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Microbial Keratitis: microbiology results of infective corneal ulcers at a tertiary Hospital in South Africa

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MICROBIAL KERATITIS:

Microbiology results of infective corneal ulcers at a tertiary hospital in South Africa

Karen Monica Koetsie

A dissertation submitted to the Faculty of Health Sciences, University of the Witwatersrand, in the fulfillment of the requirements for the degree of Master of Medicine in Ophthalmology. August 2011

DECLARATION

I hereby declare that this dissertation is my own unaided work. It is being submitted for the degree of Master of Medicine in the branch of Ophthalmology at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination in any other University.

Signed_____

This _____day of _____2010

The work reported in this dissertation was carried out in the Department of Ophthalmology, St John Eye Hospital (Chris Hani Baragwanath Hospital), Johannesburg, South Africa.

The project was approved by the Ethics Committee, University of the Witwatersrand.

DEDICATION

This dissertation is dedicated to my mother, Monica Koetsie, who has been my guardian angel and who has never left my side throughout this journey. My strength has come from the knowledge of your constant presence in my life. I wish that you were here today to share in my joy. I love and miss you so much.

PUBLICATIONS AND PRESENTATIONS ARISING FROM THIS STUDY

1. Presentation at the Ophthalmological Society of South Africa (OSSA) Congress at Sun City, South Africa, February 2010

   Title: MICROBIAL KERATITIS: Microbiology results of infective corneal ulcers at a tertiary hospital in South Africa

   Presenter: Karen Monica Koetsie

   Prize: Best Registrar Presentation

2. Poster presentation at the Department of Health Sciences Postgraduate Research Day, University of the Witwatersrand, Johannesburg, South Africa, 22 September 2010.

   Title: MICROBIAL KERATITIS: Microbiology results of infective corneal ulcers at a tertiary hospital in South Africa

   Authors: Karen Monica Koetsie and Susan Williams

   Prize: Best poster in the Infectious Diseases category

ABSTRACT

Purpose: To describe the microbiology results of corneal scrapings and morphology results of corneal ulcers over a one year period at the St John Eye Hospital with the following objectives: (i) to describe the positive culture results (ii) to describe the commonest causative organisms (iii) to describe resistance patterns to antibiotics (iv) to correlate the positive culture results with the clinical characteristics of the ulcer.

Methods: A retrospective cross sectional review of patient medical records and microbiology reports of patients who presented with corneal ulcers at the St John Eye Hospital between October 2007 and October 2008. One hundred and fifty one (151) corneal scrapings submitted to the National Health Laboratory Services (NHLS) for microbiology, culture and sensitivity testing were analyzed. The following information was extracted from the microbiology reports and patient medical records: patient demographics, microbial isolations, antibiotic sensitivity and resistance, and corneal ulcer morphology (central versus peripheral).

Results: Of the 151 patients who had corneal scrapings, 63(42%) were female and 88(58%) were male. The median age was 39.6(range 1-95; SD 19.3). An organism was identified in 78(52%) of the samples. Of the 93 pathogens isolated, 78(83.9%) were gram positive, 10(10.8%) were gram negative, and 5(5.4%) were fungi. Mixed isolates were found in 15 of the 151 corneal scrapings. The most common gram positive isolates were *Staphylococcus aureus* 23(29.5%), coagulase negative *Staphylococcus* 18(23.1%),

and *Streptococcus pneumoniae* 16(20.5%). The two most commonly isolated gram negative organisms were *Pseudomonas aeruginosa* 3(30%) and *Haemophilus influenza* 3(30%). A total of 5 fungi were isolated from the 151 corneal scrapings with *Fusarium* 3(60%) being the most common fungus isolated. Antibiotic resistance patterns were as follows: Gram positive isolates (73) consistently showed 100% sensitivity to vancomycin. A small number of gram positive organisms showed *in vitro* resistance to the second generation fluoroquinolone ciprofloxacin. This was, however only a small number of gram positive isolates and therefore the *P* value ($P<0.001$) remained significant. Overall the gram positives isolates showed a 95.3% sensitivity to ciprofloxacin. Both second and fourth generation fluoroquinolones, ciprofloxacin and moxifloxacin respectively, showed equivalent (100%) *in vitro* activity against the gram negative isolates. All gram negative isolates showed 100% laboratory susceptibility to the aminoglycosides, gentamicin and amikacin. Inpatient medical records were available for 56 of the 151 corneal ulcer scrapings. Of the 56 inpatient records reviewed 42(75%) were central ulcers. *Streptococcus pneumoniae* 10(23.8%) was the most common organism isolated in central corneal ulcers, while *staphylococcus aureus* 4(28.6%) was the most common organism isolated in peripheral corneal ulcers.

Conclusion: Compared with previous reports from the St John Eye Hospital, the spectrum of causative organisms has remained unchanged over the past 25 years. The organisms commonly responsible for microbial keratitis at the hospital are significantly susceptibility to the antibiotics currently being used as therapy.

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Chapter 1

In this chapter the background to the study will be described, as well as the overall research question and its importance. The chapter describes the field in which the research is based and the research question. The justification for the study will be stated. The relevant literature related to the topic will be presented. The aims and objectives of the study will be stated.

1. Introduction

Microbial keratitis is defined as a stromal infiltrate associated with an overlying epithelial defect with or without an anterior chamber reaction.[1] It is potentially blinding and is a major cause of ocular morbidity if appropriate treatment is not initiated promptly. Intensive broad-spectrum therapy is usually started before laboratory culture results are available.[2] It is widely accepted that the spectrum of microorganisms responsible for corneal ulceration varies in all geographic regions.[2] It is therefore crucial that empiric antimicrobial treatment used is based on the prevalence of microorganisms in the community.[2]

1.1 Problem statement

There is increasing concern that the organisms responsible for bacterial keratitis are showing resistance to the current antimicrobials in widespread use.

1.2 Justification for the study

The knowledge of the most common organisms in each region and their resistance patterns is of practical concern because of the difference in therapeutic approach. This study will show whether the more common organisms responsible for microbial keratitis in the specific setting of the St John Eye Hospital in Soweto, Johannesburg are still sensitive to the antimicrobials currently used.

1.3 Background

1.3.1 Anatomy and Physiology

The transparent cornea forms the most anterior part of the globe and is the main structure responsible for the refraction of light entering the eye. Microscopically five layers can be identified. From anterior to posterior they are as follows (1) the epithelium (2) Bowman's layer (membrane) (3) the stroma (4) Descemet's membrane (5) the endothelium.[3]

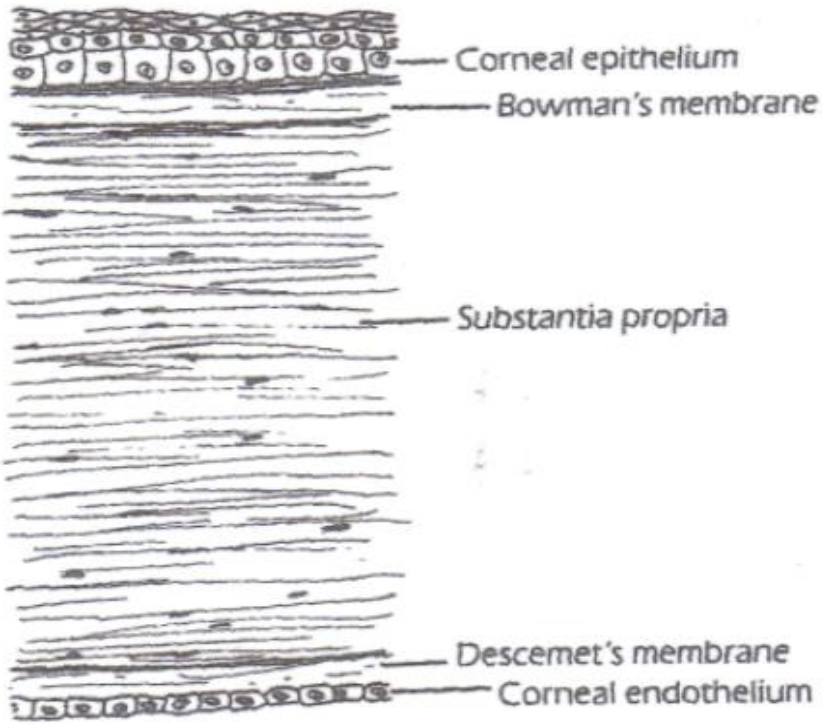


Figure 1.1: Diagram showing the anatomy of the cornea[3]

The corneal epithelium is composed of nonkeratinized, nonsecretory stratified squamous epithelium which is 4-6 layers thick. A 7μm thick tear film covers the epithelium. One of functions of the tear film is to protect the corneal surface from microbial invasion, as well as from chemical, toxic, or foreign body damage.[4] Bowman's layer lies beneath the basement membrane of the epithelium and is acellular and consists of interwoven collagen fibrils.[3] The stroma forms 90% of the corneal thickness. It is transparent, fibrous and compact and consists of many lamellae of collagen fibrils. Descemet's membrane lies on the posterior surface of the stroma and is the basement membrane of the endothelium. It is strong and homogenous and is composed of type IV

collagen fibrils. The final layer is the endothelium which is made up of a single layer of flattened cells that are polygonal in shape.[3]

1.3.2 Pathogenesis and clinical features

Infectious keratitis is one of the leading causes of blindness, but, in most cases these infections represent preventable or treatable ophthalmic disease. It is characterized by a cellular infiltration of the corneal epithelium or stroma, corneal inflammation, and necrosis. Disruption of the continuity of the epithelium is the most common event that allows the establishment of a corneal infection. In this setting the keratitis may be caused by gram positive organisms such as *staphylococci* and *streptococci*, or gram negative organisms such as *Pseudomonas aeruginosa* or fungi such as *Fusarium* species. A few organisms such as *Corynebacterium dipththeriae, Haemophilus aegyptius, Neisseria gonorrhoeae, Neisseria meningitides, Shigella* and *Listeria* species can penetrate an intact epithelium. The clinical presentation of bacterial keratitis includes decreased vision, pain, and photophobia. Ocular findings include a localized or diffuse infiltration of the epithelium or stroma, with epithelial absence. Alternatively, a stromal abscess can occur beneath an intact epithelium. Additional ocular features include lid oedema, conjunctival inflammation and chemosis, a discharge, and an anterior chamber

reaction. The anterior chamber inflammation may be so severe as to produce a hypopyon.[4]



Figure 1.2 Corneal ulcer with a hypopyon (Courtesy of Professor T R Carmichael)

1.4 Literature review

One of the challenges faced when reviewing the current literature concerning microbial keratitis is the fact that not only does the incidence vary according to the geographic location, but there are regional variations in organism type and predominance as a result of patient populations and climatic effects. The following sections will attempt to review the status of the current literature on microbial keratitis.

1.4.1 Epidemiology

The incidence of microbial keratitis varies according to the geographic location: in the United States the incidence is 11 per 100,000 persons per year, whereas in Nepal it is 799 per 100,000 persons per year.[5] Longitudinal epidemiologic studies provide information with regard to the pattern of causative organisms, changing patient demographics, and their antibiotic sensitivities.[5]

Several studies have found a male predominance when reviewing the demographic features of microbial keratitis.[1,2,5] Keay *et al* found that 63.6% microbial keratitis cases were male. [1] In another study of microbial keratitis in Sydney a 52% male predominance was found.[2] Likewise in a large retrospective study by Bharathi *et al* 1,879 (59.03%) were found to be male and 1,304 (40.97%) were females, with a male to female ratio of 1.44 to 1. More than half of the patients (51.81%) were between the ages of 21 and 50 years.[5]

Ocular trauma and contact lens wear have been identified by several studies as the most common risk factor for microbial keratitis.[1,2,5,6,7,8] Corneal infection among males could be attributed to their greater involvement in outdoor activities and outdoor occupations, thus making them prone to corneal injury with external agents.[5] In South

Africa, corneal trauma is a common cause of microbial keratitis.[6] Carmichael *et al* found a male predominance, half of whom were manual labourers.[6] Bharathi *et al* have also shown that occupation played a role in the type of responsible organism. Agricultural workers were more in number among patients with fungal keratitis, whereas non-agricultural workers were more in number among patients with bacterial keratitis.[5]

In first world countries the relatively high incidence of myopia and the popularity of contact-lens wear are commonly identified as the cause of microbial keratitis. In a study by Saeed et al, the use of disposable contact lenses was the most important cause (59.5%) of cases of microbial keratitis in Ireland.[8] Similarly, Keay et al found contact lens wear (33.7%) and trauma (36.4%) to be the two most commonly identified causes of microbial keratitis in Australia. They also found that of the traumatic cases, the majority were male (90.6%).[1]

1.4.2 Microbiological profile

The spectrum of microorganisms responsible for microbial keratitis varies in different geographical regions.[9] These variations in organism type and predominance are as a result of different patient populations and climatic effects.[5]

In a longitudinal study at a major public hospital in Australia over a 5 year period, patient demographics, corneal culture results, and keratitis risk factors were studied. A total of 253 corneal scrapings from 231 patients were included in the study. Gram positive bacteria (29%) were the most common group of organisms of which *Staphylococcus* and *Streptococcus* genera were the most common isolates. Of the Gram negative isolates (23%), *Pseudomonas aeruginosa* was the most common isolate. A fungal cause was found in 12 (5%) of cases, and *Fusarium* was the most common species.[10] They also found a significant variation in the monthly recovery of *Pseudomonas aeruginosa* and fungi which were cultured more frequently in the summer, whereas *Streptococcus pneumoniae* was more common in winter months than at other times of the year.[10]

In tropical countries such as South India, the incidence of fungal keratitis is much higher. Bharathi *et al* found that of the 3,183 patients with infective keratitis, 1,043 (33%) were bacterial, 1,095 (34%) were fungal , 33 (1%) were *Acanthamoeba*, 76 (2%) were both fungal and bacterial. The predominant bacterial pathogens isolated were *Streptococcus pneumoniae* (36%), while *Fusarium* species (42%) were the most common fungal pathogens isolated.[5]

In Taiwan, where the climate is subtropical, *Pseudomonas* species were the most commonly isolated organisms (38%), followed by fungi (14%), *Staphylococci* (8.4%), nontuberculous *Mycobacteria* (7.9%), and *Streptococci* (7.6%).[9]

In the temperate South African climate, Cockinos *et al* found that of the 52 eyes with culture positive results, 30(79%) were gram positive and 8(21%) were gram negative.[7] Of the gram positives, *Steptococcus pneumoniae* and *Staphylococcus epidermidis* were found to be the predominant gram positives, whilst *Pseudomonas aeruginosa* was the most common gram negative isolated.[7]

Therefore, the principle regional differences appear to be that filamentous fungal keratitis and gram negative keratitis are commonly seen in tropical climates, whereas gram positive bacteria are more common in temperate climates.[5]

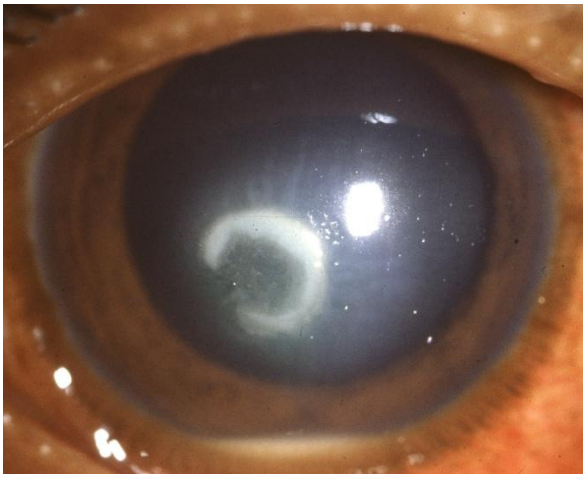


Figure 1.3 *Streptococcus pneumoniae* ulcerative keratitis with a hypopyon (Courtesy of Professor T R Carmichael)

1.4.3 Culture positive rate

The rate of positive cultures can vary between studies. The main cause of a low positive culture rate is the frequent use of antibiotics before referral in the clinical setting.[9] Also, topical anesthetic agents are known to have antimicrobial properties and therefore their use may lead to false negative culture results.[9] Table 1 below summarises the culture positive rate of studies carried out in different geographical regions. Exposure to topical antimicrobial therapy before culture may result in a delay in pathogen recovery, but not in a difference in the rate of sterile samples.[1]

Table 1.1: Culture positive rate and organisms isolated in microbial cases in different regions

	South Africa[6]	South Africa[7]	Paraguay[11]	South Florida[12]	Australia[1]	Taiwan[13]
Number of corneal scrapings	283	93	660	2920	267	501
Culture positive rate	66%	63%	79%	50%	49%	50.7%
Most common pathogen isolated	Gram positive	Gram positive	Gram positive	Gram negative	Gram Positive	Gram negative

To determine the causative organism, corneal ulcers are scraped for microscopy, culture and drug sensitivity.[14] Microbial culture is slow, with growth and identification taking approximately three to five days for bacteria and even longer for fungi.[15] Newer modalities for detection of microbial keratitis include molecular diagnostic techniques such as Polymerase Chain Reaction (PCR).[15] Kim *et al* prospectively compared microbial culture and PCR in the diagnosis of corneal ulcer. A total of 108 corneal ulcers were cultured and analyzed by PCR. Of the 108 samples, 56 (51.9%) were culture-positive. After elimination of false-positive PCR products, 94 of 108 corneal scrapings (87%) were positive by PCR.[15] The authors concluded that practical use of the technique is limited by

artefactual amplification of nonpathogenic organisms. They added that PCR may be used as an adjunct to culture to identify potential pathogens in microbial keratitis.[15]

Microbial culture therefore remains the gold standard for the detection of pathogens causing corneal ulcers. Finally, apart from its diagnostic value, corneal scraping allows improved antibiotic penetration and therapeutic debridement of necrotic tissue.[14]

1.4.4 Treatment

Microbial keratitis should be treated as an ocular emergency due to its rapid progression and devastating complications.[17] Empirical antimicrobial therapy should be promptly started and should be based on the prevalence of microorganisms in the community.[2] The initial treatment regimen is usually a broad-spectrum antibiotic followed by more specific therapy once MC&S results of corneal scrapings become available.[12]

In the early and mid-1980's, the combinations of a cephalosporin(cefazolin) and an aminoglycoside (either gentamycin or tobramycin) were probably the most widely used therapeutic regimen for the initial treatment of infectious keratitis.[18] The commercial

introduction of the second generation fluoroquinolones (ciprofloxacin and ofloxacin) led to the evaluation of the "standard" fortified eyedrop approach. Their appeal was the ease of administering a single antibiotic eyedrop without the need to prepare fortified drops and their wide gram positive and gram negative spectrum.[18] The second generation fluoroquinolone ciprofloxacin has for many years been widely accepted for the first-line treatment of bacterial keratitis.[19]

At the St John Eye Hospital in Soweto, South Africa, all patients with suspected microbial keratitis are empirically started on an intensive regimen of a broad-spectrum second generation fluoroquinolone, ciprofloxacin, and chloramphenicol ointment. Treatment is then adjusted once culture sensitivity results are known.

Past studies have shown that ciprofloxacin is effective against most bacterial keratitis. A retrospective study was undertaken by Cockinos and Carmichael at the St John Eye Hospital.[7] They evaluated the management strategies of infectious keratitis as topical ciprofloxacin monotherapy had largely replaced the fortified antibiotic drops regimen which was previously used to treat bacterial keratitis. Ciprofloxacin as empirical therapy was used successfully in 22 of the 24 patients.[7]

Ly *et al* performed quantitative susceptibility testing to six antibiotics on all bacteria isolated from 112 patients who presented to the Sydney Eye Hospital Emergency Department with presumed bacterial keratitis. Ninety per cent (90%) of coagulase-negative *Staphylococci* were susceptible to ciprofloxacin. *Pseudomonas aeruginosa* had a 100% susceptibility to gentamicin, tobramycin and ciprofloxacin. *Staphylococcus aureus* strains showed 100% sensitivity to cephalothin, the aminoglycosides and ciprofloxacin. All *Streptococcal* isolates were sensitive to cephalothin, chloramphenicol and ciprofloxacin, but showed resistance to the aminoglycosides.[2]

Recently, studies have shown an emergence of resistance to the second generation fluoroquinolones.[12] Emerging resistance of *Staphylococcus aureus*, *Streptococcal* species and *Pseudomonas* to the earlier generation fluoroquinolones has raised concern.[17] The topical fourth generation fluoroquinolones (gatifloxacin and moxifloxacin) have recently been developed in response to the emerging resistance to the older generation of fluoroquinolones.[17] Parmar *et al* showed that gatifloxacin had a significantly better action against gram positive *cocci* both *in vitro* and *in vivo* compared with ciprofloxacin.[19] This finding is important since gram positive *cocci* are the most common causes of bacterial keratitis worldwide and therefore gatifloxacin may be a preferred alternative to cipfloxacin as the first-line monotherapy in bacterial keratitis.[17]

This emergence of drug resistance and availability of newer antimicrobials has made it essential for us to update our knowledge and review our treatment guidelines.

1.4.5 Corneal ulcer morphology

The morphology of a corneal ulcer is defined by the location of the principal infiltrate. The severity of corneal ulcers usually depends on the underlying condition of the cornea and the pathogenicity of the infecting organism.[12] Bourcier *et al* looked at the clinical characteristics of bacterial keratitis. Of the 300 cases with presumed bacterial keratitis examined, they found a predominance of the inferior cornea (46%) as the location for the principle corneal infiltrate. They also found that central corneal ulcers made up 23% of the corneal infiltrate location, 24% and 22% made up the inferior nasal and temporal locations respectively, and only 4% of the infiltrates were diffuse.[20] Carmichael *et al* found that central bacterial ulcers caused by *Streptococcus pneumoniae* constituted the largest group of ulcers seen. *Staphylococcus aureas* was the most common organism isolated from the marginal catarrhal group of ulcers as a result of chronic staphylococcal lid disease.[6]

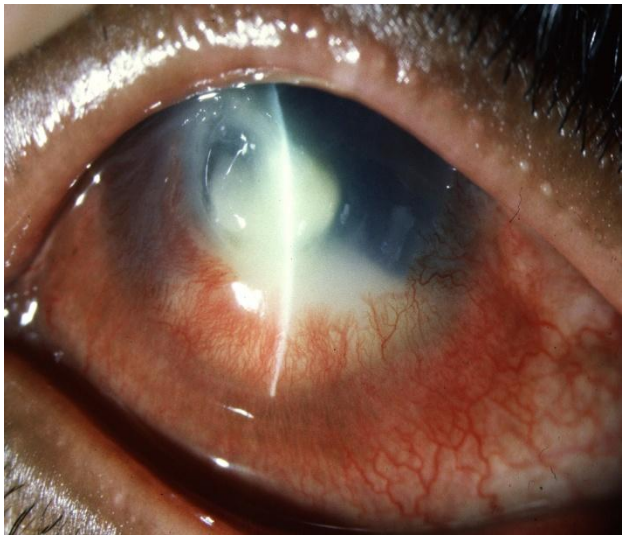


Figure 1.4 Centrally located stromal necrotic *Herpes simplex* keratitis with a hypopyon and peripheral vascularization (Courtesy of Professor T R Carmichael)

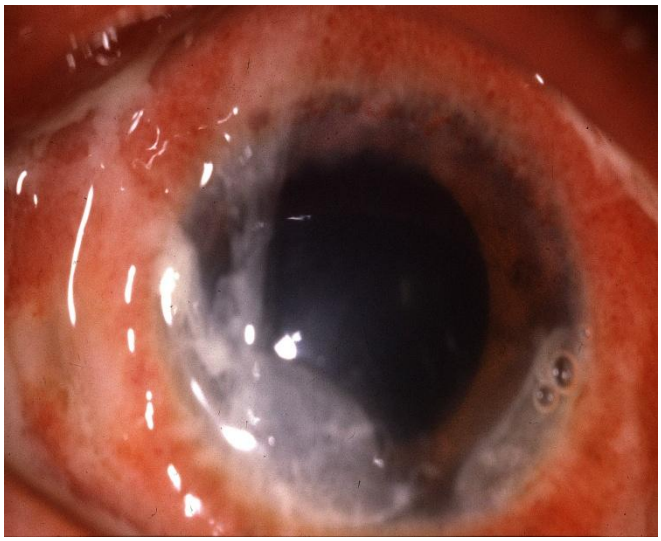


Figure 1.5 Peripheral ulcerative keratitis secondary to *Neisseria gonorrhoeae* (Courtesy of Professor TR Carmichael)

1.5 Research aim

The aim of the study was to describe the microbiology results of corneal scrapings and morphology results of corneal ulcers over a one year period at the St John Eye Hospital.

1.6 Research objectives

Objectives:

- To describe the positive culture results
- To describe the commonest causative organism
- To describe resistance patterns to antibiotics
- To correlate the positive culture results with the clinical characteristics/ morphology of the ulcer

Chapter 2

In this chapter the methodology of the study will be described. The study design, the study population and the method of data collection will be described. The management and analysis of the data will be described and ethical considerations will be mentioned.

2. Methodology

2.1 Study design

This was a retrospective cross sectional study of patient medical records and microbiological reports of patients who presented with corneal ulcers at the St John Eye Hospital between October 2007 and October 2008.

2.2 Data collection

The data were collected in two stages.

Review of microbiology reports:

All patients' microbiology reports of corneal scrapings were retrieved from the National Health Laboratory Services (NHLS). This was done by searching the NHLS data base for all corneal scrapping specimens that were sent in for microbiology, culture and sensitivity (MC&S). The relevant information was extracted from the reports and recorded on the data capture sheet (appendix A). This included patient demographics, the culture result and the antibiotic sensitivity and resistance.

Review of inpatient medical records:

The inpatient progress notes of all patients who were admitted for the treatment of their corneal ulcers were retrieved from the records room at St John Eye Hospital. Information extracted from the treating doctors notes related to the corneal morphology and was recorded on the data capture sheet (appendix A). For clinical features of the corneal ulcer, the location of the ulcer was classified into central or peripheral. A central zone was defined as the central 5mm diameter of cornea. The peripheral zone was the remaining annulus of cornea.

2.3 Study population

This consisted of one hundred and fifty one (151) corneal scrapings sent in to the National Health Laboratory Services between October 2007 and October 2008 for microbiology, culture and sensitivity testing.

2.4 Data management

Each patient was assigned a unique number to ensure anonymity. These unique case numbers were correlated to their microbiology reports and inpatient medical records. All patient information collected during the course of the research was kept strictly confidential and only information directly relevant to the study was extracted.

Data from the data capture sheets were recorded in Microsoft Excel 2003.

2.5 Data analysis

The data were analyzed using STATA 10. Descriptive analysis was done using means to describe data normally distributed and mediums where the data were skewed. *P*-values were calculated to determine the statistical significance of the findings.

2.6 Ethical considerations

Ethical approval was obtained from the Human Research Ethics Committee of the University of the Witwatersrand, Johannesburg. The ethics protocol number is M090216 (Appendix B). Information was kept strictly confidential.

Chapter 3

3. Results

During the one year period from October 2007 and October 2008, 151 corneal scrapings microbiology results were retrieved from the National Health Laboratory Services. During this period 56 inpatient records were retrieved from the St John Eye Hospital records room.

3.1 Patient demographics

There were 151 records retrieved from the NHLS records and of these 88(58%) were males. The median age was 39.6(range 1-95; SD 19.3).

The medical records of 56 patients admitted to the St John Eye Hospital for treatment of their corneal ulcers were reviewed for the same study period. Of the 56 inpatient records reviewed 37(66.1%) were male. The median age was 37.5(range 2-80; SD 18.6).

3.2 Culture yield

There were 93 organisms isolated from the 151 corneal scrapings sent for MC&S. There was no growth in 73(48.3%) of the specimens. Mixed isolates were found in 15 specimens. In the 78 specimens where there was growth 93 organisms were isolated. Of the 93 pathogens isolated, 78(83.9%) were gram positive, 10(10.8%) were gram negative and 5(5.4%) were fungi.

3.3 Gram positive organisms

Table 3.1 Number and types of gram positive organisms isolated from 151 corneal scrapings

Organism	Number of isolates
Staphylococcus aureus	23
*CNS**	18
Streptococcus pneumoniae	16
Streptococcus viridians	6
Streptococcus pyogenes	4
Corynebacterium species	3
Staphylococcus epidermidis	3
MRSA†	3
Staphylococcus warrii	1
Streptococcus milleri	1
Total	78

**Coagulase negative staphylococcus*

†*Methicillin resistant staphylococcus aureus*

A list of the gram positive organisms isolated from the 151 corneal scrapings is shown in Table 3.1. The three most commonly isolated gram positive microorganism were *staphylococcus aureus* 23(29.5%) followed by *coagulase negative staphylococcus* 18(23.1%) *and streptococcus pneumoniae* 16(20.5%).

3.4 Gram negative organisms

Table 3.2 outlines the 10 gram negative organisms isolated from the 151 corneal scrapings sent in for MC&S. The two most commonly isolated gram negative organism were *Pseudomonas aeruginosa* 3(30%) and *Haemophilus influenzae* 3(30%).

Table 3.2 Number and type of gram negative organisms isolated from 151 corneal scrapings

Organism	Number of isolates
Pseudomonas aeruginosa	3
Haemophilus influenza	3
Pantoea species	1
Serratia marcescens	1
Moraxella	1
Proteus mirabilis	1
Total	10

3.5 Fungi

A total of 5(3%) fungi were isolated from the 151 corneal scrapings sent in for MC&S with Fusarium 3(60%) being the most common fungus isolated.

Table 3.3 Number and type of fungal pathogens isolated from 151 corneal scrapings

Fungal isolate	Number of isolates
Fusarium	3
Candida albicans	1
Candida parapsilosis	1
Total	5

3.6 Antibiotic susceptibility: Gram positives

Table 3.4 details the comparisons of the antibiotic susceptibilities of the more popular antibiotics used in the treatment of microbial keratitis. Gram positive isolates (73) consistently showed 100% sensitivity to vancomycin. A small number of gram positive organisms showed some *in vitro* resistance to the second generation fluoroquinolone ciprofloxacin: Methicillin resistant *Staphylococcus aureus* (3), *Staphylococcus warrii (1*) and *streptococcus milleri* (1). This was, however, only a small number of gram positive isolates and therefore the *P* value ($P<0.001$) remained significant. *Streptococcus pnemoniae* demonstrated greatest susceptibility (100%) to Chloramphenicol than any

other gram positive organism. All of the 16 *Strep. pneumoniae* tested for susceptibility against the fourth generation fluoroquinolone moxifloxacin were 100% sensitive. The third generation cephalosporin cefotaxime significantly (*P*=0.051) showed 100% laboratory activity against all the gram positive isolates. In contrast the second generation cephalosporin cefoxitin showed a statistically significant (*P*=0.005) resistant patterns to coagulase negative *staphylococcus* (17.6%) as well as a 42.9% resistance towards other gram positives such as *MRSA, Staph. warrii and Strep. milleri.*

Table 3.4 Number and percentage of gram positive organism's sensitivity profile

Antibiotics	*Staph. Aureus*	*CNS**	*Strep. pneum.*	*Strep. viridans*	*Strep. pyogenes*	*Other†*
Ciprofloxacin *P*=0.000	22 (100%)	15 (100%)	‡	‡	‡	4 (66.7%)
Moxifloxacin	‡	‡	16 (100%)	‡	‡	‡
Vancomycin	22 (100%)	17 (100%)	16 (100%)	6 (100%)	4 (100%)	8 (100%)
Chloramphenicol *P*=0.108	17 (89.5%)	12 (75.0%)	13 (100%)	‡	‡	1 (50%)
Cefotaxime *P*=0.051	1 (100%)	1 (100%)	‡	4 (100%)	3 (100%)	1 (100%)
Cefoxitin *P*=0.005	23 (100%)	14 (82.4%)	‡	1 (100%)	‡	4 (57.1%)

**Coagulase negative staphylococcus*

†*Methicillin resistant staphylococcus aureus, Staphylococcus warrii, Streptococcus melleri*

‡Susceptibility testing not done

3.7 Antibiotic susceptibility: Gram negatives

The susceptibility profile of the gram negative organisms isolated is shown in Table 3.5. Both second and fourth generation fluoroquinolones, ciprofloxacin and moxifloxacin respectively, showed equivalent (100%) *in vitro* activity against the gram negative isolates. All gram negative isolates exhibited 100% laboratory susceptibility to the aminoglycosides, gentamicin and amikacin. Community acquired organisms such as *Haemophilus influenzae* showed 100% in *vitro* susceptibility against third generation cephalosporins, ceftazidine and cefotaxime. *Pseudomonas aeruginosa,* however, showed marked resistance (33.3%) against ceftazidine.

Table 3.5 Number and percentage of gram negative organism's sensitivity profile

Antibiotics	*Pseudo. aerug.*	*Pantoea* Species	*Proteus mirabilis*	*Haem. influen*	*Serratia Marcescens*
Gentamicin	2 (100%)	1 (100%)	1 (100%)	*	1 (100%)
Amikacin	3 (100%)	1 (100%)	1 (100%)	*	1 (100%)
Chloramphenicol *P*=0.753	*	1 (100%)	1 (100%)	2 (66.7%)	1 (100%)
Ciprofloxacin	3 (100%)	1 (100%)	1 (100%)	3 (100%)	1 (100%)
Moxifloxacin	1 (100%)	1 (100%)	*	*	*
Ceftazidine P=0.817	2 (66.7%)	1 (100%)	1 (100%)	1 (100%)	1 (100%)
Cefotaxime	*	1 (100%)	1 (100%)	3 (100%)	1 (100%)

*Susceptibility testing not done

3.8 Corneal ulcer morphology

Inpatient medical records were available for 56 of the 151 corneal ulcer scrapings sent in for MC&S. In the 56 medical records reviewed 42 (75%) were central ulcers (involved the central 5mm of the cornea). The organisms isolated from corneal scrapings of central and peripheral ulcers are shown in Table 3.6. *Streptococcus pneumoniae*

10(23.8%) was the most common organism isolated in central corneal ulcers, while *staphylococcus aureus* 4(28.6%) was the most common organism isolated in peripheral corneal ulcers. Second to this was *Staphylococcus epidermidis* which was isolated in 14.3% of peripheral corneal ulcers.

A fungal isolate, *Candida parapsilis*, was found in 1(2.4%) of the central ulcers while scrapings taken from peripheral ulcers did not demonstrate any fungal growth. In the 56 medical records reviewed 17 (30.4%) cases were associated with a hypopyon and 15 of these were central lesions.

Table 3.6 Number and type of organisms isolated from 56 central and peripheral ulcers

Isolate	Central ulcers n=42(%)	Peripheral ulcers n=14(%)
No growth	17 (40.5)	6 (42.9)
Pseudomonas aeruginosa	1 (2.4)	0
*MRSA**	1 (2.4)	0
Staph aureus	2 (4.8)	4 (28.6)
CNS†	5 (11.9)	1 (7.1)
Strep. Pneuemoniae	10 (23.8)	1 (7.1)
Proteus mirabilis	1 (2.4)	0
Strep viridians	3 (7.1)	0
Staphylococcus warii	1 (2.4)	0
Staph epidermidis	0	2 (14.3)
Candida parapsilis	1 (2.4)	0

**Methicillin resistant staphylococcus aureus*

†Coagulase negative staphylococcus

Chapter 4

Discussion

The St John Eye Hospital is the eye unit of the Chris Hani Baragwanath Hospital, a university hospital in Soweto. St John serves the population of Soweto and is a referral centre for the greater Johannesburg. The empirical approach in the treatment of microbial keratitis at St John is based on studies performed at the hospital over the past 25 years. This study sought to justify that approach since in the intervening time new antibiotics have emerged, antibiotic resistance has increased, and the high incidence of Human Immunodeficiency Virus (HIV) may have altered the prevalence pattern of microbials in the community.

In this study, a male predominance was found similar to previous studies[6,7,21] conducted at the St John Eye Hospital. Trauma has previously been reported as the most frequent cause for microbial keratitis at this hospital[7,21] and this may still be the case. As this was a retrospective review of patients' medical records, data was not available regarding any predisposing factors leading to corneal ulceration.

Microorganisms were isolated in 78 (51.6%) of the 151 corneal scrapings in this study. This corresponds to many similar studies conducted globally in which positive growth is variable and occurs in 50-70% of cases.[1,6,7,11,12,13] Despite the relatively low culture yield, microbial culture remains the gold standard for the detection of pathogens causing corneal ulcers as it is highly specific.[14,15] Newer modalities for detection of microbial keratitis such as Polymerase Chain Reaction (PCR) is an alternative to standard microbiologic testing, but has its limitations.[14] It yields a high rate of false positives for nonpathogenic organisms and is vulnerable to airborne contamination.[15] In some parts of the developing world the annual incidence of corneal ulceration has reached epidemic proportions.[15] Practical use of the technique in a developing country such as South Africa will be limited by cost. Therefore, the practice of routine microbiological analysis for all corneal ulcers is still the investigation of choice at the St John Eye Hospital.

The aetiology and epidemiology of corneal ulcers vary with the patient population, geographic location and climate, and tends to vary somewhat over time.[5,9,10,22] South Africa's weather is largely temperate and even more so at the high altitude of Johannesburg. Of the 93 pathogens isolated in this study, 78(83.9%) were gram positive, 10(10.8%) were gram negative and 5(5.4%) were fungi. The most commonly isolated gram positive bacterium was *Staphylococcus aureus* (29.5%). *Pseudomonas aeruginosa* (30%) and *Haemophilus influenzae* (30%) were the two most common gram

negative organisms isolated. Of the fungi, *Fusarium* (60%) was the most common fungus isolated. This microbial profile is in keeping with the cooler temperate climate found in Johannesburg. Similarly, in certain regions of Australia, gram positives followed by gram negatives are the most commonly isolated organisms responsible for microbial keratitis.[1,10] This is in contrast to tropical regions such as South India[5], Taiwan[9], Thailand[22] and Ghana[23], where *Pseudomonas* and *Fusarium* species are the most common causes of bacterial and fungal keratitis respectively. *Pseudomonas aeruginosa* is also more likely to be isolated from contact lens-related keratitis in areas with higher maximum and minimum temperatures.[24]

Empiric antimicrobial treatment may be used based on the prevalence of microorganisms in the community. The initial treatment regimen is usually broad-spectrum intensive treatment followed by more specific therapy once MC&S results of corneal scrapings become available.[12] One hundred percent of gram positive organisms isolated from the 151 corneal scrapings showed susceptibility to vancomycin. Of the gram positives, *Streptococcus pneumoniae* was the only organism which showed 100% sensitivity to chloramphenicol (*P*=0.108). Thirty seven of forty one gram positives showed 100% sensitivity to the second generation ciprofloxacin. Overall the gram positive isolates showed 95.3% sensitivity to ciprofloxacin. Ciprofloxacin previously found widespread acceptance for the treatment of bacterial keratitis,[2,19] however, some studies have shown an emergence of resistance to the second generation

fluoroquinolones.[12] This is important as gram positive bacteria made up 83.9% of the pathogens isolated in this study. Several studies have compared the bacteriologic and clinical efficacy of second generation and fourth generation fluoroquinolones. Kowalski *et al* showed that the fourth generation fluoroquinolones had increased susceptibility for *staphylococcus aureus* isolates that were resistant to second generation fluoroquinolones ciprofloxacin and ofloxacin and to the third generation fluoroquinolone levofloxacin.[25] Alexandrakis *et al* showed a three fold increase in the percentage of the second generation fluoroquinolones ofloxacin and ciprofloxacin resistant *S. aureus* isolates over their entire study period (11% in 1990 vs. 28% in 1998).[12] Similarly Mather *et al* showed that the fourth generation fluoroquinolones were more potent than second and third generation fluoroquinolones for gram positive bacteria and were equally potent for gram negative bacteria.[26] Since an older generation of fluoroquinolone, ciprofloxacin, is used as part of our empirical treatment regimen at the St John Eye Hospital, it is imperative that we continue to monitor the *in vitro* performance of this antimicrobial. The newer fourth generation fluoroquinolones (moxifloxacin and gatifloxacin) are not available at our hospital for routine treatment of corneal ulcers. Judicious use of them is needed to avoid future bacterial resistance.

The modern role of fortified antibiotics in the treatment of bacterial keratitis has been evaluated by several studies.[2,27] This study showed that the fortified third generation cephalosporin (cefotaxime) has 100% statistically significant (*P*=0.051) activity towards

gram positives compared to the second generation cephalosporin cefoxitin which showed an overall 87.5% activity towards gram positive *cocci*. First generation cephalosporins have a narrower spectrum of antibacterial activity than second or third generation cephalosporins.[27] Second and third generation cephalosporins also have a broader spectrum against gram negative bacteria.[26] Fortified antibiotics, however, have a number of disadvantages when compared to the fluoroquinolones.[23] They are more expensive, need to be prepared, require refrigeration, have a shorter half life and are more toxic.[17,27] Ganopadhyay *et al* found no significant treatment difference between fluoroquinolone and fortified therapy in terms of final visual outcome, but concluded that fluoroquinolones have the advantage of decreased toxicity and duration of treatment.[27] As preferred practice at our hospital, fortified antibiotics are started when laboratory results show microbial resistance to ciprofloxacin. The choice of fortified antibiotic depends on the organism cultured and its sensitivity pattern.

During the one year period of this study aminoglycosides consistently showed a 100% activity towards the gram negative isolates. As fortified antibiotics the aminoglycosides give excellent gram negative coverage and are also active against *staphylococci* and some *streptococci*, but not against *pneumococci*.[22] Once again moxifloxacin showed 100% susceptibility to the gram negatives.

The inpatient records of 56 of the 151 corneal scraping patients were retrieved from the records room. *Staphylococci* were mostly isolated from peripheral corneal ulcers. This may be secondary to the huge number of untreated staphylococcal lid disease seen in Africa.[6,7,21] Once again *Streptococcus pneumoniae* was by far the most common pathogen isolated from central ulcers.[7]

As with all retrospective studies, there are several shortcomings to this study. The first limitation is that it is cross sectional and therefore causality cannot be determined. Often a clue to identifying the most likely causative organism lies in the knowing of the patient's predisposing factors. Ocular trauma, ocular surface disease, and contact lens wear are major predisposing causes of corneal ulceration and important in the clinical diagnosis of the causative organism.[22] Another possible limitation is the relatively small sample size. Many of the more popular antibiotics used in the treatment of microbial keratitis were tested against a small number of organisms *in vitro*. This resulted in a few significant *P*- values. Another shortcoming is that the results can only be generalised to the source of the study and not to the general population. Previous studies conducted at this hospital have also used the population of greater Johannesburg and Soweto as their study group.[6,7,21] This study defines the pattern of keratitis in the same population group. Perhaps a study of microbial keratitis in another region of South Africa would reveal a different microbial profile. Finally, data on visual and clinical outcome was not included in this study and therefore treatment recommendations cannot be made.

Conclusion

Microbiology, culture and sensitivity (MC&S) remains the gold standard for the identification of pathogens causing microbial keratitis.[14,15] Factors such as ulcer size, operator skill in producing a quality sample, and culture technique are a few reasons for the low culture yield. Culturing, however, allows sensitivity testing so that treatment modifications can be made in an informed manner if the clinical response to the initial treatment is inadequate. The newer generation of fluoroquinolones is likely to play an important role in the future treatment of microbial keratitis because of an increasing resistance to the second generation fluoroquinolones currently being used. This study, however, has shown that the commonly cultured microorganisms are still significantly sensitive to the second generation fluoroquinolone ciprofloxacin. It is therefore still justifiable that it be used for treatment of suspected bacterial keratitis as first line empirical therapy.

References

1. Keay L, Edwards K, Naduvilath T, Taylor HR, Snibson GR, Forde K, Stapleton F. Microbial keratitis: Predisposing Factors and Morbidity. *Ophthalmol* 2006; 113:109-116.

2. Ly CN, Pham JN, Badenoch PR, Bell SM, Hawkins G, Rafferty DL, McClellan A. Bacteria commonly isolated from keratitis specimens retains antibiotic susceptibility to flouroquinolones and gentamicin plus cephalothin. *Clin Experiment Ophthalmol* 2006;34:44-50.

3. Snell RS, Lemp MA. Clinical anatomy of the eye. 2nd ed. United States of America: Blackwell Science, Inc, 1998.

4. Yanoff M, Duker JS. Ophthalmology. 2nd ed. Spain: Mosby, Inc, 2004.

5. Bharathi MJ, Ramakrishnan, R, Meenakshi R, Padmavathy S, Shivakumar C, Srinivasan M. Microbial Keratitis in South India: Influence of risk factors, climate, and geographical variation. *Ophthalmol Epidemiol* 2007;14:61-69.

6. Carmichael TR, Wolpert M, Koornhof HJ. Corneal ulceration at an urban African hospital. *Br J Ophthalmol* 1985;69:920-926.

7. Cockinos C, Carmichael TR. Causation and management of suppurative keratitis in Soweto, South Africa. *S Afr Optom* 2002;61:79-83.

8. Saeed A, Arcy FD, Stack J *et al.* Risk factors, microbiological findings, and clinical outcomes in cases of microbial keratitis admitted to a tertiary referral center in Ireland. *Cornea* 2009;28:285-292.

9. Fong C, Tseng C, Hu F, Wang I, Chen W, Hou Y. Clinical Characteristics of microbial keratitis in a university hospital in Taiwan. *Am J Ophthalmol* 2004;137:329-336.

10. Green M, Apel A, Stapleton F. A Longitudinal study of trends in Australia. *Cornea* 2008;27:33-39.

11. Laspina F, Samudio M, Cibils D, Ta CN *et al*. Epidemiological characteristics of microbiological results on patients with infectious corneal ulcers: a 13-year survey in Paraguay. *Graefes Arch Clin Exp Ophthalmol* 2004; 242:24-209.

12. Alexandrakis G, Alfonso EC, Miller D. Shifting trends in bacterial keratitis in South Florida and emerging resistance to fluoroquinolones. *Ophthalmol* 2000;107:1497-1502.

13. Fong C, Hu F, Tseng C *et al* Antibiotic susceptibility of bacterial isolates from bacterial keratitis cases in a university hospital in Taiwan. *Am J Ophthalmol* 2007;144:682-689.

14. Gupta N, Tandon R. Investigative modalities in infectious keratitis. *Indian J Ophthalmol* 2008;56:209-203.

15. Kim E, Chidambaram JD, Srinivasan M *et al*. Prospective comparison of microbial culture and polymerase chain reaction in the diagnosis of corneal ulcer. *Am J Ophthalmol* 2008;146:714-723.

16. Daniell M, Mills R, Morlet N. Microbial keratitis: what's the preferred initial therapy? *Br J Ophthalmol* 2003;87:1167-1169.

17. Gokhale NS. Medical management approach to infectious keraitis. *Indian J Ophthalmol* 2008;56:215-220.

18. Baum J, Barza M. The evolution of antibiotic therapy for bacterial conjunctivitis and keratitis: 1970-2000. *Cornea* 2000;19:659-672.

19. Parmar P, Salman A, Kalavathy CM *et al*. Comparison of topical gatifloxacin0.3% and ciprofloxacin 0.3% for the treatment of bacterial keratitis. *Am J Ophthalmol* 2006;41:282-286.

20. Bourcier T, Thomas F, Borderie V, Chaumeil C, Laroche L. bacterial keratitis: predisposing factors, clinical and microbiological review of 300 cases. *Br J Ophthalmol* 2003;87:834-838.

21. Omerod LD. Causation and management of microbial keratitis in subtropical

Africa. *Ophthalmol* 1987;94:1662-1668.

22. Sirikul T, Prabriputaloong T, Smathivat A *et al*. Predisposing factors and etiologic diagnosis of ulcerative keratitis. *Cornea* 2008;27:283-287.

23. Hagan M, Wright E, Newman M *et al*. Causes of suppurative keratitis in Ghana. *Br J Ophthalmol* 1995;79:1024-1028.

24. Green A, Apel A, Stapleton F. Risk factors and causative organisms in microbial keratitis. *Cornea* 2008;27:22-27.

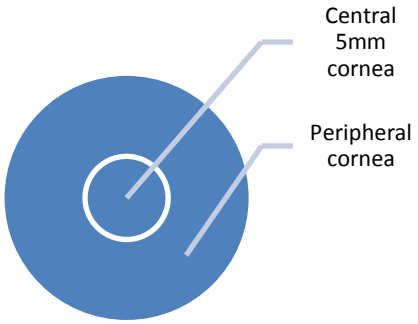
25. Kowalski RP, Dhaliwal DK, Karenchak LM *et al.* Gatifloxacin and moxifloxacin: An *in vitro* susceptibility comparison to levofloxacin, ciprofloxacin, and ofloxacin using bacterial keratitis isolates. *Am J Ophthalmol* 2003;136:500-505.

26. Mather R, Karenchak LM, Romanowski EG *et al.* Fourth generation fluoroquinolones: New arsenal in ophthalmic antibiotics. *Am J Ophthalmol* 2002;133:463-466.

27. Gangopadhyaya N, Daniell M, Weih L *et al*. Fluoroquinolone and fortified antibiotics for treating bacterial corneal ulcers. *Br J Ophthalmol* 2000;84:378-384.

Appendix A: Data Capturing Sheet

1. Corneal scraping sample number	
2. Gender	
3. Age	
4. Organism identified: 4.1 Gram positive bacteria 4.2 Gram negative bacteria 4.3 Mixed isolates 4.4 Fungi 4.5 Contaminant 4.6 Sterile/Culture Negative	
5. Antibiotic Susceptibility: 5.1 Amikacin 5.2 Ampicillin/Pen 5.3 Cefazolin 5.4 Cefepime 5.5 Cefotaxime 5.6 Cefoxitin 5.7 Ceftazidime 5.8 Cefuroxime 5.9 Chloramphenicol 5.10 Ciprofloxacin 5.11 Clindamycin 5.12 Co-amoxiclav 5.13 Colistin 5.14 Cotrimoxazole 5.15 Ertapenem 5.16 Erythromycin	Sensitive or Resistant:

5.17 Fusidic Acid 5.18 Gentamycin 5.19 Imipenem 5.20 Linezolid 5.21 Meropenem 5.22 Minocycline 5.23 Moxifloxacin 5.24 Nalidixic Acid 5.25 Pip-Taz 5.26 Rifampicin 5.27 Streptomycin 5.28 Synercid 5.29 Telithromycin 5.30 Tetracycline 5.31 Tobramycin 5.32 Vancomycin	
6. Culture morphology:	

Appendix B: Ethics Certificate

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Dr Karen M Koetsie

CLEARANCE CERTIFICATE	**M090216**
PROJECT	Microbial Keratitis: Microbiological Results of Patients with Infectious Corneal Ulcers at a University Hospital in South Africa
INVESTIGATORS	Dr Karen M Koetsie.
DEPARTMENT	Division of Opthalmology
DATE CONSIDERED	09.02.27
DECISION OF THE COMMITTEE*	Approved unconditionally

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 09.03.02 **CHAIRPERSON** .....
(Professor P E Cleaton Jones)

*Guidelines for written 'informed consent' attached where applicable

cc: Supervisor : Dr SEI Williams

DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10004, 10th Floor, Senate House, University.
I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES...

.....