

Corneal Ulcers:  
Culture isolates and antibiotic  
susceptibility of microbial  
keratitis in KwaZulu-Natal,  
South Africa

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## **Dedication**

To those that have come before me, those that have paved the way in ophthalmology, thank you. You have allowed me to work in a profession I love.

To my family and my wife: Your patience and support have given me the strength to complete this project.

## Acknowledgements

Dr Carl-Heinz Kruse, my supervisor, for your guidance and inspiration, without your direction this project would not have been possible.

The Department of Ophthalmology UKZN, thank you for having faith in me, and giving me the chance to work in this field.

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## Abstract

**Aim:** To determine the causative organisms for corneal ulcers in patients in KwaZulu-Natal, culture positivity rates and antibiotic sensitivity for the organisms cultured.

**Method:** A retrospective chart review of laboratory results of patients presenting with a corneal ulcer to St Aidan's Hospital and Addington Hospital in Durban, KwaZulu-Natal for the year 2012. Twenty eight records were received from the NHLS. The following information was extracted: age, sex, microbial isolate and antibiotic sensitivity and resistance.

**Results:** All specimens were culture positive, 3 showed mixed growth. Of the 31 organisms cultured 71% were Gram positive, 25.8% were Gram negative and 3.2% were fungal. *Streptococcus pneumoniae* (59%) and *Staphylococcus aureus* (22.7%) were the common Gram positive organisms, *Pseudomonas* was the most common Gram negative organism. Gram positive organisms were 100% susceptible to Cephalothin and Ciprofloxacin. Gram negative organisms were 88% ( $p = 0.53$ ) and 100% susceptible to Tobramycin and Ciprofloxacin respectively.

**Conclusion:** This is the first study describing sensitivities for microbial keratitis in Durban, South Africa. Similar results have been published in Johannesburg. The current treatment protocol at the UKZN Department of Ophthalmology for corneal ulcers is appropriate.

# Chapter 1

## 1. Introduction

Microbial keratitis is defined as a microbial infiltration of the corneal epithelium and/or stroma with resultant corneal inflammation and necrosis.<sup>1</sup> Bacterial keratitis is often a devastating condition and is a leading cause of monocular blindness in the developing world.<sup>2</sup> Timely use of appropriate antibiotics is therefore of utmost importance, especially in severe corneal ulcers.<sup>3</sup> These antibiotics are empirically started while awaiting microbiological culture results. Geographic location influences microbial patterns, which in turn will affect the choice of first line antibiotics.

### 1.2 Justification for the Study

Current published data on the organisms responsible for microbial keratitis in South Africa is sparse. Studies done in Johannesburg have found common organisms to be *Streptococcus pneumoniae* and *Pseudomonas aeruginosa*.<sup>4,5</sup> This holds true for KwaZulu-Natal as well.<sup>6</sup> This study will aim to identify the organisms responsible and whether current antibiotic protocols are appropriate.

## **1.3 Background**

### **1.3.1 Anatomy**

The cornea is composed of 5 layers, from external to internal being:<sup>7</sup>

1. Epithelium
2. Bowman's layer
3. Stroma
4. Descemet's membrane
5. Endothelium

The epithelium consists of 5 layers of stratified squamous epithelium. The basal layer is made up of a single layer of columnar cells, as these cells progress superficially they become flattened, non-keratinised and lose their nuclei. Desmosomes hold these cells tightly together. The Bowman's layer lies below the epithelium, is acellular and composed of collagen fibres that run in an interwoven fashion. The stroma forms the largest part of the cornea. The collagen fibrils are arranged parallel to the surface to assist in transparency. Descemet's membrane is secreted by and serves as a scaffold for the underlying endothelium. The innermost layer, the endothelium, consists of a single layer of polygonal cells. This layer plays an active role in the transport of fluid within the cornea.

### **1.3.2 Clinical Features**

For the majority of keratitis that occurs, an epithelial defect is required for the organism to infiltrate and start growing in the stroma. This is the case for streptococcus, staphylococcus, pseudomonas, mycobacterium, serratia and moraxella to name but a few. Organisms that can directly penetrate the cornea include *Listeria monocytogenes*, *Neisseria gonorrhoea*, *Haemophilus influenza* and *Corynebacterium diphtheriae*



## **1.4 Literature Review**

### **1.4.1 Epidemiology**

Extrapolating the incidence of microbial keratitis is challenging. Geographic location as well as the state of development of a country plays a major role. In the United States of America reported incidence rates are 11 per 100 000 population. In a developing country such as Nepal the rate is much higher at 799 per 100 000.<sup>8</sup> Incidence rates in Africa have not been reported on.

Multiple studies have shown microbial keratitis to be more common in males, this holds true for both developed and developing countries, including South Africa.<sup>5,8,9,10</sup> Male predominance ranged between fifty five to sixty five percent.

### **1.4.2 Organisms Identified**

On the tropical island of Taiwan a 12-year retrospective cross-sectional study was performed where *Pseudomonas* (Gram-negative) 46.7% and *Staphylococcus* (Gram-positive) 11% were the organisms most commonly identified.<sup>11</sup> A 9-year study in Miami, found similarly that *Pseudomonas* (25.7%) and *Staphylococcus aureus* (19.4%) were most commonly identified.<sup>12</sup> In south India, also a tropical area, fungi (32.77%) were the most common organism cultured. With regard to bacterial isolates *Streptococcus pneumoniae* (35.95%) and *Pseudomonas* (19.92%) were commonly isolated.<sup>7</sup>

In the United Kingdom, where a more temperate climate is experienced, bacteria are cultured more commonly than fungi, and gram positive organisms predominate.<sup>13</sup> In Johannesburg, South Africa, a region with a temperate climate,

Koetsie et al, found gram positive organisms (83.9%) were more commonly cultured as compared to gram negative (10.8%) and fungi (5.4%).

In KwaZulu-Natal, Bridgens et al found gram negative organisms (48.6%) were more common as compared to gram negative organisms (40.5%) and fungi (18%), in those corneal scrapes that were positive for growth. Peters et al found fusarium and aspergillus were the common fungal organisms cultured in the 20 patients assessed.<sup>14</sup>

These results further support the fact that geographic location as well as the state of development of an area influences the type of organism causing microbial keratitis and as such a sound knowledge of local microbiological profile is of paramount importance when determining which antibiotics to initiate.

### **1.4.3 Treatment**

Microbial keratitis is an ophthalmic emergency and is potentially sight threatening. Corneal scrapes are taken but broad spectrum antibiotics are empirically started before the culture results are available. Treatment can then be tailored as laboratory results become available.

Empirical treatment with a combination of two fortified antibiotic preparations, selected to cover the entire range of common Gram positive and Gram negative pathogens has been the mainstay of treatment for many years.<sup>15</sup> Antibiotics of choice against Gram-positive organisms are the first generation cephalosporins and an aminoglycoside is selected for Gram-negative cover. Fluoroquinolone use has become increasingly popular; it has both Gram-positive and negative cover, and therefore can be used as monotherapy. It also has low toxicity and is commercially

available. The equivalence of dual therapy using fortified antibiotics and monotherapy using a fluoroquinolone has been demonstrated in controlled trials.<sup>13</sup> Conflicting reports have shown worrying trends in microbial keratitis. Some have found resistance to fortified dual therapy antibiotics<sup>16</sup>, others have shown resistance to fluoroquinolones.<sup>12</sup>

The protocol at our department in KwaZulu-Natal for a patient with a corneal ulcer employs an intensive regimen of fortified Tobramycin (an aminoglycoside) and Cefazolin (a cephalosporin), until such time as laboratory results become available and therapy can then be directed more accurately.

## **1.5 Research Aim**

To determine the causative organisms for corneal ulcers in patients in Durban, KwaZulu-Natal, culture positivity rates and antibiotic sensitivity for the organisms cultured. This would assist to identify the most appropriate empirical therapy for microbial keratitis in this coastal city.

## **1.6 Research Objectives**

- Number of positive findings
- Number of fungal vs. bacterial keratitis
- Which bacterial organisms were identified
- Overall resistance and sensitivity to specific drugs

## **Chapter 2**

### **2. Methodology**

#### **2.1 Study design**

A retrospective chart review of laboratory results of patients presenting with a corneal ulcer to St. Aidan's Hospital and Addington Hospital in Durban, KwaZulu-Natal for the year 2012.

#### **2.2 Data Collection**

All microbiology results were received from the National Health Laboratory Service (NHLS). The information was extracted on to a data capture sheet (Appendix A).

#### **2.3 Data Management**

All patient laboratory results were assigned a corresponding study number, this assured patients' anonymity. In addition only relevant information was extracted from the laboratory files.

The data was exported to Microsoft Excel 2008. This electronic document was stored on an encrypted portable storage device with password protection.

## **2.4 Data Analysis**

Data was analysed using Intercooled Stata version 11 software. P values were generated using the Fisher's exact test

## **2.5 Ethical Considerations**

Ethical approval was obtained from the Biomedical Research Ethics Committee of the University of KwaZulu-Natal. Protocol number: BE 197/12 (Appendix B)

## Chapter 3

### 3. Results

#### 3.1 Patient Demographics

There were 28 records received from the NHLS. Of these 15 (53.6%) were male. The mean age was 40.6 years.

#### 3.2 Culture Yield

Culture results of 28 eyes from 28 patients were obtained. All specimens were culture positive, 3 showed mixed growth. Of the 31 organisms cultured 22 (71%) were Gram positive, 8 (25.8%) were Gram negative and 1 (3.2%) was a fungus.

#### 3.3 Gram-Positive organisms

Of the Gram-positive organisms cultured, the common organisms identified included *Streptococcus pneumoniae* 13 (59%), *Staphylococcus aureus* 5 (22.7%) and *Staphylococcus epidermidis* 2 (9%)

Organism	Number of Isolates
<i>Streptococcus pneumoniae</i>	13
<i>Staphylococcus aureus</i>	5
<i>Staphylococcus epidermidis</i>	2

Streptococcus viridans	1
Corynebacterium Pseudodiphtheriticum	1

*Table 1: Gram-positive organisms cultured*

### 3.4 Gram-negative Organisms

The commonest Gram-negative organism cultured was Pseudomonas 5 (62.5%).

Organism	Number of Isolates
Pseudomonas	5
Acinetobacter	1
Haemophilis influenza	1
Serratia marcescens	1

*Table 2: Gram-negative organisms cultured*

### 3.5 Fungal Organisms

One fungus was cultured which belonged to the Alternaria species.

### 3.6 Antibiotic Susceptibility

	Chloramphenicol	Cephalothin	Ciprofloxacin
Streptococcus	12 (100%)	10 (100%)	12 (100%)

<b>pneumoniae - 13</b>			
<b>Staphylococcus aureus - 5</b>	5 (100%)	5 (100%)	5 (100%)
<b>Staphylococcus epidermidis - 2</b>	2 (100%)	1 (100%)	2 (100%)
<b>Streptococcus viridans - 1</b>	1 (100%)	1 (100%)	1 (100%)
<b>Corynebacterium Pseudodiphtheriticum - 1</b>	1 (100%)	1 (100%)	1 (0%)

***Table 3: Antibiotic sensitivity of Gram-positive organisms***

	<b>Chloramphenicol</b>	<b>Tobramycin</b>	<b>Ciprofloxacin</b>
<b>Pseudomonas -5</b>	3 (1 Sensitive)	5 (5 sensitive)	4 (4 sensitive)
<b>Acinotobacter -1</b>	Resistant	Resistant	Sensitive
<b>Haemophilis influenza - 1</b>	Sensitive	Sensitive	Sensitive
<b>Serratia marcescens - 1</b>	Sensitive	Sensitive	Sensitive

***Table 4: Antibiotic sensitivity of Gram-negative organisms***



## Chapter 4

### 4. Discussion

St Aidan's Hospital and Addington Hospital are large referral centres draining the greater Durban area in KwaZulu-Natal. There have been no previous studies documenting the organisms antibiotic susceptibility profile in this province.

Durban, a region with a subtropical climate, has been found in this study to yield results where the predominant organisms cultured were Gram positive 71% and gram negative 25.8%. These results parallel those found in more temperate regions like Johannesburg and Oxford<sup>5,12</sup>. More tropical regions of Taiwan and Miami show a higher propensity for fungal and Gram negative organisms<sup>7,11,12</sup>. This was also demonstrated in a study performed at King Edward Hospital.<sup>6</sup> In this study of a 131 corneal ulcers, 84% were bacterial, of these 48.6% were gram negative and 40.5% were gram positive. There were however a large proportion of culture negative specimens which might account for the difference in data with respect to this study.

Males were found to be more affected than females, as has been described in other studies<sup>5,6,8</sup>.

The protocol at our department prescribes an intensive regimen of fortified Tobramycin (an aminoglycoside) and Cefazolin (a cephalosporin), until such time as laboratory results become available and therapy can then be directed more accurately.

Cefazolin is the fortified antibiotic that is used as primary treatment, the lab however tested for Cephalothin sensitivities, both drugs are 1<sup>st</sup> generation cephalosporins. Hsieh et al showed that both antibiotics showed good bacterial suppression, however cephalothin was more potent than cefazolin.<sup>17</sup> These sensitivities were tested at 3.13µg/ml. Topical cefazolin eye drops are administered at 50mg/ml which is a much more potent dose and this may negate the difference described by Hsieh.

Conflicting literature has arisen. Worryingly suggestions are that organisms over time have become resistant to these above antibiotics, others have shown an emerging resistance to fluoroquinolones.<sup>12</sup> Of the Gram positive organisms cultured 100% were sensitive to Cephalothin and Ciprofloxacin. Of the Gram negative organisms 88% (p = 0.53) were sensitive to Tobramycin and 100% was sensitive to Ciprofloxacin. It is heartening to see that the current protocol at our department is correct and therefore can be continued without alteration. Antibiotic resistance monitoring however should be continued as an ongoing process to obviate any change. Ciprofloxacin is a relatively old antibiotic and newer fourth generation antibiotics (moxifloxacin and gatifloxacin) are currently available in the private sector, it will be interesting to know if or how these antibiotics will change the landscape of microbial sensitivity in the future.

This study has added to the current paucity of knowledge related to microbial keratitis in South Africa. It will assist in the appropriate care for patients and will serve as a yardstick pertaining to organisms responsible for keratitis and more importantly their antibiotic sensitivities and any future changes thereof.

## 4.1 Limitations

This was a retrospective study and inherently has some shortcomings. This study has a small sample size. Details on the predisposing factors related to ulcer formation were not available and these may influence the type of organism cultured. Differences between in-vivo tests and clinical response to antibiotic treatment do exist, however as outcomes were not assessed this could not be determined. The difference of the 1<sup>st</sup> generation cephalosporin used as treatment versus that used for sensitivity testing may also act as a confounding factor.

## Chapter 5

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## Appendix A

1. Corneal Scrape Number	
2. Date	
3. Institution	
4. Age	
5. Gender	
6. Organism Isolated i. Gram +ve ii. Gram -ve iii. Fungi iv. Culture -ve v. Contaminant	
7. Antibiotic sensitivity or resistance	

## Appendix B

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**14 March 2013**

Dr. V Dullabh  
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University of KwaZulu-Natal

Dear Dr Dullabh

**PROTOCOL: Culture isolates and antibiotic susceptibility of microbial keratitis in KwaZulu-Natal, South Africa. REF: BE197/12**

### **EXPEDITED APPLICATION - RATIFICATION**

This letter serves to notify you that at a full sitting of the Biomedical Research Ethics Committee meeting held on **12 March 2013**, the Committee **RATIFIED** the sub-committee's decision to approve the above study.

Yours sincerely

A handwritten signature in black ink, appearing to read 'A Marimuthu', written over a horizontal line.

**Mrs A Marimuthu**  
*Senior Administrator: Biomedical Research Ethics*

**Plagiarism:**

DECLARATION

I...Viresh Dullabh.....declare that

(i) The research reported in this dissertation, except where otherwise indicated, is my original work.

(ii) This dissertation has not been submitted for any degree or examination at any other university.

(iii) This dissertation does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.


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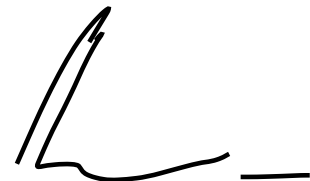
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Signed:  Date: 26/8/14

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