ADDIS ABABA UNIVERSITY

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DEPARTMENT OF MEDICAL LABORATORY SCIENCE

BACTERIAL PROFILE AND ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF EXTERNAL OCULAR INFECTIONS WITH ASSOCIATED RISK FACTORS IN ALERT CENTER, ADDIS ABABA ETHIOPIA.

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OPERATIONAL DEFINITIONS

Blepharitis: - is a chronic inflammation of the eyelids, a common problem in both children and adults.

Blepharoconjunctivitis: - is a condition that causes swelling of the outer eyelids and the conjunctiva, the thin mucous layer that acts as a protective layer for the inner eyelids and front of the eyeball.

Dacryocystitis: - is an inflammation of the lacrimal sac (tear sac), which often occurs due to an obstruction of the nasolacrimal duct (tear duct).

ABSTRACT

Background: An ocular infection is public health problem in developing countries like Ethiopia. Bacteria are major causative agents that frequently cause infections in the eyes and possibly lead to loss of vision. Resistance of bacteria isolated from the ocular to antimicrobial agents is a Global concern. Thus the present study will provide essential information on the prevalence of bacterial infection and antibiotic susceptibility pattern of bacterial isolate. The result of the study will help to formulate a policy for treatment and prevention of external ocular infections.

Objectives: To assess bacterial profile and antimicrobial susceptibility pattern of external ocular infections with its associated risk factors in ALERT Center, Addis Ababa Ethiopia.

Methods: A cross sectional study was conducted from May, 2015 to August, 2015. A total of 288 samples were collected and inoculated on Blood agar, Chocolate agar, MacConkey agar and Mannitol salt agar. Presumptive isolates were further identified by a series of biochemical tests. Antimicrobial susceptibility pattern was performed for all isolated bacteria according to the criteria of the Clinical and Laboratory Standard Institute (CLSI, 2014) by disk diffusion method. Data were analyzed using (SPSS) version 20.0 software.

Results: A total of 288 patients were enrolled. From the total of external ocular infections cases, bacterial origins were isolated among 59.4% (n=171/288). The majority of the study subjects were males 53.1% (n=153/288). Gram positive bacteria were the most dominant isolate accounting 70.2% (n=120/171). The most frequent pathogens isolated were *S. aureus* 36.8% (n=63/171), followed by Coagulase negative *Staphylococci* (CoNS) 31.6% (n=54/171). Most of the bacteria isolated showed high resistance to Penicillin 91.6% and Tetracycline 70.6% while, Gentamicin 94% was the most effective antibiotic against Gram positive and Gram negative bacteria. The overall prevalence multiple drug resistance was 93% (n=159/171) Gram positive 97.5% (n=117/120) and Gram negative 82% (n=42/51). Most of the variables were not statistically significant except for repeated infection.

Conclusion: The prevalence of bacterial pathogens among external ocular samples was high. Most of the isolates were drug resistance for commonly used antibiotics. Gentamicin and Ciprofloxacin was the most effective antimicrobial agents for both Gram positive and Gram negative bacterial from external ocular infections.

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LIST OF ABBREVIATIONS

AAERC	AHRI/ALERT Ethical Review Committee
AHRI	Armauer Hansen Research Institute
ALERT	All African Leprosy, Tuberculosis and Rehabilitation Training Center
API	Analytical profile indexes
AST	Antimicrobial Susceptibility Testing
ATCC	American Type Culture Collection
BHIB	Brain heart infusion broth
CLSI	Clinical and Laboratory Standard Institute
CoNS	Coagulase-negative *Staphylococci*
FMOH	Federal Ministry of Health
HTM	*Haemophilus* Test Medium
MHA	Muller Hinton Agar
MIC	Minimal Inhibitory Concentration
MRSA	Methicillin resistance *S. aureus*
NCCLS	National Committee for Clinical Laboratory Standards
SOP	Standard operating procedure
SPSS	Statistical Package for Social Science
WHO	World Health Organization

1. INTRODUCTION

1.1. Background

Eye infections are one of the most common diseases that affect many people, and bacteria are the first causative agents, followed by fungus and viruses (1). Although ocular infection is considered to be a minor infection, it can be vision threatening leading to possible loss of vision. (1, 2, 3). The external ocular surface acquires microbial flora at birth and some of the commensal flora may become resident in the conjunctiva and eyelids with a potential to become pathogenic. Moreover, all microorganisms derived from the environment can also transiently colonize the eye and, when given the opportunity, can invade the ocular tissues (2, 3, and 4). They can form transient flora or invade the tissue and cause infection. Pathogenic microorganisms cause ocular disease due to virulence and host's reduced resistance because of the factors like personal hygiene, living conditions, socio-economic status, decreased immune status, trauma, surgery, and systemic diseases. The parts of the eye that are frequently infected are the conjunctiva, eyelid and cornea. Clinically external eye infections present as: conjunctivitis, keratitis, blepharitis, canaliculitis, dacryocystitis, external hordeolum and cellulites. The ocular findings may be part of a widespread systematic infection (5).

Bacteria, such as *S. aureus*, *S. pneumoniae*, *P. aeruginosa*, and even *N. meningitides*, have been reported as greater virulence (6). The eyelid and conjunctiva have a normal microbial flora controlled by its own mechanism and by the host. Modification of this normal flora contributes to ocular infections such as blepharitis, conjunctivitis, dacryocystitis, canaliculitis, orbital cellulitis, endophthalmitis, etc. (4, 7).

The most common ocular infection seen by primary care physicians worldwide is bacterial conjunctivitis, which is self-limiting and largely presents as an acute infection approximately 78% to 80% of cases being bacterial in origin (8, 9, and 10). Majority of publications are related to acute conjunctivitis in which, *S. aureus*, *S. pneumoniae*, and *H. influenzae* are the most common pathogens. CoNS and *S. aureus* are most frequently isolated in chronic conjunctivitis, with a tendency for an increased antibiotic resistance in recent years (3, 9, 11-12). Gram positive pathogens are responsible for 60% to 80% of acute infections (13, 14).

The development of bacterial resistance to specific antibiotics is an important consideration for clinicians treating ocular infections. Bacterial resistance has been emerging globally, likely due to widespread and inappropriate dosing of broad-spectrum antibiotics for systemic infections; which is intensified by inadequate compliance to full treatment duration on the patient side (9, 15).

External ocular infections are usually treated on empirical basis with topical broad spectrum antibacterial drugs because clinicians do not send the patients to laboratories since microbiological and susceptibility tests are time consuming and expensive (16, 17).

Bacteria causes eye disease because of their virulence and host's condensed fighting from various factors such as socio-economic status, individual hygiene, lifestyle, nutrition, inheritance, physiology, and age. Eye may be infected by being exposed to outside influences and internal invasion of bacteria that are transported by the blood stream. External microbial infections of the eye are usually centralized in one place but may frequently distributed to other tissues (4).

1.2. Statement of the problem

Bacterial agents are known to cause external ocular infections such as conjunctivitis, keratitis, blepharitis, hordeolum, dacryocystitis, etc. which are responsible for increased incidence of morbidity and blindness worldwide (2, 17). There are 1.4 million blind children estimated worldwide, of whom about 320,000 live in Sub-Saharan Africa (16).

In Ethiopia the prevalence of blindness was reported about 1.6% and it was estimated that 87.4% of the cases were due to avoidable causes of a variety of factors that determined the clinical outcome in microbial causes of eye infections, where bacteria ocular infections is one of the factors (18).

Microbial resistance to antibiotic agents has become increasingly prevalent in ocular infections on a Global basis; the past two decades have witnessed changes in antibiotic susceptibility patterns in all systemic infections (2, 17).

In Ethiopia a common practice that antibiotics can be purchased without prescription, which led to misuse of antibiotics. This may contribute to the emergence and spread of antimicrobial resistance. Moreover, poor hygienic and infection control practice in the area may play a major role in an increased prevalence of resistant bacteria in a community (2, 15, and 17).

In the study site patients with different types of eye diseases were managed; an external ocular infection was the major where the clinicians and ophthalmic nurses treat most of the external ocular infections empirically. The statistics of the unit reported 4332 new cases and repeated cases 2556 per year in 2014. Bacterial conjunctivitis was the predominant cases around 55% from all cases of the external ocular infections caused by bacteria.

Most of cases were still treated using commonly known drugs. This study was conducted to determine the Profile of isolates from external ocular infections and the susceptibility pattern of bacterial isolates from patients who visited ALERT Center ophthalmology section.

Published information about the prevalence of bacterial profile and antimicrobial susceptibility pattern of external ocular infections in Ethiopia is limited and only few studies were identified. Most of these studies were not supported by laboratory diagnosis and the etiologic agents were not known and lack of updates on trends in antibiotic resistance

patterns of ocular pathogens to inform treatment guidelines in the care of eye infected patients. Therefore antibiotic treatment is empirical including the use of systemic antibiotics in addition to the topical ones. This developing resistance increases the risk of treatment failure with potentially serious consequences (14).

1.3. Significance of the study

- Findings from this study will help to assess changes in the prevalence of external ocular infections and their susceptibility pattern through time.
- Knowledge of the etiologic agents causing these infections and their resistant pattern is crucial in proper management of the cases.
- It will provide bacterial isolate and prevalent in external ocular infection and their antimicrobial susceptibility pattern to formulate policy for treatment and prevention regarding external ocular infection.
- Provide provision of up-to date status on the local etiologic patterns and antibiotic sensitivities to guide the clinician in the initial treatment of patients with ocular infection.
- Revise and or develop guidelines for empirical therapy based on evidence based information.

2. LITERATURE REVIEW

2.1. Epidemiology of Bacterial profile and Antibiotic Susceptibility Pattern of ocular infection

Globally, purulent bacterial conjunctivitis is mainly caused by Gram positive organisms. The most common causative agents reported were *S. epidermidis* 39%, *S. aureus* 22%, and *S. pneumoniae* 6%. The most common Gram negative microorganism found in acute conjunctivitis was *H. influenzae* 9% (3). A study conducted in USA in 2009 the emerging resistance of ocular pathogens to topical antimicrobial agents is a worldwide problem. The emergence of bacterial resistance is influenced by characteristics of the pathogens, antibiotic prescribing practices including the widespread use of systemic antibiotics, and health care guidelines (9).

A study conducted in India, the rate of culture-positivity was found to be 88%, the most common bacterial species isolated were *S. aureus* 26.7% followed by *S. pneumoniae* 22.1%. *S. aureus* was more prevalent more in eyelid infections 51.2% CoNS in endophthalmitis 53.1%; *S. pneumoniae* in lacrimal apparatus and corneal infections 64.2%; *Corynebacterium* spp. in blepharitis and conjunctivitis 71%; *P. aeruginosa* in keratitis and dacryocystitis 66.5%; *Haemophilus* spp. in dacryocystitis and conjunctivitis 66.7%; *M. lacunata* in blepharitis 54.2%; and *M. catarrhalis* in dacryocystitis 63.83%. The largest number of Gram-positive isolates was susceptible to Moxifloxacin 98.7% and Vancomycin 97.9%, and gram-negative isolates to amikacin (93.5%) and Gatifloxacin 92.7% (7).

In 2013 a study in Uganda involved 138 patients with the ulcerated eyelid margin and conjunctiva. Culture positivity was observed in 59.5% and 45.8% respectively. The findings were similar to most of the studies done elsewhere and the most common organisms identified were CoNS 65.9% and *S. aureus* 21.0% CoNS showed that the highest resistance to Tetracycline 58.2%, and to Erythromycin 38.5 %, whereas in *S. aureus* the resistance to Tetracycline and Erythromycin were 55.2% and 31.0% respectively (13).

Study in Libya in 2014 showed as the order of decreasing frequency, the isolated bacteria from anterior blepharitis in order of decreasing frequency were *S. aureus* 25%,

S. epidermidis 25%, *Klebsiella* spp. 18%, *P. aeruginosa* 9%, *Proteus* spp.7% and *E. coli* 2%. The common isolates observed in both samples were *S. aureus*, *S. epidermidis* and *Proteus* spp. Pattern of antibiotic susceptibility may vary in different geographical areas. The problem of antibiotic resistance is very serious in Libya and appears to be on the rise. High resistance rates were observed among Gram negative bacteria against commonly used drugs (i.e., Ampicillin, Trimethoprim-sulphametoxazole, and Cephalosporin) (14).

Study conducted in Nigeria in 2011, *Staphylococcus* spp. 22.6% accounted for 50.3% of conjunctivitis cases, followed by Gram positive bacilli 22.6%, Gram negative bacilli 21.3%, and Gram negative cocci 4.5%. *Corynebacterium* spp. was the most commonly isolated Gram positive bacilli, accounting for 16.1% of conjunctivitis cases. *P. aeruginosa* topped with 9.7% as the most common Gram negative bacillus. Other Gram negative bacilli in order of their isolation rates were *E. coli* 6.5%, *Proteus* spp., 3.2%, *Klebsiella* spp. 1.9%, and *Entrobacter* spp. 1.9%. *Moraxella* spp. was the only Gram negative cocci isolated, and they accounted for 4.5% of the total conjunctival infections and antibiotic susceptibility testing revealed Chloramaphenicol and Ofloxacin as the least and most active antibiotics tested as 63.9% and 96.1% of the 155 recovered isolates were sensitive to them. On the whole, the least susceptible pathogen was *P. aeruginosa* with sensitivities ranging from 20% to 80%, while *Moraxella* spp. represented the most sensitive pathogen with sensitivities ranging from 71.4% to 100%. Other bacterial isolates also elicited antibiotic sensitivities in the range of 33.3–100% (19).

Another study conducted in Nigeria in 2010, of the pathogens, 74.9% were *S. aureus*, 10.2% CoNS, 6.4% *P. aeruginosa*, 3.2% *E. coli*, 2.1% *Klebsiella* spp., 1.5% *S. pneumoniae*, 1.2% *H. influenzae*, 0.3% *P. mirabilis* and 0.3% *N. gonorrhoeae*. The highest rate of conjunctivitis is 26.3% The pathogens demonstrated relatively good susceptibilities to Erythromycin 57% and Ceftriaxone 67% but susceptibilities to the remaining antibiotics were rather poor, 31.3% to Amoxicillin, 42.7% to Amoxicillin- Clavulinic acid, 39.2% to Chloramaphenicol, 38.6% to Gentamicin, 29.5% to Ofloxacin and 32.2% to Cloxacillin (20).

A study conducted Gondar in 2015, from the total of 51 dacryocystitis cases; bacterial origins were isolated among 60.8% cases. The dominant isolates were 29.0%, *S. aureus* 19.4%, and

Pseudomonas spp. 9.7%. *S. pneumoniae*, *Entrobacter* spp., *K. pneumoniae* and *H. influenzae* were each accounted 6.5% isolation rate. Among the commonly prescribed antimicrobials tested for susceptibility pattern; amoxicillin 38.7%, Ciprofloxacin 25.8%, Chloramaphenicol 25.8%, Trimethoprim Sulphametoxazole 25.8%, and Ampicillin 19.4% were resistant to the overall bacterial isolates identified. Only *Citrobacter* spp. were sensitive to all antibiotics tested but the rest bacterial isolates were resistant for at least to one, two, three, four and more antibiotics tested. Overall, 29.0% of the bacterial isolates were resistant to only one antibiotics and resistance to two, three and four antibiotics each accounted 16.1% rate (21).

Another study conducted in Gondar, Ethiopia showed 60.8% bacterial growth among 102 eye discharges. The most frequently isolated bacterial isolates were Gram positive bacteria 74.2%. The predominant bacterial species isolated was CoNS 27.4%. Most of the bacterial isolates were resistance to Ampicillin 71%, Amoxicillin 62.9%, Erythromycin 43.5%, Gentamicin (45.2%), Penicillin 71%, Trimethoprim Sulphametoxazole 58.1%, and Tetracycline 64.6% while Ceftriaxone and Ciprofloxacin showed 75.8% and 80% susceptibility respectively. From the total bacterial isolates, 87.1% showed MDR to two or more drugs (4).

Another different study conducted in Gondar from September 2004 to August 2008, among the 236 eye swabs cultured, 54.2% were positive for different types of bacterial pathogens. Gram negative bacteria accounted for 44.5% and the predominant isolate was *E. coli* 14.8%. The Gram positive bacteria comprised 55.5% and the predominant isolate was *S. aureus* 21.1%. Multiple antibiotic resistances were observed in 77.3% of bacterial isolates to the commonly prescribed antibiotics (15).

Study conducted in Addis Ababa by Nigatu *et al*., in 2004, the most common etiologic agents isolated were *S. aureus* 24.3%, followed by *S. pneumoniae* 21%, CoNS 10.6%, *H. influenzae* 9.4%, *Psuedomonas* spp. 8.5%, *H. aegyptius* 5.1%, *K. pneumoniae* 4.7%, *Moraxella* spp. 3.4%, *N. meningitides* 1.7% and other bacteria 6.3%. The Gram positive bacteria constituted 57.9% of the total bacterial isolates. All strains from both hospitals were susceptible to Ciprofloxacin. In general rates of susceptibilities to all antibiotics tested for gram positives were lower as compared to gram negatives. More than 75% of the *Pseudomonas* spp. isolates

from this study was resistant to almost all antibiotics tested except for Ciprofloxacin, gentamicin and Norfloxacin. (16).

Another study conducted by Aweke *et al.,* in 2014 in Hawassa, the most common bacterial isolates were Gram positive cocci 61.5%. The predominant bacterial species isolated was *S. aureus* 21.0% followed by CoNS 18.2% and *S. pneumoniae* 14.0%. The most common bacterial isolates were Gram positive cocci 61.5%. The predominant bacterial species isolated was *S. aureus* 21.0% followed by CoNS 18.2% and *S. pneumoniae* 14.0%. *In vitro* Ciprofloxacin was effective against 86% of isolated pathogen. MDR was observed in 69.9% of the bacterial isolates. *S. aureus* was the overall predominant isolated pathogen followed by CoNS, *S. pneumoniae and Klebsiella* spp. Gram positive isolates were more susceptible to Amoxicillin-Clavulinic acid and Vancomycin, whereas Gram negative isolates were more susceptible to Ciprofloxacin and Gentamicin. Relatively, Ciprofloxacin is effective against most isolated pathogen (17).

A study conducted in Hawassa University among a total of 281 ocular specimen submitted to culture 48.8% were positive culture, the most common bacterial isolate were gram positive cocci 61.5% and the predominant bacterial species isolated was *S. aureus* 21% and followed CoNS 18.2% and *S. pneumoniae* 14.0%. *In vitro* ciprofloxacin was effective against 86% of isolated pathogen. Multi-drug resistance was observed in 69.9% of the bacterial isolates. Gram positive isolates were more susceptible to amoxicillin-Clavulinic acid and Vancomycin, whereas Gram negative isolates were more susceptible to Ciprofloxacin and Gentamicin. Relatively, Ciprofloxacin is effective against most isolated pathogen (22).

A study conducted in Dessie among external ocular samples was 59.4%. The majority of the isolates 93.7% were Gram positive and the other 6.3% were Gram negative bacteria. The proportion of CoNS among the Gram positive bacterial isolates was 53.7%. All Grams positive isolates were susceptible for Vancomycin but 67.4% of them were resistant against Amoxicillin (18).

2.2. Risk Factors for Colonization by Bacteria

Major risk factors for bacterial ocular infections with external sources are surgical and non-surgical trauma and use of contact lenses. Contact lenses use was found to be the most common predisposing factor for corneal infection caused by *P. aeruginosa* (23-32). In

Gujarat, Western India, the study that has been conducted on 200 cases described predominant outdoor agricultural activity as the principal causative factor for corneal injury (25).

Infectious keratitis is a potentially blinding ocular condition of cornea which can cause severe visual loss if not treated at early stage. If the appropriate antimicrobial treatment is delayed, only 50% of the eyes gain good visual recover (33).

Pediatric bacterial keratitis is an uncommon but potentially devastating condition particularly in children because they are at greater risk of developing irreversible ocular sequela, most notably secondary amblyopia (unclear vision). In addition, infectious inflammation in ocular surface appears to be more severe in pediatric patients (34).

There have been an increasing number of Methicillin resistance *S. aureus* (MRSA) cases reported in postsurgical and external ocular infections, with no known risk factors for MRSA colonization, such as admission to a hospital, surgery and contact with a MRSA-colonized patient, intravenous drug use, or previous antibiotic exposure and with more than half demonstrating both multidrug resistance and resistance to ophthalmic antibiotics (35).

A study shows among a total of 155 neonates with conjunctivitis admitted into the neonatal unit at the Lagos University Teaching Hospital were microbiologically investigated. The incidence of conjunctivitis in the newborn was 18 per 1000 live births. Predisposing factor noted were vaginal delivery, asphyxia neonatorum and prolonged rupture of membrane. Pathogens predominantly isolated were *S. aureus* 37.4%, CoNS 12.3%, *K. pneumoniae* 12.9% and *P. aeruginosa* 8.2%. Antimicrobial susceptibility results revealed varied degrees of susceptibility to Ofloxacin 75%, Cloxacillin, erythromycin, Gentamicin and Amoxicillin-Clavulinic acid 30% by the gram positive bacteria while most of the gram negative were susceptible to Colistin and Ofloxacin above 90% (36).

Positive bacterial cultures in children with acute conjunctivitis have been associated with a history of sticky eyelids/eyelashes in the morning, mucoid or purulent eye discharge, and physical examinations of the children proved the presence of crusting or gluing of the eyelids/eyelashes, lack of sensation of burning eyes, and the absence of watery discharge (37).

Severe bacterial conjunctivitis is a sight-threatening ocular infection that warrants immediate ophthalmic work-up and management. Bacterial conjunctivitis is usually bilateral. It may

start in one eye and later spread to the other. The spectrum of organisms causing conjunctivitis varies around the world. Bacterial infection of the conjunctival sac can be secondary to a foreign body, dry eye, trichiasis, or accumulation of mucus (16).

In Ethiopia, it is in common practice that antibiotics can be purchased without prescription, which leads to misuse of antibiotics. This may contribute to the emergence and spread of antimicrobial resistance. Other factors may include availability of the suboptimal quality or substandard antimicrobial drugs, increased usage of a particular antimicrobial agent, poor sanitation, contaminated food and cross-contamination from humans or animals (1).

3. OBJECTIVES OF THE STUDY

3.1. General Objective

To assess the bacterial profile and antimicrobial susceptibility pattern of external ocular infections with its associated risk factors in patients with external ocular infection at ALERT Center from May, 2015- August, 2015.

3.2. Specific Objectives

- To determine the prevalence of bacterial pathogens for external ocular infections.
- To determine the antimicrobial susceptibility pattern of the bacterial isolates to the commonly used antimicrobial agents.
- To assess risk factors associated with external ocular infections.

3.3. Hypothesis

The bacterial profile and antimicrobial susceptibility pattern of external ocular infections in ALERT Centre could be the same with previous similar studies conducted in, Ethiopia.

4. MATERIALS AND METHODS

4.1. Study Area

The study was conducted in patients with only external ocular infections attending eye clinic of ALERT Center. Addis Ababa is the capital city of Ethiopia, with a population of 3,384,569 according to the 2007 population census conducted by the Central Statistical Agency of Ethiopia, 2007 with annual growth rate of 3.8%. Based on this estimation, the population in the year 2015 would be 4,478,127. Addis Ababa lies at an altitude of 2324 m (7625 ft.) above sea level and located at 8°58'N, 38°47'E and has a mean annual temperature and rainfall of 15.9^{o}c and 1089 mm, respectively. ALERT Center is one of the specialized tertiary referral hospitals in the country. It is located in Addis Ababa at 7 kilometers south west on the way to Jimma. ALERT Center was established in 1965 with the main mission of providing training for both genders in multiple aspects of Leprosy disease including prevention, treatment and rehabilitation in an African context. Currently its mission is extended which includes the provision of quality service and training for Leprosy, Tuberculosis, Rehabilitation, Surgery, Tropical dermatology, Ophthalmology and other infectious diseases as well. The Ophthalmology clinic in the year 2013/2014 had given services for an average of around 54,312 (new cases=29,580 and repeated cases=24,732) patients who came with different complaints of eye diseases that included all age groups and both sexes.

4.2. Study design and period

A cross sectional study design was conducted from May, 2015-August, 2015.

4.3. Source of population

All patients who were visiting eye clinic of ALERT Center in the study period.

4.4. Study Population

Patients who were visiting eye clinic of ALERT Center and suspected with external ocular infections during the study period.

4.5. Selection and evaluation of study subjects

All Patients with external ocular infection that fulfill the eligibility criteria during the study period were recruited prospectively based on clinical examination by clinicians.

Inclusion criteria:

- Presence of the clinical symptoms over a period of at least 3 weeks: red eye, discharge, mucoid or mucopurulent secretion, thickening of the conjunctiva, in one or both eyes.
- All ages and sex group.

Exclusion criteria:

- Patients on antibiotics should be excluded.
- Patients unwilling to give consent to participate in the study.

4.6. Study Variables

4.6.1. Dependent variables

- Bacteria profile of external ocular infections
- Antimicrobial susceptibility pattern

4.6.2. Independent variables

- Age
- Sex
- Previous antibacterial therapy
- Occupation
- Address
- The use of contact lenses
- Repeated infections
- Duration of stay in hospital
- Trauma
- Surgery
- Systemic diseases

4.7. Sample size and Sampling Technique

A consecutive sampling Technique was used. Sampling size was calculated based on the prevalence 21% indicated in the study done in 2014 Hawassa University Teaching and Referral Hospital, Southern Ethiopia (17). With expected margin of error (d) was 0.05 and confidence interval (z) is 95%. 10% Contingency for the unknown circumstance was taken.

Sample size, $\frac{(Z\alpha/2)^2 * p(1-p)}{d^2}$ Where:

n= the required sample size

1-p=Q= negative proportion There by n = ((1.96)2 x 0.21x (1-0.21))/ (0.05)2=255

d= Expected margin of error =0.05

Z α/2= 95%confidence interval (C.I) =1.96

p=prevalence of previous study found from literature review=21%

10% non-response rate=33

Therefore, at least 288 samples are needed to determine the prevalence within ±0.05.

4.8. Data collection procedures

4.8.1. Socio demographic and clinical characteristics

External ocular examination using a slit lamp biomicroscope to rule out any focus of infection or inflammation was done thoroughly in all patients by ophthalmologist and then, demographic data were collected using structured questionnaire. Standard Operating Procedures (SOPs) were prepared by the principal investigator to be followed throughout the study. Physical diagnosis and sample collection were done by ophthalmologists/experienced ophthalmic Nurse and diagnosis were recorded on the request form and specimens were collected only from those patients presenting with external ocular infections. The Questionnaire was filled by data collector and the clinical and demographic data were entered into the questionnaire.

4.8.2. Laboratory diagnosis

4.8.2.1. Collection, handling and transport of specimen

Ophthalmologist/experienced nurse were took the swabs from eyelid and conjunctiva using sterile cotton swab moistened with sterile saline. This was rolled over the eye lid margin from medial to lateral side and back again. Pus from lacrimal sac (dacryocystitis) and blepharitis was collected using dry sterile cotton tipped swab either by applying pressure over the lacrimal sac to allow the purulent material to reflux through the lacrimal punctum or by irrigating the lacrimal drainage system. Using with sterile saline, collecting the sample from the refluxing material ensuring that the lid margins, the conjunctiva were not touched.

Two swabs were collected, labeled and transported immediately to the Microbiology Laboratory of ALERT Center and inoculated right away into various culture media.

4.8.2.2. Direct microscopy

4.8.2.2.1. Gram stain

Gram staining was done from one swab of the primary sample for identification of Gram positive and Gram negative bacteria and for the presence of polymorph nuclear cells.

4.8.2.3. Culture

The remaining swabs were inoculated on 5% Sheep's blood agar, MacConkey agar, chocolate agar and Mannitol salt agar and incubated at 37°C for 24hrs-48hrs. After inoculating the swab to the media then we put in sterile BHIB (Brain Heart Infusion Broth) for re-inoculations. Aerobic atmospheric condition was maintained for the MacConkey agar and Mannitol Salt Agar, while the chocolate agar and 5% Sheep's blood agar was incubated at 5-10% CO_2 atmosphere. All plates were initially examined for growth after 24 hours and cultures with no growth were incubated for further for 48 hours.

4.8.2.4. Identification

After getting pure colonies, further identification were conducted using standard microbiological techniques, which include Gram stain, colony morphology, and biochemical tests. Presumptive

Gram negative bacteria were identified using Kligler iron agar, citrate utilization test, lysine decarboxylase test, urease test, motility test, indole test, oxidase test, tributyrin, X and V factors and Analytical profile indexes (API 20 E and API NH) were used for further identification of some bacteria that were difficult to characterize using conventional method.

Gram positive bacteria were identified using hemolytic activity on sheep blood agar, catalase, coagulase test, bile solubility and optochin disk test (2, 41).

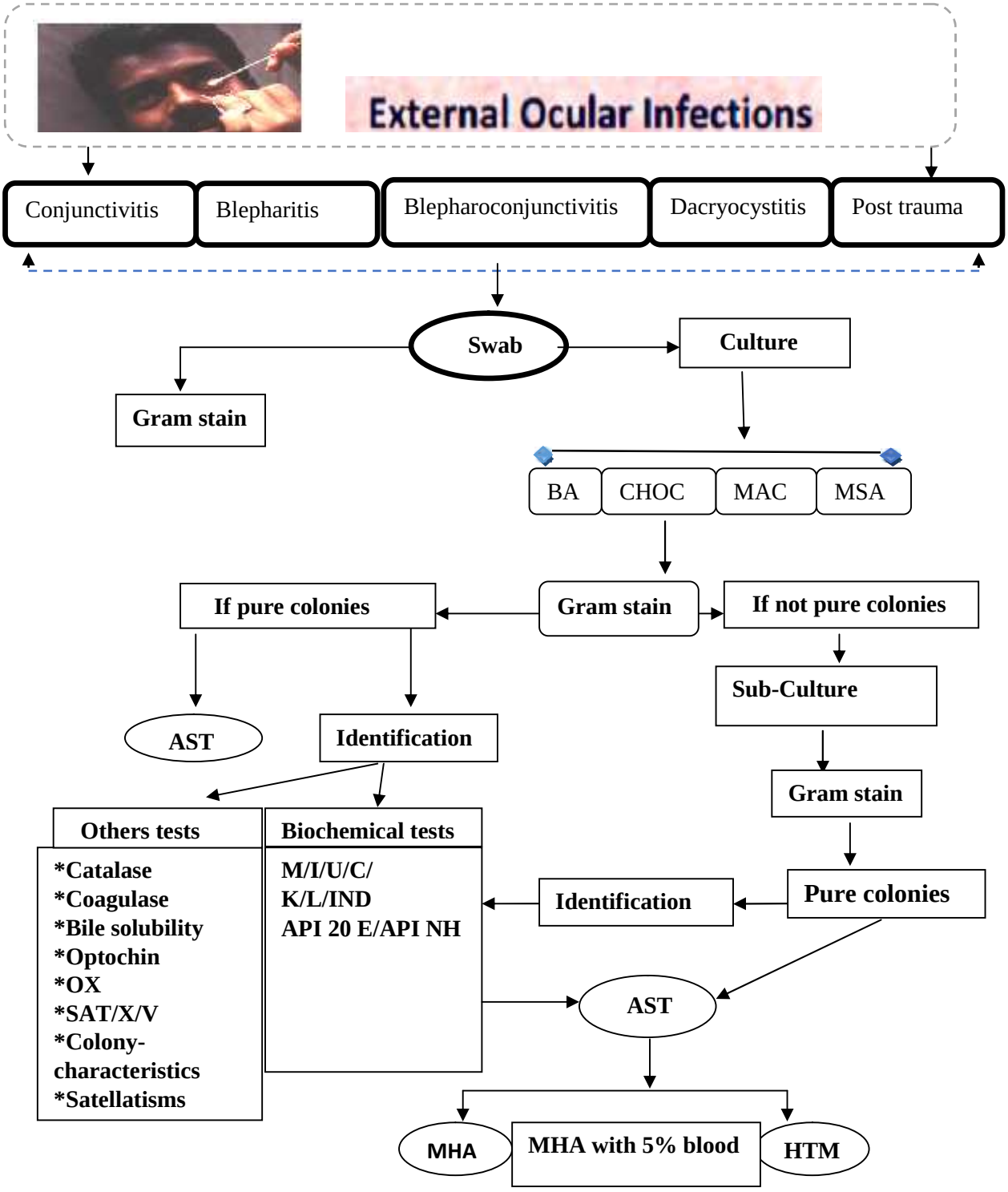

Figure 1 Flow chart for bacterial identification of external ocular infection

N.B.GPB=Gram positive bacilli, GNB=gram negative bacilli, MIUCKL=motility/Indole/Urea/Citrate utilization test/ Kligler iron agar/lysine decarboxylase test, *OX=Oxidase test, X/V/SAT=X/V Factors/satellitism,* API 20 E/API NH=Analytical profile indexes *BA=Blood agar, CHOC=chocolate agar, MSA= mannitol salt agar, MAC=MacConkey agar, Muller Hinton agar Haemophilus test medium AST=Antimicrobial susceptibility test.*

4.8.2.5. Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was carried out on each identified bacterium using disc diffusion method on Muller Hinton agar (Oxoid Ltd Basingstoke, Hampshire, UK) based on CLSI 2014 guideline (39). Briefly, 3–5 colonies of the test organism was transferred into a tube containing 1 ml of sterile normal saline(direct colony suspension) and mixed gently until the suspension becomes turbid then it was adjusted to 0.5 McFarland standards. The suspension was uniformly swabbed on to Muller Hinton agar (MHA) for non-fastidious organisms, for fastidious organisms like *Neisseriae* spp. MHA with defibrinated sterile 0.5% sheep blood was used and *Haemophilus* Test Medium (HTM) was used for *H. influenzae*. The antimicrobial impregnated disks were placed using disc dispenser on the MHA plate's surface and the plates were incubated at 37°C for 18-24 hours and the zone of inhibition around the disc were measured to the nearest millimeter using a graduated caliper in millimeters, and the isolates were classified as sensitive, intermediate, and resistant according to CLSI, 2014.

Thirteen Antibiotic disks (Oxoid Ltd, Basingstoke, and Hampshire, UK) were used and impregnated on the surface of the plate. The drugs for disc diffusion testing were in the following concentrations: Ampicillin (AMP) 10µg, Amoxicillin-Clavulinic acid (AMC) 20µg, Ceftazidime (CAZ) 30µg, Ceftriaxone (CRO) 30µg, Ciprofloxacin (CIP) 5µg, Trimethoprim-sulphametoxazole (SXT) 25µg, Erythromycin (E) 15µg, Gentamicin (CN) 10µg, Cefoxitin (FOX) 30µg, Tetracycline (TE) 30µg, Chloramaphenicol (30µg) Penicillin (P) 10U and Clindamycin (DA) 2ug.

4.9. Quality control

All specimens were collected following SOPs for Ophthalmic specimen collection. The sterility of culture media was assured by incubating 5% of each batch of the prepared media at 35-37 ºC for 24 hours. Performances of catalase reagent were checked by known *S. aureus* (positive control) and *S. pyogene* (negative control). And coagulase test was also checked by known *S. aureus* (positive control) and *E. coli* (negative control). For oxidase test the positive control *P. aeruginosa* and negative control *E. coli* was used. Before using the culture media any physical change like cracks, excess moisture, color, hemolysis, dehydration,

contamination and expiration dates were checked. Temperature of incubator and refrigerator were monitored daily. All prepared media were also checked for performance by inoculating standard strains such as *S. aureus* (ATCC 25923), *E. coli* (ATCC 25922) and *P. aeruginosa* (ATCC 27853), *H. influenzae* (ATCC 49247), *N. meningitides* sero group-A (ATCC 13077), *S. pneumoniae* (ATCC 49619) and *N. gonorrhea* (ATCC 49226). The strains were used as a quality control throughout the study for culture, Gram stain and antimicrobial susceptibly testing.

4.10. Data quality management

Training on data collection procedures was given for the data collector. Pre-testing of the questionnaire was done to assure the quality of data and for improvement of data collection tool. Supervision during data collection was done to understand how the data collectors handle the questionnaire and each filled questionnaire was checked for its completeness, accuracy, clarity, consistency on daily basis. Corrective measures were taken accordingly, then special care were given during data entry, and data cleaning and the whole data were cross checked for reliability before analysis. The data obtained were kept on a secured, password protected computer and back up data were kept at Armauer Hansen Research Institute (AHRI/ALERT). Hard copies of the data collection worksheets were kept securely locked for the duration of the study after which it was archived to protect client confidentiality.

4.11. Data Analysis

Demographic data and patients' history were obtained from laboratory request form. Data analysis and cleaning were done using SPSS version 20.0 software. Frequency count and percentage were used to clean and check the accuracy of data entry. Prevalence figures were calculated for the total study population and separately by age groups. P-value less than 0.05 were considered statistically significant.

4.12. Ethical Consideration

Ethical approval was obtained from Department of Research and Ethical Review Committee (DRERC) of Medical laboratory Science, School of Allied Health Science, College of Health Science, Addis Ababa University and AHRI/ALERT Ethical Review Committee (AAERC).

Written informed consent was obtained from each individual after the purpose of the study was explained. For children, consent was obtained from the guardian of the child who came to the hospital together with the child (Annex V-VIII). Participants were notified about the purpose of the study, their right to refuse to participate in the study, and anonymity and confidentiality of the information gathered. Study participants were given detailed information concerning the study, and for those who were literate the information sheet that had full information about the study was given and they were asked about the study to check if they have understood it correctly or not and their questions that was cleared. Those patients in the age group of (12-17) were asked for their verbal assent (Annex IX-X) and if the children agreed their parents/guardians signed for them to participate in the study.

5. RESULTS

5.1 Socio-demographic characteristics and clinical features of study subjects

A total of 288 patients were enrolled in this study. The majority of the study subjects were males 53.1% (n=153/288). The mean age of the study participants was 25.13 (SD± 19.65) years. Most of the participants were between the ages of 18-39 years 30.9% (n=89/288). The majority of the study participants were living in urban 74.3% (n=214/288). Based on occupation, most of the study participants were underage 28.5% (n=82/288). Regarding to their education majority of the study participants were elementary 33% (n=95/288). Only 4.5% (n=13/288) participants were used traditional eye medicine to treat of the external ocular infection before they have come to the study sites. 8.7% (n=25/288) of study participant were associated with systemic disease like high blood pressure, diabetes mellitus and rheumatoid factors. Few of study participant were 3.5% (n=10/288) had surgery before took sample (**Table 1**).

Table 1 Socio demographic Characteristics of study participant presenting with external ocular infections in ALERT Center, May, 2015- August, 2015.

Variables	Total Percentage
Age in years	
0-2	44(15.3)
3-11	48(16.7)
12-17	34(11.8)
18-39	89(30.9)
>=40	73(25.3)
Residence	
Rural	74(25.7)
Urban	214(74.3)
Sex	
Male	153(53.1)
Female	135(46.9)
Occupation	
Student	47(16.3)
Farmer	6(2.1)
Business man	73(25.3)
Civil servant	27(9.4)
Housewives	35(12.1)
Underage	82(28.5)
No job	18(6.25)
Educational Status	
Illiterate	32(11.1)
Elementary	95(33.0)
High school	48(16.7)
Under age	79(27.4)
College and above	34(11.8)

In this study, 59% (n=170/288) patients were suffering from conjunctivitis followed by blepharitis 20.8% (n=60/288) and Blepharoconjunctivitis, 13.9% (n=40/288). The dominant ocular infection among male and female patients was conjunctivitis **(Figure 2).**

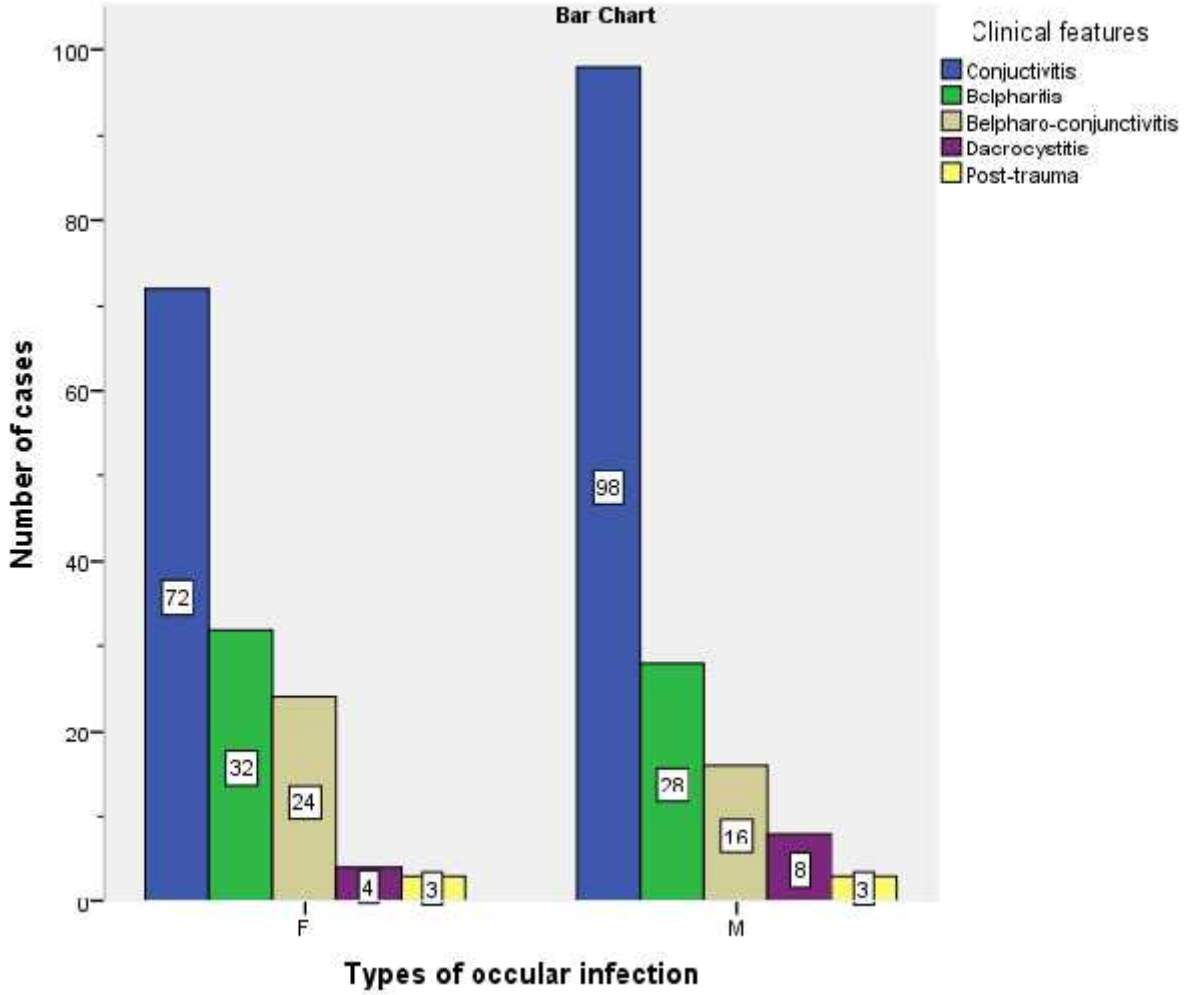

Figure 2 Prevalence of the different types of external eye infection by sex, May, 2015-August, 2015.

5.2 Prevalence of bacterial pathogens

From 288 study subjects a total of 59.4% (n=171/288) bacteria were isolated. Among the culture positive isolates, 70.2% (n=120/288) were Gram positive bacteria and 29.8% (n=51/171) were Gram negative bacteria. *S. aureus* was the predominant pathogen accounting 36.8% (n=63/171) followed by CoNS accounted for 31.5% (n=54/288). Majority of the bacteria 30.9% (n=89/288) were isolated from the age group 18-39. Bacteria from Male were high 53.2% (n=91/171). Among patients clinically categorized as conjunctivitis (n=170) and blepharitis (n=60) CoNS were the most common isolates 30.9% (n=29/288) and 17.1% (n=6/288) respectively. *S. pneumoniae* isolates were found among patients clinically categorized as conjunctivitis 3.2% (n=3/288).

Table 2 Prevalence of bacterial pathogens across the different clinical features of external ocular infections in ALERT Center, May, 2015- August, 2015.

Bacterial isolates	Conjunctivitis (N=170)	Blepharitis (N=60)	Blepharoconjunctivitis (N=40)	Dacryocystitis (N=12)	Post-trauma (N=6)	Total (N=288)
Gram positive cocci						
S. aureus	29(30.9%)	6(17.1%)	18(64.3%)	8(72.7%)	2(66.7%)	63(36.8%)
CoNS	30(31.9%)	16(45.7%)	6(21.4%)	2(18.2%)	0(0.0%)	54(31.6%)
S. pneumoniae	3(3.2%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	3(1.8%)
Gram Negatives cocci						
Moraxella spp.	8(8.5%)	3(8.6%)	0(0.0%)	0(0.0%)	0(0.0%)	11(6.4%)
N. gonorrhoeae	0(0.0%)	0(0.0%)	1(3.6%)	0(0.0%)	0(0.0%)	1(0.6%)
N. meningitides	2(2.1%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	2(1.2%)
Gram Negatives Rods						
Pseudomonas spp.	0(0.0%)	0(0.0%)	1(3.6%)	0(0.0%)	0(0.0%)	1(0.6%)
H. influenzae	2(2.1%)	1(2.9%)	2(7.1%)	0(0.0%)	0(0.0%)	5(2.9%)
E. coli	12(12.8%)	4(12.4%)	0(0.0%)	1(9.1%)	1(33.3%)	18(10.5%)
K. pneumoniae	6(6.4%)	3(8.6%)	0(0.0%)	0(0.0%)	0(0.0%)	9(5.3%)
p. mirabilis	1(1.1%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	1(0.6%)
Citrobacter spp.	1(1.1%)	2(66.7%)	0(0.0%)	0(0.0%)	0(0.0%)	3(1.8%)
Total	**94**	**35**	**28**	**11**	**3**	**171**

Table 3 Age distribution of bacterial isolates of external ocular infections in ALERT Center, May, 2015- August, 2015.

Bacterial Isolates	Age years					
	0-2 (N=44)	3-11 (N=48)	12-17 (N=34)	18-39 (N=89)	≥40 (N=73)	Total (288)
Gram positive cocci						
S. aureus	10	8	9	21	15	63
CoNS	6	8	3	16	21	54
S. pneumoniae	1	1	0	0	1	3
Gram Negatives cocci						
Moraxella spp.	0	4	0	2	5	11
N. gonorrhea	0	0	0	1	0	1
N. meningitides	1	0	0	1	0	2
Gram Negatives Rods						
Pseudomonas.spp	0	1	0	0	0	1
H. influenzae	1	1	0	2	1	5
E. coli	3	5	3	3	4	18
K. pneumoniae	4	1	1	3	0	9
P. mirabilis	0	0	0	0	1	1
Citrobacter spp.	0	0	0	0	3	3
Total	26	29	16	49	51	171

CoNS= Coagulase Negative Staphylococcus

5.3. Antimicrobial susceptibility patterns of bacterial isolate from Gram positive bacteria

The antimicrobial susceptibility patterns of bacterial isolates from external ocular infections patients showed that a significant number of bacterial isolates were resistant to one or more than one antimicrobials, but most isolated bacteria were sensitive to Gentamicin, Clindamycin, Ceftriaxone and Ciprofloxacin. The drug susceptibility pattern of the Gram positive bacteria showed that 83% (n=97/117) isolates was sensitive to Ciprofloxacin.

S. aureus strains were resistant to Penicillin 94.7% and Tetracycline 75.4%, but sensitive to Ciprofloxacin 90.5%, Clindamycin 92% and Gentamicin 98.2%. CoNS also showed similar

susceptibility pattern to *S. aureus*. *S. pneumoniae* were sensitive to Erythromycin 100%, Penicillin 100%, Chloramaphenicol 66.7% and Tetracycline 66.7% while resistant for Trimethoprim-sulphametoxazole 33.3% (**Table 4**).

5.4. Antimicrobial susceptibility patterns of bacterial isolate from Gram negative bacteria

Over 78% Gram negative bacteria isolates were found effective to Ciprofloxacin 96.1%, Amoxicillin-Clavulinic acid 58.3%, Ceftazidime 82.3%, Ceftriaxone 92.2%, Gentamicin 90.1%, and Chloramaphenicol 78%. However, Ampicillin 58.3%, Tetracycline 47% and Trimethoprim-sulphametoxazole 38% were showed resistance. All isolated Gram negative except *E. coli* and *Moraxella* spp. were highly sensitive to Ceftriaxone 100%, Ciprofloxacin 100%. *N. gonorrhea* was showed 100% sensitive for all antibiotics tested and *H. influenzae* 100% sensitive to Chloramaphenicol and Ceftazidime (**Table 5**).

Table 4 Antimicrobial susceptibility pattern of Gram positive bacteria isolated from external ocular infections ALERT Center, May, 2015- August, 2015.

Organisms isolated (n=171)		**Antibiotics tested**							
		CIP	E	DA	SXT	CN	C	P	TE
S. aureus	S	57(90.5 %)	55(87.3%)	58(92.1%)	44(69.8%)	62(98.2%)	46(73.0%)	3(5.3%)	3(5.3%)
	I	4(6.3 %)	2(3.2%)	4(6.3%)	11(17.5%)	0(0.0%)	12(19.0%)	0(0.0%)	11(19.3%)
	R	2(3.2%)	6(9.5%)	1(1.6%)	8(12.7%)	1(1.8%)	5(8.0%)	60(94.7%)	49(75.4%)
CoNS	S	40(74%)	48(88.9%)	52(96.2%)	32(59.3%)	87(98.2%)	42(77.8%)	0(0.0%)	3(5.6%)
	I	5(9.3 %)	1(1.9%)	1(1.9%)	7(13%)	3(5.6%)	8(14.8%)	1(1.9%)	5(9.2%)
	R	9(16.7%)	5(9.2%)	1(1.9%)	15(27.8%)	4(7.4%)	4(7.4%)	52(98.1%)	46(85.2%)
S. pneumoniae	S	ND	3(100%)	ND	1(33.3%)	ND	2(66.7%)	3(100%)	2(66.7%)
	I	ND	0(0.0%)	ND	0(0.0%)	ND	0(0.0%)	0(0.0%)	0(0.0%)
	R	ND	0(0.0%)	ND	2(66.7%)	ND	1(33.3%)	0(0.0%)	1(33.3%)

**CoNS: Coagulase negative Staphylococci, Amp- Ampicillin, AMC-Amoxicillin-Clavulinic acid, CRO- Ceftriaxone, C- Chloramaphenicol, CIP-Ciprofloxacin, CN- Gentamicin, DA-Clindamycin, TE- Tetracycline, SXT-* Trimethoprim-sulphametoxazole, *E- Erythromycin, P- penicillin, Amp-Ampicillin, CRO-Ceftriaxone, ND-not done. S sensitive, I Intermediate, R Resistance,*

Table 5 Antimicrobial susceptibility Pattern of Gram negative bacteria isolated from external ocular infections ALERT Center, May, 2015- August, 2015.

Organisms isolated (n=171)		Antibiotics tested									
		CIP	CRO	AMP	AMC	SXT	CN	C	CAZ	TE	FOX	P
E .coli	S	17(94.4%)	15(83.3%)	2(11.1%)	9(50.0%)	5(27.8%)	17(94.4%)	13(72.2%)	15(83.3%)	2(11%)	ND	ND
	I	0(0.0%	2(11.1%)	6(33.3%)	6(33.3%)	4(22.2%)	1(5.6%)	3(16.7%)	3(16.7%)	3(16.7%)	ND	ND
	R	1(5.6%	1(5.6%)	10(55.6%)	3(16.7%)	9(50.0%)	0(0.0%)	2(11.1%)	0(0.0%)	13(72.2%)	ND	ND
K. pneumoniae	S	9(100%)	9(100%)	0(0.0%)	5(55.6%)	5(55.6%)	9(100%)	7(77.8%)	8(88.9%)	3(33.3%)	ND	ND
	I	0(0.0%)	0(0.0%)	3(33.3%)	1(11.1%)	0(0.0%)	0(0.0%)	2(22.2%)	0(0.0%)	1(11.1%)	ND	ND
	R	0(0.0%)	0(0.0%)	6(66.7%)	3(33.3%)	4(44.4%)	0(0.0%)	0(0.0%)	1(11.1%)	5(55.6%)	ND	ND
Pseudomonas.spp.	S	1(100%)	1(100%)	0(0.0%)	1(100%)	0(0.0%)	0(0.0%)	1(100%)	1(100%)	0(0.0%)	ND	ND
	I	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	1(100%)	0(0.0%)	0(0.0%)	0(0.0%)	ND	ND
	R	0(0.0%)	0(0.0%)	1(100%)	0(0.0%)	1(100%)	0(0.0%)	0(0.0%)	0(0.0%)	1(100%)	ND	ND
Citrobacter spp	S	3(100%)	3(100%)	0(0.0%)	2(66.7%)	2(66.7%)	2(66.7%)	1(33.3%)	2(66.7%)	3(100%)	ND	ND
	I	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	1(33.3%)	0(0.0%)	0(0.0%)	ND	ND
	R	0(0.0%)	0(0.0%)	3(100%)	1(33.3%)	1(33.3%)	1(33.3%)	1(33.3%)	1(33.3%)	0(0.0%)	ND	ND
P. mirabili	S	1(100%)	1(100%)	0(0.0%)	0(0.0%)	1(100%)	1(100%)	1(100%)	1(100%)	1(100%)	ND	ND
	I	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	ND	ND
	R	0(0.0%)	0(0.0%)	1(100%)	1(100%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	ND	ND
H. influenzae	S	5(100%)	5(100%)	1(20.0%)	2(40.0%)	3(60.0%)	ND	5(100%)	5(100%)	3(60.0%)	ND	ND
	I	0(0.0%)	0(0.0%)	2(40.0%)	3(60.0%)	1(20.0%)	ND	0(0.0%)	0(0.0%)	0(0.0%)	ND	ND
	R	0(0.0%)	0(0.0%)	2(40.0%)	0(0.0%)	1(20.0%)	ND	0(0.0%)	0(0.0%)	2(40.0%)	ND	ND
N. gonorrhoeae	S	1(100%)	1(100%)	ND	ND	ND	ND	ND	1(100%)	1(100%)	1(100%)	1(100%)
	I	0(0.0%)	0(0.0%)	ND	ND	ND	ND	ND	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
	R	0(0.0%)	0(0.0%)	ND	ND	ND	ND	ND	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
N. meningitides	S	2(100%)	2(100%)	ND	ND	2(100%)	ND	1(50.0%)	ND	ND	ND	ND
	I	0(0.0%)	0(0.0%)	ND	ND	0(0.0%)	ND	1(50.0%)	ND	ND	ND	ND
	R	0(0.0%)	(0.0%)	ND	ND	0(0.0%)	ND	0(0.0%)	ND	ND	ND	ND
Moraxella spp.	S	10(90.9%)	10(90.9%)	1(9.0%)	9(82.0%)	5(45.5%)	10(90.9%)	10(90.9%)	9(82.0%)	6(54.50%)	ND	3(27.3%)
	I	0(0.0%)	0(0.0%)	5(45.5%)	1(9.0%)	3(27.25%)	0(0.0%)	1(9.1%)	2(18.0%)	3(27.25%)	ND	0(0.0%)
	R	1(9.1%)	1(9.1%)	5(45.5%)	1(9.0%)	3(27.25%)	1(9.1%)	0(0.0%)	0(0.0%)	2(18.25%)	ND	8(72.7%)

♣ND-not done

Multiple drug resistances to two or more drugs was observed in 93% (n=159/171) of the isolates. However, only 7% (n=12/171) of bacteria were resistant to one antibiotic. Gram positive resistant were 97.5% (n=117/120) and Gram negative bacteria were 82% (n=42/51) (**Table 6**).

Table 6 Multiple antibiotic resistance pattern of bacterial isolate from external ocular infection, ALERT Center, May, 2015- August, 2015.

Bacterial isolate	Antibiotic resistance patterns					Total
	R1	R2	R3	R4	R5 and more	
S. aureus	1	14	29	11	8	63
CoNS	1	9	21	10	13	54
S.pneumoniae	1	1	0	1	0	3
H. influenzae	2	2	0	1	0	5
P. aeruginosa	0	0	0	0	1	1
E. coli	2	3	7	3	3	18
K. pneumoniae	0	4	2	2	1	9
N. gonorrhoeae	1	0	0	0	0	1
N. meningitides	1	0	0	0	1	2
Moraxella spp.	3	5	2	1	0	11
Citrobacter spp.	0	0	1	1	1	3
P. mirabilis	0	0	1	0	0	1
Total	12	38	63	30	28	171

R1: resistance to one drug, **R2**: resistance to two drugs, **R3**: resistance to three drugs, **R4**: resistance to four drugs, **≥R5**: resistance to five and above drugs.

5.5. Assessment risk factors

In this study, occupation, residence, education, surgery, previous antibiotic use, using traditional eye medicine, history of chronic illness, contact lens, presence any underlying disease, the occurrence of systemic disease and repeated infections were used as possible risk and predisposing factors for ocular infection. However, bivariate logistic regression analysis showed that only repeated infection had a significant association (P=0.012) (**Table 7).**

Table 7 Bivariate analysis of possible risk factors and their association with the prevalence of bacterial infection of the eye among external ocular infections in ALERT Center, May, 2015-August, 2015.

Variable	Number positive (%)	COR (CI 95%)	P-value	AOR (CI 95%)	P-value
Age in years					
0-2	26(59.1)	0.623(0.285-1.362)	0.236	1.604(0.320-8.042)	0.566
3-11	29(60.4)	0.608(0.307-1.144)	0.284	1.082(0.273-4.283)	0.910
12-17	16(47.1)	0.83(0.166-0.887)	0.025	0.424(0.149-1.206)	0.108
18-39	49(55.1)	0.528(0.275-1.014)	0.055	0.603(0.291-1.249)	0.173
>=40	51(69.9)	1		1	
Residence					
Rural	49(66.2)	1.478(0.851-2.568)	0.166	1.309(0.699-2.452)	0.401
Urban	122(57.0)	1		1	
Repeated infection					
Yes	75(68.8%)	0.524(0.318-0.865)	0.011	0.492(0.282-0.856)	**0.012**
No	96(53.6%)	1		1	
Educational Status					
Illiterate	44(68.8)	1		1	
Elementary	64(67.2)	1.700(.850-3.398)	0.133	2.069(0.465-9.213)	0.340
High school	19(39.6)	1.582 (.796-3.146)	0.191	3.198(0.824-12.415)	0.093
Under age	44(56.4)	0.506(0.244-1.052)	0.068	0.858(0.188-3.907)	0.843
College and above	21(61.8)	1.248(0.548-2.845)	0.598	2.324(0.477-11.325)	0.297
Systemic Disease					
Blood pressure	14(4.9)	0.697(0.238-2.044)	0.510		
Diabetes	4(1.4)	2.090(0.215-20.4)	0.526		
Rheumatoid and Arthritis	7(2.4)	0.83(0.496-35.2)	0.188		
Other's	263(91.3)	1			
Surgery					
Yes	10(3.5)	2.822 (0.588-13.54)	0.205		
No	278(96.5)	1			
Hospitalized					
Yes	22(7.6)	0.404 (0.145-1.129)	0.30		
No	266(92.4)	1			

6. DISCUSSION

An external ocular infection is one of the public health problems in Ethiopia (2). In the current study, the prevalence of bacterial external ocular infections was 59.4% (n=171/288) which is in line with previous studies done in northwest Ethiopia by different authors (4, 15 and 18) who reported prevalence of 60.8%, 54.2% and 59.4% respectively. However, our finding is low compared to a study conducted in India 88%, Nigeria 74.9% and Southwest Ethiopia which is 74.7% (1,7 and 20) but it is higher than a study conducted in Bangalore, Gondar and Addis Ababa which showed a prevalence of 34.5%, 47.4% and 54.2% respectively (2,15 and16). The varied rate of isolation from one place to another might be due to different distribution of bacterial aetiology with geographic variation, study period, difference with the study population and infection prevention practices.

In this study, Gram positive cocci are the most common isolates, 70.2% (n=120/171) which is in line with several other studies in Ethiopia (1, 4, 16 and 18) and in Nigeria (20). In the current study the predominant bacterial isolates were *S. aureus* 36.8% (n=63/288) followed by CoNS 31.6% (n=54/171). There were similarities with previous works conducted in Ethiopia, (1, 15, and 16), in Nigeria (20) and in India (7).

Because of their ubiquitous nature and relatively low virulence were reported as CoNS in microbiology laboratory. However, over the past 15 years, there has been an increase in the documentations of ocular infections caused by CoNS evidence indicates that these bacteria play a primary role in the production of disease in clinical staphylococcal blepharitis and mixed seborrhoeic/staphylococcal blepharitis. Antonio *et al*., (42). In other studies the predominant isolates were CoNS. The increased prevalence of Gram positive cocci may be due to contamination of the eye from skin normal flora as a result of touching eyes with hands, cataract extraction, lens implantation, and use of contact lens (4, 13).

The prevalence of Gram negative bacterial pathogens among patients suffering from external ocular infections in our study was 29.8% (n=51/171) the low prevalence of Gram negative enteric bacteria in the present study could be due to effective personal hygiene as the most important mode of transmission for enteric pathogens is fecal-oral contamination of the eye.

Moreover, there are reports that documented the main cause for Gram negative bacteria caused ocular infection is contact lens wearing (27). In the present study, none of the patients had contact lens wearing history.

Other Gram negative bacteria isolated in this study include *H. influenzae, P. mirabilis, Moraxella* spp. and *N. gonorrhea* where studies done in Nigeria by Okesola *et al.,* (20) also showed similar isolation of Gram negative bacteria. Even *N. meningitides* was isolated in this study and similar result was found in Addis Ababa in the work of UBANI *et al*., and Nigatu *et al.*, (16, 38).

The current study showed that external ocular infections were predominantly seen among male patients which was 53.2% (n=91/171). The prevalence of conjunctivitis was also found higher among male patients, this might be due to their outdoor activities, where as a higher cases of blepharitis observed among female patients and that was also supported by similar reports from Gondar and Nigeria, However low cases of dacryocystitis and trauma in both male and female have been reported (18, 20).

Organisms causing conjunctivitis may be components of the normal eye lid flora (e.g., *S. aureus*) or nasopharyngeal flora (e.g., *S. aureus* and *H. influenzae*), or may be introduced by hand or acquired from the aerosol. The degree of inflammation, eye discharge, and symptoms of ocular infections vary. In most cases, up to 80% of the ocular infections become bilateral, justifying bilateral treatment even if the infections are present unilaterally (38).

In the current study, conjunctivitis was the dominant type of eye infection 59% (n=170/288) followed by blepharitis 20.8% (n=60/288), Blepharoconjunctivitis 13.9% (n=40/288), dacryocystitis 4.2% (n=12/288) and post trauma 2.1% (n=6/288). Male and Female in the present study suffering in conjunctivitis 34% (n=98/288) and 25% (n=72/288) respectively. Similar study done in Gondar, by Shiferaw et al., 43.1% (n=69/160) patients were suffering from conjunctivitis followed by blepharitis 29.4% (n= 47/160). The dominant type of ocular infection among male patients was conjunctivitis 43.6% (n=41/94) were as in female patients a higher cases of dacryocystitis 12.3% (n=7/66) was observed. When the different types of eye infection were stratified by sex, higher prevalence of blepharitis and conjunctivitis cases were observed among male patients than females (18).

In this study *CoNS* was the most common isolate in conjunctivitis 31.9% (n=30/94) and blepharitis 45.7% (n=16/35). This is consistent with similar studies conducted in Jimma (1) India (7) and Nigeria (38), However, *S. aureus* was found to be the predominant isolate in cases of Blepharoconjunctivitis which accounted 64.3% (n=18/28) and dacryocystitis 72.7% (n=8/11). This finding was supported by similar studies conducted in Dessie (18). The two Ethiopian studies performed in Jimma and Gondar by Tesfaye and his colleagues (1), Aweke *et al.,* (17) had shown the same results. The reason for high rate of CoNS and *S. aureus* among blepharitis cases may be virulence factor such as exo-enzymes, a surface slime and that might have been introduced by hand or acquired from the aerosol that might have a role in the pathogenesis.

The rate of isolation in this study was higher among the age group ≥40 years which was 29.8% (n=51/171). This might be due to dry eye and weaning immunity. It was supported by Shiferaw *et al.,* 2015 (18).

Immediate prescription of antibiotics without susceptibility testing for severe ocular infections is routine in ophthalmic practice resulting in increased drug resistance. In our study, among the commonly used topical antibiotics 70.6% and 91.6% of all strains were resistant to Tetracycline and Penicillin respectively. While Ciprofloxacin were found to be susceptible in 80-100% of all strains except for CoNS which was 74%. These findings were in agreement with the study conducted in Gondar (15), Jimma (1) and Uganda (13). The reason for increased resistance for, Penicillin and Tetracycline may be earlier exposure of the isolates to these drugs (allocated as first line drugs). Moreover, these drugs are very common and patients can access them easily with low price and often can be purchased without prescription over the counter in different pharmacies (2).

In this study, most of bacterial isolates have shown high resistance to penicillin 91.6%, Ampicillin 58.3% and Tetracycline 70.6%. Similar findings have been reported by Tesfaye *et al.,* and Anagaw *et al.,* (1, 15). However, Ceftriaxone 90.2%, Chloramaphenicol 75.3%, Ceftazidime 84.3%, Erythromycin 88.4%, Clindamycin 93.4% and Ciproflocacilin 86.9% showed susceptibility. In this study, the presence of higher percentage of single and multiple antibiotic resistance patterns were common. The reason for the observed resistance might be the empirical prescription of broad spectrum anti-microbial agents to treat bacterial infections

without definite diagnosis. In the community side, irrational use of antibiotics was also a common practice. It was supported study done by Tesfaye *et al.*, Jimma (1).

Demographic data showed us, in this study 35.1% of the external ocular infection patients had a history of antibiotic usage of before they attended ALERT Center for their external ocular infection management. These uncontrolled uses of antibiotics are fuelling the already existing natural antibiotic resistance mechanisms of bacteria and which might be responsible for the relatively higher prevalence rate of resistance to Tetracycline, Ampicillin and Penicillin which was similar to the study done in Gondar by Assefa and his colleagues (21).

Prevalence of high MDR to two or more of the commonly prescribed antimicrobials were isolated of Gram positive 97.5% (n=117/120) among Gram positive isolates CoNS 44.2% (n=53/120), *S. aureus* 51.7% (n=62/120) and *S. pneumoniae* 1.7% (n=2/120) in the present study and Gram negative 82% (n=42/51). The over all of observed was 93% (n=159/171) of the bacterial isolates resistant to multiple drug resistant in our study. This is in agreement with the previous studies conducted by Muluye *et al.*, (4), Barnabas *et al*., (13), Anagaw *et al.*, (15) and Aweke *et al.*, (17). Similarly high prevalence of MDR was previously reported in Gondar which was 87.1% (4). Although it is difficult to compare our MDR results with other reports China by Wang *et al*., rate of MDR *S. epidermidis* and *P. aeruginosa* accounted for approximately 22.5% and 11.2%, respectively. It has been suggested that the indiscriminate, prolonged use of a wide range of antibiotics may be a major factor in the development of drug resistance. The combined use of antibiotics could provide broader coverage against infection prior to susceptibility testing (43).

In our study the prevalence of bacterial isolation in both sexes (p-value = 0.533), various age groups (p-value = 0.186), occupation, residence, education, surgery, previous antibiotic use, using traditional eye medicine, history of chronic illness, contact lens and the occurrence of systemic disease were not statistically significant, the reason might be due to the small sample size and most of our study participants came from urban area; they had high probability of having good sanitation because they have access to information than the patients who came from rural areas that were studied in other sites. Similar findings were found from previous studies conducted in India (7), Gondar (15) and Dessie (17). However significant association was found in repeated infection (p-value = 0.012). This is presence of

bacteria from infected participants during early infection. Other studies conducted in Gondar and India had shown statistically significant association in the age group ≤ 2 years (4, 7). The reason for increased susceptibility to infection in infants may be that they are at a greater risk after their maternal immunity has disappeared and before their own immunity system had matured (38).

Other factors may include availability of the suboptimal quality or substandard antimicrobial drugs, increased usage of a particular antimicrobial agent, poor sanitation and cross-contamination from humans or animals. Other contributing factors may include improper dosage regimen during administration which includes difficulty of administration of drops of antibiotics in day time use for adult populations and children (18).

In this study post-trauma (2.1%) as predisposing factors for the external ocular infections which was not show a significance association, however, a history of trauma was the major predisposing factor, in Mexico City 25%. This result is consistent with multiple microbial keratitis studies involving children where ocular trauma has been associated in up to two thirds of the cases (44).

7. LIMITATION OF THE STUDY

1. Anaerobic bacteria and *Chlamydia trachomatis* were not included in the study because of budget constraints and lack of anaerobic system facilities.
2. Few antibiotics sensitivity tests were not done due to unavailability of the discs.

8. CONCLUSIONS AND RECOMMENDATIONS

Based on the results from the present study, it is concluded that both Gram positive and Gram negative bacteria were responsible for external ocular infections and most of the strains were multi-drug resistant. The most common isolated bacteria from cultures were *S. aureus,* CoNS, *E, coli, Moraxella* spp. and *Klebsiella* spp. The antibiotics; Gentamicin and Ciprofloxacin were comparatively more effective antibiotics against both Gram positive and negative bacteria while Gentamicin and Clindamycin were more effective against Gram positive bacteria. Ceftriaxone which is the other empirically used antibiotic therapy was more effective than the first line (Ampicillin and Tetracycline) antibiotics.

Based on these findings the following recommendations are made:

- The first-line empirical antibiotics therapy (Ampicillin, Tetracycline and Penicillin) showed resistance for the commonly isolated bacteria. Thus reconsideration of its effectiveness seems necessary in the study hospital, ALERT Center.
- Empirical treatment to external ocular infections provoke drug resistance, therefore rational use of antibiotics according to the standard antimicrobial susceptibility testing is essential employing the appropriate microbiological culturing techniques.
- Continuous surveillance would be very helpful on large samples that involve anaerobic bacteria, viral, parasite and other fungi which have a role in eye infections.

Therefore large scale study with maximum sample size and wide range of geographical area will be required.

9. REFERENCES

1. Tesfaye T, Beyene G, Gelaw Y, Bekele S, Saravanan M. Bacterial profile and antimicrobial susceptibility pattern of external ocular infections in Jimma University Specialized Hospital. American Journal of Infectious Diseases and Microbiology. 2013;1(1):13-20.

2. HeMavatHi PS, SHenoy P. Profile of Microbial Isolates in Ophthalmic Infections and Antibiotic Susceptibility of the Bacterial Isolates: A Study in an Eye Care Hospital, Bangalore. Journal of clinical and diagnostic research: JCDR. 2014;8(1):23.

3. Bremond-Gignac D, Chiambaretta F, Milazzo S. A European perspective on topical ophthalmic antibiotics: Current and evolving options. Ophthalmology and eye diseases. 2011;3:29.

4. Muluye D, Wondimeneh Y, Moges F, Nega T, Ferede G. Types and drug susceptibility patterns of bacterial isolates from eye discharge samples at Gondar University Hospital, Northwest Ethiopia. BMC Research Notes. 2014;7(1):292.

5. Castañeda-Sánchez JI, García-Pérez BE, Muñoz-Duarte AR, Baltierra-Uribe SL, Mejia-López H, López-López C, et al. Defensin Production by Human Limbo-Corneal Fibroblasts Infected with Mycobacteria. Pathogens. 2013;2(1):13-32.

6. Schaefer F, Bruttin O, Zografos L, Guex-Crosier Y. Bacterial keratitis: a prospective clinical and microbiological study. British Journal of Ophthalmology. 2001;85(7):842-7.

7. Bharathi MJ, Ramakrishnan R, Shivakumar C, Meenakshi R, Lionalraj D. Etiology and antibacterial susceptibility pattern of community-acquired bacterial ocular infections in a tertiary eye care hospital in south India. Indian journal of ophthalmology. 2010;58(6):497.

8. Sharma S. Diagnosis of infectious diseases of the eye. Eye. 2011;26(2):177-84.

9. Bertino Jr JS. Impact of antibiotic resistance in the management of ocular infections: the role of current and future antibiotics. Clinical ophthalmology (Auckland, NZ). 2009;3:507.

10. Abdullah FE, Khan MI, Waheed S. Current pattern of antibiotic resistance of clinical isolates among conjunctival swabs. Pakistan journal of medical sciences. 2013;29(1):81.

11. Blanco C, Núñez MX. Antibiotic Susceptibility of *Staphylococci* Isolates from Patients with Chronic Conjunctivitis: Including Associated Factors and Clinical Evaluation. Journal of Ocular Pharmacology and Therapeutics. 2013;29(9):803-8.

12. Marquart ME, O'Callaghan RJ. Infectious Keratitis: Secreted Bacterial Proteins That Mediate Corneal Damage. Journal of ophthalmology. 2013;2013.

13. Mshangila B, Paddy M, Kajumbula H, Ateenyi-Agaba C, Kahwa B, Seni J. External ocular surface bacterial isolates and their antimicrobial susceptibility patterns among pre-operative cataract patients at Mulago National Hospital in Kampala, Uganda. BMC ophthalmology. 2013;13(1):71.

14. Musa A, Nazeerullah R, Sarite S. Bacterial profile and antimicrobial susceptibility pattern of anterior blepharitis in Misurata region, Libya. Dentistry and Medical Research. 2014;2(1):8.

15. Anagaw B, Biadglegne F, Belyhun Y, Mulu A. Bacteriology of ocular infections and antibiotic susceptibility pattern in Gondar University Hospital, north west Ethiopia. Ethiopian medical journal. 2011;49(2):117-23.

16. Alemayehu N. Pattern of microbial agents of external ocular infections in federal police Hospital and Minilik ii memorial Hospital, Addis Ababa, Ethiopia. 2004.

17. Aweke T, Dibaba G, Ashenafi K, Kebede M. Bacterial pathogens of exterior ocular Infections and their antibiotic vulnerability pattern in Southern Ethiopia. African Journal of Immunology. 2014; 1(2)019-025.

18. Shiferaw B, Gelaw B, Assefa A, Assefa Y, Addis Z. Bacterial isolates and their antimicrobial susceptibility pattern among patients with external ocular infections at Borumeda hospital, Northeast Ethiopia. BMC ophthalmology. 2015;15(1):103.

19. Iwalokun BA, Oluwadun A, Akinsinde KA, Niemogha MT, Nwaokorie FO. Bacteriologic and plasmid analysis of etiologic agents of conjunctivitis in Lagos, Nigeria. Journal of ophthalmic inflammation and infection. 2011;1(3):95-103.

20. Okesola A, Salako A. Microbiological profile of bacterial conjunctivitis in Ibadan, Nigeria. Annals of Ibadan Postgraduate Medicine. 2010;8(1):20-4.

21. Assefa Y, Moges F, Endris M, Zereay B, Amare B, Bekele D, et al. Bacteriological profile and drug susceptibility patterns in dacryocystitis patients attending Gondar University Teaching Hospital, Northwest Ethiopia. BMC ophthalmology. 2015;15(1):34.

22. Amsalu A, Abebe T, Mihret A, Delelegne D, Tadesse E. Potential bacterial pathogens of external ocular infections and their antibiotic susceptibility pattern at Hawassa University

Teaching and Referral Hospital, Southern Ethiopia. African Journal of Microbiology Research. 2015;9(14):1012-9.

23. Callegan MC, Gilmore MS, Gregory M, Ramadan RT, Wiskur BJ, Moyer AL, et al. Bacterial endophthalmitis: therapeutic challenges and host pathogen interactions. Progress in retinal and eye research. 2007;26(2):189-203.

24. Gupta N, Tandon R, Gupta SK, Sreenivas V, Vashist P. Burden of corneal blindness in India. Indian journal of community medicine: official publication of Indian Association of Preventive & Social Medicine. 2013;38(4):198.

25. Kumar A, Pandya S, Kavathia G, Antala S, Madan M, Javdekar T. Microbial keratitis in Gujarat, Western India: findings from 200 cases. Pan African Medical Journal. 2011;10.

26. Knop E, Knop N. The role of eye associated lymphoid tissue in corneal immune protection. Journal of anatomy. 2005;206(3):271-85.

27. Havaei SA, Azimian A, Fazeli H, Naderi M, Ghazvini K, Samiee SM, et al. Isolation of Asian endemic and livestock associated clones of methicillin resistant *S. aureus* from ocular samples in Northeastern Iran. Iranian journal of microbiology. 2013;5(3):227.

28. Nadig S, Velusamy N, Lalitha P, Kar S, Sharma S, Arakere G. *S. aureus* eye infections in two Indian hospitals: emergence of ST772 as a major clone. Clinical ophthalmology (Auckland, NZ). 2012;6:165.

29. Sadaka A, Durand ML, Gilmore MS. Bacterial endophthalmitis in the age of outpatient intravitreal therapies and cataract surgeries: Host–microbe interactions in intraocular infection. Progress in retinal and eye research. 2012;31(4):316-31.

30. Bourcier T, Thomas F, Borderie V, Chaumeil C, Laroche L. Bacterial keratitis: predisposing factors, clinical and microbiological review of 300 cases. British Journal of Ophthalmology. 2003;87(7):834-8.

31. Morlet N, Minassian D, Butcher J. Risk factors for treatment outcome of suspected microbial keratitis. British journal of ophthalmology. 1999;83(9):1027-31.

32. Tam C, Mun JJ, Evans DJ, Fleiszig SM. The impact of inoculation parameters on the pathogenesis of contact lens related infectious keratitis. Investigative ophthalmology & visual science. 2010;51(6):3100-6.

33. Wong RL, Gangwani R, Yu LW, Lai JS. New treatments for bacterial keratitis. Journal of ophthalmology. 2012;2012.

34. Hong J, Chen J, Sun X, Deng S, Chen L, Gong L, et al. Paediatric bacterial keratitis cases in Shanghai: microbiological profile, antibiotic susceptibility and visual outcomes. Eye. 2012;26(12):1571-8.

35. Hesje CK, Sanfilippo CM, Haas W, Morris TW. Molecular epidemiology of methicillin-resistant and methicillin-susceptible *S. aureus* isolated from the eye. Current eye research. 2011;36(2):94-102.

36. Iroha E, Kesah C, Egri-Okwaji M, Odugbemi T. Bacterial eye infection in neonates, a prospective study in a neonatal unit. West African journal of medicine. 1997;17(3):168-72.

37. Cervantes LJ, Mah FS. Clinical use of gatifloxacin ophthalmic solution for treatment of bacterial conjunctivitis. Clinical ophthalmology (Auckland, NZ). 2011;5:495.

38. Ubani U. Common bacterial isolates from infected eyes. Journal of the Nigerian Optometric Association. 2009;15(1):40-7.

39. Clinical and Laboratory Standard Institute. Performance standards for antimicrobial susceptability testing; twenty-forth informational supplement CLSI document M100-S24. CLSI 2014.

40. Kumar *N. gonorrhoeae* presenting as red eye: rare case. Indian journal of sexually transmitted diseases. 2012; 33(1):47.

41. Kulkarni S, Bala M, Risbud A. Performance of tests for identification of *N. gonorrhoeae.* The Indian journal of medical research. 2015;141(6):833.

42. Pinna A, Zanetti S, Sotgiu M, Sechi LA, Fadda G, Carta F. Identification and antibiotic susceptibility of coagulase negative staphylococci isolated in corneal/external infections. British journal of ophthalmology. 1999;83(7):771-3.

43. Wang N, Yang Q, Tan Y, Lin L, Huang Q, Wu K. Bacterial Spectrum and Antibiotic Resistance Patterns of Ocular Infection: Differences between External and Intraocular Diseases. Journal of ophthalmology. 2015;2015.

44. Chirinos-Saldaña P, de Lucio VMB, Hernandez-Camarena JC, Navas A, Ramirez-Miranda A, Garcia LV, et al. Clinical and microbiological profile of infectious keratitis in children. BMC ophthalmology. 2013;13(1):54.

10. ANNEXS

ANNEX I ENGLISH VERSION PARTICIPANT INFORMATION SHEET

Introduction

Hello, how are you?

My name is Sebsib Neway and I am MSc student of Addis Ababa University, School of Medical laboratory Sciences. I am doing a research entitled "Bacterial Profile and Antimicrobial Susceptibility Pattern of External Ocular Infections in ALERT centre, Addis Ababa Ethiopia".

Purpose of the study

The objective of this research is to determine profile of bacteria on external ocular surface and susceptibility pattern.

Duration: The duration of this study depend upon the availability of study subjects. It might take about three months or more.

Risk associated with the specimen collection: The risk associated with the specimen collection is minimal since the collection of these specimens would follow the routine procedures for the laboratory investigation. There will be a little discomfort during sample collection.

Procedure of the study

If you agree to participate in the study, sample will be collected from the lower eye lid with moistened swab by attending ophthalmologist and experience nurse.

Confidentiality

All the data obtained will be kept strictly confidential and locking the data, only study personnel will have access to the files. Anonymous testing will be undertaken, that means samples will be coded and positive results will not be identified by names.

Benefit

There will not be any payment or direct benefit for participating and you are not asked to pay for the laboratory examination. The result will be given to you and if your result is clinically significant, it will help you for further diagnosis and treatment.

Withdrawal rights

Your participation in this study is purely voluntary, and you may stop the participation at any time or you may refuse to answer some of the questions if you feel uncomfortable. You are free to refuse to participate in the study or you can withdraw your consent at any time, without giving reasons and this will not involve any penalty or loss of benefits to which you are entitled such as proper care and treatment. Your access to treatment will not be dependent on your participation in the study.

If you are not comfortable please feel free to stop it at any level of the study. I appreciate your cooperation greatly.

If you have questions regarding this study or would like to be informed of the results after its completion, please contact through the following address.

1. Addis Ababa University, College of Health Sciences, School of Allied Health Sciences, Department of Medical Laboratory Sciences

   Tel: +251-112-75-51-70

   Fax: +251-112-75-46-69

   E-mail: SMLT@ethionet.et

   P.o.Box: 1176, Addis Ababa, Ethiopia

-If you have question about the study, the address of the principal investigator is:

2. Principal Investigator: **Sebsib Neway**

   Tel: +251-91398438/0922191780

   Email: sebsibneway2152@gmail.com/ or titayseb27@gmail.com

ANNEX II AMHARIC VERSION PARTICIPANT INFORMATION SHEET

አዲስ አበባ ዩንቨርስቲ

የጤና ሳይንስ ኮሌጅ

የህክምና ላቦራቶሪ ሳይንስ ትምህርት ቤት ከ አርማዎር ሀንሰን የጥናትና ምርምር ተsም ጋር በመተባበር

በአማርኛ የተዘጋጀ የተሳታፊዎች መረጃ

የጥናቱ ርዕስ፡ የዉጪኛው የአይን ኢንፌክሽን ተህዋስያን የሚያመጣውን ህመም እና የተህዋስያኑ መድሃኒት የመቋቋም ያለዉን

የስርጭት መጠን በአለርት ማእከል ለማወቅ፡፡

ዋና ተመራማሪ፡ ሰብስብ ነዋይ

አመካረዎች፡ 1. አቶ ካሱ ደስታ (መ/ር፣ አአዩ) 2. አቶ ዋለልኝ ደሴ (መ/ር፣ አአዩ)

3. አቶ ብሩክ የሺጥላ (የአህሪ ሰራተኛ) 4. ወ/ሮ ፀሀይነሽ ለማ(የአህሪ ሰራተኛ)

የምርምሩ ወጪ የሚሸፈነዉ፡ አህሪ

መግቢያ፡

ሰላም እንደምን አሉ?

ስሜ----------እባላለሁ:: የአ.አ.ዩ. የላበራቶሪ ሳይንስ የትምህርት ክፍል የማስተርስ ድግሪ ተማሪ ነኝ በአሁኑ ሰአት የዉጪኛው የአይን ኢንፌክሽን ተህዋስያን የሚያመጣውን ህመም እና የተህዋስያኑ መድሃኒት የመቋቋም ያለዉን የስርጭት መጠን በአለርት ማእከል ለማወቅ ጥናት እያካሄድኩ ነው፡፡

የጥናቱ ዋና አላማ፡

የጥናቱ አላማ የዉጪኛው የአይን ኢንፌክሽን ተህዋስያን የሚያመጣውን ህመም እና የተህዋስያኑ መድሃኒት የመቋቋም ያለዉን ስርጭት በአይን ህሙማን ለይ ምን ያህል እንደሆነ ለማውቅ ነው፡፡

የጥናቱ ጊዜ፡ ክትትል በሚያደርጉ የአይን ታካሚዎች ብዛት የሚወሰን ሲሆን 3 ወር እና ከዛም በላይ ለወስድ ይችላል፡፡

ሊከሰቱ ስለሚችሉ ስጋቶችና የምችት መጓደሎች፡ለጥናቱ በሚወሰደዉ ናሙና ምክንያት የተለየ ችግር አይከሰትም፡፡ የሚያሰጋ ምንም ነገር የለዉም ምክንያቱም የጥናቱ ናሙና አወሳሰድ ከወትሮዉ በሸተኛዉ ለራሱ ብሎ ከሚሰጠዉ የተለየ አይደለም፡፡ ናሙና በሚወሰድበት ሂደት ከትንሽ የህመም ስሜት ውጪ ይሄ ነው የሚባል ችግር የሚያስከትል ወይም የሚያሰጋ አይደለም፡፡

የጥናቱ ሂደት፡

እርስዎ በጥናቱ ለመሳተፍ ፈቃደኛ ከሆኑ ክአይኖት ላይ ከትትል በሚያደርግሎት ሀኪም፣ልምድ ባላቸዉ አፕቶሞሎጂስት ዶክተር ወይም ነርስ ናሙና ይሰጣሉ፡፡

የጥናቱ ሚስጢራዊነቱ፡

የሚሰጡት መረጃ ሚስጥራዊነቱ የተጠበቀነው፡፡በስም አይጻፋም የዚህ ኮድ መፍቻ በፋይል ተቆልፎ የሚቀመጥ ሲሆን የተፈቀደለት ሰው ብቻ ፋይሉን ማየት ይችላል፡፡ ከዚህ ጥናት በሚወጡ ዘገባዎች ወይንም የህትመት ውጤቶች ላይ ስምም ወይም ሌላ የእርስዎን ማንነት የሚገልጽ መረጃ አይኖርም፡፡ ከምርመራ የሚገኘውም ውጤት ወይም ሌላ መረጃ

ለሚመለከታቸው አካላት ለምሳሌ፤ እርስዎን የሚንከባከቡ የህክምና ባለሙዎች እና ጥናቱን ለሚያካሄዱት ባለሙያዎች እንዲሁም ጥናቱ ስነምግባርን ጠብቆ መከናወኑን ለሚከተሉት የኮሚቴ አባላት ብቻ ይገለፃል፡፡ ኮምፒውተር ላይ ያሉ መርጃዎች ምስጢራዊነታቸው የተጠበቀ ሲሆን በወረቀት ያሉ መረጃዎችም ደህንነቱ በሚጠበቅ ቦታ የሚቆለፉና የተፈቀደለት ሰው ብቻ ሊያያቸው እንዲችል ተደርጎ ይጠበቃሉ፡፡

የሚስገኘው ጥቅም፡

በጥናቱ በመሳተፈዎ ምንም አይነት ክፍያ አይጠየቁም ወይም የሚያገኙት ገንዘብ አይኖርም ነገር ግን የአይን ኢንፌክሽን ተህዋስያን ህመም ካለዉ ወይም የምርመራ ውጤቱ ህክምና የሚያስፈልገው ከሆነ ተጨማሪ ምርመራ እና ህክምና እንዲያገኙ የረዳዎታል፡፡ ስለሆነም ከጥናቱ በሚገኘው እውቀት የአይን ኢንፌክሽን ተህዋስያን ባክቴሪያ አማካኝነት የሚመጣውን በሽታ በተሻለ ደረጃ ለመቆጣጠርና ለበሽታው ትክክለኛውን ፀረ ባክቴሪያ ለመምረጥ ሀኪሞችን ይረዳል፡፡

ከጥናቱ ስለማቋረጥ፡

በጥናቱ የሚሳተፉት ፈቃደኛ ከሆኑ ብቻ ነው፡፡ ስለዚህ መሳተፍ አለመሳተፍ ከጀመሩ በኃላ ማቋረጥ ወይም መመለስ የማይፈልጉት ጥያቄ ከሆነ ይለፈኝ ማለት ሙሉ መብትዎ ነው፡፡ በጥናቱ መሳተፍ ወይም አለመሳተፍ አገልግልት ላይ ምንም አይነት ጥቅምም ሆነ ጉዳት አይኖረውም፡፡ ጊዜዎትን መሰዋት አድርገው ስለተባበሩኝ ከልብ አመሰግናለሁ፡፡

ስለ ጥናቱ ሕጋዊነት ለመጠየቅ ከፈለጉ፡

ይህንን ጥናት አስመልክቶ ጥያቄ ካለዎት ወይም የጥናቱ የመጨረሻ ዉጤት ምን እንደሆነ ለማዉቅ ከፈለጉ በሚከተለዉ አድራሻ ሊያገኙን ይችላሉ፡፡

1. አዲስ አበባ ዩኒቨርሲቲ ፣ የጤና ሳይንስ ኮሌጅ፣በስኩል ኦፍ አላይድ ሄልዝ ሳይንስ፣ ህክምና ላቦራቶሪ ሳይንስ ዲፓርትመንት

   ስ.ቁ.፡- +251-112-75-51-70

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   ኢ-ሜል፡: SMLT@ethionet.et

   ፒ.ኦ.ቦክስ፡ 1176 ፣አዲስ አበባ፣ኢትዮጲያ.

2. *የጥናቱአስከያጅ፡ሰብስብነዋይ*

   ስ.ቁ፡-0913198438/0922191780

   ኢሜል: Email: sebsibneway2152@gmail.com/ or titayseb27@gmail.com

ANNEX III CONSENT FORM FOR ADULT PATIENTS

I have been requested to participate in this study, which plans to determine bacterial profile and antimicrobial susceptibility pattern of external ocular infections attending eye clinic in ALERT Centre, Addis Ababa, Ethiopia in which I will be benefited from study. I have been informed this study which involves collecting swab from conjunctiva and eyelid specimen. During collection of the specimen I have been told that there is no harm except little discomfort and I have also read the information sheet or it has been read to me. I have been also informed that all information contained within the questionnaire is to be kept confidential. Moreover, I have also been well informed of my right to keep hold of information, decline to cooperate and drop out of the study if I want and that none of my actions will have any bearing at all on my overall health care and hospital access.

It is therefore with full understanding of the situations that I agreed to give the informed consent voluntarily to the researcher to use the specimen taken from conjunctiva and eyelid for the investigation. Moreover I have had the opportunity to ask questions about the project and I have received clarification to my satisfaction. I was also told that results would be reported timely to the requesting physicians for the appropriate treatment and management of the external ocular infection.

I agree that I am contributing to the treatment of my fellows by participating in this project. I have asked some questions and clarification has been given to me. I have given my consent freely to participate in the study, and I_____ hereby to approve my agreement with my signature.

I _____, after being fully informed about the detail of this study, hereby give my consent to participate in this study, if the participants are volunteer.

_____	_____	____/____/___
Name of study participant	Signature	Day/month/year
_____	_____	____/____/___
Witness (Illiterate)	Signature	Day/month/year
_____	_____	____/____/___
Name of the researcher	Signature	Day/month/year

ANNEX IV CONSENT FORM IN AMHARIC

በዚህ ጥናት ለሚዳሰሱ ጥናቱች ሀሳባቸዉን መግለጽ ለሚችሉ

እኔ----------በአለርት ማዕከል በተመላላሽ የኢይን ታካሚዎች መሀከል የዉጪኛው የኢይን ኢንፌክሽን ተህዋስያን የሚያመጣውን ህመም እና የተህዋስያኑ መድሃኒት የመቋቋም ያለዉን ስርጭት በኢይን ህሙማን ላይ ምን ያህል እንደሆነ ለማውቅ የተዘጋጀ ጥናት ላይ እድሳተፍ ተጠይቄ ስለጉዳዩም ለመረዳት በቂ መረጃ አግኝቻለሁ፡፡ ስለሆነም ናሙና የሚሰበሰበው ከታችኛው የኢይን ሽፋን ዉስጥ እና ከኮንጀቲቫ መሆኑን ስለተርዳሁኝ ናሙና ወስዶ መመርመር አስፈላጊ ስለሆነ ናሙናዉን በመስጠት ልተባበር ሙሉ ፈቃደኛ መሆኔን ገልጫለሁ፡፡ ናሙና በሚወስድበት ወቅት ከትንሽ የህመም ስሜት ውጪ ምንም አይነት ጉዳት እንደሌለው ተነግሮኛል እንዲሁም ከመጠይቁ አንብቢያለሁ ወይም ተነቦልኛል፡፡ ከምርመሩ መሳተፍ ወይም አለመሳተፍ መብቴ የተጠበቀ መሆኑን እና ላለመሳተፍ ብወስን በአለርት ሆስፒታል በሚደረግልኝ ህክምና ላይ ምንም ተፅዕኖ እንደማይኖረዉ ተረድቻለሁ፡፡

ስለዚህ የጥናቱን ጠቃሚነት አምኜበት የስምምነት ቃሌን የሰጠሁት በፍፁም ፈቃደኝነት ነዉ፡፡ በመጨሻም እኔ ከጥናቱ ዉጤት ተጠቃሚ ልሆን እንደሚችል ተገልፆልኝ በመሳተፌና በመተባበሬ ወገኖቼን ልረዳ በመቻሌ ደስተኛ መሆኔን ገልጨ፤ ግለፅ ያልሆኑ ጥያቄዎች ላይ ማብራርያ እንዲሰጠኝ ጠይቄ መልስ ተሰጥቶኛል፡፡ እንዲሁም በጥናቱ ሂደት እንድሳተፍ ፍቃደኝነቴን በፊርማዬ አረጋግጠለሁ፡፡

_____	_____	_____/_____/____
የተሳታፊው ሥም	ፊርማ	ቀን /ወር/ዓ.ም
_____	_____	_____/_____/____
ምስክር (ማንበብና መፃፍ ለማይችሉ)	ፊርማ	ቀን /ወር/ዓ.ም
_____	_____	_____/_____/____
የተመራማሪው ስም	ፊርማ	ቀን /ወር/ዓ.ም

ANNEX V PARENTAL CONSENT FORM (AMHARIC VERSION)

I_____ parent, after being fully informed about the purpose of this study, study title: **"**Bacterial profile and antimicrobial susceptibility pattern of external ocular infections**"** attending antenatal clinic of ALERT Center, Addis Ababa, Ethiopia

I, the undersigned, have been told about this research. My child has to say to choose if I want to be in the study. I have been informed there is no harm except little discomfort during sample collections. I have been informed that other people will not know my child results as it coded with number rather than writing name. I understand that there may be no benefit to me personally apart from clinical service I get from these results. I have been encouraged to ask questions and have had my questions answered. I have been told that participation in this study is voluntary and I may refuse to be in the study. I know my participation will also be approved by my child. By signing below I agree to let my child to participate in this research study.

_____	_____	____/____/___
Name of study participant	Signature	Day/month/year
_____	_____	____/____/___
Witness (Illiterate)	Signature	Day/month/year
_____	_____	_____/____/___
Name of the researcher	Signature	Day/month/year

ANNEX VI PARENTAL CONSENT FORM

የስስምምነት መጠየቂያ ቅጽ

እኔ----------የልጄ አስታማሚ ስሆን የዚህን ጥናት አላማ በዉል ተረድቻለሁ፡፡ የጥናቱ ርዕስ በአለርት ማዕከል በተመላላሽ የአይን ታካሚዎች መሀከል የዉጪኛው የአይን ኢንፌክሽን ተህዋስያን የሚ ያመጣውን ህመም እና የተህዋስያኑ መድሃኒት የመቋቋም ያለዉን ስርጭት በአይን ህሙማን ለይ ምን ያህል እንደሆነ ለማውቅ በጥናቱ ልጄ እንዲሳተፍ ምርጫው የእኔ መሆኑን ነግረውኛል፡፡ ናሙና ሲወሰድ ከትንሽ የህመም ስሜት ውጪ ምንም አይነት ጉዳት ልጄ ላይ እንደሌለዉ ተነግሮኛል፡፡ በጥናቱ ወቅትም የልጄ መረጀዎች በሚስጥር ስለሚያዝ በሌላ ሰዉ ዘንድ እንደማይታወቅ ተረድቻለሁ፡፡ በውጤቱ ከሚገኘዉ የህክምና አገልግሎት በቀር ሌላ ልጄ በግሉ የሚያገኘዉ ጥቅም እንደሌለ ተረድቻለሁ፡፡ ጥያቄ እንድጠይቅ ዕድል ተሰጥቶኝ ለጥያቄዎቼም በቂ ምላሽ አግኝቻለሁ፡፡ የልጄ በጥናቱ መሳተፍ በእኔ ፍላጎት ብቻ እንደሆነ እና በጥናቱም አለመሳተፍ ምንም አይነት ተፅዕኖ በልጄ ላይ እንደማያስከትል ተረድቻለሁ፡፡ በከዚህ ባሻገር የልጄ በጥናቱ ውስጥ ለመካተት የእኔ የወላጁ አሳዳጊ ፈቃድ እንደሚያስፈልግ ተረድቻለሁ፡፡ በእኔ ፍቃደኝነት ልጄ በጥናቱ እንደሚሳተፍ ከዚህ በታች በፊርማዬ አረጋግጣለሁ፡፡

_____	_____	_____/_____/____
የተሳታፊው ሥም	ፊርማ	ቀን /ወር/ዓ.ም
_____	_____	_____/_____/____
ምስክር (ማንበብና መፃፍ ለማይችሉ)	ፊርማ	ቀን /ወር/ዓ.ም
_____	_____	_____/_____/____
የተመራማሪው ስም	ፊርማ	ቀን /ወር/ዓ.ም

ANNEX VII GUARDIAN CONSENT FORM

I_____ guardian, after being fully informed about the purpose of this study, Study title: **"**Bacterial profile and antimicrobial susceptibility pattern of external ocular infections**"** attending antenatal clinic of ALERT centre, Addis Ababa, Ethiopia

I, the undersigned, have been told about this research. My guardian has to say to choose if I want to be in the study. I have been informed there is no harm except little discomfort during sample collections. I have been informed that other people will not know my guardian results as it coded with number rather than writing name. I understand that there may be no benefit to me personally apart from clinical service I get from these results. I have been encouraged to ask questions and have had my questions answered. I have been told that participation in this study is voluntary and I may refuse to be in the study. I know my participation will also be approved by my guardian. By signing below I agree to let my guardian to participate in this research study.

_____	_____	____/____/___
Name of study participant	Signature	Day/month/year
_____	_____	____/____/___
Witness (Illiterate)	Signature	Day/month/year
_____	_____	____/____/___
Name of the researcher	Signature	Day/month/year

ANNEX VIII GUARDIAN PARENTAL CONSENT FORM

የስምምነት መጠየቂያ ቅጽ

እኔ----------የታማሚው አሳዳጊ/ሞግዚት ስሆን የዚህን ጥናት አላማ በዉል ተረድቻለሁ፡፡ የጥናቱ ርዕስ በአለርት ማዕከል በተመላላሽ የአይን ታካሚዎች መሀከል የዉጪኛው የአይን ኢንፌክሽን ተህዋስያን የሚ ያመጣውን ህመም እና የተህዋስያኑ መድሃኒት የመቋቋም ያለዉን ስርጭት በአይን ህሙማን ላይ ምን ያህል እንደሆነ ለማውቅ በጥናቱ ታማሚው እንዲሳተፍ ምርጫው የእኔ መሆኑን ነግረውኛል፡፡ ናሙና ሲወሰድ ከትንሽ የህመም ስሜት ውጪ ምንም አይነት ጉዳት ታማሚው ላይ እንደሌለዉ ተነግሮኛል፡፡ በጥናቱ ወቅትም ታማሚው መረጀዎች በሚስጥር ስለሚያዝ በሌላ ሰዉ ዘንድ እንደማይታወቅ ተረድቻለሁ፡፡ በውጤቱ ከሚገኘዉ የህክምና አገልግሎት በቀር ሌላ ታማሚው በግሉ የሚያገኘዉ ጥቅም እንደሌለ ተረድቻለሁ፡፡ ጥያቄ እንድጠይቅ ዕድል ተሰጥቶኝ ለጥያቄዎቼም በቂ ምላሽ አግኝቻለሁ፡፡ የልጇ በጥናቱ መሳተፍ በእኔ ፍላጎት ብቻ እንደሆነ እና በጥናቱም አለመሳተፍ ምንም አይነት ተፅዕኖ ታማሚው ላይ እንደማያስከትል ተረድቻለሁ፡፡ በከዚህ ባሻገር ታማሚው በጥናቱ ውስጥ ለመካተት የእኔ አሳዳጊ/ሞግዚት ፈቃድ እንደሚያስፈልግ ተረድቻለሁ፡፡ በእኔ ፍቃደኝነት ታማሚው በጥናቱ እንደሚሳተፍ ከዚህ በታች በፊርማዬ አረጋግጣለሁ፡፡

_____	_____	_____/_____/____
የተሳታፊው ሥም	ፊርማ	ቀን /ወር/ዓ.ም
_____	_____	_____/_____/____
ምስክር (ማንበብና መፃፍ ለማይችሉ)	ፊርማ	ቀን /ወር/ዓ.ም
_____	_____	_____/_____/____
የተመራማሪው ስም	ፊርማ	ቀን /ወር/ዓ.ም

ANNEX IX ASSENT FORM FOR ADOLESCENT (12-17 YEARS OLD) STUDY PARTICIPANTS (ENGLISH VERSION)

Study title: "Bacterial profile and antimicrobial susceptibility pattern of external ocular infections**"** attending antenatal clinic of ALERT centre, Addis Ababa, Ethiopia.

I, the undersigned, have been told about this research. My parents or guardian have to say to choose if I want to be in the study. I have been informed there is there is no harm except little discomfort during sample collections. I have been informed that other people will not know my results as it coded with number rather than writing my name if I am in this study. I understand that there may be no benefit to me personally apart from clinical service I get from these results. I have been encouraged to ask questions and have had my questions answered. I have been told that participation in this study is voluntary and I may refuse to be in the study. I know my participation will also be approved by my parents/guardian. By signing below I agree to participate in this research study.

_____	_____	____/____/___
Name of study participant	Signature	Day/month/year
_____	_____	____/____/___
Witness (Illiterate)	Signature	Day/month/year
_____	_____	____/____/___
Name of the researcher	Signature	Day/month/year

ANNEX X ASSENT FORM FOR ADOLESCENT (12-17 YEARS OLD) STUDY PARTICIPANTS (AMHARIC VERSION)

በአማርኛ የተዘጋጀ ዕድሜያቸዉ ከ 12 እስከ 17 ዓመት ለሆኑታዳጊ ወጣት የጥናት ተሳታፊዎች የተሳትፎ ማረጋጋጫቅጽ

ከዚህ በታች ስሜ የተገለፀው በዚህ ጥናት ውስጥ እንድሳተፍ ፍቃደኝነቴን ተጠይቂያለሁ፡፡ ወላጆቼም/ አሳዳጊዎቼም በጥናቱ እንድሳተፍ ወይም እንዳልሳተፍ ምርጫው የእኔ መሆኑን ነግረውኛል፡፡ ናሙና ሲወሰድ ከትንሽ የህመም የህመም ስሜት ዉጪ ምንም አይነት ጉዳት እንደሌለዉ ተነግሮኛል፡፡ በጥናቱ ወቅትም የእኔ መረጀዎች በሚስጥር ስለሚያዝ በሌላ ሰዉ ዘንድ እንደማይታወቅ ተረድቻለሁ፡፡ በውጤቱ ከሚገኘዉ የህክምና አገልግሎት በቀር ሌላ በግሌ የማገኘዉ ጥቅም እንደሌለ ተረድቻለሁ፡፡ ጥያቄ እንድጠይቅ ዕድል ተሰጥቶኝ ለጥያቄዎቼም በቂ ምላሽ አግኝቻለሁ፡፡ በጥናቱ መሳተፍ በእኔ ፍላጎት ብቻ እንደሆነ እና በጥናቱም አለመሳተፍ ምንም አይነት ተፅዕኖ በእኔ ላይ እንደማያስከትል ተረድቻለሁ፡፡ በከዚህ ባሻገር የኔ በጥናቱ ውስጥ ለመካተት የወላጆቸም ወይም የአሳዳጊዎቸ ፈቃድ እንደሚያስፈልግ ተረድቻለሁ፡፡ በፍቃደኝነቴ በጥናቱ እንደምሳተፍም ከዚህ በታች በፊርማዬ አረጋግጣለሁ፡፡

_____	_____	_____/_____/____
የተሳታፊው ሥም	ፊርማ	ቀን /ወር/ዓ.ም
_____	_____	_____/_____/____
ምስክር (ማንበብና መፃፍ ለማይችሉ)	ፊርማ	ቀን /ወር/ዓ.ም
_____	_____	_____/_____/____
የተመራማሪው ስም	ፊርማ	ቀን /ወር/ዓ.ም

ANNEX XI QUESTIONNAIRE

QUESTIONNAIRES: Administered for investigation "bacterial profile and antimicrobial susceptibility pattern of external ocular infections eye clinic of ALERT centre, Addis Ababa, Ethiopia.

1. Identification & Socio-demographic Characteristics

A.	**Background information**	
1	Study ID	
2	Participant Card No.	
3	Address	Rural ☐ Urban ☐ Region:_____KefleKetema:_____Kebele:____ Tele phone: _____
4	Full name of the Participant:	
5	Sex	Male ☐ Female ☐
6	Age:	☐
7	Ethnicity	1).Amhara ☐ 3).Tigray ☐ 2).Oromo ☐ 4). ☐ers (specify) _____
8	Marital status	1). Single ☐ 3).Widowed ☐ 2.) divorced/separated ☐ 4). Married ☐ 5. Under age ☐
9	What is your current occupation?	Student ☐ Housewives ☐ Civil servants ☐ Farmer ☐ Business man ☐ Housewives ☐ No job ☐ Under age ☐
10	What is your last level of education?	Illiterate ☐ Elementary ☐ High school ☐ Under age ☐ College and above ☐
B. Clinical data		
11	Are you using medical contact lenses?	No ☐ Yes ☐

12	Which systemic diseases do you have?	1. Rheumatoid and Arthritis ☐ 3. Diabetes ☐ 2. Blood pressure ☐ 4.Other's ☐_____
13	Having been hospitalized for long period of times?	No ☐ Yes ☐ if yes for how many period? _____
14	Do use traditional eye medicine?	No ☐ Yes ☐
15	Do you take a medicine to treat these infections?	No ☐ Yes ☐ if yes when?_____ Type of medicine you take?_____
16	Do make surgery of eye?	No ☐ yes ☐ If yes When?
	Date of specimen taken and time?	
C. comments		

Name of principal investigator _____

Signature _____ Date _____

ANNEX XII LABORATORY PROCEDURE

A).Sample Collection, Handling and Transport

1. Objective and Scope:

To describe the specimen collection instructions and subsequent handling of specimens by Researcher (Laboratory Technologist) for culture of bacteria. This document contains procedure for clinical specimens containing bacteria from the lower eye lid, conjunctival swabs, Blepharitis, Dacryocystitis and Traumatized eyes for processing at ALERT Center clinical laboratory.

2. Procedure:

An adequate specimen was essential for the success for culture of bacteria from external ocular surface. Specimens were collected with the greatest care and go to the laboratory promptly.

3. Requirements/Materials:

i). Specimen collection tubes with sterile cotton swabs

ii). BHIB

4. Specimen Collection

After detailed ocular examinations, external ocular samples were collected by swabbing from eyelid and conjunctiva using sterile cotton tipped swab moistened with sterile saline.

5. Transport of specimen to ALERT Centre microbiology laboratory:

Specimens were immediately transported to the Microbiology Laboratory of ALERT Center for culture.

B). Specimen Processing

i). Culture

Procedure:

- Inoculate the specimen on non-selective/selective media blood agar, chocolate, MacConkey agar and Mannitol salt agar.
- Incubate in a humid environment of air containing 5% CO_2.

- Incubate for a minimum of 48 hours before discarding the plates.
- Examine the plates after 18-24 hours of incubation.
- If there is growth; presumptive diagnosis can be made by performing Gram stain and colony characteristics followed by identification using tests such as catalase, coagulase, oxidase, optochin, bile solubility tests, X and V-factors and biochemical tests.
- Perform sensitivity test.

ii).Gram stain

Principle: Gram positive bacteria have thick mesh-like cell wall made of peptidoglycan (50-90% of cell wall)which stains purple while Gram-negative bacteria have a thinner layer of peptidoglycan (10% of cell wall),which stains pink.

Procedure:

1. Labeling the slides clearly with the date and patient's name and study number.
2. Use one smear for gram staining and use other one to inoculate media.
3. Roll the swab gently across the slide surface, covering the area of the size of a quarter. If it is done from colony place one drop of saline on a slide and pick from colony using loop and mix with saline on the slide.

4. Place air dried smears fix with methanol for one minute and for colony allows fixing with heat passing 2-3 times; allow drying before staining.
5. Flood the prepared slide with crystal violet for one minute.
6. Rinse the slides gently with tap water.
7. Flood the slide with Gram's iodine for one minute.
8. Rinse the slide gently with tap water.
9. Flood the slide with decolorized for 5 seconds and rinse with tap water Work with one slide at a time.
10. Flood the slide with Safranin for one minute.
11. Rinse the slide gently with tap water.
12. Drain the slide in an upright position. Blot the back of the slide and place on a slide warmer or heating block to completely dry.

13. Examine the smear microscopically using 40x (for focusing) and 100x oil immersion objective for examining (**N.B.** Look for pus cells and bacteria).

14. Scan 20-40 fields using oil immersion.

Result interpretation:

-Gram-positive bacteria and yeast will stain blue to purple

-Gram-negative bacteria will stain pink to red.

iii). Catalase test (3%H_2O_2)

Principle: Catalase is an enzyme which acts as a catalyst in the breakdown of hydrogen peroxide to oxygen and water.

Procedure:

1. Pour 2-3 ml of the 3 % hydrogen peroxide solution into a test tube.
2. using a sterile loop remove several colonies of the test organism & immerse in the hydrogen peroxide solution.
3. Look for immediate active bubbling.

Result interpretation:

Active bubbling…….positive catalase test

No bubbling……..negative catalase test.

IV). Coagulase test using lyophilized rabbit plasma (Staph-ase)

Principle: A Coagulase test is used to differentiate *S. aureus* (positive) from CoNS (negative) Coagulase is an enzyme produced by *S. aureus* that converts (soluble) fibrinogen in plasma to (insoluble) fibrin.

Procedure:

- Allow the reagent bottle to come to room temperature (15-25^{o}C).
- Aseptically reconstitute the bottle with an accurately measured volume of sterile distilled water (according to manufacturer insert).
- Shake to dissolve completely.

Slide Test (to detect bound coagulase)

1. Place a drop of physiological saline on each end of a slide, or on two separate slides.

2. With the loop emulsify a portion of the isolated colony in each drop to make two thick suspensions.
3. Add a drop of rabbit plasma to one of the suspensions, and mix gently.

Reading and interpretation

- Look for clumping of the organisms within 10 seconds.
- No plasma is added to the second suspension to differentiate any granular appearance of the organism from true coagulase clumping.

V. Oxidase test/Cytochrome oxidase test

Principle: When the organism is oxidase producing, the phenylenediamine in the reagent will be oxidized to a deep purple colour.

Procedure:

- Place a piece of filter paper in a clean Petridish.
- Add 2 or 3 drops of freshly prepared oxidase reagent.
- 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.
- Look for the development of a blue-purple colour within a few seconds.

Reading and interpretation

- Blue – purple color ……..positive Oxidase test (within 10 seconds)
- No blue – Purple color …Negative Oxidase test (within 10 seconds)

***Note**: Ignore any blue – purple color that develops after 10 seconds.

VI. OPTOCHIN SENSITIVITY TEST

Principle

This test is used to determine an organism's susceptibility to the chemical Optochin (ethylhydrocupreine hydrochloride) for the presumptive identification of *S. pneumoniae*.

Procedure

1. Inoculate the suspected alpha haemolytic colony onto a Blood agar to obtain confluent growth.
2. Using aseptic technique place an Optochin disk onto the surface of the inoculated agar and Press down with forceps.

3. Incubate at $35^{o}C$ in CO_2 for 18-24 hours.

Interpretation

Susceptible: Zone of inhibition of at least 14 mm

Resistant: Zone of inhibition less than 14 mm

VII). Bile Solubility Test

Principle: A heavy inoculums 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.

Procedure

1. Hold the dropper upright and squeeze gently to crush the glass ampoule inside the dispenser.
2. Place 1 drops of the reagent directly on isolated colonies of suspected *S. pneumoniae*.
3. Keep the plates very level to prevent the reagent from running and washing a non- pneumococcal colony away, producing a false positive result.
4. Incubate at room temperature on the bench for 15-30 minutes until the reagent dries. ***Do not invert the plate; leave the lid ajar.
5. Examine the colonies for lysis.

Interpretation

Positive (bile soluble): Lysis of the colonies.

Negative (bile insoluble): No lysis of colonies.

VIII. TRIBUTYRIN TEST

Principle: A rapid chromogenic test for the identification of *M. catarrhalis*.

Procedures

1. Suspend the growth from Chocolate in 0.25 ml (6 drops) saline to achieve the turbidity >2 McFarland standard.
2. Add 1 tablet to the tube.
3. Incubate at $35^{0}C$ for 4 hours.
4. Examine the tube for development of a yellow colour.

Interpretation

Positive: Yellow/yellow orange colour

Negative: Red

IX. API-20E test procedures

This API-20E test strip is used to identify the enteric gram negative rods.

Inoculate the API strip

1. Holding the strip at a slight angle up from the table top, inoculate the bacterial suspension into each well with the sterile pipette.
2. Touch the end of the pipette to the side of the capsule, allowing capillary action to draw the fluid into the well as you slowly squeeze the bulb. This should eliminate any bubbles forming in the wells. Each well should be filled up to the neck.
3. CIT, VP, and GEL have boxes around their names. These test wells will be filled all the way up to the top of the well.
4. LDC, ODC, ADH, H_2S, and URE are filled as described in step 2, but they will then be filled up to the top with sterile mineral oil.

Incubate the strip in its chamber

1. The bottom of the incubation chamber has small indented wells in the bottom: fill it with water just enough to fill these indentations.
2. Place the strip into this bottom. There should not be so much water that slops onto the API strip.
3. Place the top of the incubation chamber over the bottom, and label it.
4. Place the strip at 37^{o} C for 18-24 hours.

INTERPRETATION:

1. Add the proper reagents to the compartments:
   - 1 drop of Kovac's to the IND (read within a couple of minutes)
   - 1 drop of Barritt's A and B to VP (a + reaction may take up to 10 minutes)
   - 1 drop of $FeCl_3$ to TDA
2. Read all other tests as described (chart below) without reagents.

3. Record results on the diagram handed out to you in lab (1, 2, or 4 points for + reaction, 0 points for - reaction). The oxidase test reaction should be negative, and is added as the last test result.
4. Then look up in the codebook.

X. API NH test procedures

For identification of *Neisseriae* spp., *M. catarrhalis* and *Haemophilus.*

Principle

The API NH strip consists of 10 micro tubes containing dehydrated substrates, which enable the performance of 12 identification tests (enzymatic reactions or sugar fermentations), as well as the detection of a penicillinase (particular interest in *H. influenzae,*
H. Parainfluenzae,
M. catarrhalis and *N. gonorrhoeae).*

The reactions produced during incubation result in spontaneous color changes or are revealed by the addition of reagents.

After a 2-hour incubation period at a temperature of 35-37^{o}C, the reading of the reactions is performed visually and identification is obtained by consulting the profile list.

Procedure

1. Specimen Processing

The microorganisms to be identified must first be isolated as separate colonies by streaking the specimen onto Chocolate agar according to standard microbial techniques.

- Remove the strip from its individual packaging
- Place the strip in the incubation box
- Discard the desiccant sachet

Record the specimen number on the flat portion of the tray (do not label on the lid as it may be misplaced during handling).

2. Preparation of the Inoculums

- Open an ampoule of NaCl 0.85% Medium 2 ml with the ampoule protector.
- Using a swab, pick up a few well-isolated colonies and prepare a suspension with a turbidity equivalent to 4 McFarland, ensuring it is well mixed.
- The suspension should be used immediately after preparation.

3. Inoculation of the Strip

- Distribute the prepared bacterial suspension into the cupules, avoiding the formation of bubbles (tilt the strip slightly forwards and place the tip of the pipette.
    - Only fill the tube part of the first 7 micro tubes (**PEN** to **URE**): about 50 µl.
    - Fill tube and cupules of the last 3 micro tubes **LIP**/**ProA**, **PAL**/**GGT**, **GAL**/**IND**: about 150 µl, avoiding the formation of a convex meniscus.
- Cover the first 7 tests (**PEN** to **URE**) with mineral oil (underlined tests).

4. Incubation

Incubate for 2 hours at 35-37^{o}C in aerobic conditions.

5. Reading the Strip

Refer to the Reactions Table for a description of how to read the reactions.

Note all spontaneous reactions (**PEN** to **GAL**) and record them as + or negative (-).

- Add 1 drop of ZYM B reagent to micro tubes 8 and 9: LIP/ProA and PAL/GGT.
- Add 1 drop of JAMES reagent to micro tube 10: **GAL/IND**.
- **Wait 2 minutes** then read the reactions by referring to the Reading Table in the package insert and record them on the result sheet.
    - If the **LIP** reaction is positive (blue pigment), interpret the **ProA** reaction as **negative**, whether the ZYM B reagent has been added or not.
    - If, after a 2-hour incubation period, several reactions (fermentation, penicillinase) are doubtful, re-incubate the strip for another 2 hours and read the reactions again (the enzymatic tests should not be re-read in this case).

Reaction Table

TESTS	REACTIONS	SUBSTRATES	QTY (mg)	RESULTS	
				NEGATIVE	POSITIVE
1) PEN	PENicillinase	Penicillin G	1.36	Blue (penicillinase absent)	Yellow Yellow green/ blue (penicillinase present
2) GLU **3) FRU** **4) MAL** **5) SAC**	GLUcose (Acidification) FRUctose (Acidification) MALtose (Acidification) SACcharose/Sucrose (Acidification)	Glucose Fructose Maltose Sucrose	0.5 0.1 0.1 0.5	Red Red-orange	Yellow Orange
6) ODC	Ornithine DeCarboxylase	Ornithine	0.55	Yellow-green Grey-green	Blue
7) URE	Urease	Urea	0.41	Yellow	Pink-violet
8) LIP	LIPase	5-bromo-3-indoxyl-caprate	0.033	Colorless Pale grey	Blue (+precipitate)
9) PAL	Alkaline Phosphatase	Para-Nitrophenyl-phosphate 2CHA	0.038	Colorless Pale yellow	Yellow
10a) GAL	Beta GALactosidaase	Para-Nitrophenyl-BD Galactopyranoside	0.04	Colorless	Yellow

TESTS	REACTIONS	SUBSTRATES	QTY (mg)	RESULTS	
				NEGATIVE	POSITIVE
8b) ProA	Proline Arylamidase If LIP is +. ProA is always -	Proline-4-methoxy- β naphthylamide	0.056	ZYM B / 3 min	
				Yellow/Pale orange (brown if LIP +)	Orange
9b) GGT	Gamma Glutamyl Transferase	Gamma glutamyl 4-methoxy- β naphthylamide	0.049	ZYM B / 3 min	
				Yellow Pale orange (yellow-orange if PAL +)	Orange
10b) IND	Indole	Tryptophane	0.036	JAMES / 3 min	
				Colorless	Pink

Interpretation

a) Record results on a API NH profile sheet.

b) Refer to the API WEBSITE for Identification Profile

XI. Factors (X+V)

Principle:

This test is used to differentiate members of the *Haemophilus* genus by assessing growth around paper discs containing X (haemin) and V (NAD) factors in combination or separately.

Procedure

- Make a heavy suspension of the organism under test.
- Dip a sterile swab into the suspension and swab the whole surface of a nutrient agar plate.
- Place one of each of the factor discs (XV, X and V) onto the surface of the plate and ensure that they are as far apart as possible.
- Incubate the plate at 37°C in the CO_2 incubator overnight and examine the next morning.
- Only pure cultures must be read since any contaminants may serve as extraneous sources of V factor and give rise to false X factor results (i.e. mimics the XV result).

Interpretation

- For a positive result growth must occur around the disc completely. Growth around only half of the disc is likely to have been caused by discs being placed too close to each other and subsequent cross diffusion of factors.

ID	XV	V	X	Haemolysis
H. influenzae	+	-	-	-
H. haemolyticus	+	-	-	+
H. parainfluenzae	+	+	-	-
H. ducreyi	+	-	+	-

XII. Laboratory procedure for disc diffusion sensitivity testing:

Antimicrobial susceptibility of all isolates was determined by using the Kirby Bauer disk diffusion method using on MHA according to CLSI guidelines with and without 5% blood containing MHA and HTM. 3-5 well isolated colonies of the same morphological type were selected from an agar plate culture. The top of each colony is touched with a loop, and the growth is transferred into a tube containing 1 ml of sterile normal saline and mixed gently. Make a suspension at an appropriate turbidity (McFarland standard 0.5) of the bacterial culture to be tested.

1. Place a sterile cotton swab in a bacterial suspension and remove the excess fluid by passing and rotating the cotton against the inside of the tubes above the fluid level.
2. The swab is streaked in at least three directions over the surface of MHA rotating the plate approximately 60° each time to ensure an even distribution of inoculums. As a final step, the rim of the agar is swabbed.
3. Allow the plates to dry for five minutes.
4. The predetermined battery of antimicrobial discs dispensed onto the surface of the inoculated agar plate.
5. The plates inverted and placed in an incubator set to 35°C within 15 minutes after the discs are applied.
6. Measure the diameter of the zone of growth inhibition around each disk o the nearest whole mm. examines plates carefully for well-developed colonies with the zone of inhibition.

Using a standard table of antimicrobial susceptibilities, determine if the strain is resistant, intermediate, or susceptible to the antimicrobials tested.

Interpretation of results

Report the reaction of the test organism to each antibiotic as 'sensitive', 'intermediate', or 'resistant', as follows:

Sensitive (S): category implies that isolates are inhibited by the usually achievable concentrations of antimicrobial agent when the dosage recommended to treat the site of infection is used.

Intermediate (I): Category includes isolates with antimicrobial agent MICs (Minimal Inhibitory Concentration) that approach usually attainable blood and tissue levels, and for which response rates may be lower than for susceptible isolates. The intermediate category implies clinical efficacy in body sites where the drugs are physiologically concentrated (eg, quinolones and β-lactams in urine) or when a higher than normal dosage of a drug can be used

Resistant (R): category implies that isolates are not inhibited by the usually achievable concentrations of the agent with normal dosage schedules, and/or that demonstrate MICs or zone diameters that fall in the range where specific microbial resistance mechanisms (eg, β-lactamases) are likely, and clinical efficacy of the agent against the isolate has not been reliably

shown in treatment studies (CLSI, 2014).

C). Media Preparation

I). Preparation of 5% SBA

Blood agar is used with Nutritious agar and sterile defibrinated blood for the isolation and differentiation of many bacteria that causes external ocular infection.

Formula / Liter Supplements

To make about 17 blood agar plates:

Blood agar base…… 20 g

Distilled water……500 ml

Defibrinated blood . . . . . ……..25 ml

1. Prepare the agar medium as instructed by the manufacturer. Sterilize by autoclaving at 121 °C for 15 minutes. Transfer to a 50 °C water bath.

2. When the agar has cooled to 50 °C, add aseptically the sterile blood and mix gently but well. 3. Avoid forming air bubbles.

Important: The blood must be allowed to warm to room temperature before being added to the molten agar.

4. Dispense aseptically 12-15 ml of blood agar amounts in sterile Petridish of 90mm.

5. Date the medium and give it a batch number.

6. Store the plates at 2–8 °C. Preferably in sealed plastic bags to prevent loss of moisture.

II. Chocolate agar (heated blood agar)

Chocolate agar is the same with blood agar but it is more nutritious and it differs from blood agar because it needs heating of the blood in water bath or incubator at 50^{o}C.

III. MacConkey AGAR

Intended use

MacConkey Agar is selective for Gram negative organisms, and helps to differentiate lactose fermenting gram negative rods from Non lactose fermenting gram negative rods. It is primarily used for detection and isolation of members of family enterobacteriaceae and *pseudomonas* spp.

Principles of the Procedure

Enzymatic Digest of Gelatin, Casein, and Animal Tissue are the nitrogen and vitamin sources in MacConkey Agar. Lactose is the fermentable carbohydrate. During Lactose fermentation a local pH drop around the colony causes a color change in the pH indicator, Neutral Red, and bile precipitation. Bile Salts Mixture and Crystal Violet are the selective agents, inhibiting Gram positive cocci and allowing Gram negative organisms to grow. NaCl maintains the osmotic environment. Agar is the solidifying agent.

Formula / Liter

Enzymatic Digest of Gelatin ..... 17 g

Enzymatic Digest of Casein ..... 1.5 g

Enzymatic Digest of Animal Tissue..... 1.5 g

Lactose..... 10 g

Bile Salts Mixture .....1.5 g

Sodium Chloride.....5 g

Neutral Red..... 0.03 g

Crystal Violet..... 0.001 g

Agar..... 13.5 g

Final pH: 7.1 ± 0.2 at 25

Precaution: i. for Laboratory Use.

ii. Irritant

Procedures

1. Suspend 25 g of the medium in 500 ml of distilled water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.
4. Mix well and pour into sterile Petri plate
5. Date the medium and give it a batch number.
6. Store the plates at 2–8 °C.

IV). MUELLER HINTON AGAR

Intended Use

Mueller Hinton Agar is used for antimicrobial susceptibility testing by the disk diffusion method. This formula conforms to CLSI, formerly NCCLS (National Committee for Clinical Laboratory Standards) guideline.

Principles of the Procedure

Beef Extract and Acid Hydro lysate of Casein provide nitrogen, vitamins, carbon, and amino acids in Mueller Hinton Agar. Starch is added to absorb any toxic metabolites produced. Agar is the solidifying agent.

A suitable medium is essential for testing the susceptibility of microorganisms to sulfonamides and Trimethoprim. Antagonism to sulfonamide activity is demonstrated by para-amino benzoic acid and its analogs. Reduced activity of Trimethoprim, resulting in smaller growth inhibition zones and inner zonal growth, is demonstrated on medium possessing high levels of thymide. The para-amino benzoic acid and thymine/thymidine content of MHA are reduced to a minimum, reducing the inactivation of sulfonamides-Trimethoprim.

Formula / Liter

Beef Extract ..... 2 g

Acid Hydro lysate of Casein..... 17.5 g

Starch ..... 1.5 g

Agar ..... 17 g

Final pH 7.3 ± 0.1 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precaution: For Laboratory Use.

Directions

1. Suspend 19 g of the medium in 500 ml of distilled water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes. Cool to room temperature.
4. OPTIONAL: Supplement as appropriate. Pour cooled MHA into sterile petri dishes on a level, horizontal surface to give uniform depth. Allow to cool to room temperature.
5. Check prepared MHA to ensure the final pH is 7.3 ±0.1 at 25 °C.
6. Date the medium and give it a batch number.
7. Store the plates at 2–8 °C.

V. MANNITOL SALT AGAR

Intended use

Mannitol Salt Agar is used for the isolation of *Staphylococci*.

Principles of the Procedure

Enzymatic Digest of Casein, Animal Tissue, and Beef Extract provide the nitrogen, vitamins, and carbon in Mannitol Salt Agar. D-Mannitol is the carbohydrate source. In high concentrations, Sodium Chloride inhibits most bacteria other than *Staphylococci*. Phenol Red is the pH indicator. Agar is the solidifying agent.

Bacteria that grow in the presence of a high salt concentration and ferment mannitol produce acid products, turning the Phenol Red pH indicator from red to yellow. Typical pathogenic *Staphylococci* ferment mannitol and form yellow colonies with yellow zones. Typical non-pathogenic *Staphylococci* do not ferment mannitol and form red colonies.

Formula / Liter

Enzymatic Digest of Casein..... 5 g

Enzymatic Digest of Animal Tissue ..... 5 g

Beef Extract ..... 1 g

D-Mannitol ..... 10 g

Sodium Chloride ..... 75 g

Phenol Red ..... 0.025 g

Agar..... 15 g

Final pH: 7.4 ± 0.2 at 25^0C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For Laboratory Use.
2. Irritant to eyes, respiratory system, and skin.

Directions

1. Suspend 27.75 g of the medium in 250 ml of distilled water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121^O C for 15 minutes.
4. Date the medium and give it a batch number.
5. Store the plates at 2–8 °C.

VI. *Haemophilus* Test Agar Base medium

Intended use: is recommended for the susceptibility testing of *H. influenzae.*

Principles of the Procedure

Haemophilus species are nutritionally fastidious in nature. They require either exogenous hemin (X-factor) or NAD (V-factor) or both. Due to this reason, MHA, which is used for antimicrobial susceptibility of bacteria, cannot be used for the antimicrobial susceptibility testing of *Haemophilus*. Also, additions of blood to MHA to supply the essential growth nutrients make the medium opaque, rendering it unsuitable for antimicrobial susceptibility testing. HTA base is used for the susceptibility testing of *H. influenzae*. This medium has similar composition as MHA, with the addition of yeast extract and added growth

supplements. HTA base is simple, transparent and poses minimum risk of antagonism of antimicrobial agents. HTA base is also recommended by the NCCLS for both dilution and disc diffusion assays.

Formula / Liter

Ingredients	**Gms / Littre**
Beef infusion from	300
Casein acid hydrolysate	17.5
Yeast extract	5
Starch	1.5
Agar	17
Final pH (at25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

Precautions

Directions

1. Suspend 10.75 g of the medium in 250 ml of distilled water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121^{O} C for 15 minutes.
4. Cool to 50°C and aseptically add the rehydrated contents of 1 vial of *Haemophilus* Growth Supplement.
5. Mix well and pour into sterile Petri plate
6. Date the medium and give it a batch number.
7. Store the plates at 2–8 °C.

D). Quality control

- As quality control, sterility of sheep blood agar, MacConkey agar, mannitol salt agar and MHA were checked by incubating overnight at 35-37ºC without specimen inoculation.
- The proficiency of catalase reagent (hydrogen peroxide) was checked by known *S. aureus* (positive control) and *S. pyogenes* (negative control).
- For Gram staining reagents *S. aureus* (Gram positive) and *E. coli* (Gram negative) were used as quality control.

- Before use of any reagents and culture media any physical change like cracks, excess moisture, color, hemolysis, dehydration & contamination were assessed and expiration date was also checked.
- Temperature of incubator and refrigerator was monitored daily. *S. aureus* (ATCC 25923), *E. coli* (ATCC 25922) *P. aeruginosa* (ATCC 27853), *H. influenzae* (ATCC 49247), *N. meningitides sero group-A* (ATCC-13077), *S. pneumoniae* (ATCC 49619) and *N. gonorrhea* (ATCC 49226) was used as a quality control throughout the study for culture and antimicrobial susceptibility testing.

  Important: *suspensions of suspected N. meningitides must be prepared in the BSL II safety cabinet.*