MICROBIAL ETIOLOGY AND ANTIMICROBIAL RESISTANCE AMONG PATIENTS WITH INFECTIVE KERATITIS AT TWO TERTIARY EYE HOSPITALS IN DAR-ES-SALAAM

PAUL BARTHALOME, (MD)

A Dissertation submitted in partial fulfillment of the requirements for the Degree of Master in Medicine (Mmed Ophthalmology) at Muhimbili University of Health and Allied Science

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MUHIMBILI UNIVERSITY OF HEALTH AND ALLIED SCIENCES

SCHOOL OF MEDICINE

DEPARTMENT OF OPHTHALMOLOGY

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CERTIFICATION

The undersigned certify that she has read and hereby recommend for examination of dissertation entitled "**Microbial Etiology and Antimicrobial Resistance among Patients with Infective Keratitis at Two Tertiary Eye Hospitals in Dar-Es-Salaam, i**n fulfillment of the requirement for the degree of Master of Medicine (Ophthalmology) of Muhimbili University of Health and Allied Sciences.

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DECLARATION AND COPYRIGHT

I, **Dr. Paul Barthalome** declare that this **dissertation** is my original work and that it has not been presented and will not be presented to any other university for a similar or any other degree award.

Signature……..……..

Date…….

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Firstly, I would like to thank the Almighty GOD for his endless blessing and guidance from the start of my research and throughout until it is completed.

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DEDICATION

I dedicate this work to my lovely mother for her tender love and patience through out my life.

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LIST OF ABBREVIATIONS

1. AIDS	Acquired Immunodeficiency Syndrome
2. AST	Antimicrobial susceptibility test
3. CCBRT	Comprehensive community based rehabilitation in Tanzania
4. CLSI	Clinical laboratory standard institute
5. FBG	fasting blood glucose
6. HIV	Human immunodeficiency virus
7. KIA	Kigler iron agar
8. KOH	Potassium Hydroxide
9. KCMC	Kilimanjaro Christian Medical Centre
10. MIC	Minimum inhibitory concentration
11. MNH	Muhimbili National Hospital
12. MUHAS	Muhimbili University of Health and Allied Sciences
13. NPL	No perception of light
14. PITC	Provider initiated testing and counseling
15. RBG	Random blood glucose
16. SDA	Sabouraud's dextrose agar
17. SIM	Sulfide Indole motility
18. SPSS	Statistical package for social sciences
19. VA	Visual acuity
20. WHO	World health organization

ABSTRACT

Background

Infective keratitis is a commonly encountered blinding ocular emergency; it is a major cause of corneal related blindness. The etiology can be polymicrobial and tend to vary with time and geographic location. Knowledge of local microbial etiology and drug resistance on infective keratitis is essential for selection of appropriate antimicrobial agents there by improving quality of care and outcomes of treatment for patients with infective keratitis.

Aim To determine microbial etiology and antimicrobial resistance among patients with infective keratitis at Muhimbili national hospital (MNH) and Comprehensive Community Based Rehabilitation in Tanzania (CCBRT) Hospital.

Methodology; Hospital based cross sectional study was conducted at MNH & CCBRT hospitals among adult patients with infective keratitis from July to December 2020. Consecutive sampling was used to recruit 58 participants. Infective keratitis was defined as an inflammation of the cornea characterized by an ulcer or epithelial defect with infiltrates associated with signs and symptoms of ocular inflammation**.** Structured questionnaire was used to obtain the demographics and associated factors for infective keratitis of the participant and cornea-scrapping specimens were taken for microbiology laboratory tests. Data was analyzed using SPSS version 23.

Results; This study involved 58 participants attended at MNH and CCBRT during the study period who were diagnosed to have infective keratitis, majority of the participants were males 65.5% .The median age of the participant was 36.30 years with a range of 18years to 80years, majority were from Dar es salaam 74.1% and most of the participants 56.9% came late with blind eyes VA less than 1.301logMAR. Microbial etiology for infective keratitis in this study was bacteria in 84.5% of the participants. The commonest bacteria isolate was staphylococcal aureus in 36.1% of the participants. The leading associated factor for infective keratitis was history of topical steroid use in 41.4% of the participants. The sensitivity of antimicrobial was highest with floroquinolone by 100%, whereas the highest resistance was shown by penicillin's by 100%.

Conclusion and Recommendations. Microbial etiology for infective keratitis at Dar-es-salaam is mostly bacterial with variable sensitivity to the commonly used Antibiotics and the leading associated factor for infective keratitis was topical steroid use. Therefore Floroqunolone or aminoglycoside monotherapy should be used in the initial ttreatment of infective keratitis in Dar es Salaam, due to their higher senstivity levels and initiatives should be taken to control over the counter corticosteroid use for ocular pathologies.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Infective keratitis is considered as a loss of superficial corneal tissue due to necrosis secondary to an infective process(1).It is the leading cause of cornea related blindness worldwide and is the leading cause of prolonged ocular morbidity and visual loss in low resource countries(2)(3). According to World health organization (WHO) report, corneal opacity which is one of the sequelae of infective keratitis ranks sixth in the common causes of uniocular blindness (4). Corneal blindness is responsible for 1.5-2 million new cases of monocular blindness every year(2).In Africa and Tanzania the magnitude of infective keratitis is less known. There are limited and very old studies on infective keratitis in Tanzania, in the study that was done at MNH 10years ago the most common etiological agents of infective keratitis were bacteria 42% followed by fungal 41 %(17). In another study that was done at KCMC 11 years ago the prevalence of infective keratitis was 54% (16). Either record review at MNH for the past 4 years (from 2015 to 2018) indicated an average of 50 adult patients were admitted with infective keratitis annually.

Bacteria, fungi and acanthamoeba are important etiological agents in the developing world. Whereas viral infections are the leading causes of infective keratitis in the developed nations(2)(5). This varies with time and by geographical area(1).

Like microbial etiologies, the associated risk factors for infective keratitis also vary with time and by geographical area. The Major risk factors for infective keratitis in developed countries are contact lens wear, history of keratoplasty and ocular trauma(6). On other hand vegetative ocular trauma, use of contaminated eye medication, use of traditional eye medications, and HIV immunosuppression are the common predisposing factors for infective keratitis in developing world(3)(7).The other predisposing factors include ocular surface disorders such as blepharitis, dacryocystitis ,dry eyes ,allergies and diabetes mellitus(6)(7).

The pathogenesis of infective keratitis involves adhesion of offending organisms (in most cases on violated cornea epithelia of different causes) after which they multiply and destroy the deep layers of the cornea either through cytotoxic effects of their toxins and /or through inflammation

set in response to these organisms and their toxins. These lead to stromal necrosis and formation of ring infiltrates. The depth of invasion, affected cornea layers and symptomatology vary based on the virulence of the causative organisms together with status of local and systemic immunity of the patient. Most fungi penetrate the anterior chamber whereas most of bacteria and viruses are limited within the cornea stroma by the descement membrane (8).

Almost any organism can invade the corneal stroma if the normal corneal defense mechanisms are compromised. However, some organisms can penetrate intact epithelium. These are *Neisseria gonorrhoeae, Corynebacteriium diphteriae, Pseudomonas aeruginosa, and Haemophilus influenza* (7).

By appearance alone, it can be difficult to determine the etiology of an infective keratitis hence the need for laboratory investigations to establish the etiology. In most cases the signs and symptoms of different types of an infective keratitis overlap.

Bacterial keratitis

Is characterized by rapid onset of pain accompanied by conjunctival injection, photophobia, decreased vision and discharges from the eyes. The rate of progression of these symptoms depends on the virulence of the infecting organism. Bacterial keratitis typically show a sharp epithelial demarcation with underlying dense, suppurative stromal inflammation that has indistinct edges and is surrounded by stromal edema. *P aeruginosa* typically produces stromal necrosis with a shaggy surface and adherent mucopurulent exudate. An endothelial inflammatory plague, marked anterior chamber reaction, and hypopyon frequently occur(9)

Fungal keratitis

Patients with fungal keratitis tend to have fewer inflammatory signs and symptoms during the initial period than those with bacterial keratitis and may have little or no conjunctival injection upon initial presentation.

Filamentous fungal keratitis frequently manifests as a gray-white, dry-appearing infiltrate that has irregular feathery or filamentous margins. Superficial lesions may appear gray-white, elevate the surface of the cornea, and have a dry, rough, or gritty texture detectable at the time of diagnostic corneal scraping. Occasionally, multifocal or satellite infiltrates may also be present. In addition, a deep stromal infiltrate may occur in the presence of an intact epithelium. An endothelial plaque

and/or hypopyon may also occur if the fungal infiltrate(s) is sufficiently deep or large. As the keratitis progresses, intense suppuration may develop and the lesions may resemble bacterial keratitis. At this point, rapidly progressive hypopyon and anterior chamber inflammatory membranes may develop. Extension of fungal infection into the anterior chamber is often seen in cases with rapidly progressive anterior chamber inflammation. Occasionally, fungus may invade the iris or posterior chamber, and angle-closure glaucoma may develop from inflammatory pupillary block. Yeast keratitis is most frequently caused by Candida species. This form of fungal keratitis frequently presents with superficial white raised colonies in a structurally altered eye. Although most cases tend to remain superficial, deep invasion may occur with suppuration resembling keratitis induced by gram-positive bacteria (10)

Acanthamoeba keratitis

Present with excruciating eye pain (a pain which is disproportion to the amount of inflammation) characterized by redness, epiphora, lacrimation, conjunctival hyperemia, foreign body sensation, and photophobia. As the disease progresses, stromal involvement results in infiltration of inflammatory cells, displaying a characteristic ring infiltrate. This progress to corneal ulceration, perforation, and ring infiltrate, stromal abscess formation, loss of visual acuity, and eventually blindness (11).

The diagnosis of infective keratitis is based on the signs and symptoms mentioned above according to etiological agents together with microbiology of the cornea scraps obtained from the patient with infective keratitis. The cornea scraps are subjected to various laboratory procedures that include gram stain, culture and antimicrobial susceptibility tests (AST)(2)(12).

Treatments of infective keratitis can be empirical where by treatment is initiated based on the clinical picture of the keratitis without knowing microbial etiology and antimicrobial susceptibility or specific treatment based on the culture and sensitivity results. Empirical treatment is normally used in the initial phase of treatment of infective keratitis and it involves the use of broad spectrum antimicrobials based on the known etiological agents and drug susceptibility of common etiological agents of a particular locality.

Ocular morbidity from infective keratitis such as corneal scarring and subsequent visual loss can be significantly reduced by prompt institution of appropriate treatment guided by the knowledge

of the microbial etiology and drug susceptibility(2)(5). Among the challenges in treatment of infective keratitis is antimicrobial resistance.

Different organisms have different modes of resistance to the antimicrobial agents. The three fundamental mechanisms of antimicrobial resistance are enzymatic degradation of antibacterial drugs, alteration of bacterial proteins that are antimicrobial targets, and changes in membrane permeability to antibiotics. Antibiotic resistance can be either plasmid mediated or maintained on the bacterial chromosome. The most important mechanism of resistance to the penicillin's and cephalosporin's is antibiotic hydrolysis mediated by the bacterial enzyme beta-lactamase(13). Phenotypic switching or morphogenesis is an adaptive mechanism, which permits fungi to survive in the presence of antifungal drugs and resist antimicrobial therapy. Studies have shown wide spread of gram negative and Gram-positive organisms resistance to various antibiotics(1)(12)(14).

The magnitude of infective keratitis at MNH and CCBRT are not known, but crude data from the registries indicate larger number of patients admitted with diagnosis of infective keratitis. Most of the patients at MNH and CCBRT) receive empirical treatments for their conditions in spite of having functional laboratories in these hospitals probably due to un-established treatment protocols for infective keratitis or low economic status of the patients to pay for laboratory investigations. Because of empirical treatment of infective keratitis drug susceptibility and pattern of antimicrobial resistance is not yet established. The available local research findings for microbial etiologies and drug susceptibility were conducted more than 10 years ago and may not be reliable any more due to rapidly growing resistance to the available antimicrobial drugs. The purpose of this study was to establish the recent microbial etiologies and to test for in vitro antimicrobial resistance at the two tertiary eye facilities of MNH and CCBRT.

1.2 Literature review

1. Microbial etiologies of infective keratitis

The etiological and epidemiological patterns of infective keratitis have been found to vary with the patient population, health of the cornea, geographic location and climate, and also tends to vary over time(15).

In a prospective hospital based study done in India, seventy one percent of the patients were culture positive of which 67.44% were positive for fungi and 32.56% gave a positive yield for bacteria. Aspergillus species was identified in 37.21% of patients, while Fusarium species was detected in 30.23% of patients. Among the patients who tested positive for bacteria, *Staphylococcus aureus* was isolated in 20.93% patients, *Streptococcus pneumoniae* was identified in 6.98% patients, while 4.65% patients tested positive for *Pseudomonas aeruginosa*(2).

In another study, which was conducted at a tertiary eye care hospital in Bangalore India, 37.5% of patients were positive for smear and culture. Microbial etiology was bacterial in 44.5% and fungal in 49.5% of cases. The most common fungus isolated was Fusarium in 31% eyes followed by Aspergillus species found in 11% of the cases. Common bacterial isolates were *Staphylococcus aureus* in 18% of the subjects followed by *Streptococcus pneumoniae* from the Gram positive bacteria, and *Pseudomonas aeruginosa* in 8.5% cases followed by *Klebsiella pneumoniae* from the Gram negative bacteria(1).

Also a multicenter study, which was carried out in Ghana and southern India to determine the etiology of infective keratitis in two regions, fungi were identified in 40% of patients in southern India and 37.6% of patients in Ghana. Bacteria were isolated from 29.3% of cases in south India and 13.8% in Ghana; In Ghana bacteria species were; Pseudomonas species 52.5%, followed by Streptococci 20% and staphylococci 10%. In India streptococci accounted for 46.8% of cases of infective keratitis followed by staphylococci 26.8% and pseudomonas 14.9%.Fusarium and Aspergillus species were isolated from 61% of all fungal infections and comprised 83% of identified fungal isolates(5).

Few studies have been conducted in East Africa and Tanzania. In a retrospective review of all individuals admitted to Kilimanjaro Christian Medical Centre (KCMC) with microbial keratitis between the 1st January 2008 and 31st March 2010, organisms were seen on 37% of cases, gram

stain examinations and cultures were positive in 54% of the cases. Two point six percentages were Gram-positive cocci, 2.6% Gram-negative rods, 5.3% Candida, and 23.7% filamentary fungi. The isolates were *Streptococcus pneumoniae1.7%, Streptococcus Viridans* 3.5%,*Pseudomonas aeruginosa* 5.3%, *staphylococcus epidermidis* 14.1%,Bacillus species.1.7% and Candida species 3.5% (16).

In another study that was conducted in Dar es salaam Tanzania at MNH 10 years ago on microbial etiology of corneal ulceration, the most common etiological agent were bacteria 42% followed by fungal 41% .The common isolated bacteria were staphylococcus species 24% followed by streptococcus 18% .Candida species was the only fungal isolate in 10% of the patients(17).

2. Factors associated with infective keratitis

Several risk factors are associated with infective keratitis depending on the study setting. Contact lens wearing is a common risk factor in developed countries whereas trauma by vegetative matter is a risk in rural populations(18)**.**

In a retrospective analysis of the hospital records of patients presenting with bacterial keratitis and treated at the Quinze-Vingts National Center of Ophthalmology, Paris, France, found Contact lens wear was the main risk factor accounting for 50.3% of the cases. Trauma and a history of keratopathy was found to be in 15% and 21% of the cases, respectively(6).

In a prospective observational cohort study conducted in Aravind Eye Hospital, India. The mentioned risk factors for microbial keratitis were cornea injury 71.5% co-existing ocular diseases 26.7% and diabetes mellitus 6.6%. Others included prior use of over the counter eye drops and traditional eye medicine use(3).

Study which was done in Ethiopia about incidence and risk factors of bacteria keratitis different predisposing factors for bacterial keratitis were identified including corneal trauma 38%, blepharitis 29%, herpetic keratitis 20%, and use of contaminated medications 20% (7).

In a prospective, cross-sectional study which was conducted in Kano, northern Nigeria from November 2014 to July 2015 about Clinical pattern and antibiotic sensitivity of bacterial Corneal Ulcers the identified risk factors included multiple risk factors 41.6%, cornea trauma 33.8%,

traditional eye medication use14.3%, prior steroid use in the eye7.8%, diabetic mellitus2.6%, contact lens use 0%, and ocular surgery 0%(20).

Descriptive cross sectional and correlation study which was done at MNH 10years ago indicated more cases of infective keratitis among HIV patients (60%) compare to (40%)of HIV negative patients(17).

3. Antimicrobial susceptibility and resistance pattern in-patient with infective keratitis

Since the causes of infectious keratitis largely vary depending on climate and geography, it is important to analyze regional microbial profiles and antibiotic susceptibility patterns for evidence-based selection of empirical treatment regime

Results from a study which was done at tertiary care teaching hospital in north Karnataka India between August 2009 and September 2011 showed most of bacterial isolates were sensitive to Amikacin by 100%, Gentamycin (50-100%) and Ofloxacin by (69-90%). Other tested antimicrobial included Neomycin, Chloramphenicol, Ciprofloxacin, Polymyxin B, Cefazolin, Norfloxacin, Tobramycin, and Fusidic acid(19).

In another two year study which was conducted at a tertiary eye care hospital in Bangalore India from June 2012 to June 2014, all isolated Gram positive cocci were susceptible to vancomycin by100% followed by, chloramphenicol 86%-100%, gatifloxacin 71%-100% and moxifloxacin 67%-100% whereas all Gram negative bacilli were susceptible to gatifloxacin by 100% followed by moxifloxacin 90%-100% and ofloxacin by 80-100%(1).

In a prospective, cross-sectional study which was conducted in Kano, northern Nigeria from November 2014 to July 2015; isolates were sensitive to ciprofloxacin 94.4%, ofloxacin 91.7%, and gentamycin 72.2 %(20).

Recently, antimicrobial resistance has emerged as a major issue in infection control. In ophthalmology, the incidence of infectious keratitis has risen in the last decade, partially due to an increasing number of contact lens users and immune-compromised patients. Also, the changes have been reported in microbial compositions responsible for infectious keratitis and antibiotic resistance patterns(14).

Results from two years study which was conducted at a tertiary eye care hospital in Bangalore India from June 2012 to June 2014; the highest resistance was seen to ciprofloxacin and gentamycin though no figures were given in the results (1).

Similarly, results of a ten-years analysis of microbiological profile and antibiotic sensitivity for bacterial keratitis in Korea, showed that all gram-negative bacteria were sensitive to ceftazidime and carbapenem (meropenem and imipenem), and most of gram-negative isolates were susceptible to tobramycin and Amikacin (96.7% and 97.2%, respectively). However, 11.5% of gram-negative isolates were resistant to gentamycin. Ciprofloxacin resistance was observed in 8.8% of gram-negative isolates(14).

In a study conducted in Kano northern Nigeria, isolates were almost completely resistant to tetracycline 91.7% which is the most easily available and commonly used drug in most African countries particularly in remote areas. Resistance to penicillin was also very high 97.2%(7)

These reports from literature indicate that until 2015, most gram negative microbes were highly susceptible to vancomycin, gatifloxacin, and moxifloxacin and there are no specific patterns of resistance .No studies which have mentioned about antifungal susceptibilities

1.3 Problem statement

Infective keratitis is the leading cause of cornea related blindness (2).Corneal blindness is responsible for 1.5-2 million new cases of monocular blindness every year(2). Infective keratitis is the second common cause of ocular blindness after cataract in the developing world(21). The associated factors for and causative organisms responsible for infective keratitis vary considerably with time and by region(2). Ocular morbidities such as corneal scarring and subsequent visual loss can be significantly reduced by prompt institution of appropriate therapy guided by the knowledge of the causative agents and drug susceptibility(1). In Tanzania there are very limited studies in this area despite of having these patients attending our facilities, according to the study which was done at KCMC more than 10 years ago the proportion of infective keratitis was 54% (16). Either in most of the hospitals with eye services including MNH and CCBRT which are considered to provide tertiary eye services, infective keratitis patients receive empirical treatment for their conditions. Cornea scrapping for culture and drug susceptibility is not routinely done, probably due to un-established treatment protocols for infective keratitis. The use of empirical treatment in treating infective keratitis often leads to treatment failure and also prolonged hospitalization.

Either the available local research findings for microbial etiologies and drug susceptibility at MNH were conducted 10 years ago and may not be reliable any more due to possibilities of having new microbial etiologies, rapidly growing resistances to available antimicrobial drugs and availability of new antimicrobial agents. This study was conducted to produce current data of microbial etiology and drug resistance pattern for patients with infective keratitis attending MNH and CCBRT.

1.4 Rationale

The findings of this study will be used to establish a protocol for treatment of patients with infective keratitis in order to achieve good post-treatment visual outcome and to improve the quality of care at MNH and CCBRT hospitals and probably beyond. Results of this study are also part of implementation of the World health organization (WHO) global action plan on antimicrobial resistance particularly on its second objective of Strengthening the knowledge and evidence based through surveillance and research.

1.5 Research questions

1. What are the factors associated with infective keratitis in Dar-es –salaam?

2. What are the microbial etiologies of infective keratitis in Dar es Salaam?

3. What is the antimicrobial susceptibility pattern in-patient with infective keratitis in Dar-es-salaam?

1.6 Objectives

1.6.1Broad objective

To determine the microbial etiology and antimicrobial resistance among patients with infective keratitis at Muhimbili national hospital (MNH) and Comprehensive Community Based Rehabilitation in Tanzania (CCBRT) Hospital

1.6.2 Specific Objectives

1. To determine the factors associated with infective keratitis among patients attending MNH and CCBRT hospitals during the study period
2. To determine the microbial etiologies of infective keratitis among patients attending MNH and CCBRT hospitals during the study period
3. To determine antimicrobial susceptibility pattern among patients with infective keratitis at MNH and CCBRT hospitals during the study period

CHAPTER TWO

2.0 Methodology

2.1 Study design

A hospital based descriptive cross sectional study.

2.2 Study area

This study was conducted at MNH and CCBRT hospitals in Dar es Salaam from July to December 2020. Data were collected from the eye departments at the two hospitals. MNH is a national referral and University teaching hospital with 1,500-bed capacity, attending 1,000 to 1,200 outpatients and admitting 1,000 to 1,200 inpatients per week. The hospital has an Ophthalmology department among the other departments which provide both outpatient and inpatient services. Review of records from year 2015 to 2018 showed the average number of admitted adult patients with a diagnosis of infective keratitis was 50 patients every year and overall average number of adult inpatient was 640 patients every year. The average numbers of adult patient attended as an outpatient are 200 patients per week. MNH has a well-equipped laboratory where a variety of investigations including microbiological tests are routinely performed.

CCBRT is a zonal referral hospital located in Dar-es-salaam city serving both in patients and outpatients from different parts across the country. Currently it serves as one of the high volume hospitals providing ophthalmic care in Tanzania with an average of 6,200 monthly eye consultations. The hospital outsources laboratory services to the Lancet laboratory located few kilometers from the hospital.

2.3 Study population

All adult patients attended at MNH and CCBRT eye clinics and wards during the study period.

2.4 Sampling technique

A consecutive sampling technique was used. The investigator visited the wards and outpatient clinics on daily basis and recruited all patients who met the inclusion criteria during the study period.

2.5 Sample size

The sample size was estimated using below formula for finite population

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

Where by

n= sample size with finite population correction

N=size of the population 50 patients, 25 patient from each center, that is an average number of infective keratitis patients admitted or attended as an outpatient for 6 months at MNH and CCBRT

z=95% confidence level which is equal to 1.96

p=proportional of patient with culture positive infective keratitis (54%) from a study which was conducted at KCMC(16)

1-p=proportion of patient without infective keratitis

d=marginal error which is taken to be 5%

n=44 Patients (minimal sample size)

2.6 Inclusion criteria

All adult patients aged 18 years and above with a provisional diagnosis of infective keratitis.

2.7 Exclusion criteria

1. Patient with interstitial keratitis with intact cornea epithelium due to difficulties in obtaining cornea scraps. Interstitial keratitis is non-suppurative keratitis characterized by cornea stromal infiltrates without epithelial defect. It can be diagnosed with the help of slit lamp examination and negative fluorescein staining.
2. Those with severe rapidly progressing infective keratitis who are already on antimicrobials empirically. These are the patients with severe signs and symptoms of infective keratitis over a very short duration that ethically withholding empirical treatment for 12 hours in order to do corneal scrapping is contraindicated
3. Those with viral keratitis. These are the patient who had classical corneal findings for viral infection including punctate epithelial defects with or without sub epithelial infiltrates, linear arborizing epithelial infiltrates with terminal buds star shaped or with

dendritic pattern, or geographical epithelial defect. Viral keratitis is in most cases diagnosed clinically and does not require routinely scrapping for culture and sensitivity.

2.8 Data collection tools

A structured questionnaire was used to collect information on social demographics, associated factors, and clinical findings. A structured laboratory form was used to collect laboratory findings for patients with infective keratitis.

2.9 Data collection procedure

The investigator with the help of an experienced ophthalmologist collected data. At the adult ophthalmology wards and outpatient clinic, patients with a clinical diagnosis of infective keratitis were approached and informed about the study. Infective keratitis was defined as an inflammation of the cornea (clear front part of the eye) characterized by an ulcer or epithelial defect with an infiltrates (sub epithelial or stromal) associated with any of the following signs and symptoms; pain, photophobia, redness of the conjunctiva, tearing, reduced vision, discharges from the eyes with or without hypopyon (pus in the anterior chamber). After signing a written informed consent, patients were consecutively recruited into the study. A Structured questionnaire was used to obtain data on social demographics and associated factors which included history of recent ocular trauma, treatment for diabetes mellitus, Human immunodeficiency virus (HIV) infection or Acquire immunodeficiency syndrome (AIDS), contact lens use, prior use of topical steroid eye drops, history of using contaminated eye drops (participants will be asked if they have used previously used eye drops by themselves or by others in the course of the illness), use of traditional eye medication, and history of recent eye surgery.

Visual acuity (VA) of the affected eye(s) were taken using illuminated Snellen charts for both literate and illiterate patients to establish the severity of visual impairment caused by infective keratitis. Recordings were as per WHO categories of visual impairment of year 2019 (normal vision [<6/18-6/6], visual impairment [6/18-6/60], severe visual impairment [<6/60-3/60] and blind<3/60-NPL). The procedure involved asking the patients to occlude the unaffected eyes and use the affected eye to identify the Snellen chart letters in the charts for literate patient or orientation of tumbling E in the chart for illiterate patients with the chart located 6 meters away. When failed examiner would assess if the patient could count fingers at 5, 4,3,2,or 1 meters whichever was possible. Again if failed a hand was waved at nearest distance or light was shown

to asses if the patient could passive. Slit lamp examination was performed using Haag Streit BM 900 to obtain the clinical status of anterior segment of the eyes with infective keratitis that were used to guide on initial treatment and during cornea scrapping procedure. Examination with slit lamp biomicroscope included cornea staining with fluorescein under cobalt blue light filter to locate and estimate the size of cornea lesion. Staining was done by applying a wet fluorescein impregnated paper strip in the inferior conjunctiva fornices and the asking the patient to blink in order to spread the dye on the ocular surface. Wetting of fluorescein impregnated paper strip was done with a drop of tetracaine 1.0% eye drop before instilling fluorescein to the patient's eyes.

The visual acuity, slit lamp examination findings and corneal staining were only used to assess the extent and severity of infective keratitis as part of routine ocular examination of all patients with infective keratitis to guide their initial treatments.

Corneal scrapings were obtained from patients while viewing through the slit lamp. A sterile cotton swab was used to clean the conjunctiva fornices. Corneal scrapings were taken before administration of any antimicrobials. Those already on antimicrobial agents were requested to withhold the medication for 12 hours prior to scrapping in order to enhance recovery of viable organisms. This is the standard procedure for all patients with infective keratitis worldwide in order to establish specific treatment in cases with no improvement with empirical treatment. Scrapings were taken from the edges and bases of the ulcers using a sterile number 15 blade after instillation of topical tetracaine 1.0% anesthesia eye drops to numb the corneal surface in order to make the procedure painless**.**

In patients with impeding corneal perforation or those with perforated corneal ulcers, scrapings were taken from the edges only. The samples were placed in a well-labeled thioglycolate broth bottles immediately and taken to Muhimbili University of Health and Allied Sciences (MUHAS) microbiology laboratory for processing. The principle investigator was responsible for the above procedures at MNH where as one trained assistant investigator performed similar procedure at CCBRT.

2.10 Laboratory procedures

In the laboratory the pre identified laboratory technician performed direct microscopy by taking the scrapings from the transport media on two glass slides, one for Grams staining (bacteria

staining to identify gram positive and gram negative bacteria) and the other for 10% KOH mount (for fungal organisms).

Corneal scrapings samples were cultured on blood agar and chocolate agar for isolation of *Staphylococcus aureus*, coagulase negative, *Neisseria gonorrhoeae* and *Haemophilus influenzae*. MacConkey agar was used to isolate pathogenic organisms (*E.colli*, *Klebsiella*, Proteus and *pseudomonas species* etc.) and Sabouraud's dextrose agar (SDA) for fungal in multiple C shaped streaks. Incubation was done at 37 °C for 48 hours, initially all plates were examined for growth after 24 hours and cultures with no growth were incubated for further 48 hours. Bacterial cultures were confirmed by growth on blood agar, chocolate agar and MacConkey agar. Cultures were considered positive if they met the following criteria: the same organism isolated on two or more media with exception of fastidious organisms, an isolate present on one media and associated with the identification of the same organism on gram stained direct smears and heavy growth at the inoculation site on one solid media [for enteric organisms]. Bacterial isolates were identified based on colonial morphology, Gram stain and biochemical tests. For gram positive; catalase, coagulase and Dnase test and for gram-negative bacteria, oxidase, Citrate, Urea, Kigler iron agar (KIA), sulfide, Indole and Motility (SIM) were performed.

Inoculated SDA were inspected daily for up to ten (10) days and declared as fungal culture negative thereafter. Fungal growths would be grossly identified by their colony morphology on obverse, pigment production on reverse, and microscopically by lacto phenol cotton blue stain. Diagnosis of fungal keratitis was to be made when any of the following criteria was met: correlation between direct KOH examination and growth on SDA and growth on more than one C streak lines.

2.11 Antimicrobial susceptibility testing

Antimicrobial susceptibility testing (AST) were performed using Kirby–Bauer's disc diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines 29th edition(23). The following antibiotics were tested: for gram-negative isolates; tetracycline, tobramycin, ciprofloxacin, moxifloxacin, gentamycin, chloramphenicol, ampicillin, doxycycline and ceftriaxone; cloxacilin was tested for gram-positive isolates only. The selected antibiotics are commonly used in empirical treatment of infective keratitis at MNH and CCBRT and probably in other centers due to their broad spectrum of activity and being cheaply available. Sensitivity testing for antifungal agents are only performed in reference laboratories but the relevance of

their results to clinical effectiveness is uncertain(24) , hence for the sake of this study it was not performed. AST results were interpreted as per CLSI guideline(23).

Quality control

The antibiotics discs were placed correctly to ensure no irregular inhibition zones and overlapping were encountered 6 discs on 100-mm plates were used and discs were placed 24 mm apart center-to-center. When Irregularities and overlapping of the zones of inhibition were observed the whole plate was discarded and repeated on the new plate with new antibiotics discs. Also to ensure standards a commercially available ready-made agar and antibiotic discs from one supplier were used.

2.12 Variables

The independent variables were social demographics i.e., [age, sex, occupation and residence] and duration of symptoms before presenting to the hospital.

Outcome variable were microbial etiologies i.e., [bacteria, fungal, and viral], antimicrobial susceptibilities; categorized as susceptible (S), intermediate (I), resistant (R), or no interpretation (NI), and associated factors for infective keratitis.

Age categories were 25 or less, 26 to 35, 36 to 45, 46 or more

Sex categories were Males and Females

Occupations were categorized as peasants, Student, Business, professionals or Unemployed

Residence were categorized as Rural or urban

Duration of symptoms before presentation was defined as early presentation <7 days, delayed presentation 7-21 days, and late presentation >21days

Antimicrobial susceptibilities; categorized as susceptible (S), resistant (R), or no interpretation (NI) was defined by the diameters of inhibition zones in millimeters (mm) around antimicrobial discs based on Kirby–Bauer's disc diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines 29th edition(23).

2.13 Data analysis

Data were analyzed with the help of statistical package for social sciences (SPSS) version 23. SPSS was used to generate frequency distribution tables for social demographic characteristics, microbial etiologies, anti-microbial susceptibility, and factors associated with infective keratitis. Associations of different factors with infective keratitis were assessed using chi-squared test. The P-value <0.05 was considered statistically significant.

2.14 Ethical Clearance

Ethical clearance to conduct the study was obtained from the Senate research and publication Committee of MUHAS. Permission to conduct the study was sought from executive directors of MNH and CCBRT. Participants were informed comprehensively about the purposes and benefits of the study. The samples of cornea scrap were taken as the part of clinical evaluation of a patient with infective keratitis in order to provide standard specific treatments for the patients. Patients were informed about minimal tolerable discomfort that might arise during scrapping procedure. Those already on antimicrobial agents were requested to withhold the medication for 12 hours prior to scrapping. This is the routine standard procedure for all patients with infective keratitis to increase the yield of microbial growth in order to establish the specific treatment required for the patients with infective keratitis except for those with severe rapidly progressing infective keratitis, which in this study were excluded**.**

All patients were required to sign the consent form after they were clearly informed and agreed to undergo the above procedures. For those who did not consent for the study they were managed empirically as per above. All personal identifiable data was kept privately in the questionnaires and patient files.

After obtaining the corneal scraping specimens, the patients were kept on empirical treatment depending on suspected clinically microbial etiology: Corneal ulcers with regular margins, wet appearance, mobile hypopyon, and with greater symptoms were primarily considered to be bacterial in nature and treated with broad-spectrum antibiotic eye drops like-ciprofloxacin, moxifloxacin and chloramphenicol. On the other hand, corneal ulcers having feathery margins, dry appearance, thick cheesy hypopyon, satellite lesions or with a history of vegetative injury were initially put on antifungal eye drops-like natamycin, econazole and terbinafine or

fluconazole tablets. The initial therapy was also guided by Gram stain or KOH mount findings. Cycloplegic drops and other supportive treatments for patients with infective keratitis were started in all patients. The anti-microbial therapy were reviewed immediately after obtaining the culture and sensitivity report. If no growth was obtained on culture, then the treatment of the patients were continued according to the clinical appearance of the ulcer as well as the clinical response to treatment. Patients with infective keratitis were discharged after ensuring the following; blunting of the perimeter of the stromal infiltrate, decreased density of the stromal infiltrate, decreased stromal edema and endothelial inflammatory plaque, and decreased anterior chamber inflammation, re epithelialization of the corneal epithelial defect and Improvement in painful symptoms.

CHAPTER THREE

3.0 RESULTS

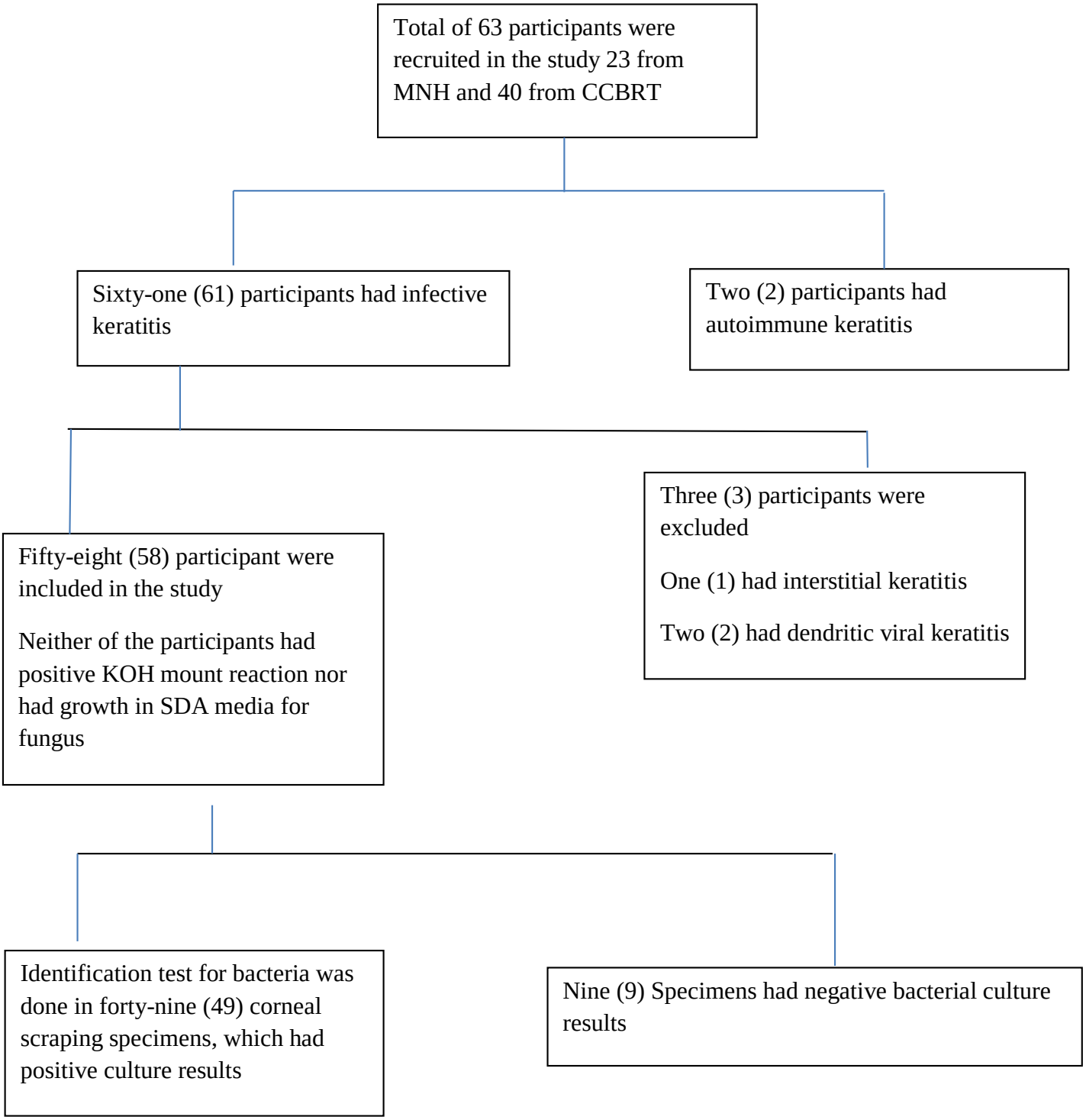

Figure 1.Flow chart illustration of recruitment process and eligibility for antimicrobial susceptibility tests.

Majority of the study participants 65.5% were male. The age range was 18 to 80 years with the median age of 36.30 years. Most participants were from Dar es Salaam (Table 1)

Table 1: The Socio-demographic characteristics of the study population (N=58)

Character	Frequency (n)	Percentage (%)
Age categories (Years)		
25 or less	15	25.9
26 to 35	13	22.4
36 to 45	13	22.4
46 or more	17	29.3
Sex		
Male	38	65.5
Female	20	34.5
Education level		
Informal	9	15.5
Primary school	34	58.6
Secondary school	11	19
University/college	4	6.9
Occupation		
Employed	13	22.4
Peasant	15	25.9
Others	30	51.7
Residence		
Dar es Salaam	43	74.1
Other regions	15	25.9

Table 2. Clinical characteristics of the study population N=58

Character	Frequency	Percentage %
Type of referral		
Self-referral	35	60.3
Referral from primary health facility	23	39.7
Duration of symptoms (weeks)		
<1	25	43.1
1 to 3	20	34.5
>3	13	22.4
History of using topical antimicrobials before cornea scraping		
Yes	**38**	65.5
No	**20**	34.5
Visual acuity of the affected eye		
<6/18 – 6/6	7	12.1
6/18 – 6/60	9	15.5
<3/60 – PL	42	72.4
Location of ulcer		
Central	36	62.1
Para central	15	25.9
Peripheral	4	6.9
Extensive ulcer	3	5.2
Depth of ulcer		
Superficial	27	46.6
Stromal	29	50
Presence of desmatocele	2	3.4
Hypopyon		
Present	19	32.8
Absence	39	67.2

Majority of the participants came more than 1 week after the onset of symptoms. Most of the affected eyes were blind (VA <3/60) with central located ulcers. (Table3)

Table 3: Factors for infective keratitis N=58

Risk factors	Frequency (n)	Percentage (%)
History of ocular trauma		
Yes	12	20.7
No	46	79.3
History of Diabetes mellitus		
Yes	6	10.3
No	52	89.7
History of using topical steroid eye-drop		
Yes	24	41.4
No	34	58.6
History of contact lens wear		
Yes	1	1.7
No	57	98.3
History of using traditional eye medication		
Yes	1	1.7
No	57	98.3
HIV serology		
Positive	2	3.4
Negative	30	51.7
Not tested	26	44.8

The leading associated factor for the infective keratitis was topical steroid use 24(41.4%) followed by ocular trauma 12(20.7%). History of contact lens wears, and the use of traditional eye medication was found in only one patient respectively, none of the participants had history of eye surgery. (Table3)

Half of cornea scraping specimens was gram positive

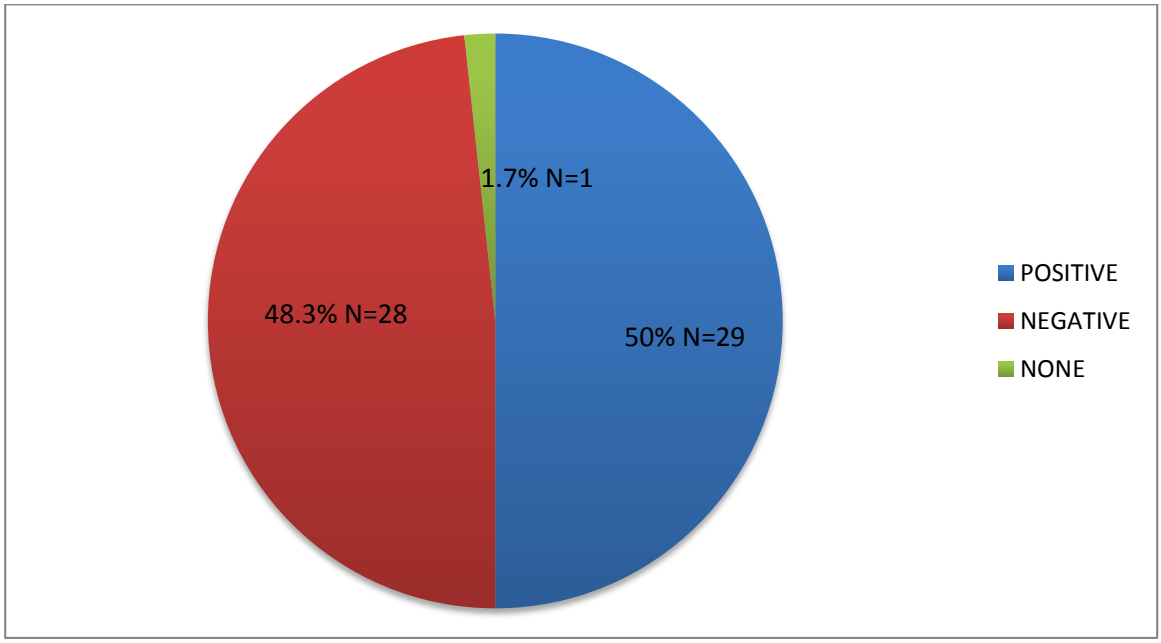

Figure 2. Gram staining for the cornea scraping specimens

Majority of the corneal scraping specimens 49 (84.5%) had bacterial growth

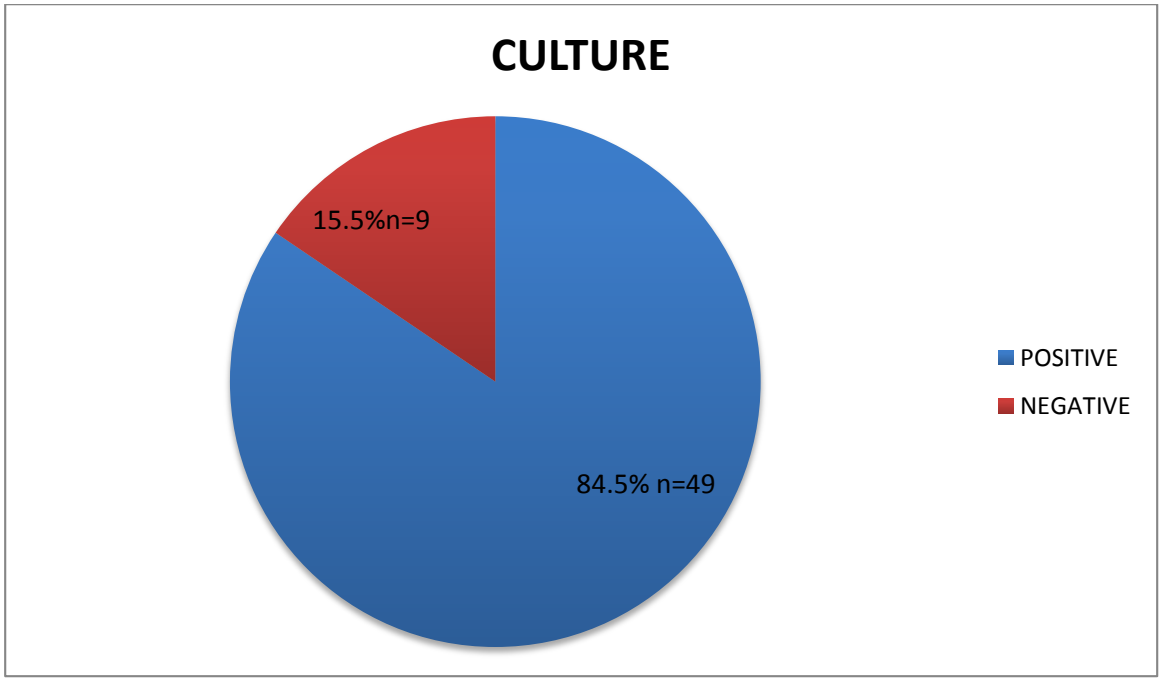

Figure 3: Bacteria culture results of study participants.

Out of 49-culture positives specimen, 37 (75.5%) cultures grew single bacterial isolates and 12 (24.5%) grew two bacterial isolates each making a total of 61 isolates. Most of the bacteria isolates were gram-positive *Staphylococcal aureus* and the least-isolated bacteria were *Enterobacter spp* (Figure 4)

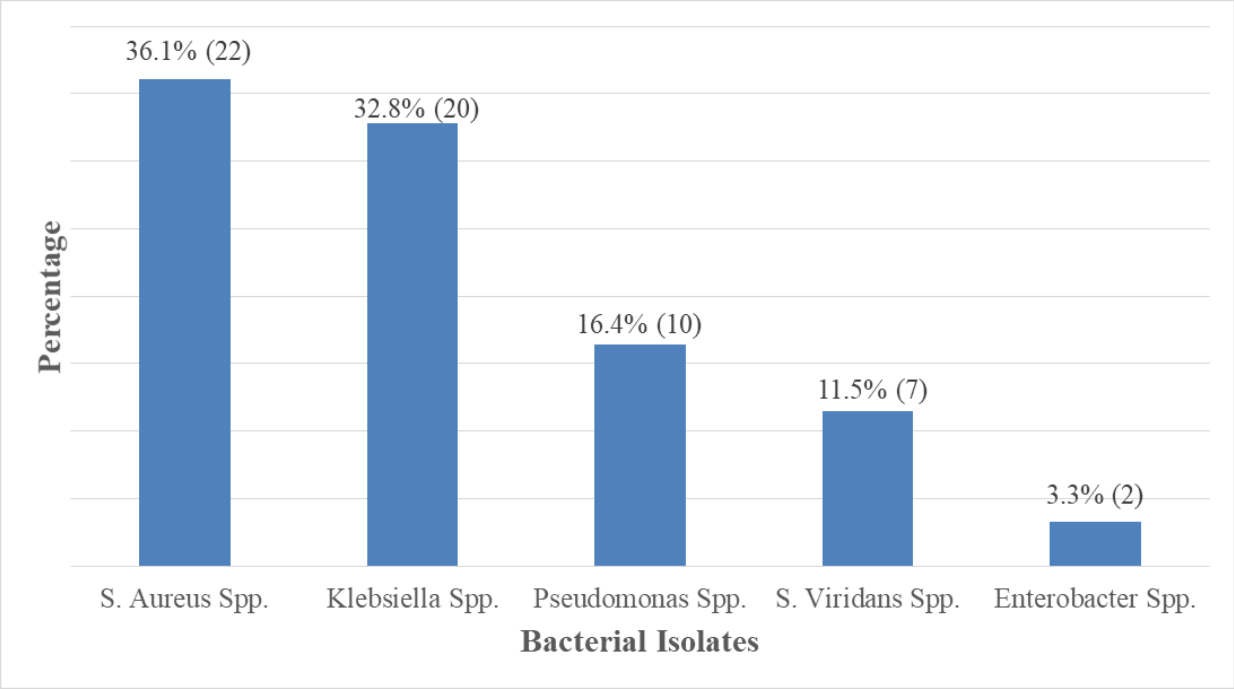

Figure 4: Bacteria isolated from corneal scrapping specimens of the study participants

Table 4: Association between bacteria culture growth and socio-demographic characteristics of participants with infective keratitis N=58.

Characteristic	Culture result		Total	p-value
Age categories (Years)	**Positive (n, %)**	**Negative (n, %)**		
25 or less	13 (86.7)	2 (13.3)	15	
26 to 35	10 (76.9)	3 (1)	13	**0.732**
36 to 45	12 (92.3)	1 (7.7)	13	
46 or more	14 (82.4)	3 (17.6)	17	
Sex				
Male	31 (81.6)	7 (18.4)	38	**0.476***
Female	18 (90)	2 (10)	20	
Education level				
Informal	8 (88.9)	1 (11.1)	9	
Primary school	29 (85.3)	5 (14.7)	34	**0.248**
Secondary school	10 (90.9)	1 (9.1)	11	
University/college	2 (50)	2 (50)	4	
Occupation				
Employed	12 (92.3)	1 (7.7)	13	
Peasant	12 (80)	3 (20)	15	**0.648**
Others	25 (83.3)	5 (16.7)	30	
Residence				
Dar es Salaam	37 (86)	6 (14)	43	**0.682***
Other regions	12 (80)	3 (20)	15	

There were no statistically significant associations (P>0.05) between social demographics and bacteria growth in the studied population.(Table4)

Table 5: Association between risk factors of infective keratitis with culture results N=58

Risk factor	Culture results		Total	P-value
	Positive (n, %)	Negative (n, %)		
History of ocular trauma				
Yes	10 (83.3)	2 (16.7)	12	**1.000***
No	39 (84.8)	7 (15.2)	46	
History of Diabetes mellitus				
Yes	6 (100)	0 (0)	6	**0.576***
No	43 (82.7)	9 (17.3)	52	
History of using topical steroid eye-drop				
Yes	21 (87.5)	3 (12.5)	24	**0.722***
No	28 (82.4)	6 (17.6)	34	
HIV serology				
Positive	2 (100)	0 (0)	2	**1.000***
Negative	25 (83.3)	5 (16.7)	30	

There were no statistically significant associations between risk factors of infective keratitis and bacteria growth in the study population. (Table 5)

Table 6. Association between clinical characteristics and culture results N=58

Characteristic	Culture results		Total	p-value
	Positive (n, %)	Negative (n, %)		
Type of referral				
Self-referral	31 (88.6)	4 (11.4)	35	**0.460***
Referral from primary health facility	18(78.3)	5(21.7)	23	
Duration of symptoms (weeks)				
<1	20 (80)	5 (20)	25	
1 to 3	17(85)	3(15)	20	**0.608**
>3	12(92.3)	1(7.7)	13	
History of using topical antimicrobials before cornea scraping	29 (76.3)	9 (23.7)	38	**0.021***
Yes	20(100)	0(0)	20	
No				
Visual acuity of the affected eye				
<6/18 – 6/6	6 (85.7)	1 (14.3)	7	**0.357**
6/18 – 6/60	9(100)	0(0)	9	
<3/60 – PL	34(81)	8(19)	42	
Location of ulcer				
Central	29 (80.6)	7 (19.4)	36	**0.543**
Para central	14(93.3)	1(6.7)	15	
Peripheral	3(75)	1(25)	4	
Extensive ulcer	3(100)	0(0)	3	
Depth of ulcer	23 (85.2)	4 (14.8)	27	
Superficial	24(82.8)	5(17.2)	29	0.801
Stromal	2(100)	0(0)	2	
Presence of desmatocele				
Hypopyon	16 (84.2)	3 (15.8)	19	**1.000***
Present	33(84.6)	6(15.4)	39	
Absence				

Prior use of antimicrobials before cornea scrapping for culture and sensitivity had statistical significant association with bacteria growth (P= 0.021). That is prior use of antimicrobials does affect the culture results.(Table 6)

Table 7: The susceptibility pattern of the isolated bacteria from participants with infective keratitis N=61.

Bacteria Isolated	**DRUG SENSITIVITY PATTERN**										
	CHLO	TETRA	TOBRA	VANCO	CIPRO	GENT	AMP	CFTR	DOXY	CLOX	MOX
	(n, %)	(n, %)	(n, %)	(n, %)	(n, %)	(n, %)	(n, %)	(n, %)	(n, %)	(n, %)	(n, %)
Enterobacter Spp N=2	2 (100)	2 (100)	2 (100)	NI	2 (100)	2 (100)	1 (50)	2(100)	2 (100)	NA	2 (100)
Klebsiella Spp N=20	19 (95)	20 (100)	20 (100)	NI	20 (100)	2(100)	0 (0)	20 (100)	19 (95)	NA	20 (100)
Pseudomonas Spp N=10	1 (10)	1 (10)	8 (80)	NI	10 (100)	8 (80)	4 (40)	9 (90.9)	0 (0)	NA	10 (100)
S. Viridans Spp N=7	7 (100)	7 (100)	7 (100)	NI	7 (100)	0 (0)	3 (42.9)	3 (42.9)	7 (100)	0 (0)	7 (100)
S. Aureus Spp N=22	18 (81.8)	16 (72.7)	22 (100)	NI	22 (100)	20 (90.9)	9 (40.9)	22 (100)	21 (95.5)	0 (0)	22 (100)

NA-Not apply; NI-No interpretation;CHLO-Chloromhenicol;CIPRO-Ciprofloxacin;TETRA-Tetracycline;TOBRA-Tobramycin;VANCO-Vancomycin;GENT-Gentamycin; AMP-Ampicilin; CFTR-Cefriaxone;DOXY-Doxycycline;CLOX-Cloxacillin;MOX-Moxifloxacilin;GENT-Gentamycin.

Highest sensitivity was shown by ciprofloxacin and moxifloxacilin by 100% each with all bacteria isolates, whereas highest resistance was shown by ampicillin and cloxacillin by 50 % and 100% respectively. Of all the bacteria isolates pseudomonas was the most resistant organism to most of the tested antibiotics. (Table7)

CHAPTER FOUR

4.0 DISCUSSION

The demographics, for infective keratitis vary considerably with time and by region, many patients who get infective keratitis are young, working adults who develop an unexpected infection from various causes .The median age of the participant in this study was 36.30 years with a range of 18 years to 80 years. This is in consistence with the Tanzania age structure distribution by age and sex where most active adults are between 25 to 54 years of age (28).

In this study topical corticosteroid use was most common associated factor for infective keratitis among the participants in 41% , probably because most of the participants were from Dar es Salaam city where there are many pharmacies from which corticosteroids are cheaply available over the counter without ophthalmologist prescription and are prescribed to most of the patients who have ocular complaints. Not only that but topical steroids are sometimes prescribed irrational by primary health care providers due to their poor knowledge on ocular pharmacology ,this is according to the pilot study which was done in Dar es salaam 2010 -2011 by Mafwiri et al(25).

Topical corticosteroids use in the eye without justifiable indications generally have a deleterious effect. A corticosteroid can enhance the stromal growth of some bacteria, such as Pseudomonas aeruginosa, but may not produce detectable effects after inoculation with staphylococci or streptococci (29). Moreover topical corticosteroid use significantly increases the risk of developing infective keratitis and result into poor subsequent outcomes of treatment (30)(31). Hence measures to counteract the use of over the counter steroids need be emphasized

The findings that the leading associated factor for infective keratitis was topical corticosteroids use in 41% of the participants and that only one participant had a history of using contact lens are different from study by Bourcier T et al which was done in Paris France in which the leading risk factor for bacterial keratitis was contact lens wear by 50.3% followed by history of keratoplasty in 21% of the participants(6). This difference can be explained by differences in treatments options for refractive errors where by there are extensive uses of contact lenses in developed countries compare to subsaharan Africa and also more keratoplasts are done in developed countries compare to developing countries.

Moreover ,the results of associated factors for infective keratitis in this study are also different from studies by Chidambaram et al and Tesfayegebremariam T et al where the leading risk factor for infective keratitis were trauma in 71.5% and 38% of the participants respectively(3)(7). These differences can be accounted for by differences in ecological distribution of the participants where by most of the participants in these two studies were from rural areas of India and Ethiopia respectively where there was high chances for sustaining farm related ocular trauma, where as most of the participants in this study were from Dar es salaam which is a city with minimal farming activities.

Cornea scrapping and culture continues to be an imperative utility in the diagnosis of infective keratitis. However because of predilection of fungi to penetrate into deeper layers of the cornea, tissue swabbing is usually inadequate in confirming a fungal agent (32). The finding that bacteria were the leading culture isolates that accounted for 84.5% of positive culture growth is similar to a multi center study by Peng et al in United states and a study by Usman et al in Kano Nigeria between 1996 and 2015 where they had predominant of bacteria growth (20)(26). However, the findings of 84.5% bacterial growth are different from studies done earlier by Burton et al and Furlanetto et al between 2001 and 2010 which showed a leading microbial isolates to be fungus (16)(27). This is probably due to changes in the predisposing factors for infective keratitis from HIV infection and Trauma in the past studies to topical corticosteroid use in this study.

Additionally, *Staphylococcal aureus* and *Klebsiella spp* were the common isolated bacteria in 36.1% and 32.8% respectively. This finding is similar to what was found by previous studies in India by Mehta et al and Ranjin et al and also in Tanzania by Burton et al at KCMC and Mafwiri et al at MNH (1)(2)(16)(17). This is probably due to similarities in geographical and climatic factors where these studies were conducted.

On antimicrobial senstivity in this study in which most of the commonly used antibiotics in which susceptability tests were carried out for the isolated bacteria showed variable senstivity. The highest senstivity of 100% were seen with floroquinolones (ciprofloxacilin and moxifloxacilin) followed by gentamycin, tobramycin and doxycline about 80-100%, and highest resistane was shown by ampicilin and cloxacilin with resistance of 50% and 100% respectively. The findings are similar to the studies which were done in India by Ranjin et al and Biradar et al. whereby by floroquinolone and aminoglycosides had high senstivity (1)(19).Also these

findings are consistance with the study which was done in Kano Nigeria by Usman et al on the senstivity of floroquinolones and aminoglycosides(20). This is probably because floroquinolones and aminoglycosides are newer drugs in our market and probably recently there has been rational use of antibiotics due to on going campaigns on proper antibiotics use to slow down the on going antimicrobial resistances. The highest resistance with penicilins (ampiclin and cloxacillin) is probably due to their prolonged extensive use in our clinical practices at MNH and CCBRT.

4.1 Limitation of the study

- Disc diffusion method (Kirby Bauer method), which was used in this study could not be used to establish vancomycin sensitivity, recommended is MIC by E-test or dilution method but are very expensive and could not be afforded in this study. However the sensitivity for other antibiotics were established and analyzed accordingly hence the missing analysis of vancomycin had insignificant effect on our results.

- The findings of AST are based on in vitro laboratory tests so they need a number of considerations before applying them in clinical practice, for example dosage adjustments and existences of ocular barriers to drug penetration.

4.2 CONCLUSION

Most infective keratitis at MNH and CCBRT are due to bacteria, the commonest bacteria being Staphylococal aureus. The leading predisposing factor for bacteria keratitis is topical steroid use. All isolated bacterial were sensitve to ciprofloxacin and moxifloxacilin. Additionally, organisms were sensitive to other commonly used antibiotics.

4.3 RECOMMENDATIONS

- Larger studies should be conducted to know the proportional of other possible etiological agents and predisposing factors for infective keratitis
- There should be public awareness rising on the safety of over the counter topical eye steroids together with continous medical education to the heath care providers on the prescription of topical steroids to the eyes
- Floroqunolone or aminoglycoside monotherapy should be used in the initial treatment of infective keratitis in Dar es Salaam, due to their higher senstivity levels.

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6.0 APPENDIX

MUHIMBILI UNIVERSITY OF HEALTH AND ALLIED SCIENCES

SCHOOL OF MEDICINE

DEPARTMENT OF OPHTHALMOLOGY

6.1 Appendix I; Informed Consent in English

MUHIMBILI UNIVERSITY OF HEALTH AND ALLIED SCIENCES DIRECTORATE OF RESEARCH AND PUBLICATIONS, MUHAS

ID-NO.....

CONSENT TO PARTICIPATE IN THE STUDY

My name is Dr. PAUL BARTHALOME; I am conducting a study on microbial etiology and antimicrobial resistance among patients with infective keratitis at two tertiary hospitals in Dar-es-salaam (MNH &CCBRT)

STUDY PURPOSE

This study is conducted to determine microbial etiology and antimicrobial resistance among patients with non-viral infective keratitis at two tertiary hospitals in Dar-es-salaam (MNH &CCBRT) It is also conducted as a partial fulfillment for the completion of Mmed Ophthalmology. Findings from this study will help to establish a protocol for treatment of patients with infective keratitis in order to achieve good post-treatment visual outcome and to improve the quality of care At MNH and CCBRT hospitals. Results of the study will also be used in implementation of the World health organization (WHO) global action plan on antimicrobial resistance particularly on a second objective of Strengthening the knowledge and evidence based through surveillance and research**.**

HOW TO BE INVOLVED

The patients who will agree to participate in this study will be required to sign the consent form, then interviewed and sample (cornea scrap) taken after that.

CONFIDENTIALITY

The information obtained from you will be confidential. No name will appear on any document of this study instead Identification numbers will be used.

PARTICIPATION AND RIGHT TO WITHDRAW

Involvement in this study is voluntary. You can participate or refuse to participate from this study. Refusal to participate from this study will not interfere with the treatment of your condition

BENEFITS

The information that you will provide will help in addressing microbial keratitis of different etiologies' after knowing drug susceptibility ,resistance patterns and the factors associated with infective keratitis

For any concern please, contact me personally or to any of the following

If you ever have questions about this study, you should contact the Principal Investigator, Dr Paul Barthalome, Muhimbili University of Health and Allied Sciences, P. O. Box 65001, Dar es Salaam. Tel. **+255629164807 .**OR in case you have questions about your rights of participation in this study you may contact Dr. Bruno Sunguya Director of Research and publications, P. O. Box 65001 DSM. Telephone**: +255 022 2152489.**

Dr. Celina Mhina, Dr Agricola Joachim and Prof Milka Mafwiri who are supervisors of this study Tel. **+255 746535289,+255717874791&+255784323250 respectively.**

Participant agreement.

I ……. have read the contents in this form. My questions have been answered. I am willing to participate in this study.

Signature of participant ……Date…….……

Signature of Researcher …….Date…….

6.2 Appendix II; Informed consent Swahili version

MUHIMBILI UNIVERSITY OF HEALTH AND ALLIED SCIENCES

SCHOOL OF MEDICINE

DEPARTMENT OF OPHTHALMOLOGY

CHUO KIKUU CHA SAYANSI ZA AFYA MUHIMBILIKURUGENZI YA TAFITI NA UCHAPISHAJI

FOMU YA RIDHAA

Namba ya utambulisho ----------

RIDHAA YA KUSHIRIKI KWENYE UTAFITI

Naitwa Dkt. Paul Barthalome, nafanya utafiti kuhusu aina ya vimelea vinavyosababisha kidonda kwenye kioo cha mbele cha jicho na usugu wa madawa mbalimbali yanayotumika katika matibabu ya vidonda hivi kwa wagonjwa wanaotibiwa katika hospitali ya taifa Muhimbili na CCBRT

DHUMUNI LA UTAFITI

Dhumuni la utafiti huu ni kutaka kujua kuhusu vimelea mbalimbali vinavyosababisha kidonda kwenye kioo cha mbele cha jicho na usugu wa madawa mbalimbali yanayotumika katika matibabu ya vidonda hivi kwa wagonjwa wanaotibiwa katika hospitali ya taifa Muhimbili na CCBRT

Dhumuni jingine la Utafiti huu ni pamoja na kutimiza sehemu ya matakwa ili kutunukiwa shahada ya uzamili wa matibabu ya Macho ya Chuo Kikuu cha Afya na Sayansi Shirikishi Muhimbili.

Matokeo ya utafiti huu yatasaidia katika kutengeneza miongozo mipya ya matibabu ya wagonjwa wenye vidonda vinavyo sababishwa na maambukizi ya vimelea mbalimbali katika kioo cha mbele cha jicho katika hospitali ya taifa Muhimbili na CCBRT na pia katika kutekeleza muongozo wa shirika la afya duniani (WHO) kuhusu tafiti za ndani kuhusu usugu wa madawa na vimelea mbalimbali vya maradhi.

JINSI YA KUSHIRIKI

Ukikubali kushiriki katika utafiti huu, utasailiwa alafu utatakiwa kujibu maswali kutoka kwenye dodoso lililo andaliwa na baadae matibabu yako yataendelea kama kawaida ikiwemo kuchukuliwa kipimo kutoka kwenye kidonda cha jicho kwa ajili ya vipimo vya vimelea na kutambua ufanisi wa madawa mbalimbali na usugu wa madawa hayo. Kabla ya majibu kuhusu aina ya vimelea na aina ya dawa yenye ufanisi kwenye kidonda chako kutolewa utapewa matibabu yanayoendana na kidonda chako kwa namna kinavyoonekana kitaalamu Baada ya majibu ya vimelea na ufanisi wa dawa kutolewa utapewa matibabu stahiki yanayo endana na majibu ya vipimo vya maabara

USIRI

Taarifa zote zitakazo kusanywa kupitia dodoso hili zitakuwa ni siri. Jina lako halitatumika

badala yake tutatumia namba yako ya utambulisho.

UHURU WA KUSHIRIKI NA HAKI YA KUJITOA

Kushiriki kwenye utafiti huu ni hiari. Unaweza kushiriki au kukataa kushiriki na hii haitaathiri matibabu yako kwa namna yoyote.

MAWASILIANO

Kama una maswali kuhusiana na utafiti huu, wasiliana na mtafiti mkuu, Dkt. Paul Barthalome, Chuo Kikuu cha Afya na Sayansi Shirikishi Muhimbili, S. L. P. 65001, Dar es Salaam. Simu**+255629164807,** Dr. Bruno Sunguya, Mkurugenzi wa Utafiti na Uchapishaji, **S.L.P 65001, Dar es Salaam, Simu+255 022 2152489** au wasimamizi wa utafiti huu Dkt . Celina Mhina;**simu +255 746535289** ,Dkt Agricola Joachimu ;simu **+255717874791** na Profesa Milka Mafwiri simu ;**+255784323250**

Kama umekubali kushiriki weka sahihi

Mimi..... nimesoma maelezo ya fomu hii nimeyaelewa na nimekubali kushiriki katika utafiti huu.

Sahihi ya mshiriki……Tarehe ya kutia sahihi…….....

Sahihi ya mtafiti…….Tarehe ya kutia sahihi…….

Appendix III Questionnaire

MUHIMBILI UNIVERSITY OF HEALTH AND ALLIED SCIENCES

SCHOOL OF MEDICINE

DEPARTMENT OF OPHTHALMOLOGY

QUESTIONAIRE SN……

DATE…../……/……

SECTION 1

(SOCIAL DERMOGRAPHIC CHARACTERISTICS)

1. Name……
2. Sex…….
3. Year of birth/Age……..
4. Residence……..
5. Occupation……
6. Level of education……..
7. Type of referral……..(self-referral from home/referred from primary health facility)
8. Duration of symptoms before reporting to the hospital……< 1week……1-<2weeks…2-3 weeks……>4weeks……

SECTION 2

(PREDISPOSING /ASSOCIATEDFACTORS)

1. History of recent ocular trauma……(YES/NO)
2. History of being treated for diabetes mellitus……(YES/NO)
3. History of being diagnosed with Human immunodeficiency virus (HIV) or Acquire immunodeficiency syndrome (AIDS)……(YES/NO)
4. History of contact lens use……..(YES/NO)
5. History of using topical steroid eye drop…… (YES/NO) If yes for what condition? Allergy, red eye etc. ……..
6. History of using contaminated eye drops……(YES/NO)
7. History of using traditional eye medication…….(YES/NO)
8. History of recent eye surgery…….(YES/NO)
9. History of recent ocular surface diseases e.g. bacteria conjunctivitis, allergic conjunctivitis, blepharitis, others …….(specify)
10. Started on topical medications/antibiotics -----YES/NO
11. Others ……..(specify)

SECTION3.

(CLINICAL PRESENTATION)

1. Visual acuity of the affected eye……..
2. Location of an ulcer, central…..paracentral…..peripheral……..extensive ulcer……
3. Appearance …..regular wet or dry margin …….irregular wet or dry margins……..

4. Depth …superficial…..…stromal.....…presence of desmatocele…... perforated……

5. Hypopyon present……YES/NO

6. HIV serology status ……1. Positive 2. Negative. 3. Unaware.

LABORATORY ANTIMICROBIAL SUSCEPTABILITY TEST TEMPLATES

		ZONE DIAMETER BREAKPOINTS(Nearest mm)		
ANTIBIOTICS	DISC CONTENT(µg)	SUSCEPTIBLE {S}	INTERMEDIATE (I)	RESISTANT (R)
Tetracycline				
Tobramycin				
Vancomycin				
Ciprofloxacin				
Moxifloxacin				
Gentamycin				
Chloramphenicol				
Ceftriaxone				
Doxycycline				
Ampicillin				