CAUSATIVE ORGANISMS IN MICROBIAL KERATITIS AND THEIR SENSITIVITY PATTERN IN KENYATTA NATIONAL HOSPITAL

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DECLARATION

I declare that this dissertation is my original work and has not been presented for award of degree in any other university.

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ABBREVIATIONS

KNH	-Kenyatta National Hospital
KOH	-Potassium Hydroxide
MOH	-Ministry of Health
PCEA	-Presbyterian Churches of East Africa
PK	- Penetrating Keratoplasty
SDA	- Sabouraud-Dextrose agar
SPSS	- Statistical Program for Social Sciences
WHO	- World Health Organization

ABSTRACT

Introduction: Microbial keratitis is a potentially vision threatening condition that requires prompt diagnosis and treatment to prevent untoward outcomes. It can be caused by bacteria, fungi or viruses.

Objective: The main objective of the study was to determine the causative organisms, predisposing factors and the sensitivity patterns of microbial keratitis in Kenyatta national hospital (K.N.H).

Justification: Knowing the common microbial causing keratitis and resistance patterns will enhance rationale use of antibiotics and decrease resistance.

Study design: This was a retrospective study using the records from the microbiology department and patient files.

Study setting: It was carried out at K.N.H microbiology department and patient files.

Data collection and analysis: Data was collected using pre designed questionnaire to record all the information needed. Descriptive statistics were used to summarize the data.

Ethical approval: The study was approved by the Ethics and Research Committee of University of Nairobi/Kenyatta National Hospital.

Results: 82 patient files were examined. The microbial growth pattern found that 56.1% had fungi and 40.2% bacteria. Further, 56.1% of fungal keratitis was due to *Fusarium spp*. 3.7% cultured both fungi and bacteria. 29.3% of the patients were farmers and 24.3% were

students. Most patients with fungal keratitis were farmers and had vegetative matter as the common predisposing factor. Most patients with bacterial keratitis were students with no specific history on predisposing factors.

81.8% of patients with bacterial keratitis had sensitivity done while for 18.2%, sensitivity was not done.

Conclusion: Fungi were the most common organism causing microbial keratitis. For bacterial keratitis cases, gram positive organisms were mainly sensitive to cephalosporins and flouroquinolones while gram negative were resistant to flouroquinolones.

Recommendation: Culture and sensitivity should be done on all patients with microbial keratitis. The protocol for empirical treatment of microbial keratitis at Kenyatta National Hospital should be adjusted based on known sensitivity patterns found in this study.

1.0 INTRODUCTION

1.1 Definition of corneal ulcer

The cornea, the clear front part of the eye through which light passes, is subject to many infections and to injury from exposure and from foreign objects. Infection and injury cause inflammation of the cornea and is called keratitis. Keratitis may involve epithelial disruption producing a corneal ulcer or affect the deeper layers of the cornea with or without ulceration. Keratitis can either be centrally located, thus greatly affecting vision, or peripherally located.[1] The risk factors for keratitis include ocular trauma, tear deficiency, trichiasis, contact lens wear, vitamin deficiency and infection.[2]

1.1.1 Bacterial Keratitis

Bacteria are a group of microorganisms all of which lack a distinct nuclear membrane and most of which have a cell wall of unique composition.[3] Most bacteria are unicellular; the cells may be spherical (*coccus), rod-shaped (*bacillus), spiral (*spirillum), comma-shaped (*vibrio) or corkscrew (*spirochaete). Generally, they range in size between 0.5 and 5 micrometer in diameter. Bacteria are usually classified by their ability to resist acetone discolouration into gram positive (which stains blue-black) and gram negative (which stains red). Their ability to form spores and oxygen also help in classification.

The pathogenesis of bacterial keratitis depends on both host defence and bacterial virulence. Bacterial keratitis usually only develops when ocular defences have been compromised.[4] However, some bacteria, including N. gonorrhea, N. meningitides, C. diphtheria and H. influenza are able to penetrate a normal corneal epithelium, usually in association with severe

conjunctivitis.[4] The invasion can either be due to direct cytotoxic action of the bacteria or more commonly via the release of toxins. In other circumstance, bacteria produce enzymes that facilitate the spread of the organism through the cornea (coagulase, collagenase, streptokinase and hyaluronidase).

1.1.2 Fungal Keratitis

Fungi are eukaryotic organisms with multiple chromosomes containing both DNA and RNA and can reproduce both sexually and asexually.[3,4] Fungi are broadly divided into filamentous (thread-like), yeast (round) and dimorphic (exist in both yeast and filamentous form). About 20-25% of individuals harbour non-pathogenic fungus saprophytes in their conjunctival sac.[5] Leck et al stated that "Infections of the cornea due to filamentous fungi are a frequent cause of corneal damage in developing countries in the tropics and are difficult to treat. Microscopy is an essential tool in the diagnosis of these infections".[6]

Filamentary keratitis may be associated with trauma, often relatively minor, involving plant matter or gardening/agricultural tools.[4]

1.2 Classification of corneal ulcers

Corneal ulcers may be classified by their anatomical position on the cornea or according to their pathogenesis.[7] Anatomical corneal ulcers can either be peripheral or central. Although the initial location of the ulcer within the cornea does not necessarily indicate the aetiology, it is usually thought that most peripheral ulcers are non-infective in nature while central ones are infective.[7] Aetiology of corneal ulcers is classified as either infectious or non-infectious. The infectious

group includes bacterial, fungal, viral and parasitic while the non- infectious group includes Mooren's, ring, marginal, phlyctenular, traumatic and nutritional.

1.3 Impact of bacterial and fungal keratitis on ocular morbidity and blindness

Corneal ulcers and their complications are among the major causes of ocular morbidity and blindness worldwide.[7] Corneal bacterial ulcer constitute the largest problem while mycotic and viral come second in infectious keratitis.[8] Studies have found that fungal and bacterial infective keratitis accounted for 80% of corneal ulcers.[4] Fungal and bacterial infective keratitis is a sight threatening condition that in some cases may have an explosive onset and rapid progression. If untreated, it may lead to progressive tissue destruction with perforation or extension into adjacent tissues.[4]

1.4 Clinical diagnosis

Bacterial and fungal ulcers can be strongly suspected on the basis of clinical features that are typical for a particular subgroup and the presence of certain risk factors.[9] This is referred as clinical diagnosis. Bacterial keratitis usually presents with relatively rapid onset, well-demarcated edges, suppurative, adherent mucopurulent exudates, purulent discharge and conjunctival injection. The common presenting features for fungal keratitis are history of trauma (especially with vegetative matter), slow progression, irregular ulcer edges, satellite lesions, endothelial plaque, and eye is less hyperaemic, elevated slough, Wesley ring and hypopyon. In both cases there is usually an epithelial defect.[11] There is little data on success of clinical diagnosis in sub-Saharan Africa.

1.5 Laboratory diagnosis

The current recommendation is that all corneal ulcers diagnosed clinically to be fungal or bacterial should be scrapped for microscopic slide and culture before initiation of the antibiotics.[12] Scrapings may be delayed off-treatment for 12 hours if antibiotics have previously been commenced. A non-preserved topical anaesthetic is instilled (preservatives may lower bacterial viability for culture). Scrapings are taken either with a disposable scalpel blade, bent tip of a larger diameter hypodermic needle or a sterile spatula like Kimura spatula. The margins and base of the lesion are scrapped. A thin smear is placed on glass slides for gram stain and potassium hydroxide (K.O.H) preparation.

Routinely, blood, chocolate and Sabouraud media are used initially. A blade or needle used for the scrapping can be placed directly into bottled media such as brain-heart infusion (BHI). Sensitivity reports are sent out at 1 or 2 days, 7 days and 2 weeks. When determining drug sensitivity for an isolated organism the results are reported as:

- Susceptible - indicating the organism is sensitive to a normal dose of antimicrobial agent
- Intermediate - indicating that the organism is likely to be sensitive to a high dose of the antimicrobial agent
- Resistant - indicating that the organism is not sensitive to the antimicrobial agent at the tested dose

Most laboratories test for antibiotic sensitivity using disc diffusion (Kirby-Bauer) method. The relevance of this to topical antibiotic instillation, where very high tissue levels can be achieved, is uncertain.[4] A culture is considered positive if it meets one of the following criteria:

- More than one confluent growth of the same organism is found at the sites of inoculation of one solid medium.

- There is growth in the liquid medium plus a positive corneal gram stain of the same organism.
- There is growth in more than one medium.

2.0 LITERATURE REVIEW

This section presents previous scientific studies reviewed in order to familiarize with the body of literature in the topics and identify the gaps based on which study will be conducted. The review is based on the study objectives.

2.1 Epidemiology of bacterial keratitis

Keratitis has since never been included in the five target diseases of WHO for blindness prevention .[13] Data regarding Keratitis is therefore mostly from individual publications. Bacterial keratitis is one of the most important causes of corneal opacifications, which is the second common cause of legal blindness world-wide after cataracts.[14] The pattern of microbial keratitis varies with geographic region. The bacteriological profile in keratitis therefore shows huge disparities amongst populations living in both western and in developing countries.[15] This variation has been explained by the fact that less industrialized countries have significantly lower number of contact lens users; hence fewer contact lenses related infections.[16] A study conducted by Dhakwa et al., compared the number of contact lens users in the US and Nepal.[17] In their study, USA was found to have an incidence of 11 for every 100,000 persons for microbial keratitis as compared to 799 per 100,000 persons in Nepal and this number corresponded to the number of contact lens user.

In North America, it is documented that *staphylococcal* species, *Pseudomonas aeruginosa* and *Streptococcus pneumoniae* as major isolates in microbial keratitis.[18] In Sweden, *Staphylococcus aureus* and *Staphylococcus epidermidis* were the most common Gram-positive bacteria in central microbial keratitis while *Pseudomonas aeruginosa* was the most common Gram-negative bacteria.[19]

2.2 Factors predisposing to microbial keratitis

According to Said et al., bacteria most frequently responsible for keratitis include *Staphylococci, Hemophilus, Streptococci,* and *Pseudomonas*.[21] Studies also reveal that if the surface of the cornea is breached, almost any bacteria, including atypical mycobacteria, can invade the cornea and result in keratitis.[22]

The usual fungal causes of microbial keratitis include *Candida, Aspergillus,* and *Norcardia*.[23] They mostly occur in people who are immunocompromised because of underlying illnesses or medications and trauma by vegetative matter. *Fusarium* keratitis, a type of fungal infection, occurs primarily in contact-lens wearers.[23] It has also been documented that physical or chemical trauma is the other frequent cause of keratitis.[24,25] The injury may become secondarily infected or remain non-infectious. Retained corneal foreign bodies are frequent sources of keratitis. Ultraviolet light from sunlight (snow blindness), a tanning light or a welder's arc, contact-lens over wear, and chemical agents, either in liquid form splashed into the eye or in gases in the form of fumes can all result in non-infectious keratitis. Chemical injury or contact lens-related keratitis often causes superficial punctate keratitis, in which the examiner notices myriads of injured surface cells on the affected cornea.[26]

Disturbances in the tear film may lead to changes in the corneal surface through drying of the corneal epithelium and loss of the protective effect of secretory immunoglobulin A (sIgA) and lysosomes. This type of keratitis is usually superficial and is known as keratitis sicca.[27] If the eyes are extremely dry, the surface cells may die and form attached filaments on the corneal surface, a condition known as filamentary keratitis.[28] In other cases, inability to close the eyelids properly can also lead to corneal drying, a condition termed exposure keratitis.[29]

2.3 Clinical Presentation

Bacterial keratitis has acute onset and presents mainly with blepharospasms, reduced vision, pain, redness, photophobia and discharge.[30] The severity of signs and symptoms depends on the virulence of the organism, the host immune status, any prior disease of the cornea, any previous therapy with corticosteroids and the duration of the infection. Gonococcal, *Haemophilus* and pneumococcal corneal infections may present with chemosis and sometimes pseudo membranes.[30] Slit-lamp examination shows cells and debris in the precorneal tear film and meniscus, absent corneal epithelium over an area of infection and focal suppurative process. It is important to document the size of any epithelial and stromal defect in at least two meridians.[30]

Several authors have given different guidelines for clinical diagnosis of keratitis. The commonly used guideline is based on Keenan & McLeod and is given in table 1 below. [31]

Table 1: Keenan & McLeod Classification of keratitis

Mild reaction	Focal, superficial suppuration
Moderate reaction	Suppuration confined to superficial two-third of the cornea
Severe reaction	Suppuration confined to posterior one-third of the cornea and may present as a ring abscess, sclera suppuration and impending perforation

Overall Gram-positive cocci form localised, round or oval, grey-white lesions with clear margins, minimal surrounding epithelial oedema and stromal infiltrates.[4] Staphylococcal ulcers are more often found in compromised corneas like bullous keratopathy, dry eyes, chronic herpetic keratitis, atopic disease and rosacea keratitis.[4] *Staphylococcus aureus* is found in 15% of

cultures from lids of normal persons and produces more severe corneal infiltration than *Staphylococcus epidermidis*.[4,6] Both these strains frequently produce indolent lesions with distinct borders, non-oedematous surrounding stroma and they tend to be localised.[4] Long standing staphylococcal ulcers dig deep into the stroma producing intra-stromal abscesses and sometimes perforation.

Mycobacterium, Nocardia and *Actinomyces* species are Gram-positive branching filamentous bacteria found in soil. The keratitis follows soil contaminated corneal injury and produces an indolent ulcer with elevated hyphae edges, often with satellite lesions, mimicking a fungal ulcer.[4,6] The cornea has a typical cracked windshield appearance. Keratitis caused by Gram-negative organisms have rapid onset and progress due to lytic enzymes like protease, lipase and elastase.[4,6] The most common and virulent Gram-negative ocular pathogens belong to *Pseudomonas* species. *Pseudomonas* can contaminate ophthalmic solutions like fluorescein, ocular cosmetics like mascara and any substance containing traces of organic carbon.[30]

Klebsiella, *Escherichia coli* and *Proteus keratitis* are common in compromised corneas with chronic epithelial disease, often without any history of trauma.[4,6] The non-spore forming anaerobes like *Peptococci, Peptostreptococci* and *Propionibacterium* form a broad group of Gram-positive and Gram-negative rods, found in mixed infections of the cornea.[4,6] They are active and invasive under compromised conditions like trauma, surgery, corticosteroids and antibiotics.[30]

2.4 Diagnosis

Microbiology remains the critical tool in the diagnosis of bacterial keratitis.[32] For diagnosis, smears, culture and sensitivity to antimicrobials form the three fundamental tools.[33] Studies suggest that cultures are preferred to smears as they are more specific and information yielding.[34] However, they take too long so treatment is initiated without waiting for results. The culture

positive rate in bacterial keratitis and ulcers is estimated to be 40–73% as compared to 0–57% in Gram's staining.[35] In polymicrobial keratitis, Gram's staining is not of much value in identifying the causative pathogen. An infection that is deteriorating despite antibiotic therapy yields a poor bacterial count for examination and diagnosis. It is important to know that, while positive cultures and smears are very useful for diagnosis, negative results may not rule out corneal infection, especially where antibiotics have already been given. In this situation, a corneal biopsy may be mandatory to establish diagnosis or the suspension of the antibiotic therapy for 72 hours to enable a repeat culture. It is wise to obtain cultures from lids and conjunctiva of both eyes, even if there is unilateral bacterial keratitis, and use blood and chocolate agar plates.[36] In spite of clinical evidence of bacterial keratitis, a methodology for diagnosis should be used. This should include aerobic bacteria, anaerobe non-spore forming bacteria, filamentous fungi and yeasts. There are occasions when fungal ulcers are infected secondarily by bacterial pathogens.

Mycobacteria (acid fast), *Actinomyces* (non-acid fast) and *Nocardia* (variable) can be identified by Carbol-Fuchsin or Ziehl-Nelson stains.[4,6] *Mycobacteria* can also be identified by fluorochrome stain and fluorescence microscopy.[4,6]

Immunological techniques available for the detection of bacterial antigens include direct immunofluorescence, immunoelectrophoresis, immunohistochemistry, fluorescent microscopy, enzyme immunoassays, agglutination, radioimmunoassay and molecular techniques.[35]

In the case of deep ulcers and abscesses without surface suppuration, it is necessary to obtain corneal fragments with a blade, microsurgical scissors or a trephine.[37] These fragments can be crushed on a glass slide for staining and also inoculated in thioglycolate and brain heart infusion (BHI). All corneal lesions due to contact lenses should be presumed to be infectious in origin,

unless proved otherwise. It is mandatory to send the contact lenses, contact lens solutions and the carrying cases for laboratory cultures.[38]

The scraping from the advancing edge and centre of the infected ulcer can be done using a modified Kimura platinum spatula. A large gauge disposable needle is a possible alternative. The use of a slit-lamp makes the procedure more scientific. The material thus obtained is to be streaked on blood agar in a C shape. Growth along the C streak is microbiologically significant while any growth away from the C streak is probably a contamination.[6] Additional specimens should be reserved for chocolate agar and Sabouraud agar without cycloheximide. Chocolate agar provides hemin (X-factor) and V-factor, essential for growth of *Haemophilus* and is ideal for isolation of *Neisseria* and *Moraxella.*[6] For anaerobic pathogens, it is ideal to use chopped meat glucose broth or thioglycolate medium with vitamin K. Thioglycolate broth also provides basic nutrients for aerobic organisms. Its sulfhydryl (SH) compound acts as an oxygen reducing agent which is suitable for anaerobic bacteria. BHI is valuable if a poor yield of organisms is expected as in patients on prior antibiotics. In patients with any signs or symptoms of dacryocystitis, fluid expressed from the lacrimal sac should be cultured.[35]

Gram stain is appropriate for bacteria and can also show dimorphic fungi in the yeast phase; however, cellular details appear better with a Giemsa stain. Gram-positive bacteria appear blue-purple and retain gentian violet while Gram-negative bacteria lose gentian violet and appear pink with safranin.[38] If done meticulously, Gram stain can identify the pathogen (single organism) in 75% of cases and in 37% of cases having mixed bacterial infections.[39] Giemsa can distinguish non-infectious keratitis by the type of inflammatory cells. In indolent corneal infections, it may be necessary to use acid fast stains for *Mycobacterium, Nocardia* and *Actinomyces* species. In order to get the maximum information, the sample should cover an area approximately 1cm in

diameter on the glass slide. Excessive decolorisation should be avoided and immersion of the slide in 95% methanol or cold acetone for 5–10 minutes is preferable to heat fixation in maintaining the morphology and staining characteristics of the pathogens.[40]

In addition to Gram and Giemsa stains, an extra slide and some specimen material should be reserved for special stains like periodic acid Schiff, calcofluor, Gomori, acid fast bacilli and methenamine silver. All refrigerated media should be warmed to room temperature before inoculation to prevent fatal cold shock to the organisms.[41]

2.5 Interpretation of Culture Media

Most aerobic bacteria in microbial keratitis appear only within 48 hours on standard culture media.[42] The plates should be examined on daily basis and liquid media observed for turbidity. The reasons for poor or negative results could be prior antibiotic therapy; an insufficient sample; excessive heat fixation; mechanical damage to cell wall architecture and a reluctance to examine the whole slide.[43]

2.7 Management

It is advisable that any doubtful microbial keratitis should be treated as bacterial keratitis unless proven otherwise.[44] It is estimated that about 87% of bacterial corneal ulcers are caused by four groups of organisms.[45] It is however worth noting that no single antibiotic is effective against all organisms.[46]

2.7.1 Corticosteroids in Bacterial Keratitis

At cellular level, corticosteroids can be accepted as damage-reducing agents in bacterial keratitis. Corticosteroids have two important actions: to decrease polymorphonuclear leukocyte activity at the level of ingestion and degranulation and, to reduce inflammation initiated by dividing bacteria and their toxins, host enzymes and hydrolytic enzymes from polymorphonuclear leucocytes.[47] Corticosteroids can be given in patients with bacterial keratitis to improve outcome.[48] Reddy et al. indicated that delayed eradication of corneal infection with combined treatment of corticosteroids and antibiotics, while Harbin reported relapse of *Pseudomonas* keratitis in a corticosteroid treated patients.[49]

2.7.2 Surgical Management of Bacterial Keratitis and Its Complications

According to Hussain et al., small corneal perforations and descemetoceles can be treated with cyanoacrylate tissue glue adhesive.[48] This has been shown to help restore anterior segment integrity. However, this may not be a permanent solution. When doing this, the stromal ulcer bed should be debrided before applying glue and a contact lens should be used over it. On the other hand, it has been shown that cyanoacrylate is toxic to endothelium and the lens.[50] A patch graft can be an alternative to cyanoacrylate glue, but it may be destroyed by bacteria, hence it should only be used after effective antimicrobial therapy. A conjunctival flap should never be used over active infected necrotic tissue or the flap will become necrotic. The flap can be used to promote healing over a debrided corneal ulcer bed, especially for peripheral ulcers.[30]

It is estimated that 10% of patients who undergo penetrating keratoplasty (PK) develop bacterial keratitis. The pathogens frequently involved in such an indication include; *Streptococcus*

pneumoniae, *Staphylococcal* species, *Pseudomonas aeruginosa*, *Moraxella*, beta-hemolytic *Streptococci* and *Pseudomonas* species.[49] The outcome of PK in bacterial keratitis depends on any previous *Herpes simplex* virus keratitis, the severity of the stromal inflammation, the size and location of the graft and any prior therapeutic measures like contact lenses or glues. The best chance of success for PK is when the procedure is done after a total bacterial kill has been achieved and before corneal vascularisation appears.[51,52]

According to Doyle et al., oral corticosteroids should be given 24 hours before and seven days after a PK to curtail inflammation.[53] To prevent suture erosion, a relapse of keratitis and wound dehiscence after PK, it is important to prepare a healthy recipient edge by debriding all necrotic corneal tissue. Bites should be taken through healthy recipient corneal tissue and excised infected cornea sent for a laboratory workup to guide post PK antimicrobial therapy.

2.7.3 Clinical Course Prognosis

Studies indicate that even with an armamentarium of highly selective and broad spectrum antimicrobials in the therapy of bacterial keratitis, about 24% of keratitis patients develop vision threatening complications like descemetoceles, perforations, endophthalmitis, atrophy and disorganisation of the affected eye.[42] Bacterial keratitis may heal with minimal or no opacification, vascularisation or visual deficit; however, inadequately, ignorantly or late treated bacterial keratitis can have an extremely dangerous clinical course because of corneal opacification, secondary glaucoma, scleral extension of infection and anterior segment disorganisation. There may be scarring of the cornea with hyalinisation, calcium and lipid deposits.[54]

Destruction of the corneal lamella may lead to corneal thinning and ectasia.[55] Corneal fistulae, anterior synechiae caused by fibrinous anterior chamber reaction, seclusion pupillae, cataract, secondary glaucoma, panophthalmitis and pthisis bulbi can be the sequalae of bacterial keratitis. Amongst Gram-negative infections of the cornea, *Moraxella* keratitis has been shown to have a good visual prognosis. In the case of mixed or polymicrobial infections, perforation and pthisis is likely to develop. *Pseudomonas* infections invariably lead to corneal perforation and loss of the eye if untreated, especially in immunocompromised patients with a history of contaminated traumatic bacterial keratitis.[56]

Knowing the specific organisms and their sensitivity pattern is important to encourage rationale use of antibiotics. Most bacterial causes of keratitis respond well to broad spectrum flouroquinolones.[51] Organisms may be resistant to more than one antimicrobial agent. The mechanism of resistance may be intrinsic, de novo or acquired.[57] Acquisition of new genetic material may occur through conjugation, transformation or transduction. Irrational use of antimicrobials creates selective pressure for the emergence of resistant strains.[57]

2.8 Statement of the problem

Microbial keratitis is a potentially sight threatening disorder and considered to be the leading cause of monocular blindness worldwide. Reviewed literature has indicated that there has been an increase in the cases of microbial keratitis worldwide. While most of the factors associated with microbial keratitis can be avoided, the apparent increase in the cases of microbial keratitis can only be explained by lack of adequate data and information regarding the disease. Knowing the common microorganisms causing corneal ulcers and their drug sensitivity pattern will enable

rationale antimicrobial usage and decrease resistance patterns especially in resource-limited setting.

3.0 JUSTIFICATION

Microbial keratitis has been managed in Kenya for a long time but there has been no published study on the causative organisms and their sensitivity patterns. This study was expected to provide vital information for both the Ophthalmologists and patients with regard to the organisms in our set up and the sensitivity to the drugs available. This may enable the Ophthalmologists to better manage microbial keratitis. It may also help to come up with a protocol on the management of microbial keratitis based on their sensitivity patterns.

It was anticipated that this study will provide the baseline information needed for future research in this area and hopefully enable evidence-based practice in the management of microbial keratitis.

Most of the causes of poor eye health causing microbial keratitis are either preventable or treatable. It is therefore important that measures towards diagnosis and treatment of microbial keratitis be enhanced to address the economic and social challenges caused by the disease.

4.0 STUDY OBJECTIVES

4.1 Broad objective

1. To determine causative organisms and predisposing factors in microbial keratitis and their sensitivity pattern

4.2 Specific objectives

2. To determine causative organisms in microbial keratitis
3. To assess the sensitivity patterns

5.0 MATERIALS AND METHOD

5.1 Study design

A hospital based retrospective study

5.2 Study period

January 2010 to December 2015

5.3 Study setting

Kenyatta National Hospital

5.4 Study population

All files of patients diagnosed with microbial keratitis with positive laboratory results

5.5 Sample size

The following sample size determination formula for finite population correction (Wanga & Lemeshow)[58] was used to estimate the proportion of population study size.

$$n^1 = \frac{NZ^2\mathrm{P}(1-P)}{d^2(N-1)+Z^2\mathrm{P}(1-P)}$$

Where

n' = sample size with finite population correction,

N = size of the target population = 75 (15patients per year for 5 years)

Z = statistic for 95% level of confidence

P = estimated proportion of patients with microbial keratitis – 4.0% [2]

d = margin of error = 2.1%

n^1 = 61.2

62 Patients (minimal sample size)

All the files of patients diagnosed with microbial keratitis with positive laboratory results between the month of January 2010 and December 2015 were included in the study.

5.6 Inclusion and Exclusion criteria

All patients diagnosed to have either fungal or bacterial keratitis with positive microbial culture results during the study period were included in the study. Patients with incomplete laboratory records of corneal scrapings corneal scrapping results which are inconclusive will be excluded in the study.

5.7 Case definition

A corneal epithelia defect of any size confirmed to be bacterial or fungal by positive laboratory results.

5.8 Study procedure

Records reviewed from the microbiology laboratory to identify patients who had corneal scrapings done then the files traced at the records department to get more data.

5.9 Data collection and Analysis

The data collection form in Annex V was used to retrieve the information needed from the microbiology laboratory and the patients file. The records were retrieved first from the general records book for all corneal scrapings done in the microbiology laboratory. The patients file number was identified and the file retrieved from the central records. Results was analysed with help of a qualified statistician using Statistical Packages for Social Sciences (SPSS 21). Descriptive statistics such as percentages, proportions, means and frequencies were used to summarize the data and draw conclusions.

5.10 Data management and confidentiality

Data was coded, entered and validated. It was checked for any wrong entry and double entry and corrected. Back-ups were created in an external hard disk in case of damage and/or loss of original data and password protected. All data was stored under lock and key and with password protected files under the custody of the principal investigator to prevent any illicit access to the data. Use of coded data was done to ensure maximum confidentiality. At the end of the study, the raw data will be destroyed and deleted from any existing hard copies by paper shredding and formatting and secure deleting and formatting of any soft copy storage devices including flash discs external hard drives and/or computer hard disks.

5.11 Ethical consideration

This was a retrospective study and therefore involved abstraction of existing records without changing the clinical practice. Confidentiality was maintained on information regarding the patient since names of clients was not be sought. The research proposal was submitted to the

University of Nairobi and Kenyatta National Hospital for clearance and approval. Permission was also sought from the administration of Kenyatta National Hospital to allow publication of the results. The results will be shared with all concerned parties and appropriate recommendations made to the hospital to enhance better patient management

6.0 RESULTS

Figure 1 below shows a total of 88 files retrieved out of which six had incomplete records. 82 files were analysed.

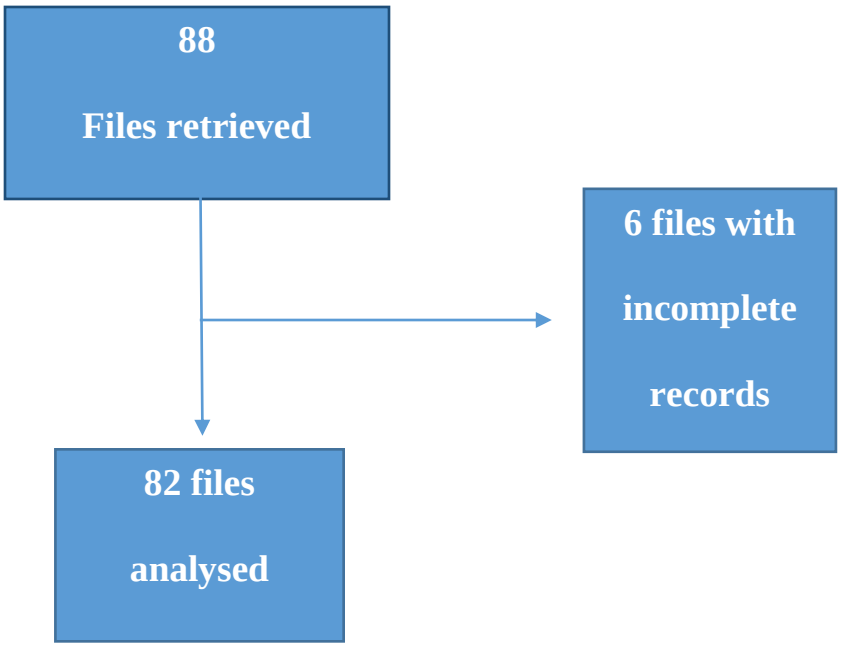

Figure 1: Flow diagram

6.1 Demographic data

Figure 2 below shows the demographic data for the study. Majority of patients were in the 21-40 age category (59.8%).

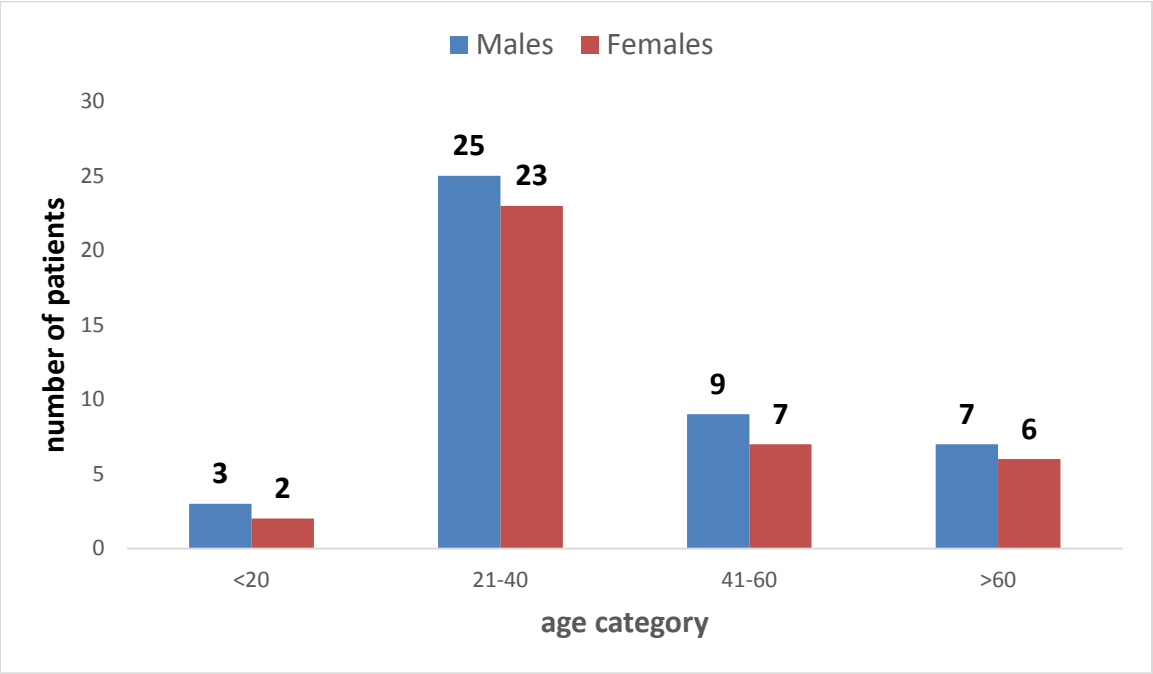

Figure 2: Distribution by age and sex (N=82)

The distribution of patients by age and sex is given in table 2 below.

Table 2: Demographic Characteristics (Age and Sex, N=82)

Parameter	Number of patients (%)
Age (Years)	
Mean (SD)	38.8 (15.3)
Median (IQR)	37 (28-43)
Range	13-85
Sex	
Male	48 (58.5%)
Female	34 (41.5%)

Table 3 below shows the distribution of patients by residence. 50% of patients were from urban setting while 45.1% were from rural areas. There were prisoners and a street boy.

Table 3: Distribution of patients by residence (N=82)

RESIDENCE	**NUMBER**	**PERCENTAGE**
RURAL	42	51.2
URBAN	37	45.2
PRISONERS	2	2.4
STREET MAN	1	1.2
TOTAL	**82**	**100**

Figure 3 below shows the duration the patient stayed before presenting to hospital. 70.7% of the patients presented within one week of symptoms.

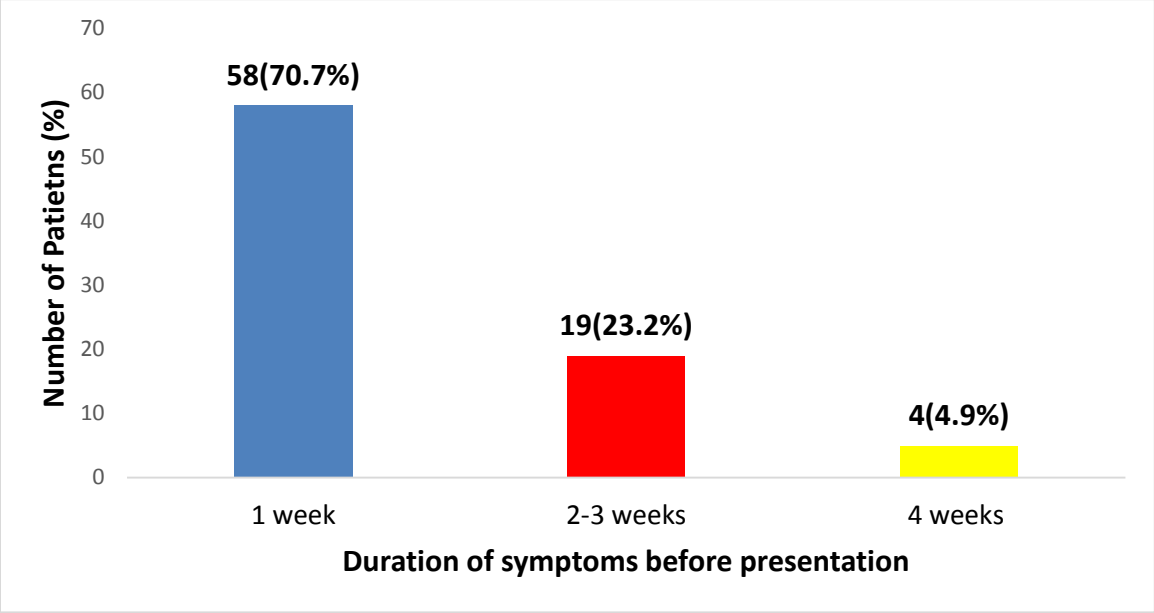

Figure 3: Duration until first presentation (N=82)

Table 4 below displays the interval between onset of symptoms and presentation to hospital for patients with bacterial and fungal ulcers.

Table 4: Duration of Symptoms until First Presentation for Patients with Bacterial and Fungal Corneal Ulcers

Duration before presentation	Bacterial Ulcer	Fungal Ulcer
1 week	29 (87.8%)	31 (67.4%)
2-3 weeks	2 (6.1%)	13 (28.3%)
≥4 weeks	2 (6.1%)	2 (4.3%)
Total	**33 (100%)**	**46 (100%)**

For both types of ulcers, most patients presented within the first week of onset of symptoms. However, fungal ulcers were more likely to delay compared to bacterial ulcers.

6.2 Microbial growth pattern (N=82)

Figure 4 below shows the microbial growth pattern. 56.1% was Fungi. 40.2% grew bacteria while 3.7% cultured both bacteria and fungi.

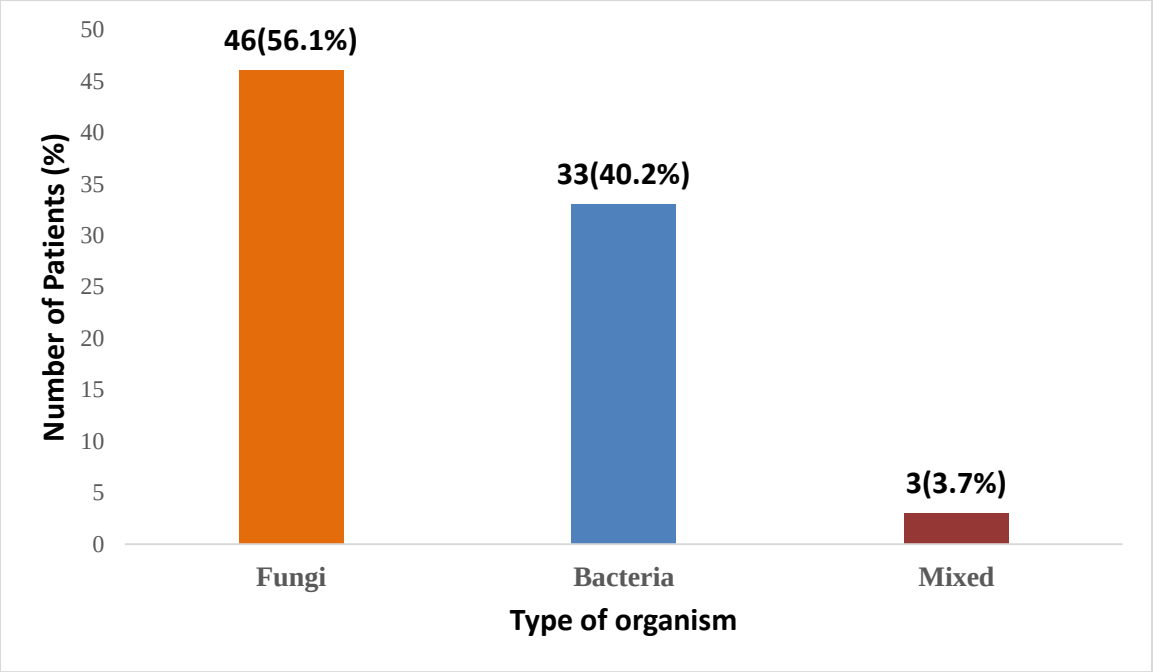

Figure 4: Microbial growth pattern (N=82)

6.3 Occupation

Table 5 below shows the occupation of the patients. Majority of patients were farmers and students.

Table 5: Occupation of patients (N=82)

Occupation	Number	Percentage
Farmers	24	29.3%
Student	20	24.3%
Business/ professionals	14	17.1%
Labourers	11	13.4%
Unemployed	5	6.1%
Children	4	4.9%
Prisoner	3	3.7%
Street boy	1	1.2%
Total	**82**	**100**

Most of the patients with fungal keratitis were farmers and casual labourers as shown in table 6 below. There was one prisoner with fungal keratitis.

Table 6: Occupation of Patients with Fungal Keratitis (N=46)

Occupation	Number	Percentage
Farmer	22	47.8
Labourers	8	17.4
Business/professionals	7	15.2
Students	6	13.0
Unemployed	2	4.4
Prisoner	1	2.2
Total	**46**	**100**

Table 7 below shows that most of the patients with bacterial keratitis were students mainly from the local colleges and universities. Only two were farmers.

Table 7: Occupation of patients with bacterial keratitis (N=33)

Occupation	Number	Percentage
Student	15	45.5
Business/professional	6	18.2
Unemployed	4	12.0
Labourer	3	9.1
Farmer	2	6.1
Prisoner	2	6.1
Street man	1	3.0
Total	**33**	**100**

6.4 Predisposing factors

48.8% of patients had no specific history on the predisposing factors before onset of the microbial keratitis as shown in Table 8 below. 18.3% reported history of specific vegetative matter entering the eye before the onset. 1 patient was being treated after corneal graft with steroids then developed microbial keratitis.

Table 8: Predisposing factors

Factor	Number	Percentage
No specific history	40	48.8
Vegetative matter	15	18.3
Soil/sand	12	14.6
Trauma	10	12.2
Animal matter	4	4.9
Steroid use	1	1.2
Total	82	100

Table 9 below shows that patients with fungal keratitis mostly had no specific history on the predisposing factors. Vegetative matter entry to the eye is a common predisposing factor in fungal keratitis.

Table 9: Predisposing Factors for Fungal Keratitis (N=46)

Factor	Number	Percentage
No specific history	18	39.1
Vegetative matter	15	32.6
Sand/soil	6	13.0
Animal matter	3	6.5
Trauma	2	4.4
Stick	2	4.4
Total	**46**	**100**

Table 10 displays the predisposing factors for bacterial keratitis. Trauma was a common predisposing factor for bacterial keratitis although most of the patients did not report any specific history.

Table 10: Predisposing Factors for Bacterial Keratitis (N=33)

Factor	Number	Percentage
No specific history	22	66.7
Trauma	4	12.1
Sand	3	9.1
Wood	3	9.1
Vegetative matter	1	3.0
Total	**33**	**100**

6.5 Type of organism

6.5.1 Pure fungal growth

Figure 5 illustrates the type of fungi that were isolated from patients with pure fungal growth on microbial culture. Fusariam species were the most grown fungi (56.1%) as pure fungal growth. Aspergillus species was 43.9%

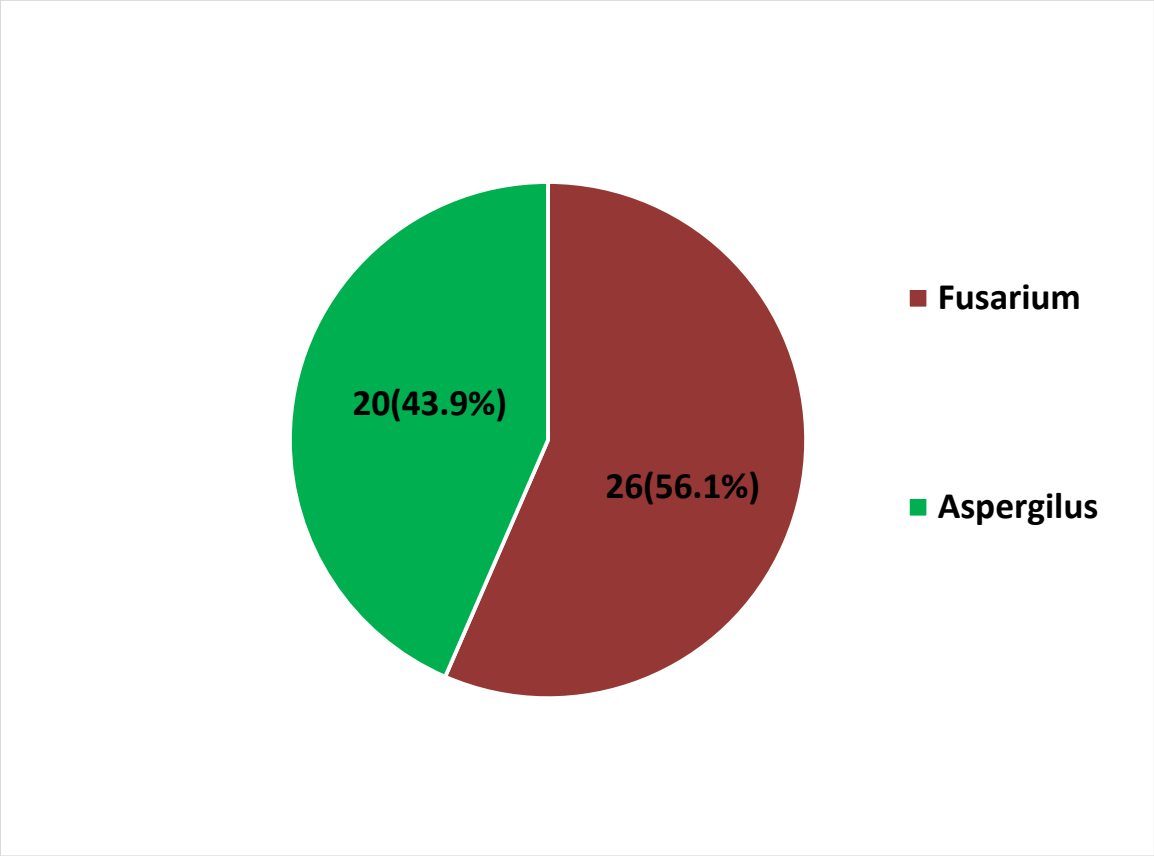

Figure 5: Pure fungal growth n=46

6.5.2 Pure bacterial growth

Figure 6 below indicates the type of bacteria isolated in patients with pure bacterial growth on microbial culture. 90.9% was of gram positive cocci, of which 45.4% were not specified as to which particular organism. 30.3% of the bacterial cultures grew Staphylococcus aureus, 15.2% grew Pseudomonas species and 9.1% grew Streptococcus pneumonia.

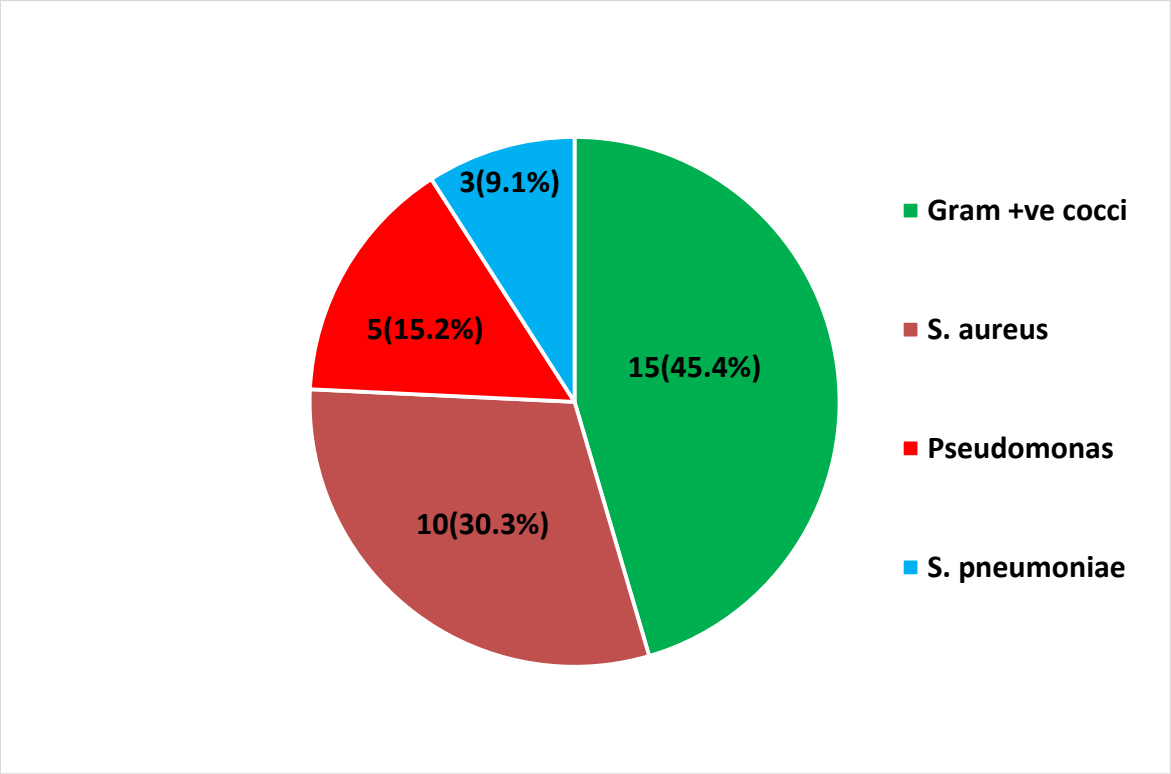

Figure 6: Pure bacterial growth n=33

6.5.3 Mixed culture growth

Table 11 shows the type of microorganisms isolated from cultures with mixed bacteria and fungi growth.

Table 11: Mixed culture growth

Organism	Number
Pseudomonas/ fusarium	1
E. Coli/ candida	1
Staph aureus/aspergillus	1
Total	**3**

6.6 Sensitivity patterns of bacterial keratitis

Table 12 and 13 describe the sensitivity patterns of the bacterial organisms to drugs.

Table 12: Sensitivity pattern of bacterial organisms (N=21)

Drugs	E. Coli (1)	Gram+ cocci (10)	Staph aureus (6)	Pseudomonas (4)
Meropenem	**S**	**S**	**S**	**S**
Ceftazidime	**S**	**S**	**S**	**S**
Ceftriaxone	**S**	**S**	**S**	**R**
Cefuroxime	**S**	**S**	**S**	**R**
Augmentin	**R**	**R**	**S**	**R**
Doxycycline	**R**	**R**	**R**	**R**

Table 13: Sensitivity pattern of bacteria (N=6)

Drugs	S. Pneumoniae (1)	Pseudomonas (1)	Gram + cocci (4)
Imipinem	-	**S**	-
Morepenem	-	**S**	-
Gentamycin	-	**R**	-
Levofloxacin	**S**	**R**	**S**
Ofloxacin	**S**	**R**	**S**
Ampicillin	-	-	**S**
Doxycycline	**S**	-	-
Vancomycin	**S**	-	-
Cotrimazole	**R**	-	-
Chloramphenicol	**S**	-	-
Timentin (ticarcillin+clavulanate)	-	**S**	-

There were total of 33 patients with bacterial keratitis. 81.8% had sensitivity patterns done and in 18.2% it was not done. All the bacteria were sensitive to meropenem and ceftazidime. Resistance was more to doxycycline and augmentin.

7. DISCUSSION

The patients in this study were mostly from rural areas. This is because Kenyatta National hospital is a national referral hospital receiving patients from all over the country. Most of the patients are usually farmers. This study found that the most common causative organism of microbial keratitis in our setting was fungi at 56.1%. This could be because most of the patients were farmers and were prone to trauma by vegetative matter. This finding is similar to a study done by Dhakwa et al. in Western Nepal, who found the commonest organism of microbial keratitis to be fungi predisposed by agricultural trauma.[17]

In this study, keratitis due to a single-bacterial infection is mainly caused by gram positive cocci (90.9%). This finding is much higher compared to that of other studies. For example, Dhakhwa et al. found that gram positive cocci accounted for 56.6% of culture-positive bacterial keratitis.[17] In our study, 45.5% culture results were recorded as just gram positive cocci and 30.3% recorded as staph aureus.

The sensitivity pattern in the study was only done for bacterial organisms. This is because during the study period the fungal sensitivity kit was out of order and when it was eventually acquired, there were no trained personnel to use it. Out of all the patients with bacterial keratitis, 81.1% had their sensitivity patterns determined. There were intervals during which bacterial sensitivity kits had to be changed which could have contributed to why the others were not done. Overall, most of the gram positive cocci were sensitive to cephalosporins. There was an increase in resistance to the third generation quinolones and all were resistant to doxycycline. Pseudomonas species were only sensitive to meropenem, imipenem and timentin. This may be due to the fact

that most patients were started on empirical treatment of quinolones mainly before getting laboratory confirmation of the causative organism, hence promoting antibiotic resistance.

The sensitivity discs that were used were the same as the ones used for systemic diseases. There was no dose adjustment for the ocular diseases and no consideration for the fact that topical medications were used. The drugs available for sensitivity were not the common ones used in ocular diseases. They were based on the current drug formulations for systemic diseases which were available in the hospital. This may have limited the study's ability to pick up the current sensitivity patterns for microbial keratitis and the resistance of the available drugs.

In this study, doxycycline was included in the drug sensitivity analysis. The role of doxycycline in microbial keratitis is usually mainly as a modulator of corneal damage, not as a part of the specific treatment regimen. Therefore, it does not add value to include it in the sensitivity discs to get the resistance patterns in microbial keratitis.

8. STUDY LIMITATIONS

Due to the design of the study, the following limitations were encountered in this study:

1. This was a retrospective study as such some of the data from patients' files were unavailable or incomplete.
2. The sensitivity pattern for fungal organisms was not done because the sensitivity disc was not working. The problem has been fixed now.
3. Not all the patients who had their cultures done had their drug sensitivity done.
4. Some of the culture results did not specify exactly which organism was cultured. They were just reported as gram positive cocci.
5. There was a likelihood of missed diagnosis of bacterial infection in sterile cultures for patients who had received treatment in other facilities prior to the time the corneal scrapping was done.

9. CONCLUSIONS

1. The most common causative organism for microbial keratitis was the fungi *Fusariam* species.
2. Most patients with fungal keratitis were farmers and casual labourers. The predisposing factor for fungal keratitis was trauma by vegetative matter while for bacterial keratitis most of them had no specific history.
3. Gram positive organisms were mainly sensitive to cephalosporins and flouroquinolones while the gram negative organisms were sensitive to ceftazidime and meropenem and resistant to the flouroquinolones.

10. RECOMMENDATIONS

1. Corneal scrapping for culture and sensitivity should be done to all patients with microbial keratitis.
2. Proper protocol based on sensitivity patterns should be adopted on empirical treatment of microbial keratitis.

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12. APPENDICES

12.1 Appendix I: Consent Form

Consent information

I, Dr Felix Ongango of the Department of Ophthalmology, University of Nairobi, am conducting a study to establish the causative organisms and their sensitivity pattern in Kenyatta National Hospital. Microbial keratitis is one of the potentially vision threatening condition that requires prompt diagnosis and treatment to prevent untoward outcomes. The purpose of the study is to find out the causative organisms in microbial keratitis and their sensitivity patterns among patients at Kenyatta national hospital.

The study will be a retrospective study using the records from the microbiology department and patient files and will be conducted at the Kenyatta National Hospital microbiology department and patient files. This study will be beneficial since information obtained will be useful for rationale use of antimicobials and management of microbial keratitis. Permission and authorization for the study will be sought from all the responsible authorities before commencement of the study and therefore all the risks of data abstraction will be dealt with. The data required will include the patients' demographic information, medical history and predisposing factors with regards to microbial keratitis and laboratory results of the corneal scrapings and culture. Throughout the study, the data obtained will be treated with strict confidentiality and will not be accessed by a third party.

Any question and concern can be addressed to Felix Ong'ang'o at felixongango@gmail.com / 0720950171 or Dr. Gichuhi at drgichuhi@yahoo.com/0722873059 or Prof. Jefitha Karimurio at

jkarimurio@gmail.com/0718057138 . Questions can also be addressed to the University of Nairobi/ Kenyatta National Hospital Review committee

By signing below, you indicate your permission for the data abstraction

_____ _____

Sign Date

12.2 Appendix II: Study Budget

MMed Thesis Budget

TITLE: Causative organisms in microbial keratitis and their sensitivity patterns in Kenyatta National Hospital

Principal Investigator: Felix Ongango

Item	Quantity	Unit Cost	Total Cost
Proposal/Ethical approval and ministry of Education approval			
Proposal writing & printing	6 copies	Ksh 10 per page	4000
Binding Proposal	6 copies	100	600
Ethics	1	2000	2000
Airtime		Ksh. 3 per minute	2000
		Subtotal	**8600**
Data Collection			
Typing and Printing of Questionnaires		60 per copy	300
Photocopy of questionnaires		18 per copy	10000
Stationary –pens, rubbers etc			2000
Flash Disc 16GB Hp	1	4500	4500
Box files for filing questionnaires	10	450 each	4500

		Subtotal	**21300**
Contracted services			
Statistician	1		50000
Research assistant	1		25000
	1		
		Subtotal	**75000**
Printing costs and binding of Final book			
Finished book printing(120 pages approximately)	8 copies- 100 pages	Ksh 10 per page	8000
	8 copies- coloured20 pages	Ksh 30 per page	4800
Binding Finished book	2 copies- marking	100 per book	200
	8 final copy(black cover)	300	2400
		Subtotal	**15400**
TOTAL BUDGET			**120300**

Signature: ---------- Date: …….

12.3 Appendix III: Itemized Consent

Title of Study: Causative Organisms in Microbial Keratitis and their sensitivity patterns in Kenyatta National Hospital

Sponsor: SELF

1. Principal Investigator

   Dr Felix Ongango

   University of Nairobi

2. Supervisor

   Dr. Gichuhi

   University of Nairobi

3. Supervisor

   Prof. Jefitha Karimurio, PhD Trachoma control expert,

   University of Nairobi, Kenya

Introduction

My name is Dr Felix Ongango. I am doing my post graduate masters in Ophthalmology at the University of Nairobi. My post graduate thesis is on Causative organisms in microbial Keratitis and their sensitivity patterns at Kenyatta National Hospital. It is a retrospective study from January 2010 to December 2015.

Microbial keratitis is the second cause of legal blindness by cornea opacification world-wide. It is caused by microbial organisms such as bacteria and fungi. Other causes include viruses, parasites, trauma and autoimmune disorders.

The purpose of this consent form is to give you information that might help you to decide whether to participate in the study or not. You are allowed to ask questions related to the study and implications on your part. **The consenting process will take place in a private place that is comfortable to you.**

Purpose of study

The results of this study will enable us to know the common microbial organisms that cause keratitis and their sensitivity patterns in Kenyatta National Hospital and enhance rationale use of antibiotics and know the predisposing factors.

Study design and site

The study will be a retrospective study done at the Microbiology department and Records department at the Kenyatta National Hospital.

Procedures to be followed

The principal investigator together with the research assistants will examine the corneal scrapping records from the microbiology laboratory and obtain data on the patients who had corneal scrapings done and their patient numbers from January 2010 to December 2015. They will then use the patient numbers to trace their files from the main records department and obtain other relevant data from the patients' files.

Benefits

The results of the study will enable us to better manage microbial keratitis and help prevent it by knowing the predisposing factors.

Risks of accessing records

There is no risk if we access the records in this study. We will maintain privacy and confidentiality of all information obtained.

Assurance of confidentiality

The information given and records will remain confidential and will not appear when we present this study or publish its results. You will receive a copy of the consent form.

Storage of data

The data will be stored in secure cabinets and computers with password/s and will only be accessible to the investigators.

Range of information desired

Patient demographic data, their predisposing factors to microbial keratitis and laboratory results of corneal scrapings and sensitivity patterns.

Right to refuse or withdraw

It is important that you understand the following general principles that will apply to all participants in the study:

1. Participation is entirely voluntary.

2. You may withdraw from this study at any time without penalty or loss of benefits.

Please feel free to ask any questions that you may have. Do you agree to participate?

I acknowledge that this consent form has been fully explained to me in a language that I understand and had the opportunity to ask questions which have been answered to my satisfaction. I agree voluntarily to participate in this study and understand that I have the right to withdraw at any time without penalty.

Participant's name (optional): _____

Participant's signature or thumb print: _____

Date: _____

Study No.:

Name of witness: _____

Signature of witness: _____ Date: _____

Investigator's signature: _____ Date: _____

Contact: If you have questions in future, please contact **The Secretary, University of Nairobi, College of Health Sciences Ethical Review Committee, P. O. Box 19676-00202, Nairobi, Telephone: 020-2726300-9 ext 44355, email** uonknh_erc@uonbi.ac.ke

12.4 Appendix IV: Work plan

	Jan	Feb	Mar	Apr	Mai	Jun	Jul	Aug	Set	Oct	Nov	Dec
Proposal presentation												
Ethics approval												
Training of research assistant (experienced ophthalmic assistant)												
Data collection												
Data analysis												
Report writing												
Dissemination of the result												

12.5 Appendix V: Study tool

Demographics	Particulars	Number
Sex	Male	
	Female	
Age in years	<20	
	21-40	
	41-60	
	>60	
Residence	Rural	
	Urban	
Occupation	Labourers	
	Homemakers	
	Business/professionals	
	Students/children	
	Others	
First presentation	Within 1st week	
	2nd -3rd week	
	>4 weeks	

Prior antimicrobial use	Yes (specify and duration)	
	No	

Predisposing factors

Trauma	
Vegetative matter	
Animal matter	
Sand/stone	
Wooden material	
Miscellaneous	
Coexisting ocular disorder	
Coexisting systemic disorder	
Inadvertent use of steroids	
No specific history	

Growth pattern of microorganisms

Type of organism	Number
Definite bacterial growth	
Definite fungal growth	
Mixed microbial growth	
Patients with positive cultures	
Patients with negative cultures	

Identification of bacterial isolates

Gram positive organisms	Pure isolates	Mixed
Staph aureus		
Strep pneumo		
Gram negative organisms		
Pseudomonas spp		
Actinobacter		
Citrobacter		
Nocardia		

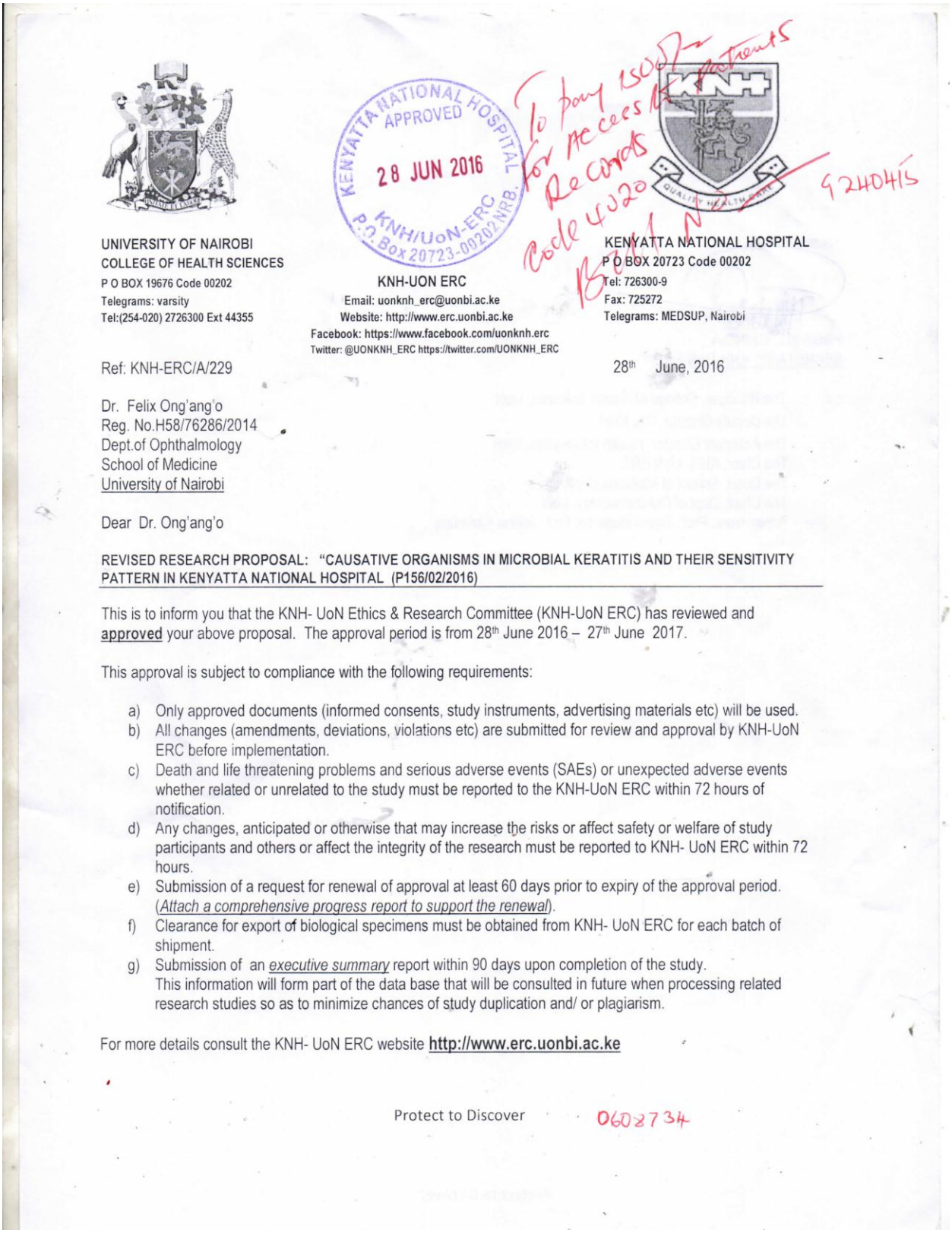
Identification of fungal isolates

Type of organism	Pure isolate	Mixed
Aspergillus spp		
Fumigates		

Identification of sensitivity pattern

Organism/drug	Susceptible	Intermediate	Resistant

12.6 Appendix VI: Ethical Approval certificate



KENYATTA NATIONAL HOSPITAL APPROVED 28 JUN 2016 KNH/UoN-ERC P.O. Box 20723-00202 NAIROBI

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Ref: KNH-ERC/A/229

28th June, 2016

Dr. Felix Ong'ang'o
Reg. No.H58/76286/2014
Dept.of Ophthalmology
School of Medicine
University of Nairobi

Dear Dr. Ong'ang'o

REVISED RESEARCH PROPOSAL: "CAUSATIVE ORGANISMS IN MICROBIAL KERATITIS AND THEIR SENSITIVITY PATTERN IN KENYATTA NATIONAL HOSPITAL (P156/02/2016)

This is to inform you that the KNH- UoN Ethics & Research Committee (KNH-UoN ERC) has reviewed and approved your above proposal. The approval period is from 28th June 2016 – 27th June 2017.

This approval is subject to compliance with the following requirements:

a) Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
b) All changes (amendments, deviations, violations etc) are submitted for review and approval by KNH-UoN ERC before implementation.
c) Death and life threatening problems and serious adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH-UoN ERC within 72 hours of notification.
d) Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH- UoN ERC within 72 hours.
e) Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. (*Attach a comprehensive progress report to support the renewal*).
f) Clearance for export of biological specimens must be obtained from KNH- UoN ERC for each batch of shipment.
g) Submission of an *executive summary* report within 90 days upon completion of the study. This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/ or plagiarism.

For more details consult the KNH- UoN ERC website **http://www.erc.uonbi.ac.ke**

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