ADDIS ABABA UNIVERSITY
COLLAGE OF HEALTH SCIENCE
SCHOOL OF ALLIED HEALTH SCIENCE
DEPARTMENT OF MEDICAL LABORATORY SCIENCES

DEMOGRAPHIC PATTERN, RISK FACTORS, CLINICAL AND MICROBIOLOGICAL CHARACTERISTICS OF FUNGAL AND BACTERIAL KERATITIS IN MENILIK II MEMORIAL HOSPITAL, ADDIS ABABA, ETHIOPIA

By: - Munira siraj

Advisors

Adane bitew(MSC,PhD)

Menen ayalew (MD)

Gebreabiezgi Teklebirhan (MSC)

A thesis submitted to the department of medical laboratory science, school of allied health science, collage of health science, Addis Ababa University in partial fulfilment of the requirements for the degree of masters of clinical laboratory sciences speciality in diagnostic and public health microbiology.

ADDIS ABABA, ETHIOPIA

OCTOBER, 2016.
About authors

**Munira Siraj:** - is an assistant lecturer in college of health science, Arba Minch University with MSC degree in microbiology.

**Adane Bitew:** - is an associate professor of microbiology and consultant health science specialist with a PhD degree. He has been working as a researcher and instructor offering courses to graduate students in the college of health sciences, Addis Ababa University for more than 20 years. He has published many original articles in peer-reviewed international journal.

**Menen Ayalew (MD):**-is Assistant Professor, in Department of Ophthalmology, Addis Ababa University and cornea and anterior segment surgeon. She is also working as Secretary for ophthalmological society of Ethiopia from 2009 to date and Medical Director of the Eye Bank of Ethiopia from January, 2011.

**Gebreabie Zgi Teklebirhan (MSC):**- is a lecturer in the college of health sciences, Addis Ababa University with MSC degree in microbiology, and he was working as a laboratory technologist in Tikur Anbessa teaching hospital.
ADDIS ABABA UNIVERSITY,

SCHOOL OF GRADUATE STUDIES

Demographic pattern, risk factors, clinical and microbiological characteristics of fungal and bacterial keratitis in Menilik II memorial hospital, Addis Ababa, Ethiopia.

Department of medical laboratory science, college of health science, Addis Ababa University

By

Munira Siraj

Approved by the examining board

Chairman Dep. graduate committee  signature

Advisor  signature

Adane bitew (Msc,PhD)

Menen Ayalew (MD)

Gebreabiezgi Teklebirhan (MSC)

External examiner  signature

Internal examiner  signature
ACKNOWLEDGMENT
First I would like to thank almighty Allah for his grace. Then I would like to humbly acknowledge my advisor Dr Adane Bitew who helped me in every step I take in this study, starting from title selection, through the end. I’ve learned a lot because of him.

I wish to express my deep affection and appreciation to my advisor Dr Menen Ayalew, for being always kind and giving positive energy for me.

I would like to thank all resident doctors who worked in anterior segment section in Menilik II hospital, ophthalmology department in the study period for their cooperation.

I would like to extend my profound gratitude to my friends who always share me their point of view. Last but not least, I thank my family for their love and support.
# Table of Contents

ACKNOWLEDGMENT ............................................................................................................. i
LIST OF TABLE ......................................................................................................................... iv
LIST OF FIGURE ......................................................................................................................... v
ABBREVIATIONS ....................................................................................................................... vi
ABSTRACT ................................................................................................................................. vii
OPERATIONAL DEFINITION ...................................................................................................... viii

1. INTRODUCTION .................................................................................................................. 1
   1.1 background ..................................................................................................................... 1
   1.2 statement of the problem ............................................................................................... 2
   1.3 Significance of the study ............................................................................................... 3

2. LITREATURE REVIEW ....................................................................................................... 4

3. OBJECTIVE .......................................................................................................................... 8
   3.1 General objective .......................................................................................................... 8
   3.2 Specific objectives ......................................................................................................... 8

4. METHODS .............................................................................................................................. 9
   4.1 study area ....................................................................................................................... 9
   4.2 study design and period ............................................................................................... 9
   4.3 Population ..................................................................................................................... 9
      4.3.1 Source population .................................................................................................... 9
      4.3.2 Study population .................................................................................................... 9
   4.4 Eligibility ....................................................................................................................... 9
      4.4.1 Inclusion criteria ..................................................................................................... 9
      4.4.2 Exclusion criteria ..................................................................................................... 9
   4.5 sample size and sampling technique ............................................................................ 9

4.6 Variables ........................................................................................................................... 10
   4.6.1 Dependent variables ............................................................................................... 10
   4.6.2 Independent variables ............................................................................................. 10

4.7 Data collection and processing ....................................................................................... 10
   4.7.1 Socio demographic data ........................................................................................... 10
   4.7.2 Specimen collection ................................................................................................. 10
   4.7.3 Laboratory procedure .............................................................................................. 11
4.8 Data quality assurance.................................................................12
4.9 Data analysis.............................................................................12
4.10 Ethical consideration...............................................................12
4.11 Dissemination of result............................................................12
5. RESULT..........................................................................................13
  5.1. Demographic characteristics of study participants...............13
  5.2 Risk factors for microbial keratitis........................................14
  5.3. Prevalence of fungal and bacterial characteristics..............15
  5.2 clinical characteristics of fungal keratitis.............................19
  5.3 clinical characteristics of bacterial keratitis.........................20
  5.4 Comparison of direct microscopy result with culture result.....21
  5.5 Microbiological profile............................................................22
6. DISCUSSION.......................................................................................24
7. Limitation Of the study..............................................................27
8. Conclusion......................................................................................27
8. Recommendations..........................................................................29
REFERENCE..........................................................................................30
ANNEXES................................................................................................34
  Annex I:-English version of written consent........................................34
  Annex II Amharic version of written consent...............................36
  Annex III check list to collect data.................................................37
  Annex IV laboratory request form for corneal scrapping...............40
  Annex V SOP for corneal scrapping.................................................40
  Annex VI SOP for culture media preparation and identification....41
LIST OF TABLE

Table 1: Demographic characteristics of study participants (N=60) at Menilik II memorial hospital from January to September 2016

Table 2: Demographic characteristic of patients and association of fungal keratitis with sex, residence, age, occupation and risk factor (n=29) at Menilik II memorial hospital from January to September 2016

Table 3: Demographic characteristic of patients and association of bacterial keratitis with sex, residence, age, occupation and risk factor (n=18) at Menilik II memorial hospital from January to September 2016

Table 4: Association of clinical manifestation with fungal keratitis (n=29) at Menilik II memorial hospital from January to September 2016

Table 5: Association of clinical manifestation with bacterial keratitis (n=18) at Menilik II memorial hospital from January to September 2016

Table 6: Comparison of Direct microscopy and culture result (n=60) at Menilik II memorial hospital from January to September 2016

Table 7: Spectrum of fungal isolates from patients with fungal keratitis (n=29) at Menilik II memorial hospital from January to September 2016

Table 8: Spectrum of bacterial isolates from patients with bacterial keratitis (n=18) at Menilik II memorial hospital from January to September 2016
LIST OF FIGURE

Figure 1 traumatic agent in patients with corneal ulceration, (N=60), at Menilik II memorial hospital from January to September 2016.
## ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AAU</td>
<td>Addis Ababa university</td>
</tr>
<tr>
<td>BHI</td>
<td>brain heart infusion</td>
</tr>
<tr>
<td>BK</td>
<td>bacterial keratitis</td>
</tr>
<tr>
<td>CONS</td>
<td>coagulase negative staphylococcus</td>
</tr>
<tr>
<td>DMLS</td>
<td>department of medical laboratory science</td>
</tr>
<tr>
<td>DRERC</td>
<td>Department Research and Ethical Review Committee</td>
</tr>
<tr>
<td>FK</td>
<td>fungal keratitis</td>
</tr>
<tr>
<td>H$_2$O$_2$</td>
<td>hydrogen per oxide</td>
</tr>
<tr>
<td>KOH</td>
<td>potassium hydroxide</td>
</tr>
<tr>
<td>MK</td>
<td>microbial keratitis</td>
</tr>
<tr>
<td>HIV</td>
<td>human immune deficiency virus</td>
</tr>
<tr>
<td>PK</td>
<td>penetrating keratopathy</td>
</tr>
<tr>
<td>SDA</td>
<td>sabroud dextrous agar</td>
</tr>
<tr>
<td>SOP</td>
<td>standard operating procedure</td>
</tr>
<tr>
<td>WHO</td>
<td>world health organization</td>
</tr>
</tbody>
</table>
ABSTRACT

Background: Microbial keratitis is an infection of the cornea that is associated with a risk of permanent visual impairment. It can be caused by bacteria, virus, fungi, protozoa and other parasites. The incidence of corneal blindness caused by microbial keratitis in the developing world is far higher than that in the developed world.

Objective: The aim of this study was to determine the demographic pattern, risk factors, clinical and microbiological characteristics of fungal and bacterial keratitis in Menilik II memorial hospital, Addis Ababa, Ethiopia.

Methods and materials: A cross sectional study was conducted at Menilik II memorial hospital, a convenient sampling technique was used from January to September 2016. Sixty patients who were suspected to have microbial keratitis and fulfilled the requirements were included in the study. After taking a detailed history, corneal scrapping was taken by the ophthalmologist after installation of preservative free anaesthesia. Scrumpled was smeared on two slides for preparation of gram stain and 10% KOH wet mount and further scrapping was inoculated in to 0.5ml BHI broth media. Then from the broth media the sample were inoculated on bacterial and fungal solid media in the main laboratory as soon as possible. On each sample Comprehensive microbiologic studies were performed. Data were entered in to SPSS version 20 software and analysis was done.

Result: 60 patients of presumed microbial keratitis were included in this study. Of sixty cases of microbial keratitis investigated, fungi and bacteria were recovered from 29 and 18 patients giving fungal and bacterial keratitis prevalence of 48.3% and 30% respectively. Potential risk factors identified were in 80% of cases. Corneal trauma was the main risk factor (55%). The most common Fungi pathogen isolated were Aspergillus spp (41.4%) followed by Fusarium spp (24.1%). Coagulase negative staphylococci and staphylococci aureus were the common bacterial agent accounting 44.4% and 22.2% respectively from the total bacterial isolates.

Conclusion
Corneal trauma was the major predisposing factor for microbial keratitis. *Aspergeilius* and coagulase negative *Staphylococci* were the most common isolates.

**OPERATIONAL DEFINITION**

**Microbial keratitis:** a loss of the corneal epithelium with underlying stromal infiltration and suppuration associated with signs of inflammation with or without hypopyon.

**Hypopyon** :-an accumulation of white blood cells in the anterior chamber of the eye.

**Anterior chamber reaction:** - presence of white blood cells and/or proteinaceous fluid in the anterior chamber

**Endothelial plaque:** - abnormal collections of a mixture of mucus, epithelial cells, and proteinaceous and lipoidal material that adhere firmly to the corneal surface.

(www. Medical dictionary.thefreedddictionery.com)

**Positive culture result:** A culture media was considered as positive when the following criteria was fulfilled

- Growth of a microorganism on one media with consistent microscopic finding
- Growth of a known ocular pathogen at the site of inoculation on a solid media
- When the type of microorganism isolated consistent with clinical sign
- Growth of the same microorganism on two or more media
1. INTRODUCTION

1.1 Background

Microbial keratitis is an infection of the cornea that is associated with a risk of permanent visual impairment. It can be caused by bacteria, virus, fungus, protozoa and parasites (1).

The severity of corneal infection depends on the underlying condition of the cornea and pathogenicity of the infecting organism (2). The spectrum of microbial agents associated with corneal ulcer is wide and varies from one geographical location to another (3).

Gram positive bacterial species are more frequently recovered in temperate zones, and gram-negative species in tropic climates and tropical countries. Humid atmosphere and outdoor occupations make the population more vulnerable to fungal infections (2).

The presence of a predisposing factor renders the corneal epithelium more vulnerable and more susceptible to microbial infections. The majority of cases have a clearly identifiable risk factor for infection (4).

Several risk factors such as contact lens wear, trauma, ocular surface disease, ocular surgery, and systemic disease have been reported to predispose patients to corneal infections (5).

On a global level, predisposing risk factors for microbial keratitis vary tremendously with geographical location. Although trauma to the eye accounted for 48 to 65 % of all corneal ulcers in developing countries, at a large trauma referral centre in the United States, trauma accounted only for 27% of all cases (6).

Untreated infectious keratitis may result in corneal perforation, with the potential for development of endophthalmitis and the loss of the eye. Infectious keratitis can occur in any part of the cornea, but the infection involving the central cornea is of paramount importance. Scarring in this location has the potential to cause visual loss, even if the infecting agent is successfully eradicated, while some bacteria(e.g. Gonococcus) can invade intact epithelium, most infectious keratitis develop at the site of an abnormality or defect in the corneal surface (7) .
Adequate ocular prevention, identification of the pathogen, initiation of immediate therapy, modification based on the organism identified and consideration of drug toxicity and bacterial resistances represent the keys to success in the management of microbial keratitis (8).

1.2 statement of the problem

A report on the causes of blindness worldwide consistently lists corneal ulceration second only to cataract as the major aetiology of blindness and visual disability in many of the developing nations in Asia, Africa, and the Middle East (9), and microbial keratitis is a major cause of ocular morbidity and unilateral blindness (3).

With the worldwide decrease in trachoma and other traditional causes of blindness such as onchocerciasis, corneal blindness caused from microbial keratitis is emerging as an important cause of visual leprosy, World Health Organization (WHO) recognize that, there is a “silent epidemic” of blindness that is occurring unnoticed worldwide (10).

Ocular trauma and corneal ulcers annually results in 1.2 to 2 million cases of corneal blindness globally with 90% of them occurring in developing countries (4). The incidence of this condition varies from 11 per 100,000 in the United States to 1 - 299 per 100,000 in developing countries (8).

High rainfall, longer rainy season, and high humidity throughout the year have been identified as favourable environmental conditions for bacterial fungal and growth in countries located in tropical and sub-tropical regions such as Ethiopia. Moreover, eighty five percent of the population of the country is engaged in agriculture including the labour-intensive tea and coffee plantation industries that exposes them to trauma which is the major pre-disposing risk factor for microbial keratitis. These make people more vulnerable for corneal ulceration and microbial infection. Due to lack of routine culture and experts in the field in health institutions the actual magnitude of MK is unknown in Ethiopia.
1.3 Significance of the study

To be able to diagnose and manage properly, conducting research to understand the actual magnitude of microbial keratitis and its etiological agents from patients referred all over the country to the only referral eye clinic in the country appears to be timely and appropriate. Thus, this study was designed to determine the magnitude of microbial keratitis and the spectrum of microorganisms implicated in causing the ulceration. Findings from this study will provide up-to-date information on microbial keratitis for evidence-based action aimed at reducing the morbidity of the infection.
2. LITREATUER REVIEW

There is a wide variation in the causative organisms for microbial keratitis in different parts of the world. To some degree this variation is explained by economic factors as well as contact lens wear. Shah et al (11) reviewed 37 research articles around the world (India, North America, Canada, Australia, Europe, Far East, Africa and South America). According to this review bacteria were the most frequent isolates 95% of which *staphylococcal* species accounted 79%, followed by *pseudomonas* species 55% and *streptococcal* species 47%.

A longitudinal study consisting of 253 patients with corneal ulcer was conducted at a major public hospital in Australia over a 5 year period by Green et al(12). The result of this study revealed that Gram positive bacteria were the most common group of organisms isolated, 29% of which were *Staphylococci* and *Streptococci*. Of the Gram negative bacteria, 23% was accounted by *Pseudomonas aeruginosa*. Fungi accounted 5% of the isolates of which *Fusarium* species were the commonest isolates.

A total of 1,786 patients were enrolled in study conducted in Ports mouth, England by Youhanna et al (4). Corneal scrapes was taken from 1,254 patients, pathogens were isolated in 800 (63.8%) patients. Pure bacterial growth was detected in 54.5% patients. The majority of the isolated bacterial were Gram-positive (71.1%) with a predominance of *Staphylococcus epidermidis* (31.7%). Furthermore, Gram-negative bacteria isolate accounted 28.9% of the total 979 bacterial isolates with the predominance of *P. aeruginosa* (12%).

A retrospective study of 300 cases presenting with bacterial keratitis was performed in Paris France by Bourcier et al (13), among them 207 (68.2%), were culture positive 83% of them being Gram positive bacteria, coagulase negative *Staphylococcus* species was the commonest isolate. Gram negative bacteria accounted 17% being dominated by *Pseudomonas* and *Serratia* species. Poly bacterial infection was noted in 2% of cases while contact lens wear was the most important risk factor.
Another retrospective study was carried out in Thailand. The study consists of 127 ulcerative keratitis cases. Among the enrolled in this study, bacterial keratitis accounted 60% followed by fungal keratitis (38%) and Acanthamoeba keratitis (2%). *Pseudomonas* species were the most common bacterial isolate followed by *streptococcus* and *staphylococcus* species. *Fusarium* spp were the most common fungal isolate followed by *Aspergillus*. Ocular trauma showed strong association with fungal keratitis while contact lens wearer and ocular disease were associated with bacterial keratitis (14).

Similar study conducted in Malaysia consisting of 42 ulcerative keratitis cases. Bacteria were recovered in 79.3% patients, fungi isolated in 13.8%. *Pseudomonas aeruginosa* was the commonest bacteria isolated, followed by *Streptococcus pneumonia*, A total of four (*Fusarium* spp., *Aspergillus* spp., and *Penicillium* spp) fungal specious isolated. Corneal trauma due to different agricultural practices was the major risk factor (15).

Several studies have been done in India on microbial keratitis in different regions. A study aimed to identify the etiologic agents and predisposing factor responsible for microbial keratits in Maduaria, South India. In this study, about 434 patents with corneal ulceration were evaluated of which 297 (68.4%) were culture positive. Among culture positive patients bacteria were isolated in 47.1 % while fungal isolates accounted 46.8 % and 5.1% mixed infection consisting of bacteria and fungi. The most common bacterial pathogen isolated was *Streptococcus pneumoniae*, (44.3%), followed by *Pseudomonas* spp (14.4%). The most common fungal pathogen isolated were *Fusarium* spp, representing 47.1% followed by *Aspergillus* spp (16.1%). Corneal injury was the major predisposing risk factor (16).

Another study consisting of 200 cases was conducted by Kumar et al (7). Among the study subjects 110 (55%) were culture positive. Pure bacterial growth was present in 53 (26.5%) and pure fungal growth in 45 (22.5%). Mixed microbial growth was present in the cultures of detected in 12 (6%).
Epidemiological characteristics, microbiological profile, and treatment outcome of patients with suspected microbial keratitis was carried out in Hyderabad, India. Microorganisms were detected in 3563 (60.4%) among 5897 cases. Of the cases 2884 (80.9%) were direct microscopy positive and 3563 was culture positive. Among culture positive corneal scarping bacteria, fungi, Acanthamoeba accounted 2115 (59.3%), 1598 (44.8%) and 118 (3.3%) of all cases respectively. Among bacterial isolates 42.3% represented by *Staphylococcus epidermidis* and *Fusarium* species were the leading cause of fungal infections accounting 36.6%. More number of patients with fungal, Acanthamoeba and polymicrobial keratitis (bacteria and fungus; bacteria and parasite) were found to be involved in agriculture-related activities as compared to other occupations; this feature was not evident in patients with pure bacterial keratitis (17).

Similar study was conducted in north Karnataka, aiming to study the incidence and microbiological profile of keratitis. The most common predisposing factor associated with infectious keratitis was trauma accounting 72%. Among the bacterial etiology *Pseudomonas aeruginosa* is the most common agent followed by *Streptococcus pneumoniae*. Among the fungal isolates *Fusarium* spp was common followed by *Aspergillus* spp. (10).

Another study in eastern Nepal, examined 447 corneal scraping, Growth of etiologic agents was found in 303 (67.8%) samples. Of these 145 (47.8%) had pure fungal growth, 103 (34%) had pure bacterial growth and 55 (18.2%) had mixed fungal and bacterial infection. The commonest fungal pathogen was *Aspergillus* spp in 78 (38.4%) followed by *Fusarium* spp. *Staphylococcus aureus* ((56.7%) was the predominated bacterial agent. *Streptococcus pneumoniae* (33, 20%) was second in the list (3).

A retrospective study was conducted in south east Brazil consisting of 190 patients with confirmed microbial keratitis. The predominant bacterial species isolated was *Staphylococcus epidermidis* (23.21%), followed by *Staphylococcus aureus* (10 of 56; 18%). The predominant fungal species isolated was *Fusarium* spp. (11 of 17; 65%), followed by *Aspergillus* (24%). Injury was significantly more frequent for fungal keratitis than bacterial keratitis and when the trauma involved a vegetative matter; injury was highly correlated with fungal keratitis. On the other hand, features such as the presence of ocular or systemic disease as well as previous ocular surgery were more frequently correlated with bacterial keratitis (18).
Microbial keratitis in Africa including Ethiopia is poorly known. A study in South Africa, aimed to describe microbial etiological agents and clinical manifestation of microbial keratitis was carried out by Monica and Williams (19). Among 151 patients with microbial keratitis, they isolated 93 pathogens among them 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 bacteria isolated. Of the fungi, *Fusarium* (60%) was the most common isolate.

In Ghana, a total of 207 consecutive cases presenting with suppurative keratitis were studied. Of 114 culture positive case 199 isolates were recovered patients. Of which the most common isolate being *Fusarium* species, *Pseudomonas aeruginosa*, and *Staphylococcus epidermidis*. Fungi, alone or in combination, were isolated from 56% of the patients who had positive cultures. An eye injury during the previous 3 months was reported by 77 (39-2%) of the patients. The most common causes of eye trauma were wood, sticks, and twigs (18 patients), other vegetation (10 patients), and stones, sand, and dirt (17 patients). No eye injury was reported by 122 (60.8%) patients (20).

A cross sectional study was carried out by Gebremariam et al in Jimma university specialized hospital aimed to identify bacterial aetiology and risk factor of bacterial keratitis from 24 corneal scrapping 20 were culture positive and *pseudomonas aeruginosa* was the commonest isolate. Corneal trauma was identified as the commonest identified risk factor (37.5%) for bacterial keratitis (21). another study done by Tesfaye et al, from the same institution reported bacterial infection in 74.7% of external ocular infection and *staphylococcus aureus* was the commonest bacterial isolate (22).
3. OBJECTIVE

3.1 General objective
To assess the Demographic Pattern, Risk Factors, Clinical and Microbiological Characteristics of Fungal and bacterial Keratitis in Menilik II Memorial hospital, Addis Ababa, Ethiopia.

3.2 Specific objectives
- To determine the demographic pattern of fungal and bacterial keratitis
- To determine prevalence of fungal and bacterial keratitis
- To determine risk factor responsible for fungal and bacterial keratitis
- To determine clinical manifestation of fungal and bacterial keratitis
- To determine microbiological profile of fungal and bacterial keratitis
- To compare the sensitivity of direct microscopy with culture
4. METHODS

4.1 study area
The study was conducted at Menilk II memorial hospital from January to September 2016. The hospital was built in 1909. It is the oldest hospital in our country. It is the only referral clinic for the management of infectious keratitis.

4.2 study design and period
Hospital based cross sectional study was conducted from January to September 2016, at Menilik II memorial hospital, Addis Ababa, Ethiopia.

4.3 Population

4.3.1 Source population
All patients with external ocular infection attending ophthalmology clinic of Menilik II memorial hospital during the study period.

4.3.2 Study population
Patients with ulcerative cornea and presumed to have microbial keratitis.

4.4 Eligibility

4.4.1 Inclusion criteria
- Willingness to participate in the study,
- presumptive diagnosis of infectious keratitis

4.4.2 Exclusion criteria
- Patients who are under antibiotic and anti-fungal treatment at presentation.
- Noninfectious corneal ulceration such as viral ulcer, Mooren’s ulcer, sterile neurontropic ulcers, marginal keratitis, ulcers associated with autoimmune disorders and healing ulcer.

4.5 sample size and sampling technique
A convenient sampling technique was used. All patients who fulfilled the inclusion criteria during the study period (January to September, 2016) were included in the study. A total of 60 patients who fulfilled the inclusion criteria were included in the study.
4.6 Variables

4.6.1 Dependent variables
- Microbiological profile

4.6.2 Independent variables
- predisposing factor
- Clinical manifestation
- Age
- Sex
- Occupation
- Residence

4.7 Data collection and processing

4.7.1 Socio demographic data
The requisition form was a standard proforma filled up by the ophthalmologist, documenting socio-demographic information, presence of risk factors, history of trauma, traumatic agents, associated ocular conditions (use of contaminated ocular medication, contact lens wear, aqueous tear deficiency, recent corneal disease (neurotropic keratopy), chronic dacryocystitis) The use of topical medication including topical corticosteroids, and other systemic disease (including patients who had a deficiency of immune response, by either systemic immunosuppressive drugs or some disease process (e.g. AIDS, diabetes)) was also noted. Visual acuity at the time of presentation and all clinical findings were collected.

4.7.2 Specimen collection
Before the procedure patients were informed about the purpose of the study and written consent was obtained. Then the patient undergoes full bio microscopic examination to record the size, location and type of the edge of ulcer. Clinical findings such as the presence of hypopyon, anterior chamber reaction, and upper eye lid oedema and endothelial plaque was also recorded. Corneal scraping was collected under aseptic conditions with a sterile 21 gauge needle, following the instillation of a local preservative free anaesthetic (TETRACANE HYDROCLORIDE 0.5%, Alcon laboratories Inc) from the base and leading edge of the ulcer by an ophthalmologist under the magnification of a slit lamp.

4.7.3 Laboratory procedure
The material scraped from corneal ulcer was initially directly smeared onto two separate labelled slides in a thin, even manner to prepare a 10% potassium hydroxide wet mount
and a gram stain. The consequent scrape was inoculated in to 0.5 ml brain heart infusion broth media. The transport media was transported to the main laboratory within 2-3 hours (23). Brain-heart infusion broth was then inoculated onto Sabouraud Dextrose Agar (SDA) supplemented with chloramphenicol (Oxoid, Basingstoke, UK) and incubated at 25°C for 4 weeks aerobically checking for fungal growth at two days interval. Brain infusion broth was also inoculated onto Blood Agar base (Oxoid, Basingstoke, Hampaire, UK) to which 10% sheep blood is incorporated, MacConkey agar, (Oxoid, Basingstoke, Hampaire, UK) and chocolate agar. All plates except MacConkey agar were incubated at 35–37°C for 18 to 24 hours in 5% carbon dioxide incubator. MacConkey agar was incubated at 35–37°C for 18 to 24 aerobically. Plates with no growth after 24 hours were re incubated for a further 24 hours. Preparation and performance evaluation of culture media were done as per the instruction of the manufacturer.

4.7.3.1 Identification of microorganism

**Fungal identification**

Fungi were identified by studying their microscopic, macroscopic characteristics and by using an array of biochemical and assimilation tests. Briefly, cultures of mycelia fungi (molds) were identified by examining macroscopic and microscopic characteristics of their colony. Texture, rate of growth, topography and pigmentation of the front and the reverse side of the culture were employed for macroscopic identification. Microscopic identification of mold isolates was performed by placing pieces of a colony from SDA to clean microscopic slide and staining with lactophenol cotton blue. After placing a cover slip, the characteristics of conidia and mycelia of each isolate were studied microscopically.

**Bacterial Identification**

Pure isolates of bacterial pathogen were preliminary characterized by colony morphology, gram stain and haemolytic reactions on blood agar plates. Identification of bacteria down to genus and/or species level was done by employing an array of routine biochemical tests such as catalase test, indole production test, H₂S production test, DNASe or coagulase test, gas production test, motility tests urease test citrate utilization test.

4.8 Data quality assurance

Standard operating procedures (SOPs) were strictly followed in all stages (pre-analytical, analytical and post-analytical stages). Adequate specimen was collected using appropriate equipment and method. The specimen was kept free of contamination. All materials, equipment and procedures were adequately controlled. Culture media was tested for sterility
and performance. The performance of equipments (autoclave, incubators) was monitored by using standard procedures. All patient information collected during the course of the research was kept strictly confidential and only information directly relevant to the study was extracted.

4.9 Data analysis
Clinical data and laboratory result was compiled and entered in to SPSS version 20 software and analysis was done. Tables and graphs was used to describe the results. Percentage, frequency, chi-square and liner regression test was performed to check the presence of association between dependant and independant variables.

4.10 Ethical consideration
The study was conducted after it is ethically reviewed and approved by the Department of Research and Ethical Review Committee (DRERC) of Department of Medical Laboratory Sciences, College of allied Health Sciences, Addis Ababa University and the Addis Ababa city health bureau. Ethical clearance was also obtained from Menilik memorial II hospital research committee. Informed Written consent was obtained from each participant before data collection and when they found to be positive for microbial keratitis they were informed by the clinician and received appropriate treatment.

4.11 Dissemination of result
After conducting the research, the results of the study was submitted to Department of Medical Laboratory Sciences (DMLS) Addis Ababa University (AAU). Results were also submitted to Menilik II memorial hospital. And whenever necessary the research will be presented to other concerned bodies like professional associations. At the end the paper will be submitted to peer reviewed journals for publication.

5. RESULT

5.1. Demographic characteristics of study participants
In this study 60 patients were included among this 38 (63.3%) were male and 22 (36.7%) were female. The median age was 46 years with a range of 16 to 75 years. Male patients with the age group of over 45 years were more affected (60%) than other age groups. Thirty three (55 %) patients were farmers, 14 (23.3%) were labourers, 9(15%) were housewives. the
remaining 4 (6.7%) patients were students/office workers or unemployed. There were 37(61.7%) rural residents and 23(38.3%) urban residents.

**Table 1**: Demographic characteristics of study participants (N=60) at Menilik II memorial hospital from January to September 2016.

<table>
<thead>
<tr>
<th>Demographic characteristics</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>38</td>
<td>63.3</td>
</tr>
<tr>
<td>Female</td>
<td>22</td>
<td>36.7</td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15-24</td>
<td>5</td>
<td>8.3</td>
</tr>
<tr>
<td>25-34</td>
<td>9</td>
<td>15</td>
</tr>
<tr>
<td>35-44</td>
<td>10</td>
<td>16.7</td>
</tr>
<tr>
<td>&gt;45</td>
<td>36</td>
<td>60</td>
</tr>
<tr>
<td><strong>Resident</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urban</td>
<td>23</td>
<td>38.3</td>
</tr>
<tr>
<td>Rural</td>
<td>37</td>
<td>61.7</td>
</tr>
<tr>
<td><strong>Occupation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Farmers</td>
<td>33</td>
<td>55</td>
</tr>
<tr>
<td>Laborers</td>
<td>14</td>
<td>23.3</td>
</tr>
<tr>
<td>Housewives</td>
<td>9</td>
<td>15</td>
</tr>
<tr>
<td>Student/office worker/unemployed</td>
<td>4</td>
<td>6.7</td>
</tr>
</tbody>
</table>

**5.2 Risk factors for microbial keratitis**

Possible risk factors for microbial keratitis were identified in 48 (80%), among those 3 patients report more than one possible risk factor for an infection, (trauma and decrease immunity in 2 patients, steroid usage and trauma in 1 patient).

Corneal trauma was the most common risk factor, which is caused by vegetative material in 20 patients (33.3%), by stone, sand and soil in 8 patients (13.3%), thermal burn in 3 patients (5%) and by miscellaneous material in 2 patients (3.3%). Totally corneal trauma accounted 55% of the predisposing factor.
Systemic illness was the second most common reported risk factor in 7 patients (11.7%), followed by previous corneal surgery in 5 patients (8.3%) and previous use of drug like steroid in 3 (5%). Since there were no patients using contact lenses, contact lens infection was not a predisposing factor.

![Pie chart showing causes of corneal trauma](image)

**Figure 1** Traumatic agent in patients with corneal ulceration (n=60) at Menilik II memorial hospital from January to September 2016.

### 5.3. Prevalence of fungal and bacterial characteristics

Sixty patients suspected of microbial keratitis were examined for fungal and bacterial keratitis. The overall prevalence of microbial keratitis were 78.3%. Out of 60 cases of microbial keratitis investigated, fungi and bacteria were recovered from 29 and 18 patients giving fungal and bacterial keratitis prevalence of 48.3% and 30% respectively. Subgroup prevalence of rates of bacterial and fungal keratitis is presented in table 1 and 2.

**Table 2** Demographic characteristic of patients and association of fungal keratitis with sex, residence, age, occupation and risk factor (n=29) at Menilik II memorial hospital from January to September 2016

<table>
<thead>
<tr>
<th>Demographic Variable</th>
<th>number</th>
<th>Fungal</th>
<th>P value</th>
<th>COR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>
Demographic characteristics of study subjects and association of fungal keratitis with sex, age, residence, occupation and risk factors are depicted in Table 1. Of 29 patients with fungal keratitis, 7(24.1%) were female and 22 (75.9 %) were male patients. The association of fungal keratitis with gender was statistically significant [(COR = 3.667, 95 % CI, 1.175-11.442) (P=0.022)]. Eighteen (62.1%) and 11 (37.9 %) patients with fungal keratitis were inhabitants of rural and urban areas respectively, there is no statistically significant association between residence and fungal keratitis. Fungal keratitis was the highest (55.2 %) in patients over 45 years old followed by age groups of 35–44 (20.6%). The association of fungal keratitis with age was not statistically significant. Similarly, fungal keratitis was higher in farmers
accounting 58.6%, followed by daily laborers (31%). Fungal keratitis was not statistically associated with occupation. Trauma was the predominant risk factor reported from 55% of the total patient but it was not significantly associated with fungal keratitis.

Table 3 Demographic characteristic of patients and association of bacterial keratitis with sex, residence, age, occupation and risk factor (n=18) at Menilik II memorial hospital from January to September 2016.

<table>
<thead>
<tr>
<th>Demographic Variable</th>
<th>Number</th>
<th>Bacterial keratitis</th>
<th>P value</th>
<th>COR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Yes</td>
<td>No</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex (n=60)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>38</td>
<td>14</td>
<td>24</td>
<td>0.257</td>
<td>1.983</td>
</tr>
<tr>
<td>Female</td>
<td>22</td>
<td>4</td>
<td>18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Residence (60)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rural</td>
<td>37</td>
<td>12</td>
<td>25</td>
<td>0.603</td>
<td>1.360</td>
</tr>
</tbody>
</table>
As shown in table 2, the prevalence of bacterial keratitis was greater in male (77.8%) than female (22.2%) study subjects and in the inhabitants of rural (66.7%) than inhabitants of urban (33.3%). Bacterial keratitis was not significantly associated neither with gender (p=0.257) nor residences (p= 0.603%). Bacterial keratitis was significantly associated in

<table>
<thead>
<tr>
<th>Age(60)</th>
<th>Urban</th>
<th>23</th>
<th>6</th>
<th>17</th>
<th>15-24</th>
<th>5</th>
<th>1</th>
<th>4</th>
<th>0.808</th>
<th>0.750</th>
<th>0.740-7.613</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>25-34</td>
<td>9</td>
<td>6</td>
<td>3</td>
<td>0.026</td>
<td>6.000</td>
<td>1.238-29.069</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>35-44</td>
<td>10</td>
<td>2</td>
<td>8</td>
<td>0.744</td>
<td>0.134</td>
<td>0.134-4.203</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&gt;45</td>
<td>36</td>
<td>9</td>
<td>27</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Occupation(60)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Agriculture</td>
<td>33</td>
<td>11</td>
<td>22</td>
<td>0.99</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Laborers</td>
<td>14</td>
<td>4</td>
<td>10</td>
<td>8</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Housewife</td>
<td>9</td>
<td>1</td>
<td>8</td>
<td>0.99</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Student</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>8</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Office- worker</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>0.99</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Unemployed</td>
<td>1</td>
<td>-</td>
<td>8</td>
<td>-</td>
<td>1</td>
<td>0.98</td>
</tr>
<tr>
<td>Risk factor(48)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Trauma</td>
<td>33</td>
<td>10</td>
<td>8</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Systemic</td>
<td>7</td>
<td>3</td>
<td>23</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Illness</td>
<td>5</td>
<td>3</td>
<td>4</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Ocular surgery</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>0.99</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Steroid usage</td>
<td>1</td>
<td></td>
<td></td>
<td>9</td>
<td>0.99</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>9</td>
<td>0.99</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>9</td>
<td>0.99</td>
<td>9</td>
</tr>
</tbody>
</table>
patients with age groups of 25-34 (p= 0.026). With regards to occupations, farmers were more affected (61.1%) than patients engaged in other activities. The association of bacterial keratitis, however, was not significant in patients engaged in farming (P=0.998). Of predisposing factors, trauma was the predominate predisposing factor accounting 55%, but bacterial keratitis was not significantly associated with trauma (p= 0.999)

5.2 clinical characteristics of fungal keratitis

The association of clinical manifestation with fungal keratitis is shown in Table 3. As shown in table 3, hypopyon was the predominant clinical manifestation with fungal keratins accounting 79.3% followed by upper eye lid edema (55.2%), anterior chamber reaction (48.3%), and endothelia plague (24.1%). None of the clinical manifestations were significantly associated with fungal keratitis.
Table 4 Association of clinical manifestation with fungal keratitis (n=29) at Menilik II memorial hospital from February to September 2016

<table>
<thead>
<tr>
<th>Clinical manifestation</th>
<th>Number</th>
<th>fungal keratitis</th>
<th>P value</th>
<th>COR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>yes</td>
<td>No</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypopyon</td>
<td>44</td>
<td>23</td>
<td>21</td>
<td>0.314</td>
<td>0.548</td>
</tr>
<tr>
<td>Anterior chamber reaction</td>
<td>31</td>
<td>14</td>
<td>17</td>
<td>0.611</td>
<td>1.301</td>
</tr>
<tr>
<td>Upper eyelid edema</td>
<td>28</td>
<td>16</td>
<td>12</td>
<td>0.307</td>
<td>0.587</td>
</tr>
<tr>
<td>Endothelial plaque</td>
<td>9</td>
<td>7</td>
<td>2</td>
<td>0.466</td>
<td>0.177</td>
</tr>
</tbody>
</table>

5.3 clinical characteristics of bacterial keratitis

The association of clinical manifestation with bacterial keratitis is shown in Table 4. As shown in table 4, hypopyon was the predominant clinical manifestation accounting for 77.8% followed by upper eye lid edema (44.4%), anterior chamber reaction (38.9%), and endothelia plaque (5.6%).respectively. None of the clinical manifestations were significantly associated with bacterial keratitis.

Table 5 Association of clinical manifestation with bacterial keratitis (n=18) at Menilik II memorial hospital from January to September 2016.

<table>
<thead>
<tr>
<th>Clinical</th>
<th>Number</th>
<th>bacterial keratitis</th>
<th>P value</th>
<th>CO</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>manifestation</td>
<td>yes</td>
<td>No</td>
<td>R</td>
<td></td>
<td></td>
</tr>
<tr>
<td>----------------------</td>
<td>-----</td>
<td>-----</td>
<td>----------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypopyon</td>
<td>44</td>
<td>30</td>
<td>0.611</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.71</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.195-2.614</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anterior chamber</td>
<td>31</td>
<td>24</td>
<td>0.199</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2.09</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.489-5.052</td>
<td></td>
<td></td>
</tr>
<tr>
<td>reaction</td>
<td></td>
<td></td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Upper eyelid edema</td>
<td>28</td>
<td>20</td>
<td>0.693</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.25</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.412-3.790</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endothelial plaque</td>
<td>9</td>
<td>8</td>
<td>0.232</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3.75</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.429-32.760</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

5.4 Comparison of direct microscopy result with culture result

Of a total of 60 patients, 47 (78.3%) case were culture positive among this 27 (45%) were correlated with direct microscopy, in 3 (7.3%) cases organisms were seen under direct microscope but culture were negative. Although 20 (33.3%) cases were negative under microscope their culture yielded positive result. Among the study population fungi and/or bacteria were neither detected nor showed visible growth in culture in 13(21.7%) samples despite being obtained from lesions compatible to keratitis (Table 5).

**Table 6** Comparison of Direct microscopy and culture (n=60) at Menilik II memorial hospital from January to September 2016
5.5 Microbiological profile

Table 7 Spectrum of fungal isolates from patients with fungal keratitis (n=29) at Menilik II memorial hospital from January to September 2016

<table>
<thead>
<tr>
<th>Fungal isolates</th>
<th>Number</th>
<th>% of the total</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aspergillus</em></td>
<td>12</td>
<td>41.4</td>
</tr>
<tr>
<td><em>Fusarium</em></td>
<td>7</td>
<td>24.1</td>
</tr>
<tr>
<td><em>Pencillum</em></td>
<td>3</td>
<td>10.3</td>
</tr>
<tr>
<td><em>Orobasidium</em></td>
<td>2</td>
<td>6.9</td>
</tr>
<tr>
<td><em>Acromonium</em></td>
<td>2</td>
<td>6.9</td>
</tr>
<tr>
<td><em>S. dimunatum</em></td>
<td>1</td>
<td>3.5</td>
</tr>
<tr>
<td><em>Cladospora</em></td>
<td>1</td>
<td>3.5</td>
</tr>
<tr>
<td><em>Rizopus</em></td>
<td>1</td>
<td>3.5</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>29</td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>
A total 47 fungal and bacterial isolates were recovered, of which 29 isolates were fungi and the remaining 18 isolates were bacteria (Table 6 and 7). Of the total fungal isolates *Aspergillus* and *Fusarium* species accounted for 41.4% and 24.1% the total fungal isolates respectively. Of 18 bacterial isolates gram positive bacteria accounted for 83.3% while gram negative bacteria consisted of 16.7%. Of gram positive bacteria coagulase negative staphylococci accounted for 44.4%.

**Table 8** Spectrum of bacterial isolates from patients with bacterial keratitis (n=18) at Menilik II memorial hospital from January to September 2016

<table>
<thead>
<tr>
<th>Bacterial isolates</th>
<th>Number</th>
<th>% of the total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gram positive</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>coagulase negative</td>
<td>8</td>
<td>44.4</td>
</tr>
<tr>
<td><em>staphylococcus</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>4</td>
<td>22.2</td>
</tr>
<tr>
<td><em>Streptococcus</em> spp</td>
<td>3</td>
<td>16.7</td>
</tr>
<tr>
<td><strong>gram negative</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>klebsella</em> spp</td>
<td>2</td>
<td>11.1</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>1</td>
<td>5.6</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>18</td>
<td>100</td>
</tr>
</tbody>
</table>
6. DISCUSSION

Both fungi and bacteria agents are implicated as causative agents of microbial keratitis. A number of studies on the prevalence of different isolates in corneal ulcers occurring in various parts of the world have shown a striking difference in the frequency of different pathogens.

Out of 60 patients with infectious keratitis attending Minilik II Memorial Hospital over 8 month period, the prevalence of bacterial keratitis was found out to be 30%. Our finding was not in line with previous local studies that reported a prevalence of bacterial keratitis in the range of 59.4-83% (21-24). Variation in the prevalence rate of bacterial keratitis in our study with earlier local studies could have resulted from regional difference of bacterial keratitis attributed to culture, methodology, geographical location and/or seasonal variations.

The result of this study showed that the highest frequency of bacterial keratitis (50 %) was in the age groups of over 45. Age groups of 25-34 (p= 0.026) has statistically significant
association with bacterial keratitis. Our findings in this regard was inconstant with the findings of Shiferaw et al, (24) that reported of bacterial keratitis was significant (p=0.009) in patients over 55 years age. This could be explained by the fact that patients in the age group of 25–34 years in this study are the main force of manual works, especially in agricultural and outdoor activities.

Our study revealed that 66.7 % of bacterial isolates were recovered from rural population. Furthermore, patients engaged in farming accounted 61.1% isolates Followed by daily laborer (22.2 %) together accounting 83.3 % of the total bacterial isolates. This is obvious because the rural parts of Ethiopia are inhabited by farmers and daily laborer.

We found that corneal trauma was the commonest predisposing factor for bacterial keratitis, out of 18 patients with bacterial keratitis, trauma was a predisposing risk factor in 10 (55.6%) patients. This is consistent with similar studies (21, 23, 25). In contrast studies done by Bourcier, et al, (13) and sirkuhal, et al, (14) reported contact lens wear as the major predisposing factor for bacterial keratitis. Hypopyon was the predominant clinical manifestation associated with bacterial keratitis.

Out of 18 bacterial isolates, gram positive bacteria accounted for 83.3%. This is in good agreement with the findings of studies conducted in France (13), Nigeria (26) and Ethiopia (27). Suggestive of Gram positive cocci as a primary cause of external ocular infection. Of gram positive bacteria coagulase negative *staphylococci* accounted for 44.4 %. Earlier studies in different parts of the world showed that coagulase negative *staphylococci* were the most predominant isolated pathogen from keratitis (7, 13, 18). Other study depicted that *Staphylococcus aureus* as predominant isolate (2, 3, 26). *Streptococcus pneumoniae* was also reported as commonest Gram positive bacterial pathogen in external ocular infections from India, Malaysia, Nigeria, and Ethiopia (16, 27, 28,29).

The prevalence of gram negative bacteria in the present study was 16.7% which is much less than the prevalence of gram negative in a study conducted in Ethiopia (48%), India (39.7%) and Nigeria (23,30).

Although fungal keratitis accounts for about 50 % of all cases of culture-proven microbial keratitis the magnitude of the problem and its etiologic agents are poorly known in Ethiopia.
In the present study out of 60 patients with infectious keratitis the prevalence of fungal keratitis was found out to be 48.3 %. Though the prevalence of fungal keratitis in our study was within the reported range, a comparatively high prevalence rate was achieved. Prevalence of fungal keratitis of 30.4 %, 37.5% and 36.8 % were reported in similar studies conducted by Garg et al (31), Sirikul et al (14) and Shokohi et al. (32). In contrast to our study, Mirshahi et al. (33) reported quite higher prevalence rates (83%) of fungal keratitis. Variation in the prevalence rate of fungal keratitis in our study with earlier studies could have resulted from regional difference of fungal keratitis attributed to culture, geographical location and/or seasonal variations.

The result of this study showed the higher frequency of fungal keratitis (20.8 %) in the age groups of 36-44. Our finding with regard to age was also comparable to the findings reported in many developing countries such as South India (16), North China (34) and Southeast Brazil (35). This could be explained by the fact that patients in the age group of 35-44 in this study are the main force of manual works, especially in agricultural and outdoor activities.

Residents of rural areas were significantly affected by fungal keratitis than urban residents even if there is no statistically significant association between residence and fungal keratitis. Similarly, fungal keratitis was higher in farmers accounting 58.6 %, followed by daily laborers (48.2 %). This could be resulted from the rural parts of Ethiopia are inhabited by farmers and daily laborer. Higher incidence of fungal keratitis has also been reported among farmers in South India (16).

Corneal trauma with vegetable contaminated matter has always been identified as a major cause of fungal keratitis from regions with a warm, humid climate and/or with an agricultural economy (25). This was evident by the present study in which, out of 29 patients with fungal keratitis, trauma was a predisposing risk factor in 19 (65.5%) patients. Hypopyon was the predominant clinical manifestation with fungal keratins like bacterial keratitis.

Of a total of 29 mold isolates Aspergillus and Fusarium species accounted for 41.4% and 24.1% the total fungal isolates respectively, together comprising 65.2% of the total isolates. More or less similar findings of Fusarium sp and Aspergillus species as the leading filamentous fungal pathogen have been described in South Florida and Ghana (35), Southeast
Brazil (18), North China (34) and Malaysia (36) where the climate is warm and humid like Ethiopia.

7. Limitation Of the study
   - Comparatively the number of sample size was a medium one.
8. Conclusion

Microbial keratitis was found out to be high. Male patients at the middle age were more affected than female patients. Similarly, patients from rural areas were more affected than from urban areas. Trauma was the most common predisposing risk factor. The infection was more prevalent in farmers. Patients in their productive age were significantly affected by the infection. High prevalence of microbial keratitis, highlights the need for nationwide Study of the infection.

*Aspergillus* specious was the commonest fungal isolate followed by *Fusarium* and *Pencillium* specious. Gram positive bacteria were the major isolates from bacterial group and coagulase negative *staphylococcus* specious were the commonest bacterial isolate from corneal scrapping followed by *s.aureus*.
8. Recommendations

Despite collecting data for 8 months, the total number of cases was relatively small. Because of the small sample size our results should be considered as exploratory and further studies are required on large number of size so that the real association can be found to confirm the reported association.

We also recommend Menilik II memorial hospital to have microbiological laboratory to perform tests on eye samples because it is working as the only referral centre in the country for the management of infectious keratitis.
REFERENCE


ANNEXES

Annex I: English version of written consent

My name is Munira Siraj. I am a laboratory technologist postgraduate student at Addis Ababa University. Now I am conducting a study in titled “Demographic pattern, risk factor clinical and microbiological characteristics of fungal and bacterial keratitis in menilik ii memorial hospital Addis Ababa, Ethiopia”. Even though there is no direct benefit due to participation in this study, the findings of the study is useful for better understanding of the problems of microbial keratitis infection which is the major health problems in our country, the result of the study can be helpful in planning and intervention to solve the problem.

You are invited to participate in this study. By participating, you will get no financial benefits and at the same time you will not get any risk. If you agree to be included in the study, I
would like to ask you to sign on a document to show your agreement; participate accordingly, and give corneal scraps sample. Being asked to give sample does not necessarily mean that you have the disease. When you are found to be positive for the micro-organism, you will be informed by the health worker and receive proper treatment.

Your participation is completely voluntary, and you can refuse to participate or stop and withdraw from the study at any time. Refusal to participate will not result in loss of medical care provided or any other benefits. The information in your records is strictly confidential. The participants name or any identification number is never be used in connection with any of the information you give.

If you have any question about the study, you will have the right to ask anytime during the study. Your signature below indicates that you have read /or listened, and understand the information provided for you about the study. Before you sign, please understand purpose of the study, procedure, risks and benefits of participation, right to refuse or withdraw, confidentiality and privacy, and who to contact if you have question.

I have read /or listened to the description of the study and I understand what procedures are and what will happen to me in the study. I agree to participate in it.

Signature ___________________________ Date __________________

Communication

In case participants have any questions, unclear ideas and doubt about the project, contact addresses are:

Name of PI: Munira Siraj (Bsc)DMLS; AAU          +251913845091

Advisor: Adane Bitew (PhD), DMLT, AAU              +251911039162
Annex II Amharic version of written consent

እኔ መሪ እበራዊ እ袤ል። ከሰኔ እኔ የምስክርን የጤና ያለው ከአስ جاء ይጋገራ የህክምና ያለው ከተምህርት ከስኔ ያለ ይጋገራ የሆኑ ያለ የሚያሳይ ያለ ይጋገራ የሚያሳይ ያለ ይጋገራ የሚያሳይ ያለ ይጋገራ የሚያሳይ ያለ ይጋገራ የሚያሳይ ያለ ይጋገራ የሚያሳይ ያለ ይጋገራ የሚያሳይ ያለ ይጋገራ የሚያሳይ ያለ ይጋገራ የሚያሳይ ያለ ይጋገራ የሚያሳይ ያለ ይጋገራ የሚያሳይ ያለ ይጋገራ የሚያሳይ ያለ ይጋገራ የሚያሳይ ያለ ይጋገራ የሚያሳይ ያለ ይጋገራ የሚያሳይ ያለ ይጋገራ የሚያሳይ ያለ ይጋገራ የሚያሳይ ያለ ይጋገራ የሚያሳይ ያለ ይጋገራ የሚያሳይ ያለ ይጋገራ የሚያሳይ ያለ ይጋገራ የሚያሳይ ያለ ይጋገራ የሚያሳይ ያለ ይጋገራ የሚያሳይ ያለ ይጋገራ የሚያሳይ ያለ ይጋገራ የሚያሳይ ያለ ይጋገራ የሚያሳይ ያለ ይጋገራ የሚያሳይ ያለ ይጋገራ የሚያሳይ ያለ ይጋገራ የሚያሳይ ያለ ይጋገራ የሚያሳይ ያለ ይጋገራ የሚያሳይ ያለ ይጋገራ የሚያሳይ ያለ ይጋገራ የሚያሳይ ያለ ይጋገራ የሚያሳይ ያለ ይጋገራ የሚያሳይ ያለ ይጋገራ የሚያሳይ ያለ ይጋገራ የሚያሳይ ያለ ይጋገራ የሚያሳይ ያለ ይጋገራ የሚያሳይ ያለ ይጋገራ የሚያሳይ ያለ ይጋገራ የሚያሳይ ያለ ይጋገራ የሚያሳይ ያለ ይጋገራ የሚያሳይ ያለ ይጋገራ የሚያሳይ ያለ ይጋገራ የሚያሳይ ያለ ይጋገራ የሚያሳይ ያለ ይጋገራ የሚያሳይ ያለ ይጋገራ የሚያሳይ ያለ ይጋገሪ የሚያሳይ ያለ ይጋገራ የሚያሳይ ያለ ይጋገራ የሚያሳይ ያለ ይጋገራ የሚያሳይ ያለ ይጋገራ የሚያሳይ ያለ ይጋገራ የሚያሳይ ያለ ይጋገራ የሚያሳይ ያለ ይጋገራ የሚያሳይ ያለ ይጋገራ የሚያሳይ ያለ ይጋገራ የሚያሳይ ያለ ይጋገራ የሚያሳይ ያለ ይጋገራ የሚያሳይ ያለ ይጋገራ የሚያሳይ ያለ ይጋገራ የሚያሳይ ያለ ይጋገራ የሚያሳይ ያለ ይጋገራ የሚያሳይ ያለ ይጋገራ የሚYa

"Demographic pattern, risk factor, clinical and microbiological characteristics of fungal and bacterial keratitis in Menilik II memorial hospital Addis Ababa, Ethiopia"
ማቀወ ይችላሉ፡፡ ተሳትፎን መንፈግ ማለት የጤና አንክብካቤ ያጣሉ ወይም ሌላ ጥቅም ያጣሉ ማለት አይደለም፡፡ መረጃዎ በሚስጥር ይጠበቃል፤ በጥናቱ ላይ ጥያቄ ካልዎት በማንኛውም ጊዜ የጥናቱን እስተባባሪ መጠየቅ ይችላሉ፡፡ ከዚህ በታች የሚገኝው ፊርማዎ ለእርስዎ የተሰጠውን መረጃ ማንበብዎን፣ መስማትዎን እና መገኝብዎን የሚያሳይ ነው፡፡ ከመፈረምዎ በፊት እባክዎትን የጥናቱን ዓላማ፣ የተሳትፎጉዳትና ጥቅሙ፣ የመተው፣ የማቋረጥ፣ የማቆም መብትና ነፃነት እንዳለዎት ይረዱ፡፡ ጥያቄ ካልዎት ማንን እንደሚያናግሩየሚየሳይነው፡፡

ተስማምተዋል ይታችላል ይስምረታል ይቀቅለት ይረድቻለሁ፡፡ መመሪያው ምን እንደ ሊነናበእኔምን በሰትእንድን ይችልተረድቻለሁ፡፡ በጥናቱ በላይለመሳተፍ በተስማምቻለሁ፡፡ ያርማ፡-

አንስነት ዴን-

Annex III check list to collect data

Check list

1. Serial number…………………
2. Card number…………………
3. Age…………………………
4. Sex……………………………
5. Occupation……………………
6. Address

   Urban□ Rural□

Part II predisposing factor

1. trauma
   -metallic□
2. previous ocular surgery
3. previous use of drugs like steroids
4. use of contaminated ocular medication
5. contact lens wear
6. Aqueous tear deficiency
7. Recent corneal diseases (neurotropic keratopy)
8. Chronic dacryocystitis
9. Decreased immunologic defense
10. Structural alteration or malposition of the eye lid

Part III. Physical examination

1. Affected eye
   OD……….. OS………

2. VA
   OD_____ OS_____ 

3. Epithelial ulcer size _________________
4. Stromal infiltrate
   Size _______________
   Location - central  
   - Para central  
   - peripheral  
   Edge - distinct  
   - feathery edge  

4. Anterior chamber reaction
5. hypopyon
6. Upper eye lid edema
7. Endothelial plaque
Part IV laboratory result

<table>
<thead>
<tr>
<th></th>
<th>positive</th>
<th>negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Gram stain</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. KOH wet mount</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Type of microorganism isolated

<table>
<thead>
<tr>
<th>Culture result</th>
<th>pure growth</th>
<th>mixed growth</th>
<th>no growth</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Sabrouad dextrose agar</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Blood agar</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Chocolate agar</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. Maconkeg agar</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Visit to hospital

First time
Previously treated

Treatment history

Duration of visit

Last visit visual acuity

OD
OS
Annex IV laboratory request form for corneal scrapping

Menilik II referral hospital, department of ophthalmology

Laboratory Request form

Date ___________________

Name...................................................           age...................            Sex………

Serial number…………………                    Card number………………….

Type of specimen corneal scrape

Microscopic result

3.  Gram stain…………………………………………………..
4.  Koh wet mount……………………………………………...

Culture result

Type of microorganism isolated………………………………………………………………..
Annex V SOP for corneal scrapping

Corneal scrapping

The cornea is the most exposed surface of the eye and, as such, is vulnerable to external trauma and the risk of infection. Many corneal diseases alter shape, surface, and transparency and thus result in reduced vision.

Principle

Corneal debridement, swabs, and/or corneal biopsy provide the specimens required for direct microscopic detection of the causative organism, for culture, and to identify the sensitivity to antibiotic agents.

Procedure

1. The procedure is explained to the patient.

2. The patient is positioned comfortably at the slit-lamp.

3. The patient must be instructed to keep both eyes open during the procedures as blinking will only add to discomfort.

4. Local anaesthetic eye drops are instilled to the affected eye to minimise ocular discomfort and facilitate the corneal scraping procedure.

5. A sterile platinum loop or a sterile needle is used to scrape the base of the ulcer with care. This is to ensure that the infective material is reached as the micro-organisms may lie deep or at the edge of the ulcer.

6. The collected material is plated on the growth media and/or carefully spread on a glass slide. The area around the material is marked with a permanent marker if a Gram staining test has been requested.
7. At the end of the procedure, the patient is given instruction in appropriate care, i.e., hand washing, lid hygiene and instillation of an antibiotic.

8. All specimens are clearly and correctly labelled before being sent to the microbiological laboratory.

**Annex VI SOP for culture media preparation and identification**

**5.1 Preparation of culture media**

Materials needed for culture media preparation (some of them)

- Culture media - bio safety cabinet
- Distilled water - auto clave
- Petri dish - balance
- Tube - measuring cylinder
- incubator

**Procedure**

1. Weighing and dissolving:- weighing is based on the manufacturer’s direction and then multiply or reduce based on the amount of media needed. Dissolving is mediated by mixing very well by rotating 360ºc until all the powder mixed and finally boiling to get homogeneous solution.

2. Sterilization majority of culture media are sterilized by being autoclaved.it is performed by placing in autoclave of 121 for 15 minute this ensures the destruction of bacterial endospores as well as vegetative cells it is important to sterilize a medium at the correct temperature and for the correct length of time as instructed in the method of preparation

3. Add heat susceptible chemicals or substance such as blood in case of blood agar and certain antibiotic supplement

4. PH testing:-the pH of most culture media is near neutral. The simplest way of testing PH of culture media is to use narrow ranged PH paper in to a sample of the medium when it is at room temperature before dispensing.

5. Dispensing: - before this we have to cool the media based on the manufacture’s direction but in case of agar media we have to cool a temperature of between because agar I nature is solidifying agent so below this temperature will solidify the media before dispensing, dispensing should be until it covers the surface of the plate on flat and sterile surface to get uniform depth at room temperature. Wait minute it solidify and invert the media and wait hours (6 hours most of the time)
6. sterility and performance test: - performance test is used to check the quality of culture media prepared using control organism

7. Labeling and storage all culture media must be clearly labeled with its name preparation and expiration date. Plates of culture media should be stored at 2-8°C preferably in sealed plastic bags to prevent loss of moisture.

Inoculation

Inoculation is the next step in culturing of samples where placing the sample on appropriate culture media takes place. In this study SDA, maconky agar, blood agar, chocolate agar and brain heart infusion broth media was used.

5.2 Microscopic examination

5.2.1 Gram stain

Principle

Differences in Gram reaction between bacteria is thought to be due to differences in the permeability of the cell wall of Gram positive and Gram negative organisms during the staining process. Following staining with a triphenyl methane basic dye such as crystal violet and treatment with iodine, the dye iodine complex is easily removed from the more permeable cell wall of Gram negative bacteria but not from the less permeable cell wall of Gram positive bacteria.

Required

- Crystal violet
- Lugoi’s iodine
- Acetone alcohol
- Safranin
Staining procedure

1. Air-dry and heat-fix specimen using a Bunsen burner or spirit lamp

   Note: When the smear is for the detection of gonococci or meningococci, it should be fixed with methanol for 2 minutes (avoids damaging pus cells).

2. Cover the fixed smear with crystal violet stain for 30–60 seconds.

3. Rapidly wash off the stain with clean water. Note: When the tap water is not clean, use filtered water or clean boiled rainwater.

4. Tip off all the water, and cover the smear with Lugol’s iodine for 30–60 seconds.

5. Wash off the iodine with clean water.

6. Decolorize rapidly (few seconds) with acetone–alcohol. Wash immediately with clean water.

7. Cover the smear with neutral red stain for 2 minutes.

8. Wash off the stain with clean water.

9. Wipe the back of the slide clean, and place it in a draining rack for the smear to air-dry.

10. Examine the smear microscopically, first with the 40 objective to check the staining and to see the distribution of material, and then with the oil immersion objective to report the bacteria and cells.

5.2.2 KOH wet mount

Principle

Potassium hydroxide is used as a mounting fluid for visualization of fungal filaments as it helps in the clearance or lyses of all surrounding tissues. In addition *Acanthamoeba* cysts and *Nocardia* filaments can also be visualised.
Preparation of 10% KOH

1. Approximately 1 gm of KOH is weighed
2. It is dissolved in 10 ml of distilled water
3. One drop of 10% glycerol is added

KOH wet mount

1. Take a clean glass slide and place the sample on the center
2. Add a drop of 10% KOH and place a cover slip
3. Take special care to avoid any air bubbles
4. Observe under low and high power

5.3 Biochemical tests for gram positive bacteria

Catalase test

**Principle**

Catalase acts as a catalyst in the breakdown of hydrogen peroxide to oxygen and water. An organism is tested for catalase production by bringing it into contact with hydrogen peroxide. Bubbles of oxygen are released if the organism is a catalase producer. The culture should not be more than 24 hours old.

**Required**

- \( \text{H}_2\text{O}_2 \)

**Procedure**

1. Pour 2–3 ml of the hydrogen peroxide solution into a test tube.
2. Using a sterile wooden stick or a glass rod (not a nichrome wire loop), remove several colonies of the test organism and immerse in the hydrogen peroxide solution.
3. Look for immediate bubbling

**Result**

Active bubbling . . . . . . . . . . Positive catalase test

No bubbles . . . . . . . . . . . . . Negative catalase test

DNA-ase test
**Principle**

Deoxyribonuclease hydrolyzes deoxyribonucleic acid (DNA). The test organism is cultured on a medium which contains DNA. After overnight incubation, the colonies are tested for DNA-ase production by flooding the plate with a weak hydrochloric acid solution. The acid precipitates unhydro-lyzed DNA. DNA-ase-producing colonies are therefore surrounded by clear areas due to DNA hydrolysis.

**Procedure**

1. Divide a DNA-ase plate into the required number of strips by marking the underside of the plate.
2. Using a sterile loop or swab, spot-inoculate the test and control organisms. Make sure each test area is labelled clearly.
3. Incubate the plate at 35–37 C overnight.
4. Cover the surface of the plate with 1 mol/l hydrochloric acid solution. Tip off the excess acid.
5. Look for clearing around the colonies within 5 minutes of adding the acid,

**Results**

Clearing around the colonies . . . . . . . . . DNA-ase positive strain
No clearing around the colonies . . . . . . . DNA-ase negative strain

**Coagulase test**

**Principle**

Coagulase causes plasma to clot by converting fibrinogen to fibrin. Two types of coagulase are produced by most strains of S. aureus:

- Free coagulase which converts fibrinogen to fibrin by acti-vating a coagulase-reacting factor present in plasma. Free coagulase is detected by clotting in the tube test.
• Bound coagulase (clumping factor) which converts fibrinogen directly to fibrin without requiring a coagulase-reacting factor. It can be detected by the clumping of bacterial cells in the rapid slide test.

Slide test method (detects bound coagulase)

1 Place a drop of distilled water on each end of a slide or on two separate slides.

2 Emulsify a colony of the test organism (previously checked by Gram staining) in each of the drops to make two thick suspensions.

3 Add a loopful (not more) of plasma to one of the suspensions, and mix gently. Look for clumping of the organisms within 10 seconds.

Results

Clumping within 10 secs . . . . . . . . . . . . . S. aureus

No clumping within 10 secs . . . . . . . . No bound coagulase

Bile solubility test

This helps to differentiate S. pneumoniae, which is soluble in bile and bile salts, from other alpha-haemolytic streptococci (viridans streptococci) which are insoluble.

**Principle**

A heavy inoculum of the test organism is emulsified in physiological saline and the bile salt sodium deoxycholate is added. This dissolves S. pneumoniae as shown by a clearing of the turbidity within 10–15 minutes. Viridans and other streptococci are not dissolved and therefore there is no clearing of the turbidity.

**Required**

— Sodium deoxycholate,
— Physiological saline

**Procedure**

1. Emulsify several colonies of the test organism in a tube containing 2 ml sterile physiological saline, to give a turbid suspension

2. Divide the organism suspension between two tubes.

3 To one tube add 2 drops of the sodium deoxy-cholate reagent and mix.

4 To the other tube (negative control), add 2 drops of sterile distilled water and mix.

5 Leave both tubes for 10–15 minutes at 35–37 C.
6 Look for a clearing of turbidity in the tube containing the sodium deoxycholate,

Results

Clearing of turbidity . . . . . . . . . . . . . . . . . S. pneumoniae

No clearing of turbidity . . . . Organism is probably not S. pneumoniae

Biochemical tests for gram negative bacteria

1. Indole test

Testing for indole production is important in the identification of enterobacteria. Most strains of *E. coli*, *P. vulgaris*, *P. rettgeri*, *M. morganii*, and *Providencia* species break down the amino acid tryptophan with the release of indole.

**Principle**

The test organism is cultured in a medium which contains tryptophan. Indole production is detected by Kovac’s or Ehrlich’s reagent which contains 4 (p)-dimethylaminobenzaldehyde. This reacts with the indole to produce a red colored compound. Kovac’s reagent is recommended in preference to Ehrlich’s reagent for the detection of indole from enterobacteria.

**Procedure**

**Detecting indole using tryptone water**

1. Inoculate the test organism in a bijou bottle containing 3 ml of sterile tryptone water.
2. Incubate at 35–37°C for up to 48 h.
3. Test for indole by adding 0.5 ml of Kovac’s reagent. Shake gently. Examine for a red color in the surface layer within 10 minutes.

**Results**

Red surface layer . . . . . . . . . . Positive indole test
No red surface layer . . . . . . . . . . Negative indole test
1. **Citrate utilization**

This test is one of several techniques used occasionally to assist in the identification of enterobacteria. The test is based on the ability of an organism to use citrate as its only source of carbon.

**Citrate utilization using a Simmon’s citrate agar**

**Principle**

The medium contains citrate, ammonium ions, and other inorganic ions needed for growth. It also contains Bromothymol blue, a pH indicator. Bromothymol blue turns blue at a pH of 7.6 or greater. When an organism catabolizes citrate, it produces alkaline waste products, causing the medium to turn blue. Furthermore, only an organism that can utilize citrate will produce visible growth on the citrate slant.

**Result**

Positive: Blue colored growth

Negative: No growth/ no color change

2. **Oxidase test**

The oxidase test is used to assist in the identification of *Pseudomonas, Neisseria, Vibrio, Brucella*, and *Pasteurella* species, all of which produce the enzyme cytochrome oxidase.

**Principle**

A piece of filter paper is soaked with a few drops of oxidase reagent. A colony of the test organism is then smeared on the filter paper. Alternatively an oxidase reagent strip can be used. When the organism is oxidase-producing, the phenylenediamine in the reagent will be oxidized to a deep purple colour. Occasionally the test is performed by flooding the culture plate with oxidase reagent but this technique is not recommended for routine use because the reagent rapidly kills bacteria. It can however be useful when attempting to isolate *N. gonorrhoeae* colonies from mixed cultures in the absence of a selective medium. The oxidase positive colonies must be removed and subcultured within 30 seconds of flooding the plate.

**Procedure**

1. Place a piece of filter paper in a clean petridish and add 2 or 3 drops of freshly prepared oxidase reagent.
2. Using a piece of stick or glass rod (not an oxidized wire loop), remove a colony of the test organism and smear it on the filter paper.
3. Look for the development of a blue-purple colour within a few seconds.
3. **Urease test**

Testing for urease enzyme activity is important in differentiating enterobacteria. *Proteus* strains are strong urease producers. *Y. enterocolitica* also shows urease activity (weakly at 35–37 °C). Salmonellae and shigellae do not produce urease.

**Principle**

The test organism is cultured in a medium which contains urea and the indicator phenol red. When the strain is Urease producing, the enzyme will break down the urea (by hydrolysis) to give ammonia and carbon dioxide. With the release of ammonia, the medium becomes alkaline as shown by a change in colour of the indicator to pink-red.

**Urease test using Christensen’s (modified) urea**

1. Inoculate the test organism in a sterile Christensen’s modified urea
2. Incubate at 35–37 °C for 18hrs
3. Look for a pink colour in the medium

**Results**

- Pink colour . . . . . . . . . . . Positive urease test
- No pink colour . . . . . . . . . Negative urease test

---

4. **Motility test**

This medium is used for checking the motility of organisms. Low agar concentration allows free movement of bacteria.

**Principle**

Bacteria are motile by means of flagella. This test is done to determine whether an organism is motile or non-motile.

**Procedure**

The test isolate is inoculated by stabbing in the center of media in the tube with straight wire.

**Result**

1) Non motile-- growth restricted to stab line
2) Motile-- Diffused growth or swarm extends as a zone of turbidity from the stab line.

5. **Triple sugar iron agar**

Triple sugar iron agar is used for the differentiation of enteric pathogens by ability to determine carbohydrate fermentation and hydrogen sulphide production.
Results

<table>
<thead>
<tr>
<th>Butt colour</th>
<th>Slant colour</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yellow</td>
<td>Red</td>
<td>Glucose only fermented</td>
</tr>
<tr>
<td>Yellow</td>
<td>Yellow</td>
<td>Glucose fermented, also lactose and/or sucrose</td>
</tr>
<tr>
<td>Red</td>
<td>Red</td>
<td>No action on glucose, lactose or sucrose</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bubbles or cracks present: gas production</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Black precipitates present: hydrogen sulphide gas production</td>
</tr>
</tbody>
</table>
Declaration

I the undersigned declare that this is my original work and has not been reported for a degree in this or any other university and all sources of materials used for this thesis have been acknowledged.

Principal investigator: Munira Siraj

E mail: munirasiraj988@gmail.com phone 251 913845091

Signature ________________________________

Approval of advisors

1. Adane Bitew (PHD, AAU)

Email bitewadane@gmail.com Phone 0911039162

Signature __________________________ date __________________________

2. Menen Ayalew(MD)

Email menenashi@yahoo.com phone 0911405251

Signature __________________________ date __________________________

3. Gebreabiezgi Teklebirhan (MSC)

Email Phone 0912860318

Signature __________________________ date __________________________