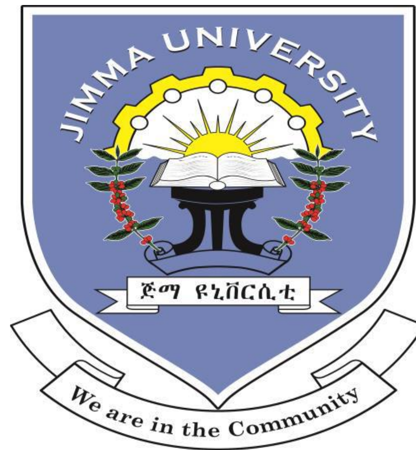
ANTIMICROBIAL SUSCEPTIBILITY PATTERN AND BIOFILM FORMING POTENTIAL OF BACTERIA ISOLATED FROM SUSPECTED EXTERNAL OCULAR INFECTED PATIENTS ATTENDING JIMMA UNIVERSITY MEDICAL CENTER EYE CLINIC, SOUTH WEST ETHIOPIA



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Antimicrobial Susceptibility Pattern and Biofilm Forming Potential of Bacteria Isolated from Suspected External Ocular Infected Patients Attending Jimma University Medical Center Eye Clinic, Southwest Ethiopia

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ABSTRACT

Background: Ocular disease and its complications are a significant health problem worldwide with particular significance to developing countries that impacts greatly on quality of life. Ocular infections due to bacteria can cause damage to structure of the eye, which can leads to reduced vision and blindness. The emergence of antimicrobial resistance and development of bacterial biofilm in ocular infection is currently increasing the risk of treatment failure with potentially serious consequences.

Objective: The study was aimed to assess the antimicrobial susceptibility pattern and biofilm forming potential of bacteria isolated from suspected external ocular infected patients at Jimma University Medical Center, Jimma, Ethiopia.

Method: A cross sectional study was conducted on 319 suspect patients with external ocular infections from March 2017 to June 2017 at Jimma University Medical Center, department of ophthalmology. External ocular specimens were collected using sterile swabs after patients were examined by ophthalmologists. The specimens were shipped in Amiens transport media to Microbiology Laboratory for isolation and identification. Samples were inoculated onto Blood agar, Chocolate agar, MacConkey agar and Mannitol salt agar. Presumptive isolates of gram positive and gram negative bacteria were further identified by a series of biochemical tests. The antimicrobial susceptibility pattern of the isolates was determined by disk diffusion method according to CLSI 2015. Microtiter (96 wells) plate method was used to screen the biofilm formation by measuring optical density at 570nm using ELISA reader.

Result: Out of 319 study participants with external ocular infection, prevalence of bacterial pathogens was 46.1%. The predominant bacterial isolate were Coagulase negative staphylococcus (CoNS) (27.7%) followed by *Staphylococcus aureus* (19.7%). Among gram negative, *Pseudomonas aeroginosa* (6.8%) was the leading isolate. Increased antimicrobial resistance was observed in tetracycline (64%), erthromycin (66.7%) and penicillin (77.1%). Amoxicillin-clavulanic acid, ciprofloxacin and gentamicin were the most effective drugs for both gram negative and gram positive ranging from 69-100%. About 13.8% of *S. aureus* was methicillin resistant (MRSA). Multidrug resistance accounted for 68.7%. The overall biofilm formation rate of isolates was 66.1% with *P. aeroginosa* (40%), CoNS (34.1%) and *S. aureus* (31%) formed strong biofilm.

Conclusion: The prevalence of bacterial isolates among external ocular infection was high. Almost all bacterial isolate were resistant to atleast one or more drugs. Multidrug resistance pathogens were observed increasingly biofilm formers. Therefore, antimicrobial susceptibility testing should be practiced to guide treatment of patients and to control the emergence of drug resistant bacteria.

Key word: *External ocular infections, Bacterial isolate, Biofilm formation, Microtiter plate assay, Drug susceptibility pattern.*

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LIST OF ABBREVIATIONS

AST	Antimicrobial Susceptibility Testing
ATCC	American Type Culture Collection
BF	Biofilm formation
BHIB	Brain heart infusion broth
BSc	Bachelor of Science
CLSI	Clinical Laboratory Standard Institute
CoNS	Coagulase-negative staphylococci
ELISA	Enzyme Linked Immuno Sorbent Assay
H_2O_2	Hydrogen Peroxide
H_2S	Hydrogen Sulfide
JUMC	Jimma University Medical Center
JURERC	Jimma University Research and Ethical Review Committee
KIA	Kligler iron agar
MDR	Multiple drug resistance
MHA	Muller Hinton Agar
MIC	Minimal Inhibitory Concentration
MRSA	Methicillin resistance *Staphylococcus aureus*
MRCoNS	Methicillin resistance coagulase negative staphylococcus
MSA	Manitol salt agar
MSc	Master of Science
NCCLS	National Committee for Clinical Laboratory Standards
NT	Not tested
OD	Optical density
PhD	Doctor of Philosophy
SOP	Standard operating procedure
SPSS	Statistical Package for Social Science
TSB	Trypton soya broth
WHO	World Health Organization

1. INTRODUCTION

1.1 Background

The human eye is a unique and necessary organ that is constantly exposed to the external environment. It is the window of our body. Ocular infections are common and vary from self-limiting to sight-threatening conditions. Ocular infection can cause damage to structure of the eye, which can lead to reduced vision and even blindness with devastating consequences if not diagnosed and treated properly. Pathogenic micro-organisms cause ocular disease and the most frequently affected parts of the eye are the conjunctiva, eyelid and cornea [1]. Conjunctivitis is the most common cause of "red eye". Blepharitis is an inflammation of the eyelid margins which can decline in visual function. Dacryocystitis is an inflammation of the lacrimal sac and duct [2]. The cause of ocular infections can be bacteria, fungi, viruses and parasites [3].

The external ocular surface acquires a microbial flora at birth and some of the commensal flora may become resident in the conjunctiva and eyelids with a potential to become pathogenic. Microbial aetiologies around the eye can form transient flora or invade the tissue and cause infection. Eventhough the eye protected by number of natural defence mechanism, it suffers from infections caused by adapted microorganisms. Pathogenic microorganisms cause ocular disease due to virulence factor acquisition and/or host's reduced resistance [4]. Among microbial aetiologies, bacteria are major causative agents that frequently cause infections at ocular surface or invasion of the blood-eye barrier and possible loss of vision [5].

The spectrum of organisms causing ocular infection varies around the world. Bacterial agents known to cause external ocular infections include *Staphylococcus aureus*, Coagulase negative staphylococcus (CoNS), *Streptococcus pneumoniae, Pseudomonas aeruginosa,* and even *Niesseriae. meningitidis*, have been reported as greater virulence [6]. In infants and children, the most common ocular pathogens are *Staphylococcus aureus, Haemophilus influenzae, Streptococcus pneumoniae*, and also *Moraxella species.* 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 [3, 7].

The most common ocular infection seen by primary care physicians worldwide is bacterial conjunctivitis, which is usuaaly self-limiting and largely `presents as an acute infection approximately in 78 to 80% of cases being bacterial in origin [8]. Majority of reviews [5, 9] are related to acute conjunctivitis in which, *S. aureus, S. pneumoniae* and *H. influenzae* are the most common implicated pathogens. CoNS and *S. aureus* are most frequently isolated in chronic conjunctivitis, with a tendency for an increased antibiotic resistance in recent years. Gram positive pathogens are responsible for 60 to 80% of acute infections [10].

External ocular infections are usually treated on empirical basis with topical broad spectrum antibacterial drugs. Eventhough those broad spectrum antimicrobial drugs are available for ocular management, the development of bacterial resistance to specific antibiotics are becoming an important consideration for clinicians treating ocular infections. This may be due to indiscriminate use of antibiotics, which results from patients' incompliance to recommended treatment and demand, wrong prescription and guidelines from unskilled health practitioners, irrational use of antibiotics in human, poor quality antibiotics, inadequate surveillance and susceptibility testing which takes time and expensive and also when the organism once forms biofilm [11].

Biofilm is the assemblage of microbial cells that are irreversibly associated with biotic and abiotic surfaces and is usually enclosed in the self-secreted extracellular polymeric substances. Biofilm formation is a well-known pathogenic mechanism, where bacteria are successful at colonization and persistence over their free living planktonic counterparts because of active cell division and recruitment of secondary invader pathogens. They are more tolerant to commonly used antimicrobial agents [12]. Bacterial biofilms are increasingly being recognized as significant enhanced virulance underlying the development of certain chronic infections when some medical equipment implanted in human eye and play great role in bacterial resistance [13].

1.2 Statement of the problem

Ocular disease and its complications are a significant health problem worldwide that impacts greatly on quality of life. For 2010, WHO estimates that 285 million people are visually impaired, of whom 39 million were blind worldwide and that 90% of these individuals live in low-resource countries. The estimated prevalence of blindness ranged from 0.08% of children to 4.4% of persons aged over 60 years. It was also estimated that the number of blind people worldwide was increasing by 1–2 million per year [14, 15]. In the case of sub-Saharan Africa, an estimated 26 million individuals live with visual impairment, of which 5.9 million individuals are classified blind facing many problem [2, 16]. The cost of treating bacterial conjunctivitis alone was estimated to be around 377 million USD to 857 million USD per year in USA [17].

In Ethiopia the prevalence of blindness was reported about 1.6% and that of low vision is 3.7% [18]. It was estimated that 80% of the cases were as a result of avoidable causes of a variety of factors like personal hygiene, living conditions, socio-economic status, decrease immune status, etc. that determine the clinical outcome in microbial causes of eye infections. Visual impairment has profound human and socioeconomic consequences in the whole societies and brings a significant economic burden for the individual, the family and society. Bacterium is major causative agents that frequently cause infections in eye and possible loss of vision. Hence, there is a need for an immediate investigation and treatment for the serious bacterial eye infection that threatens the cornea of eye [18, 19].

The management of bacterial eye infections may involve treatment with broad spectrum antibiotics; however, microbial resistance to antimicrobial agents is emerging and increasing worldwide [20]. Inappropriate and irrational use of antimicrobial medicines provides favorable conditions for resistant microorganisms to emerge, spread and persist. The past two decades have witnessed changes in antibiotic susceptibility patterns in all systemic infections [21].

The development of bacterial biofilms is presently recognized as one of the most relevant drivers of persistent infections, and constitutes a serious challenge for clinical microbiologists and clinicians being 100-1000 fold more resistant to antimicrobial agents than normal(planktonic) cells [13]. Phenotypic and physiological change in biofilm, restricted penetration of antibiotic

into biofilms and expression of resistance genes were some of the factor that provides a higher resistance to antimicrobial treatment because antimicrobials have been developed against planktonically grown bacteria [22].

For specific antibacterial treatment, isolation and identification of bacterial pathogens along with antibiotic susceptibility spectrum is essential [23]. In most developing countries like Ethiopia, it is a common practice that antibiotics is purchased without prescription order, which led to underuse, overuse and misuse of antibiotics [12]. This may contribute to the emergence and spread of antimicrobial resistance in addition to biofilm formation. Moreover, poor sanitary and infection management (control practice) in the area may play a serious role in an increased prevalence of resistant bacteria in a community. This developing resistance increases the risk of treatment failure with potentially serious consequences [24, 25].

Even though study conducted in 2012 in Jimma area, the bacterial aetiology and sensitivity to different categories of antimicrobial agents varies from time to time and from place to place as indicated in different reviews [7, 26]. Therefore, the changing spectrum of microorganisms involved in ocular infections and the emergence of acquired microbial resistance needs continuous surveillance to guide empirical therapy. Thus, the updated knowledge of the aetiologic agents causing these ocular infections and their susceptibility is crucial in proper management of the cases. Moreover, bacterial biofilm development which is one of the important features of bacteria in external ocular infections was not addressed in previous works [5].

Hence the present study is designed to update profile of bacteria present in external ocular infection, their antibiotics susceptibility pattern along with biofilm forming potential of the bacterial isolates at Jimma University Medical Center, Southwest Ethiopia.

1.3 Significance of the study

To be able to diagnose and manage properly, Ophthalmologists have to be aware of the types of micro-organisms prevalent in the local community and their susceptibility against different antibiotics. Sensitivity of bacterial aetiology to antimicrobial agents may differ from place to place and from time to time. So, the current study was undertaken to fill this gap.

This research provides up-to date status on the local aetiologic agents and their antimicrobial susceptibility pattern in external eye infection and their biofilm forming capability was also assessed. This supports clinicians by aiding in the selection of proper and effective drugs for external ocular infection.

Targeted treatment allows for a shorter time to clinical and microbiological resolution which may decrease the mild morbidity, decrease health care costs of visits and potential complications, return patients back to school or the work force, and limit the potential spread of this communicable infection among susceptible population.

In addition to generating data on the distribution of bacterial isolates from external ocular specimen and their antimicrobial susceptibility pattern, related risk factors can give appropriate information for policy makers. The data that can be obtained from this study can be part of the solution for current program or components for future studies.

Moreover, it helps to revise or develop guidelines for empirical therapy based on evidence based information that guide the clinician in the initial treatment of patients with external ocular infection

2. LITERATURE REVIEW

2.1 Review on bacteria from ocular infections and drug susceptibility pattern

Bacteria become one of the most widespread causes of ocular infection worldwide. The prevalence and isolation rate of bacteria in samples collected from eye and drug susceptibility pattern have been studied in different part of the world [27-30]. Frequently reported clinical manifestations include conjunctivitis, scleritis, keratitis, blepharitis and dacryocystitis [21].

Conjunctivitis has worldwide distribution, affecting persons of all ages, races, social strata, and both genders. 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%). On the otherhand, the most common gram negative bacteria found in acute conjunctivitis was *H. influenzae* [31]. The more recent antibiotics used in Europe were aminoglycosides (gentamicin, tobramycin, amikacin, netilmicin). Gentamicin and tobramycin are active against most Staphylococci, Proteus and enterobacteriaceae, but resistant strains are now reported. Penicillin, cephalosporin (second generation), fluoroquinolones and macrolides also commonly used in ophthalmology [31].

Staphylococci have a special relationship with the eye. They may be present in the lid margins or conjunctiva as normal flora without causing disease or they may cause severe eye infections which may result in irreversible blindness. CoNS commonly isolated mixed with more typical ocular flora leads to major infections including keratitis, conjunctivitis and endophthalmitis. Endophthalmitis caused by bacteria is the most severe form of vision threatening ocular infection. CoNS are the most common cause of postoperative endophthalmitis worldwiden [32]. Study conducted in USA [33] reported 49.9% of postoperative endophthalmitis is caused by CoNS. Another study conducted in Singapore [34] and India [35] showed high prevalence of CoNS (57% and 62.6% respectively) in postoperative endophthalmitis.

A retrospective study was conducted in New Zealand between January 2013 and December 2014 and reported bacterial keratitis where staphylococcus was identified to be the most common isolate (38.2%), followed by *P. aeroginosa* (21.3%). Aminoglycosides, cefazolin, ceftazidime,

erythromycin, tetracycline, and doxycycline were 100% effective against tested isolates *in vitro*. Amoxicillin (41.6%), cefuroxime (33.3%), and chloramphenicol (94.7%) showed reduced efficacy against Gram-negative bacteria, whereas penicillin (51%) and ciprofloxacin (98.8%) showed reduced efficacy against Gram-positive bacteria [36].

A study conducted in China between 1 January 2005 and 31 December 2010 reported on bacterial keratitis for culture results and *in vitro* antibiotic susceptibility. Eighty consecutive cases of paediatric bacterial keratitis cases were included, among which 59 (73.75%) were identified as having positive culture. *Staphylococcus epidermidis* was the most commonly isolated organism (39%), followed by *Streptococcus pneumoniae* (18.6%) and *Pseudomonas aeruginosa* (10.2%). Antibiotic sensitivities revealed that bacteria had low resistance rates to fluoroquinolones and aminoglycosides (18.4% and 24.4%) respectively [30].

A retrospective study conducted in India from 2005-2012 report that corneal ulceration due to bacterial isolates accounted for 1205 (55.5%) out of the 2170 ulcers. The most common bacterial pathogens isolated were various species of Staphylococcus, representing (64.5%), followed by *Streptococcus- species* (12.3%) and *P. aeruginosa* (9.7%). High percentages of Gram-positive bacteria were susceptible to gatifloxacin (>94%), followed by ofloxacin and moxifloxacin. Almost 90% of *P. aeruginosa* isolates were susceptible to ciprofloxacin and moxifloxacin [37]. In another study conducted in India the rate of culture-positivity was found to be 88% in eyelids' infection, 70% in conjunctival and 69% in lacrimal apparatus. The most common bacterial species isolated were *S. aureus* (26.69%) followed by *S. pneumoniae* (22.14%) [38].

A study conducted in India showed, the predominant bacterial isolate was *S. aureus* (27.4%) followed by *P. aeruginosa* (22.15%). Overall prevalence of methicillin-resistant *S. aureus* was 12.82% [39]. Another study conducted in UK reported that, a total of 548 external eye infections were caused by *S. aureus*, of these, 17 (3%) were MRSA positive [40]. Various studies reported that MRSA was more sensitive to chloramphenicol but highly resistant to clindamycin, tetracycline and gentamicin [41, 42]. Examining various commercially accessible eyelid cleaning products, povidone–iodine was the most effective agent for decreasing the bacterial load that can exist on the eyelid margin. The effects of riboflavin and UV light also most effective in completely eradicating the bacteria [43, 44].

A cross-sectional study conducted in Libya from May 2012 to November 2012 showed that bacteria from anterior blepharitis was isolated including *S. aureus* (25%), *S. epidermidis* (25%), *Klebsiella species* (18%), *P. aeruginosa* (9%), *Proteus species* (7%) and *E. coli* (2%) in order of decreasing frequency. High resistance rates were observed among Gram negative bacteria against commonly used drugs (i.e., Ampicillin, Trimethoprim-sulphametoxazole, and Cephalosporin) [10].

A cross-sectional study conducted in Lagos, Nigeria in 2011 indicated that all ocular specimens were culture-positive. Gram-positive cocci comprising *S. aureus* (27.7%) and CoNS (22.6%) followed by gram-positive bacilli (22.6%), gram-negative bacilli (21.3%), and gram negative cocci (4.5%). *Corynebacterium species* accounts for 16.1% of conjunctivitis cases, *P. aeruginosa* (9.7%) was the most commonly isolated gram negative bacilli. Others were *E. coli* (6.5%), *Moraxella species* (4.5%), *Proteus species* (3.2%), *Klebsiella species* (1.9%) and *Enterobacter aerogenes* (1.9%). Antibiotic susceptibility testing revealed chloramphenicol 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 respectively. Another study conducted in Nigeria reported *S. aureus* (23.7%) as a leading isolate among gram positives and *P. aeruginosa* (10.1%) as a leading isolate among gram negatives. The bacterial isolates were more susceptible to the 2nd generation quinolones than the 1st generations [45, 46].

A cross-sectional study conducted in Hawassa University Hospital from Dec 2012 to Apr 2013 among a total of 281 ocular specimens revealed that, 48.8% was culture positive. The most frequent isolates were gram positive cocci (61.5%) and the predominant isolate was *S. aureus* (21%) followed by CoNS (18.2%) and *S. pneumoniae* (14.0%). 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 was effective against most isolated pathogens [9].

A retrospective study was conducted in Gondar University Hospital from Sept, 2009 to Aug, 2012 with a total of 102 eye discharges. From the total, 60.8% had bacterial growth. The most frequent isolates were gram-positive bacteria (74.2%). The predominant bacterial species

isolated was CoNS (27.4%) followed by *S. aureus* (21%). Most of the bacterial isolates were resistance to ampicilin (71%), amoxicilin (62.9%), erythromycin (43.5%), gentamicin (45.2%), penicillin (71%), trimethoprim-sulphamethoxazole (58.1%), and tetracycline (64.6%) while Ceftriaxon and Ciprofloxacin showed (75.8%) and (80%) susceptibility respectively. From the total bacterial isolates, (87.1%) were showed multi drug resistance to two or more drugs [11].

A cross-sectional study was conducted in Gondar from Febr to May 2015 on 51 dacryocystitis cases. Bacterial aetiologies were isolated among 60.8% cases with dominant isolates of *S. aureus* and *P. aeroginosa* followed by *S. pneumoniae*, *Entrobacter species*, *K. pneumoniae* and *H. influenzae*. 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 species* were sensitive to all antibiotics tested but the rest bacterial isolates were resistant for at least to one or more antibiotics tested [47].

A cross sectional study conducted among a total of 160 patients with external ocular infections at Borumeda, Dessie in 2014 showed that from the total ocular samples collected, 59.4% was culture positive. The majority of the isolates (93.7%) were gram positive and the remaining (6.3%) isolates were gram negative bacteria. CoNS (31.9%) were the leading isolate among gram positive bacteria followed by *S. aurues* (13.1%) and *S. pneumoniae* (6.2%). All Grams positive isolates were susceptible for Vancomycin but most were resistant to ampicillin and amoxicillin. Most gram negative were sensitive to gentamicin but highly resistant to tetracycline, norfloxaxine, ceftriaxone and ciprofloxacin [48].

A cross sectional study conducted in Jimma University Hospital from Feb, 2012 to Oct, 2012 showed that among 198 ocular samples cultured, 74.7% was positive. The predominant isolate among gram-positive were *S. aureus* (28.4%) followed by *S. pneumoniae* (13.5%). From gram-negatives *P. aeruginosa* (20.9%) was the leading isolate followed by *H. influenzae* (8.8%). Majority of gram-positive cocci were susceptible to ciprofloxacin (92.2%) and vancomycin (90.9%) and gram-negative isolates to amikacin (94.4%) and ciprofloxacin (91.5%). These findings indicated that gram-positive cocci were the most common bacteria isolated from

external ocular infections and were more susceptible to ciprofloxacin and vancomycin, whereas gram-negative isolates were more susceptible to amikacin and ciprofloxacin [5].

A hospital based cross-sectional study conducted from September 2015 to December 2015 in Tigray showed that, among 270 ocular infected subjects, two third of them were culture positive for different bacterial isolates. The predominant bacterial isolates were *S. aureus* (22.2%), CoNS (17.2%) and *P. aeruginosa* (11.7%). Ocular surface disease, ocular trauma, hospitalization and cosmetic application practices were significantly associated with the occurrence of bacterial infection. Concerning antimicrobial susceptibility, most isolates were susceptible to amikacin (93.2%), gentamicin (89.1%) and ciprofloxacin (89.2%). Overall, (22.5%), (19.1%) and (34.8%) isolates were resistant to one or more antimicrobials, respectively [49].

2.2 Review on bacterial biofilm formation on external ocular infections

Biofilms are defined as a normal pattern of microorganisms organized in microbial communities that are attached on living and non-living surfaces. Bacterial biofilms are formed within the self-secreted extracellular chemical compound containing polysaccharides, proteins and extracellular microbial DNA [50]. This social behaviour arises as an adaptation strategy for survival in hostile environments, including the human host. Additionally it may promote the reinfection of colonized sites. Likewise the matrix confers a protection against biocides and medicine. Biofilm formation contributes to drug resistance development [51], thus 100,000 estimated hospital deaths were caused due to biofilm per year in the United States and 80% of human microbial infections.

According to the accessible information, up to 65–80% of all infections are related to biofilm formation that plays a vital role in pathologic process. Biofilms are generaly related to chronic infections, in distinction to the planktonic bacteria involved in acute processes [52]. In staphylococci, it seems that polysaccharide intercellular adhesin, matrix macromolecules (proteins) as well as the accumulation-associated protein, and presumably the biofilm-associated homologues protein contribute to this matrix. Commensal isolates of coagulase-negative staphylococci (CoNS), especially *S. epidermidis* recovered from healthy conjunctiva carry most

of the genes associated with biofilm maturation, suggesting that the ability to form biofilms is an integral part of their life-style [29].

The increased risk for the development of microbial keratitis in contact lens wearers has been related to the power of the lens to induce modification of the corneal epithelium to hold organisms to the ocular. Tear fluid exchange is also compromised between the anterior and posterior sides of the lens, changing the composition of the tear fluid on the ocular surface and limiting its antimicrobial properties. Additionally, contact lenses help a surface where microorganisms might attach and colonize the surface as a biofilm, that represents a source for microorganisms to disribute to an antecedently broken membrane epithelial tissue [54].

The placement of permanent scleral buckles between the conjunctiva and sclera may be a common surgical operation for retinal detachment. Scleral buckle-associated infections are mostly caused by gram positive cocci, particularly coagulase-negative staphylococci. The presence of a biofilm within the explanted material has been assumed to play a crucial role in its pathologic proceess. Punctual plugs are products of silicone polymer, hydrophobic acrylic, collagen and hydrogel. It is often helps to treat ocular surface dryness unresponsive to topical medication. Bacterial biofilm is observed on punctual plugs following implantation [54]. Lacrimal intubation devices as well as lacrimal stents and Jones tubes are mainly used during to treat nasolacrimal duct obstruction. These materials may facilitate biofilm formation on their surface. Most of these polymer (silicone) stents were culture positive for *S. epidermidis* and *P. aeruginosa* [55].

Study conducted in different part of the world showed different bacterial biofilm formation rate. Study conducted in Chicago [56], Egypt [57] and Saudi Arabia [58] reported bacterial biofilm formation rate of 57.7%, 33.3% and 90% respectively. Biofilm forming potential of different bacteria varied in different bacterial species and in different places. Study conducted in different places reported that *P. aeroginosa*, *E.coli*, CoNS and *S. aureus* as higher biofilm former [59-61]. Another study showed that *S. aureus* and CoNS which form biofilm was shown to be resistant to aminoglycosides, penicillins, fluoroquinolones, folate pathway inhibitors and tetracycline. Most gram negative bacterial isolates which form biofilm were shown to be resistant to aminoglycosides, cephalosporin, folate pathway inhibitors and phenicols [62-64].

The increasing prevalence of antimicrobial resistance is one of the ultimate fears to the important public health issue in the modern world. Although several new antibiotics were developed in the last few decades none of them have improved potential activity against multidrug-resistant bacteria. Most of the pathogenic microorganisms are able to develop protection against those particular compounds by the development of microbial biofilm [51]. No study conducted on determination of bacterial capability to form biofilm on ocular related infection and their antibiofilm susceptibility tests in Ethiopia. The result obtained from this study showed that bacterial biofilm formation on ocular infection is high. This may be one of the reasons for increased antimicrobial resistance in ocular infections and needs further study on large population on the potential of bacteria to form biofilm with their antibiofilm susceptibility tests.

3. OBJECTIVE OF THE STUDY

3.1 General Objective

To assess the magnitude of bacterial pathogen isolated from external ocular infected suspect patients and the bacterial biofilm forming capability and antimicrobial susceptibility pattern of the isolates at Jimma University Medical Center, from March, 2017 to June, 2017.

3.2 Specific Objectives

- To assess the magnitude of bacterial isolates from external ocular infection at Jimma University Medical Center.
- To determine the antimicrobial susceptibility pattern of bacterial isolates.
- To assess the magnitude of biofilm formation capability of bacterial isolates.

4. MATERIALS AND METHODS

4.1 Study Area

The study was conducted at Jimma University Medical Center among patients with external ocular infections attending at ophthalmology clinic. Jimma town is located 354 Km away from Addis Ababa, in the South Western direction of Ethiopia. Jimma Zone is found in Oromia Regional state with a total estimated population of 2,486,155 according to 2007 (Ethiopian calander) population census conducted by the Central Statistical Agency of Ethiopia [53]. It covers an area of 199,316.18 square kilometer and an average altitude of about 1780 meters above sea level. The various section of the university hospital provides service for an inpatient and outpatient department for a 15 million projected population in the catchment area of southwestern parts of Ethiopia. The department of ophthalmology in the year 2015/2016 had given services for an average of around 26,228 patients (new cases=15,818, repeat cases=5272 and by outreach=5138) who have come with different complaints of eye diseases (data is from JUMC ophthalmic clinic archived records and personal communication). The patients attended include all age groups and both sexes.

4.2 Study period

The study was conducted at Jimma University Medical Center (JUMC), Department of Ophthalmology, Southwest Ethiopia from March 2017 to June 2017

4.3 Study Design

A cross-sectional study was conducted to determine the prevalence of bacterial profile, their antimicrobial susceptibility pattern and biofilm forming potential of the isolate.

4.4 Population

4.4.1 Source population

The source population was all patients who have visited Jimma University Medical Center, department of ophthalmology during the study period.

4.4.2 Study Population

All patients who have visited JUMC, department of ophthalmology and suspected with external ocular infections during the study period.

4.5 Eligibility

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

4.5.1 Inclusion criteria

- All patients examined and diagnosed with slit-lamp bio-microscope and have an external ocular infection with red eye, discharge, mucoid or mucopurulent secretion, thickening of the conjunctiva, in one or both eyes.
- Patients or other guardian agreed to participate and give informed consent including all ages and sex group.

4.5.2 Exclusion criteria

- Patients on antibiotics within the last 5 days prior to sample collection date were excluded.

4.6 Sample size determination and Sampling technique

In this cross sectional study, a convienent sampling technique was used. Sample size was calculated based on single sample size estimation using prevalence of 74.7% as indicated in the previous study done in Jimma University Medical Center in 2012 [5]. With expected margin of error (d) taken at 5% and confidence interval (z) of 95% and 10% contingency for the non-respondent and unknown circumstance was used:

The sample size $n = z(\alpha/_2)^2 \, p\,(1-p)/d^2$

Where

n = Sample size

α = level of significance

z = at 95% confidence interval Z value (α = 0.05) =>Z $\alpha/2$ = 1.96

p = prevalence of previous study found from literature review=74.7%

d = Margin of error at (5%) (0.05)

$n = ((1.96)^2 \times 0.747(1-0.747))/(0.05)^2$

n = 290

10% non-response rate=29, so the total sample size (n) was

n = 29 + 290 = 319

4.7 Measurement

4.7.1 Dependent variables

- Bacterial isolate
- Antimicrobial susceptibility pattern
- Biofilm forming capability of isolate

4.7.2 Independent variables

Socio-demographic characteristics and clinical feature

- Age
- Sex
- Monthly income
- Educational level
- Occupation
- Address
- Repeated infections
- Duration of stay in hospital
- The use of contact lenses
- Previous eye surgery
- Previous antibacterial therapy
- Systemic diseases
- Use of traditional medicine
- Biofilm formation

4.8 Data collection procedures and process

4.8.1 Socio demographic and clinical characteristics

Socio-demographic (age, sex, monthly income, educational level, occupation and address) and clinical data (repeated infections, duration of stay in hospital, the use of contact lenses, surgery, previous antibacterial therapy, systemic diseases and use of traditional medicine) and others data like source of light and fire wood used at home were collected by trained optometrist or

ophthalmologist from each study participants using structured questionnaire (Annex V). To identify the clinical picture of external ocular infections, all patients were examined using a slit-lamp bio-microscope and diagnosed by an ophthalmologist. Standard Operating Procedures (SOPs) were prepared to handle specimens collected from patients by the principal investigator and followed throughout the study period (Annex VII). The questionnaire was prepared in English and translated to Afan Oromo and Amharic and again back translated to English. Specimens were collected only from those patients presenting with external ocular infection.

4.8.2 Sample collection, handling and transport of specimen

All consecutive patients examined with the slit-lamp bio-microscope were set apart for suspected infection of bacterial disease clinically by ophthalmologists. Then, specimens from an external ocular sample of the eyelid and conjunctiva were collected from consented patients. Briefly, patients were requested to look up while, lower eye lid was pulled down and then samples were collected from one or both eyes based on the nature of the infection. The sample collector holds the palpebra apart and gently collects the discharge from the surface of the eye using sterile cotton swab that has been pre-moistened with sterile physiological saline. The sterile normal saline moistened swab was rubbed over the lower conjunctival sac from medial to lateral side and back again. Purulent material in cases of dacryocystitis was collected by everted puncta then applying pressure over the lacrimal sac area from the infected eye [65, 66]. In cases of ulcerative blepharitis, lashes deposit, tear film foaming content, and corneal punctuate erosions was swabbed. From each patient, two swabs were collected; one for gram stain and the other for culture. The swabs were immersed in 3 ml of Amiens transport media with charcol, placed in a cold box and transported to Jimma University Medical Microbiology Laboratory for bacterial isolation, identification and further analysis.

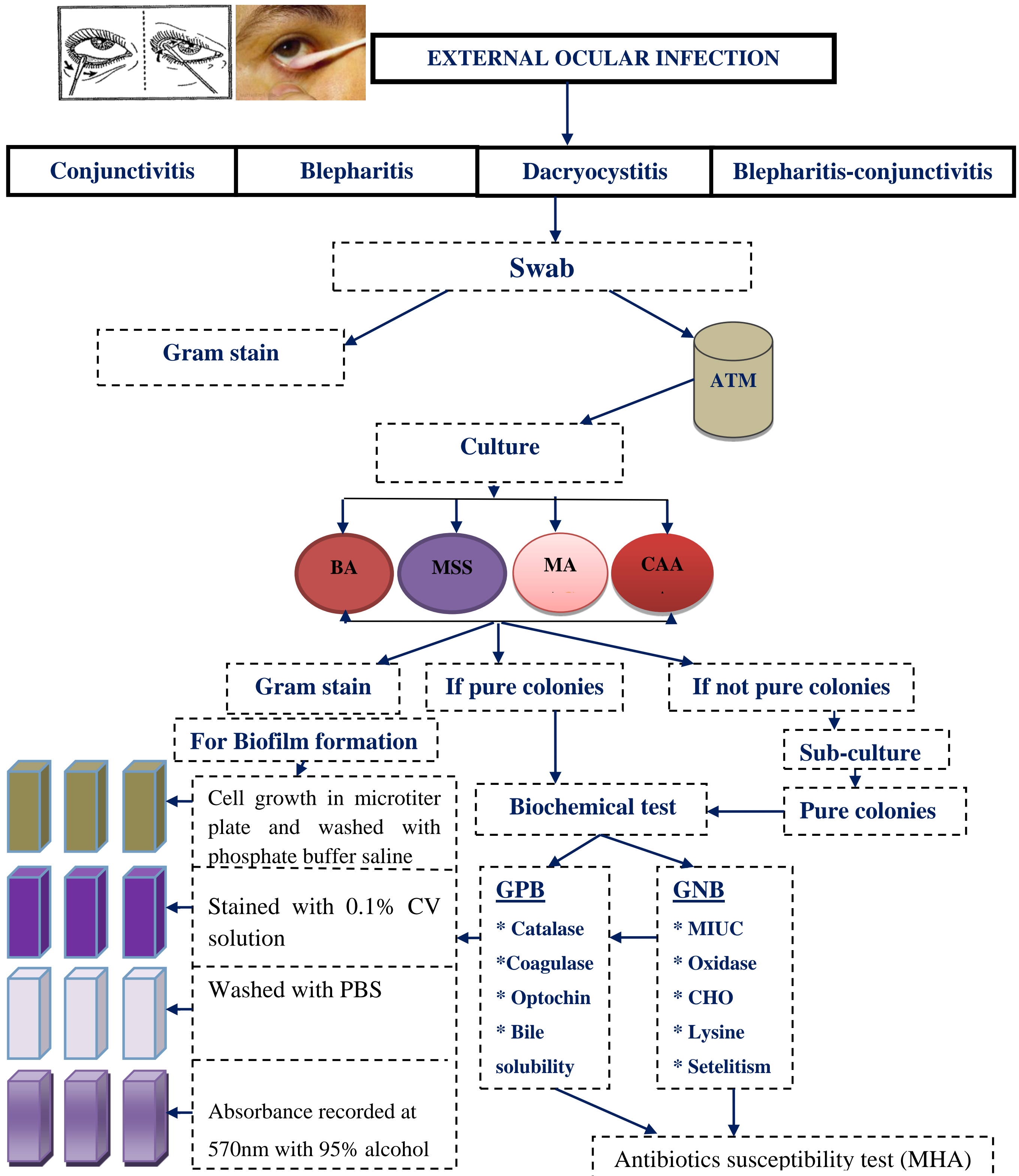


Figure 1: Flow chart for bacterial identification and biofilm formation in eye infection, March 1/2017 to June 30/2017

GPB=Gram positive bacteria, GNB=Gram negative bacteria, MIUC=Motility/Indole/Urea/Citrate utilization test, BA=Blood agar, CAA=Chocolate agar, MSS= Mannitol salt agar, MA=MacConkey agar, MHA= Muller Hinton agar, ATM= Amiens transport media, PBS=Phosphate buffer saline, CV=Crystal violet

4.9 Direct microscopy

4.9.1 Gram stain

Gram staining was done from swab of the primary sample for presumptive identification of gram positive and/or gram negative bacteria following standard procedure (Annex VII).

4.9.2 Culture and Biochemical tests

4.9.2.1 Isolation and Identification of bacterial pathogens

Ocular specimens were inoculated onto MacConkey agar, Mannitol Salt Agar, Blood agar and Chocolate agar plates (all media were from Oxoid, Hampshire, UK). The plates were incubated at 37 °C for 24 to 48 hours aerobically. For fastidious organism, Chocolate agar (heated 5% Sheep's blood agar) was incubated at 37 °C for 24 to 48 hours in a 5-10% CO_2 atmosphere. All plates were initially examined for growth after 24 hours and cultures with no growth were further incubated for another 24 hours. For mixed colonies sub-culture was performed to get pure colonies. After obtaining pure colonies, further identification were conducted using standard microbiological techniques including gram reaction, colony morphology and biochemical tests.

Gram-negative bacteria were identified on the basis of phenotypic and by performing a series of biochemical tests namely, carbohydrate utilization tests, indole production, urease test, manitol fermentation, citrate utilization, lysine decarboxylation, oxidase test, activity on Kligler iron agar (KIA), H_2S production and motility testing. Gram positive bacteria were identified using hemolytic activity on sheep blood agar, catalase and coagulase test, optochin disk sensitivity, bile solubility and Bacitracin disk. For *Hemophilus species*, satelitism test was done in which *hemophilus species* grown on blood agar was streaked with *S.aureus* that provide a growth factor required for *Haemophilus species* (small colonies surrounding *S. aureus* colonies [25].

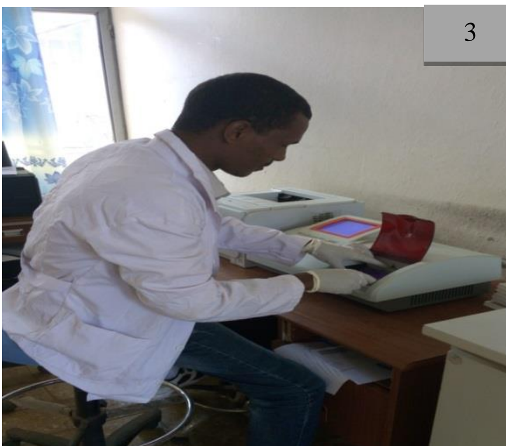
For biofilm formation testing, about ten colonies of bacteria, isolated from fresh agar plates was inoculated into tube filled with sterile trypton soya broth (TSB) with 1% glucose and incubated at 37°C for 24 hours. The overnight culture was diluted by 1:100 into fresh media for biofilm assay. Then, 200 µL of diluted suspension was added into 96 wells of sterile flat-bottom microtiter plate and incubated for 48 hours at 37°C. After incubation, the bacterial suspension of

each well was gently removed. The wells were washed three times with phosphate buffer saline with pH 7.3 to remove free-floating ‘planktonic’ bacteria. After the plates were fixed with methanol (99%), then, they were stained with 220 μL of crystal violet (CV) (0.1%, w/v) and allowed to stand at room temperature for 10-15min. Wells were washed three times with phosphate buffer saline to remove unbound CV dye. After drying, 220 μL of ethanol (95%) was added to each well in order to solubilize the CV. Finally, 200 μL of solubilized CV was transfered to new flat bottomed microtiter plate. The optical density (OD) of the solubilized biofilm stain was determined by a microplate reader (HumaReader HS, German) at a wavelength of 570 nm. The experiment was performed in triplicate separately for each strain and the average values were calculated [67].

For classification of adherence, the mean values of OD obtained for blank tests were subtracted from the mean values of OD obtained for each test strain to correct the background staining of microtiter plate. Then biofilm formations of the isolates were classified into four classes as stated in previous study [68]: non-adherent (OD < ODc); weakly-adherent (ODc < OD < 2xODc); moderately-adherent (2xODc < OD < 4xODc); strongly-adherent (4xODc < OD); with ODc: The cut off value of absorbance (ODc) was proof of the biofilm formation and was defined as the sum of the arithmetic mean of negative control and a triple value of its standard deviation (ODc $=\ddot{x}+3\ \sigma$). TSB with 1% glucose without bacterial suspension incubated in microtiter plate was used as negative control.

While 95% alcohol added to microtiter plate stained with 0.1% CV

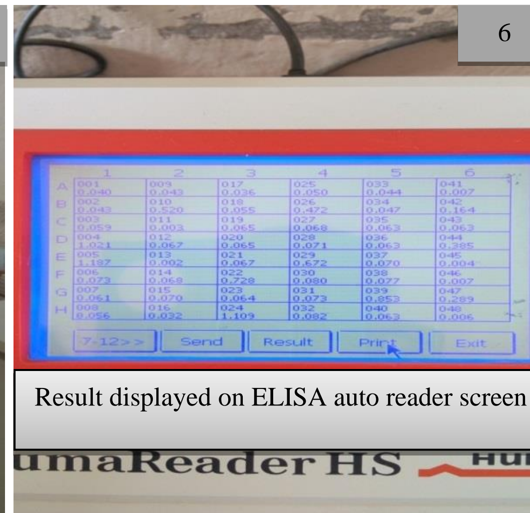
While dissolved CV transferred to new flat bottomed microtiter plate



Insertion of microtiter plate into ELISA auto reader to measure absorbance

Set the wavelength, Control and labeling the whole samples accordingly

ELISA auto reader read the absorbance and quantified



Result displayed on ELISA auto reader screen

Figure: 2 Picture showed biofilm formation test of bacterial isolates by using ELISA auto reader, March 1/2017 to June 30/2017

4.10 Antimicrobial susceptibility testing

For every identified bacteria, antimicrobial susceptibility test was carried out on Muller Hinton agar (MHA) (Oxoid, Hampshire, UK) using the disk diffusion method described by Clinical Laboratory Standard Institute (CLSI) 2015 guideline [69]. From a pure culture, three to five colonies of the test organisms were emulsified in 3 ml of sterile nutrient broth and mixed gently. The suspension was diluted and incubated at 37 °C till the turbidity of the suspension becomes adjusted to 0.5 McFarland standards. The suspension was swabbed uniformly onto MHA agar entirely by rotating the plate 60 degree between streak for non-fastidious organisms and MHA with defibrinated sterile sheep blood (5%) for fastidious organisms. The antimicrobial impregnated disks (from Oxoid, Hampshire, UK and Hi-MEDIA) were placed using sterile forceps on the MHA plate's surface and the plates were incubated (plate placed side up or inverted) at 37°C for 18-24 hours and the zone of inhibition around the disk was measured to the nearest millimeter using a graduated caliper in millimeters, and the isolates were classified as sensitive, intermediate and resistant according to CLSI, 2015. Methicillin resistant isolates were determined by cefoxitin disk which is a better inducer of the mecA gene and was determined by inoculation of *S. aurues* or CoNS on MHA and incubated against cefoxitin (30 µg) at 33-35°C for full 24 hours before reading which is recommended by CLSI [69].

The following antibiotics with the respective concentrations were used to determine the antibiogram of the strains: Fifteen impregnated antibiotic disks were used in the following concentrations: Amikacin (AK) 30 µg, Ampicillin (AMP) 10µg, Amoxicillin-Clavulinic acid (AMC) 20µg, Cefoxitin (FOX) 30µg, Ceftazidime (CAZ) 30µg, Ceftriaxone (CRO) 30µg, Chloramphenicol (C) 30µg, Ciprofloxacin (CIP) 5µg, Clindamycin (DA) 2µg, Erythromycin (E) 15µg, Gentamicin (CN) 10µg, Penicillin-G (P) 10IU, Tetracycline (TE) 30µg, Trimethoprim-sulphametoxazole (SXT) 1.25/23.75µg and Tobramycin (TOB) 10 µg

4.11 Data quality assurance

All ophthalmic specimens were collected following standard operating procedure by professional ophthalmologists. The sterility of culture media was ensured by incubating 5 % of each batch of the prepared media at 37 °C for 24 hours. A performance of catalase reagent was checked by

known *S. aureus* (positive control) and *S. pyogene* (negative control). The test for coagulase was also checked by known *S. aureus* (positive control) and *S. epidermidis* (negative control). For better results, any physical change like cracks, excess moisture, color, hemolysis, dehydration, contamination, deterioration and expiration dates were checked before using the culture media. Temperature of incubator and refrigerator were monitored daily. The quality and performance of culture media and antimicrobial susceptibility were checked using standardized reference strains of *E. coli* (ATCC 25922), *S. aureus* (ATCC 25923), *P. aeruginosa* (ATCC 27853) and *S. pneumoniae* (ATCC 49619) that were obtained from Ethiopian Public Health Institute, Addis Ababa, Ethiopia. The qualities and performance of biochemical tests were also checked by these reference strains. The information obtained from each consented patients were filled on the prepared format by optometrists at Jimma University Medical Center ophthalmology department.

4.12 Data quality management

Cross-checking and data cleaning were done on daily basis. Missing information obtained was checked by going back to the questionnaire and corrective measures were taken accordingly. All laboratory and clinical data were recorded on appropriate record during the study period and the data were stored on a CD, external memory flash and hard copy as back up.

4.13 Data processing and analysis

Demographic data and patients' history were checked daily for the fulfilmement of information on the day of data collection from laboratory request form. Data entry, data analysis and data cleaning were done using Epi-Data 3.1 and SPSS version 21.0 software. Frequency count and percentage were used to present the finding. Prevalence figures were calculated for the total study population and separately by clinical feature of the disease. Bivariate and multivariate logistic regression were used to asses the significantly associated variable with bacterial prevalence. P-values less than 0.05 were considered statistically significant.

4.14 Ethical Consideration

The study was conducted after it was ethically reviewed and approved. Ethical approval was obtained from Research and Ethical Review Committee (RERC) of the school, followed by approval by Institutional Review Board (IRB), Institute of Health, Jimma University. Permission was also obtained from Jimma University Medical Center clinical director and ophthalmology department. Written informed consent was obtained from each individual after the purpose of the study was explained using the common language they speak and hear. For children, consent was obtained from the guardian of the child who comes to the hospital. 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 whether they have understood it correctly or not and their questions were cleared. Those patients grouped in childhood/adolescent age group were asked for their verbal assent (Annex V) and if they agreed, their parents/guardians signed consent sheet for them to participate in the study.

4.15 Dissemination of results

After conducting the research, results will be presented to the school of medical laboratory sciences, Institute of health, Jimma University and other concerned bodies such as professional societies conferences and workshops. The manuscript can be also submitted to peer reviewed journals for publication.

4.16 Operational definition

Conjunctivitis: - Termed as "red eye" or "pink eye", is a nonspecific term used to describe an inflammation of the conjunctiva, which can be caused by infectious, allergic or toxic.

Blepharitis: - It is inflammation of the eyelid margin / infections of the glands of the eyelid, a common problem in both children and adults.

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

Hordeolum: - Acute infections of the glands of Zeis (sebaceous gland) characterised by redness, pain, and swelling of the eyelid.

Multi drug resistance: non-susceptible to ≥1 agent in three or more than three different classes of drugs.

Repeated infection: - When a person came to health institution more then two times due to ocular is infection.

5. RESULTS

5.1 Socio-demographic and clinical feature of study participant

A total of 319 study participants clinically diagnosed with external ocular infection were included in this study, out of which, 172(53.9%) were male and 147(46.1%) were female with sex distribution ratio of 1.2:1. The age of study participant ranged from 1 month to 95 year with the median age of 21. The majority of study subjects were between the age group of 0-2 years which accounts 103(32.3%) followed by >45 years age groups accounting 74(23.2%). Most of the study participants were living in urban 177(55.5%). From the total study participant, 184(57.7%) were Oromo followed by Amhara 55(17.2%) and Kefa 47(14.7%). Majority of the study subjects were preschool children 109(34.2%) followed by elementary school students which account 87(27.3%). Most of the study subjects were unmarried 168(52.7%) and 88(27.6%) of study participant were farmers. More than half of study participants (199/319, 62.4%) have monthly income less than 1000 Ethiopian Birr.

On the clinical feature, few of the study participants had additional chronic disease otherthan ocular infection like hypertension 18(5.6%), diabetes 17(5.3%) (AOR= 0.09, 95% CI: 0.02-0.43, P= 0.002) and rheumatoid arthritis 11(3.4%). The remaining study participants were only with external ocular infection or unidentified cases which accounts 273(85.6%). From the total, 21(6.6%) of study participant were previously hospitalized for eye infection (AOR= 0.10, 95% CI: 0.03-0.42, P = 0.001) and 31(9.7%) took medicine for eye treatment. About 5(1.6%) of study participant had eye surgery in previous time and 23(7.2%) were used traditional eye medicine. only 5(1.6%) of study subjects were used contact eye lenses. From the total, 255(80%) had infections on both of their eyes while 33(10.3%) infected on their left eyes and 31(9.7%) had infections only on their right eyes. Fire woods were used as the source of power for cooking among 190(59.6%) of the study participants at their home while 153(48.0%) of them were using electric power as source of light at their home (Table 1).

In this study, all sociodemographic variables listed including sex, age, residence, occupation and education were not significantly associated with external ocular bacterial infections ($p > 0.05$).

Table 1: Socio-demographic characteristics, bivariate and multivariate logistic regression analysis of factors associated with ocular bacterial infections at Jimma University medical center eye clinic March 1/2017 to June 30/2017

Variables		**Frequency (%)(N=319)**	**Positive isolate (%) (N=147)**	**COR (95% CI)**	**P-value**	**AOR (95% CI)**	**P-value**
Gender	Male	172(53.9)	79(53.7)	1		1	
	Female	147(46.1)	68(46.3)	0.99(0.64-1.54)	0.953	0.97(0.57-1.62)	0.891
Age in year	0-2	103(32.3)	44(29.9)	1.49(0.82-2.72)	0.190	1.99(0.47-8.48)	0.351
	3-16	48(15.0)	20(13.6)	1.56(0.75-3.25)	0.235	1.78(0.47-6.76)	0.395
	17-30	46(14.4)	22(15.0)	1.22(0.58-2.54)	0.604	1.33(0.50-3.56)	0.566
	31-45	48(15.0)	22(15.0)	1.32(0.64-2.73)	0.459	1.36(0.59-3.13)	0.468
	>45	74(23.2)	39(26.5)	1		1	
Residence	Rural	142(44.5)	72(49.0)	0.72(0.46-1.11)	0.138	0.64(0.34-1.21)	0.169
	Urban	177(55.5)	75(51.0)	1		1	
Educational status of study participant	Illiterate	69(21.6)	36(24.5)	0.86(0.36-1.04)	0.725	2.04(0.49-8.53)	0.329
	Preschool age	109(34.2)	45(30.6)	1.33(0.58-3.02)	0.500	1.25(0.25-6.38)	0.787
	Elementary school	87(27.3)	41(27.9)	1.05(0.45-2.43)	0.915	1.43(0.38-5.33)	0.598
	Secondary school	25(7.8)	11(7.5)	1.19(0.41-3.48)	0.753	1.28(0.32-5.12)	0.725
	College and above	29(9.1)	14(9.5)	1		1	
Chronic disease	Rheumatoid arthritis	11(3.4)	5(3.4)	0.96(0.29-3.21)	0.941	0.94(0.26-3.39)	0.919
	Hypertension	18(5.6)	6(4.1)	1.59(0.58-4.37)	0.366	2.18(0.68-6.97)	0.189
	Diabetes	17(5.3)	15(10.2)	0.11(0.02-0.47)	0.003	0.09(0.02-0.43)	0.002
	No Chronic diseases	273(85.6)	123(83.7)	1		1	
Previous hospitalization	Yes	21(6.6)	18(12.2)	0.13(0.04-0.44)	0.001	0.10(0.03-0.42)	0.001
	No	298(93.4)	129(87.8)	1		1	
History of medicine intake	Yes	31(9.7)	16(10.9)	0.78(0.37-1.64)	0.516	0.89(0.38-2.13)	0.806
	No	288(90.3)	131(89.1)	1		1	
Medical contact lenses used	Yes	5(1.6)	4(2.7)	0.21(0.02-1.89)	0.164	0.13(0.01-1.46)	0.098
	No	314(98.4)	143(97.3)	1		1	
Previous eye surgery	Yes	5(1.6)	3(2.0)	0.57(0.09-3.43)	0.534	3.09(0.30-31.7)	0.342
	No	314(98.4)	144(98.0)	1		1	
Traditional eye medicine used	Yes	23(7.2)	15(10.2)	0.43(0.18-1.04)	0.062	0.45(0.17-1.21)	0.115
	No	296(92.8)	132(89.8)	1		1	

COR= Crude odds ratio; CI= Confidence interval; AOR= Adjusted odds ratio; N= Number

In respect of diagnosis of eye infection types, patients suffering from conjunctivitis were 165(51.7%); blepharoconjunctivitis, 74(23.2%); blepharitis, 52(16.3%); dacryocystitis, 13(4.1%) and others external ocular infection accounts for 15(4.7%). The most dominant external ocular infection among different age group and sex was conjunctivitis. Among different age groups, within 1 month to 2 years age group (which account 68, 66.0%) and 3-16 age group (which account 33, 68.8%) were the major age groups suffering from conjunctivitis and among gender, 76(46.1%) females and 89(53.9%) males were suffering from conjuncitivitis (Figure 2).

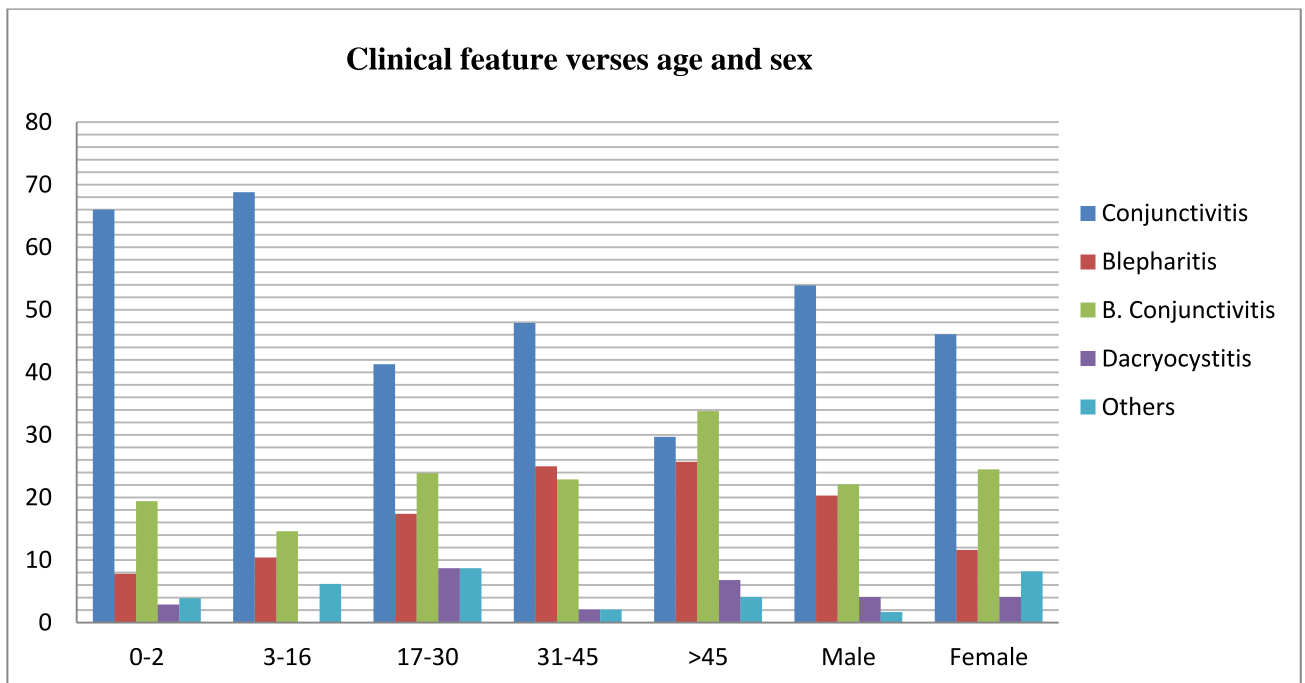


Figure 3: Prevalence of different external ocular infection among different age group and sex at Jimma University medical center eye clinic, March 1/2017 to June 30/2017

5.2 Prevalence of bacterial isolate

Out of 319 samples, bacteria were isolated from 147 giving overall prevalence of 46.1% with 79(53.7%) occurring in male and 68(46.3%) in female. No mixed bacteria found in a single sample in this study. Among the bacterial isolates, 96(65.3%) were gram positive and 51(34.7%) were gram negative with the ratio of 1.9:1. Among gram positive, CoNS 41(27.9%) were the most frequent bacterial isolates followed by *S. aureus* with 29(19.7%) and *S. pneumoniae* with 13(8.8%). From gram negative, *P. aeroginosa* was the predominant bacterial isolate with 10(6.8%) followed by *K. pneumoniae* with 9(6.1%). From gram positive, Streptococcus viridians recovered from 3(2.0%) cases and from gram negative, *N.meningitidis* isolated from 2(1.4%) cases were the least bacterial isolate identified in this study. The spectrum of bacterial isolate varies with the age of patients. Most of the bacterial isolates were found among infants and children subjects between 1 month to 2 years of age group with 44(29.9%). (Table 2).

Table 2: Prevalence of bacterial isolate within different age group at Jimma University medical center eye clinic, March 1/2017 to June 30/2017

Name of bacterial isolate	Age in years					Total (N=319)
	0-2 (N=103)	3-16 (N=48)	17-30 (N=46)	31-45 (N=48)	>45 (N=74)	
Gram positive bacteria						
S. aureus	9(20.5)	2(10.0)	7(31.8)	2(9.1)	9(23.1)	**29(19.7)**
CoNS	10(22.7)	5(25.0)	4(18.2)	11(50.0)	11(28.2)	**41(27.9)**
S. pneumoniae	4(9.1)	1(5.0)	2(9.1)	1(4.5)	5(12.8)	**13(8.8)**
S. pyogenes	1(2.3)	3(15.0)	1(4.5)	0(0.0)	0(0.0)	**5(3.4)**
S. agalactiae	1(2.3)	1(5.0)	0(0.0)	0(0.0)	3(7.7)	**5(3.4)**
S. viridians	2(4.5)	0(0.0)	0(0.0)	0(0.0)	1(2.6)	**3(2.0)**
Gram negative bacteria						
P. aeroginosa	1(2.3)	2(10.0)	2(9.1)	3(13.6)	2(5.1)	**10(6.8)**
K. pneumoniae	2(4.5)	2(10.0)	1(4.5)	1(4.5)	3(7.7)	**9(6.1)**
P. mirabilis	1(2.3)	1(5.0)	2(9.1)	0(0.0)	1(2.6)	**5(3.4)**
P. vulgaris	1(2.3)	1(5.0)	0(0.0)	1(4.5)	1(2.6)	**4(2.7)**
S. marcescens	2(4.5)	0(0.0)	1(4.5)	0(0.0)	0(0.0)	**3(2.0)**
Citrobacter species	2(4.5)	0(0.0)	0(0.0)	1(4.5)	2(5.1)	**5(3.4)**
Enterobacter species	2(4.5)	0(0.0)	0(0.0)	0(0.0)	1(2.6)	**3(2.0)**
E. coli	1(2.3)	1(5.0)	1(4.5)	2(9.1)	0(0.0)	**5(3.4)**
H. influenzae	4(9.1))	0(0.0)	1(4.5)	0(0.0)	0(0.0)	**5(3.4)**
N. meningitidis	1(2.3)	1(5.0)	0(0.0)	0(0.0)	0(0.0)	**2(1.4)**
Total	**44(29.9)**	**20(13.6)**	**22(15.0)**	**22(15.0)**	**39(26.5)**	**147(100)**

CoNS=Coagulase negative staphylococcus

Regarding the bacterial profile in different clinical features (diagnosis), most of the isolates were recovered from conjunctivitis 75(51.0%) followed by blepharitis 32(21.8%) and blepharoconjunctivitis 27(18.4%). The least bacterial isolates were found in dacryocystitis with 8(5.4%). The predominant bacterial isolates observed in conjunctivitis cases were CoNS, 16(21.3%) followed by *S. aureus*, 15(20%); in blepharitis CoNS, 10(31.2%) followed by *S. aureus*, 8(25%); in blepharoconjunctitivis CoNS, 10(37%) followed by *H. influenzae*, 3(11.1%) and in dacryocystitis CoNS, 3(37.5%) followed by *S. pneumoniae*, 2(25%) and *S. aureus*, 2(25%). Among gram negative, *P. aeroginosa,* 5(6.7%) and *K. pneumoniae*, 4(5.3%) were predominant bacterial isolate in conjunctivitis (Table 3).

Table 3: Prevalence of bacterial profile from different clinical feature of external ocular infection at Jimma University medical center eye clinic, March 1/2017 to June 30/2017

Name of bacterial isolate	**Types of diagnosis**					**Total**
	Conjunctivitis (N=165)	Blepharitis (N=52)	Blepharo Conjunctivitis(N=74)	Dacryocystitis (N=13)	Others (N=15)	(N=319)
Gram positive bacteria						
S. aureus	15(20.0)	8(25.0)	1(3.7)	2(25.0)	3(60)	**29(19.7)**
CoNS	16(21.3)	10(31.2)	10(37.0)	3(37.5)	2(40)	**41(27.9)**
S. pneumoniae	9(12.0)	2(6.2)	0(0.0)	2(25.0)	0(0.0)	**13(8.8)**
S. pyogenes	4(5.3)	0(0.0)	1(3.7)	0(0.0)	0(0.0)	**5(3.4)**
S. agalactiae	2(2.7)	2(6.2)	1(3.7)	0(0.0)	0(0.0)	**5(3.4)**
S. viridians	2(2.7)	1(3.1)	0(0.0)	0(0.0)	0(0.0)	**3(2.0)**
Gram negative bacteria						
P. aeroginosa	5(6.7)	3(9.4)	2(7.4)	0(0.0)	0(0.0)	**10(6.8)**
K. pneumoniae	4(5.3)	2(6.2)	2(7.4)	1(12.5)	0(0.0)	**9(6.1)**
P. mirabilis	3(4.0)	1(3.1)	1(3.7)	0(0.0)	0(0.0)	**5(3.4)**
P. vulgaris	2(2.7)	0(0.0)	2(7.4)	0(0.0)	0(0.0)	**4(2.7)**
S. marcensens	3(4.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	**3(2.0)**
Citrobacter species	3(4.0)	1(3.1)	1(3.7)	0(0.0)	0(0.0)	**5(3.4)**
Enterobacter species	2(2.7)	0(0.0)	1(3.7)	0(0.0)	0(0.0)	**3(2.0)**
E. coli	2(2.7)	2(6.2)	1(3.7)	0(0.0)	0(0.0)	**5(3.4)**
H. influenzae	2(2.7)	0(0.0)	3(11.1)	0(0.0)	0(0.0)	**5(3.4)**
N. meningitides	1(1.3)	0(0.0)	1(3.7)	0(0.0)	0(0.0)	**2(1.4)**
Total	**75(51.0)**	**32(21.8)**	**27(18.4)**	**8(5.4)**	**5(3.4)**	**147(100)**

CoNS=Coagulase negative staphylococcus

5.3 Antimicrobial susceptibility pattern among gram positive bacterial isolates

Antimicrobial susceptibility pattern for gram positive bacteria were done on twelve antibiotics belonging to nine categories. Among the isolates, *S. aureus* showed high susceptibility to clindamycin 24(82.8%) followed by ciprofloxacin and amoxicillin clavulinic acid each account 22(75.9%) and gentamicin 20(69%). On the other hand, *S. aureus* was highly resistant to penicillin 25(86.2%), erythromycin 24(82.8%) and tetracycline 22(75.9%). Among *S. aureus* isolate, 4(13.8%) were MRSA. CoNS almost showed comparable susceptibility to *S. aureus* and 12(29.3%) of isolated CoNS were methicillin resistant (MRCoNS). *S. pneumoniae* showed high susceptibility to amoxicillin clavulinic acid 13(100%) and ciprofloxacin 11(84.6%) but highly resistant to penicllin and trimethoprim-sulphamethoxazole each account 9(69.2%). Others gram positive bacterial isolates were highly sensitive to amoxicillin clavulinic acid (100%), clindamycin (93.3%) and gentamicin (86.7%) while less susceptible to trimethoprim-sulphamethoxazole (31.1%), tetracycline (37.8%) and ampicillin (48.9%). By antimicrobial susceptibility test, some gram positive bacterial isolates were grouped under intermediate to antibiotics like tetracycline and erythromycin than others (Table 4).

5.4 Antimicrobial susceptibility pattern among gram negative bacterial isolates

Among gram negative bacterial isolate, *P. aeroginosa* showed high susceptibility to ceftriaxone, ciprofloxacin and amoxicillin clavulinic acid each account 8(80%), gentamicin and amikacin each account 7(70%). On the other hand, among ten *P. aeroginosa* isolated, high resistance was observed to ceftazidime 9(90%), tetracycline 8(80%), trimethoprim-sulphamethoxazole and tobramycin each account 7(70%). *K. pneumoniae* showed high susceptibility to ceftriaxone, amoxicillin clavulinic acid and ciprofloxacin each accounts 8(88.9%); but it was less susceptible to tobramycin, trimethoprim-sulphamethoxazole and tetracycline (33.3-44.4%). Others higher sensitive gram negative bacteria isolated from external ocular infection to different antibiotics were: ceftriaxone 31(96.9%), ciprofloxacin 32(100%), amoxicillin clavulinic acid 28(93.3%), gentamicin 25(83.3%) and amikacin 19(76%). On the other hand they were less susceptible to tobramycin, trimethoprim-sulphamethoxazole and tetracycline with in the range of 0-66.7%. Some gram negative bacterial isolates were intermediate to antibiotic like trimethoprim-sulphamethexazole and tetracycline by antimicrobial susceptibility test (Table 5).

Table 4: Antimicrobial susceptibility patterns of gram positive bacterial isolate from external ocular infection at Jimma University medical center eye clinic, March 1/2017 to June 30/2017

			Antimicrobial agents tested											
Bacterial isolate	Total	Pattern	**AMC** No(%)	**AMP** No(%)	**CIP** No(%)	**AK** No(%)	**C** No(%)	**CLN** No(%)	**TE** No (%)	**SXT** No(%)	**ERY** No(%)	**CN** No(%)	**FOX** No(%)	**PE** No(%)
S. aureus	29	S	22(75.9)	5(17.2)	22(75.9)	19(65.5)	16(55.2)	24(82.8	5(17.2)	7(24.1)	4(13.8)	20(69.0)	25(86.2)	4(13.8)
		I	0(0.0)	0(0.0)	4(13.8)	1(3.4)	0(0.0)	0(0.0)	2(6.9)	2(6.9)	1(3.4)	1(3.4)	0(0.0)	0(0.0)
		R	7(24.1)	24(82.8)	3(10.3)	9(31.0)	13(44.8)	5(17.2)	22(75.9)	20((69.0	24(82.8	8(27.6)	4(13.8)	25(86.2)
CoNS	41	S	37(90.2)	7(17.1)	32(78.0)	27(65.9)	23(56.1)	33(80.5	9((22.0)	12(29.3)	8(19.5)	29(70.7)	29(70.7)	7(17.1)
		I	0(0.0)	0(0.0)	0(0.0)	3(7.3)	0(0.0)	0(0.0)	3(7.3)	1(2.4)	3(7.3)	3(7.3)	0(0.0)	0(0.0)
		R	4(9.8)	34(82.9)	9(22.0)	11(26.8)	18(43.9)	8(19.5)	29(70.7)	28(68.3)	30(73.2	9(22.0)	12(29.3)	34(82.9)
S. pneum Oniae	13	S	13(100)	5(38.5)	11(84.6)	10(76.9)	10(76.9)	8(61.5)	3(23.1)	4(30.8)	7(53.8)	10(76.9)	NT	4(30.8)
		I	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	2(15.4)	0(0.0)	0(0.0)	0(0.0)	NT	0(0.0)
		R	0(0.0)	8(61.5)	2(15.4)	3(23.1)	3(23.1)	5(38.5)	8(61.5)	9(69.2)	6(46.2)	3(23.1)	NT	9(69.2)
S. pyogen es	5	S	5(100)	2(40.0)	4(80.0)	4(80.0)	3(60.0)	4(80.0)	2(40.0)	1(20.0)	3(60.0)	4(80.0	NT	3(60.0)
		I	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(20.0)	0(0.0)	0(0.0)	1(20.0)	0(0.0)	0(0.0)	NT	0(0.0)
		R	0(0.0)	3(60.0)	1(20.0)	1(20.0)	1(20.0)	1(20.0)	3(60.0)	3(60.0)	2(40.0)	1(20)	NT	2(40.0)
S.agalact iae	5	S	5(100)	2(40.0)	5(100)	4(80)	3(60.0)	5(100)	2(40.0)	2(40.0)	3(60.0)	4(80.0)	NT	2(40.0)
		I	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(20.0)	0(0.0)	0(0.0)	1(20.0)	1(20.0)	0(0.0)	NT	1(20.0)
		R	0(0.0)	3(60.0)	0(0.0)	1(20.0)	1(20.0)	0(0.0)	3(60.0)	2(40.0)	1(20.0)	1(20.0)	NT	2(40.0)
S.viridian s	3	S	3(100)	2(66.7)	3(100)	3(100)	3(100)	3(100)	1(33.3)	1(33.3)	2(66.7)	3(100)	NT	1(33.3)
		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)	0(0.0)	NT	0(0.0)
		R	0(0.0)	1(33.3)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(33.3)	2(66.7)	1(33.3)	0(0.0)	NT	2(66.7)

CoNS=Coagulase negative Staphylococci, S=Sensitive, I=Intermediate, R= Resistance, AMC = Amoxicillin-clavulanic acid, AMP = Ampcillin, CIP = Ciprofloxacin, AK = Amikacin, C = Chloramphenicol, CLN=Clindamycin, TE = Tetracycline, SXT = Trimethoprim-sulphamethoxazole, ERY = Erythromycin, CN = Gentamicin, FOX=Cefoxitin, P = Penicillin, TOB=Tobramycin, No=Number, NT=Not tested

Table 5: Antimicrobial susceptibility patterns of gram negative bacterial isolate from external ocular infection at Jimma University medical center eye clinic, March 1/2017 to June 30/2017

			Antimicrobial agents tested										
Bacterial isolate	Total	Pattern	**AMC** No(%)	**AMP** No(%)	**CIP** No(%)	**CRO** No(%)	**C** No(%)	**CAZ** No(%)	**TE** No(%)	**SXT** No(%)	**CN** No(%)	**TOB** No(%)	**AK** No(%)
P. aeroginosa	10	S	8(80.0)	5(50.0)	8(80.0)	8(80.0)	4(40.0)	1(10.0)	2(20.0)	2(20.0)	7(70.0)	3(30.0)	7(70.0)
		I	0(0.0)	0(0.0)	1(00.0)	0(0.0)	1(10.0)	0(0.0)	0(0.0)	1(10.0)	1(10.0)	0(0.0)	1(10.0)
		R	2(20.0)	5(50.0)	1(10.0)	2(20.0)	5(50.0)	9(90.0)	8(80.0)	7(70.0)	2(20.0)	7(70.0)	2(20.0)
K. pneumoniae	9	S	8(88.9)	6(66.7)	8(88.9)	8(88.9)	7(77.8)	5(55.6)	4(44.4)	3(33.3)	7(77.8)	3(33.3)	7(77.8)
		I	0(0.0)	0(0.0)	1(11.1)	1(11.1)	1(11.1)	1((11.1)	0(0.0)	1(11.1)	1(11.1)	1(11.1)	1(11.1)
		R	1(11.1)	3(33.3)	0(0.0)	0(0.0)	1(11.1)	3(33.3)	5(55.6)	5(55.6)	1(11.1)	5(55.6)	1(11.1)
P. mirabilis	5	S	5(100)	4(80.0)	5(100)	4(80.0)	4(80.0)	3(60.0)	2(40.0)	3(60.0)	4(80.0)	2(40.0)	4(80.0)
		I	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(20.0)	1(20.0)	0(0.0)	1(20.0)	0(0.0)	1(20.0)
		R	0(0.0)	1(20.0)	0(0.0)	1(20.0)	1(20.0)	1(20.0)	2(40.0)	2(40.0)	0(0.0)	3(60.0)	0(0.0)
P. vulgaris	4	S	4(100)	3(75.0)	4(100)	4(100)	4(100)	3(75.0)	2(50.0)	2(50.0)	3(75.5)	1(25.0)	3(75.0)
		I	0(0.0	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(25.0)	1(25.0)	0(0.0)	0(0.0)	0(0.0)
		R	0(0.0)	1(25.0)	0(0.0)	0(0.0)	0(0.0)	1(25.0)	1(25.0)	1(25.0)	1(25.0)	3(75.0)	1(25.0)
S. marcescens	3	S	3(100)	1(66.7)	3(100)	3(100)	3(100)	3(100)	1(33.3)	0(0.0)	3(100)	1(33.3)	2(66.7)
		I	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(33.1)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
		R	0(0.0)	1(33.3)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	2(33.3)	3(100)	0(0.0)	2(66.7)	1(33.3)
Citrobacter species	5	S	4(80.0)	4(80.0)	5(100)	5(100)	4(80.0)	4(80.0)	1(20.0)	3(60.0)	4(80.0)	3(60.0)	4(80.0)
		I	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	2(40.0)	2(40.0)	0(0.0)	0(0.0)	0(0.0)
		R	1(20.0)	1(20.0)	0(0.0)	0(0.)	1(20.0)	1(20.0)	2(40.0)	0(0.0)	1(20.0)	2(40.0)	1(20.0)
Enterobacter species	3	S	3(100)	2(66.7)	3(100)	3(100)	3(100)	3(100)	1(33.3)	2(66.7)	2(66.7)	1(33.3)	2(66.7)
		I	0(0.0)	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)	0(0.0)
		R	0(0.0)	1(33.3)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	2(66.7)	0(0.0)	1(33.3)	2(66.7)	1(33.3)
E. coli	5	S	4(80)	3(60.0)	5(100)	5(100)	3(60.0)	3(60.0)	1(20.0)	2(40.0)	5(100)	2(40.0)	4(80.0)
		I	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(20.0)	0(0.0)	1(20.0)	0(0.0)	0(0.0)	0(0.0)
		R	1(20.0)	2(40.0)	0(0.0)	0(0.0)	2(40.0)	1(20.0)	4(80.0)	2(40.0)	0(0.0)	3(60.0)	1(20.0)
H. influenza	5	S	4(80.0)	3(60.0)	5(100)	5(100)	4(80.0)	4(80.0)	3(60.0)	3(60.0)	4(80.0)	NT	NT
		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)	NT	NT
		R	1(20.0)	2(40.0)	0(0.0)	0(0.0)	1(20.0)	1(20.0)	2(40.0)	2(40.0)	1(20.0)	NT	NT
N. meningitides	2	S	NT	NT	2(100)	2(100)	1(50.0)	NT	NT	1(50.0)	NT	NT	NT
		I	NT	NT	0(0.0)	0(0.0)	0(0.0)	NT	NT	0(0.0)	NT	NT	NT
		R	NT	NT	0(0.0)	0(0.0)	1(50.0)	NT	NT	1(50.0)	NT	NT	NT

5.5 Biofilm formation test result by microtiter plate method

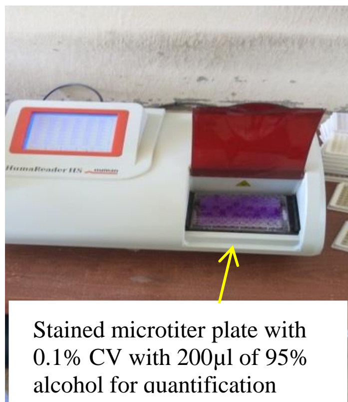


Figure 4: Screening of biofilm formation by microtiter plate method March1/ 2017 to June 30/2017

From the total of 127 bacterial isolates screened for biofilm formation by microtiter plate method, 84(66.1%) strains were observed to form biofilms *in vitro*. The rates of bacterial biofilm formation were categoriezed into four: 31(24.4%) of them as strong former; 39(30.7%) as moderate; 14(11.0%) as weak and 43(33.9%) as non-biofilm former. Among 83 gram positive isolates, 14(34.1%) CoNS followed by 9(31.0%) *S. aureus* were the frequent strong biofilm formers. On the otherhand, among 44 gram negative isolates, 4(40.0%) *P. aeroginosa* was the most strong biofilm former followed by 2(22.2%) *K. pneuminiae* (Table 6).

Table 6: Biofilm forming capability and adherence classification of bacteria isolated from ocular infected patients at Jimma University medical center eye clinic March 1/2017 to June 30/2017

Bacterial isolates	**Biofilm formation classification**				
	Total	Strong	Moderate	Weak	Non- adherent
S. aureus	29	9(31.0)	10(34.5)	2(6.9)	8(27.6)
CoNS	41	14(34.1)	13(31.7)	4(9.8)	10(24.4)
S. pyogenes	5	0(0.0)	1(20.0)	1(20.0)	3(60.0)
S. agalactiae	5	0(0.0)	1(20.0)	1(20.0)	3(60.0)
S. viridians	3	0(0.0)	0(0.0)	0(0.0)	3(100)
P. aeroginosa	10	4(40.0)	3(30.0)	1(10.0)	2(20.0)
K. pneumoniae	9	2(22.2)	3(33.3)	2(22.2)	2(22.2)
P. mirabilis	5	1(20.0)	1(20.0)	1(20.0)	2(40.0)
P. vulgaris	4	0(0.0)	0(0.0)	2(50.0)	2(50.0)
S. marcescens	3	0(0.0)	1(33.3)	0(0.0)	2(66.7)
Citrobacter species	5	0(0.0)	3(60.0)	0(0.0)	2(40.0)
Enterobacter species	3	0(0.0)	1(33.3)	0(0.0)	2(66.7)
E. coli	5	1(20.0)	2(40.0)	0(0.0)	2(40.0)
Total	**127**	**31(24.4)**	**39(30.7)**	**14(11.0)**	**43(33.9)**

Non-adherent (OD < ODc); Weakly-adherent (ODc < OD < 2xODc); Moderately-adherent (2xODc < OD < 4xODc); Strongly-adherent (4xODc < OD); with ODc: The cut off value of absorbance (ODc) was proof of the biofilm formation and was defined as the sum of the arithmetic mean of negative control and a triple value of its standard deviation (ODc $=\ddot{x}+3\ \sigma$). The calculated ODc measured by ELISA auto reader was 0.231. **NB:** For fastidous organisms like *S. pneumoniae* (n=13), *H. influenzae* (n=5) and *N. meningitidis* (n=2), their biofilm formation capability were not detected due to the reqiurment of special condition for their growth.

5.6 Antibiotics resistance patterns of bacterial isolate

Among the total isolates (n=147), multidrug resistance (MDR ≥3 different classes of drugs) were recorded in 101 (68.7%) of all bacterial isolates. From the total 96 gram positive isolates, 75(78.1%) of them were MDR while from the total 51 gram negative isolates, only 26(51.0%) were MDR. Among gram positive organisms, *S. aureus* 25(86.2%) followed by CoNS 34(82.9%) showed a high level of multi-drug resistance while among gram negatives *P. aeroginosa* 10(100%) followed by *K. pneumoniae* 6(66.7%) and *E. coli* 3(60.0%) showed increased level of multi-drug resistance.

Table 7: Multidrug resistance pattern of bacteria isolated from external ocular infected patients at Jimma University medical center eye clinic, March 1/2017 to June 30/2017

Bacterial isolates	**Total**	**Antibiotics resistance pattern**					
		Ro	**R1**	**R2**	**R3**	**R4**	**≥ R5**
S. aureus	29	0(0.0)	0(0.0)	4(13.8)	5(17.2)	7(24.1)	13(44.8)
CoNS	41	0(0.0)	2(4.9)	5(12.2)	8(19.5)	10(24.4)	16(39.0)
S. pneumoniae	13	0(0.0)	1(7.7)	3(23.1)	3(23.1)	6(46.2)	0(0.0)
S. pyogenes	5	0(0.0)	1(20.0)	0(0.0)	2(40.0)	1(20.0)	1(20.0)
S. agalactiae	5	1(20.0)	1(20.0)	1(20.0)	1(20.0)	0(0.0)	1(20.0)
S. viridians	3	0(0.0)	2(66.7)	0(0.0)	1(33.3)	0(0.0)	0(0.0)
P. aeroginosa	10	0(0.0)	0(0.0)	0(0.0)	3(30.0)	2(20.0)	5(50.0)
K. pneumoniae	9	1(11.1)	1(11.1)	1(11.1)	4(44.4)	2(22.2)	0(0.0)
P. mirabilis	5	0(0.0)	2(40.0)	2(40.0)	0(0.0)	0(0.0)	1(20.0)
P. vulgaris	4	0(0.0)	2(50.0)	1(25.0)	1(25.0)	0(0.0)	0(0.0)
S. marcescens	3	0(0.0)	0(0.0)	2(66.7)	1(33.3)	0(0.0)	0(0.0)
Citrobacter species	5	1(20.0)	2(40.0)	0(0.0)	2(40.0)	0(0.0)	0(0.0)
Enterobacter species	3	0(0.0)	1(33.3)	1(33.3)	1(33.3)	0(0.0)	0(0.0)
E. coli	5	0(0.0)	1(20.0)	1(20.0)	1(20.0)	1(20.0)	1(20.0)
H. influenzae	5	1(20.0)	2(40.0)	1(20.0)	1(20.0)	0(0.0)	0(0.0)
N. meningitidis	2	0(0.0)	2(100)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Total	**147**	**4(2.7)**	**20(13.6)**	**22(15.0)**	**34(23.1)**	**29(19.7)**	**38(25.9)**

CoNS= Coagulase negative Staphylococci, Ro= bacterial isolate sensitive to all antibiotics, R1=bacterial isolate resistance to 1 antibiotics, R2= bacterial isolate resistance to 2 antibiotics of different classes, R3= bacterial isolate resistance to 3 antibiotics of different classes, R4= bacterial isolate resistance to 4 antibiotics of different classes and ≥R5= bacterial isolate resistance to 5 and above antibiotics of different classes.

5.7 The correlation of antimicrobial resistance and biofilm formation of bacterial isolate

From the total bacterial isolate, higher biofilm former bacteria were observed in multidrug resistance. In bacterial isolates resistance to 1 and 2 antibiotics, higher non biofilm formers bacterial isolate were seen. But in bacterial isolates resistance to 3 and above antibiotics, higher biofilm formers bacterial strains were observed. The highest (48.4%) strong biofilm former bacterial strains were seen in bacterial isolate that are resistant to five and above antimicrobial agents. In this study, Chi- square analysis revealed significant correlation between multidrug resistant and biofilm formers bacterial isolates ($p<0.05$, correlation coefficient = 0.491). Eventhough there is significant correlation between bacterial biofilm formation and antibiotic resiatance of isolates, the correlation coefficient (0.491) from the Chi- square analysis not showed strong correlation. This may be due to the *in vitro* susceptibility tests and screening of biofilm formation of bacterial isolates.

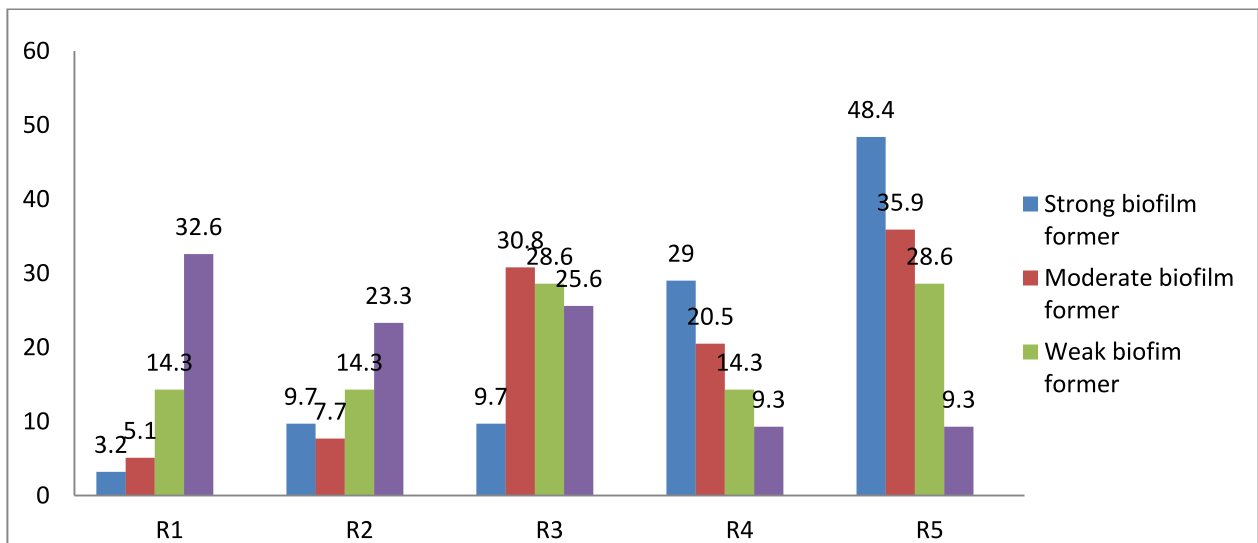


Figure 2: The correlation of antimicrobial resistance and biofilm formation of bacterial isolate at Jimma University medical center eye clinic, March 1/2017 to June 30/2017

6. DISCUSSION

An external ocular infection and its complications are a significant health problem worldwide and in Ethiopia that impacts greatly on quality of life [25]. In this study, a total of 319 patients suffering from external ocular infections were included. The prevalence of bacterial isolate of external ocular infection was more common in males (53.7%) than in females (46.3%). This is in agreement with studies done in Ethiopia [48], Hong Kong [70] and Nigeria [71] with prevalence of 57%, 57% and 59.2% respectively in male. This might be explained by the fact that traditionally, in these countries, mainly males are involved in outdoor activities such as farming, transportation, construction work and industry works where they likely exposed to contaminated environment.

The prevalence of bacterial isolates was higher among <2 years age group followed by >45 years age groups with prevalence of 29.9% and 26.5% respectively. This is similar with previous study conducted in Ethiopia [12], India [20] and Nigeria [71] with prevalence of 66.1%, 46.2% and 26.3% respectively in child. However, different from study conducted in Ethiopia [5] in which higher bacterial isolates were reported among the age group of 18-39 (39.2%). The variation might be due to differences in study period, inclusion of varied cases and differences in sample size. The explanation for enhanced susceptibility to ocular infection in babies might be due to lack of matured immunity in babies following the disappearance of maternal immunity whereas in old age it may be due to dry eye and decline or reduction of immunity [46].

In this study, the socio-demographic and clinical feature including age, sex, residence, monthly income, occupational status, educational status, source of light at home, previous medicine taken, contact lenses used, previous eye surgery and traditional eye medicine used had no significant association with isolated bacterial prevalence ($P>0.05$). Similar finding reported in Ethiopia [48] and India [38]. This might be due to small sample size and also showed that these were not the only determinant factors for the epidemiology of the isolated bacteria rather presence of ocular surface disease, ocular trauma and cosmetic application practices as suggested in previous studies done in different areas [49, 72, 73] that play the major role in the distribution of bacteria cause ocular infection. In present study, only patients with diabetes mellitus and previous history of hospitalization were significantly associated with bacterial prevalence ($P < 0.05$). This is in

agreement with previous study done in Ethiopia [49], India [74], Spain [75] and elsewhere [76]. This might be explained by in repeated hospitalization the probabilities of acquiring bacterial is high and in diabetic mellitus the infected area might be takes prolonged time for healing

In our study, conjunctivitis was the predominant external ocular infection accounting 51.7% followed by blepharoconjunctivitis (23.2%), blepharitis (16.3%), dacryocystitis (4.1%) and other eye infection accounts 4.7%. This is similar with previous study conducted in Ethiopia [9, 48] and India [70] reported that conjunctivitis was the leading cases of ocular infection with prevalence of 46.9%, 41% and 52% respectively. In contrast to our study; however, one finding in southwest Ethiopia [5] reported blepharoconjunctivitis (48.6%) as the predominant types of external ocular infection. The differences within the same area might be due to differences in study period, sample size and varied inclusion of cases.

The overall prevalence of 46.1% bacterial external ocular infections was observed in this study. This result is comparable with other previous study reports conducted in Southern Ethiopia [9] with prevalence of 48.8 %. However, our result is lower than the prevalence of 88%, 74.9% and 74.7% conducted in India [38], Nigeria [7] and Southwest Ethiopia [5] respectively. But it is higher than the study conducted in Bangalore [25] and Japan [27] with prevalence of 34.5% and 32.2% respectively. The varied rate of isolation from one place to another or within the same place might be due to different factors related to (sample collection, sample transportation, standard of media used and culturing technique), different distribution of bacterial aetiology with geographic variation, differences in study period with the study population, varied cases inclusion and infection prevention practices.

In this study, the most common isolates observed were gram positive cocci (65.3%). This result is very comparable with other previous study conducted in Ethiopia [12], Nigeria [46] and India [77] with prevalence of 74.2%, 62.6% and 51.4% respectively, indicative of gram positive cocci as primary cause of external ocular infection. In our study, CoNS (27.9%) were the predominant isolates followed by *S. aureus* (19.7%) and *S. pneumoniae* (8.8%). This result is in line with previous study conducted in Ethiopia [48, 78], India [79] and Uganda [80] , where CoNS were the predominant isolates (29-66%). However, study conducted in Ethiopia [5, 9] and Nigeria [45] reported *S. aureus* (28.4%, 21%, and 27.7%) respectively as a predominant isolate while higher

prevalence of *S.pneumoniae* (26.4%) in India [81] and *P. aeroginosa* (52.5%) in Ghana [81] were reported. This difference might be due to variation in environmental conditions, the standard of personal hygiene, limited sample size and differences in study period.

In our study, high prevalence of *S. aureus* and CoNS were observed. Both coagulase negative and positive staphylococci are responsible for a variety of anterior and posterior segment of eye infections. Over the past 15 years, there has been an increase in the documentations of ocular infections caused by CoNS [82]. In recent years, CoNS become an important nosocomial pathogen and health-care related infections partly as results of the increasing use of medical devices [83]. Bacterial endophthalmitis, the most severe form of vision threatening ocular infection may follow surgery, trauma or may be of endogenous origin. CoNS are the most common cause of postoperative endophthalmitis worldwide. Study done in USA [33], Singapore [34] and India [35] showed high prevalence of CoNS (49.9%, 57% and 62.6%) respectively in postoperative endophthalmitis. CoNS also rank first among bacteria causing post-traumatic endophthalmitis [84].

In the present study, the prevalence of gram negative bacterial isolate among patients suffering from external ocular infection was 34.7%, with *P. aeroginosa* (6.8%) as the leading gram negative bacterial isolate followed by *K. pneumoniae* (6.1%). Similar studies in different area [5, 30, 36, 45] also reported *P. aeroginosa* as the most frequent isolate with prevalence ranged from 10-20%. In contrast to our finding; however, other studies reported *K. pneumoniae* as leading isolate in Libya [10] with prevalence of 18%. The differences might be due to geographical variation, the standard of personal hygiene, limited sample size, differences in study period and inclusion or exclusion of different cases.

Among the clinical features, the predominant isolate observed in the cases of bacterial conjunctivitis was, CoNS (21.3%) followed by *S. aureus* (20.0%) which are responsible to variety of eye infection including self-limiting, an acute and chronic infection of conjunctiva worldwide. This is in agreement with study conducted in Ethiopia [9, 48], Uganda [80] and India [77] where high isolation rate of CoNS and *S. aureus* were reported in conjunctivitis with prevalence ranged from 13-50%. However, previous studies conducted in southwest Ethiopia [5] showed *S. aureus* (22.9%) and *S. pneumoniae* (20%) as the predominant isolate in cases of

bacterial conjunctivitis. This variation might be due to differences in study period, sample size and inclusion of varied cases in study population.

In our study, CoNS (31.2%) and *S. aureus* (25%) were predominantly isolated in blepharitis. This is consistent with study done in Southern Ethiopia [9], India [39], Nigeria [46], Libya [10] and Iran [85], reported high isolation rate of CoNS and *S. aureus* in blepharitis with prevalence ranged from 22-45%. In dacryocystitis CoNS (37.5%) followed by *S. pneumoniae* (25%) and *S aureus* (25%) were the predominant isolate in our study. Similar study conducted in Ethiopia [9, 78], Malaysia [86] and Nepal [87] reported CoNS, *S. pneumoniae* and *S. aureus* as predominant isolate in dacryocystitis with prevalence ranged from 12-33%. However, study conducted in Tigray [49] and Gondar [48] reported *S. aureus* (25%, 20%) and *S. pyogenes* (25%, 20%) respectively as predominant isolate in dacryocystitis. The variation might be due to different distribution of bacterial aetiology with geographic variation, differences in study period, study population and sample size.

The current study showed high rate of resistance to different antimicrobial agents in both gram positive and gram negative bacterial isolate, which is consistent with study done in Ethiopia [5, 48], Libya [10] and Uganda [80]. Remarkably high frequency of resistance to ampicillin, penicillin, erythromycin, trimethoprim sulphamethoxazole, tobramycin and tetracycline has been observed in our study. This might be due to availability (easily purchased), indiscriminate (unrestricted) use of the drugs without prescription, prescription of antibiotics without susceptibility testing for severe ocular infections, the cost and shortage of diagnostic laboratory services for susceptibility test and or unavailability of updated guideline regarding the selection of drugs are some of the factor which lead to th development of high resistance rate.

In our study, the overall resistance rate of isolated *staphylococcus species* to commonly prescribed antibiotics were: ampicillin (82.8%), tetracycline (73.3%), erythromycin (78%) and penicillin (84.3%). This is consistent with previous study conducted in Ethiopia [9, 12] ranged from (65-95%) but lower resistance rate of tetracycline and erythromycin (31-64%) were reported in Ethiopia [5, 12] and Uganda [80]. Higher resistance rate of *S. pneumoniae* to tetracycline (61.5%) was also observed in our study when compared to previous study report [5, 9] (20% and 30%) respectively. In this study, *P. aeroginosa* was highly resistant to ceftazidime

(90%) and tetracycline (80%). This is consistent with study done in Ethiopia [5] where *P. aeroginosa* was highly resistant to tetracycline (71%) but higher resistant rate of *P. aeroginosa* to chloramphenicol (80%) was reported in Nigeria [45]. Others gram negative isolates were highly resistance to tetracycline and tobramycin ranged from 40-80%. Epidemiological factors, local antibiotic policies, patients' characteristics, origin of the strains, study period and geographic location are among the factors contributing to highly variable resistance rates.

In the present study, all gram positive bacterial isolate were highly sensitive to amoxicillin-clavulanic acid, ciprofloxacin and gentamicin within the susceptibility range of 69-100%. This is comparable with previous study done in Ethiopia [9, 48, 49] and India [88] with susceptibility rate ranged from 50-100%. In contrast to our finding; however, previous study conducted in southwest Ethiopia [5] reported gram positive bacterial isolates as greater sensitive to tetracycline (60-73%) than our study (17-40%). This variation might be due to differences in study period, indicative of increasing resistance to earlier generation ocular antibiotics. Most gram negative bacteria isolated in our study were sensitive to Amoxicillin-clavulanic acid, ciprofloxacin, ceftriaxone and gentamicin within the range of 66-100%. This is in agreement with previous study done in different area [9, 48, 89] with susceptibility rate 60-100%.

In current study, 13.8% of *S. aureus* strain was MRSA and 29.3% of Coagulase negative staphylococcus was MRCoNS (Cefoxitin disk used). Our finding is comparable with other study reported in India [39] where 12.8% of *S. aureus* was MRSA in ocular infection. In contrast to our finding, study done in Uganda [80] reported higher MRSA (27.6%) but comparable MRCoNS (31.9%). Lower MRSA was reported in UK [40] with prevalence of 3%. The variation might be due to different distribution of bacterial strains with geographic variation and study period. The most common ocular infections resistant to methicillin were conjunctivitis. This is similar with previous study done in UK [90]. In our study, MRSA was highly sensitive to chloramphenicol (75%) but highly resistant to clindamycin, tetracycline and gentamicin. Similar study reported chloramphenicol as clinically effective in MRSA (>81%) isolated from conjunctivitis cases and highly resistant to clindamycin, tetracycline and gentamicin [41, 42].

The ophthalmologist's practice in view of increasing bacterial resistance should be changed. Health care workers, including those in outpatient settings, must remove transient

microorganisms from hands by using hand washing or hand antisepsis between all patient contacts and after contact with inanimate objects in the immediate vicinity of patients. Eye-lane surfaces and hand instruments should be cleaned periodically in order to prevent and control MRSA and other bacterial transmission [89]. Povidone–iodine is the most effective agent for decreasing the bacterial load that can exist on the eyelid margin and it is better to use for the eradication of microbes including MRSA [43]. By *in vitro* study, riboflavin, and UV light were also effective in eradicating MRSA [44].

In this study, about 66.1% of the isolates showed ability to produce biofilm that ranged from weak to strong forming ability. This can be contribute to drug resistance development and plays a vital role in pathologic processes. This finding is comparable with study conducted in Chicago [56] with biofilm formation rate of 57.7%. However, lower biofilm formation rate was reported in Egypt [57] (33.3%). Higher biofilm formation rate was reported in Saudi Arabia [58] (90%). The variation might be due to differences in geographical location, differences in study period with the study population and variety of origin of the strains. Biofilm producing bacteria are responsible for many recalcitrant infections and are notoriously difficult to eradicate. It is a well-known pathogenic mechanism in most bacterial microorganism. They exhibit resistance to antibiotics by various methods like restricted penetration of antibiotic into biofilms, decreased growth rate and expression of resistance genes.

In our study, *P. aeruginosa* (80%), *K. pneumoniae* (77.8%), CoNS (75.6%), *S. aureus* (72.4%) and *E. coli* (60%) were the leading biofilm former. This is comparable with study done in other place [59, 60], where higher biofilm formation rate of *S. aureus* (51.9%) *and* CoNS (66%) respectively. In contrast to our finding, however; lower biofilm formation rate of *P. aeroginosa* (60%), *S,aureus* (36.4%) and *K. pneumoniae* (11.1%) were reported in Egypt [57]. Higher biofilm formation rate of *E.coli* (80%) was reported in India [61]. The variation might be due to differences in geographical location, study period and variety of origin of the strains.

Formation of biofilms allows organisms to survive and thrive in hostile environment, disperses to form new niches and gives them significant advantages in protection against environmental fluctuations. Bacteria in biofilms display different patterns of gene expression and phenotypes, reducing their metabolic rate and increasing cell-to-cell communication while becoming less

sensitive to chemical and physical stresses, and they show increased chances of developing new antibiotic resistances and may further complicate patient treatment. The exopolysaccharide component of the biofilm matrix can function to impair antibiotic penetration and provide a barrier against phagocytosis by host immune cells. Oxygen does not diffuse freely through the biofilm structure, leading to the formation of an oxygen concentration gradient, which generates anaerobic microenvironments [50, 51].

In our study multiple drug resistance (MDR) to three or more antimicrobial agents were observed in 101(68.7%). This is comparable with study conducted in northern Ethiopia [47] and southern Ethiopia [9] with resistance rate of 71% and 69.9% respectively. In contrast to our finding; however, study done in Ethiopia [41] reported higher MDR (87.1%). Lower MDR was reported in Ethiopia [49], China [42] and Uganda [80] with resistance rate of 34.8%, 13% and 28% respectively. The variation might be due to differences in study period or the difference in type and generation of antibiotic that we used for susceptibility testing or differences in number of antibiotics classes to be considered as MDR. It has been suggested that the indiscriminate and prolonged use of a wide range of antibiotics, lack of infection control and increasing frequency and speed of travel might be a major factor leads to emergency of drug resistance strains

In the current study, we observed that strains capable of forming biofilms were more frequently observed to be an MDR phenotype. MDR in biofilm forming strains shows that; biofilms play a great role in antimicrobial resistance. Similar study [75, 76] reported biofilm formation is higher in MDR. Phenotypic changes, Physiological heterogeneity, Low diffusion of antibiotics across the matrix, elevated expression of efflux and quorum-sensing may be some of the reason for this high resistance. *S. aureus* and CoNS ability to form biofilm was observed in strains to be resistant to aminoglycosides, penicillins, fluoroquinolones, folate pathway inhibitors and tetracycline. Similarly, most of gram negative bacterial isolates ability to form biofilm were observed in species to be resistant to aminoglycosides, cephalosporin, folate pathway inhibitors and phenicols. Similar previous studies report have shown that biofilm formation is higher in similar antimicrobial agents [62-64].

7. Limitation of the study

1. Tests for anaerobic bacteria and *Chlamydia trachomatis* were not included in the current research. Moreover, viral, fungi and parasite that bring ocular infection were not performed.
2. Anti-biofilm susceptibility of the biofilm former bacterial isolates were not done due to lack of antimicrobial powder constituent for agar dilution.

8. Conclusion and Recommendation

8.1 Conclusion

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.

- ☞ The most common isolate from external ocular infection was Coagulase negative staphylococcus followed by *Staphylococcus aureus, Streptococcus pneumoniae, Pseudomonas aeroginosa* and *Klebsiella pneumoniae*
- ☞ High resistant rate was observed to ampicillin, penicillin, erythromycin, trimethoprim sulphamethoxazole, tobramycin and tetracycline.
- ☞ Amoxicillin-clavulanic acid, ciprofloxacin and gentamicin were the most effective against gram positive bacterial isolate
- ☞ Ceftriaxone, gentamicin, amoxicillin-clavulanic acid and ciprofloxacin were the most effective against gram negative bacterial isolate.
- ☞ The overall bacterial isolate resistant to three or more antibiotics (MDR) was 68.7%. Gram positive bacterial isolates account 74.3% while 25.7% of gram negative bacterial isolates showed MDR.
- ☞ The first-line empirical antibiotics therapy used for ocular infection treatment like penicillin, tetracycline, erythromycin and ampicillin should be taken into consideration because they showed high resistance to the commonly isolated bacteria
- ☞ By cefoxitin disk test, 13.8% of *Staphylococcus aureus* strain was MRSA and 29.3% of coagulase negative staphylococcus was methicillin resistant (MRCoNS).

- *Pseudomonas aeruginosa* (80%) was the leading biofilm former followed by *Klebsiella pneumoniae* (77.8%), Coagulase negative staphylococcus (75.6%) and *Staphylococcus aureus* (72.4%) including weak to strong biofilm former.

8.2 Recommendation

- The antibiotic sensitivity profile suggests that ciprofloxacin, gentamicin, amoxicillin-clavulanic acid and ceftriaxone were more effective for the treatment of external ocular infection.
- Identification of the specific aetiologic agent along with their antibiotic resistance patterns should be practiced during the management of ocular infections to reduce further emergence of multidrug-resistant bacteria.
- An updated local antibiotics policy and guidelines should be established or revised from the previous and distributed to all responsible body to preserve and increase the effectiveness and rational use of antibiotics for better management of ocular infection.

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ANNEXS

ANNEX I፡ Participant information sheet: English, Oromic and Amharic version

A. English Version

Name of the Organization: Jimma University, Institute of Health, School of Medical Laboratory Science, Department of Medical Microbiology.

Title of the Research Project: 'Antimicrobial susceptibility pattern and biofilm forming potential of bacteria isolated from suspected external ocular infected patient in Jimma University Medical Center, Jimma, Ethiopia" among patients with external ocular infection at Jimma University Medical Center Ophthalmology Department, Jimma, Ethiopia.'

Name of Investigator: Kuma Diriba (BSc, MSc candidate)

Introduction

You are invited to participate in a study to be conducted by MSc student at Jimma University, Institute of Health, School of Medical Laboratory Science. It is aimed at determining the spectrum of bacterial isolates, biofilm forming potential of the isolated bacteria and their drug susceptibility pattern among patients with external ocular infection at Jimma University Medical Center eye clinic Jimma, Ethiopia.

Objective of the study

The objective of this research is to determine profile of bacterial isolate, biofilm forming potential of the isolated bacteria and their antibiotics susceptibility pattern on external ocular surface at Jimma University medical center eye clinic.

What will be expected from you as a participant of the study?

As a participant of this study you will be expected to agree to give sample. Then external ocular sample will be collected for diagnostic purpose by responsible ophthalmologist or trained nurse from your eyes. In addition you will be expected to give answers for some questions about your health and socio demographic conditions. You need to know that the results might be diskussed

with appropriate individuals out of this hospital. But your name, address and phone number will not be disklosed and rather than identification code will be used in such conditions.

How much time the participant spent to participate in this study?

You will spend about 10-15 minutes until the specimen will be collected, the questionnaire will be filled and the consent will be signed.

What will be the risks of participating in this study?

The risk associated with the specimen collection will be 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 that will not harm the eye at all.

How our information will be kept in secret? (Confidentiality)

All information that you give and the results from you or your child's specimen will be used for this study only. Only limited number of professionals will have access to the information. All the information will be encoded in a computer and will be password protected.

What will be the benefits from participation?

Since this study is MSc student research, there will be no payment for participants and you will be not asked to pay for the laboratory examination. The result will be given to you and if your result will be clinically significant, it will help you for further diagnosis and treatment.

What will be your rights as a participant of this study?

Participation in this study is exclusively voluntarily. If you are not interested to participate or if you once decide to participate and with draw at any time, there will be no consequences and you or your child will get all the services provided in the hospital will not be diskontinued. You have also welcomed if you have any question for further explanations about the study. You can get the results of the analysis.

What can I do if I have a problem or question?

Please direct any questions or problems you may encounter during this study to (investigator, advisors and co-advisors)

1. Kuma Diriba (BSc)
   Cell phone: +251-913-38-45-50 [Email: kumadiriba47@gmail.com]
2. Dr. Tesfaye Kassa (MSc, PhD)
   Cell phone: +251-931-05-71-95 [Email: ktes@gmail.com]
3. Mr. Yared Alemu (BSc, MSc)
   Cell phone: +251-917-80-25-31 [Email: yared.alemu6@gmail.com
4. Dr.Sisay Bekele (MD)
   Cell phone: +251-920-22-75-17 [Email: sisayop@gmail.com]

B. Garagalcha Afaan Oromo

Maqaa dhaabbataa: Yuuniveersiitii Jimmaatti Muummee fayyaa
Mata duree Qorannichaa: Baakteriyaa dhukkuba ijaa fidan adda baasuu fi qorannoo qoricha isaan balleessu irratti gochuu, Kibba Lixa Itoophiyaa, 2017.
Maqaa qorataa: Kumaa Diribaa
Maqaa Ispoonseeraa: Yuuniveersiitii Jimmaa.
Unka kun fedhii maamiltootni dhukkuba ijaa qaban qorannoo kana irratti fedhii isaaniin, dhimma kana keessa beekuun irratti hirmaachuuf waadaa seenanii dha.

seensa

Kaayyoon Unki kun qophaa'eef inni guddaan hirmaattootni qorannoo mata duree "Baakteriyaa dhukkuba ijaa fidan adda baasuu fi qorannoo qoricha isaan balleessu irratti gochuu" jedhu fi bara 2017 geggeffamu irrati namootni hirmaatan fedhii isaanii kan ittiin mirkanneffatanii dha.

Yeroo qo'annichaa

Qorannoon kun kan gaggeeffamu ji'a sadiif yemmuu ta'uu, akkaataan funaansa isaa maamila irraa saamuda fudhachuun Laaboraatooriin qorachuun raawwatama. Qorannoon tokkoo sa'aatin inni fudhatu sa'a 24 hanga sa'a 48 ti.

Miidhaa inni geessu

Qorannoo kanatti hirmaachuun miidhamni gama fayyaan mul'atuu fi isin irraa ga'uu danda'u tokko illee kan hin jirree fi yeroo saamuda fudhatamu miira dhukkubbii xiqqoo irra kan hafe rakkoo biroo kan hin qabneedha.

Bu'aa

Bu'aan adda yookin kafaltiin hirmaachuun argamu hin jiru. Haa ta'uu malee qorannoon kun kafalti malee bakteeri'a dhukkubicha ijaa fiduu fi qorichi isa balleessu adda baafame kan isinitti humamu akkassumas qorannoon kun tajaajilli isin argachaa jirtanii akka kana caalaa fooyya'uuf shoora olaanaa taphata.

Iccitii

Mirgi sagalee keessan bilisan kennuu fi Iccitiin isaa sirriitti eegama. Tarii dhoksaatti sagalee keessan lachuu yoo barbaadan mirga guutuu qabachuu keessan isinii mirkaneessaa odeeffannoon isin irraa argamu lakk. dhoksaa (koodii) waan funaanamuuf odeeffannoo isin laatan eenyuu illee adda baasee beekuu hin danda'u.

Beenyaa

Beenyaan adda yeroo keessaniifis ta'ee haala biraaf kaffalamu hin jiru. Garuu qorannoon laaboratoori tola kan isini hojjattamuu fi hirmaachuun keessan tajaajilichaa fooyyessuu keessatti qooda bakka hin buune qaba.

Fedhii hirmaachuu

Qorannoo kana irratti hirmaachuu dhiisuuf mirga guutuu qabdu. Kana malees erga jalqabdan giddutti kutuuf mirgi keessan eegama.

Teessoo

Yoo gaaffii qabatan amma bilisa taatanii nagaafachuu ni dandeessu. Kana malees gaaffii kamiyyuu yoo qabbattan namoota armaan gadi dubbisu dandeessu.

1. Obbo Kuma Diriba (BSc)
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C. **የአማርኛ ግልባጭ**

የድርጅቱ ስም :- ጂማ ዩኒቨርሲቲ የእክምና መእከል

የጥናቱ ርእስ፡- የዉጪኛው የአይን ኢንፌክሽን ተህዋስያን የሚያመጣውን ህመም እና የተህዋስያኑ መድሃኒት የመቋቋም ያለዉን የስርጭት መጠን በጅማ ዩኒቨረሲቲ የእክምና መእከል በአይን ኪሊኒኪ ለማወቅ፡፡

የተመራማሪ ስም፡ ኩማ ዲሪባ

የአማካሪዎች ስም፡ ዶ/ር ተስፋየ ካሳ
አቶ ያረዲ አለሙ
ዶ/ር ስሳይ በከለ

የእስፓንሰር ስም :- ጂማ ዩኒቨርሲቲ

የጥናቱ ዓላማ

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

ጥናቱ የሚያስገኘው 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፡፡

ስጋትና ጉዳት

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

ምስጢራዊነት

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፡፡

ከጥናቱ ስለማቋረጥ፡

በጥናቱ የሚሳተፉት ፈቃደኛ ከሆኑ ብቻ ነው፡፡ ስለዚህ መሳተፍ ከጀመሩ በኋላ ማቋረጥ ወይም አለመሳተፍ የማይፈልጉት ከሆነ ይለፈኝ ማለት ሙሉ መብትዎ ነው፡፡ በጥናቱ መሳተፍ ወይም አለመሳተፍ አገልግሎት ላይ ምንም አይነት ጥቅምም ሆነ ጉዳት አይኖረውም፡፡ ጊዜዎትን መሰዋት አድርገው ስለተባበሩኝ ከልብ አመሰግናለሁ፡፡ በተጨማሪ መረጃ ማነጋገር ብትፈልጊ ማንኛውም ጥያቄ ቢኖርሽ አሁን ወይም ሌላ ጊዜ የሚከተሉትን ሠዎች በሚከተለው አድራሻ ማግኘት ትችያለሽ፡፡

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ANNEX II: Consent form for adult patients: English, Oromic and Amharic version

A. English Version

Participant Code Number__________

I have been informed fully in the language I understand about the aim of this research. I understood the purpose of the study entitled with "bacterial profile, biofilm forming potential and antimicrobial susceptibility pattern of external ocular infections attending department of ophthalmology in Jimma University Medical Center. 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 informed that medical history and ocular sample will be taken and there will be interview. In addition I have been told all the information collected throughout the research process will be kept confidential. I understood my current and future medical services will not be affected if I refused to participate or with draw from the study. I ____________________, after being fully informed about the detail of this study, hereby give my consent to participate in this study and approve my agreement with signature.

Patient Name ___________________ signature ______ Date___________
Investigator name _________________signature _______Date__________

B. Garagalcha Afaan Oromo

Lakkoofsa hirmaataaf kenname ………………

Yommuun qorannoo kana irratti hirmaadhu afaan naaf galuun natti himameera ykn naaf ibsameera.Faayidaa qorannoo kanaatis ''Baakteriyaa dhukkuba ijaa fidan adda baasuu fi qorannoo qoricha isaan balleessu irratti muummee fayyaa Jimmaa Universiititti godhamu'' naaf galeera. Waa'ee dhukkubbii ijaa akkan gaafatamuu fi naamuda ija irraa akka kennamu naaf himameera. Odeeffannoo qorannoo kana irraa argamu hunduu iccitiin akka kaa'amus irratti walii galleerra. Qorannoo kana hirmaachuu yoon hin barbaadne ykn yoon addaan kute, ammas ta'ee

fulduraaf fayyadamummaa kiyyarratti rakkoo tokkoollee akka hin uumnee naaf himameera. Ani ____________________________ erga naaf gale booda mallattoo kootin nan mirkaneessa.

Maqaa dhukkubsataa………………………. Mallattoo ……… Guyyaa……….
Maqaa qo'ataa……………………………Mallattoo……… Guyyaa………

C. የአማርኛ ግልባጭ

የተሳታፊው ልዩ መለያ ቁጥር __________

እኔ ስሜ ከታች የተጠቀሰው ተሳታፊ የዉጪኛው የአይን ኢንፌክሽን ተህዋስያን የሚያመጣውን ህመም እና የተህዋስያኑ መድሃኒት የመቋቋም ያለዉን ስርጭት በአይን ህሙማን ላይ ምን ያህል እንደሆነ ለማውቅ የተዘጋጀ ጥናት ላይ እንድሳተፍ ተጠይቄ ስለጉዳዩም ለመረዳት በቂ መረጃ አግኝቻለሁ፡፡ ስለሆነም ናሙና የሚሰበሰበው ከታችኛው የአይን ሽፋን ዉስጥ እና ከኮንጃቲቫ 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 ፡፡ በተጨማሪም የሚወሰዱ ማናቸውም መረጃዎች በሚስጥር እንደሚያዙ ተነግሮኛል፡፡ እንዲሁም የምጠየቀውን መረጃ ያለመስጠትና በጥናቱ ያለመሳተፍ ከጥናቱ በማናቸውም ወቅት ራሴን ማግለል እንደምችል የተገለፀልኝ ሲሆን ይህንንም በማድረጌ ወደፊትም ሆነ አሁን የማገኛቸውን የህክምና ግልጋሎቶች እንደማይጓደሉብኝ ተነግሮኛል፡፡ እንዲሁም በጥናቱ ሂደት እንድሳተፍ ፍቃደኝነቴን በፊርማዬ አረጋግጠለሁ፡፡

የታካሚ/ የተሳታፊ ስም --------------------------------ፊርማ --------------- ቀን ----------------
የተመራማሪ ስም --ፊርማ --------------- ቀን ----------------

ANNEX III: Parental/guardian consent form: English, Oromic and Amharic version

A. English Version

I_______________________ parent/guardian, after being fully informed about the purpose of this study, titled "Antimicrobial susceptibility pattern and biofilm forming potential of bacteria isolated from suspected external ocular infected patient" attending JUMC, department of ophthalmology. I, the undersigned, have been told about this research. My child/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 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/guardian. By signing below, I agree to let my child to participate in this research study.

Name of participant__________________ Signature _______________ Date ____/____/___

Witness (Illiterate) ___________________ Signature _______________ Date ____/____/___

Name of the researcher ________________ Signature _______________ Date ____/____/___

B. Garagalcha Afaan Oromo

Ani ____________________ Kan daa'ima yookin kan guddise qabadhe Yommuun qorannoo kana irratti hirmaadhu afaan naaf galuun natti himameera ykn naaf ibsameera.Faayidaa qorannoo kanaatis ''Baakteriyaa dhukkuba ijaa fidan adda baasuu fi qorannoo qoricha isaan balleessu irratti, muummee fayyaa Jimmaa Universiititti godhamu '' naaf galeera. Waa'ee dhukkubbii ijaa akkan gaafatamuu fi saamuda ija daa'ima yookin kan guddise irraa akka fudhatamu naaf himameera. Odeeffannoo qorannoo kana irraa argamu hunduu iccitiin akka kaa'amus irratti walii galleerra. Qorannoo kana irratti daa'ima koo yookin kan guddise hirmaachisuu yoon hin barbaadne ykn yoon addaan kute, ammas ta'ee fulduraaf fayyadamummaa kiyyarratti rakkoo

tokkoollee akka hin uumnee naaf himameera. Ani erga naaf gale booda mallattoo kootin nan mirkaneessa.

Maqaa hirmaata______________________ Mallattoo _______________ Guyyaa ____/____/___
Ragaa (kan hinbaranne) ________________ Mallattoo _______________ Guyyaa ____/____/___
Maqaa qorata ________________________ Mallattoo _______________ Guyyaa ____/____/___

C. የአማርኛ ግልባጭ

እኔ--የልጄ አስታማሚ ቤተሰብ ወይም የታማሚው አሳዳጊ/ሞግዚት ስሆን የዚህን ጥናት አላማ በዉል ተረድቻለሁ፡፡ የጥናቱ ርዕስ በጅማ ዩኒቨረሲቲ የእክምና መእከል በአይን ኪሊኒክ በተመላላሽ የአይን ታካሚዎች የዉጪኛው የአይን ኢንፌክሽን ተህዋስያን የሚያመጣውን 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፡፡ ከዚህ ባሻገር የልጄ በጥናቱ ውስጥ ለመካተት የእኔ የወላጁ/አሳዳጊ ፈቃድ እንደሚያስፈልግ ተረድቻለሁ፡፡ በእኔ ፍቃደኝነት ልጄ በጥናቱ እንደሚሳተፍ ከዚህ በታች በፊርማዬ አረጋግጣለሁ፡፡
የተሳታፊው ሥም________________ ፊርማ _______ ቀን ___/___/__
ምስክር (ማንበብና መፃፍ ለማይችሉ) _________ ፊርማ ____ ቀን ___/___/__
የተመራማሪው ስም_______________ ፊርማ ______ ቀን ___/___/__

ANNEX IV: Assent form for adolescent: English, Oromic and Amharic version

A. English Version

Study title: "Antimicrobial susceptibility pattern and biofilm forming potential of bacteria isolated from suspected external ocular infected patient" attending Jimma University Medical Center, department of ophthalmology. I, the undersigned, have been got full information 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 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 participant__________________ Signature _______________ Date ____/____/___
Witness (Illiterate) ___________________ Signature _______________ Date ____/____/___
Name of the researcher ________________ Signature _______________ Date ____/____/___

B. Garagalcha Afaan Oromo

Ani kan armaan gaditti mallatteesse akkan qorannoo kana keessatti hirmaadhu gaafatameera. Maatiin koos akkan itti hirmaadhu yookin akkan hin hirmaanne filannoo mataakoo naaf kennaniiru. Yommuun qorannoo kana irratti hirmaadhu dhukkubbii xiqqoon miira iirratti dhagahamuun alatti miidha kan hinqabne ta'uun afaan naaf galuun natti himameera ykn naaf ibsameera.Faayidaa qorannoo kanaatis ''Baakteriyaa dhukkuba ijaa fidan adda baasuu fi qorannoo qoricha isaan balleessu irratti gochuu'' naaf galeera. Waa'ee dhukkubbii ijaa akkan gaafatamuu fi saamuda ija irraa akka fudhatamu naaf himameera. Odeeffannoo qorannoo kana irraa argamu hunduu iccitiin akka kaa'amus irratti walii galleerra. Qorannoo kana irratti hirmaachuu yoon hin barbaadne ykn yoon addaan kute, ammas ta'ee fulduraaf fayyadamummaa

kiyyarratti rakkoo tokkoollee akka hin uumnee naaf himameera. Ani erga naaf gale booda fedha kootiin hirmaachuu mallattoo kootin nan mirkaneessa.

Maqaa hirmaata______________________ Mallattoo _______________ Guyyaa ____/____/___
Ragaa (kan hinbarannef) ________________ Mallattoo _______________ Guyyaa ____/____/___
Maqaa qorata _______________________ Mallattoo _______________ Guyyaa ____/____/___

C. የአማርኛ ግልባጭ

በአማርኛ የተዘጋጀ ለወጣት ታዳጊ የጥናት ተሳታፊዎች የተሳትፎ ማራጋጋጫ ቅጽ፡፡ ከዚህ በታች ስሜ የተገለፀው በዚህ ጥናት ውስጥ እንድሳተፍ ፍቃደኝነቴን ተጠይቂያለሁ፡፡ ወላጆቼም/ አሳዳጊዎቼም በጥናቱ እንድሳተፍ ወይም እንዳልሳተፍ ምርጫው የእኔ መሆኑን ነግረውኛል፡፡ ናሙና ሲወሰድ ከትንሽ የህመም ስሜት ዉጪ ምንም አይነት ጉዳት እንደሌለዉ ተነግሮኛል፡፡ በጥናቱ ወቅትም የእኔ መረጃዎች 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፡፡ ከዚህ ባሻገር የኔ በጥናቱ ውስጥ ለመካተት የወላጆቸም ወይም የአሳዳጊዎቸ ፈቃድ እንደሚያስፈልግ ተረድቻለሁ፡፡ በፍቃደኝነቴ በጥናቱ እንደምሳተፍም ከዚህ በታች በፊርማዩ አረጋግጣለሁ፡፡
የተሳታፊው ሥም______________ፊርማ ________ ቀን ___/___/__
ምስክር (ማንበብና መፃፍ ለማይችሉ) _________ ፊርማ _____ ቀን ___/___/__
የተመራማሪው ስም______________ ፊርማ ________ ቀን ___/___/__

ANNEX V: Questionnaire :- English, Oromic and Amharic version

Questionnaire on Socio-demographic characteristics and clinical feature of the study participants on the title "antimicrobial susceptibility pattern and biofilm forming potential of bacteria isolated from suspected external ocular infected patients attending Jimma University Medical Center, department of ophthalmology", Southwest Ethiopia, 2017.

A. Questionnaire English Version

Socio-demographic Characteristics and Patient Identification

Instruction: Mark or response to the given question on the space provided

Sr. No	I. Background information	
	Questions	**Response**
1	Patient code/ID	
2	Patient Card No.	
3	Age in years	
4	Sex	1. Male 2. Female
5	Salary(Income)/Month	
6	Residence	1. Rural 2. Urban
7	Ethnicity	1. Oromo 4. Kefa 2. Amhara 5. Dawuro 3. Tigray 6. Other (specify)_________
8	Educational status	1. Illiterate 5. Collage and above 2. Preschool 3. Elementary school 4. Secondary school
9	Marital status	1. Single 4. Divorced 2. Married 5. Widowed 3. Separated
10	Occupational status	1. Civil servant 5. Student 2. Farmer 6. Under age 3. Merchant 7. Others 4. House wife
11	What is the source of light in your home?	1. Use of wood for light 2. Use of kerosene for light 3. Use of electric for light

		4. other source of light
12	What is the source of fire used for cooking in your home	1. Wood 3. Kerosine 2. Electric 4. Other_____________
II. Clinical data		
13	Which systemic (chronic) diseases do you have?	1. Rheumatoid and Arthritis 3. Diabetes 2. Blood pressure 4. Others_____
14	Types of diagnosis	1. Conjunctivitis 3. Blephero-conjunctivitis 2. Blepharitis 4. Dacryocystitis
15	Having been hospitalized in previous time for eye infection?	1. Yes If yes, when __________ 2. No
16	Did you take a medicine to treat these infections?	1. Yes if yes, when ___________ 2. No
17	Are you using medical contact lenses?	1. Yes 2. No
18	Did you make surgery of eye in previous time?	1. Yes If yes, when _________ 2. No
19	Did you use traditional eye medicine?	1. Yes 2. No
20	Which eye is involved	1. Right 2. Left 3. Both
Date of specimen collected and time _______________/_______________		

III Comments

____

____

Name of principal investigator ____________________ Signature ________ Date _________

B. Garagalcha Gaaffii Afaan Oromo

Gaaffilee waa'ee odeeffannoo hawwasummaa fi ragaa fayyummaa hirmaataa qorannoo kanaatif mata duree "Baakteriyaa dhukkuba ijaa fidan adda baasuu fi qorannoo qoricha isaan balleessu irratti gochuu" jedhu irratti muummee fayyaa Jimmaa Universititiif dhiyaate, Kibba Lixa Itoophiyaa, 2017.

Ajajaa: Gaaffilee armaan gadiitif deebii sirrii deebisi yookin filadhu

Sr. No	**I. Odeeffannoo hawwasummaa**	
	Gaaffii	**Deebii**
1	Koodii dhukkubsata	

2	Lakk. Kaardii dhukkubsata	
3	Umurii	
4	Saala	1. Dhiira 2. Dubara
5	Galii ji'aan argatu/ttu	
6	Bakka jireenya	1. Magaalaa 2. Baadiyyaa
7	Sab-lammii	1. Oromoo 4. Kefa 2. Amaara 5. Dawuro 3. Tigrayi 6. Kan 68iro______
8	Sadarkaa barumsaa	1. Kan hinbaranne 2. Kan umuriin hin geenye 3. Sadarkaa tokkoffaa 4. Sadarkaa lamaffaa 5. Kolleegii fi isa ol
9	Haala fuudhaa fi heerumaa	1. Kan hineerumne 4. Kan wal hiikan 2. Kan heerumte 5. Kan irra du'e 3. Kan gargar jiratan
10	Haala hojii	1. Hojjataa mootummaa 2. Qotee bulaa 3. Daldalaa 4. Hojattu mana keessa 5. Barataa 6. Umuriin kan hin geenye 7. Kan biro
11	Maddi ifa mana keetti fayyadamtu maali?	1. Muka akka madda ifaatti fayyadama 2. Kuraazi akka madda ifaatti fayyadama 3. Elektirikii akka madda ifaatti fayyadama 4. Madda ifaa kan biro _______________
12	Maddi annisa hoo'a mana keettiti fayyadamtu maal?	1. Muka irra 3. Gaazii irraa 2. Elektiriki 4. Kan biro
II.	**Ragaa fayyaa**	
13	Dhukkuba qaama miidhan kam qabda?	1. Dhukkuba qurxumaata 3. Dhibee sukkaara 2. Dhiibbaa dhiigaa 4. Others_____
14	Gosoota dhukkuba ijaa	1. Konjaktivayiti 3. Bilefarayiti-konjaktivayiti 2. Bilefarayiti 4. Daakirosistayiti
15	Kana dura ija dhukkubsattee mana yaalaa deemtee beetta?	1. Eeyyee Yoo ta'e, yoom? _________ 2. Lakki

16	Dhibee ijaatif qoricha fudhattee beetta?	1. Eeyyee Yoo ta'e, yoom? ________ 2. Lakki
17	Leensis ijaa fayyadmte beekta	1. Eeyyee 2. Lakki
18	Ijji kee kana dura opireshiini ta'e beeka?	1. Eeyyee Yoo ta'e, yoom? _________ 2. Lakki
19	Qoricha aadaa fayyadamte beettaa?	1. Eeyyee 2. Lakki
20	Ija naamudi irra fudhatame	1. Kan mirgaa 2. Kan bitaa 3. Lamanuu irra
Guyyaa fi sa'aati naamudaan itti fudhatame _______________/_______________		

III. Yaada

____

____

Maqaa qorataa ______________________ Mallattoo ___________Guyyaa___________

C. የአማርኛ ግልባጭ

"የዉጪኛው የአይን ኢንፌክሽን ተህዋስያን የሚያመጣውን ህመም እና የተህዋስያኑ መድሃኒት የመቋቋም ያለዉን የስርጭት መጠን" በምል ርእስ ላይ ለጅማ ዩኒቨርሲቲ የእክም መእከል የአይን ኪሊኒኪ የተዘጋጀ ማህበራዊ መረጃዎች እና የእክምና ማረጃ የምሰበሰቢበት ጥያቄ፡፡

ትሂዛዝ፡ ከተዘጋጁ ምረጫዎች አንዱን ምረጥ ወይም በተዘጋጀ በዶ ቦታ ለይ ፀፍ

ተ.ቁ	1. ማህበራዊ መረጃዎች	
	ጥያቄ	**መልስ**
1	የህመምተኛዉ መለያ ቁጥር	
2	የህመምተኛዉ.ካረዲ ቁጥር	
3	ዕድሜ	
4	ፆታ	1. ወንድ 2. ሴት
5	አማካይ ወርሀዊ ገቢ	
6	የመኖረያ አድራሻ	1. ገጠር 2. ከተማ
7	ብሔር	1. ኦሮሞ 4. ጉራጌ 2. አማራ 5. ዳዉሮ 3. ትግሬ 6. ሌላ (ይገለፅ)____
8	የትምርት ሁኔታ	1. ያለተማሬ 2. ዕድሜ ያልደረሰ 3. አንደኛ ደረጃ

		4. ሁለተኛ ደረጃ 5. ኮሌጅና ከዛ በላይ
9	የትደር ሁኔታ	1. ያላገባች 4. የተለያዩ ቦታ የምኖሩ 2. ያባች 5. ባል የሞተባት 3. የፋታች
10	የሥራ ሁኔታ	1. የመንግስት ሠራተኛ 6. ዕድሜ ያልደረሰ 2. ገበሬ 7. ሌላ (ይገለፅ)___ 3. ነጋዴ 4. የቤት እመቤት 5. ተማሪ
11	በቤታቸዉ ለብረሃን ምንጭ የምጠቀሙ ምንዲነዉ?	1. እንጨት 2. ኩራዝ 3. የኤሌክትሪክ ማብራት 4. ሌላ (ይገለፅ)_______
12	በቤታቸዉ ለማብሰል የምጠቀሙ ዓይል የምንዲነዉ?	2. የእንጨት 3. ከጋዝ 2 የኤሌክትሪክ ዓይል 4. ሌላ_______
II.	**የእክምና ማረጃ**	
13	ከነዚ በሽዎች የትኘዉ አለ?	1. ሬሁማቶይዲና አረትራይት 4. የሱካር በሽታ 2 የደም ግፍት 5. ሌላ (ይገለፅ)____
14	የዓይን በሽታ ዓይነት	1. ኮንጀክትቭይትስ 3. ቢሌፈሮ-ኮንጀክትቭይትስ 2. ቢሌፈራይትስ 4. ዳክራዮስታይትስ
15	ከዚህ በፍት ለዓይን እክምና ሆስፒታል ተኘተው ያውቃሉ?	1. አዎን ______ መቼ?_______ 2. አይደለም
16	ለዓይን እክምና መዲሃንት ወስደ ተጠቅመው ያውቃሉ?	1. አዎን _______ መቼ? ________ 2. አይደለም
17	ለዓይን የእክምና ሌንስ ይጠቀማሉ?	1. አዎን 2. አይደለም
18	ከዚህ በፍት የዓይን ቀዶ እክምና ተደርጎለት ያውቃል?	1. አዎን _______ መቼ? ________ 2. አይደለም
19	ባህላዊ የዓይን እክምና ተጠቅመል?	1. አዎን 2. አይደለም
20	ናሙና የተወሰደበት ዓይን	1. ቀኘ ዓይን 2. ግራ ዓይን 3. ከሁለቱም ዓይን
ናሙና የተወሰደበት ቀንና ሰዓት ___________/___________		

III. አስተያት

____

የተመራማሪ ስም ________________ፊርማ ______ ቀን _________

Annex VI: Laboratory data collection format

1. Patient ID. ---------------- Sample ID -----------------
2. Date of sample collection -----------/-----------/-----------
3. Source of specimen: Conj. □ Lids □
4. Type of diagnosis: --
5. Gram stain result--
6. Culture growth: Yes □ No □, BA____, MA_____, CA____, MS_____
   Hemolysis on blood agar: Alpha _____, Betta_____, No hemolysis _____
   Optochin disk________, Bile solubility __________, Bacitracin disk_______
7. Name of bacteria, if isolated (1) _______________ (2) ______________(3) _______________
8. Biochemical identification test results: Lactose ___, Gluc___, Gas___, Indole___, Citrate___
   Urease___, Lysine___, Oxidase____, Catalase______, Coagulase _____Others ______
9. Biofilm formation for isolated bacteria: Yes □ No □
10. If yes for question number 9, (1) Strong, (2) Moderate, (3) Weak
11. Antimicrobial susceptibility testing

Antimicrobial	S (mm)	I (mm)	R (mm)
▪ Ampicillin (AMP) (10µg)	------------	-------------	----------
▪ Amoxicillin-Clavulinic (AMC)(20µg)	------------	--------------	---------
▪ Amikacin (AK) (30 µg)	--------------	-------------	---------
▪ Ceftazidime (CAZ) (30µg)	-------------	------------	--------
▪ Cefoxitin (FOX) (30µg)	------------	--------------	---------
▪ Ceftriaxone (CRO) (30µg)	------------	--------------	---------
▪ Chloramphenicol (C) (30µg)	------------	--------------	----------
▪ Ciprofloxacin (CIP) (5µg)	------------	--------------	----------
▪ Clindamycin (CLN) (2µg)	------------	--------------	----------
▪ Erythromycin (ERY) (15µg)	------------	--------------	---------
▪ Gentamicin (CN) (10 µg)	------------	--------------	----------
▪ Penicillin (PE) (10 IU)	------------	--------------	---------
▪ Tetracycline (TE) (30µg)	------------	--------------	--------
▪ Tobramycin (TOB) (10 µg)	------------	--------------	-------
▪ Trimethoprim-Sulfamethoxazole (SXT) (1.25µg)	----	-----------	--------

ANNEX VII: SOP for preparation of culture media, collection and processing of specimens, Culturing and Identification

A. Sample Collection, Handling and Transport

1. Objective and Scope:

To describe the specimen collection instructions and subsequent handling of specimens by Researcher (BSc, MSc candidate) for identification of the bacteria; This document contains procedure for clinical specimens collection containing bacteria from the lower eye lid, conjunctival swabs, Blepharitis and Dacryocystitis for processing at Jimma University Microbiology laboratory.

2. Procedure:

An adequate specimen is essential for the success of culture of bacteria from external ocular surface. Specimens was collected with the greatest care and taken to the laboratory properly. Each and every procedure should undertake accordingly per standard.

3. Materials and Equipment needed (some of them)

- Culture media
- Petri dish
- Culture tube
- Inoculating loop
- Straight wire loop
- Bunson burner
- Sample of bacteria (control strains)
- Bio-safety cabinet
- Boiler
- Adjustable micropipette
- Incubator
- Refrigerator
- Autoclave
- PH meter
- Flask
- Graduated cylinder
- Aluminum foil
- Balance
- Microtiter plate
- Microtiter plate reader

B: Preparation of culture media

1. **5% Blood agar**

Purpose and principle: Blood agar is used for isolation and cultivation of many types of non-fastidious and fastidious bacteria. It is also used to differentiate bacteria by their hemolysis capabilities. Several species of gram-positive cocci produce exotoxins called hemolysins able to destroy red blood cells and hemoglobin. Blood agar, which is a mixture of defibrinated sheep blood, allows differentiation of bacteria based on their ability to hemolyze RBCs. Hemolysis, the enzymatic breakdown of red blood cells, is performed by different bacteria (mainly among the gram-positive cocci). Hemolytic ability can be classified into three. Those are beta-hemolysis (complete clearing of the agar), alpha-hemolysis (partial clearing of the agar) and gamma-hemolysis (no change in the coloration of the agar).

Procedure

1. Suspend blood agar base in distilled water according to manufacturer instructions.
2. Sterilize by autoclaving at 121°C for 15 minutes. Transfer to a 50°C water bath.
3. When the agar has cooled to 50°C, add aseptically 5% defibrinated sterile blood and mix gently but well.
4. Avoid forming air bubbles. Important: The blood must be allowed to warm to room temperature before being added to the molten agar.
5. Dispense aseptically 15 ml of blood agar amounts in sterile Petri dish of 90mm.
6. Date the medium and give it a batch number.
7. Store the plates upside down at 2–8°C. Preferably in sealed plastic bags to prevent loss of moisture.

2. 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 70°C for hemolysis of red blood cells. It is used for the growth of fastidious organism.

3. MacConkey Agar

Intended use and principle

MacConkey agar is selective for gram negative organisms. The selective property of the medium comes from the presence of bile salts and crystal violet that inhibit most gram positive bacteria.

It 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 species*. The principle behind is, during Lactose fermentation a local pH drop around the colony results a color change within the pH indicator, neutral red, and bile precipitation. Bile salts mixture and crystal violet inhibit the growth of gram positive cocci and only allow gram negative to grow. When lactose is fermented, acid products lowers pH below 6.8, neutral red causes the medium to turn pinkish-red. Colonies of non-lactose fermenting bacteria will be colorless

Procedures

1. Suspend the medium in distilled water according to manufacturer instructions.
2. Heat with frequent agitation and boil for one minute to completely dissolve.
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.

4. Mueller Hinton Agar

Intended use and principle

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 thymidine. The para-amino benzoic acid and thymine/thymidine content of MHA are reduced to a minimum, reducing the inactivation of sulfonamides-trimethoprim. The pH should be within the range of 7.2 to 7.4 at 25°C

Procedure

1. Suspend a commercially available dehydrated Müeller-Hinton base/agar according to the manufacturer's instructions.

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 in refrigerator.

5. Mannitol Salt Agar.

Principles

This medium is both selective and differencial media. It contains Mannitol, phenol red which is pH indicator and high concentrations of sodium chloride (7.5%) salt which is primarly selective for *Staphylococci species* but inhibits the growth of other bacteria. Agar is the solidifying agent. *Staphylococcus species* that grow in the presence of a high salt concentration and ferment mannitol produce acid products, changes the phenol red pH indicator from red to yellow. The pathogenic *Staphylococci aureus* ferment mannitol and form yellow colonies with yellow zones; Typical nonpathogenic Staphylococci do not ferment mannitol and form red colonies but grow on this medium. The medium surrounding these colonies does not change color. Final pH 7.4 ± 0.2 at 25^0C

Procedure

1. Suspend the medium in distilled water according to manufacturer instructions.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121^oC for 15 minutes.
4. Date the medium and give it a batch number.
5. Store the plates at 2–8 °C in refrigerator.

C. Specimen Collection

After detailed ocular examinations, have patient, while facing straight ahead, look up. This facilitates exposure of the lower palpebral conjunctiva. Pull down lower lid to expose the conjunctiva and pass the moistened swab back and forth twice over the greater part of the tarsal conjunctiva, carefully avoiding the eyelid border and eye lashes. Specimens were collected from one or both of the infected eyes according to the nature of the infections. Ophthalmologist/residents or experienced ophthalmic nurse took the swabs from the infected eyelid and conjunctiva using sterile cotton swab moistened with sterile saline. It 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, the sample was collected from the refluxing material ensuring that the lid margins, the conjunctiva was not touched.

Transport of specimen to Jimma University microbiology laboratory:

Following collection, specimens were immediately transported to Jimma University Microbiology Laboratory for bacterial isolation, identification and further analysis.

Specimen Processing

A. Culture

Procedure:

1. Inoculate the specimen on non-selective/selective media blood agar, chocolate, MacConkey agar and Mannitol salt agar and incubate at 35-37°C.
2. Incubate fastidious organism in a humid environment of air containing 5-10% CO_2.
3. Incubate for a minimum of 48 hours before discarding the plates.
4. Examine the plates after 18-24 hours of incubation.
5. 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 and biochemical tests.
6. Perform sensitivity test

B. Gram stain

Purpose: Used to classify bacteria as gram positive and gram negative based on their gram reaction. Gram staining detects a fundamental difference in the cell wall composition of bacteria.

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.

Required materials

- Crystal violet (initial stain), Gram's iodine (mordant or binding agent), Acetone–alcohol (decolorizer) and Safranin (counter stain) were the reagents commonly used for gram staining technique,
- Immersion oil,
- Slides, Forceps, Washer, Staining bottles
- Flame (bunson burner), Cotton, Match and Microscope

Procedure:

1. Labeling the slides clearly with the date and patient's ID and study number.
2. 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 pure colony using loop and mix with saline on the slide.
3. Place air dried smears fix with methanol for one minute and for colony, allow fixing with heat passing 2-3 times; it should be dried before staining.
4. Flood smear with crystal violet for one minute, then rinse gently with tap water.
5. Flood the slide with Gram's iodine for one minute and then rinse gently with tap water.
6. Flood the slide with decolorizer for 30 seconds and rinse with tap water.
7. Flood the slide with Safranin for one minute and then rinse gently with tap water.
8. 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.

9. Examine the smear microscopically using 40x (for focusing) and 100x oil immersion objective for observation.

Result interpretation:

-Gram-positive bacteria and yeast will stain blue to purple
-Gram-negative bacteria will stain pink to red.

C. Biochemical testing procedures

I. Identification of gram positive bacteria

1. **Catalase test**

Principle: Catalase is an enzyme which acts as a catalyst in the breakdown of hydrogen peroxide to oxygen and water. An organism is tested for catalase production by bringing it into contact with hydrogen peroxide. The formation of bubbles (O_2) is evidence of catalase activity. As a differential test, the catalase test is used to distinguish *staphylococci species* which have catalase enzyme from streptococci which donot. 3% hydrogen peroxide (H_2O_2) required for this purpose.

Procedure:

1. Using aseptic technique, transfer a loopful of organism from a solid culture. Be careful not to pick up any agar
2. Using a dropper or Pasteur pipette, place 1 drop of 3% H_2O_2 onto the organism on the microscope slide. Do not mix
3. Look for immediate active bubbling.

Result interpretation:
Active bubbling………. Positive for catalase test
No bubbling………….. Negative for catalase test

2. Coagulase test

Purpose: The coagulase test is used to differentiate *staphylococcus aureus* (coagulase positive) from other coagulase negative staphylococcus species (CoNS)**.**

Principle: In the presence of the coagulase enzyme produced by *S. aureus,* the addition of commercial rabbit plasma produces a clumping reaction in which fibrinogen (soluble) in plasma converted to fibrin (insoluble). Coagulase acts as a thrombinase-like action. The coagulase test can be performed using two different procedures: slide test and tube test. During tube test, as the bacteria multiply in the plasma, they secrete staphylo-coagulase. Staphylo-coagulase initiates blood coagulation by activating prothrombin. Staphylo-coagulase adheres to fibrinogen, forming a complex that cleaves fibrinogen into fibrin, bypassing the blood clotting cascade and directly causing a clot of fibrin to form.

Required: Rabbit plasma, the plasma should be allowed to warm to room temperature before being used

Procedure:

1. Allow the reagent bottle to come to room temperature (15-25°C).
2. Place a drop of physiological saline on two separate slides.
3. Emulsify the test organism in each of the drop to make thick suspension.
4. Add one drop of rabbit plasma to one of the suspensions and mix gently.
5. Look for clumping of the organism within 10 seconds.

Interpretation:

Clumping within 10 seconds ------------------ *S. aureus*

No clumping within 10 seconds ------------- other *staphylococcus species*

3. **Optochin sensitivity test**

Principle: This test is used to detect an organism's susceptibility to the chemical Optochin (Ethylhydrocupreine hydrochloride) for the presumptive identification of *S. pneumoniae* strain which is sensitive to the chemical Optochin disk.

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^oC in ~5-10% CO_2 for 18-24 hours.

Interpretation

Susceptible: Zone of inhibition of at least 14 mm
Resistant: Zone of inhibition less than 14 mm

4. **Bile Solubility Test**

Principle: A heavy inoculums of isolated pure colony of test organism is emulsified in physiological saline and then the bile salt sodium deoxycholate is added. This dissolves *S. pneumoniae* as shown by a clearing of the turbidity within 10-15 minutes or addition of drops of the reagent directly on isolated colonies of suspected *S. pneumoniae* results in lysis of the colonies within 15-30 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 plate's 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 jar.
5. Examine the colonies for lysis.

Interpretation

Positive (bile soluble): Lysis of the colonies.
Negative (bile insoluble): No lysis of colonies

II. Identification of gram negative bacteria

Identification of gram negative bacteria was undertaken based on isolation of pure colonies with a series of biochemical tests.

Procedure

1. Prepare a suspension of the 3-5 pure colony of test organism within 5ml of nutrient broth or normal saline and adjusted to 0.5 McFarland standards.
2. A loop full of the bacterial suspension is inoculated in to citrate agar, triple sugar iron agar, lysine decarboxylase agar, oxidase, urea agar and motility medium (SIM). See in detail below one by one.
3. Incubate at 35-37°C for 18-24 hours.
4. Look for color change (turbidity for motility) of the medium.
5. Identify the test organism by considering the result of biochemical tests.

1. Indole test

Principle: Some bacteria can produce indole from amino acid tryptophan using the enzyme tryptophanase. Production of indole is detected using Ehrlich's reagent or Kovac's reagent.This reagent is not a dye or stain but reacts with indole to produce an AZO dye. Indole reacts with the aldehyde in the reagent to give a red color. An alcoholic layer concentrates the red color as a ring at the top.

Required:-Kovac's reagent, Tubes, Inoculating loop and Incubator.

Procedure:

Bacterium to be tested is inoculated in peptone water or sulphur-indole-motility, which contains amino acid tryptophan and incubated overnight at 37°C. Following incubation few drops of Kovac's reagent are added. Kovac's reagent consists of para-dimethyl aminobenzaldehyde, isoamyl alcohol and concentrated HCl. Ehrlich's reagent is more sensitive in detecting indole production in aerobes and non-fermenters. Formation of a red or pink colored ring at the top is taken as positive. Example: *Escherichia coli*: Positive; *Klebsiella pneumonia*: Negative

2. Citrate utilization test

Principle: This test detects the ability of an organism to utilize citrate as the sole source of carbon and energy. Bacteria are inoculated on a medium containing sodium citrate and a pH indicator bromothymol blue. The medium also contains inorganic ammonium salts, which is utilized as sole source of nitrogen. Utilization of citrate involves the enzyme citritase, which breaks down citrate to oxaloacetate and acetate. Oxaloacetate is further broken down to pyruvate and CO_2. Production of Na_2CO_3 as well as NH_3 from utilization of sodium citrate and ammonium salt respectively results in alkaline pH. This results in change of medium's color from green to blue.

Procedure: Bacterial colonies are picked up from a straight wire and inoculated into slope of Simmon's citrate agar and incubated overnight at 37^oC. If the organism has the ability to utilize citrate, the medium changes its color from green to blue.

3. Triple sugar iron agar

Triple Sugar Iron Agar (TSI) used to determine if bacteria can ferment glucose, and/or lactose and if it can produce hydrogen sulfide or other gases. In addition, TSI detects the ability to ferment sucrose. These characteristics help distinguish various enterobacteriacae, including *Salmonella* and *Shigella,* which are intestinal pathogens.

Procedure: Bacterial colonies are picked up from a straight wire and the tube is inoculated by stabbing into the agar butt (bottom of the tube) with an inoculating wire and then streaking the slant in a wavy pattern. Results are read at 18 to 24 hours of incubation. TSI contains three sugars: glucose, lactose and sucrose. Lactose and sucrose occur in 10 times the concentration of glucose (1.0% versus 0.1%). Ferrous sulfate, phenol red (a pH indicator that is yellow below pH 6.8 and red above it), and nutrient agar are also present. A yellow slant on TSI indicates the organism ferments sucrose and/or lactose. A yellow butt shows that the organism fermented glucose. Black precipitate in the butt indicates hydrogen sulfide production. Production of gases other than hydrogen sulfide is indicated either by cracks or bubbles in the media or the media being pushed away from the bottom of the tube.

4. Oxidase test (Filter Paper Method)

Purpose and principle: The oxidase test is a biochemical reaction that assays for the presence of cytochrome oxidase. This test determines the presence of cytochrome oxidase enzymes.When the organism is oxidase producing, the phenylenediamine in the reagent will be oxidized to a deep purple color.

Procedure:

1. Place a piece of filter paper in a clean petri dish.
2. Add 2 or 3 drops of freshly prepared oxidase reagent.
3. Using a piece of stick or wire loop, pick a well isolated colony of the test organism and rub/smear it on the filter paper.
4. Look for the development of a blue-purple color within 5-10 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

5. Lysine decarboxylase agar (LDC)

Lysine decarboxylase agar is selective isolation medium for *Salmonellae* and *Shigella*. Low in nutrients this medium relies on a small amount of Sodium deoxycholate for selectivity. The indicator system is complex. Most enteric organisms except *shigella* will ferment xylose to produce acid however the *salmonellae* will also decarboxylate the lysine to keep the pH neutral. At near neutral pH the *salmonella* can produce H_2S from the reduction of thiosulphate producing black or black centered colonies. *Citrobacter species* can also decarboxylate lysine, however the acid produced by fermentation of both lactose and sucrose will keep the pH too acid for H_2S to be produced

6. **Urease test**

Some bacteria produce the enzyme urease, which catalyzes the hydrolysis of urea to form ammonia and carbon dioxide. Organisms that do not produce this enzyme cannot metabolize urea. Urea broth has a minimal amount of yeast extract along with urea. Organisms that cannot metabolize urea will have insufficient nutrients for growth. Urea hydrolysis will result in a pH

increase because of the production of ammonia. The pH indicator phenol-red, will turn pink with this pH increase. However, the presence of strong buffers in the medium requires a large amount of ammonia production to cause a color change. Thus, only strong hydrolyzers of urea will turn the broth pink (indicating a positive result). This should happen within 24 hours.

Procedure

1. Obtain two urease broths from the refrigerator.
2. Inoculate one broth using aseptic technique. Leave the other broth uninoculated (this will be used as a control).
3. Incubate at an appropriate temperature (whatever temperature your organism grows well at 37°C). Incubate for 24 to 48 hours (do not exceed 48 hours for this test).
4. Obtain your broths from the incubator and observe the color.

Results: Positive (+) = Pink coloration within 24 to 48 hours

Negative (-) = Orange coloration after 24 to 48 hours

7. Motility testing

To test for motility, use a sterile straight wire to pick a well-isolated colony and stab the motility medium within 1 cm of the bottom of the tube. Be sure to keep the straight wire in the same line it entered as it is removed from the medium. Incubate at 35°C for 24 hours or until growth is evident. A positive motility test is indicated by a red turbid area extending away from the line of inoculation. A negative test is indicated by red growth along the inoculation line but no further extension from the line of inoculation.

Procedure

1. Obtain a motility agar tube from the back shelf.
2. Use an inoculating pick. Straighten the pick as much as possible.
3. Make a stab inoculation (about 2/3 of the way into the agar) from your unknown stock culture. Try to make the stab (in and out) as straight as possible. A messy stab will be difficult to evaluate.
4. Incubate at an appropriate temperature for 24 to 48 hours (up to 72 hours).

5. Observe your culture by holding it up to a light source.

8. H_2S production

Principle:- Hydrogen sulfide (H_2S) is produced by bacterial anaerobic degradation of the two sulfur-containing amino acids, cysteine and methionine. Hydrogen sulfide is released as a by-product when carbon and nitrogen atoms in the amino acids are consumed as nutrients by the cells. Under anaerobic conditions the sulfhydryl (-SH) group on cysteine is reduced by cysteine desulfurase. The agar contains high levels of peptones (sources of cysteine and methionine) and ferrous sulfate as an indicator. When H_2S is produced, the ferrous ion reacts with it to give ferrous sulfide, an insoluble black precipitate.

Required material

- TSI Agar Slant, Inoculating loop and Incubator.

Procedure

1. The triple sugar iron agar slant was inoculated by stabbing the butt and drawing the stick over the surface of the slope.
2. Incubated at 35-37°C for 18 to 24 hours.
3. Looked for black precipitate formed.

Result

- Acid deep (yellow)/alkaline slant (red):- glucose fermented, lactose and/or sucrose not fermented.
- Acid deep (yellow)/acid slant (yellow):- glucose, lactose and/or sucrose fermented.
- Alkaline deep and slant (all red):- glucose, sucrose, and lactose not fermented.
- Deep split or displaced: - gas production.
- Deep blackened: - H_2S production.

9. Methicillin resistant *Staphylococcus aureus* (MRSA)

Methicillin-resistant *S. aureus* strains have acquired the mecA gene, which is carried on a large mobile genetic element called the staphylococcal chromosomal cassette mec (SCCmec). This gene codes for a penicillin binding protein, PBP2a, which interferes with the effects of beta

lactam antibiotics (e.g. penicillins and cephalosporins) on cell walls. Oxacillin disk diffusion testing is not reliable for detecting oxacillin/ methicillin resistance. Cefoxitin should be used as a surrogate for disk diffusion testing. Cefoxitin is better inducer of the mecA gene, and tests using cefoxitin give more reproducible and accurate results than tests with oxacillin. Cells expressing oxacillin-resistance grow more slowly than the oxacillin-susceptible population and may be missed at temperatures above 35°C. This is why CLSI recommends incubating isolates being tested against oxacillin or cefoxitin at 33-35° C (maximum of 35°C) on MHA for a full 24 hours before reading.

Interpretive Criteria (in mm) for Cefoxitin disk diffusion test

	Susceptible	Intermediate	Resistant
S. aureus	≥ 22 mm	N/A	≤ 21 mm
CoNS	≥ 25 mm	N/A	≤ 24 mm

D. Antibiotic susceptibility testing

Antimicrobial susceptibility of all isolates was determined by using the Kirby Bauer disk diffusion method on MHA according to CLSI guidelines. For fastidious organism, 5% sheep blood containing MHA was used after heating. 3-5 well isolated colonies of the same morphological type (pure colony) 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 3ml of sterile normal saline and mixed gently. The suspension was made at an appropriate turbidity (adjusted to 0.5 McFarland standard) of the bacterial culture to be tested.

Procedure

1. Prepare a suspension of the test organism by emulsifying several pure colony of test organism in a small volume of nutrient broth or normal saline.
2. Much the turbidity of suspension with 0.5 McFarland turbidity standards.
3. With a sterile swab take sample from the suspension (squeeze the swab against the side of the test tube to remove the excess fluid).
4. Spread the inoculums evenly over the Muller-Hinton agar plate with the swab by rotating 60°C three times.

5. Using a sterile forceps or needle, place the antimicrobial disk on the inoculated plate. Incubate the plate aerobically at 35-37°C for 18-24 hours by inverting the plates. For fastidious organism, heated 5% Sheep's blood agar with MHA was incubated at 37 °C for 24 to 48 hours in a 5-10% CO_2 atmosphere.
6. Read the test after checking that the bacterial growth is neither heavy nor light. Measure the diameter of the inhibition zone.
7. Interpret the reaction of the test organism to each antibiotics used as sensitive, intermediate, or resistance as per the standard.

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 usual 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 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, lactamases) are likely, and clinical efficacy of the agent against the isolate has not been reliably shown in treatment studies (CLSI, 2015).

E. Quality control

As quality control, sterility of sheep blood, Blood agar, MacConkey agar, mannitol salt agar and MHA were checked by incubating 5% 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) and *P. aeruginosa* (ATCC 27853) was used as a quality control throughout the study for culture and antimicrobial susceptibility testing.

F. Biofilm formation testing protocol

1. Growing a Biofilm

1. Inoculate minimum of ten colonies isolated from fresh agar plates in sterile trypticase soy broth
2. Incubate the inoculated trypticase soy broth at 37° C for 24 h.
3. Dilute the overnight culture 1:100 into fresh medium for biofilm assays.
4. Add 200 µL of the dilution per well in a 96 wells of flat bottom microtiter plate.
5. Incubate the microtiter plate for 48hrs at 37°C.

2. Staining the Biofilm

1. After incubation, the microtiter plate content of each well was removed by tapping the bottom plates using micropipete.
2. Wash the wells with 0.2 mL of phosphate buffer saline (pH 7.3) four times. This step helps remove unattached cells and media components that can be stained in the next step, and significantly lowers background staining.
3. Fix adherent organisms forming-biofilms in the microtiter plate with 99% methanol.
4. Add 220 µL of a 0.1% solution of crystal violet into each well to stain the biofilm formed on the surface of microtiter plate.
5. Incubate the microtiter plate at room temperature for 10-15 min.
6. Wash the plate 3-4 times with phosphate buffer saline
7. Turn the microtiter plate upside down and dry for a few hours or overnight.

3. Quantifying the Biofilm

1. Add 220µL of decoloring solution (95% ethanol alcohol) into each well of the microtiter plate to solubilize the CV.
2. Incubate the microtiter plate at room temperature for 10-15 minute. Don't allow to stay more time to prevent ethanol evaporation.

3. Transfer 200µL of the solubilized CV to a new flat bottomed microtiter plate.
4. Briefly mix the content of each well by blowing up and down using pipet.
5. Measure the absorption at 570nm to quantify the biofilm formed.
6. Make microtiter plate incubated with sterile TSB as negative control.

NB: For each isolate biofilm formation tests were carried out in triplicate and the results were averaged.

Annex VIII: Declaration Sheet

I, the undersigned, MSc Medical Microbiology student declare that this Thesis paper is my original work in partial fulfillment of the requirement for the degree of master science in Medical Microbiology. Where others work has been used, it has been carefully acknowledged and referenced in accordance with the requirements.

Name of principal investigator

2. Kuma Diriba Signature _________Date______________

Approved by my advisors;

1. Dr. Tesfaye Kassa Signature _________Date______________
2. Mr. Yared Alemu Signature _________Date______________

Co-advisor

1. Dr. Sisay Bekele Signature _________Date______________

Eximiners

1. Name _________________________ Signature ___________ Date ___________
2. Name _________________________ Signature ___________ Date ___________