marital status	Unmarried*	66	(35)		29	(23)		0.025
	Married	122	(65)		96	(77)		
Household SES ǂ	Poor	51	(28)		34	(29)		0.520
	Middle	116	(64)		72	(60)		
	Upper	13	(7)		13	(11)		

SES: Socioeconomic status.

*Unmarried included-single, divorced, widowed. ǂ This was relative self-reported economic status compared to the neighbours.

Table 2. Baseline clinical characteristics of participants (n=313), comparing traditional eye medicine (TEM) users to non-users.

Variable		TEM Users (188)			TEM Non-Users (125)			
		Median	(IQR)	(Total range)	Median	(IQR)	(Total range)	P value
Presentation time in days		18	(12–35)	(1–274)	14	(5–32)	(0–370)	0.005
Infiltrate size in mm*		5.6	(3.8–8.1)	(0.5–11)	4.3	(2.4–6.8)	(0.6–12)	0.0005
Epithelial defect size in mm*		4.2	(2.5–11)	(0–14)	3.6	(2.2–5.1)	(0–11)	0.0105
Presenting Vision (Log MAR)		1.5	(0.3–2.5)	(0–4)	0.6	(0.2–2.5)	(0–4)	0.005
		Count	**(%)**		**count**	**(%)**		**P value**
Visual Acuity	> 6/18	50	(27)		52	(42)		0.011
	6/18 – 6/60	24	(13)		18	(14)		
	< 6/60	113	(60)		55	(44)		
Eye discharge	Yes	107	(57)		60	(48)		0.122
History of Trauma	Yes	42	(22)		49	(39)		0.001
Presence of lid swelling	Yes	85	(46)		45	(36)		0.097
Slough ǂ	None	31	(17)		30	(24)		0.246
	Flat	77	(41)		47	(38)		
	Raised	78	(42)		46	(37)		
Infiltrate colour	White	77	(44)		71	(63)		0.005
	Cream	76	(43)		30	(27)		
	Other	23	(13)		11	(10)		

Variable		TEM Users (188)			TEM Non-Users (125)			
		Median	(IQR)	(Total range)	Median	(IQR)	(Total range)	P value
Hypopyon	Yes	66	(35)		28	(22)		0.014
Perforated at admission	Yes	29	(15)		16	(13)		0.517
Microbiology	Unknown	38	(23)		27	(25)		0.089
	Bacteria	10	(6)		10	(10)		
	Fungus	108	(67)		60	(55)		
	Mixed	6	(4)		11	(10)		

Log MAR: Logarithm of the minimum angle of resolution.

*These were calculated as the geometrical means using the MUTT protocol[15]. The upper limits exceeded normal corneal diameter for some lesions, which extended up to the sclera. ǂ Raised slough was when the corneal infiltrate profile was raised, flat slough was when the profile was flat while no slough is when there was no debris noted. The difference in presenting vision and infiltrate sizes remained significant even after adjusting for delayed presentation.

Table 3. Univariable and multivariable logistic regression for factors associated with traditional eye medicine use (n=313).

Variable	Univariable Analysis			Multivariable Analysis		
	Crude OR	(95% CI)	p-value	Adjusted OR	(95% CI)	p-value
Age in years	1.002	(0.988-1.016)	0.699			
Distance to Eye hospital (for every km)	1.005	(1.001-1.0090	0.009	1.004	(1.001-1.008)	0.035
Distance to the nearest Health Centre (for every km)	1.028	(0.971-1.089)	0.332			
Sex (Being male)	0.82	(0.52-1.30)	0.415			
Occupation (Being a farmer)	1.64	(1.01-2.68)	0.048			
Married	0.55	(0.33-0.93)	0.026	0.54	(0.31-0.95)	0.035
Education level						
None	1		0.016	1		0.059
Primary	0.64	(0.36-1.14)		0.71	(0.38-1.30)	
Secondary	0.44	(0.20-0.93)		0.44	(0.20-1.00)	
Tertiary	0.24	(0.09-0.65)		0.28	(0.09-0.83)	
Household economic status						
Low	1		0.526			
Middle	1.07	(0.63-1.81)				
Upper	0.66	(0.27-1.61)				
Presentation time						
0–3 days	1		<0.001	1		0.002
4–7 days	2.17	(0.72-6.53)		1.50	(0.46-4.83)	
8–14 days	6.03	(2.10-17.3)		4.76	(1.55-14.6)	
15–30 days	5.77	(2.03-16.4)		4.37	(1.44-13.2)	
>30 days	4.89	(1.75-13.6)		3.74	(1.27-11.1)	
History of trauma	0.44	(0.26-0.72)	0.001	0.43	(0.25-0.74)	0.003

Table 4. Baseline characteristics of people who participated in the in-depth interviews, including traditional healers and patients with microbial keratitis (both traditional eye medicine (TEM) users and non-users).

Participant	Age	Sex	Marital status	Occupation	Household size	Education	Religion
Traditional Healers (n=11)							
1	70	Male	Divorced	Farmer	1	None	Christian
2	56	Female	Married	Farmer	4	None	Christian
3	52	Female	Widowed	Farmer	3	None	Christian
4	76	Female	Married	Farmer	8	Primary	Christian
5	78	Female	Married	Farmer	5	-	-
6	53	Female	Widowed	Farmer	2	-	Christian
7	72	Female	Widowed	TBA	4	Primary	Christian
8	82	Male	Divorced	Farmer	8	None	Christian
9	59	Male	Married	Carpenter	18	Secondary	Christian
10	69	Female	Married	TBA	6	Primary	Christian
11	60	Female	Widowed	TBA	5	Primary	Christian
TEM Users (n=21)							
1	42	Male	Married	Farmer	7	Primary	Christian
2	46	Male	Married	Charcoal maker	8	Primary	Christian
3	26	Male	Married	Mechanic	4	Primary	Christian
4	53	Female	Married	Farmer	5	Primary	Christian
5	38	Female	Married	Farmer	3	Primary	Christian
6	26	Male	Single	Graduate	5	Tertiary	Christian
7	18	Female	Single	Farmer	6	Secondary	Christian
8	39	Male	Married	Farmer	5	None	Muslim
9	85	Female	Widowed	Farmer	18	None	Christian
10	60	Female	Married	Business	5	None	Christian
11	72	Female	Married	Farmer	8	None	Christian
12	29	Male	Married	Teacher	3	Tertiary	Christian
13	60	Male	Married	Farmer	6	Primary	Muslim
14	39	Female	Married	Farmer	5	Primary	Christian
15	54	Male	Married	Guard	4	Primary	Christian
16	58	Female	Married	Farmer	4	Primary	Christian
17	30	Female	Divorced	Farmer	4	Primary	Christian
18	81	Male	Married	Farmer	9	None	Christian
19	81	Male	Married	Farmer	5	Primary	Christian
20	69	Male	Married	Farmer	17	Primary	Christian
21	20	Male	Single	Shop keeper	20	Primary	Muslim
TEM Non-Users (n=6)							
1	56	Male	Married	Teacher	6	Tertiary	Christian
2	25	Male	Married	Bike rider	6	Primary	Christian
3	39	Male	Married	Accountant	1	Tertiary	Christian
4	30	Female	Single	Hairdresser	1	Primary	Christian
5	20	Male	Single	Farmer	10	Secondary	Christian
6	19	Female	Single	Student	4	Tertiary	Muslim

TBA: Traditional Birth Attendant;

TEM, Role of Traditional Healers, personal circumstances and Ignorance.

Lack of confidence in conventional medicine

While some participants reported visiting health centres for treatment, many talked of resorting to TEM with the persistence in pain after use of conventional medicine. A 26-year male mechanic said "*At first, I got some relief when I put the eye drop, but later, it pained me severely and I was advised to use herbs. Having seen no great improvement, I started using herbs*." A participant in an IGD told us "*We are using western medicine to no avail. You can use western medicine for a week or a month but don't get healed.*" A 75-year male traditional healer reported that "*many people with eye problems come to me because some even fail to get cured from Mbarara hospital and are referred to me. I then put my traditional eye medicine like twice and they gain or enjoy life again.*" These statements supported the observation above that the majority (73%) of the TEM users had applied it after they had visited a health facility.

Lack of service in health facilities

Inadequate care including lack of medicines, rude health workers, unskilled health workers and poorly equipped health facilities, especially government owned ones, were reported as major drivers to use of TEM by a majority of patients. *"There are no experts or doctors experienced in treating eye diseases in Health Centres within our vicinities. When you find a doctor at a Health Centre, they say that they don't know such an eye disease you are suffering from"* (a 28-year unemployed man). The majority of primary health facilities do not have trained primary eye care workers. Eye patients are reviewed by general health workers who may have limited experience with managing ophthalmic condition. Eye care workers are nurses who have received an ophthalmic certificate course in examination and management of common eye conditions. In addition, as an 81-year-old farmer put it *"Health facilities within our areas don't have eye medicine, examination machines and they are also unwelcoming to a person who has gone there. One just looks at the eye, prescribes the medicine and start treating the illness. Or, you hear medicine has been brought but when you go there the next day, you are told there is no medicine."*

Poverty as a barrier to access care

With subsistence farmers constituting the major part of the population, poverty was reported as a key barrier to accessing eye care, encouraging people to opt for TEM. This was expressed as being unable to afford transport to eye hospitals and treatment. In an IGD1, one respondent told us *"Those of us who are able to afford treatment are very few you can count them; many people who have the same problem have turned blind because they cannot afford treatment."* Another person added *"It's a result of poverty! Many people in the village have no money. Even sometimes you don't have money in the pocket, so you pick the herb and apply it to the sick eye. You get to come here at the facility when you can't count the types of herbs you have tried just because of poverty."* Compared to going to hospital and the costs involved, TEM was a far cheaper option: the majority of the patients had obtained it from within their homesteads and had not spent any money on it.

Fear of the eye hospital

Most people lived far from the eye hospital and fear of travelling long distances, which was reported as a constraint. *"One can be having money but chooses not come to the hospital fearing how he will reach. Not all people are poor, but one just wonders where he is to pass and continue to Mbarara eye hospital. There are reluctant for example one says he won't be able to reach the place he has never gone to"* (an 81-year old male farmer from a distant village). We found that most of the patients travelled l distances (about 90 km) to reach the only referral eye hospitals in Mbarara town. Another form of fear was of what treatment would be offered; some people thought that this would make them go blind. For example, a participant in IGD2 told us *"What stops them from going to the hospital is that one is told they are going to operate your eye and after that it means that it is damaged completely you will never see again. That is the reason many people fear coming to the hospital, they say when you are operated the eye ends up getting damaged. They say when you reach in the hospital and get operated, it doesn't get well"*

Cultural understanding of MK and its treatment

Use of TEM in general is viewed as an acceptable practice and as part of culture in the community. It was revealed by several participants that MK is culturally understood as a disease to be treated locally. Almost all participants talked of receiving advice to use TEM from fellow community members who attest that it cured them. An 81-year old female farmer told us *"People in communities don't know that MK as an eye disease is treated in hospitals or that there are hospitals that can treat it. People say it is cured by traditional eye medicine."* Another 42-year old farmer said *"The old people we live with know those medicines and they testify that they cured them. Therefore, they encourage one who is suffering from an eye disease to keep using them saying he too will get well.*" Most of the people came from rural settings where there is a strong sense of community.

Belief in TEM

From the experience of previous TEM users and personal experience of use, it was not surprising that almost all participants who had used TEM believed it was effective. They attributed their failure to heal to their body makeup. "*The old people believe and know that traditional eye medicine cures eye diseases. There are people, they identified for me who used the same medicine and got well. Even themselves, they told me that they used it and got cured"* (a 42-year male farmer). "*The person who gave me traditional eye medicine told me she too suffered from the same disease and got healed by the same herbs*" (a 60-year old butter maker). On being asked why it had not worked for them, a 53-year old female farmer responded "*those who don't heal I think the condition of the eye might have needed medical attention from doctors as genetically people are different. There is one who heals by traditional eye medicine and another who doesn't and is only treated by modern medicine from hospitals."*

Role of traditional healers

With the belief and acceptance that use of TEM is within their culture, many had confidence in traditional healers. The

traditional healers themselves also had a strong confidence in their medicine and reported remarkable cure rates. One 56-year old traditional healer said: "*They go to the hospitals and come back to me when they have failed to heal with modern medicine. I give them traditional eye medicine and they get healed, none that I have treated or given my medicine has failed to get well*" Another 75-year old male healer reported "*There are many people I have treated; none I gave my medicine has ever complained that it failed to heal her or him. Whoever I meet just praises God and prays for me to be blessed. I treat people with faith in God.*"

Personal circumstances

Desperation due to the pain of the condition and the view of TEM as a form of first aid was mentioned as a prompt to use traditional medicine. This was mostly reported among patients who used TEM before presenting to health facilities. Participants explained that with the pain, one can use anything recommended to him or her to the extent of accepting TEM containing needle prick blood from another person without being afraid of contracting HIV. A 42-year male farmer told us *"This disease is so painful. No one should suffer from it because, with pain you can use anything given to you. You are not mindful of HIV, you only want the pain gone"*. A 85-year female farmer wondered, "*Can anyone who has been found in pain and recommended an herb fail to use it? Pain can make you do anything*".

Lack of awareness to the dangers of TEM

Interestingly, most participants did not think using TEM could be dangerous. "*Traditional eye medicine doesn't damage the eye, it just rinses or cleanses it*" (a 46-year old male charcoal burner). "*There are no risks of using traditional eye medicine because when one fails to get healed, she or he goes somewhere else or to hospitals*" (an 85-year female farmer). In addition, some thought it was better than conventional medicine and did not have any side effects like most conventional medicines. A 59-year old traditional healer said, "*Our herbal medicine is fresh not preserved.*"

Discussion

This study investigated the extent of TEM use by people with microbial keratitis, and how this impacts their clinical presentation and outcome. We went on to explore more deeply the specific practices and the reasons and beliefs behind using TEM. The use of TEM in Southern Uganda in the treatment of MK is common (60%), and more frequent than that previously reported from Malawi (34%) and Tanzania (25%)[9,10]. Importantly, we found that people who used TEM presented later with a more severe clinical picture and they ended up with worse final visual acuity outcomes at 3-months, compared to those who had not used TEM.

Our findings are similar to previous reports from Malawi, which found that patients who had used TEM presented later than those who had not used TEM[9,16]. The previous studies, however, did not examine final outcomes, after the infection had been treated. MK is a disease where prompt treatment is critical if one is to improve the likelihood of a good outcome. We know from prior literature that once an infection is advanced, treatment does relatively little to change its course[17]. The clear conclusion from earlier studies from South Asia and East Africa is that effective treatment of MK should be started as early as possible to save the eye and achieve the best possible outcomes[18,19].

In this study we combined both quantitative modelling approaches and complementary qualitative approaches to investigate not only "what" but also "why" people use TEM. In the explanatory multivariable model, increasing distance to the eye hospital, lower education level, an onset not linked to trauma and not being married were associated with TEM use. These were explored further in the informal group discussions (IGDs). These discussions the major reported reasons for using TEM were around consumer confidence in the health system, access, poverty and cultural influence.

Importantly, we found that most people who used TEM did so after first visiting a government health facility. This is consistent with the IGDs, in which people felt that conventional medicine was not helping, leading them to resort to alternative approaches. This conclusion could be a result of inappropriate treatment. However, even with appropriate treatment, the clinical response can be slow, especially for fungal keratitis. Patients need to be properly counselled to manage expectations. Another important aspect is good pain management on top of the anti-microbial treatment. Patients reported that desperation due to pain made them more likely to try many options to find relief. This initial early contact point with the formal health system represents an opportunity to improve the diagnosis and treatment of people with MK, through providing enhanced training, diagnostic tools and medication in the primary care setting.

Lack of appropriate ophthalmic medicines is a major challenge. For example, the best current evidence indicates that topical natamycin is the treatment of choice for filamentous fungal keratitis[20]. However, this is currently not readily available in the main ophthalmic units Uganda or elsewhere in SSA. It is certainly not available in more isolated locations. Therefore, patients with a fungal MK will not access effective treatment until they arrive in a major eye unit. Natamycin was added to the WHO Essential Medicines List in 2018, which will hopefully result in greater availability soon.

Limited access to eye care was a major driver of TEM use. This was evident in the regression modelling, with increasing TEM use with increasing distance to the eye hospitals. The majority of TEM users came from districts relatively far away where no eye care facilities were situated. This was a strong and frequently articulated theme in the interviews and discussions. Multiple people commented on the lack of eye health services in the nearby health facilities, the long distances to the eye hospital and poverty is a major barrier to access (because of the high transport and other direct costs). Several people also highlighted that government health centres near to them have no eye specialists or treatment and do not treat eye conditions. Pharmacies simply sell available eye drop medication, with no examination; frequently these are steroid and antibiotic combinations which may result in more harm than good in fungal keratitis. Unfortunately, Uganda still grapples with a severe shortage of human resources and infrastructure for eye health[21].

Although the regression model did not demonstrate a relationship between economic status and TEM Use, during the IGDs poverty was reported to be a major driver for using TEM. In the model, there were only a handful of people in the upper economic status which may have obscured this relationship. The majority of the patients were subsistence farmers and therefore not able to readily afford the cost of medicines and transportation. In contrast, TEM could be accessed closer to home at almost no cost. Most of the patients used got the TEM from their nearby gardens or from the neighbour and applied it freshly squeezed into the eye. People who are married may have access to greater household financial resources, possibly explaining why being married was associated with less TEM use.

We found that TEM use was linked to strong cultural beliefs and this seemed related to the level of education. In the model, people with no or little education were more likely to use TEM. It was worrying that people did not perceive TEM use as potentially dangerous. This was also reinforced by messages from traditional healers and older members of the community who carry a high level of respect. Public health orientated messaging and health education need to particularly focus on and work with these groups. There is some evidence from Malawi and Nigeria, where ophthalmologists worked with traditional healers to lower the use of TEM, that changes are possible[7,16]. Although, in our context, only 3% of TEM users consulted a traditional healer, their place in society cannot be underestimated and it would be in our best interest to bring them on board.

Strengths/limitations

The use of a mixed methods approach provided a more informative data on reasons for using TEM for MK in Uganda. To the best of our knowledge, this was the first study in SSA that looked at 3-month outcomes of people who had used TEM for treatment of MK. Although a sensitive topic, it was noted that participants and traditional healers were willing to talk about their TEM experiences. We did not have any evidence that people withheld information. The large numbers were enough to have a well powered study to explore factors associated with TEM use. Inclusion of children would have provided a more overall understanding of this topic, however, this was not practical in out setting.

Conclusion

TEM use is an important factor in the presentation and outcome of MK in Uganda, leading to delayed presentation to hospital, a poor presentation and a worse outcome. Cultural beliefs, access to the health system (due to poverty and long distances) and inherent challenges in the primary health centres (lack of knowledge, medicines, equipment and supplies) are major drivers of TEM use. Sensitisation of the people and capacity building in the primary health centres will be a step in the right direction to mitigate these effects.

Data availability

Underlying data

Havard dataverse: Traditional Eye Medicine use in Microbial Keratitis in Uganda. https://doi.org/10.7910/DVN/5GOPKZ[14].

This project contains the following underlying data:

- tem_data_descriptive_5May2019.tab (quantitative underlying data)
- tem_coding_framework_May2019.tab (codes of qualitative data responses)

Extended data

Havard dataverse: "Topic guides for exploring Traditional Eye Medicine Use for treatment of Microbial Keratitis in Uganda.docx", Traditional Eye Medicine use in Microbial Keratitis in Uganda, https://doi.org/10.7910/DVN/5GOPKZ[14].

This project contains the following underlying data

- Topic guides for exploring Traditional Eye Medicine Use for treatment of Microbial Keratitis in Uganda.docx (Topic guides that were used to probe respondents to talk about their understanding, opinions and experiences of using Traditional Eye Medicine)
- Quantitative questionnaire on use of Traditional Eye Medicine.docx (A of a quantitative questionnaire that was used to collect information from all the patients with MK on their history of use of Traditional Eye Medicine)

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PubMed Abstract | Publisher Full Text | Free Full Text

Open Peer Review

Current Peer Review Status: ✔ ✔

Version 2

Reviewer Report 08 October 2019

https://doi.org/10.21956/wellcomeopenres.16901.r36670

✔ **Savitri Sharma**
Jhaveri Microbiology Center, Brien Holden Eye Research Centre (BHERC), L V Prasad Eye Institute (LVPEI), Hyderabad, Telangana, India

Revision satisfactory.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: I am a clinical microbiologist in an academic tertiary care eye centre with over 25 years experience in diagnosing and researching microbial keratitis cases in India. I have published extensively and written book chapters in the area of ocular infections including microbial keratitis. My research areas include fungal keratitis, Acanthamoeba keratitis, antibiotic susceptibility, infection control, molecular diagnosis of eye infections, infectious endophthalmitis etc.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Reviewer Report 24 July 2019

https://doi.org/10.21956/wellcomeopenres.16656.r35735

✔ **David Yorston**
Tennent Institute of Ophthalmology, Gartnavel Hospital, NHS Greater Glasgow and Clyde, Glasgow, UK

This is a useful addition to the mounting evidence that improving the early treatment of microbial keratitis should be a priority for prevention of blindness programmes.

- The authors conclude that TEM is more likely to be used if patients have less access to effective eye care facilities. Although poverty was cited by many participants as a driver for TEM use, it was not significant in the multivariate analysis. This may be explained by the paucity of higher SES patients in both groups. I think it is likely that poverty does contribute to TEM use, alongside the other factors.

- Although the text of the results section states that TEM users had worse presenting acuity than non TEM users, the data in Table 2 appears to contradict this, and I suspect there may be an error in the table.

- This study confirms the finding of previous authors who noted that TEM use is associated with a greater risk of hypopyon. The underlying assumption of this article is that all patients had microbial keratitis prior to TEM use. However, it is possible that some may have had self-limiting, or minor conditions, such as a corneal abrasion or conjunctivitis. The introduction of unsterile preparations on to a compromised ocular surface may have led to *de novo* development of microbial keratitis.

- An unexpected finding is that TEM use in this population was usually independent of traditional healers. I have always assumed that TEM use is partly driven by a desire for answers that western medicine is not good at providing, particularly "Why has this happened to me?". This study would seem to indicate that the main motivation for most patients was a simple desire for faster and greater improvement in their symptoms.

- A less surprising finding is that outcomes were significantly worse for patients using TEM. Previous studies have not been able to obtain outcome data, as it can be difficult for these patients to return for review. It is valuable to have clear evidence that TEM use is harmful.

- One significant weakness in the study is the exclusion of children. In Tanzania we found that 50% of TEM users were aged 11 or younger. I suspect that the findings would be similar in children and adults, but the authors should acknowledge this weakness in the discussion.

- The ready availability of TEM in people's homes and gardens means that campaigns to reduce the use of TEM are unlikely to be successful. Prevention of blindness programmes would be better to focus on improving the delivery of eyecare, and raising the quality of the care delivered. Anecdotally, I can report that TEM use was widespread in a poor part of rural Tanzania, but almost non-existent in the relatively developed Central Province of Kenya. My experience would appear to support the authors' conclusion that improving rural eye care will lead to a decline in the harms caused by TEM.

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Vitreoretinal surgery, public health ophthalmology in developing countries

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 02 Sep 2019

SIMON ARUNGA, London School of Hygiene & Tropical Medicine, London, UK

Comment: The authors conclude that TEM is more likely to be used if patients have less access to effective eye care facilities. Although poverty was cited by many participants as a driver for TEM use, it was not significant in the multivariate analysis. This may be explained by the paucity of higher SES patients in both groups. I think it is likely that poverty does contribute to TEM use, alongside the other factors.
Response: We agree with the reviewer that poverty does contribute to TEM use and was indeed reported by many participants. In the multivariable model, there were only a handful of people in the upper economic status which may have obscured this relationship. We have added this comment in lines 339-341. Also to note is that SES/Access/poverty are all on a similar/same causal path and do not function independently of each other.

Comment: Although the text of the results section states that TEM users had worse presenting acuity than non TEM users, the data in Table 2 appears to contradict this, and I suspect there may be an error in the table.
Response: We thank the reviewer for spotting this. We noticed that the data had been accidentally interchanged. It has been corrected in table 2.

Comment: This study confirms the finding of previous authors who noted that TEM use is associated with a greater risk of hypopyon. The underlying assumption of this article is that all patients had microbial keratitis prior to TEM use. However, it is possible that some may have had self-limiting, or minor conditions, such as a corneal abrasion or conjunctivitis. The introduction of unsterile preparations on to a compromised ocular surface may have led to de novo development of microbial keratitis.
Response: We agree with the reviewer and feel the same way. However, there was no way of objectively ascertaining this fact. We intend to explore this in our future studies.

Comment: An unexpected finding is that TEM use in this population was usually independent of traditional healers. I have always assumed that TEM use is partly driven by a desire for answers that western medicine is not good at providing, particularly "Why has this happened to me?". This

study would seem to indicate that the main motivation for most patients was a simple desire for faster and greater improvement in their symptoms.
Response: Indeed, this was surprising. Only 3% of the participants visited a traditional healer to obtain TEM. From our further exploration of this in the qualitative studies, our impression is that *"everyone in the community is a traditional healer"* since the knowledge of the herbs is common among the community members. However, this does not negate the role of the healers since they are strong advocates for TEM use.

Comment: A less surprising finding is that outcomes were significantly worse for patients using TEM. Previous studies have not been able to obtain outcome data, as it can be difficult for these patients to return for review. It is valuable to have clear evidence that TEM use is harmful.
Response: We thank the reviewer for acknowledging this new contribution.

Comment: One significant weakness in the study is the exclusion of children. In Tanzania we found that 50% of TEM users were aged 11 or younger. I suspect that the findings would be similar in children and adults, but the authors should acknowledge this weakness in the discussion.
Response: We thank the author for this comment. Although we provided care for children who presented with Microbial Keratitis, the design of our study enrolled only adults due to pragmatic reasons such as being able to test people for HIV, subjecting children under general anaesthesia for corneal scrapping and ethical approvals for a vulnerable group. In addition, we found out during the pilot phase that microbial keratitis was not very common among children in our setting, accounting for only about 3% of all microbial keratitis cases. However, this point has been acknowledged in the limitation. lines 364-365.

Comment: The ready availability of TEM in people's homes and gardens means that campaigns to reduce the use of TEM are unlikely to be successful. Prevention of blindness programmes would be better to focus on improving the delivery of eyecare, and raising the quality of the care delivered. Anecdotally, I can report that TEM use was widespread in a poor part of rural Tanzania, but almost non-existent in the relatively developed Central Province of Kenya. My experience would appear to support the authors' conclusion that improving rural eye care will lead to a decline in the harms caused by TEM.
Response: We thank the reviewer for this comment.

Competing Interests: n/a

Reviewer Report 01 July 2019

https://doi.org/10.21956/wellcomeopenres.16656.r35736

?

Savitri Sharma
Jhaveri Microbiology Center, Brien Holden Eye Research Centre (BHERC), L V Prasad Eye Institute (LVPEI), Hyderabad, Telangana, India

This report gives an account of the "mystery" surrounding the traditional eye medicine usage in the treatment of microbial keratitis. The practice is rampant in some of the developing and underdeveloped countries and the more we know and understand this practice better we can get at influencing people to make a distance from them. Social, cultural, economical and emotional factors - all seem to be responsible for continued presence of this unwanted practice. This reviewer appreciates the efforts of the authors in putting up this paper together which is very well written. Following are minor comments that may help make the paper even better:

1. Abstract: Results begins with digits which in good writing should be avoided and replaced with words.

2. Methods: Clinical examination and microbiological methods are not described at all. A description would allow better understanding of how the data was collected.

3. Analysis, Page 4, results, last but one line: The word farmer is spelt wrongly with one "r" missing.

4. There is no data on what type of organisms were involved in the microbial keratitis in the two study groups. If microbiology was done, as is claimed in methods, there should be results of the same. Similarly, how were the patients treated in the control group that did not receive traditional eye medicine? These are important determinants of the outcome in the two groups that have been compared. My comments of "partly satisfied" are related to these issues.

5. Discussion: Para 2, line 6: “...if one is improve the likelihood of a good outcome.” This sentence is incorrect with a missing word "to".

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Partly

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: I am a clinical microbiologist in an academic tertiary care eye centre with over 25 years experience in diagnosing and researching microbial keratitis cases in India. I have published

extensively and written book chapters in the area of ocular infections including microbial keratitis. My research areas include fungal keratitis, Acanthamoeba keratitis, antibiotic susceptibility, infection control, molecular diagnosis of eye infections, infectious endophthalmitis etc.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 02 Sep 2019

SIMON ARUNGA, London School of Hygiene & Tropical Medicine, London, UK

Comment: Abstract: Results begins with digits which in good writing should be avoided and replaced with words.
Response: We thank the reviewer for spotting this. We have revised this sentence to read "Out of 313 participants enrolled, 188 reported TEM use".

Comment: Methods: Clinical examination and microbiological methods are not described at all. A description would allow better understanding of how the data was collected.
Response: We thank the reviewer for this comment. The detailed assessment of the patients has been described in a different report (under review), however, we have revised the manuscript and summarised patient assessment. Line 48-55.

Comment: Analysis, Page 4, results, last but one line: The word farmer is spelt wrongly with one "r" missing.
Response: We thank the author for spotting this. It has been corrected. Line 115.

Comment: There is no data on what type of organisms were involved in the microbial keratitis in the two study groups. If microbiology was done, as is claimed in methods, there should be results of the same. Similarly, how were the patients treated in the control group that did not receive traditional eye medicine? These are important determinants of the outcome in the two groups that have been compared. My comments of "partly satisfied" are related to these issues.
Response: We would like to draw the attention of the reviewer to the last section of table 2 which summarises the types of organisms in the two groups. Although the proportion of fungal keratitis was more common among the people who had used TEM, the evidence of this difference was weak. We agree with the reviewer that treatment for people with keratitis should consider the history of use of TEM since that could influence the organisms involved, especially in the absence of a good microbiology support. However, treatment of the participants in our study was dependant on the microbiological findings.

Comment: Discussion: Para 2, line 6: "...if one is improve the likelihood of a good outcome." This sentence is incorrect with a missing word "to"
Response: We thank the author for spotting this. It has been corrected. Line 296.

Competing Interests: n/a

A Table showing the list of herbs used as Traditional Eye Medicine in Uganda

LOCAL BANTU NAME	PLANT FORM	ENGLISH NAME	*BOTANICAL NAME*
Akatooma			*Erlangea cordifolia*
Omujaja			*Ocimum grattisimum*
Akanyunyambuzi			*Oxalis latifolia*
Omubarama			*ClutiaAbyssinca*
Amampera	Tree	Guava	*Psidium guajava*
Omuhukye	Shrub		*Lantana trifolia*
Eshwinga	Herb		*Solanum nigrum (sensu lato)*
Omufumbagesi			*Rumex abyssinica*
Omuyora Kahoo			*Leucaena leucocephala*
Ekyoganyaja			*Erlangea tomentosa*
Entoobo			*Solanum incanum*
Akajwamante	Herb/shrub		*Lactuca capensis*
Akacumucumu	Herb/shrub		*Leonotis nepetifolia*
Enyabarashana	Herb	Black jack	*Bidens pilosa*
Ekarwe	Herb		*Melanthera scandens*
Akacumita Mbongo			*Oxygonum sinuatum*
Ekihindihindi	Climbing herb	Climbing bean	*Phaseolus lunatus*
Akajongojongo			
Omubirizi			*Vernonia amygdalina*
Oburabyo Bwekiko	Tree	Flame tree	*Flowers of Erythrina abysinica*
Entabee	Herb	Tobacco	*Nicotiana tabacum*
Akabindizi	Herb		*Zehneria scabra*
Orumbungu	Grass	Cough grass	*Digitaria abysinica*
Kanyoro			
Rukaka			*Aloe sp.*
Amashanda G`Omutooma	Tree	Sap of	*Ficus natalensis*
Enkoninyabato			*Klanchoe tetraphylla*
Ekinami			*Crasocephalum bauchiense*
Obushaza	Herb/crop	Cow Peas	*Pisum sativum*
Ekijamba	Crop	Bean leaves	*Phaseolus vularis leaf*
Omwihura	Climbing herb		*Momordica foetida*
Akayenje			*Euphorbia tirucali*
Omuturashonga			
Oruhingura	Woody herb		*Triumpheta rhomboidae*
Omukogorane			*Pseundarthria hookeri*
Omusoroza	Herb	African Indigo	*Indigofera arrecta*
Akanyamafundo			*Leucas marticensis*
Omubarama			*ClutiaAbyssinca*
Akaitsire Nkore			
Omuziranfu			*Tetrorchidium didymostemon*
Nyakasambu			
Ekyoganyaja	Woody herb /shrub		*Erlangea tomentosa*
Akatunguru		Onion	*Allium cepa*

Omuhukye	Shrub		*Lantana trifolia*
Ekyiyondo			*Kalanchoe luciae*
Ekiteezi			*Commelina bengalensis*
Ekihabukuru			*Desmodium intortum*
Eteija	Creeping herb	Wandering jew	*Commelina bengalensis*
Omuherere	Shrub		*Vernonia cistifolia*
Ekihungunga			
Orunokwo			
Omuhe			*Microglossa angolensis*
Bukabuka	Herb		*Ageratum conyzoides*
Ereka	Herb		*Kalanchoe Pinnata*
Omuzabibu	Vine		*Cardiospermum grandifloum*

This table represents a collection of herbs reported by the patients with Microbial Keratitis MK) enrolled in the main cohort study who had used Traditional Eye Medicine (TEM). In this cohort, 188/313 patients reported to have used TEM. The patients were asked to bring in a sample of the herbs they had used on their subsequent visits, there were then taken to the taxonomy department at Mbarara University of Science and Technology for identification. For some who were unable to bring the samples, the taxonomy department would obtain these samples from a local network of herbalists.

This work is still ongoing, and a separate manuscript will be prepared to present these findings of the different herbs used as TEM for treatment of microbial Keratitis in South Western Uganda.

Chapter 10. Impact of Microbial Keratitis on Quality of Life in Uganda

A technician fits a prosthetic eye in a post enucleation patient at Ruharo Eye Centre, Uganda

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RESEARCH PAPER COVER SHEET

Please note that a cover sheet must be completed for each research paper included within a thesis.

SECTION A – Student Details

Student ID Number	LSH1511754	**Title**	Dr
First Name(s)	Simon		
Surname/Family Name	Arunga		
Thesis Title	Epidemiology of Microbial Keratitis in South Western Uganda		
Primary Supervisor	Prof Matthew Burton		

If the Research Paper has previously been published please complete Section B, if not please move to Section C.

SECTION B – Paper already published

Where was the work published?			
When was the work published?			
If the work was published prior to registration for your research degree, give a brief rationale for its inclusion			
Have you retained the copyright for the work?*	Choose an item.	Was the work subject to academic peer review?	Choose an item.

*If yes, please attach evidence of retention. If no, or if the work is being included in its published format, please attach evidence of permission from the copyright holder (publisher or other author) to include this work.

SECTION C – Prepared for publication, but not yet published

Where is the work intended to be published?	BMJ Open Ophthalmology
Please list the paper's authors in the intended authorship order:	Simon Arunga, Geoffrey Wiafe, Esmael Habtamu, John Onyango, Stephen Gichuhi, Astrid Leck, David Macleod, Victor H. Hu, Matthew J. Burton
Stage of publication	Submitted and peer reviews addressed. Awaiting acceptance

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I designed the study, collected the data, conducted the analysis with guidance from David Macleod, Victor Hu, and M J Burton, prepared and submitted the final manuscript to BMJ Open Ophthalmology in consideration of comments from all co-authors.

SECTION E

Student Signature	AS
Date	19/9/19

Supervisor Signature	MJB
Date	20/9/19

BMJ Open Ophthalmology

The impact of microbial keratitis on quality of life in Uganda

Journal:	*BMJ Open Ophthalmology*
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Complete List of Authors:	Arunga, Simon; London School of Hygiene and Tropical Medicine Department of Clinical Research, ; Mbarara University of Science and Technology, Ophthalmology Wiafe, Geoffrey; Mbarara University of Science and Technology, Ophthalmology Habtamu, Esmael; London School of Hygiene and Tropical Medicine International Centre for Eye Health; Carter Center Onyango, John; Mbarara University of Science and Technology, Ophthalmology Gichuhi, Stephen; University of Nairobi, Ophthalmology; London School of Hygiene & Tropical Medicine, Clinical Research Leck, Astrid; LSHTM, ITD/CRU Macleod, David; London School of Hygiene & Tropical Medicine, Medical Statistics Hu, Victor; London School of Hygiene and Tropical Medicine, International Centre for Eye Health Burton, Matthew; London School of Hygiene and Tropical Medicine, Clinical Research Department
Twitter User Name:	
Keywords:	Cornea, Epidemiology, Infection

The impact of microbial keratitis on quality of life in Uganda

Authors

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Conflict of interest

None of the authors have any proprietary interests or conflicts of interest related to this submission.

Submission statement

This submission has not been published anywhere previously and that it is not simultaneously being considered for any other publication.

Keywords: Microbial Keratitis, Bacterial keratitis, Fungal keratitis, Blindness, Quality of Life, Uganda

Word count: Abstract 240, Text 2879

Precis

Microbial keratitis in Uganda leads to a reduction in the quality of life in affected individuals, compared to unaffected individuals, matched for age, sex and location of residence.

ABSTRACT

Background: Microbial Keratitis (MK) is a frequent cause of sight loss in sub-Saharan Africa. However, no studies have formally measured its impact on Quality of Life (QoL) in this context.

Methods: As part of a nested case control design for risk factors of MK, we recruited patients presenting with MK at two eye units in Southern Uganda between December 2016 and March 2018 and unaffected individuals, individually matched for sex, age and location. QoL was measured using WHO Health-Related and Vision-Related QoL tools (at presentation and 3-months after start of treatment in cases). Mean QoL scores for both groups were compared. Factors associated with QoL among the cases were analyzed in a linear regression model.

Results: 215 case-controls pairs were enrolled. The presentation QoL scores for the cases ranged from 20-65 points. The lowest QoL was visual symptom domain; mean 20.7 (95% CI 18.8-22.7) and the highest was psychosocial domain; mean 65.6 (95% CI 62.5-68.8). At 3-months, QoL scores for the patients ranged from 80-90 points while scores for the controls ranged from 90-100. The mean QoL scores of the cases were lower than controls across all domains. Determinants of QoL among the cases at 3-months included visual acuity at 3-months and history of eye loss.

Conclusion: MK severely reduces QoL in the acute phase. With treatment and healing, QoL subsequently improves. Despite this improvement, QoL of someone affected by MK (even with normal vision) remains lower than unaffected controls.

Key messages

What is already known about this subject?

- Quality of life is affected in many bilateral ocular conditions such as cataract, glaucoma and trichiasis.
- The impact of Microbial Keratitis on Quality of Life compared to unaffected individuals has not been previously reported.
- Microbial Keratitis is a common cause of blindness in Sub Saharan Africa

What are the new findings?

- Microbial Keratitis severely reduces Quality of Life in the acute phase of the disease.
- Quality of life improves with treatment and healing.
- Despite improvement, QoL of someone affected by MK (even with normal vision) remains lower than unaffected controls.

How might these results change the focus of research or clinical practice?

- The focus of this study is to make the case that Microbial Keratitis, although usually a uniocular disease severely reduces the Quality of Life of the affected individuals.
- Treatment strategies for Microbial Keratitis should include deliberate interventions to address Quality of Life.

BACKGROUND

Microbial keratitis (MK) has been described as a “silent epidemic”, which leads to substantial morbidity, related to sight loss, pain and stigma.[1] It is the leading cause of unilateral blindness after cataract in tropical regions, estimated at 2 million cases of monocular blindness per year.[2] In 2017, 1.3 million individuals were bilaterally blind from corneal opacity globally (excluding trachoma and vitamin A deficiency), accounting for 3.2% of binocular blindness.[3]

Quality of Life (QoL) is a very important consideration in the management of any disease and treatments should ultimately aim to maintain or restore QoL.[4] Few studies from sub-Saharan Africa (SSA) have examined the effect of cataract and trachomatous trichiasis on QoL.[5-7] These have generally examined both the general Health Related Quality of Life (HRQoL) and more specific Vision Related Quality of Life (VRQoL).

However, there is no published data on the impact of MK on QoL from SSA, and very little from other World regions. In the Mycotic Ulcer Treatment Trial 1 (MUTT1) in India which compared topical natamycin to topical voriconazole for the treatment of fungal keratitis, QoL scores at 3 months were compared between the two treatment arms. However, there was no comparison group of unaffected individuals.[8] There is a need to better understand how MK and its outcomes affect people, to develop improved management, counselling and support.

In order to investigate the impact of MK on QoL, we conducted this study in South-Western Uganda. Here we describe the QoL among patients with MK, at presentation and three months after presentation compared to the QoL of unaffected individuals recruited from the community who were individually matched for age, sex and location of residence.

METHODS

Ethical Statement

This study adhered to the tenets of the Declaration of Helsinki. It was approved by the London School of Hygiene & Tropical Medicine Ethics Committee (Ref 10647), Mbarara University Research Ethics Committee (Ref 10/04-16) and Uganda National Council for Science and Technology (Ref HS-2303). Written, informed consent in the local language, was obtained before enrolment. If the potential participant was unable to read, the information was read to them, and they were asked to indicate their consent by application of their thumbprint, which was independently witnessed.

Study Design and Participants

This study of the impact of MK on QoL was nested within a case-control study of MK in Uganda. We prospectively enrolled patients with MK that presented to Ruharo Eye Centre and Mbarara University and Referral Hospital Eye Centre from December 2016 to March 2018. These are the tertiary referral centres for South-Western Uganda. The case definition of MK was the presence of a corneal epithelial defect (of at least 1mm diameter) with an underlying stromal infiltrate, associated with signs of inflammation (conjunctival hyperaemia, anterior chamber inflammatory cells, +/- hypopyon).[9] We excluded those not willing to participate or to return for follow-up, pregnant women, lactating mothers and those under 18 years. All the questions in the tools were responded to directly by the study participants.

Assessment of Cases

At presentation we documented basic demographic information and ophthalmic history. Presenting LogMAR (Logarithm of Minimum Angle of Resolution) visual acuity at 2 meters in a dark room was measured using the Peek Acuity smartphone application.[10] Cases were examined at a slit lamp to assess the anterior segment using a structured protocol. Corneal scrape specimens were collected from the ulcer at a slit lamp or an operating microscope and samples were processed in the department of microbiology laboratory at Mbarara University of Science and Technology. Following corneal scrapping, immediate Calcofluor White staining was done in the side lab at the eye hospital on a fluorescence microscope (Zeiss Primostar ILED) by the attending ophthalmologist to rule out fungal Keratitis. Additional microscopy (Gram staining and KOH staining) was done in the main University microbiology laboratory and results became available within 24 hours. Agar plates and broths (Blood, Chocolate, Potato Dextrose and Brain Heart) were incubated at 35-37°C for bacteria for up to 7 days and at 25°C for up to 21 days for fungi. Organism identification and sensitivity testing were performed

using standard microbiological techniques. Cases were treated empirically at presentation and the treatment was reviewed when the microbiology results became available. Patients with fungal keratitis were treated with Natamycin 5% eyedrops (Zonat Sunways India), those with bacterial keratitis were treated with Ofloxacin 0.3% eyedrops (Biomedica Remedies-India). They were reviewed on days 2, 7, 21 and 90 (3-months). At 3-months, the cases were followed-up in their homes for a final assessment.

Control Recruitment:

We recruited healthy community controls during the 3-month follow-up of cases in their home village. The controls were individuals without any current eye complaints and with normal vision. The controls were individually matched to the cases. They had to be living in same village as the case, be of the same gender and in the same age group (+/- 5 years). Enrolment followed a similar approach to that previously used in study in Ethiopia.[6] The research team visited the villages (typically 50-100 households), the local village head was asked to write down all individuals they thought would meet the matching criteria for a particular case in that village. One person was randomly selected from this list using a lottery method. They were approached and provided with details of the study and invited to participate if eligible. If a selected individual refused or was ineligible, another person was randomly selected and approached. Both cases and controls were asked about their social economic status, this was a self-reported question compared to their neighbours on a 5-scale level (5 “very rich”, 4 “rich”, 3 “neither rich nor poor”, 2 “poor”, 1 “very poor”).[6, 7, 11] In this 5-point scale, participants were asked “*Compared to your neighbours, how do you rate your household wealth status?”*

Quality of Life Instruments

To measure QoL we used two instruments: the general health WHOQOL-BREF and the vision related WHO/PBD-VF20. These were both initially independently translated by two translators into Runyankole, the local language. Any discrepancies were discussed with a third party and a merged final agreed version produced. Both instruments were administered to cases at presentation and at the 3-month follow-up. They were administered to the control group only once, during the 3-month assessment of the matched case.

VRQoL: the WHO/PBD-VF20 tool measures vision related quality of life. It assesses the impact of visual impairment in several domains including mental wellbeing, dependency and social functioning. The WHO/PBD-VF20 consists of 20 questions divided into four sub-scales: “General Vision” subscale (1 question); “Visual Symptoms” subscale (3 questions); “General Functioning” subscale (12 questions); and “Psychosocial” subscale (4 questions). It begins by asking the patient *“Overall, how would you rate your eyesight using both*

eyes?"; and uses a five-point scale answer option such as *"very good", "good", "moderate", "bad", "very bad"*. Each subsequent question also has a 5-point response option: one indicates the highest and five the lowest score.

HRQoL: the WHOQOL-BREF (WHOQOL Group, 1998) has good applicability in low and middle-income countries (LMIC) as it was developed simultaneously from concept across 18 countries in Africa, Asia and Latin America.[12] It measures 4 domains of health: Physical Health, Psychological Health, Social Relationships, and Environment. It asks respondents 26 questions. These include the frequency they have experienced issues and/or were able to do things (e.g. feel safe, able to concentrate, enjoy life) in the past 4 weeks and how satisfied they are with certain aspects of their lives (e.g. sleep, capacity for work)[12].

Sample size

Based on the effect sizes found in previous work on cataract and trichiasis, a sample size of 215 pairs would have 80% power to detect a moderate effect of MK on QoL with an effect size of 0.27 (effect size = QoL score difference (3) / SD 11) with a Type 1 error of 5%.[5, 7]

Analysis

Data were managed in Access (Microsoft), and transferred to Stata 14 (StataCorp) for analysis. Data were analysed using a previously described methodology, applied in other QoL studies.[6, 7, 13]

VRQoL. All items were grouped, and scores added into their respective subscales: "General Vision" subscale (1 question); "Visual Symptoms" subscale (3 questions); "General Functioning" subscale (12 questions); and "Psychosocial" subscale (4 questions). The subscale scores were then converted into a scaled value out of one hundred, using the formula: ([individual score—lowest possible score]/[highest possible score—lowest possible score]) x 100. Therefore, the person with the lowest possible VRQoL score would receive a scaled value of "0" and the person with the highest possible VRQoL score receives a scaled value of "100".

HRQoL. Data were analysed following the WHOQOL-BREF protocol.[12] Three negatively framed items were reversed into a positive frame so higher scores denote higher QoL. To generate domain scores, questions were grouped into their respective domains and their scores totalled. The mean score of all items included in the domain was calculated and then multiplied by four. These scores then transformed to a 0 to 100 scale with

the formula specified in the manual to allow comparison between domains made of unequal number of items.[14]

Psychometric property evaluation. Construct validity of the VRQoL and HRQoL data was assessed through known-group difference and convergence validity using a linear regression model. Cronbach's alpha was used to test for internal consistency and reliability of the VRQoL and HRQoL data.

Cases and controls were compared for baseline characteristics. However, we noticed that not all the pairs had been correctly matched for age because the village heads had subjectively guessed the ages of the controls. We thus adjusted for age throughout the analysis. The VRQoL and HRQoL analysis compared the cases to the controls at 3-months using a linear regression random effects model, which was adjusted for age and socio-economic status, as these factors may confound the association between MK and QoL. A linear regression analysis was used to determine vision related factors associated with QoL among the cases at 3 months adjusted for baseline QoL, age, sex, education and economic status.

Patient and public involvement

Apart from helping to provide information during the piloting and data collection phase, we did not explicitly involve patients or the public in the designing and implementation of our work

RESULTS

A total of 313 MK cases presented and were enrolled. We were able to follow-up 260 cases at 3-months. It was not possible to enrol a control for 45/260 cases. Therefore, the analysis of QoL at 3-months comprises 215 pairs. The baseline characteristics were comparable among the cases and control group: median age was 47 years (IQR 35-60, total range 18-96 years), and 120 (56%) were male. (Table 1)

Table 2 shows the baseline and 3-month VRQoL and HRQoL scores for the cases and the scores for the control group. The mean baseline VRQoL scores among the cases were all low (<50) except the psychosocial domain which had a score of 65 points. The most affected domain was visual symptoms, with a mean score of 20.7 (95% CI 18.8-22.7). The mean baseline HRQoL scores among the cases were all low (<50). At 3-months, all the case VRQoL and HRQoL scores had increased and were relatively high (between 80-91). Despite this increase, there was still very strong evidence ($p<0.0001$ in all domains) that QoL scores among MK cases at 3-months were lower than the controls, after adjusting for age, and economic status.

Table 3 shows the presenting vision, microbiology and 3-months outcomes among the 260 cases. Majority (137/260) presented with vision worse than 6/60 in the affected eye. Microbiology results were available for 226/260 participants out of which the majority (63%) showed fungal keratitis. At 3-months, 138/260 had vision of better than 6/18 in the affected eye. Vision had improved in 137 individuals, remained the same in 56 and worsened in 66 participants (sign rank $p<0.0001$).

To investigate if the difference in QoL between the cases and controls was due to factors in addition to impaired vision in the MK group, a separate sub-group analysis was performed comparing only MK cases with normal vision in the affected eye (better than 6/18) to their paired controls (Supplementary table 1). It was observed that the differences in QoL was similar to that obtained when using all the cases.

Tables 4 and 5 shows factors associated with a good VRQoL and HRQoL among the cases at 3 months. This analysis was among all the 260 MK cases who were followed up at 3 months. Analysis was restricted to variables related to the disease such as vision at 3 months and whether the person had lost their eye. They were adjusted for sex, age, education, baseline QoL and socioeconomic status. Vision and eye loss were the both found to be associated with VRQoL and HRQoL.

Validity of the data was found to be good. Satisfying the known-groups difference criteria, the cases had significantly lower VRQoL and HRQoL scores in all domains ($p<0.0001$) than the controls (Table 2). The VRQoL data were reliable after being assessed for internal consistency with a Cronbach's alpha: coefficients of >0.80 (visual symptom 0.90, general functioning 0.98, psychosocial 0.87). The overall HRQoL data had a Cronbach's alpha of 0.98 (physical health 0.96, psychological 0.89, social 0.91 and environment 0.95).

Table 1: Baseline characteristics among the 215 case-control pairs. (matched on gender and village and adjusted for age)

Exposure	Cases (215)		Controls (215)		P-value
	n	(%)	n	(%)	
Married (yes) ɬ	154	(72)	143	(67)	0.215
Head of household (yes) ǂ	146	(68)	140	(65)	0.441
Education status §					
None	60	(28)	48	(22)	0.148
Primary	110	(51)	114	(53)	
Secondary	31	(14)	32	(15)	
Tertiary	14	(7)	21	(10)	
Farmer (yes) §	157	(73)	168	(78)	0.144
Size of the household ǁ					
Small (1-4 people)	50	(23)	109	(51)	0.05
Medium (5-10 people)	115	(54)	94	(44)	
Large (>11 people)	50	(23)	12	(5)	
Self-reported wealth status *					
Poor	36	(17)	20	(9)	0.003
Middle	158	(73)	188	(88)	
Upper	21	(10)	6	(3)	

ɬ People who were married, or cohabiting were considered as married while those who were divorced, single or widowed were considered as not married. ǂ Being head of the household meant people who were responsible for the overall care of the family, this was regardless of gender: among the cases and controls, 31% and 23% were female heads of households respectively. § Majority of the participants had no or minimal education (primary level) which is not uncommon for a predominantly rural population in Uganda. Subsistence farming is the main occupation for this population. ǁ Majority of the household sizes were medium to large (5 people or more). This is not uncommon since most of the living in rural Uganda is largely in an extended family setting. * Self-reported wealth status was classified as poor (1" very poor" 2" poor"), middle (3 "neither poor nor rich") upper (4 "rich" 5 "very rich"). There was one missing value among the control group. Participants were asked to compare themselves to their neighbours and give a score of their economic status.

Table 2: Vision-Related (VRQoL) and general Health-Related Quality of Life (HRQoL) among cases (baseline and 3 months) and controls (215 pairs).

Domain	Cases at Baseline		Cases at 3 Months		Controls at 3 Months		Adjusted mean difference at 3 months		
	Mean	(95%CI)	Mean	(95%CI)	Mean	(95%CI)	Mean ł	(95%CI)	p-value ǂ
VRQoL									
Overall Sight	32.9	(30.6-35.2)	86.3	(83.3-89.2)	98.6	(97.5-99.6)	11.6	(8.8-14.5)	<0.0001
Visual Symptom	20.7	(18.8-22.7)	88.3	(85.5-91.1)	99.4	(98.9-99.8)	10.5	(7.7-13.3)	<0.0001
General Functioning	42.8	(40.6-45.1)	89.0	(86.1-91.9)	99.6	(99.3-100)	9.9	(7.1-12.7)	<0.0001
Psychosocial	65.6	(62.5-68.8)	90.7	(88.1-93.3)	99.8	(99.4-100)	8.5	(6.0-11.0)	<0.0001
HRQoL									
General facet items									
Overall quality of life	40.3	(39.0-41.6)	86.6	(83.9-89.4)	97.2	(96.4-97.9)	10.2	(7.6-12.8)	<0.0001
Overall Health	31.0	(29.0-33.0)	85.6	(82.7-88.5)	98.2	(97.3-99.1)	12.0	(9.1-14.9)	<0.0001
Domains									
Physical health	28.4	(26.5-30.3)	86.1	(83.1-89.2)	98.3	(97.6-98.9)	11.5	(8.6-14.5)	<0.0001
Psychological	49.2	(47.5-50.9)	84.4	(81.9-86.9)	94.0	(93.4-94.7)	9.1	(6.7-11.5)	<0.0001
Social	48.5	(46.5-50.5)	88.2	(85.2-91.2)	98.5	(97.6-99.3)	9.9	(6.9-12.9)	<0.0001
Environment	43	(41.7-44.3)	84.8	(82.0-87.6)	96.3	(95.2-97.3)	10.9	(8.2-13.6)	<0.0001

Only the cases who had controls were included in this analysis (215 pairs).
ł mean difference between cases and controls adjusted for age, sex and wealth status.
ǂ Linear regression random effects model was used to test for significance of the differences among the cases and controls adjusted for age, sex and wealth status.

Table 3: Presenting vision, microbiology and 3 months outcomes for the cases (n=260)

Variable	n/260	(%)
Presenting Visual Acuity in the affected eye (Snellen) ł		
> 6/18	86	(33%)
6/18-6/60	36	(14%)
< 6/60	137	(53%)
Presenting Visual Acuity in the non-affected eye (Snellen) ł		
> 6/18	232	(90%)
6/18-6/60	14	(5%)
< 6/60	13	(5%)
Microbiology *		
Fungal	143	(63%)
Bacterial	18	(8%)
Mixed	13	(6%)
Unknown	52	(23%)
Visual Acuity in the affected eye (Snellen) at 3 months		
> 6/18	138	(53%)
6/18-6/60	37	(14%)
< 6/60	85	(33%)
Visual acuity in the non-affected eye at 3 months		
>6/18	229	(90%)
6/18-6/60	11	(4%)
< 6/60	15	(6%)
Outcome at 3 months		
Healed no scar	34	(12%)
Healed Mild scar	83	(30%)
Healed moderate scar	65	(24%)
Healed dense scar	46	(17%)
Eviscerated	24	(9%)
Not healed	20	(7%)
Staphyloma	4	(1%)

ł There was one missing value (n=259). * Corneal scrapping was performed on 226/260 participants; it was not possible to obtain corneal scrapping samples in 34 participants either due to uncooperative patient, declining consent, deep infiltrates with intact epithelium, such patients were treated based on clinical impression. 52 samples returned negative, no organism detected on microscopy or culture, these were also managed based on clinical impression.

Table 4: Univariable and multivariable linear regression for factors associated with Vision-Related Quality of Life (VRQoL) among cases only (n=260) seen at 3 months.

Variable	Overall Sight		Visual symptom		General functioning		Psychosocial	
	Mean	**(95%CI)**	**Mean**	**(95%CI)**	**Mean**	**(95%CI)**	**Mean**	**(95%CI)**
Visual acuity at 3 months								
> 6/18	89.7	(86.6-92.7)	90.9	(87.6-94.2)	93.3	(90.5-96.1)	93.3	(90.9-96.1)
6/18 - 6/60	78.4	(69.1-87.6)	82.7	(73.5-91.8)	82.2	(73.3-91.1)	83.3	(74.9-91.7)
< 6/60	77.4	(71.6-83.1)	82.9	(78.3-87.6)	81.3	(76-86.6)	83.8	(78.7-88.9)
p-value [a] ǂ	<0.0001		0.006		<0.0001		<0.0001	
p-value [b] ǂ	0.001		0.044		0.006		0.003	
Eye removal ǂǂ								
No	83.6	(80.7-86.6)	86.5	(83.5-89.3)	87.5	(84.6-90.3)	88.6	(86-91.2)
Yes	87.5	(76.3-98.7)	93.1	(86.3-99.8)	90.8	(81.6-100)	90.8	(81.2-100)
p-value [a]	0.406		0.168		0.356		0.619	
p-value [b]	0.030		0.025		0.054		0.111	

[a] P-values from univariable linear regression analysis.
[b] P-values from multivariable linear regression analysis, adjusted for age, education status, wealth category and baseline QoL.
ǂ For visual acuity at 3 months (ordinal exposures with three categories), the p-values were calculated for trend.
ǂǂ Eye removal was a priori

Table 5: Univariable and multivariable linear regression for factors associated with Health-Related Quality of Life (HRQoL) among cases only (n=260) seen at 3 months.

Variable	Overall QoL		Overall health		Physical health		Psychological		Social		Environment	
	Mean	(95%CI)	Mean	(95%CI)	Mean	(95%CI)	Mean	(95%CI)	Mean	(95%CI)	Mean	(95%CI)
Vision outcome at 3 months												
> 6/18	88.7	(85.5-91.9)	88.8	(85.6-91.6)	89.4	(86.2-92.5)	87.7	(85.1-90.3)	90.6	(87.4-93.7)	87.9	(84.9-90.9)
6/18 - 6/60	82.1	(73.7-90.5)	82.0	(74.2-90.0)	81.9	(73.6-90.0)	80.9	(74.3-87.5)	84.8	(76.8-92.8)	78.8	(70.6-87)
< 6/60	78.4	(73.3-83.3)	79.3	(74.0-84.5)	77.7	(72.0-83.5)	77.5	(72.9-82.1)	80.5	(74.6-86.3)	77.1	(71.9-82.3)
p-value [a] ǂ	<0.0001		0.001		<0.0001		<0.0001		0.002		<0.0001	
p-value [b] ǂ	0.008		0.011		0.007		0.003		0.015		0.013	
Eye removal ǂ												
No	84.0	(81.2-86.9)	84.5	(81.8-87.1)	84.0	(81.1-87)	83.0	(80.6-85.4)	86.4	(83.6-89.3)	82.5	(79.4-85.3)
Yes	88.0	(79.2-96.9)	88.1	(79.2-96.9)	89.1	(78.9-99.4)	86.8	(78.9-94.7)	87.8	(77.8-98.1)	88.2	(79.7-96.6)
p-value [a]	0.356		0.402		0.288		0.331		0.633		0.198	
p-value [b]	0.038		0.031		0.028		0.041		0.122		0.009	

[a] P-values from univariable linear regression analysis.
[b] P-values from multivariable linear regression analysis, adjusted for age, sex, education status, wealth category and baseline QoL.
ǂ For visual acuity at 3 months (ordinal exposures with three categories), the p-values were calculated for trend.
ǂ Eye removal was a priori

DISCUSSION

This study investigated the impact of MK on QoL in Uganda. Overall, both the VRQoL and HRQoL among MK patients at baseline was substantially reduced. The lowest scores were in the visual symptom (most had reduced vision) and physical health (most were in pain) categories. The least affected domain at baseline was psychological, which assesses ability to attend functions, feeling ashamed, feeling like a burden to others and fear of losing the other eye. In the only other published QoL study in people with MK, the MUTT1 study from India, QoL at the time of presentation was not reported.[8]

The QoL scores among the MK cases had improved greatly by 3-months. However, compared to the control group, there was strong evidence that MK results in a persistent reduction in QoL, with a mean difference in QoL scores of around 10 points. This effect remained evident even when MK cases with impaired vision were excluded from the analysis.

In looking at factors associated with QoL among MK cases at 3-months, after adjusting for other factors that may affect QoL, such as age, sex, education and economic status, visual acuity was an important determinant for both VRQoL and HRQoL. There was evidence that a history of eye loss was also associated with both VRQoL and HRQoL at 3 months. It was surprising to note that the people who had undergone evisceration had generally better QoL scores compared to those who did not. At the time of eye removal, most of the eyes were too damaged and painful that the people were “demanding” for eye removal. They received socket prostheses (artificial eyes) after their eye removal procedures, this could have led to a marked reduction in pain and other unpleasant symptoms after evisceration, which led to a less impaired QoL compared to others who were not eviscerated where there would have been some with ongoing pain and other symptoms like dense corneal scars.

Validity and reliability of QoL data

These tools have been used in a number of other vision related studies to show a difference in QoL and have been reported to be valid and reliable in studies conducted in similar settings.[5-7] Although the WHO/PBD-VF20 tool was designed to assess binocular vision, it has been demonstrated to be effective in detecting differences in monocular visual impairment in the MUTT1 trial where patients randomised to natamycin had a better 3-month visual related QoL outcome compared to patients randomised to variconazole.[8] In this study, both the VRQoL and HRQoL data measured what they were intended to measure (construct validity) by demonstrating evidence of differences in the scores between groups known to be different; MK cases and

healthy controls had lower and higher scores respectively. The VRQoL data also showed that sub-scales correlate well with measures of impact of MK on QoL such as visual acuity where worsening in these measures is associated with lower VRQoL subscale scores (construct validity). There was evidence of higher homogeneity among the items in each VRQoL and HRQoL subscale (internal consistency) than the generally accepted criteria of >0.70. Cronbach's alpha scores ranged from 0.80-0.99 across all sub scales.

Strengths/Limitations

This was the first study to look at impact of MK on QoL and compare these with unaffected controls. The control selection was good and matched for age, sex and village. We used validated tools which have been previously applied in similar settings and the sample size was adequate to test for differences in QoL. This study did not collect baseline QoL information from the control group because it was not logistically possible. Information on other ocular comorbidities such as presence of a cataract and posterior segment disease at 3 months was not collected as these examination were conducted at patients' homes and it was not practical to perform a full eye examination in such settings.

Conclusion

This study showed that MK severely affects QoL in the acute phase. With treatment, QoL improves, with the highest QoL in cases who had little or no visual impairment at 3-months. Despite this impressive improvement, the QoL at 3-months of someone previously affected by MK (even when they have normal vision) remains lower than controls.

Authors' contribution

AS, EH, AL, VHH and MJB designed the study. All authors contributed to the conducting of the study. AS, DM and MJB analysed the results. All authors contributed to the draft of manuscript and editing of the final paper.

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Chapter 11. The Management of Microbial Keratitis within Uganda's Health system

Buhweju Health Centre IV, one of the primary health facilities in Uganda

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SECTION A – Student Details

Student ID Number	LSH1511754	**Title**	Dr
First Name(s)	Simon		
Surname/Family Name	Arunga		
Thesis Title	Epidemiology of Microbial Keratitis in South Western Uganda		
Primary Supervisor	Prof Matthew Burton		

If the Research Paper has previously been published please complete Section B, if not please move to Section C.

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Where was the work published?			
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Where is the work intended to be published?	Wellcome open research
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Stage of publication	Choose an item. Submitted, awaiting peer review

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I designed the study, collected the data, conducted the analysis with guidance from, David Macleod, Victor Hu, and M J Burton, prepared and submitted the final manuscript to Wellcome Open research in consideration of comments from all co-authors.

SECTION E

Student Signature	AS
Date	19/9/19

Supervisor Signature	MJB
Date	19/9/19

RESEARCH ARTICLE

The management of microbial keratitis within Uganda's primary health system: a situational analysis [version 1; peer review: 1 approved]

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Abstract

Background: Microbial keratitis (MK) frequently leads to sight-loss, especially when the infection is severe and/or appropriate treatment is delayed. The primary health system as an entry point plays a central role in facilitating and directing patient access to appropriate care. The purpose of this study was to describe the capacity of primary health centres in Uganda in managing MK.

Methods: We carried out a rigorous assessment of primary health centres and mid-cadre training schools in South Western Uganda. Through interviews, checklists and a picture quiz, we assessed capacity and knowledge of MK management. In addition, we interviewed the heads of all the mid-cadre training schools to determine the level of eye health training provided in their curricula.

Results: In total, 163 health facilities and 16 training schools were enrolled. Of the health facilities, only 6% had an Ophthalmic Clinical Officer. Only 12% of the health workers could make a diagnosis of MK based on the clinical signs in the picture quiz. Although 35% of the facilities had a microscope, none reported doing corneal scraping. None of the facilities had a stock of the recommended first line treatment options for MK (ciprofloxacin and natamycin eye drops). Among the training schools, 15/16 had an eye health component in the curriculum. However, the majority (56%) of tutors had no formal expertise in eye health. In 14/16 schools, students spent an average of two weeks in an eye unit.

Conclusions: Knowledge among health workers and capacity of health facilities in diagnosis and management of MK was low. Training for eye health within mid-cadre training schools was inadequate. More is needed to close these gaps in training and capacity.

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Any reports and responses or comments on the article can be found at the end of the article.

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Introduction

Microbial keratitis (MK) can be caused by a range of pathogens including, bacteria, viruses, protozoa, and fungi. It is characterized by acute or sub-acute onset of pain, conjunctival hyperaemia and corneal ulceration with a stromal inflammatory cell infiltrate[1]. MK frequently leads to sight-loss from dense corneal scarring, or even loss of the eye, especially when the infection is severe and/or appropriate treatment is delayed.

MK has been described as a 'silent epidemic', which leads to substantial morbidity, related to blindness, pain and stigma[2]. It is the leading cause of unilateral blindness after cataract in tropical regions, estimated at 2 million cases of monocular blindness per year[3]. In 2017, 1.3 million individuals were bilaterally blind from corneal opacity globally (excluding trachoma and vitamin A deficiency), accounting for 3.2% of binocular blindness[4]. In Sub-Saharan Africa (SSA), MK is an important cause of binocular blindness and is responsible for about 15% of monocular blindness[5,6].

A good outcome depends on early appropriate treatment, supported by correct identification of the causative organism, and careful follow-up[7,8]. In low and middle-income countries (LMICs), these resources are not readily available and outcomes tend to be poor[9]. It is important for patients to present early when the infection can be more easily controlled; for instance, studies in Burma and Bhutan showed that if people responded within the first 24–48 hours to a corneal abrasion by applying antibacterial or antifungal medication, there would be 100% recovery without any sequalae[10,11]. Once a corneal ulcer is fully established, there is little that can be done to change its course[12].

The primary health system plays a central role in facilitating and directing patient access to appropriate care. A retrospective study from Tanzania found that having visited a health facility was, paradoxically, a risk factor for severe presentation among patients with MK[9,13]. Our previous work found that although the majority of patients lived within 5km of their nearest primary health centre (PHC) and presented early to those facilities, they ended up presenting very late to eye hospitals and with severe infection when little could be done[14]. There were several missed opportunities at this entry level into the health care system to diagnose, manage and or promptly refer these patients.

The purpose of this study was to conduct a situation analysis of knowledge, practise and capacity of the PHCs in management of MK and to determine the level of training offered on eye health to mid-level cadres in Uganda.

Methods

Ethical statement

This study followed the tenets of the Declaration of Helsinki. It was approved by the London School of Hygiene & Tropical Medicine Ethics Committee (Ref 10647), Mbarara University Research Ethics Committee (Ref 10/04–16) and Uganda National Council for Science and Technology (Ref HS-2303). Permission was sought from the District Health Offices to approach the facilities. Then, written informed consent was obtained from the facility and school heads before enrolment to participate in the study and to allow the research assistants collect information about their facilities. Written informed consent was also obtained from each facility head, school head and health worker for their personal participation in the research.

Study design and setting

The sampling frame for this study was defined by the catchment area for a related cohort study that prospectively enrolled all patients presenting with MK to the main tertiary referral centres for South Western Uganda (Ruharo Eye Centre [REC] and Mbarara University and Referral Hospital Eye Centre [MURHEC]) from December 2016 to March 2018. The districts from which these patients came from were identified by tallying the district data of MK patients and seeing which districts had large numbers of MK patients. These were then pooled into regions depending on their geographical distribution and distance from the eye hospital. One district was then randomly selected from each pool.

Uganda's Health System is a tier-based system divided into seven levels with the lowest point of care being at the village level. However, physically, a Health Centre II (HC II) is the lowest unit and is located at a parish level. These units have different staffing and capacity in terms of service provision. Patients are referred along the tier system depending on the complexity of their condition. Special Clinics are facilities that provide specialised services only, such as HIV treatment services. There is a total of 408 HC IIs, 152 HC IIIs, 43 HC IVs and 12 hospitals within the 20 districts in South Western Uganda. We randomly selected six districts, stratified by geographical distribution and accessibility from the eye hospital in Mbarara. All the health facilities within the sampled districts were enrolled. In addition, all the mid-cadre training schools (nursing and clinical officers) within South Western Uganda were enrolled.

Data collection

Quantitative interviews. Research assistants administered questionnaires (File 1, *Extended data*)[15] to facility heads and/or health workers who treat eye patients to ascertain the level of knowledge, routine practices and capacity. One health worker was interviewed per facility. Heads of mid-level cadre training schools were interviewed using questionnaires to ascertain the amount of eye health training provided (File 2, *Extended data*)[15].

Checklists. Data were collected from the health centres we visited about infrastructure, equipment and supplies relevant to managing MK. The research assistants collected this information from the facility health workers and directly tallied patient register entries to count the number of eye patients in general and MK cases that visited that unit, as well as diagnosis and treatment. A copy of the data collection tool has been provided as *Extended data* (File 1).

Picture quiz. A photograph of an eye with classic signs of MK was given to the health workers (one health worker per facility) to test their knowledge of clinical signs and ability to diagnose MK (Figure 1, File 3, *Extended data*)[15]. The quiz was a section of the general questionnaire (File 1, *Extended data*)[15] that was administered. The health workers were shown a coloured picture of an infected eye that had clinical signs of MK. They were then asked to identify the clinical signs and suggest a diagnosis and management plan.

Analysis

Data were analysed in STATA 14 (StataCorp). Summary tables were used to describe knowledge, inventory, proportion of eye patients seen. This was stratified by level of the health centres. The same analysis was used for training schools to describe training key training areas in the identification and management of MK.

Results

A total of 163 health facilities were enrolled from six districts in South Western Uganda (101 HC IIs, 45 HC IIIs, 13 HC IVs and four district hospitals). These were from Kamwengye district (27), Kisoro district (31), Ntungamo district (40), Sheema district (26), Lyantonde district (16) and Ssembabule district (23). Table 1 shows the baseline characteristics of the enrolled health facilities. Only five facilities had an Ophthalmic Clinical Officer (OCO): these included four hospitals and one HC IV[15]. Most of the facilities (59%) were headed by an enrolled nurse cadre. Most (74%) facilities had less than 50% of the expected staffing levels. The proportion of patients seen at these facilities who presented with eye problems was 2–8%.

Figure 1 illustrates, by level of the health facility, the availability of basic diagnostic tools and treatment for MK (Supplementary Table 2, File 3, *Extended data*)[15]. Overall, 14% of the facilities had an examination torch, 4% had fluorescein strips for corneal staining, 35% had laboratory microscopes and Gram staining facilities. When we looked at eyedrops relevant to MK, 29% had gentamycin eye drops available. However, none of the facilities had ciprofloxacin or natamycin eye drops. There was a systematic difference in this capacity across the level of the health centres, with higher facilities being better equipped.

Knowledge of clinical signs of MK was tested using a picture quiz of a patient's eye with MK (Figure 1, File 3, *Extended data*)[15]. The results are presented in Supplementary Table 3 (File 3, *Extended data*)[15]. Overall, 60% of the health workers identified a red eye. However, only 4% identified an epithelial defect, 23% a corneal infiltrate and 4% recognised a hypopyon (Figure 2). On being asked what the most likely diagnosis was, only 12% of the health workers could correctly identify it as MK or eye infection. There was a systematic difference of the knowledge by level of the facility.

Figure 3 shows knowledge of risk factors and complications of MK among the primary health workers (Supplementary Table 3, File 3, *Extended data*)[15]. Overall, 22% identified immune suppression, 5% identified traditional eye medicine (TEM), 5% identified steroid eye drops, 50% identified trauma. When asked to name complications of MK, 95% of the health workers reported blindness. However, only 33% mentioned eye loss. On stratifying by level, there was not much difference in this knowledge across the different level facilities.

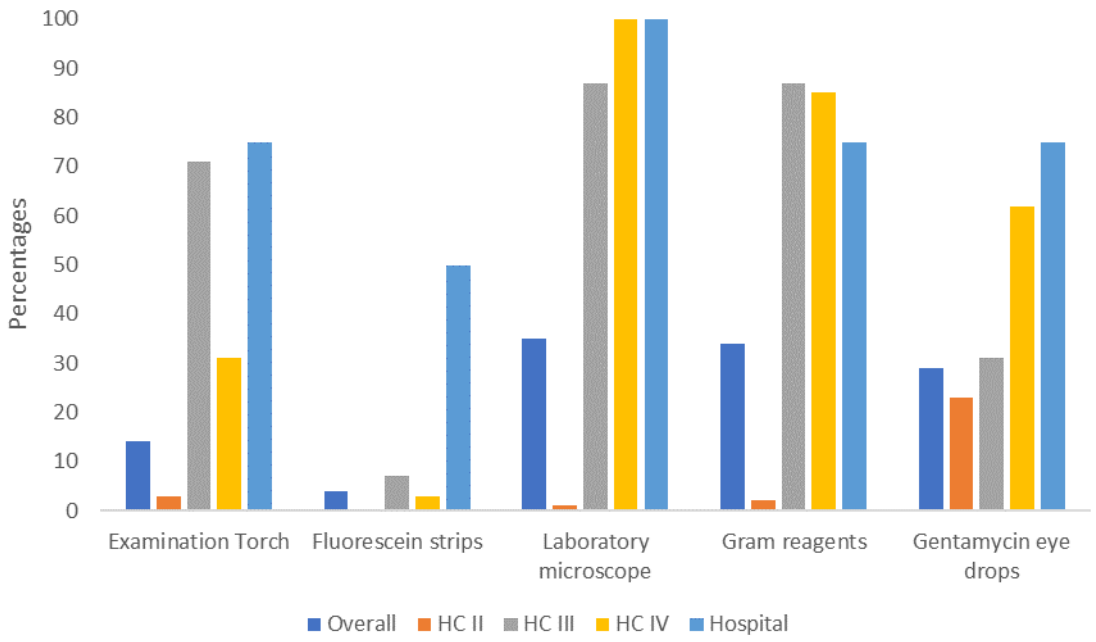

Figure 1. Basic inventory of health facilities for detecting and managing microbial keratitis (n=163).

Table 1. Baseline characteristics of the enrolled health facilities (n=163).

Variable	Count	(%)
Level of the health centres (HCs)		
HC II	101	(62%)
HC III	45	(28%)
HC IV	13	(8%)
Hospital	4	(2%)
Distance to nearest referral centre		
0–5km	27	(17%)
6–10km	72	(44%)
11–20km	43	(26%)
>20km	21	(13%)
In charge cadre type		
Enrolled nurse	96	(59%)
Clinical officer	39	(24%)
Medical officer	18	(11%)
Other	10	(6%)
Staffing levels		
0–25%	42	(26%)
26–50%	79	(48%)
51–75%	32	(20%)
>75%	10	(6%)
Patient registry data		
Variable	**Median**	**(IQR)**
Number of patients seen in last three months		
HC II	3421	(1350-4782)
HC III	3187	(2160-4213)
HC IV	4625	(2296-6251)
Hospital	8301	(6181-14146)
Proportion of eye patients in last three months in percentage (range)		
HC II	1.0%	(0.5-2)
HC III	2.5%	(1-5)
HC IV	2.0%	(1-6)
Hospital	8.0%	(3-14)

161/163 had an all-weather road access and 158/163 had a phone communication at the facility. 136/163 had a power supply.

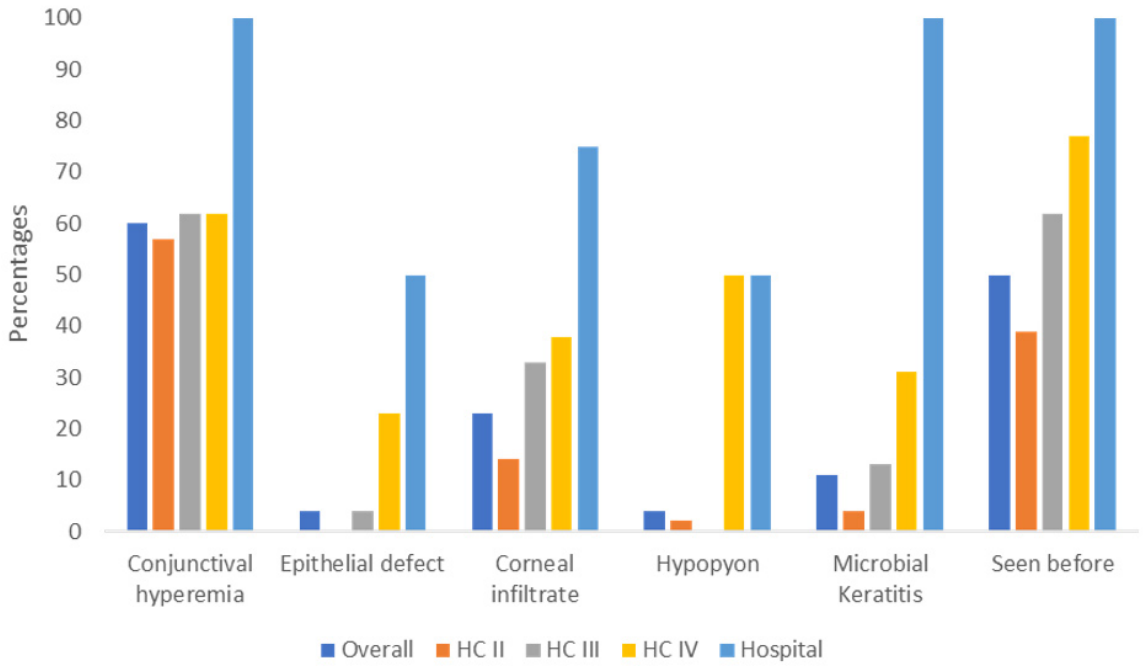

Figure 2. Knowledge of clinical signs of microbial keratitis among primary health workers (n=163).

Figure 4 shows the knowledge of management of MK among the primary health workers (Supplementary Table 3, File 3, *Extended data*)[15]. Overall, only 3% knew about staining of the cornea with fluorescein to examine for epithelial defects in the diagnosis of MK. None of the cadres, including the OCOs, showed knowledge of the role of microscopy/culture in management of MK. Antibiotic as a choice of treatment was reported in majority of the cadres across all levels. However, antifungal was reported by only the OCOs. This is not surprising as antifungal eye drops are not commonly available.

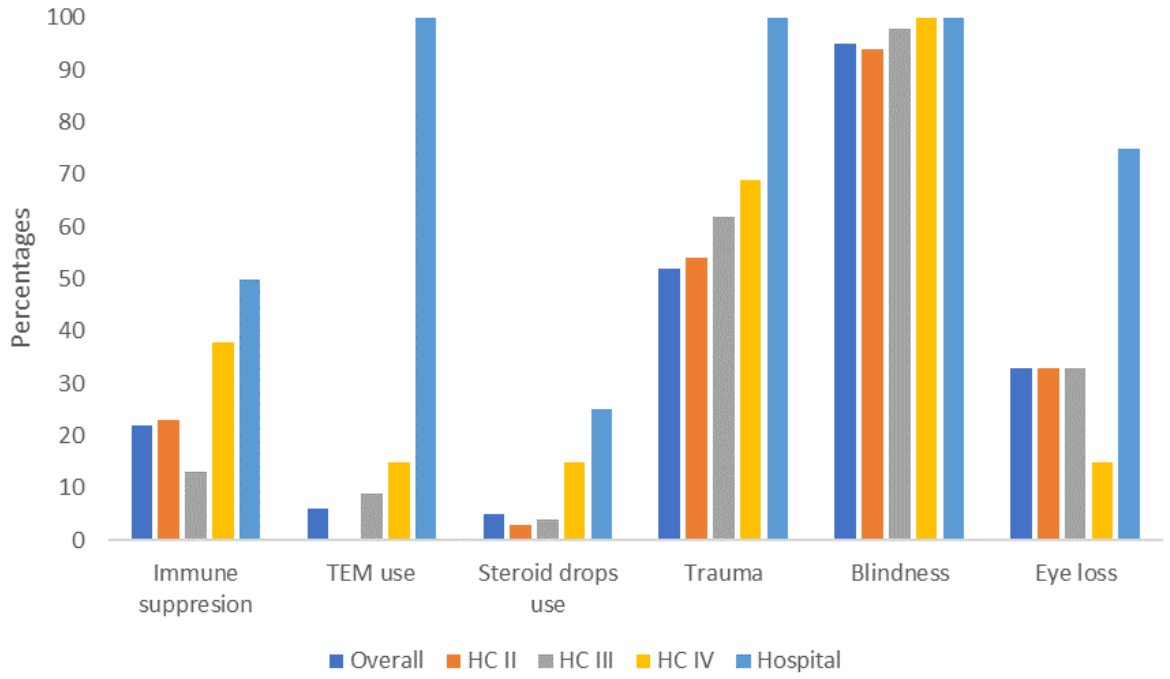

Figure 3. **Knowledge of risk factors and complications of microbial keratitis among primary health workers (n=163).**

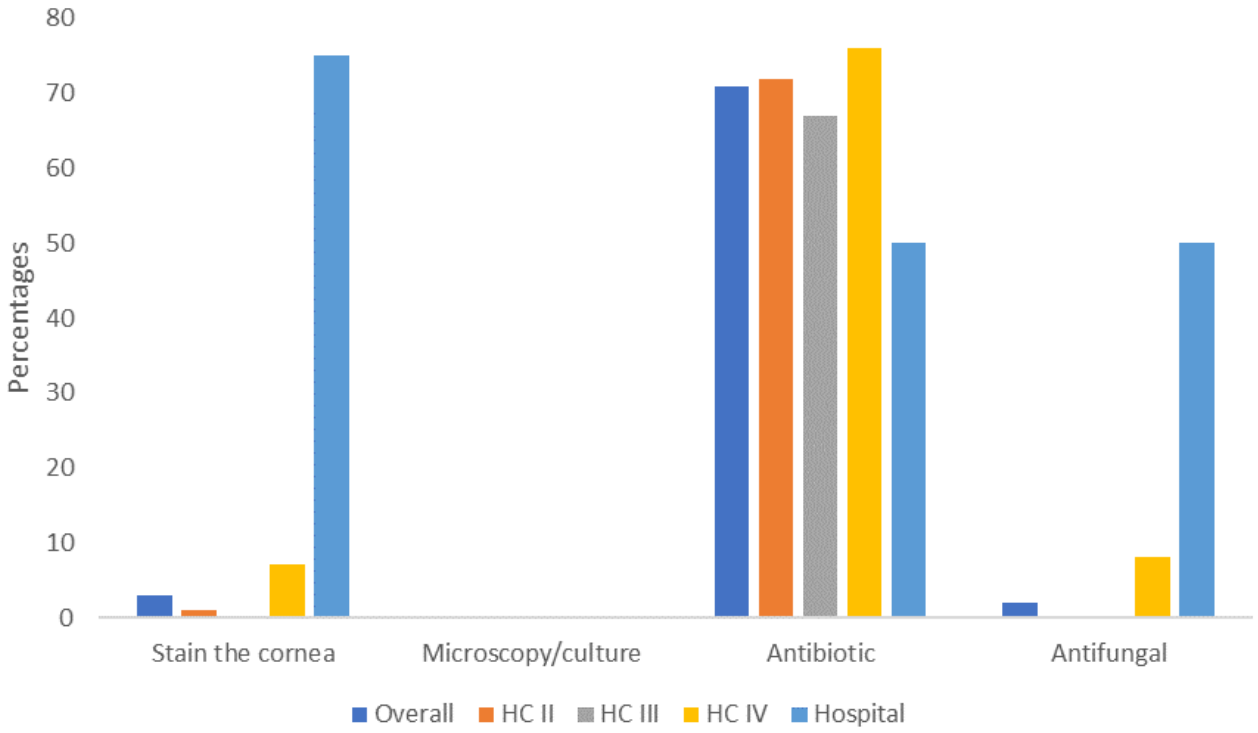

Figure 4. **Knowledge on management of microbial keratitis among primary health workers (n=163).**

There are 22 mid-cadre training schools in South Western Uganda. We included 16 in this study. The rest declined to participate. The findings are summarised in Table 2. Overall, 15/16 schools had an eye health component in their curriculum, 14/16 schools provided eye clinic rotations for their students. However, the majority (56%) of the trainers/tutors had never had any formal training in ophthalmology. Although training of practical skills during eye ward attachments was excellent for basic areas, critical skills for management of MK and other emergency conditions such as staining, removal of a foreign body, using loupes to examine eyes were not being taught.

Discussion

This study aimed to assess the capacity of the PHCs in managing MK. We found that there was a lack of essential personnel for eyecare at the PHCs. Human Resources for Health (HRH) are a huge problem for many places in SSA. This is particularly an issue for Human Recourses for Eye Health (HREH), with many countries in SSA failing to achieve the Vision 2020 staffing targets[16,17]. In our study, the majority of the facilities were substantially understaffed, with more than half having severe staffing shortages. According to the Uganda Ministry of Health staffing norms, each HC IV and above are supposed to have an Ophthalmic Clinical Officer (OCO). However, in our study, only 1/13 HC IVs had an OCO. This means that majority of the eye patients are seen by general health workers who have poor knowledge on eye care[18,19].

One study from Tanzania reported limited knowledge and productivity for eye care among primary health workers. In that study, the primary workers were found wanting in basic skills including measuring of visual acuity[19]. Another study that looked at knowledge of primary eye care among 343 general

Table 2. Assessment of capacity of eye health training in mid-cadre schools (n=16).

	Overall n=16		Certificate level, n=6		Diploma level, n=10	
Variable	**Count**	**(%)**	**Count**	**(%)**	**Count**	**(%)**
Presence of an eye health curriculum						
Yes	15	(94%)	6	(100%)	9	(90%)
Major topics covered in the curriculum (if Yes)*						
Anatomy	14	(94%)	6	(100%)	8	(89%)
Blinding conditions	12	(80%)	5	(83%)	7	(78%)
Eye infections	15	(100%)	6	(100%)	9	(100%)
Pharmacology	13	(87%)	4	(67%)	9	(100%)
Ophthalmology training level of the eye health tutor						
None	9	(56%)	5	(83%)	4	(40%)
Diploma	7	(44%)	1	(17%)	6	(60%)
Does hospital attachment include eye ward? (if Yes; median 2 weeks IQR 1-2, total range 1–6 weeks)						
Yes	14	(88%)	5	(83%)	9	(90%)
Practical skills learnt in the eye ward ǂ						
Taking ocular history	14	(100%)	5	(100%)	9	(100%)
Measuring visual acuity	14	(100%)	5	(100%)	9	(100%)
Instilling eye drops	14	(100%)	5	(100%)	9	(100%)
Eye exam with a torch	14	(100%)	5	(100%)	9	(100%)
Corneal staining	5	(36%)	2	(40%)	3	(33%)
Eye exam with loupes	1	(7%)	1	(20%)	0	(0%)
Ophthalmoscopy	4	(29%)	1	(20%)	3	(33%)
Foreign body removal	1	(7%)	1	(20%)	0	(0%)

*Only schools which had an eye health curriculum were analysed (n=15). ǂ n=14, two schools did not have students rotate in an eye ward. Certificate level means certificate in nursing, diploma level means diploma in nursing or clinical medicine.

health workers from Kenya, Tanzania and Malawi also found low knowledge levels for common conditions; only 8.2% of the workers could correctly measure visual acuity[18].

Therefore, it is not unexpected that we found that the majority of health workers could not correctly identify the signs of microbial keratitis, one of the more common ophthalmic emergencies, and provide a satisfactory management plan. This limited knowledge will most likely compromise patient care and lead to poor outcomes. For example, a very small proportion of the health workers identified use of steroids as a risk factor for MK, which might imply that they would consider treating potential MK patients with steroids, possibly making it worse. Our experience in treating MK patients presenting to us is that the majority have used a combination of eye drops, most containing a steroid.

The health facilities were poorly resourced in basic items for eye care such as examination torches, fluorescein for corneal staining and drugs for treatment. We did not find a single health facility that had a stock of ciprofloxacin and/or natamycin eyedrops, which are the recommended first line agents for bacterial and fungal keratitis, respectively[20–22].

Despite these discouraging findings, there were some positive findings that could be utilised to improve care. Most health workers were able to identify conjunctival hyperaemia (red eye) on the picture quiz. This would be a good starting point for a training pack on managing emergency eye conditions. We recently published two articles targeting primary health workers on how to identify MK and how to locally make fluorescein for corneal staining, and a more recent version of a WHO primary eye care training manual for Africa has been made available[23–25]. We hope to use these resources for training general health workers on triage and emergency management of acute ocular conditions.

In addition, we found that a modest number of facilities had microscopes and laboratory personnel. With simple training and simple tools such as magnifying spectacles/loupes, the health workers could be in a good position to do corneal scraping and microscopy to differentiate between bacterial and fungal keratitis. Laboratory diagnosis for MK is still the most reliable method since some clinical presentations are equivocal and even corneal specialists may make the wrong clinical diagnosis[26]. However, corneal scraping requires a certain level of competence and would be feasible only at a limited number of centres which have OCOs. The other health cadres not suitable to do corneal scraping can be equipped to identify MK and provide early referral. Simple tools such as corneal staining with fluorescein have been used in large studies in Bhutan and Burma among minimally trained health workers to facilitate early detection, treatment and referral of MK[10,11]. In these trials, village health workers were able to stain the cornea with fluorescein and examine for corneal abrasions using a blue light torch.

Some facilities also had a stock of gentamycin 0.3% eye drops available. This can be locally fortified to make a more potent alternative of 1.5% gentamycin, which is quite effective for managing some forms of bacterial keratitis[27,28]. In Uganda, both gentamycin eyedrops and parenteral gentamycin vails (that can be used to fortify the eye drops) are available and supplied to the facilities through the free National Medical Stores. However, gentamycin is limited to Gram negative bacteria and does not cover Gram positive bacteria and fungi. Quinolones such as ciprofloxacin have a broader coverage. These, together with natamycin for fungal keratitis, are on the WHO essential medicines list and should be availed to lower facilties[20–22].

In this study, we also assessed mid-cadre training schools to assess the scope of training on eye care. Overall, we found that most schools had an eye health component in their curriculum and students were getting some time (although little) to have hands on training rotations in an eye unit.

However, the levels of ophthalmology training among the trainers were low. The majority of the trainers, especially in certificate-level training schools, had never received any structured courses in ophthalmology except as part of the short rotations during their medical training. Therefore, this casts doubt on the quality of training they can offer on eye health.

The training of practical skills during eye ward attachments was reported to be excellent for basic areas such as taking ocular history, measuring visual acuity, eye examination with a torch and instilling eye drops. However, critical skills for management of MK and other emergency conditions such as staining, removal of a foreign body and using loupes to examine eyes were not being taught.

Limitations and strengths

This study enrolled a large sample of health facilities and used multiple sources of data collection. The findings from the training schools were reported by the school heads and therefore might have a degree of bias. We were not able to conduct an exit assessment among the students to verify if these learnings reported by the school heads were accurate.

Conclusion

The findings from this study draw attention to the very limited quality of eye care at the PHC, but it also points to several opportunities that could be utilised to improve this. The knowledge among health workers and capacity of health facilities in diagnosis and management of MK was low. Training for eye health among mid-cadre training schools was inadequate. More is needed to strengthen these gaps in training and capacity.

Data availability

Underlying data

Harvard Dataverse: The management of microbial keratitis within Uganda's primary health system: a situational analysis. https://doi.org/10.7910/DVN/MSLAOS[15].

This project contains the following underlying data

- Health Facilities data.xlsx (quantitative underlying data)
- Training schools dataset.xlsx (quantitative underlying data)

Extended data

Harvard Dataverse: The management of microbial keratitis within Uganda's primary health system: a situational analysis. https://doi.org/10.7910/DVN/MSLAOS[15].

- 7.MAES HEALTH CENTRE data collection forms-v3-31oct2018.docx (File 1: a copy of the data collection forms used on the health facilities which include sections on the checklist and picture quiz)
- 7.MAES SCHOOLS data collection forms-v1-25FEB2016.docx (File 2: a copy of the data collection forms used on the training schools)
- Capacity of the Health system in managment of MK supplementary files.docx (File 3: a copy of supplementary file data showing the picture that was used for the quiz as well as additional tables showing more detail of the results)

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The paper states microbial keratitis is still a blinding disease and efforts are needed to address this. In the developing world, models of care affect outcomes for this condition. This paper is of interest as it examines the role of appropriate care in the management of microbial keratitis. Specific comments are:

Abstract:

- "Mid-cadre" is not a term that the readership would necessarily understand, is there another term that could be used to describe this here?
- What type of microscope?

Introduction:

- Sight loss from keratitis can even result when scarring is not severe but rather when it causes irregular astigmatism.
- Clarify the link between microbial keratitis, corneal abrasion and corneal ulcer.

Methods:

- Please clarify if the tertiary referral centres, are tertiary referrals for ophthalmology or for general care.
- How representative is South Western Uganda of Uganda as a whole? Can the results be generalised to Uganda?
- Is the village level a HC I and therefore is HC II the second level?
- Are the different HC levels in order of increasing complexity?

- When it is stated that “all the health facilities within the sampled districts were enrolled” does this mean all HC levels I to VII?

- Please define what is meant by mid-cadre.

Data collection:
- Were the questionnaires specifically designed for the study?

Results:
- Are district hospitals HCVII?

- Did any of the facilities have slit lamps? Cobalt blue light?

- Table 1: please describe what “staffing levels” means.

Discussion:
- Use either microbial keratitis or the abbreviation MK consistently.

- Please elaborate further on why steroids should not be given, this will be a good educational point for the article.

- Spelling “vails”, please correct to “vials”.

- Please comment that gram positives are typically more common causes of keratitis compared to gram negative bacteria particularly in non-contact lens wearers.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: cornea, microbial keratitis, stem cells, dry eye, herpes simplex, wound healing, keratoconus, registries

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Chapter 12. Further Discussion

Beautiful mountain ranges of one of the villages in Uganda where one of the study participants came from. The photo was taken during a home visit.

This project was important in exploring foundational issues around Microbial Keratitis in Uganda. There was a significant amount of learning from this work which will shape the next steps in research to improve care of this condition and prevention of sight loss from MK. This chapter focuses on reiterating the key findings from each of the different studies and their implications to future work/practise in management of MK in Uganda and in Sub-Saharan Africa (SSA).

15.1 Epidemiology of Microbial Keratitis in Uganda

In this study, we enrolled all patients presenting with MK to the two main referral eye hospitals in South Western Uganda and followed them up for three months after the start of their treatment to determine their outcomes. To the best of our knowledge, this was the first large study to prospectively report on patient outcomes in this region. Despite the challenges of a cohort study in a resource limited setting and far distances of participants, we successfully followed up 83% of these in their homes at three months.

In our setting, the most reliable tracking method in our experience was asking participants to detail the landmark features to their homes. Instead of just asking about the villages where they came from, we asked them to state in a detailed way of how to get from the eye hospital to their house. The assistants used this method to tack them to their homes. Although there was a good coverage of telephones, these quickly became unreliable due to change in government policy that required people to register their phones, those which were not registered were disconnected. Overnight, about half of our participants were unreachable by phone. This method had been used previously by Waddell et al to track children with retinoblastoma in Uganda with 94% follow-up.[1] In the absence of proper street names and house numbers, this is a method that might be helpful in tracking people for follow up.

We found that Fungal Keratitis was the most common agent causing MK in our setting. Of all our 270 patients with microbiology data, 54% were reported as fungal cases. This was similar to what had been reported from other studies in Africa which averaged around 50%. [2-5] However, what was interesting was that the proportion of cases with microbiologically confirmed bacterial keratitis was 6%, which was low. One possible explanation for this is that bacterial infection cases less frequently “reach” the eye hospital because they are managed in the lower health centres. When we evaluated the primary health facilities, we found a modest stock level of antibiotic eye drops like gentamycin which may be an effective antimicrobial agent in some types of infection.[6,7] None of the health centres had any antifungal medicine. It is likely that the people with fungal Keratitis did not get better in the health centres

and ultimately ended up at the eye hospitals. However, there is a need to study the microbiology profile of the cases that present to the primary health facilities.

Lack of microbiology support is a serious challenge in many places in SSA. A logical treatment plan depends on identifying the causative agent. At the onset of our project, an ocular microbiology service was non-existent in our setting. In trying to set up our microbiology service, one of the things we found useful was sending a laboratory technician for an ocular microbiology course at Aravind Eye Hospitals, India. On his return, he helped to set up this service which improved our culture yield to 55% and overall composite yield to 80%.This is a relatively comparable to published microbiology yield rates.[4,8] In addition, we deployed a side lab at the eye hospital and equipped it with a fluorescence microscope for immediate microscopy to detect fungi using Calcofluor White staining. This method has been described previously and fungal hyphae can be readily detected even by non-experienced personnel.[9,10] When starting treatment, in this context the critical question is whether or not there is evidence of fungal infection.

The diagnosis was challenging for those who did not have any microbiology results. Out of the 313 MK patients, we managed to collect samples for 270 of which a further 65 were negative on both microscopy and culture. In this situation, diagnosis based on phenotypic presentation is still useful. There has been only one large multicentre prospective study that has devised a clinical scoring system to help guide the clinician as to whether or not fungal infection is present.[11] The clinical signs that were significantly indicative of fungal keratitis, as opposed to bacterial, are serrated margins, raised slough, dry texture, satellite lesions and colouration other than yellow ($p<0.05$).[11] Conversely, bacterial infections are more frequently associated with a hypopyon and fibrinous exudate ($p<0.05$).[11] A relatively recent photographic survey asking corneal specialists to diagnose bacterial versus fungal infection further supports the presence of feathery, irregular margins as indicative of fungal infection. However, the correct diagnosis was only arrived at in 66% of cases.[12] Using these, a probability of the causative infection algorithm (in a tropical environment) was proposed by some of our group in aiding clinical diagnosis.[13]

A potential solution to help improve diagnostic accuracy, especially where the microbiology is negative or not available, is to develop automated, computer-assisted image analysis of good quality digital photographs of infected corneas. Ideally this algorithm would be developed to be capable of running as an application on a smartphone, utilising the built-in digital camera with or without hardware modification. However, there would remain the need for microbiology support to help the clinicians investigate the cause particularly in patients who do not respond to treatment. Currently it is clear that no single diagnostic approach is sufficient by itself. A

study from Moorfields Eye Hospital found that the diagnosis of fungal keratitis required a number of different modalities and that it varied quite a lot as to those that were positive.[14]

Another possible solution would be molecular diagnosis especially where microscopy and culture turn out negative. Polymerase chain reaction (PCR) is useful in such settings as it can detect the presence of nucleic acid from small samples and even non-viable organisms. Previous studies employing this technique for microbial keratitis have found PCR to be effective in detecting organisms and often display higher overall pick up rates than culture or microscopy.[15]

A newer technology of *In vivo* confocal microscopy (IVCM) examines the ocular surface at the cellular level. Some of the colleagues from our group recently reported a large evaluation of IVCM for MK in India. IVCM was found to be highly sensitive and specific for fungal infection.[16,17] Being able to see the fungal hyphae in vivo would help the ophthalmologist to make an immediate diagnosis and reduce the need for corneal scrapping with its challenges. Our hospital recently acquired one IVCM machine to use in ongoing corneal infections work, however, In SSA, these options may not be a realistic option yet due to the costs involved.

15.2 Risk factors of Microbial Keratitis in Uganda

We nested this case-control study in the main cohort described above. Briefly, at 3 months of follow-up, healthy community controls were enrolled. They were 1:1 matched to the cases to test for risk factors. These included HIV, Diabetes Mellitus (DM) and other occupational factors like farming, circumstantial like poverty. To the best of our knowledge, this was the first case-control study in SSA to directly test risk factors of MK.

There was strong evidence that indeed, HIV and DM are risk factors to MK. What this means for practise is that patients presenting with MK should be offered tests for HIV and DM as this may be the entry point into care. As a matter of fact, we found close to 40% of the people who tested positive were unaware of their HIV status prior to coming to the eye hospital. In Uganda and many parts of SSA, the HIV treatment policy was recently updated to “test and treat” regardless of the person’s CD4 count.[18] This helps to mitigate the immune suppressive effects of the virus at an early stage. Therefore, people with undiagnosed HIV are at an increased risk of mortality stemming from many opportunistic infections associated with immune deficiency (AIDS). During our study, we reported a young man with undiagnosed HIV who developed a rapidly progressive form of Candida Keratitis that resulted in evisceration. He was started on Anti Retro Viral therapy (ART). A few months later, he developed Candida Keratitis in his remaining eye, he was promptly treated and resolved with good vision at 3 months.[19]

One key thing we did not explore is how HIV and DM might influence the relative susceptibility to different types of infection due to the immune suppressive nature. An older study from Tanzania (1999) had reported a possible predisposition to fungal keratitis among HIV positive patients.[20] In this study, 214 Corneal Ulcer patients presenting to Muhimbili Medical Centre, Tanzania were enrolled and corneal scrapping specimens obtained for microscopy and culture. Out of the 32 patients with confirmed fungal keratitis, 26 (80%) were HIV positive while only 33% patients with non-fungal keratitis were HIV positive. The prevalence of HIV in this study was 40%. In our study, we did not find strong evidence for such an association. The prevalence of HIV was 13% and 23/37 (62%) of the HIV positive patients had fungal Keratitis compared to 132/247 (53%) of the HIV negative patients (p=0.32). The microbiological profile of MK in HIV and DM needs to be explored in subsequent studies when sufficient patient numbers are enrolled.

Farming was implicated as an occupational risk factor for MK. Our theory is that this is mediated through trauma. However, only relatively few patients in our study reported trauma (29%). This is consistent with other studies from sub-Saharan Africa (SSA) of 20-40%.[3-5] However, these levels are lower than those from South Asia, where around 75% are associated with an injury.[4,21-24] Targeting farmers and other people predisposed to eye trauma such as bike taxi riders is a valid intervention to prevent new cases of MK.

In this case-control study, it was hard to elicit history of TEM use and trauma among the controls, perhaps due to recall bias. Because of this, it was not possible to test these factors in a statistical model.

15.3 Delayed presentation/access to care

As part of the study, we asked patients to map out the process of their journeys from the onset of symptoms to the point of coming to the hospital. This included, order of facilities where patients went to seek care, treatment advice, cost of care and use of Traditional Eye Medicine. Presentation time was noted, and predictors of delay were analysed.

We found that access to eye care is a big problem. According to the model by Levesque et al, access to health care is defined as an opportunity to have health care needs fulfilled.[25] It is an interaction between the supply side (health system) and demand side (patients).

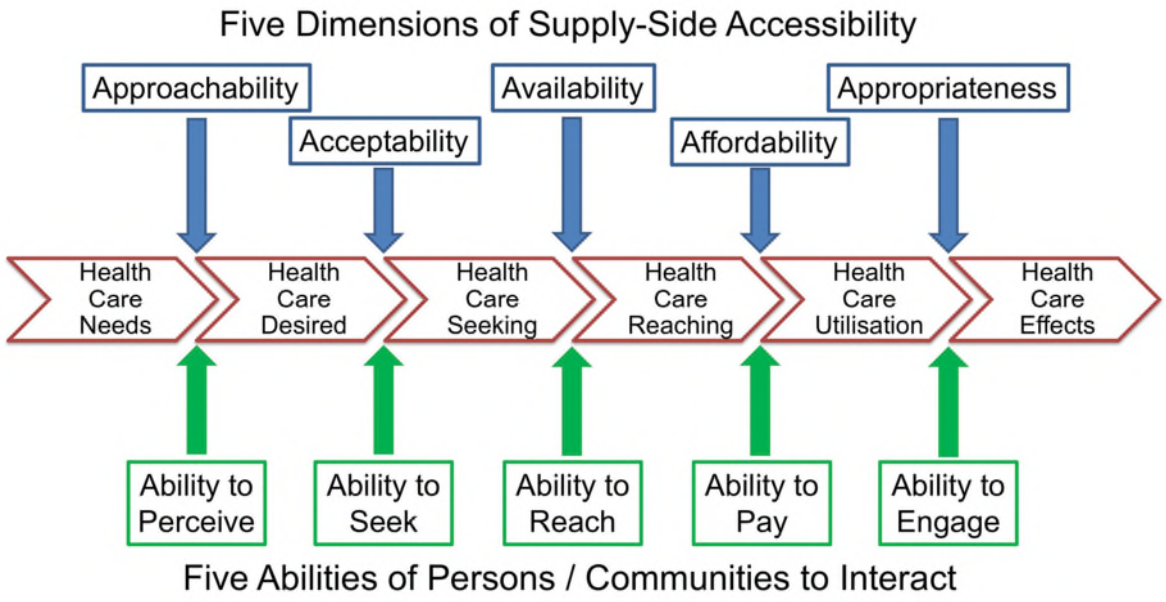

Figure 1 Levesque's model on dimensions which influence access

As shown figure 2, this model has five dimensions of interaction between the demand side and supply side which influence access. The supply side dimensions include 1) Approachability; 2) Acceptability; 3) Availability and accommodation; 4) Affordability; 5) Appropriateness. The demand side are the corresponding abilities of persons interact with the dimensions of accessibility to generate access. These include: 1) Ability to perceive; 2) Ability to seek; 3) Ability to reach; 4) Ability to pay; 5) and Ability to engage.[25]

Approachability means that people with a health condition (in this case MK) can identify a service and that this service can be reached, and it will have an impact on the health of the individual. This is complemented by ability of the patient to perceive the need for treatment. Contrary to our initial expectation that MK patients have poor health seeking behaviour (went to hospitals late), we were surprised to find that all patients who did not present directly to the eye hospital attended their nearest primary health care facility in good time (median presenting time 2 days).[26] If they had received appropriate care at this point, the infection would have been controlled quickly. There is still much to learn about the treatment received, the microbiological profile at this stage, and if the “treatment” made the infection worse. However, what was clear from this study is that although the patients perceived the need for care and approached the health system early, there was little prompt recognition and appropriate referral of MK patients to start effective treatment quickly.

Acceptability is factors which determine the possibility of people to accept the aspects of the service (social and cultural).[25] Acceptability of the health system is generally not a big problem

in Uganda, however, the bigger problem is acceptability of use of Traditional Eye Medicine (TEM). Majority of the patients reported to have used TEM as one of the "services" to treat MK and this practise was reported as a generally acceptable one. Acceptability is also influenced by the ability of the patient to seek the health services due to autonomy, gender inequality and economic empowerment.[25] Gender inequalities have been documented in other conditions like Trachoma, Cataract and glaucoma where females have poorer access than males.[27,28] In our study, although majority of the patients presenting to the eye hospital were male, we could not conclude on this partly because we did not have gender data of patients presenting at the primary health facility level.

Availability means that the health services can be reached and in a timely manner. This is restricted if the resources are unevenly distributed around a country such as distance to the facility, cost of transport, occupational flexibility.[25] Uganda faces a severe shortage of human resource for eye health just like most countries in SSA.[29,30] According to the Uganda Medical and Dental Practitioners' Council (UMDPC), there are 51 Ophthalmologists in Uganda for a population of over 40 million people.[31] Out of these, 33 (63%) practice in the capital city Kampala, 7 (14%) in the second city Mbarara where referral eye hospitals for South western Uganda are located. Patients travelled long distances to the eye hospital with a median distance of 79km (some up to almost 400km).

Affordability reflects the economic capacity for people to spend resources and time to use appropriate services. It results from direct prices of services and related expenses in addition to opportunity costs related to loss of income.[25] Although the public health system is free in Uganda, patients incurred costs on transport and cost of medicines. This was linked to several other factors such as delayed presentation, use of traditional eye medicine which cascaded into patients presenting with severe, advanced ulcers and ultimately with poor outcomes. These are discussed in subsequent sections of this chapter). In our observation, when patients presented early, response to treatment was good with total resolution of the ulcer with no or minimal scarring. In our future work, we plan to shift our efforts from downstream to upstream where we can close the gaps in access and have early interventions.

Appropriateness denotes the fit between services and clients need, its timeliness, the amount of care spent in assessing health problems and determining the correct treatment and the technical and interpersonal quality of, ability to engage.[25] Appropriate care was perceived as lacking at the primary health facilities. For example, out of all the patients that used Traditional Eye Medicine (TEM), 73% used it after they had visited their primary health facility. They did not feel that the care they had received would make them better. There was a missed opportunity for health education and counselling against TEM use. These findings were further

confirmed by a situational analysis of the health system where we found severe limitations in capacity to manage MK. This is discussed in the next section of this chapter.

As part of the next steps, we plan a series of interventions to strengthen the health system in diagnosis, triage, treatment and referral.

15.4 Primary health system

In this study, we carried out a rigorous assessment of 163 primary health centres and 16 mid-cadre training schools in South Western Uganda. Through interviews, checklist and picture quiz, we assessed capacity and knowledge of MK management. In addition, we interviewed the heads of all the mid-cadre training schools to determine the level of eye health training provided in their curricula.

What we found was a grim picture. The health facilities did not have vital equipment and consumables and the health workers lacked basic knowledge in diagnosis and management of Microbial Keratitis. This points to an overall poor care service delivery of eye health in the health system. Deliberate efforts are needed to strengthen this. We recently published two articles targeting primary health workers on how to identify MK and how to locally make fluorescein for corneal staining and a more recent version of a WHO primary eye care training manual for Africa has been made available. [32-34] We hope to use these resources for training general health workers on triage and emergency management of acute ocular conditions. This will include development of a smart phone based clinical management algorithm using the WHO primary eye care guidelines. This will be hosted on the peek vision foundation platform (https://www.peekvision.org/). Such a resource will empower the primary health workers to make logical management decisions for the patients. In parallel, more efforts are needed to improve the overall training of health workers in eye health and to deploy designated eye health workers at primary health facilities.

15.5 Role of Traditional Eye Medicine

This study was done as a mixed methods study. In addition to prospectively collecting information on history, TEM use, microbiology and 3-month outcomes on all the study participants, we conducted qualitative interviews with patients, carers and traditional healers on reasons why people use TEM. Outcome measures included presenting vision and at 3-months, comparing TEM Users versus Non-Users. A thematic coding framework was deployed to explore reasons for use of TEM.

The problem of TEM had several facets. First, TEM use was more commonly reported in our study (60%) than reported from Malawi (34%) and Tanzania (25%).[35,36] Secondly, a smaller proportion of patients used it as a primary treatment for MK (30%) while more others (70%) used it after not observing a favourable improvement to treatment obtained from the primary health facilities. This was one of the findings from the qualitative survey, where lack of confidence in the health system was one of the main drivers of TEM use. The opportunity here is that once effective care is available in the primary health facilities, it can be leveraged as one way to influence behaviour change away from TEM use.

Thirdly, TEM use was associated with delayed presentation to the hospital and independently associated with worse condition at presentation. People who used TEM had worse ulcers at presentation and subsequently worse vision outcomes compared to those who had not used TEM. Using some of these patient stories of people who used TEM and developed poor outcomes is one of the strategies of discourage this practise.

In this study, although we meticulously collected a detailed history of TEM from participants, it was not possible to localise the contribution of TEM on the causal pathway of MK: whether people had used TEM for a non-MK problem and developed MK in the process or had used it after developing a simple form of MK which became more complicated, such as fungal keratitis. In our study, there was modest evidence of higher proportions of fungal Keratitis among people who had used TEM compared to those who had not. Future animal studies would be ideal to explore these hypotheses.

15.6 Quality of Life

Quality of Life (QoL) is a very important consideration in the management of any disease and treatments should ultimately aim to maintain or restore QoL.[37]Although there had been a previous report on the QoL among MK patients in the Mycotic Ulcer Treatment Trial (MUTT1) where patients with fungal keratitis who had received Natamycin had a better quality of life compared to those who had received Voriconazole, there had been no head to head comparison of QoL of MK cases compared to healthy normal population controls.[38] As part of a nested case control design for risk factors of MK, we collected and compared mean QoL scores among MK cases and controls.

This study showed that MK severely affects QoL in the acute phase. With treatment, QoL improves, with the highest QoL in cases who had little or no visual impairment at 3-months. Despite this impressive improvement, the QoL at 3-months of someone previously affected by MK (even when they have normal vision) remains lower than controls. This makes a case for

advocacy in the care of MK and other conditions which cause monocular blindness and visual impairment.

However, one limitation was that we did not collect information from the contralateral eye of the cases as this could affect the QoL score if the eye had existing visual impairment or ocular condition.

Conclusion:

This PhD was a step in the right direction in providing background information about the epidemiology of MK in Uganda. However, more multidisciplinary work is needed to improve prevent new infections of MK and outcomes of people who develop MK. Specifically, there is need to:

- Understand the role of TEM in causing new infections
- Develop strategies to prevent new infections
- Develop sensitisation packages for people groups at increased risk of MK
- Improve diagnosis of MK
- Improve treatment options and access to treatment of infections especially fungal keratitis
- Strengthen the capacity of the primary health system to diagnose, promptly manage and or refer MK
- Improve the training of mid cadre health workers in eye health
- Develop strategies of influencing behaviour change against use of TEM
- Develop advocacy campaigns on funding for care and rehabilitation of patients with MK

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Chapter 13. Future work

Uganda Corneal Infections Study Team. Left to right Ms Allen Asiimwe (anthropologist), Ms. Pauline Boonabaana Pauline (study nurse), Prof Matthew Burton (Main supervisor, based at LSHTM), Dr Simon Arunga (Ophthalmologist, PhD student), Mr Bernard Beinomughisha (research assistant), Mr Gilbert Arinda (study coordinator)

From ongoing work, we plan to conclude the following studies:

1. Clinical and Microbiology Correlation. We shall complete an analysis comparing the correlation between the phenotypic presentation and the microbiology. The microbiology data available includes pan-fungal PCR detection and sequencing of the collected specimens. This was done at Kilimanjaro Christian Medical College laboratory.

2. Quality of life at one year. We intend to conduct a follow up evaluation of the participants and controls to compare their quality of life. The data we have now is at 3 months and we want to compare with a longer follow up data.

3. Evaluation of corneal scarring after Microbial Keratitis. We shall evaluate the profile of the corneal scarring among the participants and feasibility of corneal transplant (anterior segment OCT depth of the scars, size, limbal stem cell status, endothelial integrity and intraocular comorbidities).

4. Traditional medicine studies. We collected data on the different types of herbs that patients used to treat MK. We are working with a taxonomist to describe them and shall proceed to conduct pharmacological and microbiological tests on the most commonly reported herbs.

5. Economic Impact of MK on household wealth comparing MK cases and healthy control households. We collected household asset data among cases and controls which will be analysed in the near future.

In addition, Simon will continue to work with his supervisor Prof Matthew Burton on a 5-year Wellcome Trust funded programme on several follow up projects listed below (https://www.lshtm.ac.uk/newsevents/news/2018/lshtm-lead-vital-research-severe-corneal-infections-low-and-middle-income).

6. Randomised controlled trial of topical chlorhexidine 0.2% verses natamycin 5% for fungal keratitis. In a hospital-based randomised controlled trial (RCT), we will test the hypothesis that g-chlorhexidine 0.2% is non-inferior to g-natamycin 5% in parallel two-arm, single-masked RCTs. We will also assess superiority of either drug. We will conduct two independent trials: (i) Nepal, (ii) East Africa (Uganda and Tanzania) which have already started.

7. Randomised controlled trial of topical ilomastat 0.05% verses placebo in the treatment of microbial keratitis. We will test the hypothesis that g-ilomastat 0.08% is superior to placebo in preventing corneal perforation / TPK in parallel two-arm, double-masked

RCTs. We will conduct two independent trials: (i) Nepal, (ii) East Africa (Uganda and Tanzania). These trials will be conducted after Study 1 is completed.

8. Cluster randomised controlled trial of a complex intervention to prevent severe microbial keratitis. We will test the hypothesis that an intervention package can prevent blindness from severe MK in parallel two-arm, single-masked cluster RCTs. We will conduct two independent trials: Nepal, Uganda. This will be the core of Simon's post-doctoral research fellowship.

9. Microbial keratitis diagnostic and pathophysiology studies. We shall conduct a comparative study evaluating sensitivity/specificity of diagnostic strategies for fungal vs. bacterial infection. This shall be on specimen collected from patients from the above studies.

10. Genetics: In the previous work, we collected human genetic samples from carefully phenotyped cases. The participants recruited in these subsequent clinical trials would also be invited to contribute genetic material to a wider study of genetic risk factors in MK

11. Immunopathology: relatively little is known about the types of immune responses at the site of infection at different points in the natural history of this disease in humans. It is plausible that some types of responses are associated with more or less favourable outcomes for patients, even when timely antimicrobial is provided. We shall do corneal gene expression and Impression cytology to describe the cell types infiltrating the cornea during microbial keratitis and their secreted factors, and the association of these factors with poor visual outcomes. This will help us to better understand the immunopathophysiology of Microbial Keratitis and potential for learning new unrecognised therapeutic targets.

Appendices

Appendix 1: Mbarara Akavurugye Eye Study – Case Record Sheet:

Demographic Data

1. Study Number			
2. Hospital Number			
3. Hospital		REC=1, MURHEC=2	
4. Sur Name			
5. First name			
6. DOB		Write in this format DD/MM/YYYY	
7. Age (years)			
8. Sex		Male = 1, Female = 2	
9. Phone number – 1			
10. Whose phone number?		Please state Name and Relationship of the owner of the phone number mentioned above.	
11. Phone number – 2			
12. Whose phone number		Please state Name and Relationship of the owner of the phone number mentioned above.	
13. Occupation		0 = No job, 1 = Mainly farmer, 2 = Mainly employed (manual), 3 = Mainly employed (non-manual), 4 = Mainly self-employed (own business, merchant), 5 = Civil servant, 6 = Retired, 7 = Student, 8 = Other (specify)	
14. Education level		0 = No formal education, 1 = Lower Primary (Grade 1-4), 2 = Upper Primary (5-7), 3 = Lower secondary (1-4), 4 = Upper secondary (5-6), 5 = Certificate, 6 = Diploma, 7 = Degree and above	

15. Literacy level		0 = Illiterate, 1 = Able to read Bantu a little, 2 = Able to read Bantu well, 4 = Able to read English and Bantu, 5 = Other (specify)	
16. Ethnic group		1 = Munyankole,2 = Mukiga, 3 = Mutoro, 4 = Mufumbira,5=Mukonzo,6=Muganda,7 = Other (specify)	
17. Marital status		0 = Single, 1 = Married/cohabiting, 2 = Divorced, 3 = Widowed	
18. Address		Village	
		Parish	
		Sub-County	
		County	
		District	
19.	20. Household Head's Name		
21.	22. Name of well-known Neighbour		
23.	24. Total number of household members? Write number		
25.	26. Members of the household under 16 years of age? Write number		
27.	28. Members of the household between 16 and 60 years of age? Write number		
29.	30. Members of the household above 60 years of age? Write number		
31.	32. How many under 16 year's member of the household went to school? Write number		
33.	34. How many adult members of the household (≥16 years of age) are "literate"? Write number		
35.	36. The highest level of education achieved in the household	0 = No formal education, 1 = Lower Primary (Grade 1-4), 2 = Upper Primary (5-7), 3 = Lower secondary (1-4), 4 = Upper secondary (5-6), 5 = Certificate 6 = Diploma, 7 = Degree and above	
37.	38. What is the highest status occupation with in the household	1 = Farming, 2 = Manual employment, 3 = Non – manual employment, 4 = Self-employment, 5 =	

		Civil servant, 7 = Other (specify)	
39.	40. Are you the household head?	0 = No, 1=Yes	
41.	42. What is the literacy level of the household head?	0 = Illiterate, 1 = Able to read Bantu a little , 2 = Able to read Bantu well, 3 = Able to read English and Bantu	
43.	44. What is educational level of the household head?	0 = No formal education, 1 = Lower Primary (Grade 1-4), 2 = Upper Primary (5-7), 3 = Lower secondary (1-4), 4 = Upper secondary (5-6), 5 = Certificate, 6 = Diploma, 7 = Degree and above	
45.	46. Occupation of the household head?	0 = No job, 1 = Mainly farmer, 2 = Mainly employed (manual), 3 = Mainly employed (non-manual), 4 = Mainly self-employed (own business, merchant), 5 = Civil servant, 6 = Retired, 7 = Student 8 = Other (specify)	
47.	48. Distance to the nearest Health Centre in KM		
49.	50. Geo location of nearest Health Centre		
51.	52. Level of Nearest health Centre	1=clinic/pharmacy, 2=HC II, 3=HCIII, 4=HC IV, 5=District hospital, 6=don't know	
53.	54. Nearest source of water in KM		
55.	56. Type of main water source	0= none, 1=Well,2 = Piped, 3= Roof collected tank, 4=Borehole, 5=protected spring	
	Visual Acuity assessment		
57. Presentation Date		Write in this format: DD/MM/YYYY	

58. Presenting Visual Acuity		Right eye	
		Left eye	
59. Best corrected visual acuity		Right eye	
		Left eye	
60. Contrast sensitivity		Right eye	
		Left eye	

History

	History of the presenting Complaint		
1. Eye affected		1=Right, 2=Left, 3=Both	
	Symptoms (Ask the patient what has brought them to hospital and write 1=Yes, 0=No)		
2.	3. Do you have eye pain?		
4.	5. Do you have Reduced/loss of vision in the affected eye?		
6.	7. Is there tearing?		
8.	9. Is there eye discharge?		
10.	11. Is there photophobia?		
12.	13. Is there foreign body sensation?		
14.	15. Is there any other complaint? other (specify)		
16.	17. Out of all those symptoms, which one is the most important to you?		
18.	19. When did the symptoms begin? (Encourage the patient to pinpoint the calendar date, write in this format: DD/MM/YYYY)		
20.	21. Is there history of trauma or something falling into the eye before onset of symptoms? (1=Yes, 0=No)		
	If Qn 5 above is YES, ask Qn 6 and 7, If Qn 5 is NO, go to question 8		
22. What was the traumatising object?		1=vegetative matter, 2=stick/wood, 3=soil/sand/dust, 3=insect, 4=Other (name), 99=Not applicable	

23. What happened to the traumatising object?		1=did not “enter” the eye, 2=”entered” the eye and was removed, 3=”entered” the eye, was **NOT** removed, 99=Not applicable	
	Current medical history		
24. Have you used any treatment up to now?		1=Yes, 0=No **(If NO then move to question 11)**	
25. Which topical (eye drop) treatment have you used to-date? (Ask the patient to show you the medicine they have been using)			
		Traditional Eye Medicine	
		Antibiotic	
		Steroid	
		Antibiotic-steroid	
		Anti-viral	
		Anti-fungal	
		Unknown name	
26. If the patient can remember the specific name or shows you a bottle, write down the name		1=Chloramphenicol, 2=Ciprofloxacin, 3=Tetracycline, 4=Gentamycin,5=Iodine,6=antibiotic-steroid, 7=Acyclovir, 8=clotrimazole, 9=econazole, 10=Natamycin, 11=other (specify), 99=Not able to ascertain	
	Other ocular History		
27.	28. Did you have any symptoms in last 3 months before onset of current illness?	0=none, 1=reduced vision, 2=itching, 3=excessive tearing, 4=abnormal discharge, 5=feeling dry, 6=lid swelling, 7=foreign body	

		sensation, 8=other (specify)	
29.	30. Were you using any eye medication in the last 3 months before onset of current illness?	1=antibiotic, 2=steroid, 3=antibiotic-steroid, 4=artificial tears, 5=anaesthetic, 6=antiviral, 7=antifungal, 8=glaucoma drug, 9=other	
31.	32. Did you have any eye operation in last 3 months before onset of current illness?	0=None, 1=lid surgery, Probing and syringing=3, cataract=4, glaucoma surgery=5, DCR=6, other=7 (specify)	
	Other medical/surgical history		
33.	34. Did you have any ENT conditions in last 3 months before onset of current illness?	0=none, 1=flu like symptoms, 2=cough, 3=sinus congestion, 4=other	
35.	36. Did you have of any ENT/facial surgery in the last 3 months?	1=Yes, 0=No	
37.	38. Do you have a history of Diabetes Mellitus?	1=Yes, 0=No, 3=Don't Know	
	If the answer to Qn 17 is YES, proceed to ask Qn 18-20, if NO or DON'T KNOW, skip to Qn 21		
39.	40. How many years have you been diagnosed with DM?		
41.	42. Which treatment are you using for DM?	0=none,1=diet, 2= Oral, 3=insulin, 4=other (specify)	
43.	44. What was your Last fasting blood sugar check?		
45.	46. Do you have a history of HIV infection?	1=Yes, 0=No, 3=Don't know	
	If the answer to Qn 21 is YES, proceed to ask Qn 22-24, if NO or DON'T KNOW, skip to Qn 25		

47.	48. How many years have you been diagnosed with HIV?		
49.	50. Which treatment are you using for HIV?	0=NA, 1=No treatment, 2=Septrin/dapsone only, 3=Septrin + HAART, 4=Other (specify)	
51.	52. What was your last CD4 count?		

53. Pain Impact Questionnaire	
1=Never, 2=Occasionally, 3=Often, 4=Constantly	
In the course of your current illness, How often have you experienced eye pain?	
In the course of your current illness, how often has eye pain interfered with your personal care such as bathing, eating, and dressing?	
In the course of your current illness, how often has eye pain disturbed your sleep?	
In the course of your current illness, how often has eye pain interfered with your household work such as cooking, house cleaning, washing cloth, fetching water, fetching firewood, caring to other family members?	
In the course of your current illness, how often has eye pain affected your agricultural or paid work?	
In the course of your current illness, how often has eye pain affected your participation in social activities such as attending weddings, social meetings, and funerals?	

Study Number:

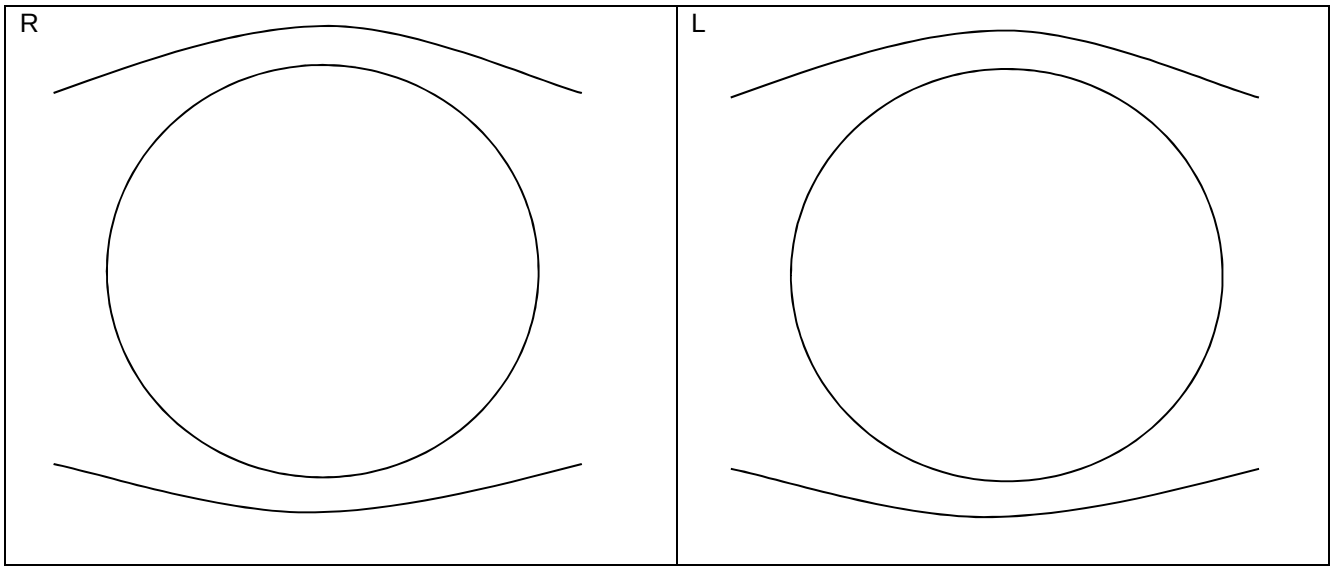
Appendix 2: Clinical examination of cases record form

1. Eye affected	1=Right, 2=Left	
2. Adnexa anatomy	1=Normal, 2=abnormal (specify)	
3. Presence of eye lid swelling	0=No, 1=Yes	
4. Presence of entropion	0=No, 1=Yes	
5. Presence of lagophthalmos	0=No, 1=Yes	
6. Presence of Trichiasis	0=No, 1=Yes	
7. Bell's phenomenon	1=Normal, 2=abnormal	
8. Regurgitation test	1=Normal, 2=abnormal	
9. Conjunctival Hyperemia	1=Not present, 2=mild, 3=moderate, Severe	
10. Corneal sensation	1=Normal, 2=Reduced, 3=Not done	
11. Slough - elevated	0=None, 1=Flat, 2=Raised	
12. Slough - texture	0=None, 1=Dry, 2=Wet	
13. Infiltrate edge	1=None, 1=Defined, 2=Serrated	
14. Satellite lesions?	0=None, 1=Yes	
15. Infiltrate colour?	0=None,1=White, 2=Cream, 3=Green, 4=Yellow, 5=Dark brown, 6=other (specify)	
16. Immune ring?	0=No, 1=Yes	
17. Corneal vascularisation	0=No, 1=Yes	
18. Hypopyon?	0=No, 1=Yes	
19. Hypopyon height (mm)	Measure from limbus	mm
20. Hypopyon shape	0=None, 1=Flattened, 2=Heaped	
21. Keratic precipitates	0=No, 1=Yes	
22. Keratic precipitate age	0=none, 1=new, 2=old	
23. Keratic precipitate size	0=none, 1=small, 2=large	
24. Perineural infiltrates	0=No, 1=Yes	

25. Fibrin	0=No, 1=Yes	
26. Flare	0=No, 1=Yes	
27. Flare grade	0=None, 1=mild, 2=moderate(iris and pupil seen), 3=moderate-severe (iris and pupil hazy), 4=severe (fibrin in AC)	
28. Cells in AC	0=No, 1=Yes	
29. Cells grade	0=None, 1=1-5 cells, 2=6-15 cells, 3=16-25 cells,4=26-50 cells, 5=Hypopyon	
30. Posterior corneal abscess	0=No, 1=Yes	
31. Endothelial plaque	0=No, 1=Yes	
32. Size of epithelial defect	Measure max. diameter	mm
33. Size of infiltrate	Measure max. diameter	mm
34. Depth of ulceration	100% minus % of remaining corneal thickness	%
35. Site of the ulcer	1=Involving the visual axis, 2=Visual axis spared	
36. Impending perforation	0=No, 1=Yes	
37. Perforation	0=No, 1=Yes	
38. Comment on the non-affected eye (if normal or not, comorbidity if present)		

Clinical Examination – Day 1		Date:
R	VA	L
R	Photo (0=No, 1=Yes)	L

Study Number:



Microbiology

Microbiology done	0=No, 1=Yes (reason if No)	
Gram stain result	Detail:	
KOH stain result	Detail:	
Blood Agar	Detail	
Potato dextrose Agar	Detail	
PCR	Detail	

Other Investigations

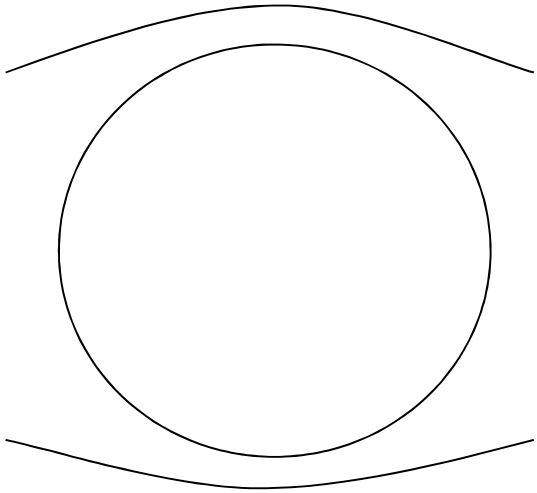
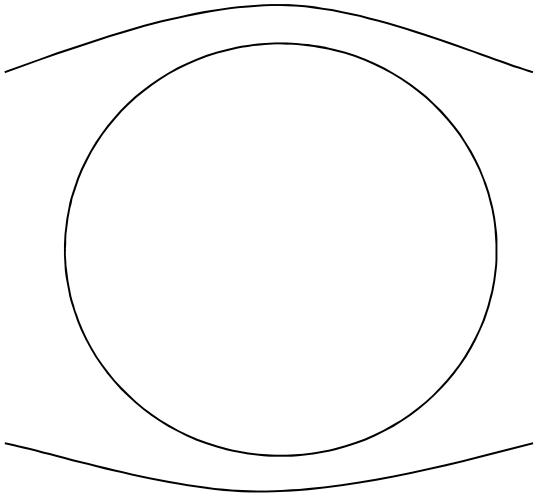
Detail
HIV
CD4
Blood sugar
Antibacterial sensitivity
Antifungal sensitivity

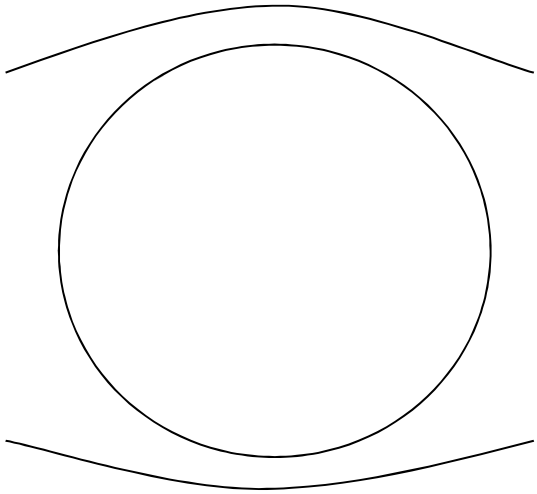
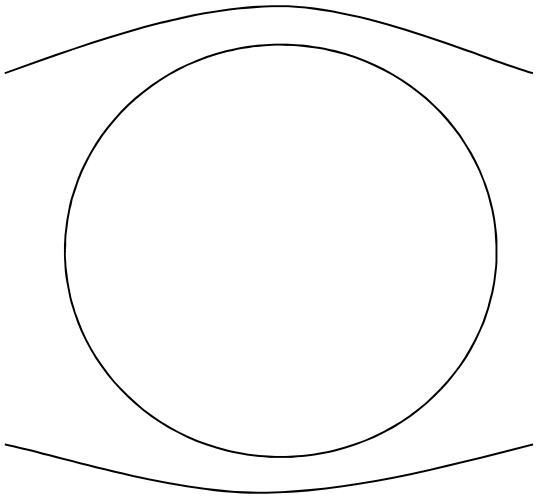
Initial Treatment:

Drug	0=No, 1=Yes	Dose	Frequency
g-Ciprofloxacin			

g-Econazole			
g-Atropine			
g-Natamycin			
Occ-aciclovir			
Itraconazole PO			
Aciclovir PO			
Ciprofloxacin PO			
Doxycycline PO			

Study Number:

Clinical Examination – Day		Date:
R	VA	L
R	Photo (0=No, 1=Yes)	L
R 		L 

Clinical Examination – Day		Date:
R	VA	L
R	Photo (0=No, 1=Yes)	L
R 		L 

Study Number:

Outcome

Change in treatment	0=No, 1=Yes	
Revised treatment	Detail	
Surgery	Detail	
Healed	0=No, 1=Yes	
Perforation after admission	0=No, 1=Yes	
Eviscerated	0=No, 1=Yes	
Final Vision	Presenting VA Right eye	
	Presenting VA Left eye	
	Pinhole VA Right eye	
	Pinhole VA Left eye	
HIV test performed	0=No, 1=Yes	
If HIV test performed, what was the result?	0=Not performed, 1=Negative, 2=Positive	
Discharge Date		

Comments:

Study Number:

Appendix 3: Mbarara Akavurugye Eye Study – Control Record Sheet:

Demographic Data

61. Study Number		
62. Hospital Number		
63. Hospital	REC=1, MURHEC=2	
64. Sur Name		
65. First name		
66. DOB	Write in this format DD/MM/YYYY	
67. Age (years)		
68. Sex	Male = 1, Female = 2	
69. Phone number – 1		
70. Whose phone number?	Please state Name and Relationship of the owner of the phone number mentioned above.	
71. Phone number – 2		
72. Whose phone number	Please state Name and Relationship of the owner of the phone number mentioned above.	
73. Occupation	0 = No job, 1 = Mainly farmer, 2 = Mainly employed (manual), 3 = Mainly employed (non-manual), 4 = Mainly self-employed (own business, merchant), 5 = Civil servant, 6 = Retired, 7 = Student, 8 = Other (specify)	
74. Education level	0 = No formal education, 1 = Lower Primary (Grade 1-4), 2 = Upper Primary (5-7), 3 = Lower secondary (1-4), 4 = Upper secondary (5-6), 5 = Certificate, 6 = Diploma, 7 = Degree and above	
75. Literacy level	0 = Illiterate, 1 = Able to read Bantu a little, 2 = Able to read Bantu well, 4 = Able to read English and Bantu, 5 = Other (specify)	
76. Ethnic group	1 = Munyankole,2 = Mukiga, 3 = Mutoro, 4 = Mufumbira,5=Mukonzo,6=Muganda,7 = Other (specify)	
77. Marital status	0 = Single, 1 = Married/cohabiting, 2 = Divorced, 3 = Widowed	
78. Address	Village	
	Parish	

Study Number:

	Sub-County	
	County	
	District	
79. Household Head's Name		
80. Name of well-known Neighbour		
81. Total number of household members? Write number		
82. Members of the household under 16 years of age? Write number		
83. Members of the household between 16 and 60 years of age? Write number		
84. Members of the household above 60 years of age? Write number		
85. How many under 16 year's member of the household went to school? Write number		
86. How many adult members of the household (≥16 years of age) are "literate"? Write number		
87. The highest level of education achieved in the household	0 = No formal education, 1 = Lower Primary (Grade 1-4), 2 = Upper Primary (5-7), 3 = Lower secondary (1-4), 4 = Upper secondary (5-6), 5 = Certificate 6 = Diploma, 7 = Degree and above	
88. What is the highest status occupation with in the household	1 = Farming, 2 = Manual employment, 3 = Non –manual employment, 4 = Self-employment, 5 = Civil servant, 7 = Other (specify)	
89. Are you the household head?	0 = No, 1=Yes	
90. What is the literacy level of the household head?	0 = Illiterate, 1 = Able to read Bantu a little , 2 = Able to read Bantu well, 3 = Able to read English and Bantu	
91. What is educational level of the household head?	0 = No formal education, 1 = Lower Primary (Grade 1-4), 2 = Upper Primary (5-7), 3 = Lower secondary (1-4), 4 = Upper secondary (5-6), 5 = Certificate, 6 = Diploma, 7 = Degree and above	
92. Occupation of the household head?	0 = No job, 1 = Mainly farmer, 2 = Mainly employed (manual), 3 = Mainly employed (non-manual), 4 = Mainly self-employed (own business, merchant), 5 = Civil servant, 6 = Retired, 7 = Student 8 = Other (specify)	
93. Distance to the nearest Health Centre in KM		

94. Geo location of nearest Health Centre		
95. Level of Nearest health Centre	1=clinic/pharmacy, 2=HC II, 3=HCIII, 4=HC IV, 5=District hospital, 6=don't know	
96. Nearest source of water in KM		
97. Type of main water source	0= none, 1=Well,2 = Piped, 3= Roof collected tank, 4=Borehole, 5=protected spring	
Visual Acuity assessment		
98. Presentation Date	Write in this format: DD/MM/YYYY	
99. Presenting Visual Acuity	Right eye	
	Left eye	
100. Best corrected visual acuity	Right eye	
	Left eye	
101. Contrast sensitivity	Right eye	
	Left eye	

Study Number:

Appendix 4: Quality of life tools

1.	**Quality of Life Questionnaire**					
The following questions ask how you feel about your quality of life, health, or other areas of your life. I will read out each question to you, along with the response options. **Please choose the answer that appears most appropriate.** If you are unsure about which response to give to a question, the first response you think of is often the best one.						
Please keep in mind your standards, hopes, pleasures and concerns. We ask that you think about your life **in the last four weeks.**						
		Very poor	Poor	Neither poor nor good	Good	Very good
1.1.	How would you rate your quality of life?	1	2	3	4	5
		Very dissatisfied	Dissatisfied	Neither satisfied nor dissatisfied	Satisfied	Very satisfied
1.2.	How satisfied are you with your health?	1	2	3	4	5
The following questions ask about **how much** you have experienced certain things in the last four weeks.						
		Not at all	A little	A moderate amount	Very much	An extreme amount
1.3.	To what extent do you feel that physical pain prevents you from doing what you need to do?	5	4	3	2	1
1.4.	How much do you need any medical treatment to function in your daily life?	5	4	3	2	1
1.5.	How much do you enjoy life?	1	2	3	4	5
1.6.	To what extent do you feel your life to be meaningful?	1	2	3	4	5
		Not at all	A little	A moderate amount	Very much	Extremely
1.7.	How well are you able to concentrate?	1	2	3	4	5
1.8.	How safe do you feel in your daily life?	1	2	3	4	5
1.9.	How healthy is your physical environment?	1	2	3	4	5
The following questions ask about how completely you experience or were able to do certain things in the last four weeks.						
		Not at all	A little	Moderately	Mostly	Completely
1.10.	Do you have enough energy for everyday life?	1	2	3	4	5
1.11.	Are you able to accept your bodily appearance?	1	2	3	4	5
1.12.	Have you enough money to meet your needs?	1	2	3	4	5
1.13.	How available to you is the information that you need in your day-to-day life?	1	2	3	4	5
1.14.	To what extent do you have the opportunity for leisure activities?	1	2	3	4	5
		Very poor	Poor	Neither poor nor good	Good	Very good
1.15.	How well are you able to get around?	1	2	3	4	5
		Very dissatisfied	Dissatisfied	Neither satisfied nor dissatisfied	Satisfied	Very satisfied
1.16.	How satisfied are you with your sleep?	1	2	3	4	5
1.17.	How satisfied are you with your ability to perform your daily living activities?	1	2	3	4	5
1.18.	How satisfied are you with your capacity for work?	1	2	3	4	5
1.19.	How satisfied are you with yourself?	1	2	3	4	5

1.20.	How satisfied are you with your personal relationships?	1	2	3	4	5
1.21.	How satisfied are you with your sex life?	1	2	3	4	5
1.22.	How satisfied are you with the support you get from your friends?	1	2	3	4	5
1.23.	How satisfied are you with the conditions of your living place?	1	2	3	4	5
1.24.	How satisfied are you with your access to health services?	1	2	3	4	5
1.25.	How satisfied are you with your transport?	1	2	3	4	5
The following question refers to how often you have felt or experienced certain things in the last four weeks.						
		Never	Seldom	Quite often	Very often	Always
1.26.	How often do you have negative feelings such as blue mood, despair, anxiety, depression?	5	4	3	2	1

2.	**Visual Functioning Questionnaire**					
The first two questions are about your overall eyesight. I will read out a choice of five answers and you will choose the one that describes you best.						
	Question	**Answer options** (Please circle the number which corresponds to the answer)				
2.1.	Overall, how would you rate your eyesight using both eyes – with glasses or contact lenses if you wear them?	1.V.good	2. Good	3. Moderate	4. Bad	5. V.bad
		1	2	3	4	5
2.2.	How much pain or discomfort do you have in your eyes (e.g. burning, itching, aching)?	1. None	2. Mild	3. Moderate	4. Severe	5. Extreme
		1	2	3	4	5
In the next section, I am going to ask you how much difficulty, if any, you have doing certain activities. I will read out choice of five answers and you will choose the one that describes you best.						
		1. None	2. Mild	3.Moderate	4. Severe	5. Extreme/ Cannot do
2.3.	Because of your eyesight, how much difficulty do you have in going down steps/stairs/ steep slopes?	1	2	3	4	5
2.4.	How much difficulty do you have in noticing obstacles while you are walking alone (e.g. animals or vehicles)?	1	2	3	4	5
2.5.	How much difficulty do you have in seeing because of glare from bright lights	1	2	3	4	5
2.6.	Because of your eyesight, how much difficulty do you have in searching for something on a crowed shelf?	1	2	3	4	5
2.7.	How much difficulty do you have in seeing differences in colours?	1	2	3	4	5
2.8.	Because of your eyesight, how much difficulty do you have in recognizing the face of a person standing near you?	1	2	3	4	5
2.9.	How much difficulty do you have in seeing the level in a container when pouring?	1	2	3	4	5
2.10.	Because of your eyesight, how much difficulty do you have in going to activities outside of the house on your own (e.g. sporting events, shopping, religious events)?	1	2	3	4	5

2.11.	Because of your eyesight, how much difficulty do you have in recognizing people you know from a distance of 20 metres? (e.g. from that building/tree – give marker of 20 meters**)**	1	2	3	4	5
2.12.	How much difficulty do you have in seeing close objects (e.g. making out differences in coins or notes, reading newsprint)?	1	2	3	4	5
2.13.	How much difficulty do you have in seeing irregularities in the path when walking (e.g. potholes)?	1	2	3	4	5
2.14.	How much difficulty do you have in seeing after a few moments when coming inside after being in bright sunlight?	1	2	3	4	5
2.15.	How much difficulty do you have in doing activities that require you to see well close up (e.g. sewing – not including threading the needle, using hand tools)?	1	2	3	4	5
2.16.	Because of your eyesight, how much difficulty do you have in carrying out your usual work?	1	2	3	4	5
In the next section, I am going to ask you how you feel because of your vision problem. I will read out a choice of five answers and you will choose the one that describes you best.						
		1. Never	2. Rarely	3. Sometimes	4.Often	5. Very often
2.17.	Because of your eyesight, how often have you been hesitant to participate in social functions?	1	2	3	4	5
2.18.	Because of your eyesight, how often have you found that you are ashamed or embarrassed?	1	2	3	4	5
2.19.	Because of your eyesight, how often have you felt that you are a burden on others?	1	2	3	4	5
2.20.	Because of your eyesight, how often do you worry that you may lose your remaining eyesight?	1	2	3	4	5
2.21.	Does your vision problem affect your life in ways we have not mentioned? If YES, describe how					
	Record as fully as possible the answer given					

3.	Activity and Participation																
		Were you involved in [activity] in the last week?		Why have you not done [Activity]? 0= Not able to do it 1=There was no need to do the activity/was not available 2= The activity was not my responsibility 99=NA				How much difficulty did you have in doing [Activity] in the last week? 0 = Extereme /not able to do 1= A lot of difficulty 2= Some difficulty 3= Little difficulty 4= No difficulty 99=NA						Did you do this activity: 1 =with no assistance 2 =with some assistance 3 =fully assisted 99 = NA			
		0=No	1=Yes														
	Household/Family																
3.1.	Cooking/washing dishes	0	1	0	1	2	99	0	1	2	3	4	99	1	2	3	99
3.2.	House cleaning	0	1	0	1	2	99	0	1	2	3	4	99	1	2	3	99
3.3.	Washing clothes	0	1	0	1	2	99	0	1	2	3	4	99	1	2	3	99
3.4.	Shopping	0	1	0	1	2	99	0	1	2	3	4	99	1	2	3	99
3.5.	Looking after children	0	1	0	1	2	99	0	1	2	3	4	99	1	2	3	99
3.6.	Looking after elderly/sick	0	1	0	1	2	99	0	1	2	3	4	99	1	2	3	99
3.7.	Travel (any purpose)	0	1	0	1	2	99	0	1	2	3	4	99	1	2	3	99
3.8.	Other Specify:…….	0	1	0	1	2	99	0	1	2	3	4	99	1	2	3	99
	Paid work																
3.9.	Paid employment	0	1	0	1	2	99	0	1	2	3	4	99	1	2	3	99
3.10.	Commission work	0	1	0	1	2	99	0	1	2	3	4	99	1	2	3	99
3.11.	Daily labour	0	1	0	1	2	99	0	1	2	3	4	99	1	2	3	99
3.12.	Self-employed/own business	0	1	0	1	2	99	0	1	2	3	4	99	1	2	3	99
3.13.	Other paid work: Specify…….	0	1	0	1	2	99	0	1	2	3	4	99	1	2	3	99
	……..																
	Work for own use																
3.14.	Farming	0	1	0	1	2	99	0	1	2	3	4	99	1	2	3	99
3.15.	Animal rearing	0	1	0	1	2	99	0	1	2	3	4	99	1	2	3	99
3.16.	Fetching firewood/charcoal	0	1	0	1	2	99	0	1	2	3	4	99	1	2	3	99

3.17.	Fetching water	0	1	0	1	2	99	0	1	2	3	4	99	1	2	3	99
3.18.	Processing agricultural products/food	0	1	0	1	2	99	0	1	2	3	4	99	1	2	3	99
3.19.	Other production own use:Specify:…… ……..	0	1	0	1	2	99	0	1	2	3	4	99	1	2	3	99
	Leisure																
3.20.	Social visits	0	1	0	1	2	99	0	1	2	3	4	99	1	2	3	99
3.21.	Attending ceremonies	0	1	0	1	2	99	0	1	2	3	4	99	1	2	3	99
3.22.	Attending meetings	0	1	0	1	2	99	0	1	2	3	4	99	1	2	3	99
3.23.	Reading, listening to radio, watching TV, games etc	0	1	0	1	2	99	0	1	2	3	4	99	1	2	3	99
3.24.	Chatting, relaxing with friends/family	0	1	0	1	2	99	0	1	2	3	4	99	1	2	3	99
3.25.	Other activities, specify	0	1	0	1	2	99	0	1	2	3	4	99	1	2	3	99
	Personal activities																
3.26.	Eating, Bathing, Dressing, sleeping	-	-	-	-	-	-	0	1	2	3	4	99	1	2	3	99

4.	**Self-Rated Wealth**		
4.1.	How well-off do you think your household is in relation to the other households in the village?	1 = Very poor, 2 = Poor, 3 = Average (neither poor nor wealthy), 4 = Wealthy, 5 = Very wealthy	

5.	**Ophthalmic Questionnaire**		
	Question	**Answer option**	**Answer**
5.1.	Do you feel ashamed or embarrassed due to the MK?	0 = No,1 = Yes	
5.2.	Do you worry that you may lose your remaining eyesight due to the MK?	0 = No 1 = Yes	
5.3.	Does your husband/wife/family member ignore you due to the MK?	0 = No 1 = Yes	
5.4.	Do you have sleeping problem? (If No, enter “99” to the next two questions and go to Q 5.7)	0 = No 1 = Yes	
5.5.	If yes, do you think your sleeping problem is related with the MK?	0 = No, 1 = Yes 3 = NA	

5.6.	How do you think your sleeping problem is related to the MK?	1 = Related to the pain 2 = Psychological (frustration, low self esteem, poor functioning ...) 3= Other (Describe) 4= NA	
5.7.	Dose the MK restricts you from doing/participating in productive activities or earn an income?	0 = No 1 = Mildly (A little bit) 2 = Moderately (Some restriction) 3 = Severely (a lot of restriction) 4 = Extremely/not able to do/participate)	
5.8.	Does the MK affect your life in any way? For instance in physical functioning, social functioning/relationship, marriage...etc	0 = No 1 = Yes	
5.9.	If yes, in what ways? describe		

6.	**Peer Rated Wealth**				
	Please randomly select three village members of the participant and ask the the following question on the wealth status of the houshold understudy		**Peer 1**	**Peer 2**	**Peer 3**
6.1.	How well-off do you think the household of [Household head] in relation to the other households in the village?	1 = Very poor, 2 = Poor, 3 = Average (neither poor nor wealthy), 4 = Wealthy, 5 = Very wealthy			

7.	**Asset (Wealth Indicators)**		
Please observe and record the following about the main building in the household			
	Question	**Answer options**	**Answer**
7.1.	What is the major construction material of the external walls?	1 = Brick, 2 = Concrete blocks, 3 = Sand and Cement , 4 = Wood, logs, 5 = Tin, 6 = Mud, 7 = Sticks and leafs, 8 = Wood and mud, 9 = Wood and animal faeces, 10 = Sticks and Animal faeces, 11 = Sticks and plastics, 12 = Rock, 77= Other (specify)	
7.2.	What is the major material of the roof?	1 = Concrete, 2 = Tin (metal sheets), 3 = Cereals Straw or grass, 4 = Plastic sheath, 5 = Wood, 6 = Wood and mud, 77 = Other (specify)	
7.3.	Number of tin the house is made	Write number	
7.4.	Does the house has a Cornish	0 = No, 1 = Yes, Plastic, 2 = Yes, Nylon, 3 = Yes, Cloth , 4 = Yes, Wood, 77 = Other (specify)	
7.5.	What is the primary material of the floor	1 = Tile, 2 = Concrete, 3 = clay/earthen floor, 77 = Other (specify)	
7.6.	How many rooms do the members of your household occupy, including bedrooms, living rooms and rooms used for household enterprises (do not include bathrooms, kitchens, balconies and corridors)	Write number of rooms	
7.7.	Location of domestic animals dwelling	0 = No domestic animals, 1 = Within the main house 2 = Outside the main house	
7.8.	Kitchen location	1 = Within the main house, 2 = Outside the main house	
7.9.	How many houses are there other than the main house (excluding, cattle dwelling and kitchen)	Write number of houses	

7.10.	What is the type of toilet that is used in your household	0 = No latrine, 1 = Traditional latrine, 2 = Improved pit latrine with ventilation (VIPL), 3 = Flush toilet, 77 = Other (specify)	
7.11.	Where is the toilet located	1 = Inside dwelling, 2 = Outside dwelling – in compound, 3 = Outside dwelling – outside compound , 99 = NA	
7.12.	Is this dwelling owned or rented?	1 = Owned, 2 = Rented, If Rented, go to Qn 7.14	
7.13.	Please estimate the amount of money you could receive as rent per month if you let this dwelling to another person	Write in Shillings or NA	
7.14.	If rented, what is the value paid per month?	Write in Shillings or NA	
7.15.	Dose the household has Electricity/solar?	0 = No 1 = Yes	
7.16.	**Dose the household own the following materials?**	**0=No, 1=Yes**	
	Radio/HiFi stereo	0=No, 1=Yes	
	TV/VCD/DVD	0=No, 1=Yes	
	Fridge/Freezer	0=No, 1=Yes	
	Electric stove	0=No, 1=Yes	
	Telephone/Cellular Phone	0=No, 1=Yes	
	Cupboard	0=No, 1=Yes	
	Sofa set	0=No, 1=Yes	

	Table small	0=No, 1=Yes	
	Table medium	0=No, 1=Yes	
	Table large	0=No, 1=Yes	
	Traditional Bed	0=No, 1=Yes	
	Metal bed	0=No, 1=Yes	
	Wood/spring bed	0=No, 1=Yes	
	Chair	0=No, 1=Yes	
	Bench	0=No, 1=Yes	
	Stool/small chair	0=No, 1=Yes	
	Showcase large	0=No, 1=Yes	
	Showcase medium	0=No, 1=Yes	
	Showcase small	0=No, 1=Yes	
	Clock	0=No, 1=Yes	
	Bicycle	0=No, 1=Yes	
	Water pump/generator	0=No, 1=Yes	
	Vehicle	0=No, 1=Yes	

	Motorbike	0=No, 1=Yes	
7.17.	Does the household own the following? (If Land, enter response by changing in to Hectar)	Mango Tree, 0=No, 1=Yes	
		Avocado Tree, 0=No, 1=Yes	
		Guava Tree, 0=No, 1=Yes	
		Lemon Tree, 0=No, 1=Yes	
		Orange Tree, 0=No, 1=Yes	
		Banana Tree land in Hectar	
		Sugarcane land in Hectar	
		Chat land in Hectar	
		Ecualiptous tree land in Hectar	
		Coffee land in Hectar	
		Vegtable land in Hectar	
		Maize and other cereals land in Hectar	
		Town land in Hectar	
7.18.	How much amount of land does the family own?	Write total amount of land in Hectar	

7.19.	How many cows does this household own in total?	Write number	
7.20.	How many goats does this household own in total?	Write number	
7.21.	How many sheep does this household own in total?	Write number	
7.22.	How many chickens/ducks does this household own in total?	Write number	
7.23.	How many pigs does this household own in total?	Write number	

Appendix 5: Health system survey tools

A. Health Centre Data Tool

Health Centre data collection tool. This tool will be used for collecting information from the health centre on knowledge, capacity, and practise in management of microbial keratitis. It has four parts; general information, knowledge on MK, capacity of the health centre and information on treatment practise for MK.		
Name of the HC		write
1. Study Number	(write)	
2. Location (district)	(write)	
3. Level of the HC	1=Clinic, 2=HCII, 3=HCIII, 4=HCIV, 5=District Hospital	
4. Geo location of HC	(write)	
5. How would you describe the road access to your HC?	1=Not all weather, 2=all weather	
6. What is the population coverage of your HC?	Write number	
7. Is there a latrine?	0 = No, 1 = Yes	
8. Is there a water source at the HC?	0 = No, 1 = Yes	
9. What type of water source do you have at the HC?	0= none, 1=Well, 2, 3 = Piped, 4= Roof collected tank, 5=Borehole, 6=protected spring	
10. How far is the nearest water source?	0= water at HC, distance in metres	
11. What is your main type of electricity supply?	0 = None, 1 = solar, 2=Hydro, 4=Generator	
12. Is there mobile phone network?	0 = No, 1 = Yes	
13. What is the distance in KM of the next referral centre		
14. Geo-location of the next referral centre		
15. What is the Level of the next referral HC	1=Clinic, 2=HCII, 3=HCIII, 4=HCIV, 5=District Hospital	
HC Capacity		
16. What is the total number of staff at your HC?		Write
17. What is the total number of staff supposed to be at your HC?		
18. What is the total number of the following types of staff cadre available at your HC? If none write 0	1=VHT	
	2=Nursing Aid	

	3=Ophthalmic assistant			
	4=Enrolled Nurse			
	5=Registered Nurse			
	6=Midwife			
	7=Laboratory officer			
	8=Nursing officer			
	9=Clinical Officer			
	10=Ophthalmic clinical officer			
	11=Medical officer			
	12=Ophthalmologist			
	13=Other (specify)			
19. What is the cadre level of the HC in charge?	Use reference numbers above			
20. What eye equipment is available to you: (If available, ask about condition and number)	Item	Available 0=No, 1=Yes	Condition 0=N/A, 1=Not working, 2=Working	Number
	Direct Ophthalmoscope			
	Magnifying loupes			
	Torch			
	Blue Light Torch			
	Eye lid retractors			
	Microscope			
	Slit lamp			
	Lab running water source			
	Staining rack			
	Bunsen burner/fire source set up			
21. Which of the following diagnostic consumables do you have currently?	Fluorescein	0=No, 1=Yes		
	21 Gauge Needles	0=No, 1=Yes		

	Microscope slides	0=No, 1=Yes	
	Amethocaine eye drops	0=No, 1=Yes	
	Sterile gloves	0=No, 1=Yes	
	Cover slips	0=No, 1=Yes	
	KOH	0=No, 1=Yes	
	Crystal Violet	0=No, 1=Yes	
	Grams iodine	0=No, 1=Yes	
	Acetone	0=No, 1=Yes	
	Carbolfuschin	0=No, 1=Yes	
	Blotting paper/cotton	0=No, 1=Yes	
22. Which of the following treatment stocks do you have currently?	Tetracycline ointment	0=No, 1=Yes	
	Chloramphenicol ointment	0=No, 1=Yes	
	Chloramphenicol eye drops	0=No, 1=Yes	
	Gentamycin eye drops	0=No, 1=Yes	
	Gentamycin IV vails	0=No, 1=Yes	
	Ciprofloxacin eye drops	0=No, 1=Yes	
	Steroid eye drops	0=No, 1=Yes	
	Steroid/antibiotic eye drops	0=No, 1=Yes	
	Iodine	0=No, 1=Yes	
	Chlorohexidine	0=No, 1=Yes	
	Antifungal eye drops (write)	0=No, 1=Yes	
	Antifungal tablets (write)	0=No, 1=Yes	

Practise (Information to be collected from the In charge/person who sees eye patients and patient/dispensing logs			
22. Total number of eye patients seen in the last 6 months			
23. Tally of the different eye diseases diagnoses and	Diagnosis (write)/	Number	Treatment given

treatments in the last 6 months			
24. Tally of eye patients referred in the last 6 months (if available)			
25. Where do you normally refer eye patients?			
Knowledge-information to be obtained from the person who usually sees eye patients or the in charge			
26. What is your training in eye care?	0=None, 1=partial (as part of my course), 2= Certificate in eye care, 3=Diploma in eye care, 4=Specialist		
27. How much in percentage time of your training was spent on eye care?			
28. List about five common eye diseases that you know?			
29. Here is a picture of an eye condition (with and without staining). What clinical signs can you see? 0=Not mentioned, 1=Mentioned		Conjunctival Hyperaemia	
		Epithelial breach	
		Corneal opacity	
		Hypopyon	
		Corneal FB	
30. What is the most likely diagnosis? (write)			
31. Assuming you had all the resources available to you, what would be your first line treatment?			
32. Which eye drops would you avoid?			
33. Have you seen such a case before? (0=No, 1=Yes)			
34. If yes, how did you manage it?			
35. What do you think are the risk factors to this condition?			
36. What do you think are the complications of this condition?			

B. Training School data collection tool

Study Number:

This tool will be used for collecting information from the training school on eye health training provided to the health workers. It has four parts; general information, knowledge content, skills content and competencies.		
General information		
1. Name of the Training school		
2. Study Number		
3. Location (district)		
4. Geo location of Training School		
5. What is the highest Level of Training school	1=Certificate nursing school, 2=diploma nursing school, 3=clinical officer school	
6. Number of Trained students per year		
7. Duration of training		
Training School Capacity		
8. What is the training level of the principal	1=certificate, 2=diploma, 3=degree, 4=masters, 5=PhD	
9. Do you have eye health within your curriculum?	1=Yes, 0=No	
10. What is the training level of the eye health tutor?	1=certificate, 2=diploma, 3=degree, 4=masters, 5=PhD	
11. What is the eye health training level of the eye health tutor?	1=None (did eye health as part of their medical training), 2=Certificate in eye health, 3=Diploma in eye health, 4=Masters in Eye health	
12. How much training in months do the trainees spend on eye health?		
13. Which are some of the topics covered under eye health training?	Are covered, 1=yes, 0=No	
	Anatomy of the eye	
	Blinding diseases	
	Eye Infections	
	Microbial keratitis	
	Examination of the eye	
	Ocular Pharmacology	
14. What is specifically taught under microbial keratitis? (write)		

Skills and competencies		
15. Do the trainees spend part of their training in hospital 1=Yes, 0=No		
16. How much time of their training do the trainees spend time in hospital? (Write in months)		
17. During the hospital rotations, do the trainees spend part of their time particularly in an eye ward/clinic/hospital?,(1=Yes, 0=No)		
18. How much time in months do the trainees spend in an eye ward/clinic/hospital?		
19. Do the trainees get hands on training while in an eye ward/clinic/hospital? (1=Yes, 0=No)		
20. While the trainees are in an eye ward/clinic/hospital, who supervises them?	1=Ophthalmic Assistant, 2=Ophthalmic Nurse, 3=Ophthalmic clinical officer, 4=Ophthalmologist 5=other (specify)	
21. Which skills do the trainees learn while in an eye ward/clinic/hospital?	skill	1=yes, 0=No
	Taking ocular history	
	Assessing visual acuity	
	Eye Examination using a torch	
	Application of eye drops	
	Staining the cornea with fluorescein	
	Eye examination using magnifying loupes	
	Removal of a corneal foreign body	
	Diagnosing a corneal ulcer	
	Eye examination using a direct ophthalmoscope	
	Other, write	

C. **Ophthalmic Assistants data collection tool**

This tool will be used for collecting information from the OAs on knowledge, capacity, and practise in management of microbial keratitis. It has four parts; general information, knowledge on MK, capacity of the health centre and information on treatment practise for MK.		
General information		
1. Study Number		
2. Name of OA		

3. Sex	1=male, 2=female	
4. Age in years		
5. Marital status	1=single, 2=married, 3=divorced, 4=widow/widowed	
6. Does your spouse stay with you?	0= if N/A, 1=No, 2=Yes	
7. How long ago (in years) was your training?		
8. Have you undertaken more training since your OA course?	0=No, 1=Yes	
9. What is your highest level of Training	1=certificate, 2=diploma, 3=degree, 4=masters	
10. Are you still in clinical practise for eye care?	0=No, 1=Yes	
11. If No, which area of practise are you now?	0=N/A (still in eye care), 1=Another area of medicine (write), 2=Area outside medical practise (write)	
Why did you decide to change to another area of practise?		
12. What is the Level of the HC where you work?	1=Clinic, 2=HCII, 3=HCIII, 4=HCIV, 5=District Hospital	
13. Geo location of HC	(write)	
14. How would you describe the road access to your HC?	1=Not all weather, 2=all weather	
15. What is your population coverage		
16. Is there a latrine?	0 = No, 1 = Yes	
17. Is there a water source at the HC?	0 = No, 1 = Yes	
18. What type of water source is available at the HC?	0= none, 1=Well, 2, 3 = Piped, 4= Roof collected tank, 5=Borehole, 6=protected spring	
19. How far is the nearest water source	0= water at HC, distance in KM	
20. Main type of electricity supply?	0 = None, 1 = solar, 2=Hydro, 4=Generator	
21. Is there mobile phone network?	0 = No 1 = Yes	
22. What is the distance in KM of the next referral centre		
23. Geolocation of the next referral centre		
Capacity available		

24. Which of the following eye equipment are currently available to you?	Item	Available 0=No, 1=Yes	Condition 0=N/A, 1=Not working, 2=Working
	Direct Ophthalmoscope		
	Magnifying loupes		
	Torch		
	Blue Light Torch		
	Eye lid retractors		
	Microscope		
	Slit lamp		
25. Which of the following diagnostic consumables do you have available currently?	Fluorescein	0=No, 1=Yes	
	Amethocaine eye drops	0=No, 1=Yes	
	Sterile gloves	0=No, 1=Yes	
26. Which of the following treatment stocks do you have currently available to you?	Tetracycline ointment	0=No, 1=Yes	
	Chloramphenicol ointment	0=No, 1=Yes	
	Chloramphenicol eye drops	0=No, 1=Yes	
	Gentamycin eye drops	0=No, 1=Yes	
	Gentamycin IV vails	0=No, 1=Yes	
	Ciprofloxacin eye drops	0=No, 1=Yes	
	Steroid eye drops	0=No, 1=Yes	
	Steroid/antibiotic eye drops	0=No, 1=Yes	
	Iodine	0=No, 1=Yes	

	Chlorohexidine	0=No, 1=Yes	
	Antifungal eye drops (write)		
	Antifungal tablets (write)		
Practise (Please refer to your log)			
27. What was the total number of eye patients you saw in the last 6 months			
28. What was the most common diagnosis?			
29. How many of those had MK?			
30. What was the most prescription given?			
31. How many eye patients did you refer in the last 6 months?			
32. What was the most commonly referred condition?			
33. Where do you normally refer eye patients?			
34. How do you usually manage MK in your setting?			
35. What do you think are the risk factors to this condition?			
36. What do you think are the complications of this condition?			
37. How do you think this condition can be prevented?			

54. Treatment chronology:

What was the order of your treatment after onset of symptoms? (Encourage the patient to carefully recollect the order of events in their treatment journey)

Study number	Event number	Treatment Event Calendar date	Where?	Treatment received: 1=Traditional Eye Medicine, 2=Chloramphenicol, 3=Ciprofloxacin, 4=Tetracycline, 5=Gentamycin,6=Iodine,7=antibiotic-steroid, 8=Acyclovir, 9=clotrimazole, 10=econazole, 11=Natamycin, 12=referred, 13=other (specify), 99=Not able to ascertain							
		DD/MM/YYYY	1=Home remedy/traditional, 2=clinic/pharmacy, 3=HC II, 4=HCIII, 5=HC IV, 6=District hospital, 99=don't know	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Approximate cost of care in UGX (write)			Did you need an escort for this visit? 0=No, 1=Yes
								consultation	Medicine	Direct cost	
	1										
	2										
	3										
	4										

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Observational / Interventions Research Ethics Committee

Dr Simon Arunga
LSHTM

20 April 2016

Dear Simon

Study Title: Microbial keratitis in Uganda

LSHTM Ethics Ref: 10526

Thank you for responding to the Observational Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Conditions of the favourable opinion

Approval is dependent on local ethical approval having been received, where relevant.

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document Type	File Name	Date	Version
Investigator CV	SIMON ARUNGA CV	25/01/2016	version 1
Investigator CV	SEELEY Janet-LSHTM 2015	25/01/2016	version 1
Investigator CV	CV AKL update Dec 2015 (1)	26/01/2016	version 1
Investigator CV	Matthew Burton Ethics CV - Corneal Study	05/02/2016	1
Protocol / Proposal	7.MAES data collection forms-v1-25FEB2016	25/02/2016	1
Information Sheet	2.MAES Information and consent forms for cases-v1-25FEB2016	25/02/2016	1
Information Sheet	3.MAES Information and consent forms for controls-v1-25FEB2016	25/02/2016	1
Information Sheet	4.MAES Information and consent forms for FGDs-v1-25FEB2016	25/02/2016	1
Information Sheet	5.MAES Information and consent forms for HCs-v1-25FEB2016	25/02/2016	1
Information Sheet	6.MAES Information and consent forms for Training Schools-v1-25FEB2016	25/02/2016	1
Protocol / Proposal	8.MAES FGDs themes on Traditional Eye Medicine and QoL-v1-25FEB2016	25/02/2016	1
Protocol / Proposal	9. MAES Topic Guide for IDIs-v1-12APRIL2016	12/04/2016	1
Protocol / Proposal	1. Microbial Keratitis in South Western Uganda - Protocol v2-12APRIL2016	12/04/2016	2
Covering Letter	Response letter	15/04/2016	1

After ethical review

The Chief Investigator (CI) or delegate is responsible for informing the ethics committee of any subsequent changes to the application. These must be submitted to the Committee for review using an Amendment form. Amendments must not be initiated before receipt of written favourable opinion from the committee.

The CI or delegate is also required to notify the ethics committee of any protocol violations and/or Suspected Unexpected Serious Adverse Reactions (SUSARs) which occur during the project by submitting a Serious Adverse Event form.

At the end of the study, the CI or delegate must notify the committee using an End of Study form.

All aforementioned forms are available on the ethics online applications website and can only be submitted to the committee via the website at: http://leo.lshtm.ac.uk

Additional information is available at: www.lshtm.ac.uk/ethics

Yours sincerely,

Professor John DH Porter
Chair

ethics@lshtm.ac.uk
http://www.lshtm.ac.uk/ethics/

Improving health worldwide

MBARARA UNIVERSITY OF SCIENCE AND TECHNOLOGY
RESEARCH ETHICS COMMITTEE
P.O. Box 1410, Mbarara, Uganda. **Tel: +256 4854 33795, ,**
E-mail: sec.rec@must.ac.ug **Fax: +256 4854 20782**

Our Ref: MUREC 1/7 **Date:** June 21, 2016

Dr. Simon Arunga
Principal Investigator
Akavurugye Eye Study

Re: **Submitted Protocol on: "Microbial Keratitis in South Western Uganda: The Mbarara Akavurugye Eye Study." No. 10/04-16**

Reference is made to the above protocol which was submitted to the Research Ethics Committee for consideration and approval.

It is noted that you have addressed all the concerns earlier raised by the Committee.

I am pleased to inform you that your study has been approved for a period of one year up to June 21, 2017

The following documents have been approved with the application:

Document	**Language**	**Version**
Proposal	English	Version 2
Protocol form	English	Version 2
Data Collection tools	English, Runyankole/Rukiiga	Version 2
Consent form	English, Runyankole/Rukiiga	Version **June 2016**

You are required to register the study with Uganda National Council for Science and Technology, and submit progress and end of study reports to MUST REC.

You can now proceed with the rest of the research activities after getting permission from Uganda National Council for Science and Technology.

I wish you all the best.

[redacted]
Dr. Francis Bajunirwe
CHAIR,
MUST RESEARCH ETHICS COMMITTEE

Uganda National Council for Science and Technology
(Established by Act of Parliament of the Republic of Uganda)

Our Ref: HS 2303 **24th May 2018**

Dr. Simon Arunga
Principal Investigator
Mbarara University of Science and Technology
Mbarara

Dear Dr. Arunga,

Re: Research Approval: **Microbial Keratitis in South Western Uganda: The Mbarara Akavurugye Eye Study (MAES)**

I am pleased to inform you that on **20/11/2017**, the Uganda National Council for Science and Technology (UNCST) approved the above referenced research project. The Approval of the research project is for the period of **20/11/2017** to **20/11/2019**.

Your research registration number with the UNCST is **HS 2303**. Please, cite this number in all your future correspondences with UNCST in respect of the above research project.

As Principal Investigator of the research project, you are responsible for fulfilling the following requirements of approval:

1. All co-investigators must be kept informed of the status of the research.
2. Changes, amendments, and addenda to the research protocol or the consent form (where applicable) must be submitted to the designated Research Ethics Committee (REC) or Lead Agency for re-review and approval **prior** to the activation of the changes. UNCST must be notified of the approved changes within five working days.
3. For clinical trials, all serious adverse events must be reported promptly to the designated local IRC for review with copies to the National Drug Authority.
4. Unanticipated problems involving risks to research subjects/participants or other must be reported promptly to the UNCST. New information that becomes available which could change the risk/benefit ratio must be submitted promptly for UNCST review.
5. Only approved study procedures are to be implemented. The UNCST may conduct impromptu audits of all study records.
6. An annual progress report and approval letter of continuation from the REC must be submitted electronically to UNCST. Failure to do so may result in termination of the research project.

LOCATION/CORRESPONDENCE

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COMMUNICATION

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Below is a list of documents approved with this application:

	Document Title	Language	Version	Version Date
1.	Research proposal	English	2.0	May 2016
2.	Mbarara Akavurugye Eye Study – Case Record Sheet	English	1.0	February 2016
3.	Clinical Examination of Cases Record Form	English	1.0	February 2016
4.	Quality of life tools	English	1.0	February 2016
5.	Health system survey tools	English	1.0	February 2016
6.	Informed consent documents	English and Runyankore	N/A	June 2016
7.	MAES Data tools	English	1.0	February 2016

Yours sincerely,

Isaac Makhuwa
For: Executive Secretary
UGANDA NATIONAL COUNCIL FOR SCIENCE AND TECHNOLOGY

Copied to: Chair, Mbarara University of Science and Technology, Research Ethics Committee

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RESEARCH PAPER COVER SHEET

Please note that a cover sheet must be completed for each research paper included within a thesis.

SECTION A – Student Details

Student ID Number	LSH1511754	**Title**	Dr
First Name(s)	Simon		
Surname/Family Name	Arunga		
Thesis Title	Epidemiology of Microbial Keratitis in South Western Uganda		
Primary Supervisor	Prof Matthew Burton		

If the Research Paper has previously been published please complete Section B, if not please move to Section C.

SECTION B – Paper already published

Where was the work published?	Medical Mycology Case reports		
When was the work published?	17 July 2018		
If the work was published prior to registration for your research degree, give a brief rationale for its inclusion			
Have you retained the copyright for the work?*	Choose an item. CCBY	Was the work subject to academic peer review?	Choose an item. YES

*If yes, please attach evidence of retention. If no, or if the work is being included in its published format, please attach evidence of permission from the copyright holder (publisher or other author) to include this work.

SECTION C – Prepared for publication, but not yet published

Where is the work intended to be published?	
Please list the paper's authors in the intended authorship order:	
Stage of publication	Choose an item.

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I treated this case, collected consent, prepared the maniscript with guidance from Astrid Leck from, Victor Hu, and M J Burton, prepared and submitted the final manuscript to Medical Mycology case reports in consideration of comments from all co-authors.

SECTION E

Student Signature	AS
Date	19/9/19

Supervisor Signature	MJB
Date	20/9/19

Medical Mycology Case Reports 22 (2018) 14–17

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journal homepage: www.elsevier.com/locate/mmcr

Bilateral *Candida* keratitis in an HIV patient with asymptomatic genitourinary candidiasis in Uganda

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ARTICLE INFO

Keywords:
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Candida keratitis
Genitourinary candidiasis
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Uganda

ABSTRACT

A 35-year-old male presented with Candida keratitis in the left eye. He was HIV positive with a CD4 of 352 cells/μL. The eye quickly deteriorated, despite intensive antifungal treatment and was eviscerated. Five months later, he re-presented with Candida keratitis in his right eye. A focal source of Candida infection was suspected and a urine culture identified Candida spp, despite being asymptomatic for genitourinary candidiasis. He was subsequently treated with good outcome (max. 75 words)

1. Introduction

Microbial keratitis (MK) is caused by a range of pathogens, including bacteria, viruses, protozoa and fungi. It is characterized by pain, conjunctival hyperemia and corneal ulceration with stromal inflammatory cell infiltrate. MK frequently leads to sight-loss from dense corneal scarring or even loss of the eye when severe.

In tropical regions approximately half of MK is attributable to fungal pathogens [1,2]. Filamentous organisms predominate, with *Fusarium spp*. and *Aspergillus spp*. accounting for the large majority [3]. Yeast infections, mostly caused by *Candida spp* are less frequent. In contrast, in temperate regions yeast often predominate, although some recent reports suggest an increasing proportion of filamentous infections [4]. Reported risk factors for fungal keratitis include trauma, ocular surface disease, contact lens use, prior surgery, traditional eye medicine (TEM), steroid use and immunosuppression [4–6].

Candida keratitis is particularly associated with chronic ocular surface disease and has been reported following various corneal procedures [4,7,8]. Although the source of the *Candida* is usually exogenous, it may sometimes have an endogenous source such as from the oral and genitourinary surfaces or a disseminated systemic infection in severely immunocompromised individuals [9,10]. Genitourinary *Candida* infection is relatively common in Africa; it can be either symptomatic or asymptomatic [11]. It is reported to contribute 30–50% of all cases treated with genitourinary infection [11–14]. However, it has not been previously reported to be associated with keratitis.

Here we report a case of a 35-year-old man with sequential bilateral *Candida* keratitis with a concomitant asymptomatic genitourinary *Candida* infection. This provides important lessons on investigation, treatment and preventative care in similar cases.

2. Case

2.1. First eye presentation

A 35-year-old male Ugandan presented to Mbarara University Referral Hospital Eye Centre (MURHEC) in June 2017 with a 10-day history of a painful, red left eye. There was no history of trauma, contact lens or TEM use. He was not aware of his HIV status at the time of presentation, but thought that he was HIV negative. He described a somewhat similar eye problem in his teenage years, which followed trauma, was treated and had healed. He had experienced no further ocular problems until this new presentation.

On this admission (day0),the left visual acuity was hand movements only, with no improvement on pinhole. There was a dense white paraxial supratemporal corneal infiltrate (2.0 mm × 1.5 mm), an overlying epithelial defect (2.0 mm × 1.5 mm), 80% corneal thinning and a 3.5 mm hypopyon (Fig. 1a). Additionally, the left cornea had an old inferior vascularized scar (7 mm × 6 mm). The right eye had an unaided visual acuity of 6/5 and normal ocular examination.

Corneal scrapings were collected for microscopy (Gram stain, Potassium Hydroxide [KOH] stain, Calcofluor White [CFW] stain, Lactophenol Cotton Blue stain[LPCB]) and culture (Blood Agar [BA], Chocolate Agar [CA], Potato Dextrose Agar [PDA] and Brain Heart Infusion [BHI]). Initial CFW slide revealed fungal elements. The Gram, KOH and LPCB tests were negative. However, *Candida spp*. grew on BA,

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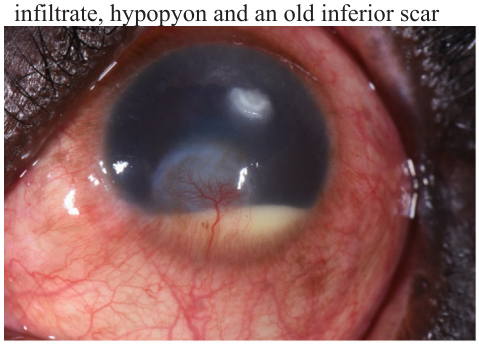
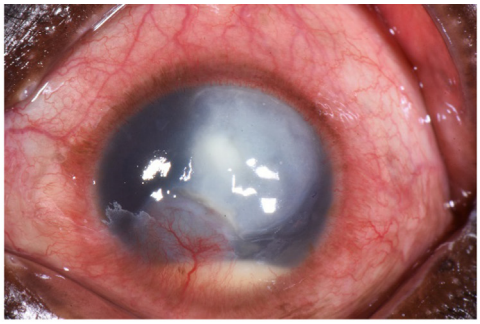
Received 2 July 2018; Accepted 16 July 2018
Available online 17 July 2018

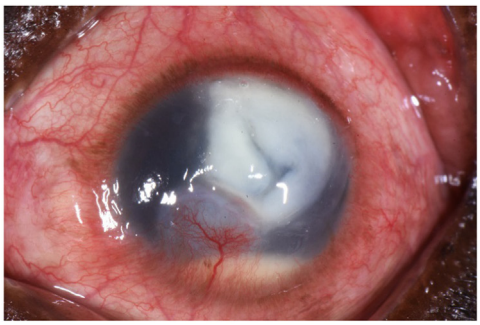
(a) Day0 Left eye had a small supra-temporal infiltrate, hypopyon and an old inferior scar



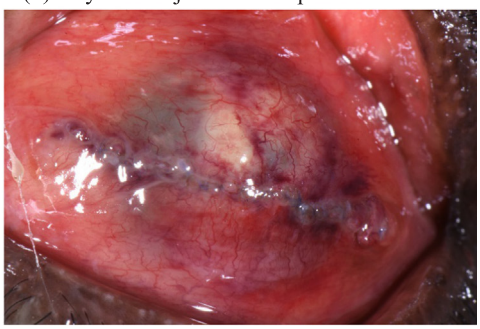
(b) Day3 Rapid deterioration with increased infiltrate size.



(c) Day7: Impending perforation



(d) Day14: Conjunctival Flap



(e) Day21: Conjunctival and corneal melt.

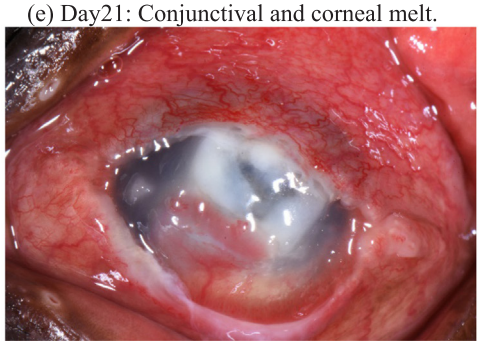


Fig. 1. (a–e) showing appearance of the left eye from presentation (day0) to day21, in June 2017.

PDA, CA and BHI subculture within 48 h.

The patient was started on hourly Natamycin 5% eyedrops (Zonat Sunways India) as well as Ofloxacin 0.3% eyedrops (Biomedica Remedies-India) 4 times/day and Atropine eyedrops. By day3, the eye had rapidly deteriorated (Fig. 1b) and hourly Chlorohexidine 0.2% eyedrops (locally formulated) was added to his treatment. By day7 the cornea had thinned further and was threatening to perforate (Fig. 1c). Corneal tissue for transplantation is currently unavailable in Uganda. On day8, a conjunctival flap procedure was performed (Fig. 1d), in conjunction with a subconjunctival injection of Fluconazole 2% (0.5 ml). On day21, he returned with a total corneal and conjunctival flap melt (Fig. 1e). At this stage further active treatment was considered futile and a decision was taken with the patient to perform an evisceration. Subsequently, a prosthetic shell was fitted.

It is our routine practice to offer HIV counselling and testing to all people presenting with MK. This individual accepted the offer and was found to be HIV positive. He was referred to HIV services and started anti-retroviral therapy. His CD4 count was 352 cells/μL around the time treatment was initiated.

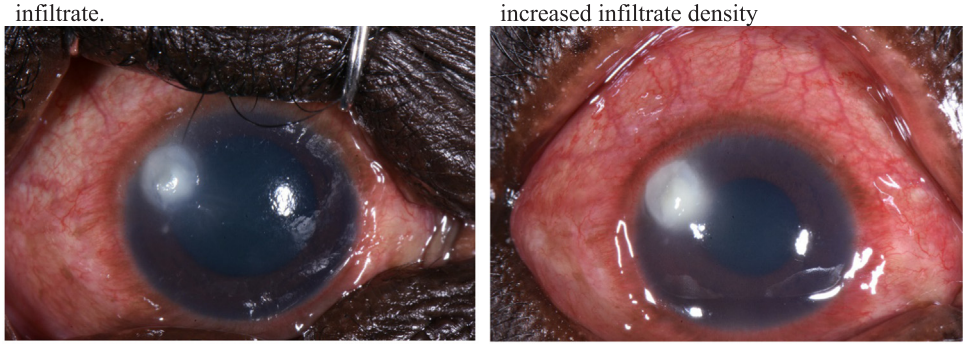
2.2. Second eye presentation

Five months later, he returned to MURHEC with a 4 day history of a painful right eye. Again, there was no history of trauma, contact lens or TEM use. On this day0 for the righteye presentation, visual acuity in the right eye was 6/12. Slit lamp examination showed a supra-temporal dense corneal infiltrate (3.1 mm × 2.8 mm), Fig. 2a. Corneal scrape samples were collected and sent for microbiological investigations, as outlined above. Gram stain showed pseudo-hyphae. CFW and KOH reported fungal hyphae and all culture plates (BHI subculture, BA, CA, PDA) grew *Candida spp*. The same first line protocol as previous (Natamycin, Ofloxacin and Atropine) was started. At this point, we were concerned that he might have a source of *Candida* elsewhere, that had led to the sequential corneal infections. He reported no systemic symptoms; specifically he did not have dysuria. As part of the assessment a urine sample was cultured, which also grew *Candida spp*.

By day3 we noted a moderate deterioration (Fig. 2b). Therefore, we added hourly Amphotericin B 0.15% eyedrops (locally formulated with a hyper methylcellolose base) and oral fluconazole 200 mg twice a day to his treatment. By day21, the ocular pain had greatly reduced and the infiltrate had transitioned into a scar extending to the visual axis (7 mm × 4 mm). He developed a small para-central perforation. This self-sealed with iris plugging; the anterior chamber was deep and Siedel's test was negative (Fig. 2c). By 3 months (day90) the scar size had reduced slightly (6 mm × 3.2 mm), and his right visual acuity was 6/24.

(a) Day1 right eye had a Supra-temporal infiltrate.

(b) Day3 Moderate deterioration, with increased infiltrate density



(c) Day21 Healed ulcer with a small self-sealing perforation and scar involving central cornea.

(d) Day90 Healed ulcer; the central component of the scar had reduced slightly.

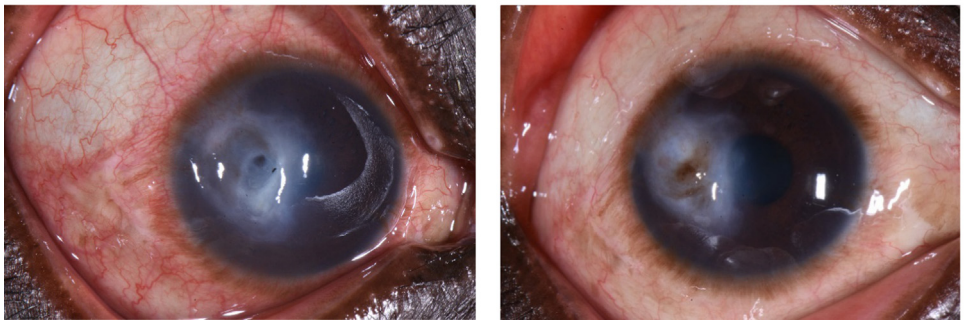


Fig. 2. (a–d) showing appearance of the right eye from presentation (day0) to day90, starting in December 2017.

3. Discussion

Although *Candida* keratitis has generally been found to be more common in temperate climates, it has been reported, albeit less frequently, in tropical regions [1,2,15]. This patient presented us a unique opportunity to reflect on the presentation of *Candida* keratitis in HIV infected patients and identify key considerations to ensure a good outcome.

Firstly, the patient had undiagnosed HIV infection with a relatively low CD4 count which could have predisposed him to the initial infection. We routinely provide HIV counselling and testing to MK patients as part of our hospital protocol, based on previous studies in the region that noted a high proportion of MK patients with HIV [16,17]. This is consistent with our experience in Uganda, where we find in ongoing case-control work HIV is more frequent in people with MK (unpublished data).

Secondly, we did not initially suspect a systemic source of the *Candida* infection. The patient was asymptomatic for this. Endophthalmitis resulting from *Candida* septicemia is well characterized [10,18,19]. Blood culture for *Candida* septicemia was not performed in our patient because he was afebrile, he did not have oral thrush and otherwise clinically well. Patients with *Candida* septicemia are usually very sick at presentation; they require hospitalization, with a majority requiring intensive care treatment [18]. Our patient was found to have asymptomatic genitourinary candidiasis on urine culture. Therefore, we think that the most likely explanation for the acquisition of his sequential case bilateral *Candida* keratitis was due to poor hygiene.

Thirdly, our patient rapidly deteriorated on the first presentation resulting loss of the eye, despite intensive treatment with two antifungal agents. *Candida* keratitis rapidly causes corneal perforations, corneal scars, endophthalmitis and loss of vision in many cases [20]. However, experience of managing his first infection helped us to aggressively manage his remaining eye when it became infected. Prompt microbiological confirmation of the *Candida* helped to initiate a dual drug combination of hourly Natamycin 5% eyedrops and Amphotericin B 0.15% eyedrops. We were able to save the eye and preserve useful vision. Molecular strain typing which would be required to validate if there was any similarity among the isolates, was not available.

This case graphically illustrates the increased risk to fungal keratitis experienced by HIV positive individuals. It highlights the need in unusual bilateral cases for careful assessment for a potential source elsewhere in the body. It is a reminder of the high ocular morbidity associated with these types of infections and the particular treatment challenges they present

Acknowledgements

The authors are grateful to the nursing staff Ms Pauline Boonabaana, Mr Gilbert Arinda, Mr Bernard Beinomugisha and Mr Martin Bukenya who collected and recorded patient data as well as Mr James Mwesigye the microbiology technician who processed all the collected samples. Dr John Onyango the Head of Department MURHEC for providing supervisory support to the staff.

Conflict of interest

None.

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