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Original Article

Identification of bacterial profile, common associated risk factors, and antimicrobial susceptibility patterns, of bacterial keratitis in community hospitals of Asmara, Eritrea

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Abstract

Background: *Bacterial keratitis causes infection and inflammation of the cornea resulting in pain, blurred vision, photosensitivity, lacrimation, eye discharge, and loss of vision in severe cases. The present study was designed to investigate the bacterial profile, associated risk factors, and Antimicrobial Susceptibility Patterns (AST) of bacterial keratitis in Berhan Aini hospital, Godaif, and Biet Mekae community hospitals of Asmara Eritrea.*

Methods: *A cross-sectional study was designed to assess the incidence of bacterial keratitis among suspected keratitis patients who visited Berhan Aini hospital and community hospitals of Godaif and Biet Mekae over the past year. The study subjects were diagnosed by an ophthalmic officer and sample was collected.*

Results: *A total of 330 suspected bacterial keratitis patients (330 eyes) were examined during the study period of more than one year. The total 220 (66.66%) cases were culture positive, the most common isolated bacteria was Staphylococcus aureus, 110 (50%); followed by Coagulase-negative Staphylococcus aureus CoNS 66 (30%), Streptococcus pneumoniae, 33(15%); and Streptococcus viridans 11 (5%). S.aureus isolate showed 99 (90%) sensitivity to ciprofloxacin, rifampin, gentamycin, vancomycin, and nitrofurantoin, 88 (80%) to chloramphenicol, 77 (70%) to clindamycin, 66 (60%) to erythromycin, and 55 (50%) to tetracycline, whereas it was 110 (100%) resistant to oxacillin and Penicillin. The most predisposing factor among the cases was trauma.*

Conclusion: *The most common bacteria causing bacterial keratitis in Asmara Ophthalmic Hospitals were Gram-positive bacteria. Trauma was found to be the most common exposing factor for bacterial keratitis which was not statistically significant associated with the culture-positive result ($p>0.05$). S. aureus was found to be highly sensitive to ciprofloxacin, gentamycin, vancomycin, rifampin, and nitrofurantoin, and 100% resistant to oxacillin and penicillin upon evaluation of antimicrobial activity of several antibiotics.*

Keywords: *Keywords: Asmara, Bacterial Keratitis, AST, Risk factors, Eritrea*

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Introduction:

Eyes, which distinguish distant objects from near objects, determine their color and shape and perceive light to see the world and understand how objects relate to each other, are complex organs of vital importance for everyday life.

The cornea protects our eyes from harmful substances with the help of eyelids, eye sockets, tears, and sclera within the white part of the eye [(1)].

The corneal and conjunctival epithelial cells are tightly bound together, providing another barrier to microbial invasion, and epithelial cells themselves can phagocyte and transport microbes [(2)]. The thing which lies between the front of the cornea and our environment is a very thin tear film [(3)].

Acute or subacute pain, conjunctival injection, and corneal ulceration with a stromal inflammatory infiltrate are characteristic signs of microbial keratitis which is an infection of the cornea [(1).]

Microbial keratitis, which can be caused by bacteria, fungi, viruses, or parasites, is a potentially vision-threatening condition and requires prompt diagnosis and treatment to prevent serious implications. The cornea provides natural resistance to infection, so microbial keratitis rarely occurs in the normal eye. However, bacteria can invade the cornea due to exposure of the eye to risk factors such as trauma, contact lens wear, dry eyes, ocular surface disorders, and immunosuppression, these risk factors may alter the defense mechanism of the outer eye, and allow microorganisms to invade the eye and cause infection [4].

Bacterial keratitis, which is an inflammation of the cornea, progresses rapidly, and corneal destruction may be completed in 24 - 48 hours with some of the more virulent bacteria. The main symptoms of bacterial keratitis are pain, redness, watering, mucopurulent or purulent discharge, photophobia, defective vision, foreign body sensation, and loss of vision in severe cases. The underlying condition of the cornea and the pathogenicity of the infected bacteria generally display the severity of corneal infection [5].

In bacterial keratitis, many patients have a poor clinical outcome if aggressive and appropriate therapy is not promptly initiated due to susceptibility of the avascular corneal stroma to bacterial infection. Bacterial keratitis is one of the most visually threatening ocular infections resulting from the high incidence and potential complications. The presence of particularly invasive pathogens such as *Pseudomonas aeruginosa* and *Staphylococcus aureus* can lead to corneal perforation in less than 24 hours. When fluoroquinolone antibiotic is used as a mono-therapeutic agent, the emergence of multiresistant strains is also a major concern.

Bacteria which cause keratitis may be Gram-positive or Gram-negative, the wide range of bacteria includes *Staphylococci, Streptococci, Pseudomonas, Enterobacteriaceae, Corynebacterium species, Moraxella species, Serratia species, Haemophilus, and N. gonorrhea* [5]. Eighty percent of bacterial corneal ulcers are caused by *Staphylococcus aureus, Streptococcus pneumoniae, and Pseudomonas species.* The most frequent and most pathogenic ocular pathogen which can cause corneal perforation in just 72 hours is *Pseudomonas aeruginosa.* Bacterial keratitis can be controlled and treated by prompt and meticulous investigation, otherwise, it becomes a serious condition and may progress to endophthalmitis, perforation, and blindness. Contact lens wears followed by a preexisting ocular disease that includes ocular trauma, ocular surgery, laser refractive surgery, and use of topical steroids are some of the predisposing factors for bacterial keratitis.

Finally, geographic and climatic factors can also influence the bacterial pattern of keratitis; rural or city area populations have also seen many differences in keratitis profile. This difference between geographic and climatic factors on the profile of bacterial keratitis can be explained by the high variation of habits, such as antibiotic use, contact lens wear, or even specific rural pathogen exposure [5].

The management of bacterial keratitis should include adequate ocular prevention, knowledge of the microbiological patterns in a given clinical practice, and a correct choice of antibiotics available for treatment, and considering drug toxicity and bacterial resistance, will also be key to success in the effective management of bacterial keratitis [6,7].

Eritrea is geographically located in Sub-Sahara Africa where there is an increased burden of microbial keratitis. Understanding the magnitude of bacterial keratitis problem as a cause of vision loss, fewer or no studies have been reported yet from Eritrea which makes our study novel. There may be many studies from surrounding countries, but with changes in geographic location and changes in the physiology of people the final outcome varies. The current study will be helpful to identify the main risk factors that predispose the Eritrean population to bacterial keratitis and increase their awareness regarding the risk factors. It will help health service providers to give appropriate treatment according to the specific cause of keratitis. It will take a sharp interest to the health service providers to understand the main etiologies. The study will pave the way for a large-scale study on all microbial keratitis in all clinical areas.

Materials and Methods:

Study design:

The current study was a cross-sectional study designed to determine the incidence-associated risk factors of bacterial keratitis with their antibiotic susceptibility pattern.

Study area:

This study was conducted in Berhan Aini National Ophthalmic Hospital and community hospitals of Godaif and Bet Mekai which are found in Asmara. All samples were processed and analyzed in the microbiology laboratories of the National Drug Quality Control Laboratories (NDQCL) and the National Health Laboratory (NHL). The study period was from July 2020 to October 2021.

Study population:

The study population were individuals of all ages who were visiting the eye hospital during the study period with signs and symptoms of infectious keratitis.

Inclusion criteria:

- All patients visiting the outpatient department with corneal infection
- Patients diagnosed with infectious keratitis and admitted to the hospital

Exclusion criterion:

- Patients under antibiotic therapy

Sample size:
Convenient sampling with inclusion and exclusion criteria was used in which the maximum number of participants was included within the time of data collection.

Data Collection Techniques:
A researcher-administered questionnaire was used to collect demographic data and risk factors associated with infectious keratitis. The questionnaire was filled out by the researcher in the form of an interview. Data obtained from the questionnaire were associated with the microbiological laboratory results.

Specimen collection:
The analytical part of the study started with the preparation of all important materials, reagents, and equipment before the process of specimen collection and transportation to the site of analysis. Each specimen was designed to be labeled with a series of numbers that contains the sample collection date and sample code.
Slit-lamp biomicroscope was used by an ophthalmologist for the examination of all patients.
The present study used a corneal swab, as it is the simplest option and is called a "**quick culture**." Corneal scrapings are the gold standard method of corneal sample collection, but due to lack of specialists and risk in the collection, the researchers were advised to go with corneal swabs.

Corneal swab specimens were collected with sterile swabs from the infected cornea after applying 2 drops of local anesthetic drop (Tetracaine 1%). The swab was labeled with the specific name of the participant and sample code after filling out the questionnaire. Then the samples were transported using the swab transport media (Stuart) to the laboratory within one hour of collection.

Specimen processing:
The samples were checked if they were properly labeled, and the media was labeled with respect to the sample code. The swab specimen was then primarily inoculated with three media that passed the quality control, i.e., chocolate agar, mannitol salt agar (MSA), and mcconkey agar (MAC) (Hi-Media, Mumbai, India) by the striking method which was under continuous sterilization. The cultured media were incubated at 37^{o}C for 18-24 hours. After the appropriate incubation time, the culture was examined for the presence of any growth, and the morphology of the growth was carefully described, depending on the size, color, and shape of the colony. This gave some clues to the bacterial identification. For example, the color changes indicated in mannitol fermenters and the possible acid production when the bacteria can utilize the supplement present in the media, the shape and size are also helpful to see if it is mixed or if two bacteria were grown in the media. Mannitol Salt Agar has mannitol and high salt concentration (i.e., 7.5% NaCl), it is selective as it selectively supports the growth of gram-positive staphylococcus SPP and differential as it differentiates mannitol fermenters from non-mannitol fermenters.

Mcconkey agar is used as a selective media which supports the growth of gram-negative bacteria, as it has crystal violet which hinders the growth of gram-positive bacteria, and as a differential media which differentiates lactose fermenters from non-lactose fermenters. Chocolate agar is an enriched media that supports the growth of microaerophilic bacteria.

Primary Isolation and Identification Tests:
Following 18 to 24 hours of incubation, the culture media were read. Gram staining and biochemical tests were done for those media which showed growth.

Gram Staining: This method was used to identify and differentiate Gram-positive and Gram-negative bacteria. A loop full sample of the bacteria was mixed with a drop of normal saline on a microscope slide and allowed to air dry. The smear was covered with crystal violet and allowed to stand for one minute. The stain was briefly washed off using distilled water. The excess water was drained off. Then the smear was covered with Gram's iodine solution for one minute, which was used as a mordant so that the gram-positive bacteria will retain the color of crystal violet. It was then washed off, and the slide was held 45- degree angle and allowed 95% (isopropyl alcohol) to flow down the surface of the slide until the alcohol turned colorless as it flowed from the smear down the surface of the slide. Decolorization was stopped by washing the slide with a gentle smear of water. Then the smear was covered with safranin stain for 1 minute, washed with water, and air-dried. The slides were examined under oil immersion [(1)]. All reagents for Gram staining were purchased from an Indian company. (Hi-Media, Mumbai, India).

Biochemical Test: These tests were performed for detailed identification of bacteria to their species level using enzymatic or color change (PH indicators). The Gram-positive isolates were differentiated using the catalase test, DNase test, and optochin test. Gram-negative isolation is more complex than Gram-positive because they require several tests like oxidase test, citrate test, urea test, indole test, methyl red test (MR test), carbohydrate tests (glucose, mannitol, sorbitol, sucrose, and triple sugar iron (TSI), (Hi-Media, Mumbai, India), etc., to identify the isolates.

Antimicrobial Susceptibility Test:
Antibiotic susceptibility testing of each isolated pathogen was done using routine antibiotic discs according to the disc diffusion technique on muller hinton agar (MHA). The organism, which was primarily inoculated was again sub-cultured for refreshing just before performing antimicrobial susceptibility tests.

A bacterial suspension was prepared from freshly isolated colonies, and a small amount of the suspension was put in a biochemical test tube. Using a sterile swab, the bacterial suspension was inoculated onto muller hinton agar using a sterile swab. The organisms were tested against different types of anti-Gram-positive drugs for susceptibility profiles. The drugs were separated into two groups of 5 and 6 to provide enough space for disc diffusion in the media, and then the discs were set in position in the media. The two media were incubated overnight (24 hours) at 37^0C. Then the results were read and the zone of inhibition was measured using a ruler and the isolates were designated as sensitive, intermediate, and resistant using the standard reference ranges mentioned in Clinical and Laboratory Standards Institute 2019 (CLSI 2019) [8].

Quality control:

To assure correct results, the working situation should appropriately be quality controlled. Quality control was started by cleaning the laboratory. Regarding the quality control of materials and equipment, the equipment which was necessary to perform the research was checked for their performance capacity.

As part of the quality assessment function of the incubator, autoclave, oven, and refrigerator were checked. The incubator was set to a temperature of 37- 38^0C using a thermometer daily. The temperature of the refrigerator was also maintained at 2 – 6^0C, and this was controlled using a thermometer each day. Then, the autoclave was checked for the presence of water and the sterility was controlled using a color tape indicator. For prevention of contamination, all materials such as test tubes, reagent containers, Petri dishes, media, etc. were autoclaved. The swabs used for sample collection were checked for their potency using a stock organism and by inoculating the swab without any usage of the sample for its sterility. Along with this, to signify the quality of the media, 10% of the prepared media were incubated at 37^0C to control the growth and predict the degree of contamination. Furthermore, the quality of the media was checked by inoculating stock organisms. The working area was disinfected before and after performing any test using 10% bleach or alcohol. Along with all this, to maintain the quality of the specimen and the viability of the microorganisms, the corneal swab was processed within one hour of collection.

Data analysis:

After the samples were processed, the results were conveyed in statistical terms using a statistical computer software version 20 SPSS. The quantitative results are given in terms of percentages, graphs, and tables. The chi-square test was used to compare the independent variables, and a p-value < 0.05 was considered significant in analyzing the relationship between the variables.

Ethical consideration:

The study was conducted according to the declaration of Helsinki; Asmara College of Health Sciences and obtained ethical approval by the ethical committee of the Research Centre of the Ministry of Health. Furthermore, it also got permission from the medical director of Berhan Aini Hospital and ophthalmic officers of Godaif and Biet Mekae Community Hospitals. A written consent was obtained from the participants, and proxy consent was obtained from parents or guardians for those participants below 18 years of age.

Results:

General description of the study:

During the study period, a total of 330 keratitis cases who visited the ophthalmic centers of different hospitals in Asmara were included. Out of the 330 participants, 132(40%) were from Berhan Aini Hospital, 110(33.3%) from Biet Mekae community hospital, and the rest 88(26.6%) from Godaif community hospital.

Patient's demographic characteristics:

Out of the 330 study participants, 165 (50%) were males and females each, so the overall male-to-female ratio was 1:1. The study participants' age ranged from 4–88 years with a mean age of 50 years (SD 23.7). Zoba Maekel had the highest number of keratitis cases with 132(40%) followed by Zoba Debub 88(26.6%), Northern Red Sea 55 (16.6%), Southern Red Sea 33(10%), Anseba and Gash Barka 11(3.3%) One hundred eighty-seven (56.6%) of the study participants were living in urban, while the remaining 143(43.3%) were from rural areas. The employment status of the study participants showed that 154(46.6%) were unemployed, 121(36.6%) farmers, 22 (6.6%) mechanics, 11 (3.3%) students, 11 (3.3%) secretaries, and 11 (3.3%) merchants. **Table 1**

Table 1: Study participants' demographic characteristics

Parameter	Frequency	Percentage
	Age	
4-24	33	10%
25-45	121	36.6%
46-66	88	26.6%
67-88	88	26.6%
	Sex	
Female	165	50%
Male	165	50%
	Occupation	
Student	11	3.3%
Mechanic	22	6.6%
Secretary	11	3.3%
Merchant	11	3.3%
Farmer	121	36.6%
Unemployed	154	46.6%
	Residence	
Urban	187	56.6%
Rural	143	43.3%
	Zone	
Maekel	132	40%
Debub	88	26.6%
NRS	55	16.6%
SRS	33	10%
Anseba	11	3.3%
Gash Barka	11	3.3%

Ethnicity distribution:
In terms of ethnicity, the majority were Tigrigna, followed by Tigre, Saho, and Bilen. No study participant was found from the remaining ethnic groups. **Figure 1**

Fig 1: Ethnicity of study participants

Sample analysis of the corneal swab specimens:
Following sample collection, the corneal swab samples were inoculated on mannitol salt agar, mcconkey agar, and chocolate agar. Following 18-24 hours of incubation at 37°C, the samples were read, and the samples which showed growth were further processed using Gram's stain, catalase test, optochin test, and dnase test to identify the isolates. Finally, the isolates were tested against different anti-Gram-positive drugs to test their antimicrobial susceptibility pattern.

Culture results of corneal swab:
Laboratory results were a vital part of this research alongside the clinical information gathered from the questionnaire. In the current study, 330 samples were obtained and processed using culture and sensitivity techniques. The outcome of the culture results indicates that 220(66.6%) of the study participants showed growth, while the remaining 110 (33.3%) showed no growth. **Figure 2**

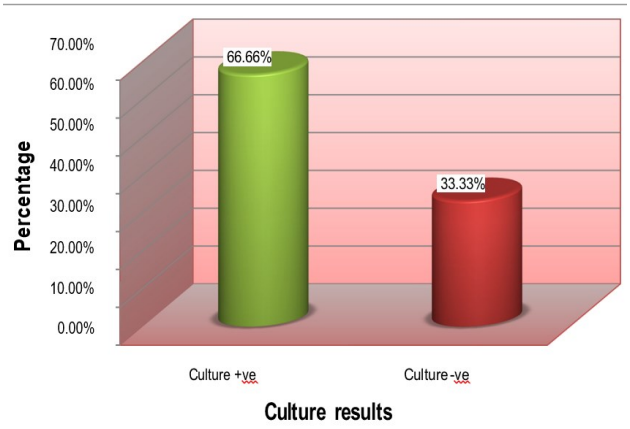

Fig 2: Culture results of the corneal swab

Identification of culture results:
Culture-positive samples were further processed for Gram staining. All isolates were found to be Gram-positive. Catalase, dnase, and optochin tests were performed. The catalase test was performed by taking a few colonies from the culture with the help of an applicator stick and dropping them on 3% hydrogen peroxide (H_2O_2). If the bacteria are catalase-positive, they will break hydrogen peroxide into oxygen and water molecules which are indicated by the production of bubbles. This type of test was used to distinguish *Staphylococcus* from *Streptococcus spp* where the former was catalase +ve, **Figure 5**. For those who were catalase-positive, the dnase test was done to differentiate *S.aureus* from other *Staphylococci spp*. This type of test was done by subculturing on dnase agar media, where a positive test was interpreted when a clear zone was observed around the colony following the flooding of HCl acid. Catalase and dnase-positive bacteria were considered clinically significant, and it was *S.aureus,* otherwise, they were *CoNS.* Based on the alpha-hemolytic pattern on chocolate agar, the optochin test was done to differentiate *S. pneumoniae* from *S.viridans*, as *S. pneumoniae* is sensitive to it. **Table 2.**

Table 2: *Identification of culture results*

	Results		
Identification Tests	Positive n (%)	Negative n (%)	Total
Mannitol Fermentation	176(80%)	44(20%)	220
Gram stain	220(100%)	0(0%)	220
Catalase Test	176(80%)	44 (20%)	220
DNase Test	176(80%)	44(20%)	220
Hemolytic pattern on Chocolate agar	Alpha hemolysis 44(20%)	176 (80%)	220
Optochin disk	33 (15%)	187 (85%)	220

Bacterial Etiologies:
After doing the appropriate culturing, subculturing, and biochemical tests for the 220 culture -positive results, our findings showed that *staphylococcus aureus* was the most dominant 110(50%), followed by *CoNS* 66(30%), *streptococci pneumoniae* 33(15%), and *streptococci viridians* 11 (5%). No gram-negative bacteria were found. **Figure 3, 4 & 6**

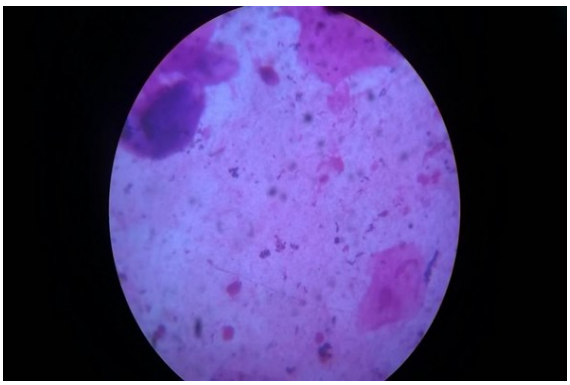

Fig 3: Gram Stain showing Gram-Positive Diplococci *Streptococcus pneumoniae*

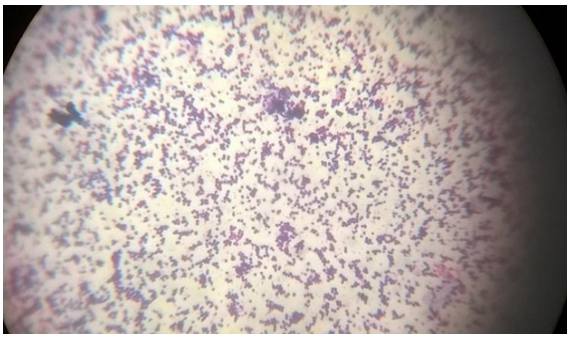

Fig 4: Gram stain showing Gram-Positive cocci in clusters *Staphylococcus aureus*

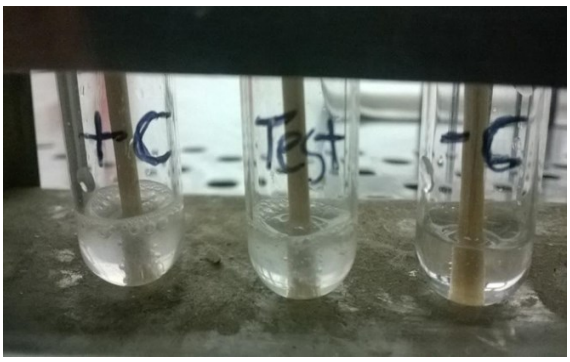

Figure 5: Tube catalase test to distinguish *Staphylococcus* from *Streptococcus spp*

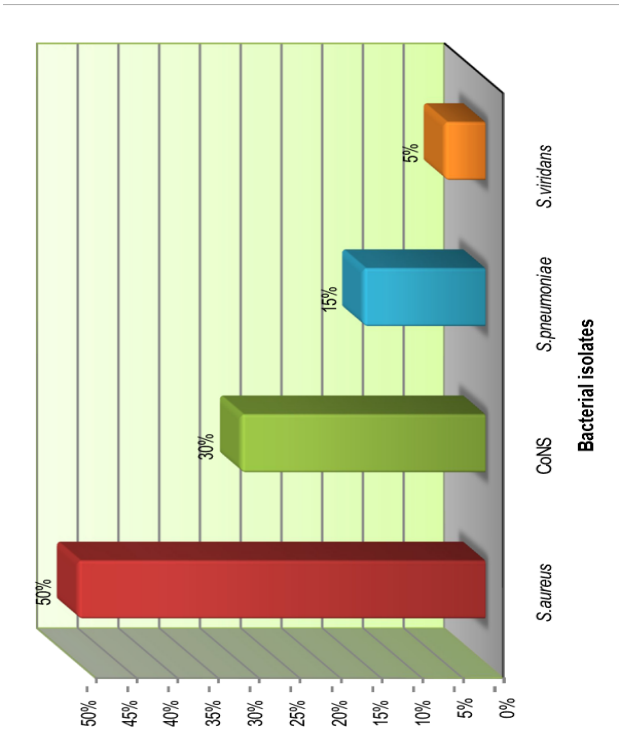

Fig 6: Identified bacterial etiologies:

Culture results in relation to demographic characteristics:

Of the 220 corneal swab samples which were culture positive, 132(60%) were male and 88(40%) female. The study participants 99 (45%) were below 40 years old and 121 (55%) above 40 years old. **Figure 7**

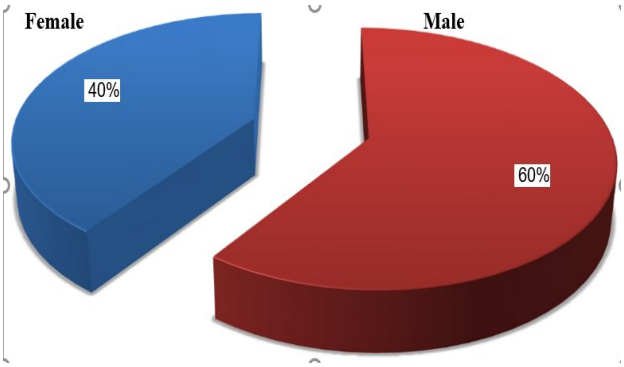

Fig 7: Culture results in relation to sex:

Culture results in relation to occupation:

The employment status of the study participants in relation to culture-positive results, 110 (50%) were unemployed, 77 (35%) farmers, 11(5%) students, 11(5%) secretaries, and 11(5%) merchants.

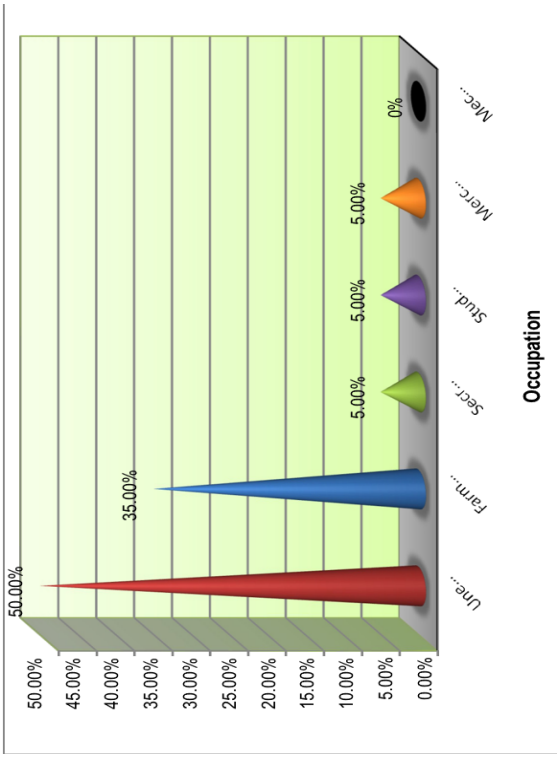

Fig 8: Culture results in relation to occupation

Risk factors predisposing to ocular conditions:

The present study also assessed the most potential risk factors that expose an individual to infectious keratitis. Generally, the possible known risk factors for infectious keratitis are trauma, foreign bodies, wearing contact lenses, ocular and eyelid surgery, other than these ocular surface diseases, corneal epithelial abnormalities and systemic diseases have a contributing factor. During the study time, a questionnaire was used to assess the risk factors of bacterial keratitis, and the questionnaire was filled out by the researchers. Trauma was the most common risk factor which was found in 165(50%) of the total cases, followed by the entrance of foreign bodies, systemic disease, and cases with unknown cause, i.e., 55(16.6%), 45(13.6%), and 65(19.6%), respectively. The participants were also assessed for their smoking status, alcohol consumption, ocular surgery, and any vitamin deficiency, however, no study subject was found with those above conditions. SPSS software version 20 was used to determine the association of risk factors with culture results. Out of the 220 culture-positive results, 132(60%) were due to trauma, 33(15%) due to systemic disease, 22(10%) due to foreign bodies, and 33(15%) due to unknown causes though no significant association was found with a *p*-value less than 0.05. **Table 3**

Table 3: Associated risk factors with culture results

Risk factors	Culture Results			
	Positive n (%)	Negative n (%)	Total n (%)	P-value
Trauma	132(60%)	33(30%)	165(50%)	0.123
Foreign bodies	22(10%)	33(30%)	55(16.6%)	0.551
Systemic disease	33(15%)	11(10%)	44(13.3%)	0.593
Unknown	33(15%)	33(30%)	66(20%)	0.306
Total	220	110	330	

Antimicrobial susceptibility results:

According to the National Health Laboratory, the sensitivities of pathogenic *S. aureus* and *S. pneumoniae* isolates were tested with different drugs for their sensitivity pattern. The panels used in AST varied based on the Gram stain characteristics and the antibiotic mode of action. Except for CoNS and *S. viridans,* all Gram-positive bacteria were subjected to antimicrobial tests because they are normal flora. The antibiotic panel used for Gram-positive bacteria were Ciprofloxacin, Clindamycin, Chloramphenicol, Erythromycin, Gentamycin, Oxacillin, Nitrofurantoin, Penicillin, Rifampin, Tetracycline, and Vancomycin. The selection of antibiotics was based on the treatment guidelines of the country for treating different bacterial infections [9,10]. The AST pattern of Gram-positive bacteria is shown in **Table 4**.

AST patterns for *S.aureus*:

S.aureus isolate showed 100% sensitivity to rifampin, while it was 90% to ciprofloxacin, gentamycin, vancomycin, and nitrofurantoin. 80% to chloramphenicol, 70% to clindamycin, 60% to erythromycin, and 50% to tetracycline, whereas it was 100% resistant to oxacillin and penicillin.

Table 4: AST patterns for *S.aureus*

	Sensitive n (%)	Intermediate n (%)	Resistance n (%)	Total
Ciprofloxacin	99(90%)	0(0%)	11(10%)	110
Clindamycin	77(70%)	11(10%)	22(20%)	110
Chloramphenicol	88(80%)	22(20%)	0(0%)	110
Erythromycin	66(60%)	0(0%)	44(40%)	110
Gentamycin	99(90%)	0(0%)	11(10%)	110
Oxacilin	0(0%)	0(0%)	110(100%)	110
Nitrofurontin	99(90%)	11(10%)	0(0%)	110
Penicillin	0(0%)	0(0%)	110(100%)	110
Rifampin	110(100%)	0(0%)	0(0%)	110
Tetracycline	55(50%)	0(0%)	55(50%)	110
Vancomycin	99(90%)	0(0%)	11(10%)	110

AST patterns for *S.pneumoniae*:

The sensitivity pattern observed for *S.pneumoniae* is displayed in **Table 5**. It showed 100% sensitivity to clindamycin, chloramphenicol, tetracycline, gentamycin, and 33.3% to vancomycin, whereas it was 100% resistant to oxacillin.

Table 5: AST patterns for *S.pneumoniae*

	Sensitive (%)	Intermediate (%)	Resistance (%)
Clindamycin	33(100%)	(0%)	0(0%)
Chloramphenicol	33(100%)	0(0%)	0(0%)
Gentamycin	33(100%)	0(0%)	0(0%)
Oxacillin	0(%)	0(0%)	33(100)
Tetracycline	33(100%)	0(0%)	0(0%)
Vancomycin	11(33.3%)	22(66.7%)	0(0%)

Discussion:

Bacterial keratitis is one of the most visually intimidating eye diseases because of its high frequency and potential problems. Gram-positive bacteria such as Staphylococcus. aureus, Staphylococcus. epidermidis, and several Streptococcus spp, as well as Gram-negative bacteria such as Pseudomonas Aeruginosa, are largely involved in bacterial keratitis infection (11). The current is a cross-sectional study conducted among suspected bacterial keratitis patients. A total of 330 swab samples were collected with the help of one ophthalmologist. Samples collected from the infected cornea were inoculated on mannitol salt agar, mcconkey agar, and chocolate agar for microbiological analysis.

Microbial keratitis is now recognized by World Health Organization (WHO) as a major cause of corneal blindness which leads to visual disability. Microbial keratitis incidence varies from one country to another, and there is regional variation in organism type within the country (12).

In this study, out of the 330 most susceptible participants for bacterial keratitis, 220 (66.7%) were found to be culture-positive and all isolates were Gram-positive. However, the remaining110 (33.3%) were culture negative. All corneal samples collected from keratitis patients were not positive for bacterial cultures, because there is a possibility of other pathogens, and our study is limited to identifying only bacterial keratitis.

The most predominant bacterial isolate was *S. aureus* (50%), followed by *CoNS* (30%), *S. pneumoniae* (15%), and *S. viridans* (5%). A Review study done in the United Kingdom (UK) revealed that *Staphylococci* (40.1%), followed by *Pseudomonas* species (28.5%), were the most common bacteria isolated from bacterial keratitis subjects, while other Gram-negative species (17.2%) and *Streptococci* was (7.1%) [13]. In another related study conducted at the University of Lausanne, Switzerland (2001), the most commonly isolated bacteria were *S. epidermidis*, 40%; *S. aureus*, 22%, and *S. pneumoniae* 8% [14]. A similar study in a tertiary eye center in India reported (64.5%) *Staphylococcus* species in total positive bacterial cultures, followed by (12.3%) *Streptococcus* spp. and (9.7%) *Pseudomonas aeruginosa*. A supporting study conducted in South Africa (2011) reported that the most commonly isolated bacteria were *S. aureus* (29.5%) and *P. aeruginosa* (30%) [15]. In Assiut University, Egypt (2010) reported *Staphylococcus* species in 45 out of 50 cases [16]. In another study of 50 cases, 30 were found positive for *S. aureus* as the most common bacteria, while an unidentified species of *Staphylococcus* was also isolated from 15 cases [17]. Unlike our results, a study conducted in Sudan 2008 showed that *Pseudomonas* was the most predominant 58.8% cases, followed by *Staphylococcus aureus* 2 (11.8%) [18]. There was no presence of Gram-negative bacteria in our study, and this could be due to regional geographical and climatic variation in the occurrence of corneal pathogens. While other studies conducted in other parts of the world showed the prevalence of both Gram-positive and negacteria [17,18].

Predisposing factors are required for bacterial keratitis to happen. Corneal ulceration risk factors are different throughout the world, ocular trauma or ocular surface disease up to recently were associated with microbial keratitis. In developed countries, contact lens wearing has been a major risk factor for bacterial keratitis compared to low-income countries where trauma is the far more common predisposing factor where it accounts for up to 77.5% of cases. [19]. As in our case, the most predisposing factor was trauma as it was seen in 132 (60%), followed by foreign bodies 22(10%), systemic disease (diabetics) 33(15%), and those with unknown cause 33(15%) all with positive culture results. There was no statistically significant association between risk factors and culture-positive results as the p-value was greater than 0.05. A study conducted at Tertiary Eye Centre in India (2013) reported that in (64%) of keratitis patients' trauma was the common predisposing factor [20] and similar figures were reported from studies conducted in Sudan, Iran, South Africa, and Qatar. The other factors which were most frequently reported were Mud, dust, and soil followed by leaf & vegetable matter which was (35.6%) and (31.6%), respectively [21]. During the windy season and the peak season of agricultural work, injury by dust particles and injury by vegetable matter is possibly more common and this injury leads to damage to the eye. Another study conducted in Egypt showed that 63.4% of the 115 cases of keratitis were due to trauma, and they concluded that trauma as the most significant risk factor, other than trauma, unknown factors, foreign bodies, wearing contact lenses, hypertension, diabetes, and cataract surgery all showed lower percentages [22].

While, in Saudi Arabia, contact lens wearing followed by corneal trauma and diabetes mellitus was a risk factor in 20 cases, the study has not reported any significant correlation between systemic risk factors and severity of clinical presentation [23]. The antimicrobial agents available today are mostly microbiostatic, requiring a prolonged course of therapy. Antibiotics susceptibility testing of each isolated pathogen was done using routine antibiotics discs according to the disc diffusion technique on muller hinton agar (MHA). The antimicrobial activities of microorganisms were assessed based on the diameters of the clear inhibition zone of adjacent paper disks. If there is no inhibition zone, it indicates no antimicrobial activity. The antimicrobial activity of *Staphylococcus aureus* and *Streptococcus pneumoniae* with various antibiotics such as tetracycline; clindamycin, ciprofloxacin; oxacillin, erythromycin, chloramphenicol, vancomycin, gentamycin, nitrofurantoin, rifampin, and penicillin were studied. From the results, it was observed that the antibiotics showed their capacity to inhibit the growth of these bacteria and some were quite resistant to antibiotics, as indicated in **Tables 4 and 5**.

The current work has shown that gentamycin, ciprofloxacin, nitrofurantoin, rifampin, and vancomycin are sensitive against *S.aureus* (90%) whereas *S.pneumoniae* is (100%) sensitive to gentamycin, chloramphenicol, and tetracycline. *S.aureus* was (100%) resistant to oxacillin and penicillin, while *S.pneumoniae* was only subjected to oxacillin as it showed (100%) resistance [24]. The results of the current study are in line with other similar studies conducted in Ethiopia that revealed *S.aureus* was sensitive to gentamycin (85.7%), vancomycin (83.3%), and ciprofloxacin (88.1%), however, it was highly resistant to penicillin (100%). In our study, we found that *S.pneumoniae* was sensitive to chloramphenicol (100%), and vancomycin (100%), our finding is in line with the study conducted in India, Iran, and Nigeria [25]. A study conducted in South Africa also showed *S.aureus* and *S.pneumoniae* were highly sensitive to vancomycin and chloramphenicol, while *S.aureus* was resistant to ciprofloxacin [26,27,28,29].

Conclusion :

Bacterial keratitis requires an initial diagnosis to ease its purpose of stopping future visual loss, and after early diagnosis, if the treatment is ineffective, again bacterial keratitis may progress to perforation and blindness. The current study concluded that the incidence of bacterial keratitis in Ophthalmic Hospitals of Asmara was found to be 50% for *S. aureus*, 30% for CoNS, 15% for *S.pneumoniae,* and 5% for *S.viridans*. The most frequent leading feature for bacterial keratitis was found to be trauma, but there was no significant association with positive culture results.

The antimicrobial activity of *S. aureus* was highly sensitive to ciprofloxacin, gentamycin, vancomycin, rifampin, and nitrofurantoin, as it was resistant to oxacillin and penicillin. Similarly, *S.pneumoniae* was found to be sensitive to clindamycin, chloramphenicol, and tetracycline, as it was resistant to oxacillin. The current findings of our study support the use of the above-mentioned antibiotics effectively for the treatment of serious bacterial keratitis.

Limitations of the Study

1. Corneal scraping was not possible because of technical problems and a shortage of expert people.
2. Limited time frame to get sufficient samples.
3. Very limited recorded data was available based on our requirement to conduct a retrospective study; therefore, we conducted a cross-sectional study.
4. Novobiocin disc, which is used to differentiate coagulase-negative Staphylococci, was not available.
5. Due to lack of funding and shortage of well-equipped labs, we focussed only on the identification of bacteria instead of identifying all causative agents of keratitis.

Recommendations:

1. This research will generate baseline data for future studies. Therefore, it is recommended that:
2. Microbiological assay should be done for patients with keratitis before starting their treatment as it will aid in prescribing the most effective antibiotics.
3. For the resistant strain of *S. aureus,* further studies which include molecular level should be conducted to find the exact resistance pattern and administration of the right treatment.
4. Continuous large-scale studies must be conducted in the future that focus on microbial keratitis.

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Data Availability: The data collected to support the results are available on different websites.

Authors'Contribution: Dr.Khawaja Shakeel Ahmed: Supervised the whole study and written the manuscript. Mr. Bharat Kumar Bhayal: Provided the necessary materials and literature and co-supervised the study. Mr. Dawit Eman: Helped in collecting samples. Miss. Yordanos Tekeste: Helped in analysing the samples. Mr. Michael Tadelle**:** Helped in analysing the samples**.** Miss. Fitsum Omaha: Analysed the samples. Miss. Freweyni Tesfay: Analysed the samples. Mrs. Kisanet Mebrahtu: Analysed the samples. Miss. Luwam Michael: Analysed the samples. Miss. Silas Amanuel: Analysed the samples.

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