ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCE, SCHOOL OF MEDICINE, DEPARTMENT OF MICROBIOLOGY, IMMUNOLOGY AND PARASITOLOGY

BACTERIAL ISOLATES FROM EXTERNAL OCULAR INFECTIONS AND THEIR ANTIBIOTIC SUSCEPTIBILITY STATUS AMONG PATIENTS VISITING MENELIK II REFERRAL HOSPITAL, ETHIOPIA

By:

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A THESIS SUBMITTED TO ADDIS ABABA UNIVERSITY, COLLEGE OF HEALTH SCIENCES, SCHOOL OF MEDICINE, DEPARTMENT OF MICROBIOLOGY, IMMUNOLOGY AND PARASITOLOGY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE MASTERS' DEGREE IN MEDICAL MICROBIOLOGY.

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List of abbreviations/Acronyms

AARHB: Addis Ababa Regional Health Bureau

AAU: Addis Ababa University

ATCC: American type culture collection

BPA: Blood plate agar

BHIB: Brain heart infusion broth

CA: Chocolate agar plate

CFU: Colony forming unit

CHS: College of Health Sciences

CI: Confidence interval

CLSI: Clinical and laboratory standards institute

CONS: Coagulase negative *Staphylococci*

DMIP: Department of Microbiology, Immunology and Parasitology

EOI:External ocular infection

EUCAST: Europian committee on antimicrobial susceptibility testing

GNB: Gram negative bacteria

GPB: Gram positive bacteria

Mac: MacConkey agar

MDR: Multi drug resistance

MSA: Manitol salt agar

SOPs: Standard operating procedures

SPSS: Statistical package for the social sciences

QC: Quality control

WHO: World Health Organization

Operational definitions (Definitions of terminology)

Blepharitis: - is a chronic inflammation of the eyelids

Blepharo-conjunctivitis: - 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.

Conjunctivitis: - is an inflammation of conjunctiva, the membrane that lines the eye lids and covers the exposed surface of the eyeball/sclera.

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

Keratitis: - is inflammation of cornea which is clear and transparent front part of the eye and is caused by an infection.

Multi drug resistance (MDR):isBacterialisolates non-susceptibility to at least one drug in three or more antimicrobial categories (classes)

Occupation: is any activity in which a person is engaged or being occupied (business)

Systemic Disease:is a disease that involves money organs/tissues or the whole body. e.g. HIV

Previous eye surgery- is a condition of patients who had eye surgery before sample collection.

Rural: - is Village's that have low population density as well as no infrastructure of the environment.

Urban: - is Town, human settlements with high population density and infrastructure of built environment.

Abstract

Background: Infection of the external structures of the eye is one of the commonest types of eye disease worldwide including Ethiopia.Generally, infection of the eye can lead loss or impairment of visual function causing major disability. Therefore, prompt isolation and testing susceptibility of bacterial isolates are necessary for bettertreatment of bacterial ocular infection.

Objective:the aim of this study was to assess the magnitude of bacterial isolates from external ocular infections and their antibiotics susceptibility status among patients visiting ophthalmology unit of Menelik II Referral Hospital, Ethiopia.

Methods: A Hospital based cross sectional prospective study was conducted at the Menelik II Referral Hospital among patients seeking health services at the department of ophthalmology from Januaryto April, 2019. All patients confirmed of external ocular infections were included. External ocular samples were collected using aseptic techniques. All samples were investigated for the presence of bacterial growth and bacteria were identified using gram stain, colony morphology, and disk sensitivity and biochemical tests. Drug susceptibility test was done using the Kirby-Bauer Disk diffusion method according to the guidelines of clinical and laboratory standard institute (CLSI).

Result: In this study,the magnitudes of bacterial isolates were 175/323 (54.2%. The proportion of gram positive bacterial isilates was 171/184 (92.9%).Coagulase negative *staphylococci* (*CoNS*)76/184 (41.3%) were the predominant bacterial isolatefollowed by *Staphylococcus aureus,Streptococcus Viridian*and *Klebsiella* spp., 67/184 (36.4%),16/184 (8.7%) and 6/184 (3.3%) respectively. The dominant clinical feature was Blepharitis accounting 122/323 (37.8%). Gram positive bacterial isolates were susceptible to, Tobramycin, Gentamycin, chloramphenicol, Vancomycin and ceftriaxone. However, 94.0% of these gram positive bacterial pathogens showed resistance to penicillin. Multidrug resistance (MDR) were observed in gram positive and gram negative bacteria isolates 136/184 (67.4%)and 12/13 (92.1%) respectively.

Conclusion: Blepharitis was the leadingEOI followed by conjunctivitis. The predominant bacteria species was *CoNS* followed by*S. aureus*. The higher prevalence of MDR 135/184 (73.4%)and increasing of *MRSA*23/67 (34.3%)bacterial pathogens dictates the need for continuous surveillance apart from routine antibiotic susceptibility testing.

Key words: External ocular infection, Bacterial isolate and Antibiotic susceptibility status

1. Introduction

1.1. Background:

Ocular infections are common in most part of the developing world [Belyhun *et al.*,2018]. External and intraocular infections can lead to visual impairments, which is a major public health problem [Teweldemedhin *et al.*,2017]. Infection of the external structures of the eye is one of the commonest types of eye disease worldwide including Ethiopia [Getahun *et al.*, 2017*;* Tesfaye*et al.*, 2013]. In addition, although relatively impermeable to microorganisms, infection within the eye can result from trauma, surgery or systemic disease [Armstrong, 2000].Variables affecting the development of bacterial external ocular infections (EOI) include poor hygiene, socio-economic status, living conditions, contact lens use, previous ocular trauma, chronic epithelial defects and immune status [Alzahrani*et al.*, 2018**].**

Ocular bacterial infections can cause a series of symptoms and signs, such as the formation of pus, conjunctival hyperemia, lid edema, and even visual impairment. The causative bacteria can come from the outside environment or from systemic infections transported by blood. Bacteria of the normal microbiome can also cause infection, especially when they enter the aqueous humor or vitreous fluid [Wang*et al.*, 2015].Even though**,** the eyelid and conjunctiva normal microbial flora is controlled by its own mechanism and by the host, modification of this normal flora contributes to ocular infections [Bharathi *et al.*,2010]. Many opportunistic pathogenic agents are increasingly encountered in ocular infections due to widespread use of topical and systemic immunosuppressive agents such as, increasing numbers of patients with human immunodeficiency virus (HIV) infection [Schaftenaar *et al.,* 2014; shabay *et al.*,2015].

The most common EOIsuch as; conjunctivitis, blepharitis, dacryocystitis, orbital and periorbital cellulitisarerecognized forms of eye infection. Blepharitis is inflammation of the eyelids which could be characterized by redness, itching andgreasy or crusty eyelashes. Keratitis is an inflammation of the cornea which is a major cause of vision loss and blindness second to cataract [Shiferaw *et al.*,2015].

In Ethiopia, eye infections caused by bacteria were important public health problems [Belyhun*et al.,* 2018]. Bacterial conjunctivitis is more common in young children and the elderly than in other age groups. The most essential pathogens in bacterial conjunctivitis are *S. aureus, S.*

pneumoniae, and *H. influenzae*. Other bacteria like; ***S****taphylococcus epidermidis*, *Enterococcus* spp., *Moraxella* spp., *streptococci viridans* group, *Escherichia coli*, *Serratia marcescens*, *Pseudomonas aeruginosa*, and *Proteus mirabilis* have also been isolated less frequently from bacterial conjunctivitis samples [Bertino, 2009; Balanco and Nunez, 2013].

The most common causal pathogens for bacterial blepharitis are *S. aureus* and*CoNS*. Likewise, dacryocystitis, the most common isolates are *CoNS*, *S. aureus* and *P. aeruginosa* [Assefa *et al*, 2015]. In case of severe acute dacryocystitis; single infection may predominate, often involving gram negative rods. Several other species of bacteria could be also involved in the pathogenesis of chronic dacryocystitis. Usually, the majority of patients harbour multiple microorganisms [Assefa *et al.*, 2015]. In case of keratitis; two of the most common bacteria to cause microbial keratitis are *Pseudomonas aeruginosa* and *Staphylococcus aureus* [Willcox*et al.*, 2011].

Generally, infection of the eye can lead to loss or impairment of visual function causing major disability. The cornea is particularly delicate and the degree of scarring or inflammation, which is relatively minor in other parts of the body, maighthas serious consequences in the cornea.Hence, there is a need for an immediate treatment for the serious bacterial eye infection that threatens the cornea of eye [Ubani, 2009; Smith and Flowers, 1995].

The spectrum of ocular infections varies from categories of EOI, so aggressive antimicrobial therapy is desirable for ocular infections.Prompt isolation and testing susceptibility of pathogensis necessary to treat these infections effectively [Singh *et al.*,2018]. Otherwise, worldwide problem regarding the emergence of bacterial resistance increases the risk of treatment failure with potentially serious consequences [Brown, 2007; Rahman *et al.*, 2013].

Bacterial resistance has been emerging worldwide, in relation to that, irrational use of antimicrobial agents is a known cause and risk factor for the emergence of antimicrobial resistance [Rahman *et al*., 2013]. Likely,a widespread and inappropriate dosing of broad-spectrum antibiotics for systemic infections and exacerbated by inadequate compliance to full treatment duration [Stratton, 2003] may help for the existence of resistance bacterial pathogens. Especially in developing countries, antibiotics can be purchased without prescription, which leads to misuse of antibiotics is the major cause of the emergence and spread of antimicrobial resistance [Shabay*et al.*,2015]. Similarly, the emergence of bacterial resistance is influenced by

characteristics of the pathogens, antibiotic-prescribing practices including the widespread use of systemic antibiotics, and health care guidelines [Bertino, 2009].In Ethiopia empirical selections of antimicrobial therapy based on clinical observations are common clinical practices [Belyhun*et al.*,2018]. Therefore, the emergence of bacterial resistance towards topical antimicrobial agents may increases the risk of treatment failure with potentially serious consequencesin the conjunctiva, cornea, and eye lids [Gaynor *et al.*, 2005; Umamageswari *et al.*,2013]. As a result, the bacterial etiology and their susceptibility as well as resistance patterns may vary according to geographical location [Umamageswari *et al.*,2013]. Hence, up to date information is essential for appropriate antimicrobial therapy and management of ocular infection [Shiferaw*et al.*,2015].

Menelik II Referral Hospital is nationally allocated referral hospital for all ophthalmology related complicated diseases in Ethiopia. However, no published studiesfound from this hospitalon this topic. Correspondingly, antibiotic susceptibility status of external ocular bacterial isolates still not enough information. This study might give highlighton the common external ocularinfections, bacteriological infections and antimicrobial susceptibility status. Therefore, the impact of this thesis might be relatively significant, that might be used as input for further study of external ocular bacterial infection.

1.2. Statement of the problem

The external ocular infections are responsible for increased incidence of morbidity and blindness world widely [Miller*et al.*, 2017;Amsalu *et al.*, 2015]. In this case, keratitis is a major cause of vision loss and blindness second to cataract [Shiferaw *et al.*,2015] and is the most common in developing countries. But the etiological cause for keratitis may vary at different geographical locations, time to time and population to population [Sherwaland Verma, 2008].

Blepharitis can also result in patient discomfort and decrease in vision. Moreover, untreated lacrimal abscess can progress to orbital cellulitis, superior ophthalmic vein thrombosis, and these may lead to life threatening condition. Infections of the conjunctiva can also spread to the cornea and can cause a perforation [Ali *et al.*, 2013].Bacterial agents are known to cause external ocular infections such as conjunctivitis, keratitis, blepharitis, hordeolum, dacryocystitis, etc. The World Health Organization (WHO) estimated that globally 285 million people were visually impairedof whom 39 million subjects were blind by the year 2010. The report also showed that 82% of the

visual impairment, including blindness was avoidable [Namitha and Mahalakshmi, 2014]. In Ethiopia, the prevalence of blindness was reported about 1.6% and it was estimated that 87.4% of the cases were due to avoidable causes of a variety of factors that determined the clinical outcome in microbial causes of eye infections, where bacteria ocular infections is one of the factors [Shiferaw *et al.*,2015].

There is a worldwide problem regarding the emergence of resistant strains toward antibiotics that have been routinely used in the hospitals [Musa *et al.*,2014]. The emergence of bacterial resistance towards antimicrobial agents may increases the risk of treatment failure with potentially serious consequences. This is because of lack of access to microbiology laboratory in most health institutions, clinicians' uses empirical therapy; that concerns' the risk of multidrug resistance expansion [Tesfaye *et al.,* 2013; Shiferaw *et al.*,2015].

Bacterial pathogens and its antibiotic susceptibilitymay vary according to geographical location [Hsiao *et al.,* 2016*;* Musa *et al.*,2014] and in the same place from time to time. Hence, the varying spectrum of bacteria involved in EOI and the emergence of acquired microbial resistance dictate the need for continuous surveillance to guide empirical therapy [Musa *et al.*,2014].

1.3. Significance of the study

- The result of this study provides up to date information about the bacterial isolates associated with external ocular infection, which might be helpful for clinicians to select the best antibacterial drug against bacterial external Ocular infection.
- Generally, the research finding may help to look for the best treatment selection in which the concerned bodies will consider it as an input in updating the treatment of EOI to be evidence-based empirical therapy.
- As a result, the result of this study may contribute to minimize the community burden of risk of visual impairment, including blindness which might be avoidable.
- In addition, it can serve as baseline information for further studies.

With this background, we have undertaken this study to characterize the bacteria isolates causing EOI; and to evaluate the antibiotic susceptibility status of external ocular bacterial infections along with identification possible associated risk factors.

2. Literature review

2.1. Etiologic agents of external ocular nfection

The most common types of external ocular infections are conjunctivitis, blepharitis, dacryocystitis, orbital, and periorbital cellulitis. Conjunctivitis (red eye) is inflammation of the conjunctiva, and bacterial conjunctivitis could be characterized by mucopurulent discharge and conjunctival hyperemia [Getahun *et al.*,2017]. Dacryocystitis is an infection of the nasolacrimal sac of an eye, frequently caused by nasolacrimal duct obstruction. It may be related to a malformation of the tear duct, injury, eye infection, or trauma [Assefa *et al.*,2015]. Keratitis is an inflammation of the cornea which may lead to corneal ulcer and corneal blindness [Getahun *et al*., 2017].

The etiology of conjunctivitis can be categorized into two broad categories namely infectious (bacteria, virus or fungi) and non-infectious (allergic, mechanical, chemical etc). Infectious conjunctivitis is mainly bacterial or viral, with approximately 78% to 80% of cases being bacterial in origin [Abdullah *et al.*,2013]. Bacterial and viral etiologies are most commonly blamed for ocular infections. Studies have shown that gram positive cocci are responsible for maximum number of infections followed by anaerobic bacteria and gram negative bacilli [Ipe *et al.*,2016].

Infectious /microbial/ keratitis is the most frequent cause of keratitis, is ability to sight threatening progression, characterized by defects of corneal epithelium with inflammation of underlying corneal stroma. Bacteria, viruses, fungi and parasitic organisms are the possible sources of this infection [Bitew *et al.*,2018]. Bacterial keratitis was the second most common manifestation keratitis [Deguchi*et al.*, 2018]. Common causal bacteria include *S. aureus*, coagulase-negative *staphylococci*, *S. pneumoniae* and *Pseudomonas aeruginosa*.*P. aeruginosa* is the most common microorganism implicated in bacterial keratitis among contact lens wearers [Watson*et al*., 2018].

Blepharitis is a common condition that causes inflammation of the eyelids. Sometimes referred to as granulated eyelids, it frequently produces flaky debris and particles at the base of the eyelashes [Getahun *et al.*, 2017*;*Musa *et al.*, 2014]. There are three forms of blepharitis such as; bacterial blepharitis, seborrheic blepharitis, and meibomian gland dysfunctional blepharitis.The

most common bacteria isolated from patients with chronic blepharitis are *Staphylococcus aureus, Staphylococcus epidermidis, Propioibacterium acnes,* and *Corynebacteria* [Musa *et al.,* 2014].

Dacryocystitis is an infection of the nasolacrimal sac of an eye, frequently caused by nasolacrimal duct obstruction. It may be related to a malformation of the tear duct, injury, eye infection, or trauma [Jawaid *et al.,*2017]. In most cases of infectious dacryocytitis; polymicrobial were common and concurrently isolated from bacterial, fungal, and viral origin. The microbiology of dacryocystitis may also differ in its acute and chronic infections. In severe acute dacryocystitis; single infection may predominate, often involving gram negative rods. Several other species of bacteria could be also involved in the pathogenesis of chronic dacryocystitis [Assefa *et al.,* 2015].

Generally, the common bacteria that cause eye infections are: *Pseudomonas aeruginosa, Proteus* spp, *Haemophilus aegyptius, Neisseria gonorrhoeae, Moraxella* sppsuch as; *Moraxella catarrhalis, Moraxella lacunata, Streptococcus pyogenes,* and *Staphylococcus aureus* [Muluye *et al.,*2014]. Bacterial isolates in acute ocular infections shows that *Staphylococcus* species were the most common isolates [Deguchi, 2018].

2.2. Risk factors for colonization by bacteria

Bacteria causes eye disease because of their virulence and host's condensed fighting from various factors such as socio-economic status, lifestyle, nutrition, inheritance, physiology, and age.The microbial etiology and drug susceptibility as well as resistance profile may differ with geographic location according to the restricted inhabitants [Muluye *et al.,* 2014].

Major risk factors for bacterial ocular infections with external sources are surgical and nonsurgical trauma, and use of contact lenses. Contact lenses use was found to be the most common predisposing factor for corneal infection caused by *P. aeruginosa* [Tam *et al.,* 2010].

The incidence of conjunctivitis in the newborn increased due to predisposing factors noted were vaginal delivery, asphyxia neonatorum and prolonged rupture of membrane. There has been increasing number of Methicillin resistance *S. aureus* (*MRSA*) cases in EOI that derived from surgery and contact with a *MRSA*-colonized patient, intravenous drug use, or previous antibiotic exposure [Cervantes and Mah, 2010]. It is a common practice that antibiotics can be purchased

without prescription, which leads to misuse of antibiotics. This may contribute to the emergence and spread of antimicrobial resistance bacteria [Shahaby, 2015]. Other factors may include availability of the suboptimal quality or substandard antimicrobial drugs, increased usage of a particular antimicrobial agent, poor sanitation, contaminated food and cross-contamination from humans or animals [Tesfaye *et al.*, 2013]. Additionally, overuse of topical antibiotics plays an important role in the development of bacterial resistance in EOI. But also an incorrect selection of topical antimicrobials, insufficient dosing or poor compliance to medications may also play a key role in the increasing of drug resistance [Alzahrani*et al*., 2018].

2.3. Epidemiology of external ocular infection associated bacterial isolates

A retrospective study report from Malaysia; a total of 1,211 isolates of identified ocular surface bacterial infection had been analyzed; gram-negative bacteria were most frequently isolated 54.8%, followed by gram-positive bacteria 40.7%. The isolation rate of gram-negative bacteria was shown higher than gram-positive bacteria within a ten years report. However, from individual isolates, the most frequently isolated organisms were *S.aureus* 17% and *Pseudomonasaeruginosa* 15.3%. Among the gram-negative isolates, *P. aeruginosa*695 (27.9%) was the predominant causative pathogen, followed by *Haemophilus influenzae*695 (14.4%) *Haemophilus parainfluenzae*84 (12%) *Enterobacter* spp. (11.6%, 695) and *Klebsiellapneumoniae*578(2%),*Staphylococcus aureus*516 (57.9%) was the major causal agent among gram-positive isolates, followed by *Streptococcus pneumoniae*516 (20.9%) and *Streptococcus pyogenes*516 (7.4%).Among *S.aureus*31.5% isolates were MRSA [Rahman *et al.*,2016].

A study conducted in King Abdullah International Medical Research Center, Saudi Arabia;by reviewing consecutive microbiological reports of EOI specimens obtained from the eyelids, conjunctiva or the cornea during the study period of January 2015 to December 2016. Gram negative bacteria represented the majority of organisms cultured from EOI specimens. With 154 of 218 cultures (71%) showing their growth. *Heamophilus influenza* was the most frequently encountered organism overall representing 57 of 218 and 26% of all cases, as well as being the most common gramnegative bacteria seen in EOI [Alzahrani*et al*., 2018].

A study done in Sudanese; out of 100 clinical presentations suggestive of acute conjunctivitis recruited samples; 65% showed a positive bacterial growth in culture and 35% showed no bacterial growth. The predominant isolate among the positive samples is *Staphylococcus aureus* 61. 5% followed by *Haemophilus influenza* 30.8% and *Klebsiella pneumoniae* 7.7% [Almajeed*et al.,* 2015].

A study in Ethiopia, Gondar hospital, clinical features of ophthalmic patients associated with EOI, can be conjunctivitis, blepharitis, dacrocystitis, keratitis, and blepharo-conjunctivitis thatconstituted 56.4%, 35.3%, 5.1%, 2.2%, and 1%, respectively. Among the 176 conjunctivitis cases, 48.9% were culture positive for bacterial pathogens and 4 patients had mixed bacterial growth. The predominant bacteria isolated from conjunctivitis cases forming the proportions indicated were *S.aureus* (51.1%), *CoNS* (31.1%), and *Klebsiella* species (5.6%). Similarly, *S. aureus* (50.6%) was the most frequently isolated bacteria followed by *CoNS* (32.9%) among blepharitis cases. In the cases of dacryocystitis, *S.aureus* and *CoNS* accounted for 50% each. Among patients suffering from keratitis, only *S.aureus* (n = 3) and *CoNS* (n = 2) were isolated [Getahun *et al.*,2017].

Another study reports in Ethiopia at Borumeda hospital; Among 160 External ocular specimens subjected to culture, 59.4 % were positive for different bacterial species. Among the culture positive isolates, 93.7 % were gram positive and 6.3 % gram negative bacteria. Coagulase negative *Staphylococci (CoNS)* was the predominant pathogen 57.3 % followed by *Staphylococcus aureus* 23.6 % and *Streptococcuspneumoniae* 11.2 %. Among patients clinically categorized as blepharitis and conjunctivitis, *CoNS* were the most common isolates 46.8 and 30.4 % respectively. Nine out of the ten *Streptococcus pneumoniae* isolates were found among patients clinically categorized as conjunctivitis and Belpharo-conjunctivitis. However, a comparatively higher prevalence of bacterial pathogens was found among patients suffering from dacryocystitis 80 % [Shiferaw*et al.*,2015].

A study done in Ethiopia, Jimma; suspected bacterial keratitis of the 24 corneal scrapings were subjected to culture, 20 patients (83.3%) had bacterial growth. The predominant isolate was *P.aeruginosa* (41.7%) and in grampositive group *S. aureus* (20.8%) was the predominant isolate [Tesfaye*et al*., 2015]

As reviewed by Mohammad Javed Ali; the general trend in chronic dacryocystitis reflects culture-positive rates ranging from 52.5% to 97.3% with isolation rates of grampositive organisms ranging from 53.7% to 75% and those of gram-negative organisms from 25% to 37.4%. The most common gram positive organisms isolates include *S. aureus* (worldwide), *S. pneumoniae (*Africa*),* and *S. epidermidis* (USA) [Ali *et al.*,2013].

Different study shown the prevalence of bacterial blepharitis like; *S. aureus* and *S. epidermidis* were isolated with higher frequency (in 89-100% of cases) from the blepharitis cases [Abdullah *et al.*,2013].*Staphylococcus* species were also the predominant bacterial species isolated from eyes with blepharitis (46.7%, 493) total blepharitis bacterial isolates [Bharathi *et al.*,2010]. On the other hand, among the 110 blepharitis cases, 67.3% were culture positive and 4 patients also had mixed bacterial pathogens.*S. aureus* (50.6%) was the most frequently isolated bacteria followed by *CoNS* (32.9%) among blepharitis cases [Getahun *et al*., 2017]. Studies results were respectively from Pakistan, India, and Ethiopia.

2.4. Exteral ocular infections bacterial isolatesandantibiotic susceptibility status

In USA, the ocular TRUST study, its objectives were to annually evaluate *in vitro* antimicrobial susceptibility of *S. aureus*, *S. pneumoniae*, and *H. influenzae* isolated from ocular infections to fluoroquinolones (ciprofloxacin, gatifloxacin, levofloxacin, moxifloxacin), macrolides (azithromycin), aminoglycosides (tobramycin), penicillins, dihydrofolate reductase inhibitors (trimethoprim), and polypeptides (polymyxin B). The initial study reported on ocular isolates collected from 2005 through 2006 from 35 institutions across the US and found 16.8% of *S. aureus* isolates to be methicillin-resistant; intermediate and full resistance to penicillin, azithromycin, and trimethoprim was seen in 18.3%, 22.4%, and 22.4% of pneumococcal isolates, respectively; and no resistance was reported among *H. influenzae* isolates [Asbel and Sanfilippo, 2017].

A study in India, a total number of organisms isolated from external eye infections was 86. Out of which bacterial isolate were (71%). The gram positive isolates were susceptible to Vancomycin 100% and Ciprofloxacin 75.25% shown. The gram negative organisms were mostly sensitive to Amikacin 100%, Imipenam 100% and fluroquinolones like ciprofloxacin 96% shown[Umamageswari *et al.*,2013].

A study from Saudi Arabia; from routinely tested for susceptibility to Ampicillin and Amoxicillin/Clavulanate, with 44 of the 57 *H. influenza* isolates 77.1% found to be susceptible to Ampicillin, while remaining isolates were resistant. 53 of 57 H. *influenza* isolates 92.9% were susceptible to Amoxicillin/Clavulanate with only 4 resistant cases. Of the drugs used in testing of all gram-positive bacteria Vancomycin had the highest susceptibility rate 100%. Gentamicin had susceptibility rates of 100% for S. *aureus* and 82% for *MRSA*. Erythromycin's susceptibility rate for S. *aureus* was 78%, 52% for S. *pneumonia* and 91% for *MRSA*. Moxifloxacin had 100% susceptibility for S. *aureus* and *MRSA*. For the most commonly encountered gramnegative organisms; *Pseudomonas aeruginosa* cultures were susceptible in 95% of cases when tested against Gentamicin, 91% when tested against ciprofloxacin and 82% when tested against ceftazidime. 85% of *Klebsiella pneumonia* isolates were susceptible to ciprofloxacin, 70% to gentamicin and 50% to trimethoprim/ sulfamethoxazole. *Escherichia coli* susceptibility was 100% to gentamicin and 83% to ciprofloxacin. For methicillin resistant *S.aureus* the highest resistance was to Gentamicin 18% while the highest resistances for *Streptococcus pneumonia* were towards Oxacillin 62% and Erythromycin 48% [Alzahrani*et al*., 2018].

A study done in Kenya, The most common bacteria found on the external ocular surface are *CoNS*and *S.aureus*, majority of bacterial isolates showed high resistances to tetracycline and erythromycin. All bacterial isolates showed the highest susceptibility rates to ciprofloxacin, gentamicin, tobramycin and vancomycin. The MDR isolates among *CoNS* (39.6*%), S.aureus* (27.6%)and other isolates (16.7%) emphasizing the need for ongoing antimicrobial resistance surveillance to influence infection control and prevention [Mshangila *et al.*, 2013].

Another study in Ethiopia at Borumeda hospital; the drug susceptibility pattern of the Gram positive bacteria showed that 89 (93.3%) and 100 % of the isolates were sensitive to cefoxitin and vancomycin respectively. However, more than half of gram positive isolates 89 (53.9 %) and others 91 (34.7 %) showed resistance against amoxicillin and ampicillin respectivelyThe drug susceptibility patterns of the gram negative bacterial isolates showed that 5 out of 6 (83.3 %) were susceptible to gentamicin. However, majority of gramnegative bacteria isolates 4/6(66.7 %) were resistance to tetracycline, norfloxacin, ceftriaxone, and ciprofloxacin [Shiferaw *et al.*, 2015].

3. Objectives of the Study

3.1. General objective

To determine the magnitude of bacterial isolates fromexternal ocular infections and their antibiotics susceptibility status among patients visiting ophthalmology unit of Menelik II Referral Hospital, Addis Ababa, Ethiopia from January to Appril, 2019 G.C.

3.2. Specific objectives

- Toassessthe prevalentcategories of EOIs
- To determine the magnitude of bacterial isolates form external ocular infections.
- To determine the antibiotic susceptibility status of bacterial isolates from EOI to thefrequently used antibiotic agents.
- To evaluatepossible associatedrisk factors ofbacterialexternal ocular infections.

4. Materials and methods

4.1. Study area

This study was conducted at Menelik II Referral Hospital, Addis Ababa, Ethiopia, which is a referral hospital administered under Addis Ababa Regional Health Bureau (AARHB). It was the first government hospital in the nation, built by Emperor Menelik II in 1902 E.C. it was established with the main mission of providing injured soldiers treatment and rehabilitation. Presently its mission extended to include provision of quality service for Tuberculosis & HIV, Forensic investigation, Rehabilitation, Surgery, Gynecology, Orthopedics, Ophthalmology and other infectious diseases as well. Eventhough, Ophthalmology and Forensic investigation departments are controlled under AARHB; these departments have been also serving as the nationally referral center in Ethiopia. Therefore, Ophthalmology department was the largest ward serving more than 120 patients per working days and around 15 ophthalmological surgeriesare performed each working day.

Nowadays, there are approximately 750 clinical and non-clinical staff members that provide medical specialty services to an estimated 70,000 people annually, who were referred from mainly Addis Ababa and all over the country to ophthalmology and other health services. Also, Addis Ababa University Ophthalmology medical doctor's specialty teaching and research activity arecoordinated there.

4.2. Study design and period

A Hospitalbased cross sectional prospective study design was conducted from Januaryto April, 2019.

4.3. Source population

All Patients confirmed of having external ocular infections who were visiting Ophthalmology department of Menelik II Referral Hospital during the study period.

4.4. Study population

All Patients confirmed of having external ocular infections and fulfill the eligibility criteria during the study period were included.

4.5. Selection and evaluation of study subjects

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

4.5.1. Inclusion criteria

All ages and sex group with the presence of the followingclinical sign and symptoms over a couple of days were recruited; red eye, discharge, mucoid or mucopurulent secretion, thickening of the conjunctiva, in one or both eyes.Only patientswho were willing to give their consent were enrolled in the study.

4.5.2. Exclusion criteria

Patients on antibiotics since the last two weeks andthoseunwilling to give consent to participate in the studywereexcluded from the study.

4.6. Study variables

4.6.1. Independent variables

- Age
- Gender
- Residence
- Geographic Location
- Occupation
- Educational Status
- House hold income
- Previous Surgery
- Systemic diseases
- Trauma
- The use of contact lenses
- Face washing frequency

4.6.2. Dependent variables

- Bacteria isolates of external ocular infections
- Antibiotic susceptibility status

4.7. Sample size and Sampling technique

Sample size was calculated based on the prevalence of 30% indicated in the previous study done in 2018 in that hospital on bacterial isolates of keratitis [Bitew *et al*., 2018]. Expected margin of error (d) 0.05 and confidence interval (z) is 95%, the calculation uses the following formula.

Sample size, where: $\mathbf{n} = \frac{(Z\alpha/2)^2 . P.Q}{d^2}$ n= the required sample size

1-p=Q= negative proportion

d= Expected margin of error =0.05

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

p=prevalence of previous study found from literature review=30%, hence, n =$\frac{(1.96)^2 \text{ x}0.3\text{x}0.7}{(0.05)^2}$

Therefore, 323Study participantswerecollected within ±0.05 degree of freedom.Sampling wasdone every other day from EOI confirmed patients who fulfill the sampling criteria conveniently.

4.8. Data collection tools

4.8.1. Socio demographic and clinical characteristics

External ocular examinationwas conducted using a slit lamp biomicroscope thoroughly in all patients by ophthalmologist to rule out whether or not the problem detected was due to EOI infection orinflammation and then, demographic data were collected using structured questionnaire (Annex VII). Physical diagnosis and sample collection were done by ophthalmologists/experienced ophthalmic nurse; specimens were collected only from those patients who werediagnosed by the examining health professional to have external ocular infections. The questionnaire was filled by Prnicipal investigator (data collector) and the clinical and demographic data were entered into the SPSS data analysis software version 25.0. (IBM, USA).

4.8.2. Collection, handling and transport of specimen

Samples were collected from prospective participant patients following the standard practice of the hospital, which is described briefly below. Corneal scrapping was collected by qualified ophthalmologist and ophthalmic nurse after instilling 2 to 3 drops of local anesthetic (Tetracaine hydrochloride 0.5%) into the conjunctiva and patient was asked to wait for 2 to 3 minutes; and corneal surface was cleaned for debris and discharge using dry sterile cotton tipped swab and with the help of slit lamb the edge of the ulcer was scraped using 21 gauge needle. Conjunctival specimens were collected using sterile cotton tipped swab, applied by passing the swab gently over the lower tarsal and fornix conjunctiva 2-times. Likewise, Ophthalmologists/experienced nurses took blepharitis specimens using sterile cotton swab by rolling it over the eye lid margin from medial to lateral side and back again. Pus from dacryocystitis and canalicultes 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.

4.9. Laboratory Investigation

4.9.1. Gram stain

Gram stainwasperformed from primary growth sample of blood culture for primary detection. Therefore, the presence of either gram positive or gram negative bacterialisolate was dictated for further procedure [Annex XVI].

4.9.2. Culture

The primary swabs wereinoculated onAmie's transport media and transported to Yikatit 12 Hospital Medical College, Microbiology department. Subsequentlythey were inoculated into BHIBfor enrichment thensub culturedto 5% Sheep's blood agar, MacConkey agar, Chocolate agar and Mannitol salt agar and incubated at 37°C for 24 hours up to 48 hours. Aerobic atmospheric condition wasmaintained for the MacConkey agar and Mannitol salt Agar, while the Chocolate agar and 5% Sheep's blood agar were incubated at 5-10% CO_2 atmosphere. Initially, all plates were examined for growth after 24 hours and cultures with no growth wereincubated further for 48 hours. Finally subculture and biochemical testswere done for species identifications.The isolate was then maintained in sterile BHIB mixed with glycerol for the purpose of storage for further work.

4.9.3. Identification

After getting pure colonies, further identification wereconducted using standard microbiological techniques, which include gram stain, colony morphology, and biochemical tests. Presumptive gram negative bacteria wereidentified using kligler iron agar, citrate utilization test, lysine decarboxylase test, urease test, motility test,indole test,oxidase test, andNutrient brothwere used for further identification of bacteria. Gram positive bacteria wereidentified using hemolytic activity on sheep blood agar, catalase, coagulase test, tellurite disk, bacitracin disk, sulfa diskand optochin disk test [Hemavathi *et al.,* 2014; Muluye *et al.*,2014].

4.9.4. Antimicrobial susceptibility testing

A modified Kirby-Bauer disk diffusion technique for drug susceptibility test (DST) was performed on all identified bacterial isolates as recommended by Clinical and Laboratory Standard Institute [CLSI, 2017]and [EUCAST] on Mueller-Hinton agar and Mueller-Hinton agar supplemented with 5% defibrinated sheep blood for fastidious bacterial isolates. The bacterial suspension equivalent to the McFarland standard (0.5 CFU) was seeded on Muller-Hinton agar and after few minutes put the paper impregnated antibiotic disks (Oxoid Ltd Basingstoke, Hampshire, UK) then incubate for 18-24hrs at 37^{o}C based on the organisms to be tested. Diameters of the zone of inhibition around the diskwas measured to the nearest millimeter using a caliper and classified as sensitive, intermediate, and resistant. The following antibiotics, which are recommended by M100, 27thedition of 2017were tested. such as: Penicillin (10µg), Erythromycin (15µg), Cotrimoxazole (1.25/23.75µg), Cefoxitin (30µg), Gentamycin(10µg),Ciprofloxacin(5µg), Tobramycin (10 µg),Chloramphenicol (30 g),Tetracycline (30µg), Ampicilin(10µg), Ceftriaxone (30µg) and Vancomycin (30µg) for gram positive bacterial islotessensitivity; and Amikacin (30g)Ampicilin(10µg)Gentamycin (10µg), Cotrimoxazole (1.25/23.75µg), Ciprofloxacin (5µg),Cefoxitin (30µg), Ceftazidime (10 µg), Ceftriaxone (30µg), Meropenum (10 µg), Tobramycin (10 µg) and Chloramphenicol (30 g) for gram negative bacterial isolates sensitivity were measured . Bacterial isolates which are resistant for at least one antibiotic from three or more classes of antibiotics was considered as multidrug resistant (MDR) [Magiorakos *et al*, 2012].

4.9.5. Quality control

Standard operating procedures (SOP) were strictly followed from sample collection up to final laboratory identification tomaintain the quality of expected outcome. All the equipments were checked for their functionality.QC was performed to check the quality of medium. Then ready culture media were checked for the sterility by incubating 5% of prepared media for overnight and observe for the presence of any growth. Visual inspections of cracks in media or plates, unequal fill, hemolysis, evidence of freezing, bubbles, and contamination were performed. Finally, abilities of the prepared media insupporting the growth of organisms were checked by inoculating control strains.Reference strains of *S. aureus* (ATCC 25923),*E. coli* (ATCC 25922), *Klebsiella pneumonia (*ATCC 700603) and*P. aeruginosa (*ATCC 27853) were used as a quality control for culture and susceptibility testing throughout the study.

4.10. Data quality assurance

To generate quality and reliable data, all quality control checks were done before, during and after data collection. All the questions in structured questionnaire were prepared in a clear and precise way and translated into local language (Amharic). Data were collected by the principal investigator. The entire questionnaires were checked for completeness, during and after data collection. The raw data (the laboratory, clinical and demographic data) were checked for completeness and representativeness prior entry to the database.

4.11. Data analysis

After data collection, the corresponding code number was written carefully at each margin. The data generated were entered in to the Microsoft-Excel spreadsheet 2010 (Microsoft Cop., USA) every day. The data were imported and analyzed by statistical package for social sciences (SPSS) version 25.0. (IBM, USA). Descriptive statistics were computed and data were presented using figures and tables. Binary logistic regression was used to show the association of different independent variables with the dependent variable (bacterial isolates). Moreover, a multivariate analysis was computed to identify factors that are independently influence the occurrence of dependent variables (bacterial isolates) by adjusted odds ratio analysis. P-value less than 0.05 wereconsidered as significant in all analysis.

4.12. Ethical considerations

The study protocol was evaluated and approved by the Research Ethics Review Committee (RERC) of DMIP, School of Medicine,CHS, AAU; Addis Ababa Regional Health Bureau Research and Emergency Management; and College of Health Science Institutional Review board(CHS-IRB), Addis Ababa University.Then ethical clearance wasobtained. Official cooperation letters wereobtained from AARHB, DRERC and CHS-IRB,Addis Ababa University. Moreover, prior to commencing the study, a written informed consent and/or assents were obtained from each participants and guardians.Subject confidentiality and any special data security requirements were maintained and assured. Results of the laboratory examinations that have a direct benefit in the health of the study participants were informed to physicians and the participants got their results when came back for appointments or retreatments.

5. Results

5.1. Socio-demographic characteristics of participants

A total of 323 patients with external ocular infection were enrolled. The majority of the participants were males [186 (57.6%)] and the mean age was 40.9 years and the range was 85.96 years. About [143 (44.3%)] of the patients were over 45 years of age and most of them [206 (63.8%)] were urban residents. Data on occupation showed that the majority [90 (27.9%)] were farmers, followed by 75 (23.2%) government or private office employee, while 4(1.2%) participants were unemployed.

Table 1 Socio-demographic characteristics of the study participants

Variables	Frequency	Percent %
Age		
≤15	45	13.9
16-30	66	20.4
31-45	69	21.4
46-60	81	25.1
≥ 61	62	19.2
Gender		
Female	137	42.4
Male	186	57.6
Residence		
Rural	117	36.2
Urban	206	63.8
Occupation		
Preschool Children	23	7.1
Student	32	9.9
Farmer	90	27.9
House Wife	33	10.2
Laborer	43	13.3
Merchant	23	7.1
Government or Private Office Employee	75	23.2
Unemployed	4	1.2
House hold Monthly Income		
≤ 600 Birr	17	5.2
601 - 1500 Birr	133	41.2
1501 - 4500 Birr	120	37.2
>4500 Birr	53	16.4
Total	323	100

Most of study participants' [150 (46.4%)] monthly household income was below 1500 Birr, while only 53 (16.4%) of them had monthly income above 4500 Birr (**Table 1**).According to their geographical location,the majority [147 (45.5%)] were from Addis Ababa, followed by Oromia [88 (27.2%)] and Amhara [55 (17.0%)], in that order **(Figure 1)**.

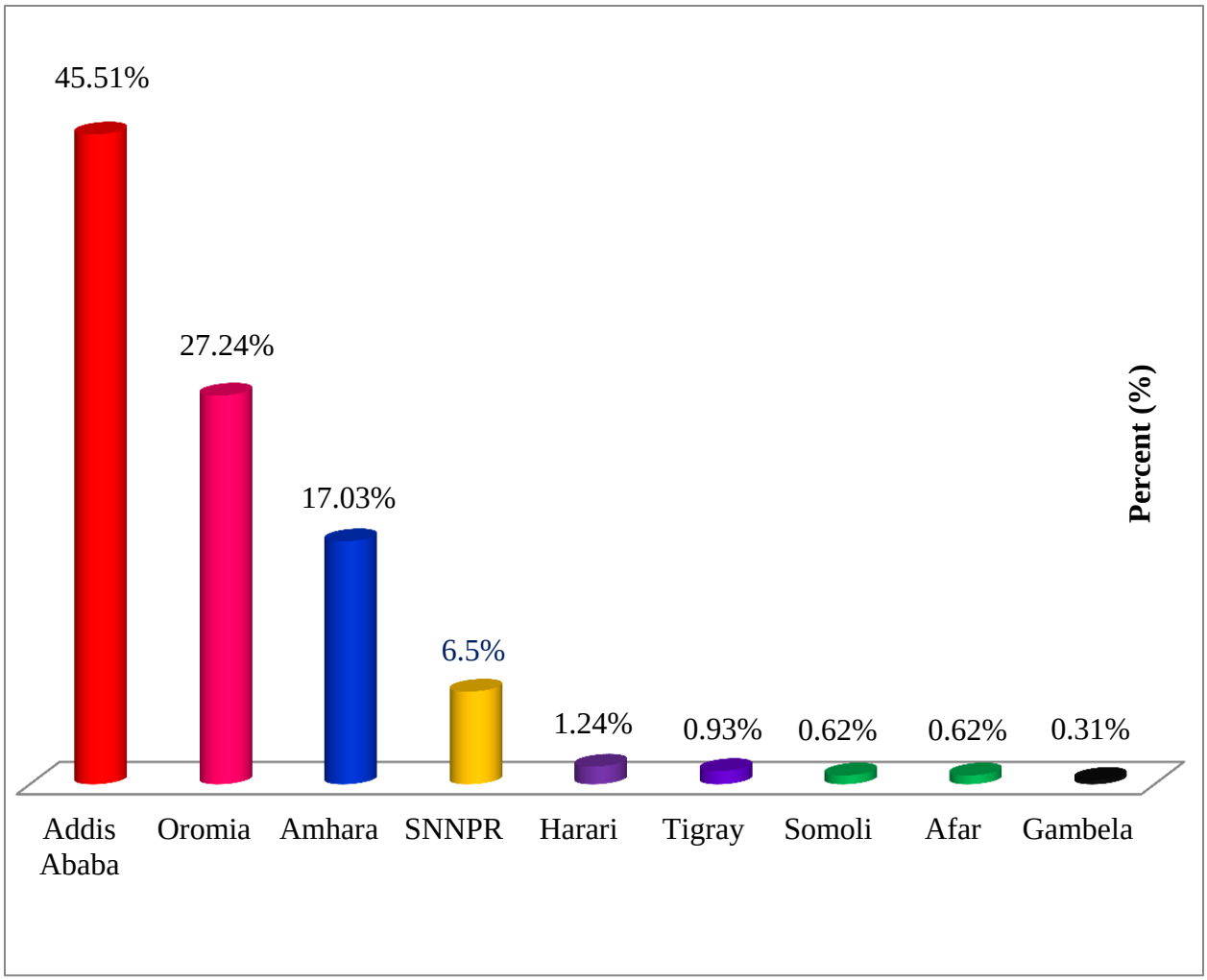

Figure 1 Study participant's distribution by their geographical location

The majority [208/323(64.4%)] of the study patients' educational status was shown to be primary school level and below. (**Figure 2**) shows detail data.

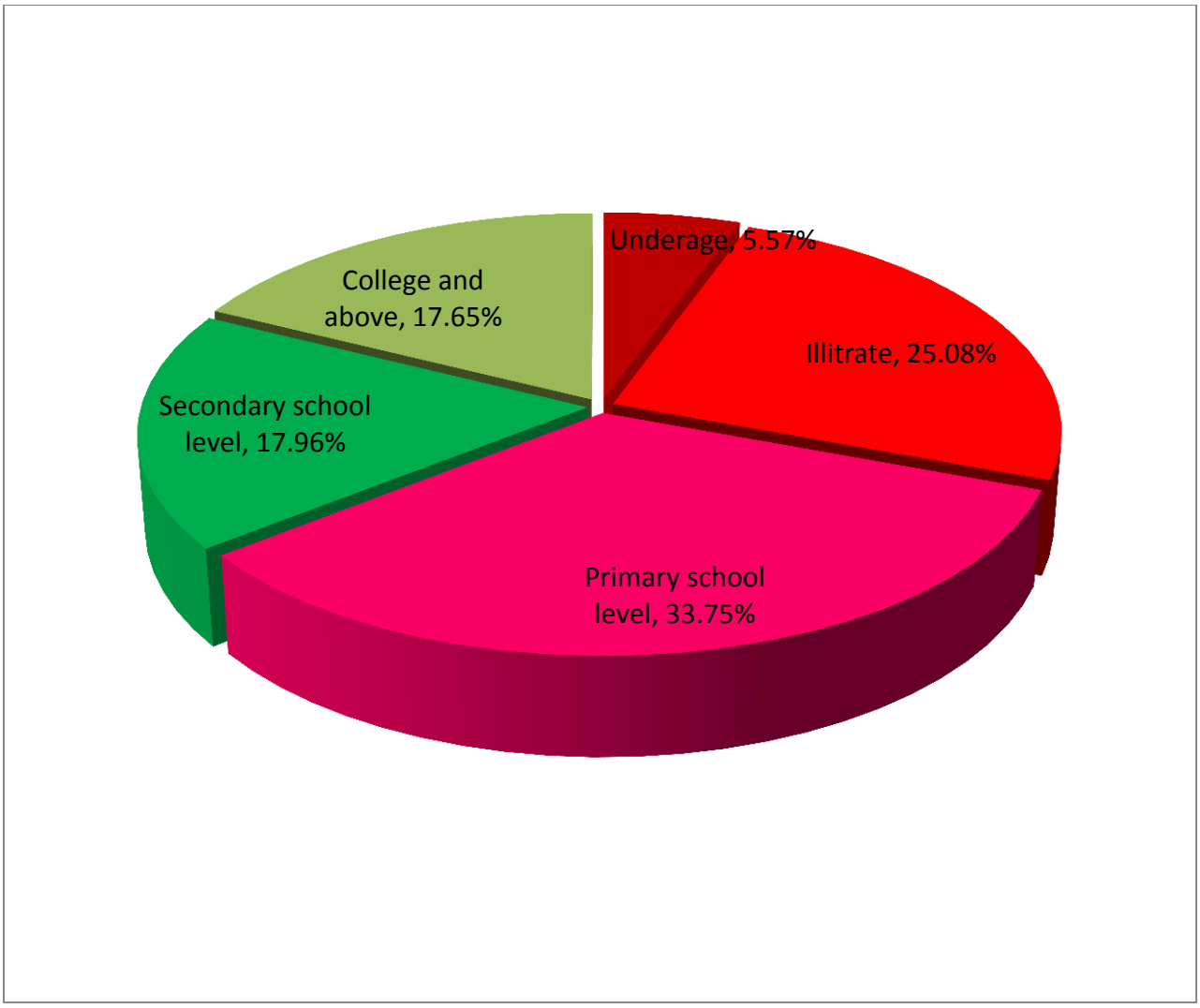

Figure 2 The distribution of study participants based on their educatitional status

5.2. Possible associated risk factors and clinical findings

In regards to participants' clinical data from 323 study participants, (59/(18.3%) patients had previous history of eye surgery, 51 (15.8%) had history of trauma but only 25 (7.7%) individuals hadsystemic disease. The data also showed that only 71 (22%) of the patients had history of contact eye lens usage. On the other hand, the study participants' face washing frequency shows the majority to practice practice twice daily 124(38.4%) followed by once daily 119 (36.8%)(**Table 2**).

Table 2 Clinical history performance of study participant

Variables	Frequency	Percent/%/
Previous eye surgery		
Yes	59	18.3
No	264	81.7
Systemic disease		
Yes	25	7.7
No	298	92.3
History of any eye trauma		
Yes	51	15.8
No	272	84.2
Uses eye glass		
Yes	71	22
No	252	78
Face washing frequency		
Sometimes	36	11.1
One times	119	36.8
Two times	124	38.4
More than two times per day	44	13.6
Total	323	100

Even though age, occupation, house hold income, use of eye glass, residence, educational status, frequency of face washing, systemic disease, and history of eye trauma were used as possible risk and predisposing factors for ocular infection in this study, multivariate logistic regression analysis showed that only educational status from demographic data (including illiterate (P = 0.005), elementary school (P = 0.002) and pre-school children (P = 0.001)); and from clinical data previous history of eye surgery (P = 0.045) and systemic disease (P = 0.006) were significantly associated with Bacterial isolates of EOI (**Table 3**).

Table 3 Possible risk factors and their association with the magnitude of bacterial isolates among patients with external ocular infection at Menelik II Referral Hospital, Ethiopia, 2019

Variables	Categories	Bacterial isolates			COR (95% CI)	AOR(95% CI)	P-value
		Yes (%)	No (%)	N (%)			
Age	≤15	28(62.2)	17(37.8)	45(13.9)			
	16-30	34(51.5)	32(48.5)	66(20.4)			
	31-45	30(43.5)	39(56.5)	69(21.4)	2.538(1.249 - 5.159)	1.18(0.508 - 2.741)	0.7
	46-60	42(51.9)	39(48.1)	81(25.1)			
	>60	41(66.1)	21(33.9)	62(19.2)			
Educational status	Illiterate	55(67.9)	26(32.1)	81(25.1)	.145(0.058 - 0.365)	.165(0.047 - 0.587)	0.005
	Preschool children	15(83.3)	3(16.7)	18(5.6)	.062(0.014 - 0.268)	.014(0.001 - 0.192)	0.001
	Elementary school level	67(61.5)	42(38.5)	109(33.7)	.193(0.08 - 0.466)	.190(0.065 - 0.552)	0.002
	Secondary School level	20(34.5)	38(65.5)	58(18.0)			
	College and Above	18(31.6)	39(68.4)	57(17.6)			
Occupation house hold monthly income		175(54.2)	148(45.8)	323(100)			
	≤ 600 Birr	13(76.5)	4(23.5)	17(5.3)	.202(0.058 - 0.704)		
	600 - 1500 Birr	89(66.9)	44(33.1)	133(41.2)	.324(0.168 - 0.627)		
	1500 - 4500 Birr	52(43.3)	68(56.7)	120(37.1)			
	>4500 Birr	21(39.6)	32(60.4)	53(16.4)			
Previous eye surgery	Yes	44(74.6)	15(25.4)	59(18.3)	.357(0.178 - 0.633)	.471(0.225 - 0.984)	0.045
	No	131(49.6)	133(50.4)	264(81.7)			
Systemic disease	Yes	20(80.0)	5(20.0)	25(7.7)	.271(0.099 - 0.741)	.205(0.066 - 0.635)	0.006
	No	155(52.0)	143(48.0)	298(92.3)			
Face washing frequency	Sometimes	26(72.2)	10(27.8)	36(11.1)	.321(0.125 - 0.821)		
	One Times	65(54.6)	54(45.4)	119(36.8)			
	Two times	64(51.6)	60(48.4)	124(38.4)			
	More than two times	20(45.5)	24(54.5)	44(13.6)			

N = number of patients, *COR* = crude odds ratio, *AOR* = adjusted odds ratio

According to their clinical features of study patients, Blepharitis was the predominant clinical manifestation but none of the clinical manifestations was significantly associated with bacterial isolates. Blepharitis 37.77% Conjunctivitis22.60%, Keratitis17.65%, Dacrocystitis 12.07%, Blepharo-conjunctivitis 5.57%and others accounted 4.33% clinical manifestations (**Figure 3**).

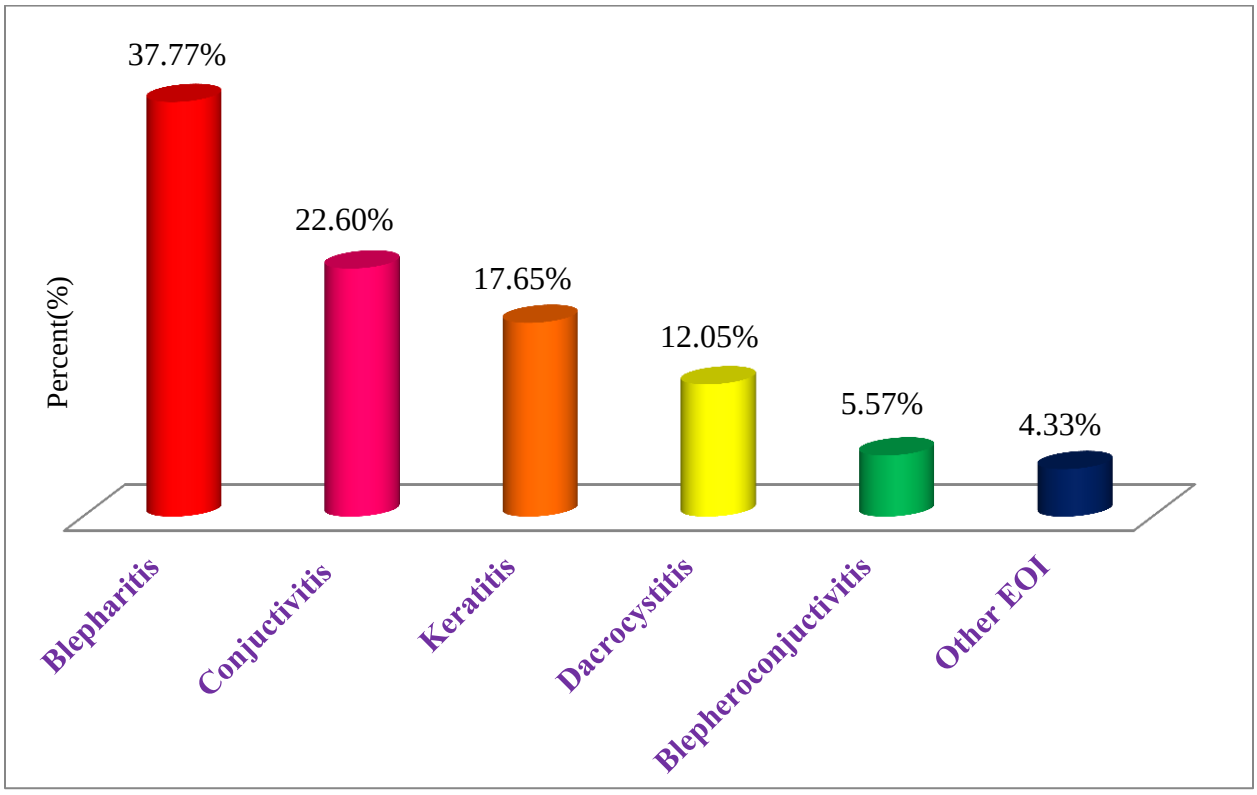

Figure 3 The distribution of external ocular infection by their clinical presentations

A total of 175 (54.2%) of the ophthalmic patients were culture positive for a single or dual bacterial isolates.Among the 122blepharitis cases, 55.7% (n= 68) were culture positive for bacterial pathogens of whom3 (4.4%) of patients had mixed bacterial growth. Among the 73 conjunctivitis cases, 38 (52.1%) were culture positive and 3 (7.9%) patients had mixed bacterial pathogens. EOI with the least number of bacterial isolates was Blephero-conjanctivitis with 10(5.4%), of whom only one case hadmixed bacterial isolates.Among the 57 keratitis cases, 32 (56.1%) were culture positive and 1 (3.1%) patient also had mixed bacterial pathogens(**Table 4**).

Table 4 The distribution and loads of bacterial isolates in external ocular ophthalmic patients at Menelik II Referral Hospital, Ethiopia, 2019

Types of external ocular infection	Loads of bacterial isolates percentage per patient				Total bacterial isolate N(%)
	Bacterial Isolates n(%)	Total cases n(%)	single bacterial Isolates n(%)	Mixed Bacterial Isolates n(%)	
Conjunctivitis	38(52.1)	73(22.6)	35(92.1)	3(7.9)	**41(22.3)**
Dacrocystitis	23(59)	39(12.1)	23(100)	0	**23(12.5)**
Blepharitis	68(55.7)	122(37.8)	65(95.6)	3(4.4)	**71(38.6)**
Keratitis	32(56.1)	57(17.6)	31(96.9)	1(3.1)	**33(17.9)**
Blephero conjunctivitis	9(50)	18(5.6)	8(88.9)	1(11.1)	**10(5.4)**
Other EOI	5(35.7)	14(4.3)	4(80)	1(20)	**6(3.3)**
Total	175(54.2)	323(100)	166(51.4)	9(2.8)	**184(100)**

n=number of participant, N= number of bacterial species, Mixed = two bacterial isolate from single sample

5.3. Magnitude of bacterial isolates

In this study, 175 (54.2%) of the 323 external ocular specimens from patients at risk of bacterial infection were positive for different bacterial species. On the other hand, the majority of the patients [166/175 (94.9%)] had single bacterial isolates while only [9/175 (5.1%)] demonstrated mixed bacterial isolates. A total of 184 bacteria species were isolated either singly or in mixed cases (**Table 4**). Among, the isolates, 171 (92.9%) were gram positive and 13 (7.1%) were gram negative bacteria species. Coagulase negative *Staphylococci* (*CoNS*) were the predominant isolate with [76/184 (41.3 %)] followed by *S. aureus* [67/184 (36.4%)] and *Streptococcus Viridian*16/184 (8.6%). Among culture positive conjunctivitis, Blepharitis and Blephero-conjuctivitis gram positive bacteria were constitutes 40/41 (98%), 67/71 (94%), and 10/10 (100%) respectively. On the other hand, higher proportions of gram negative bacterial isolates were obtained only from Dacrocystitis [6/23 (26%)] and Blepharitis [4/71 (5.6%)]. Among patients positive for bacterial isolates and clinically categorized as blepharitis (n = 71);*CoNS* were the most common isolates occurring 36/71 (50.7%) followed by*Staphylococcus aureus*23/71 (32.4%)isolates. In addition to that,*CoNS* isolates were the majority finding fromKeratitis cases[13/33 (39%)] followed by *S. aureus*[12/33(37%)]of patients.In contrast,

*S.aureus*was the most common isolates among patients withconjunctivitis 18/41 (43.9%) and Dacrocystitis 9/23 (39.1%). A sumtotal ofmore than half of *Streptococcus Viridian*bacterial isolates[9/16 (56.3%)] were found from patients with conjunctivitis and blepharitis accounting for 4/16 (25%) and 5/16 (31.3%), respectively. Five out of six (83.3%) *Streptococcus pneumoniae* isolates were found among patients clinically categorized as conjunctivitis and Blepharitis. However, a comparatively higher prevalence of gram positive bacterial isolates was found among patients suffering from Blephero-conjunctivitis [10/10 (100%)].

On the other hand, the proportion of Gram negative bacterial isolates was 13/184 (7.1%) and that of *Klebsiella species*[6/184 (3.3%)], *Proteus Species*[2/184 (1.1%)]and *Acinetobacter Species*[2/184 (1.1%)]. Among gram negative bacterial isolates, *Klebsiella spp.*[6/13 (46.2%)] was the predominant isolates **(Table 5)**.

Table 5 The distribution of bacterial species in external ocular infection at Menelik II Referral Hospital, Ethiopia, 2019

Bacterial species	Conjunctivitis (n=41) N(%)	Dacrocystitis (n=23) N(%)	Blepharitis (n=71) N(%)	Keratitis (n=33) N(%)	Blephero-conjuctivitis (n=10) N(%)	Other EOI (n=6) N(%)	**Total** n=184 N(%)
Gram positive							
Staphylococcus aureus	18(43.9)	7(30.4)	23(32.4)	12(37)	5(50)	2(33.3)	67(36.4)
CoNS	14(34.1)	7(30.4)	36(50.7)	13(39)	3(30)	3(50)	76(41.3)
β-Hemolytic Streptococci	0	0	0	1(3)	0	0	1(0.5)
Streptococcus Viridians	4(9.8)	2(8.7)	5(7)	3(9)	2(20)	0	16(8.6)
Streptococcus Pneumonia	3(7.3)	0	2(2.8)	1(3)	0	0	6(3.3)
Enterococcus Spp.	1(2.4)	1(4.3)	1(1.4)	1(3)	0	1(16.7)	5(2.7)
Total isolates	40(97.6)	17(73.9)	67(94.3)	31(94)	10(100)	6(100)	***171(92.9)***
Gram negative							
Escherchia coli	0	1(4.3)	0	0	0	0	1(0.5)
Klebsiella Spp.	1(2.4)	3(13.1)	2(2.8)	0	0	0	6(3.3)
Acinetobacter Spp.	0	1(4.3)	0	1(3)	0	0	2(1.1)
Proteus Spp.	0	1(4.3)	1(1.4)	0	0	0	2(1.1)
Pseudomonas aeruginosa	0	0	0	1(3)	0	0	1(1.1)
Providencia staurt	0	0	1(1.4)	0	0	0	1(1.1)
Total isolates	1(2.4)	6(26)	4(5.6)	2(6)	0	0	***13(7.1)***
Total bacterial species	41(22.3)	23(12.5)	71(38.6)	33(17.9)	10(5.43)	6(3.3)	**184(100)**

N= number of bacterial isolates in each clinical futures; n = number of bacterial isolates;CoNS= Coagulase negative Staphylococci; Spp. = Species

5.4. Antibiotic susceptibility status of gram positive bacterial isolate

The antibiotic susceptibility patterns of bacterial isolates from ophthalmic patients showed that a significant number of bacterial isolates were resistant to one or more antimicrobials. Among the 171 gram positive bacterial isolates, Tobramycin 94.4%, Vancomycin 92.9%, Gentamycin 76.2%, 59.4%, Ceftriaxone and Chloramphenicol 75% were susceptible (**Table 6**). On the other hand, significant amounts of gram positive isolates were lower sensitivety to penicillin (6.0%), ampicillin (20.0%), erythromycin (28.9%), and Cotrimoxazole (36%). Among the *S. aureus* isolates, 65/67 (97.0%) and 58/67 (86.5%) were susceptible to Tobramycin and Gentamycin, respectively. However, higher proportions of the *S.aureus* isolates were lower sensitivety to penicillin [2/67 (3.0%)], and Erythromycin [21/67 (31.3%)]. In this study, the prevalence of *MRSA*, determined based on the resistance pattern of cefoxitin, was 23/67 (34.3%).

Tobramycin was the only antibiotic to which *CoNS*isolates were highly sensitive acountingfor 70/76 (92.1%);and forthe rest three drugs*CoNS*isolates were sensitive in the order of to Gentamycin 50/76 (67%),to Chloramphenicol 45/76 (59.2%), and to Cifoxitin 41/76 (53.9%). All *Enterococcus spp.* and *Streptococcus pneumoniae* isolates were 6/6 (100%) sensitive to Vancomycin. On the other hand, *Streptococcus pneumoniae* isolates and *Enterococcus spp.* showed less susceptibility 3/6 (50%) and 1/5 (20%) to Cotrimoxazole and Ampicillin, respectively. Among *Streptococcus viridians*, all isolates showed more than 50 % sensitivity to all tested antibiotics. Chloramphenicol and Vancomycin were equally [14/16 (87.5%)] susceptibility to *Streptococcus viridians* isolates (**Table 6**).

Table 6 Antibiotic susceptibility pattern of gram positive bacteria isolated from external ocular infections at Menelik II Referral Hospital, Ethiopia, 2019

Bacterial isolates	Patterns N/(%)	Antibiotic susceptibility status											
		pen	Ery	Gen	Cot	Cpr	Cxt	Tob	Chl	Tet	Amp	Cft	**Van**
S.aureus	R	65(97.0 %)	37(55.2%)	3(4.5%)	31(46.3%)	11(16.4%)	23(34.3%)	2(3.0%)	11(16.4%)	-	-	-	-
	I	-	9(13.4%)	6(9.0%)	1(1.5%)	8(11.9%)	-	0(0%)	3(4.5%)	-	-	-	-
	S	2(3.0%)	21(31.3%)	58(86.5%)	35(52.2%)	48(71.6%)	44(65.7%)	65(97.0%)	53(79.1%)	-	-	-	-
CoNS	R	75 (98.7%	57(75%)	18(23.7%)	56(73.7%)	39(51.3%)	35(46.1%)	4(5.3%)	30(39.5%)	-	-	-	-
	I	-	6(7.9%)	7(9.2%)	4(5.3%)	6(7.9%)	-	2(2.6%5)	1(1.3%)	-	-	-	-
	S	1(1.3%)	13(17.1%)	50(67%)	16(21%)	31(40.8%)	41(53.9%)	70(92.1%)	45(59.2%)	-	-	-	-
Streptococcus pneumoniae	R	1(16.7%)	1(16.7%)	-	2(33.3%)	-	-	-	2(33.3%)	2(33.3%)	-	-	**0(0%)**
	I	-	0(0%)	-	1(16.7%)	-	-	-	0(0%)	0(0%)	-	-	**0(0%)**
	S	5(83.3%)	5(83.3%)	-	3(50%)	-	-	-	4(66.7%)	4(66.7%)	-	-	**6(100%)**
β hemolytic Streptococci	R	0(0%)	0(0%)	-	1(100%)	-	-	-	1(100%)	1(100%)	-	-	**0(0%)**
	I	0(0%)	0(0%)	-	0(0%)	-	-	-	0(0%)	0(0%)	-	-	**0(0%)**
	S	1(100%)	1(100%)	-	0(0%)	-	-	-	0(0%)	0(0%)	-	-	**1(100%)**
Streptococcus viridian	R	-	7(43.8%)	-	-	-	-	-	2(12.5%)	-	-	2(12.5%)	**2(12.5%)**
	I	-	1(6.2%)	-	-	-	-	-	0(0%)	-	-	2(12.5%)	**0(0%)**
	S	-	8(50%)	-	-	-	-	-	14(87.5%)	-	-	12(75%)	**14(87.5%)**
***Enterococcus* Spp.**	R	-	-	-	-	2(40%)	-	-	-	-	4(80%)	-	**0(0%)**
	I	-	-	-	-	0(0%)	-	-	-	-	-	-	**0(0%)**
	S	-	-	-	-	3(60%)	-	-	-	-	1(20%)	-	**5(100%)**
Total n= total isolates tested	**R/% n**	141(94.0%) 150	118(71.1%) 166	34(23.8%) 143	96(64.0%) 150	66(44.6%) 148	58(40.6%) 143	8(5.6%) 143	50(30.1%) 166	3(42.9%) 7	4(80%) 5	4(25%) 16	**2(7.1%)28**
	S/% N	9(6.0%) 150	48(28.9%) 166	109(76.2%) 143	54(36%) 150	82(55.4%) 148	85(59.4%) 143	135(94.4%) 143	116(69.9%) 166	4(57.1%) 7	1(20%) 5	12(75%) 16	**26(92.9%) 28**

Pen, Penicillin; Ery, Erythromycin; Gen, Gentamicin; Cot, cotrimoxazole; Cpr, Ciprofloxacin; Cxt, Cefoxitin; Tob, Tobramycin; Chl, Chloramphenicol; Tet, Tetracycline; Amp, Ampicillin; Cft, Ceftriaxone; Van, Vancomycin; N, number of isolate;S, Sensitive; R, resistant and I, Intermediate

5.5. Antibiotic susceptibility status of gram negative bacterial isolates

In this study, totally 13 gram negative bacterial isolates were obtained. All of these isolates showed [13/13 (100%)] susceptibility to Tobramycin. In addition, the gram-negative bacteria were highly susceptibility [11/13 (84.6%)] to Gentamycin. Besides, [10/13 (76.9%)] gram negative bacterial isolates were equally susceptible towards Amikacin and ciprofloxacin. But only 8/13 (61.5%) gram negative bacterial isolates were susceptible to Meropenum; and 6/10 (60%) to each of Ceftriaxone and Chloramphenicol. However, these gram negative bacterial isolates were shown to have low susceptibility rate towards Ampicillin [1/10(10%)], Cotrimoxazole [3/13 (23.1%)] and Ceftazidime [6/13 (46.2%)]. Among these gram negative bacterial isolates, *Klebsiella* spp. was [6/6 (100%)] susceptible towards Meropenum and Tobramycin but reduced susceptibility to Amikacin and Gentamycin with rate of [5/6 (83.3%)] for both antibiotics.Altogether, *Acinetobacter* spp. and *Proteus* spp. isolates while [4/4 (100%)] susceptible to both ciprofloxacin and Tobramycin, but they were [4/4 (100%)] resistant to Cotrimoxazole. Other species i.e. *E.coli, Providecia staurt, pseudomonas aeruginosa*,and*Proteus* speciesisolates were 5/5 (100%) susceptible to Gentamycin (**Table 7)**.

Table 7 Antibiotic susceptibility pattern of gram negative bacteria isolated from external ocular infections at Menelik II Referral Hospital, Ethiopia, 2019

Bacterial isolates	**Pattern N(%)**	**Antibiotic susceptibility status**									
		Amk	Amp	Gen	Cot	Cpr	Caz	Cft	Mer	Tob	Chl
Acinetobacter spp.	R	1(50%)	-	1(50%)	2(100%)	0(0%)	2(100%)	-	0(0%)	0(0%)	-
	I	0(0%)	-	0(0%)	0(0%)	0(0%)	0(0%)	-	1(50%)	0(0%)	-
	S	1(50%)	-	1(50%)	0(0%)	2(100%)	0(0%)	-	1(50%)	2(100%)	-
Escherchia coli	R	0(0%)	1(100%)	0(0%)	1(100%)	0(0%)	1(100%)	0(0%)	1(100%)	0(0%)	0(0%)
	I	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
	S	1(100%)	0(0%)	1(100%)	0(0%)	1(100%)	0(0%)	1(100%)	0(0%)	1(100%)	1(100%)
Klebsiella spp	R	1(16.7%)	6(100%)	1(16.7%)	3(50%)	0(0%)	3(50%)	1(16.7%)	0(0%)	0(0%)	2(33.3%)
	I	0(0%)	0(0%)	0(0%)	0(0%)	2(33.3%)	0(0%)	2(33.3%)	0(0%)	0(0%)	1(16.7%)
	S	5(83.3%)	0(0%)	5(83.3%)	3(50%)	4(66.7%)	3(50%)	3(50%)	6(100%)	6(100%)	3(50%)
Proteus spp.	R	0(0%)	2(100%)	0(0%)	2(100%)	0(0%)	0(0%)	1(50%)	1(50%)	0(0%)	1(50%)
	I	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	1(50%)	0(0%)	0(0%)
	S	2(100%)	0(0%)	2(100%)	0(0%)	2(100%)	2(100%)	1(50%)	0(0%)	2(100%)	1(50%)
Pseudomonas aeruginosa	R	0(0%)	-	0(0%)	1(100%)	0(0%)	1(100%)	-	0(0%)	0(0%)	-
	I	0(0%)	-	0(0%)	0(0%)	0(0%)	0(0%)	-	0(0%)	0(0%)	-
	S	1(100%)	-	1(100%)	0(0%)	1(100%)	0(0%)	-	1(100%)	1(100%)	-
Providencia staurt	R	1(100%)	0(0%)	0(0%)	1(100%)	0(0%)	0(0%)	0(0%)	1(100%)	0(0%)	0(0%)
	I	0(0%)	0(0%)	0(0%)	0(0%)	1(100%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
	S	0(0%)	1(100%)	1(100%)	0(0%)	0(0%)	1(100%)	1(100%)	0(0%)	1(100%)	1(100%)
Total n = total isolates tested	**R/%N**	3(23.1%) 13	9(90%) 10	2(15.4%) 13	10(86.9%) 13	3(23.1%) 13	7(53.8%) 13	4(40%) 10	5(38.5%) 13	0(%) 13	4(40%) 10
	S/%N	10(76.9%) 13	1(10%) 10	11(84.6%) 13	3(23.1%) 13	10(76.9%) 13	6(46.2%) 13	6(60%) 10	8(61.5%) 13	13(100%) 13	6(60%) 10

Amk, Amikacin; Amp, Ampicillin; Gen, Gentamicin; Cot, cotrimoxazole; Cpr, Ciprofloxacin; Caz, ceftazidime; Cft, Ceftriaxone; Mer, Meropenum; Tob,Tobramycin; Chl, Chloramphenicol; N, number of isolate;S, Sensitive; R, resistant and I, Intermediate

5.6. Multidrug resistance patterns of bacterial isolates

In this study, majority of the bacterial species isolated from external ocular infection exhibited different level of antimicrobial resistance. In this connection, only [2.9% (10/171)] bacterial isolates demonstrated no antimicrobial resistance to any of the antibiotics tested. The overall prevalence of multidrug resistance (bacteria resistant to at leastone antibiotic from three or more classes) was [135/184 (73.4%)]. Among gram positive isolates, [123/171 (72%)] had multidrug resistance. In addition, from those bacterial isolates 87/171 (50.9%) demonstrated resistance to four and above antibiotics. This indicates that, most of the isolates had shown higher resistance tocommonly used antibiotics which resultsan increased treatment failure. However, most of gram negative bacterial isolates [12/13 (92.3%)] demonstrated multidrug resistance, of which 9/13 (69.2%) isolates were resistant to four antibiotics and above. Among gram positives, multidrug resistances were observed [70/76 (92%)] of CoNS isolates and [67.2% (45/67)] of *S.aureus* isolates. However, among gram positive isolates, the lowest 1/5 (20%) multidrug resistance observed was in *Enterococcus* spp **(Table 8).**

Table 8 Multidrug resistance patterns of bacterial isolates from external ocular infection at Menelik II Referral Hospital, Ethiopia, 2019

Bacterial isolates	**Drug resistance status of bacterial isolates**							
	R_0 n(%)	R_1n(%)	R_2 n(%)	R_3n(%)	R_4n(%)	R_5n(%)	R_6n(%)	R_7n(%)
Gram positive isolates								
S.aureus	1(1.5)	8(11.9)	13(19.4)	22(32.8)	9(13.4)	12(17.9)	2(3.0)	0
CoNS	0	0	6(8.0)	8(10.5)	20(26.3)	25(33.0)	15(19.7)	2(2.6)
Streptococcus pneumoniae	3(49.9)	0	1(16.7)	1(16.7)	1(16.7)	0	0	
β hemolytic Streptococci Spp.	0	0	0	1(100)	0	0	0	0
Streptococcus viridian Groups Spp.	1(6.3)	6(37.5)	4(25)	3(18.7)	2(12.5)	0	0	0
Enterococcus Spp.	0	4(80)	1(20)	0	0	0	0	0
Total n/%	5(2.9)	18(10.5)	25(14.6)	35(20.5)	32(18.1)	37(21.6)	17(9.9)	2(1.2)
Gram negative isolates								
Escherchia coli	0	0	0	0	1(100)	0	0	0
Klebsiella spp.	0	0	0	2(33.3)	3(50)	1(16.7)	0	0
Proteus spp.	0	0	0	0	0	2(100)	0	0
Pseudomonas aeruginosa	0	0	1(100)	0	0	0	0	0
Acinetobacter spp.	0	0	0	1(50)	1(50)	0	0	0
Providentia staurt	0	0	0	0	1(100)	0	0	0
Total n/%	0	0	1(7.7)	3(23.1)	6(46.1)	3(23.1)	0	0
Over all total n/%	5(2.7)	18(9.8)	26(14.1)	38(20.6)	38(20.6)	40(21.7)	17(9.2)	2(1.1)

Spp. = species;*CoNS* = Coagulase negative *staphylococci*; R_0= sensitive to all antimicrobials; R_1 = resistant to 1antimicrobial; R_2 = resistant to 2 antimicrobials; R_3 = resistant to 3 antimicrobials; R_4 = resistant to 4antimicrobials; R_5 = resistant to 6 antimicrobials; R_7 = resistant to7antimicrobials

6. Discussion

There is an insufficiency published data on bacterial external ocular infection and antibiotic susceptibility status in Ethiopia, even though some studies has been done in some part of the country. The previous studies were mainly from the north, north western and south western parts of the country and recently one published study in Addis Ababa. However, the catchment area of this study was predominantly urban, located in the Addis Ababa city and all referral cases from any part of the country. Therefore, this study was conducted to evaluate the bacterial profile of EOI and antibiotic susceptibility status at a national ophthalmology referral hospital level at the center of the country. Therefore, it might help researchers to get essential information for further studies.

Generally, a total of 323 patients were involved in this study and the overall prevalence of bacterial eye infection was 175 (54.2%). Comparable finding have been reported in previous study conducted in Ethiopia, from Addis Ababa (54.9%) [Aklilu*et al*., 2018] and from Gondar (60.8%) [Muluye *et al.,* 2014];from India, (58.8%) [Bharathi, 2010]; 54.6%[Singh *et al.,* 2018] were reported. However, a relatively lower prevalence were reported in Hawassa (48.8%) [Amsalu *et al.,* 2015] and South India (46.3 %) [Rajesh*et al*., 2017].On the other hand, a relatively higher prevalence of bacterial isolate of external ocular infection was reported in Ethiopia, from Jimma (74.7%) [Tewelde *et al.,* 2013] and other countries such as Saudi Arabia(78.7%) [Shahaby*et al.*,2015].

However, the bacterial isolation rate fromour study wasamong the lower yield range compared to many other studies.The varying degree of isolation from one place to another might be due to differin distribution of bacterial etiology with the patient population, health of the cornea, geographic location and climate, and also tends to vary somewhat over time [Tesfaye*et al*., 2013].There is also possibility of inconsistency due to seasonal variation of study period since our study was conducted from January to April2019 while other studies done elsewhere in Ethiopia took place during a different time of the year: the study from Addis Ababa during Aprilto August 2016 [Aklilu*et al.,* 2018] and from Tigray, Quiha, during September 2015 to December 2015 [Teweldemedhin et al. 2017]. Moreover, most of our study participants were urban settlers so infection prevention practice in that different settings might be lower compared to the study participants in rural areas.

In the present study, demographic variables(age, gender, educational status, occupation, residence, household income) and clinical variable (systemic disease, history of previous eye surgery, eye trauma, contact eye lens usage and face washing habit) were considered as possible associated and predisposing risk factors for bacteria isolates from external ocular infection. Consequently, the data showed that statistically significant associations were observed between external ocular infection and history of systemic disease *(P* = 0.006), history of previous eye surgery (*P* = 0.045) and elementary school level(*P*=0.005), being preschool children (*P*= 0.002) and being illiterate (*p*= 0.001).

However, there were no significant associations between external ocular bacterial infection andthe remaining independent variables. Comparable findings were reported from different parts of the world. For example, the reported results from Australia [Green*et al*., 2008], Taiwan [Lin *et al.,* 2015] and India [Reddy*et al.,* 2015] showedthat systemic disease and previous ocular surgery were the most common predisposing risk factors for bacterial isolates of EOI.

Those study results on contact eye lens usage and previous eye trauma were reported as risk factors in contrast to our study result where it didn't show significant association. On the other hand, a study from Saudi Arabia [Alzahrani *etal.,* 2018], reported poor face hygiene to be a significant risk factor for lead abscess formation, for which our study found no significant association between EOI andface washing frequency.

In the case of association between educational status and external ocular infection, some literatures from Addis Ababa [Aklilu*et al*., 2018], Northeast Ethiopia [Shiferaw *et al.,* 2015] and Tigray [Teweldemedhin *et al.,* 2017] reported no significant association with bacterial isolates contrary to our study results. This difference might be due to the presence of other confounding factors such as house hold monthly income which was not tested by the othermentioned studies in Ethiopia likeGondar [Belyhun *et al*., 2018], Borumeda [Shiferaw et al., 2015] and Bahir Dar [Aweke*et al*., 2014]; and due to different way of educational status categorization,where other studies have grouped into only two categories at Addia Ababa [Aklilu*et al.,* 2018] and three categories at Hawassa [Amsalu *et al.,* 2015]. Nonetheless, one should expect better hygiene practice with educational status. However, this issue needs further study for generalization.

In this study, the predominant type of external ocular infection was Blepharitis [122 (37.8%)], followed by Conjunctivitis [73(22.6%)], Keratitis [57(17.6%)], Dacrocystitis [39(12.1%)], Blepharo-conjunctivitis 18(5.6%); and others constituted [14(4.3%)]. This is different from reports by other studies in Ethiopia such as, Hawasa [Amsalu *et al.,* 2015],Borumeda[Shiferaw *et al.,* 2015] and Gondar [Getahun *et al.,* 2017],whereconjunctivitis was reported to bethe predominant clinical future followed by Blepharitis. The reason for this discrepancy between our finding and the latter three studies could be attributed to the natureof most of conjunctivitis cases which are acute and thus requiremedication at the nearest medical center in the cases of the latter three rather than being referred to the ophthalmology referral hospital of our study site; as a result, most of the patients with blepharitis cases, being in a chronic presentation, are usually handled at the referral hospital, the reason for which Blepharitis might remain predominant at our site.

In the present study, among blepharitis cases, 68/122 (55.7%) were culture positive for bacterial pathogens but the proportion of bacterial isolates was slightlyhigher [23/39 (59%)] among patients suffering from dacrocystitis than those suffering from blepharitis. Similarly, finding from previous report of southern Ethiopia reporteda higher proportion [16/19 (84.2%)] of bacterial isolates among patients suffering from dacrocystitis [Aweke*et al*., 2014]. Another report from India also documented a significant association of bacterial infection among patients with dacrocystitis[651/930 (70%)] [Bharathi *et al.,* 2010].On the other hand, astudyfromJapan [Deguchi*et al*., 2018] reported thatbacterial isolates in patients with conjunctivitis was the most common manifestation (73%) of eye infection followed by bacterial keratitis15%, showingthat only the second one agrees with our study result.

In this study, the leading bacterial isolates from EOI were gram positive cocci. This is supported by several previous reports from Ethiopia, Jimma [Tesfaye *et al.,* 2013]; Borumeda [Shiferaw *et al.,* 2015]; Gondar [Getahun *et al.,* 2017], and countries like Nigeria [Ubani, 2009], Rwanda [Semanyenzi *et al.,* 2013] and India [Ipe *et al.,* 2016]. On the other hand, a reviewreport from South East Asia indicated isolation rate of gramnegative bacteria to be consistently higher than grampositive bacteria [Rahman *et al.,* 2013].The dominance of gram negative bacterial infection in the Taiwanese studies may reflect both geographic prevalence of the microorganism and contact lens–related because of the highly myopic population from Taiwan [Hsiao et al., 2016]

and [Lin et al., 2015].Furthermore,the dominance of gram positive bacterial isolates in our study might be due to the alteration in the normal flora, which can occur by external contamination, by infection spread from adjacent sites or via blood-born path way and disruption of epithelial layer covering the conjunctiva or eye lead.

CoNS has beenin the pastoverlookedas a cause severe infections since it is consideredas normal flora; however, nowadays because of increased use of intravascular devices and an increased number of hospitalized immunocompromised patients, *CoNS* have emerged as a major cause of nosocomial bloodstream infections [Abdullah *et al.,* 2013]. Thus, it is alarming that *CoNS*was the most predominant pathogenamong gram-positive cocci isolates in this study with overall prevalence of 76/184 (41.3%) in ophthalmic patients. This dominanceis in concordant with previous study reports fromEthiopia,Gondaracountig for 27.42% [Muluye *et al.,* 2014]; and in Uganda, the leadimg bacterial isolates were CoNS accouting for 65.9% followed by Staphylococcus aureus 21.0% [Mshangila *et al.* 2013]. Additionally,in Rwanda 51.4%[Semanyenzi *et al.,* 2013]and Iran 90% [Aghadoost, 2007]*CoNS* was the predominant isolates from EOI.

The data of the present study showed that the predominant bacteria from blepharitis cases were *CoNS* followed by *S.aureus* accounting for 36/71 (50.7%) and 23/71 (32.4%), respectively. On the other hand, *S.aureus* was the most frequently isolated bacteria among conjunctivitis cases [18/41 (43.9%)] followed by *CoNS*[14/41 (34.1%)], which is in agreement with the previous study done in Ethiopia,Hawassa,from blepharitis cases(*CoNS*35.6% and*S.aureus*32%); and from conjunctivitis cases(*S.aureus*16.4% and*CoNS* 13.4%)[Amsalu*et al.,* 2015].The finding of more bacterial isolates in the eyelid samples compared to conjunctival samples is due to colonization and recurrent introduction of bacteria from adjacent skin to the eyelid margin, whereas the presence of physical, biochemical and immunologic defensive mechanisms on the conjunctiva tend to clear microbes[Peposee*t al*., 1996].

The bacteria isolates from a dacryocystitis case were *S.aureus*[30.4% (7/23)], *CoNS*[7/23 (30.4%)] and *Klebsiella* spp[3/23 (13.1%)]. The result is supported by several reports from different parts of Ethiopia, (e.g., from Gondar*CoNS* 29.0% and *S. aureus* 29.4%[Assefa *et al.,* 2015]***;*** *CoNS*25.0%, *S.aureus* 17.1%[Belyhun *et al.,* 2018]. On the other hand, *S.*

*pneumoniae*was reported as the dominant bacterial isolates among dacryocystis cases fromHawassa study31.3%[Amsalu *et al.*, 2015].

In regards to sensitivity of isolate to tested antibiotics, significant amounts of gram positive isolates had lower sensitivety to penicillin (6.0%), ampicillin (20.0%), erythromycin (28.9%), and Cotrimoxazole (36%). Among the *S. aureus* isolates, 65/67 (97.0%) and 58/67 (86.5%) were susceptible to Tobramycin and Gentamycin, respectively. However, higher proportions of the *S. aureus* isolates hadlower sensitivety to penicillin [2/67(3.0%)], and Erythromycin [21/67(31.3%)]. In this study, the prevalence of *MRSA*, determined based on the resistance pattern of cefoxitin, was 23/67 (34.3%).

The current studyTobramycin was the only antibiotic to which *CoNS*isolates were highly sensitive acountingfor [70/76 (92.1%)];and for the rest three drugs*CoNS*isolates were sensitive in the order of to Gentamycin [50/76 (67%)], to Chloramphenicol [45/76 (59.2%)], and to Cefoxitin [41/76(53.9%)]. However, higher proportions of *CoNS* isolates indicatedto belower sensitivety to many of the commonly used antibiotics; Penicillin [1/76(1.3%)], Erythromycin [13/76(17.1%)], Cotrimoxazole [16/76(21%)], and Ciprofloxacin [31/76(40.8%)]. This is comparable withsimilar studies done in Rwanda in regards to Erythromycin (29%) but where sensitivety to Penicillin (40.6%) and Ciprofloxacin (41.2%) were reportedto be lower[Semanyenzi, 2013].A study from Jimma reported comparable low sensitivety to Cotrimoxazole[10/31 (32.3%)] but higher sensitivety [22/31 (71%)]to Erythromycin and[28/31 (90.3%)] to Ciprofloxacin [Tewoldemedhin *et al.,* 2017].In regards, resistance by grampositive bacteria, higher proportions of the S. aureus isolates [65/67 (97%)] were resistant to penicillin, and Erythromycin [46/67 (68.6%)]. The same highrate of resistance to Penicillin [42/42 (100%)] but relatively moderate rateof resistnce to Erythromycin [21/42 (50%)] was also reported from previous studyin Jimma [Tesfaye*et al*., 2013].Improper selection of antibiotics, inadequate dosing and poor compliance to therapy may play an important a role in increasing resistance [Rajesh*et al*., 2017].

Among the S. aureus isolates, 65/67 (97.0%) and 58/67 (86.5%) were susceptible to Tobramycin and Gentamycin, respectively.However, higher proportions of the *S. aureus* isolates were lower sensitivety to penicillin [2/67(3.0%)] and Erythromycin [21/67(31.3%)]. An agreement with other study report from Ethiopia, Gondar3.1% sensitivety to penicillin; but in other ways, higher sensitiviety to Erythromycin 71.9% was reported[Getahun *et al*., 2017]. The lower sensitivity to

Erythromycin in our study might be as results of time and geographic variation as well as study populations.

Furthermore, the prevalence of *MRSA* infection, as determined based onthe resistance pattern of cefoxitin, among external ocular ophthalmic patients in thisstudy was 23/67 (34.3%). Even thoughthe gold standard for identifying *MRSA* is detection of mecA gene [Brown, 2001], the study result depended only on detection of cefoxtin since genotypictests arerelatively expensive and beyond thescope of this study. Previously, one study conducted in Taiwan using molecular characterization, and antibiograms of *MRSA*demonstrated that patients with bacterial conjunctivitis simultaneously having *S. aureus* isolates, 34/59(57.6%) of them were *MRSA* positive[Kang *et al.,* 2015]. On the other hand, based on cefoxitin resistance statusa report from Brazil[Vola *et al.,* 2013] 56/566 (9.9%)S. aureus isolates were resistant to methicillin and concluded that *MRSA* is yet an infrequent cause of external ocular infections in Brazil.This illustrates the marked variation in the prevalence of *MRSA* ocular infections geographically and at different time points [Getahun *et al*., 2017].This may reflect real differences in the prevalence of *MRSA*, the standards of microbiology tests, or even epidemiological changes over time.

The drug susceptibility status of gram negative bacterial isolates to Tobramycin and Gentamycin were [13/13 (100%)] and [11/13 (84.6%)], respectively. Additionally, [10/13 (76.9%)]of gram negative bacterial isolates were susceptible toAmikacin and ciprofloxacin. This is in agreement with the previous studies from Pakistani [Abdullah *et al*., 2013]. On the other hand, the lowest susceptibility towards Ampicillin, Cotrimoxazole and Ceftazidime [1/10 (10%)], [3/13 (23.1%)], [6/13 (46.2%)] were examined respectively. Therefore, clinicians' use of empiric treatment for better medication should consider Tobramycin and Gentamycin as first choices followed by Amikacin and Ciprofloxacin in the absence of antibiotic susceptibility test.

Prevalence of multidrug resistance (MDR) is bacterial isolate at least havigone or more commonly prescribed antimicrobials resistant fromthree or more classes of antibiotis[Magiorakos *et al*., 2012]). MDR wereobserved in [135/184 (73.4%)]of the bacterial isolates. The overall MDR pattern of the gram negative bacterial isolates was [12/13 (92.3%)]. This is in agreement with the previous study reports with higher prevalence of MDR documented from Gondar [Muluye *et al*.,2014]. However, in Hawassa low prevalence of multidrug resistance [100/143 (69.9%)] was reported [Amsalu *et al.,* 2015]. Among gram positive isolates, [123/171

(72%)] had multidrug resistance. In addition, from those bacterial isolates 87/171 (50.9%) demonstrated resistance to four and above antibiotics. This indicates that, most of the isolates had shown higher resistance to commonly used antibiotics which results an increased treatment failure. Since emergence of bacterial resistance towards antimicrobial agents may increases the risk of treatment failure with potentially serious consequences.This is because of lack of access to microbiology laboratory in most health institutions, clinicians' uses broad spectrum or empirical therapy; that concerns' the risk of multidrug resistance expansion [Tesfaye *et al*., 2013; Shiferaw *et al*., 2015].Higher prevalence of MDR in our study might be due to an irrational use of antimicrobial agents and empirical therapy which can results in the emergence of bacterial strains that show multidrug resistance [Magiorakos *et al*, 2012]. Since, Menelikk II Hospital is a national referral center, where most of the patients coming for medical attention are those who have already started and failed medication to common topical antimicrobial therapy.

7. Limitations of the study

The limitation of current study were those bacteria which are not easly identfid by routine laboratory diagnosis such as; *Chlamydia trachomatis*, *Corynebacterium* species and anaerobic bacteria that cause ocular infections were not investigated in this study.Additionally genotyping analysis also not performed, due to resource problems.

8. Conclusion and recommendation

The predominant external ocular clinical future was blepharitis followed by conjuctivitis among external ocular ophthalmic patients. Lower educational status, systemic disease and previous history of ocular surgery were significantly associated with the presence of bacterial isolates. Overall, *CoNS, S. aureus, Viridian streptococci* and *Klebsiella* species werethe predominant isolates. High antibiotic resistance to commonly prescribed antibiotics was observed. The majority of the gram positive and all gram negative bacterial isolates were multidrug resistant.

- The overall antibiotic susceptibility status of bacterial isolates dictates that Tobramycin and Gentamycin, followed by chloramphenicol deserve consideration to be used as apparent drug of choice for empirical therapy.
- Microbiological bacterial isolation and antibiotic susceptibility testing should be practiced as routine diagnostic procedure for ocular infections to reduce the growing emergence of multidrug-resistant bacteria.
- Generally,increasing of drug resistance patternillustrates the need for constant bacterial surveillance and further study.

9. Reference

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10. Annexes

Annex I. Participant information sheet for adults

Introduction:

My name is Asmamaw Nitsuh and I am MSc student in Medical Microbiology at Addis Ababa University College of Health Sciences department of Microbiology, Immunology & Parasitology. I am doing a research on "Bacterial isolates from external ocular infections and antibiotic susceptibility status" among patients visiting Menelik II, Referral Hospital, Ethiopia

Purpose of the study:

The purpose of this study is to determine magnitude of bacterial isolates from external ocular infections and antibiotic susceptibility status of external eye complaints in the hospital. In order to design treatment and preventive strategies, the explanation of the magnitude, antimicrobial resistance and associated risk factors of this common infection is crucial; particularly since its prevalence in the study area is still remain poorly understood, therefore this study will assess the prevalence of external ocular bacterial infection, antimicrobial resistance status and associated risk factors.

Procedure and participation: For this study to be successful, we need your participation. And I am asking you to participate voluntarily in this study. If you are voluntary to participate in this study, you are expected to understand and sign the informed consent. Then socio demographic and clinical information related to external ocular infection will be filled on the questionnaire. Eye swap/fluid /corneal scrappy sample will be collected for laboratory analysis by attending ophthalmologist and experience nurse.

Confidentiality: All personal information you give and data obtained from laboratory analysis will be kept confidential.

Expected benefit:- Your participation in this study will benefit for the nation as a whole. positive finding in the laboratory examination after the generation of data on bacterial susceptibility will be reported to your physician. In some case, the patients may have direct benefits to guide treatment selection for those having an appointment or come back for retreatment. Other than that, there may be no benefit to you personally.

Risks: there is no any risk and no harm for participating in this study except that there is a little discomfort during sample collections.

Results dissemination: There will be a report, which is written about the finding of the study, through either publication or any other means. The result will not bear any information relevant to your personality in anyway.

Freedom to withdraw: You have the right to withdraw or leave the study. Your participation in this study is purely voluntary, and you may stop the participation at any time or you may refuse to answer some of the questions if you feel uncomfortable without giving reasons. This will not involve any penalty or loss of benefits to your medical services.

Person to contact:

If you have question or problem related with the present study, you can contact at any time using the following address:

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Ethical clearance:

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CHS-IRB, from CHS, AAU: mobile: 0941222169 and

Addis Ababa Regional Health bureau Research and Emergency Health Management: Mobile: 0911235613

Annex II. Participant information sheet for adults (Amharic version)

የጎልማሶች የጥናቱ ተሳታፊዎች መረጃ ቅጽ

መግቢያ፡ አስማማዉ ንፁህ እባላለሁ የአድስ አበባ ዩንቨርሲቲ የህክምና ማይክሮባዮሎጅ የ2ኛ ድግሪ ተማሪ ነኝ፡፡በዳግማዊ ምኒልክ ሪፈራል ሆስፒታል የሚገኙ የዉጨኛዉ የአይን በሽታ አምጭ ባክቴሪያዎች በአይን ህመምተኞች ላይ ስላለዉ ስርጭት እና ይዘት ለማወቅ የሚካሄድ ጥናት ነው፡፡

የጥናቱአላማ፡- በዳግማዊ ምኒልክ ሪፈራል ሆስፒታል የሚገኙ የአይን ህመምተኞች የዉጨኛዉ የየአይን ህመም አምጭ ባክቴሪያዎች ስርጭት ለማወቅ፣ ምን ያህሉ በባክቴሪያው ይያዛሉ እና በባክቴሪያው ለመያዝ ምክንያት የሆኑ ነገሮችን ለማዎቅ፣መድሃኒት የተቋቋሙ ባክቴሪያ የተባሉትን ረቂቅ ህዋሳት ስርጭታቸውን ለማጥናትና ለህዋሳቱ ተመራጭ የሆኑትን መድሃኒቶች ለመምረጥ ነው፡፡

ፈቃደኝነት፡- እርስዎንና ሌሎችንም በጥናቱ በሙሉ ፍቃደኝነት እንዲሳተፉ እየጠየቅን በጥናቱ ለመሳተፍ ፍቃደኛ ከሆኑ ለሚቀርብለዎት መጠይቅ ምላሽ ከሰጡ በኋላ ክአይኖት ላይ ክትትል በሚያደርግሎት ሀኪም፣ልምድ ባላቸዉ ኦፕታለሞሎጂስት ዶክተር ወይም ነርስ ናሙና ይሰጣሉ፡፡

ሚስጥራዊነት፡- የሚሰጡት መረጃ በጥናቱ ወቅትም ሆነ ከዛ በኃላ ባሉት ጊዜያት ሙሉ በሙሉ ሚስጥራዊነቱ የሚጠበቅና መረጃውም የሚያዘዉ በስም ሳይሆን በመለያ ቁጥር ይሆናል፡፡በጥናቱ ላይ እያሉ በፈለጉት ጊዜ የማቆም ወይም የማቋረጥ መብት አለዎት፡፡

የሚያገኙት ጥቅም፡- የእርሰዎ በዚህ ጥናት ተሳታፊ መሆን ለአካባቢዉ ብሎም ለሃገር ጠቀሜታ አለው፡፡ በጥናቱ ለሚሳተፉ ፍቃደኛ ተሳታፊዎች በግል ሊያስገኘዉ የሚችል ምንም አይነት ጥቅም ላይኖረዉ ይችላል:: በጥናቱ የተገኘ ፖዘቲቭ የላብራቶሪ ዉጤት ለሚከታተላቸዉ ሀኪም እንዲደርሰዉ ይደረጋል፡፡ በቀጠርዎ ቀን ወይም በድጋሜ ሲመጡ ዉጤቱ ሊያገኙ ይችላሉ፡፡ በዚህ አጋጣሚ ጥናቱ ለእርስዎ ጠቃሚ ሊሆን ይችላል፡፡

በጥናቱ ተሳታፊዎች ላይ ያለው ጉዳት እና ተዛማጅ ችግር:- በዚህ ጥናት ላይ በመሳተፍዎ ሊደርስብዎ የሚችል አንድም ጉዳት አይኖርም፡፡የሚወሰደው ናሙና ልምድ ባላቸዉ ባለሙያዎች ስለሆነ የሚያመጣዉ ችግር አይኖርም፡፡

ውጤቱን ስለመጠቀም፡- ከዚህ ጥናት በኋላ የበሽታውን ስርጭት በተመለከተ ሪፖርት ይፃፋል፡፡ሆኖም የእርስዎን ማንነት የሚገልፅ መረጃ የማይካተት ሲሆን ችግሩን ለማሳወቅ ብቻ የሚውል ነው፡፡

ከጥናቱ ስለማቋረጥ፤ በማንኛዉም ስዓት ጥናቱን ማቋረጥ ቢፈልጉ ያለምንም ማብራሪያ ከተሳታፊነት ማቆም ይችላሉ፡፡ ጥናቱን በማቆምዎ ምክንያት በህክምና አገልግሎትዎ ላይ ምንም አይነት መጉላላት አይደርስብዎትም፡፡

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ዶ/ር ዮናስ ጥላሁን ስ.ቁ፡- 09 ዘ 23 56 ዘ3፤ ኢ-ሜይል፡- yonas.tilahun@ymail.com

ህጋዊነት ማረጋገጫ፡ ከአዲስ አባባ ዩኒቨርስቲ፡- DRERC: +251910104699 እና CHS-IRB: +251941222169

Annex III. Participant information sheet for parents/guardians for participants below 12 years

Introduction:

My name is Asmamaw Nitsuh and I am MSc student in Medical Microbiology at Addis Ababa University College of Health Sciences department of Microbiology, Immunology & Parasitology. I am doing a research on "Bacterial isolates from external ocular infections and antibiotic susceptibility status" among patients visiting Menelik II, Referral Hospital, Ethiopia

Purpose of the study:

The purpose of this study is to determine magnitude of bacterial isolates from external ocular infections and antibiotic susceptibility status of external eye complaints in the hospital. In order to design treatment and preventive strategies, the explanation of the magnitude, antimicrobial resistance and associated risk factors of this common infection is crucial; particularly since its prevalence in the study area is still remain poorly understood, therefore this study will assess the prevalence of external ocular bacterial Infection, antimicrobial resistance status and associated risk factors.

Procedure and participation: For this study to be successful, we need your child participation. And I am asking you to your child participate voluntarily in this study. If you are voluntary to your child to participate in this study, your child participation will be approved by your signature by signing below "I agree to participate in this research". Then socio demographic and clinical information related to external ocular infection will be filled on the questionnaire. Eye swap/fluid /corneal scrapping sample will be collected for laboratory analysis by attending ophthalmologist and experienced ophthalmic nurse.

Confidentiality: All personal information your child give and data obtained from laboratory analysis will be kept confidential.

Expected benefits: Your child participation in this study will benefit for the nation as a whole. Positive finding in the laboratory examination after the generation of data on bacterial susceptibility will be reported to your child physician. In some case, the patients may have direct benefits to guide treatment selection for those having an appointment or come back for retreatment. Other than that, there may be no benefit to you or your child personally.

Risks: There is no any risk and no harm for participating in this study except that there is a little discomfort during sample collections.

Result dissemination: There will be a report, which is written about the finding of the study, through either publication or any other means. The result will not bear any information relevant to your child personality in anyway.

Freedom to withdraw: Your child has the right to withdraw or leave the study. Your child participation in this study is purely voluntary, and you may stop your child participation at any time or you may refuse to answer some of the questions if you feel uncomfortable without giving reasons. This will not involve any penalty or loss of benefits to your child medical services.

Person to contact:

If you have question or problem related with the present study, you can contact at any time using the following address:

Principal investigator:

Mr. Asmamaw Nitsuh, Mobile: +251929140087**,** E-mail: asmamaw.nitush76@gmail.com

Advisor's name and address:

Dr. Solomon G/Selassie, Mobile: +251 911 19 96 37, Email: solomongst@yahoo.com

Dr. Woldaregay Erku, Mobile: +251 911 45 32 97, Email: woldearegay.erku@aau.edu.et

Po Box: 9086, Addis Ababa University

Coadvisor: Dr. Yonas Tilahun**,** Mobile: +251911235613**,** Email: yonas.tilahun@ymail.com

Ethical clearance:

DRERC from DMIP, School of Medicine, CHS, AAU; Mobile: 0910104699

CHS-IRB, from CHS, AAU: mobile: 0941222169 and

Addis Ababa Health bureau Research and Emergency Health Management: Mobile: 0911235613

Annex IV. Participant information sheet for parents/guardians for participants less Than 12 years old/ (Amharic version)

ዕድሜያቸዉ ከ12 ዓመት በታች ላሉት የጥናቱ ተሳታፊዎች መረጃ ቅጽ

መግቢያ፡ አስማማዉ ንፁህ እባላለሁ የአድስ አበባ ዩንቨርሲቲ የህክምና ማይክሮባዮሎጅ የ2ኛ ድግሪ ተማሪ ነኝ፡፡በዳግማዊ ምኒልክ ሪፈራል ሆስፒታል የሚገኙ የዉጨኛዉ የአይን በሽታ አምጭ ባክቴሪያዎች በአይን ህመምተኞች ላይ ስላለዉ ስርጭት እና ይዘት ለማወቅ የሚካሄድ ጥናት ነው፡፡

የጥናቱአላማ፡- በዳግማዊ ምኒልክ ሪፈራል ሆስፒታል የሚገኙ የአይን ህመምተኞች የዉጨኛዉ የየአይን ህመም አምጭ ባክቴሪያዎች ስርጭት ለማወቅ፣ ምን ያህሉ በባክቴሪያው ይያዛሉ እና በባክቴሪያው ለመያዝ ምክንያት የሆኑ ነገሮችን ለማዎቅ፣መድሃኒት የተቋቋሙ ባክቴሪያ የተባሉትን ረቂቅ ህዋሳት ስርጭታቸውን ለማጥናትና ለህዋሳቱ ተመራጭ የሆኑትን መድሃኒቶች ለመምረጥ ነው፡፡

ፈቃደኝነት፡- የእርስዎ ልጅ እና ሌሎችንም በጥናቱ በሙሉ ፍቃደኝነት እንዲሳተፉ እየጠየቅን ልጅዎ በጥናቱ እንዲሳተፍ ፍቃደኛ ከሆኑ ፈቃደኛ መሆንዎን በወላጅ/ አሳዳጊ ፊርማ መረጋገጥ አለበት፡፡ቀጥሎም ለሚቀርብለዎት መጠይቅ ምላሽ ከሰጡ በኋላ ከልጅዎ አይን ላይ ክትትል በሚያደርግሎት ልዩ የአይን ሀኪም፣ልምድ ባላቸዉ ኦፕታለሞሎጂስት ዶክተር ወይም ነርስ ናሙና ይሰጣሉ፡፡

ሚስጥራዊነት፡- የሚሰጡት መረጃ በጥናቱ ወቅትም ሆነ ከዛ በኃላ ባሉት ጊዜያት ሙሉ በሙሉ ሚስጥራዊነቱ የሚጠበቅና መረጃውም የሚያዘዉ በስም ሳይሆን በመለያ ቁጥር ይሆናል፡፡ ልጅዎ በጥናቱ ላይ እያሉ በፈለጉት ጊዜ የማቆም ወይም የማቋረጥ መብት አለዎት፡፡

የሚያገኙት ጥቅም፡- የእርሰዎ ልጅ በዚህ ጥናት ተሳታፊ መሆን ለአካባቢዉ ብሎም ለሃገር ጠቀሜታ አለው፡፡ በጥናቱ ለሚሳተፉ ፍቃደኛ ተሳታፊዎችለልጁም ሆነ ለወላጁ በግል ሊያስገኘዉ የሚችል ምንም አይነት ጥቅም ላይኖረዉ ይችላል:: በጥናቱ የተገኘ ፖዘቲቭ የላብራቶሪ ዉጤት ለሚከታተላቸዉ ሀኪም እንዲደርሰዉ ይደረጋል፡፡ በቀጠሮዉ ቀን ወይም በድጋሜ ልጅዎን ሲያመጡ ዉጤቱን ሊያገኙ ይችላሉ፡፡ በዚህ አጋጣሚ ጥናቱ የእርስዎ ልጅ ተጠቃሚ ሊያደርግ ይችላል፡፡

በጥናቱ ተሳታፊዎች ላይ ያለው ጉዳት እና ተዛማጅ ችግር:- ልጅዎ በዚህ ጥናት ላይ በመሳተፉ/ፏ ሊያደርሽ የሚችልዉ አንድም ጉዳት አይኖርም፡፡የሚወሰደው ናሙና ልምድ ባላቸዉ ባለሙያዎች ስለሆነ የሚያመጣዉ ችግር አይኖርም፡፡

ውጤቱን ስለመጠቀም፡- ከዚህ ጥናት በኃላ የበሽታውን ስርጭት በተመለከተ ሪፖርት ይፃፋል፡፡ሆኖም የተሳታፊዉን ማንነት የሚገልፅ መረጃ የማይካተት ሲሆን ችግሩን ለማሳወቅ ብቻ የሚውል ነው፡፡

ከጥናቱ ስለማቋረጥ፤ በማንኛዉም ሰዓት የእርሰዎን ልጅ ከጥናቱ ማቋረጥ ከፈለጉ ያለምንም ማብራሪያ ልጅዎን ከተሳታፊነት ማስቆም ይችላሉ፡፡ ልጅዎን ከጥናቱ በማስቆምዎ ምክንያት በልጅዎ ህክምና አገልግሎት ላይ ምንም አይነት መጉላላት አያስከተለብዎትም፡፡

የዋናው አጥኚ አድራሻ:- አስማማዉ ንፁህ ስልክ፤**+251 929 14 00 87**፤ asmamaw.nitsuh76@gmail.com

የአጥኝዉ አማካሪዎች፡- ዶ/ር ሶሎሞን ገብረስላሴ ስ.ቁ 0911 19 96 37፤ኢ-ሜይል solomongst@yahoo.com

ዶ/ር ወልዳረጋይ እርቁ ስ.ቁ 0911 45 32 97፤ኢ-ሜይል woldearegay.erku@aau.edu.et

ዶ/ር ዮናስ ጥላሁን ስ.ቁ፡- 09 ıı 23 56 ı3፤ ኢ-ሜይል፡- yonas.tilahun@ymail.com

ህጋዊነት ማረጋገጫ፡ ከአዲስ አባባ ዩኒቨርስቲ፡- DRERC: +251910104699 እና CHS-IRB: +251941222169

Annex V. Participant information sheet for parents/guardians for participants 12- 17 years old

Introduction:

My name is Asmamaw Nitsuh and I am MSc student in Medical Microbiology at Addis Ababa University College of Health Sciences department of Microbiology, Immunology & Parasitology. I am doing a research on "Bacterial isolates from external ocular infections and antibiotic susceptibility status" among patients visiting Menelik II, Referral Hospital, Ethiopia

Purpose of the study:

The purpose of this study is to determine magnitude of bacterial isolates from external ocular infections and antibiotic susceptibility status of external eye complaints in the hospital. In order to design treatment and preventive strategies, the explanation of the magnitude, antimicrobial resistance and associated risk factors of this common infection is crucial; particularly since its prevalence in the study area is still remain poorly understood, therefore this study will assess the prevalence of external ocular bacterial Infection, antimicrobial resistance status and associated risk factors.

Procedure and participation: For this study to be successful, we need your participation. And I am asking you to participate voluntarily in this study. Your parents or guardian have to choose if you want to be in the study. If you are voluntary to participate in this study, your participation will be approved by your parents/guardian by signing below "I agree to participate in this research". Then Socio demographic and clinical information related to External ocular Infection will be filled on the questionnaire. Eye swap/fluid /corneal scrapping sample will be collected for laboratory analysis by attending ophthalmologist and experience nurse.

Confidentiality: All personal information you give and data obtained from laboratory analysis will be kept confidential.

Expected benefits: Your participation in this study will benefit for the nation as a whole. Positive finding in the laboratory examination after the generation of data on bacterial susceptibility will be reported to your physician. In some case, the patients may have direct benefits to guide treatment selection for those having an appointment or come back for retreatment. Other than that, there may be no benefit to you personally.

Risks: There is no any risk and no harm for participating in this study except that there is a little discomfort during sample collections.

Results dissemination: There will be a report, which is written about the finding of the study, through either publication or any other means. The result will not bear any information relevant to your personality in anyway.

Freedom to withdraw: You have the right to withdraw or leave the study. Your participation in this study is purely voluntary, and you may stop the participation at any time or you may refuse to answer some of the questions if you feel uncomfortable without giving reasons. This will not involve any penalty or loss of benefits to your medical services.

Person to contact:

If you have question or problem related with the present study, you can contact at any time using the following address:

Principal investigator:

Mr. Asmamaw Nitsuh, Mobile: +251929140087**,** E-mail: asmamaw.nitush76@gmail.com

Advisor's name and address:

Dr. Solomon G/Selassie, Mobile: +251 911 19 96 37, Email: solomongst@yahoo.com

Dr. Woldaregay Erku, Mobile: +251 911 45 32 97, Email: woldearegay.erku@aau.edu.et

Po Box: 9086, Addis Ababa University

Coadvisor: Dr. Yonas Tilahun**,** Mobile: +251911235613**,** Email: yonas.tilahun@ymail.com

Ethical clearance:

DRERC from DMIP, School of Medicine, CHS, AAU: Mobile:+251910104699

CHS-IRB, from CHS, AAU: mobile: +251941222169 and Addis Ababa Health bureau Research and Emergency Health Management: Mobile: +251911235613

Annex VI. Participant information sheet for adolescences /12-17 years old/ (Amharic version)

ለታዳጊ ወጣት ከ12-17 ዓመት እድሜ ያሉ የጥናቱ ተሳታፊዎች መረጃ ቅጽ

መግቢያ፡ አስማማዉ ንፁህ እባላለሁ የአድስ አበባ ዩንቨርሲቲ የህክምና ማይክሮባዮሎጅ የ2ኛ ድግሪ ተማሪ ነኝ፡፡በዳግማዊ ምኒልክ ሪፈራል ሆስፒታል የሚገኙ የዉጨኛዉ የአይን በሽታ አምጭ ባክቴሪያዎች በአይን ህመምተኞች ላይ ስላለዉ ስርጭት እና ይዘት ለማወቅ የሚካሄድ ጥናት ነው፡፡

የጥናቱአላማ፡- በዳግማዊ ምኒልክ ሪፈራል ሆስፒታል የሚገኙ የአይን ህመምተኞች የዉጨኛዉ የየአይን ህመም አምጭ ባክቴሪያዎች ስርጭት ለማወቅ፣ ምን ያህሉ በባክቴሪያው ይያዛሉ እና በባክቴሪያው ለመያዝ ምክንያት የሆኑ ነገሮችን ለማዎቅ፣መድሃኒት የተቋቋሙ ባክቴሪያ የተባሉትን ረቂቅ ህዋሳት ስርጭታቸውን ለማጥናትና ለህዋሳቱ ተመራጭ የሆኑትን መድሃኒቶች ለመምረጥ ነው፡፡

ፈቃደኝነት፡- አንተን/አንችን እና ሌሎችንም በጥናቱ በሙሉ ፍቃደኝነት እንዲሳተፉ እየጠየቅን በጥናቱ ለመሳተፍ ፍቃደኛ ከሆኑ ፈቃደኛ መሆንዎን በወላጅ/ አሳዳጊ ፊርማ መረጋገጥ አለበት፡፡ቀጥሎም ለሚቀርብለዎት መጠይቅ ምላሽ ከሰጡ በኋላ ከአይኖት ላይ ክትትል በሚያደርግሎት ሀኪም፣ልምድ ባላቸዉ ኦፕታለሞሎጂስት ዶክተር ወይም ነርስ ናሙና ይሰጣሉ፡፡

ሚስጥራዊነት፡- የሚሰጡት መረጃ በጥናቱ ወቅትም ሆነ ከዛ በኃላ ባሉት ጊዜያት ሙሉ በሙሉ ሚስጥራዊነቱ የሚጠበቅና መረጃውም የሚያዘዉ በስም ሳይሆን በመለያ ቁጥር ይሆናል፡፡በጥናቱ ላይ እያሉ በፈለጉት ጊዜ የማቆም ወይም የማቋረጥ መብት አለዎት፡፡

የሚያገኙት ጥቅም፡- የእርሰዎ በዚህ ጥናት ተሳታፊ መሆን ለአካባቢዉ ብሎም ለሃገር ጠቀሜታ አለው፡፡ በጥናቱ ለሚሳተፉ ፍቃደኛ ተሳታፊዎች በግል ሊያስገኘዉ የሚችል ምንም አይነት ጥቅም ላይኖረዉ ይችላል:: በጥናቱ የተገኘ ፖዘቲቭ የላብራቶሪ ዉጤት ለሚከታተላቸዉ ሀኪም እንዲደርሰዉ ይደረጋል፡፡ በቀጠርዎ ቀን ወይም በድጋሜ ሲመጡ ዉጤቱ ሊያገኙ ይችላሉ፡፡ በዚህ አጋጣሚ ጥናቱ ለእርስዎ ጠቃሚ ሊሆን ይችላል፡፡

በጥናቱ ተሳታፊዎች ላይ ያለው ጉዳት እና ተዛማጅ ችግር:- በዚህ ጥናት ላይ በመሳተፍዎ ሊደርስብዎ የሚችል አንድም ጉዳት አይኖርም፡፡የሚወሰደው ናሙና ልምድ ባላቸዉ ባለሙያዎች ስለሆነ የሚያመጣዉ ችግር አይኖርም፡፡

ውጤቱን ስለመጠቀም፡- ከዚህ ጥናት በኋላ የበሽታውን ስርጭት በተመለከተ ሪፖርት ይፃፋል፡፡ሆኖም የተሳታፊዉን ማንነት የሚገልፅ መረጃ የማይካተት ሲሆን ችግሩን ለማሳወቅ ብቻ የሚውል ነው፡፡

ከጥናቱ ስለማቋረጥ፤ በማንኛዉም ስዓት ጥናቱን ማቋረጥ ከፈለግህ/ሽ ያለምንም ማብራሪያ ከተሳታፊነት ማቆም ይችላል፡፡ ጥናቱን በማቆምዎ ምክንያት በህክምና አገልግሎትዎ ላይ ምንም አይነት መጉላላት አያስከተለበህም/ሽም፡፡

የዋናው አጥኚ አድራሻ:

አስማማዉ ንፁህ ስልክ፤**+251 929 14 00 87**፤

የአጥኝዉ አማካሪዎች

ዶ/ር ሶሎሞን ገብረስላሴ ስ.ቁ 0911 19 96 37፤ኢ-ሜይል solomongst@yahoo.com

ዶ/ር ወልዳረጋይ እርቁ ስ.ቁ 0911 45 32 97፤ኢ-ሜይል woldearegay.erku@aau.edu.et

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ህጋዊነት ማረጋገጫ፡ DRERC: +251910104699 እና CHS-IRB: +251941222169 ከአዲስ አባባ ዩኒቨርስቲ

Annex VII. Informed consentent form for adults

Title of the project: "Bacterial isolates of external ocular infection and their antibiotic susceptibility status among patients visiting Menelik II Referral Hospital, Ethiopia".

I have been well aware of that this research undertaking is for a partial fulfillment of MSc degree which is fully supported and coordinated by Addis Ababa University College of Health Sciences department of Microbiology, Immunology & Parasitology. I have been fully informed in the language I understand about the research project objectives that are to understand the bacterial isolates of external ocular bacterial infection and antibiotic susceptibility status of the isolates, and determining associated risk factors among external eye patients at menelik II Referral Hospital. I have been informed that all the information I shall provide to the interviewer will be kept confidential. I understood that the research has no any risk and no composition. I understand that there may be no benefit to me personally apart from Laboratory results. I also knew that I have the right to withhold information, skip questions to answer or to withdraw from the study any time. I have informed that nobody will impose me to explain the reason of withdrawal. It is also enlightening that there would have no effect at all in my health benefit. I have assured that the right to ask information that is not clear about the research before and or during the research work and to contact

I have read this form, or it has been read to me in the language I know and understood the condition stated above. Therefore, I am willing and confirm my participation by signing the consent.

Do you have agreed to participate in the study? Yes / No

Signature _____

Name of data collector _____

Signature _____ Date _____

Annex VIII. Informed consent form for adults (Amharic version)

የጎልማሶች የፈቃደኝነት መጠየቂያ ቅፅ

የጥናቱ ርዕስ፤ በዳግማዊ ምኒልክ ሪፈራል ሆስፒታል የሚገኙ የዉጨኛዉ የአይን ህመም ጋር ተዛማጂነት ያላቸዉን ባክቴሪያዎች በአይን ህመምተኞች ላይ ስላለዉ አይነት እና ስርጭት እንዲሁም ለመደሃኒት መቋቋም ሃቅም ያለዉይዘት ለማወቅ የሚካሄድ ጥናት ነው፡፡

እኔ በዳግማዊ ምኒልክ ሪፈራል ሆስፒታል የአይን ታካሚዎች መሀከል የዉጪኛው የአይን ኢንፌክሽን ተህዋስያን የሚያመጣውን ህመም እና የተህዋስያኑ መድሃኒት የመቋቋም ያለዉን ስርጭት በአይን ህሙማን ላይ ምን ያህል እንደሆነ ለማውቅ የተዘጋጀ ጥናት ላይ እድሳተፍ ተጠይቄ ስለጉዳዩም ለመረዳት በቂ መረጃ አግኝቻለሁ፡፡ በመሆኑም፤የጥናቱ ዓላማና ጥቅም ተገልጾልኛል፡፡በውጤቱ ከሚገኘዉ የህክምና አገልግሎት በቀር ሌላ በግሌ የማገኘዉ ጥቅም እንደሌለ ተረድቻለሁ፡፡ ናሙና በሚወስድበት ወቅትም ከትንሽ የህመም ስሜት ውጪ ምንም አይነት ጉዳት እንደሌለው ተነግሮኛል፤ እንዲሁም ከመጠይቁ አንብቢያለሁ ወይም ተነቦልኛል፡፡ የናሙና ውጤት በሚስጥር እንደሚያዝ ተነግሮኛል፡፡በተጨማሪም ጥናቱ ዉስጥ አለመሳተፍ መብቴ እደሆነና በማንኛዉም ጊዜ ከጥናቱ በራሴ ዉሳኔ መዉጣት እንደምችልና በዚህም ምክንያት ምንም አይነት መጉላላት እንደማይደርስብኝ በሚገባ ተረድቻለሁ፡፡ስለሆነም ሁኔታዉን በሚገባ በማጤን በፈቃደኝነት በምርምሩ ላይ ለመሳተፍ ለአጥኚው ፈቃደኝነቴን ሰጥቻለሁ፡፡በተጨማሪም የምሰጠዉ ናሙና ለባክቴሪያዎች ምርመራ ብቻ እንደሚዉል ተነግሮኝ ተስማምቻለሁ፡፡ማንኛውንም ያልገባኝን ነገር የመጠየቅ ዕድል ተሰጥቶኝ በሚገባኝ ቋንቋ መልስ አግኝቻለሁ፡፡
ማንኛውም ጥያቄ ወይም ጥርጣሬ ካለ ይህንን አድራሻ ተጠቅሜ መረጃ ማግኘት እነደምችል ተረድቻለሁ፡፡

ከላይ የተሰጠኝን መረጃ አንብቢያለሁ፤ በሚገባኝ ቋንቋ ተረድቻለሁ፡፡ ይህን ሁሉ በማገናዘብ ምርምሩ ላይ ስለኔ መረጃ እና ከዉጨኛዉ የአይን ክፍል የሚፈለገዉን ናሙና ለመስጠት ተስማምቻለሁ፣ ይህንንም በፊርማየ አረጋግጣለሁ፡፡

በጥናቱ ዉስጥ ለመካተት በፍቃደኝነት ተስማምተዋል? ተስማምቻለሁ / አልተሰማማሁም

ፊርማ _____ ቀን _____/_____/_____

የመረጃ ሰብሳቢዉ ስም _____ ፊርማ_____ ቀን _____/_____/_____

Annex IX. Parental consent form

I am the parent of the son, after being fully informed about the purpose of this study. Study title **"**Bacterial isolates from external ocular infections and antibiotic susceptibility status**"** among patients visiting Ophthalmology unit of Menelik II, Referral Hospital, Ethiopia. I, the undersigned, have been told about this research. My child has to say to choose if he/ she want to be in the study. I have been informed that there is no harm except little discomfort during sample collections. I have been informed that other people will not know my child's result as it coded with number rather than writing name. I understand that there may be no benefit to me personally apart from clinical service I get from these results. I have been encouraged to ask questions and have had my questions answered. I have been told that participation in this study is voluntary and I may refuse to be in the study. I know my participation will also be approved by my child. By signing below I agree to let my child to participate in this research study.

Study participant parent signature day/month/year

_____ ____/____/___

Witness (Illiterate) signature day/month/year

_____ ____/____/___

Name of data collector _____ signature _____ date _____

Annex X. Parental consent form (Amharic version)

የልጅ አስታማሚ የስምምነት መጠየቂያ ቅጽ

እኔ የልጄ አስታማሚ/ሞግዚት ስሆን የዚህን ጥናት አላማ በዉል ተረድቻለሁ፡፡ የጥናቱ ርዕስ በዳግማዊ ምኒልክ ሪፈራል ሆስፒታል የአይን ታካሚዎች መሀከል የዉጪኛው የአይን ኢንፌክሽን ተህዋስያን የሚያመጣውን ህመም እና የተህዋስያኑ መድሃኒት የመቋቋም ያለዉን ስርጭት በአይን ህሙማን ለይ ምን ያህል እንደሆነ ለማወቅ እንደሆነ ገለፃ ተሰጥቶኝ ግንዛቤ አግኝቻለሁ፡፡ በጥናቱ ልጄ እንዲሳተፍ ምርጫው የእኔ መሆኑን ነግረውኛል፡፡ ናሙና ሲወሰድ ከትንሽ የህመም ስሜት ውጪ ምንም አይነት ጉዳት ልጄ ላይ እንደሌለዉ ተነግሮኛል፡፡ በጥናቱ ወቅትም የልጄ መረጃዎች በሚስጥር ስለሚያዝ በሌላ ሰዉ ዘንድ እንደማይታወቅ ተረድቻለሁ፡፡ በውጤቱ ከሚገኘዉ የህክምና አገልግሎት በስተቀር ልጄ በግሉ/ሏ የሚያገኘዉ ወይም የምታገኘው ጥቅም እንደሌለ ተረድቻለሁ፡፡ ጥያቄ እንድጠይቅ ዕድል ተሰጥቶኝ ለጥያቄዎቼም በቂ ምላሽ አግኝቻለሁ፡፡ ልጄ በጥናቱ ለመሳተፍ በእኔ ፍላጎት ብቻ እንደሆነ እና በጥናቱም አለመሳተፍ ምንም አይነት ተፅዕኖ በልጄ ላይ እንደማያስከትል ተረድቻለሁ፡፡ ከዚህ ባሻገርም ልጄ በጥናቱ ውስጥ ለመካተት የእኔ የወላጁ አሳዳጊ ፈቃድ እንደሚያስፈልግ ተረድቻለሁ፡፡ በእኔ ፍቃደኝነት ልጄ በጥናቱ እንዲሳተፍ/እንድትሳተፍ ከዚህ በታች በፊርማዬ አረጋግጣለሁ፡፡

የተሳታፊው ቤተሰብ ፊርማ ቀን /ወር/ዓ.ም

_____ _____/_____/____

ምስክር (ማንበብና መፃፍ ለማይችሉ) ፊርማ ቀን /ወር/ዓ.ም

_____ _____/_____/____

የመረጃ ሰብሳቢዉ ስም _____ ፊርማ_____ ቀን _____/_____/_____

Annex XII. Assent form for adolescent /12-17 years old/

I, the undersigned, have been told about this research. Title of the thesis project: "Bacterial isolates of external ocular Infection and their antibiotic susceptibility status among Patients visiting Menelik II Referral Hospital, Ethiopia". 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. 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.

Study participant parent signature day/month/year

_____ ____/____/___

Witness (Illiterate) Signature day/month/year

_____ ____/____/___

Name of data collector _____signature _____ date _____

Annex XIII. Assent form for adolescent /12-17 years old/ (Amharic version)

ለታዳጊ ወጣት (ዕድሜያቸዉ ከ 12 እስከ 17 ዓመት የሆኑ) የጥናት ተሳታፊዎች የተሳትፎ ማራጋገጫ ቅጽ:

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

የተሳታፊው ወላጂ/አሳዳጊ ፊርማ ቀን /ወር/ዓ.ም

_____ _____/_____/___

ምስክር (ማንበብና መፃፍ ለማይችሉ) ፊርማ ቀን /ወር/ዓ.ም

_____ _____/_____/___

የመረጃ ሰብሳቢዉ ስም _____ ፊርማ_____ ቀን _____/_____/_____

Annex XIV: Questionnaire

Table 9: Questionnaire for adults (English version)

Part I: Socio-demographic characteristics			
Identification code ----------			
No.	Questionnaire on identification of the respondents	Alternative choice for responses	Remark
1	Age	_____ years	
2	Sex	1. Male 2. Female	
3	Address (Residence area)	…….. 1. Urban 2. Rural	
4	What is your occupation?	1. Farmer 2. Housewife 3. Student 4. Laborer 5. Merchant 6. Government or private office employee 7. unemploye	
5	What is your educational status?	1. Illiterate 2. Primary school level 3. Secondary school level 4. College and above	
6	How much monthly income in ETB do you have?	1. ≤600 2. 600-1500 3. 1500-4500 4. >4500	
Part II: Clinical data			

1	Types of external ocular infection presentation	1. Conjunctivitis 2. Blephartis 3. Dacrocystitis 4. Keratitis 5. Blephero-conjuctivitis 6. Others----------	
2	Do you have history of previous eye surgery?	1. Yes 1. 2. No	
3	Do you have systemic disease?	1. Yes 2. 2. No	
4	In question number (3) If yes what?	1. Diabetes mellitus 2. HIV 3. Others , Specify----------	
5	Have you used antibiotic in the last 2 weeks?	1. Yes 3. No	
6	Did you have ever any type of trauma?	1. Yes 2. No	
7	Do you have used contact eye lenses?	1. Yes 2. No	
8	How many times do you wash your face within a day?	1. Rarely 2. One times 3. Two times 4. More than two times per day	

Table 10 መጠይቅ (Amharic version questionnaire)

ክፍል **1:** የግለሰቡ ማህበራዊ እና ኢኮኖሚያዊ መረጃ የተሳታፊው መለያ ቁጥር፡ ----------			
ተ.ቁ	የግለሰቡ አካላዊ ማህበራዊ እና ኢኮኖሚያዊ መጠይቅ ዝርዝር	የመልስ አማራጮች	ይለፍ
1	እድሜ?	…….. አመት	
2	ጾታ?	1. ወንድ 2. ሴት	
3	የመኖሪያ አድራሻ?	…… 1. ከተማ 2. ገጠር	
4	የስራ ድርሻዎት ምንድን ነዉ?	1. ገበሬ 2. የቤት እመቤት 3. ተማሪ 4. የጉልበት ሰራተኛ 5. ነጋዴ 6. የመንግስት ወይም የግል የቢሮ ሰራተኛ 7. ስራ የሌለዉ	
5	የትምህርት ደረጃ/ዎት/ ሁኔታ?	1. ያልተማረ 2. የመጀመሪያ ደረጃ ት/ት 3. የሁለተኛ ደረጃ ት/ት 4. ኮሌጅ እና ከዚያ በላይ	
6	በአማካይ የቤተሰቦ ወርሃዊ ገቢ ስንት ነው?	1. ≤600 2. 600-1500 3. 1500-4500 4. >4500	
ክፍል- 2: ከጤና ጋር የተያያዙ መረጃዎች			
1	የዉጨኛዉ የአይን ህመም አይነት	1. ኮንጀክቲቫይቲስ 2. ዳክሮሳይስቲቲስ	

		3. ብሊፋርቲስ 4. ኬራቲቲስ 5. ሌላ ከሆነ ይጠቀስ……	
2	ከዚህ በፊት የአይን ቀዶ ህክምና አድርገዉ ያዉቃሉ?	1. አዎ 2. አላደረግሁም	
3	በህክምና የተረጋግጠ የህመም አይነት አለብዎት?	1. አዎ 2. የለብኝም	
4	በተራ ቁጥር 3 ጥያቄ አዎ ከከሆነ መልስዎ	1. ስኳር በሽታ 2. የኤዲስ በሽታ 3. ሌል ከሆነ ይጠቀስ……...	
5	ባለፈዉ ሁለት ሳምንት ዉስጥ የህመም መድሃኒት ተጠቅመዉ ነበር?	1. አዎ 2. አይ፤ አለተጠቀመሁም	
6	አይንዎ አካባቢ ድንገተኛ ጉዳት ገጥምዎት ያዉቃል?	1. አዎ 2. አለገጠመኝም	
7	ከዚህ ቀደም የአይን መነፀር ተጠቅመዉ ያውቃል?	1. አዎ 2. አላውቅም	
8	ፊትዎን በቀን ለምን ያህል ጊዜ ይታጠባሉ?	1. አልፎ አልፎ 2. አንድ ጊዜ 3. ሁለት ጊዜ 4. በተደጋጋሚ	

Table 11: Questionnaire for participants less Than 12 years old/ (English version)

Part I: Socio-demographic characteristics			
Identification code ----------			
No.	Questionnaire on identification of the respondents	Alternative choice for responses	Remark
1	Age	_____ years	
2	Sex	1. Female 2. Male	
3	Address (Residence area)	…….. 1. Rural 2. Urban	
4	is he a student or not?	1. yes 2. no	
5	What is his educational status?	1. Illiterate 2. Preschool children 3. Elementary school level	
6	How much is the family Monthly income in ETB does have?	1. ≤600 2. 600-1500 3. 1500-4500 4. >4500	
Part II: Clinical data			
1	Types of external ocular infection presentation	1. Conjunctivitis 2. Blephartis 3. Dacrocystitis 4. Keratitis 5. Blepheroconjuctivitis 6. Other----------	
2	Does he/she have history of previous eye surgery?	1. Yes 2. No	

3	Does he/she have systemic disease?	1. Yes 2. No	
4	In Question number (3) If yes what?	1. Diabetes mellitus 2. HIV 3. Others , Specify----------	
5	Have he/she used antibiotic in the last 2 weeks?	1. Yes 2. No	
6	Did he/she have ever any type of trauma?	1. Yes 2. No	
7	Does he/she have used contact eye lenses?	1. Yes 2. No	
8	How many times does he/she have washed his/her face within a day?	5. Rarely 6. One times 7. Two times 8. More Than two times per day	

Table 12 የህፃናት መጠይቅ (Amharic version questionnaire for participants less than 12 years)

ክፍል**1:** የግለሰቡ ማህበራዊ እና ኢኮኖሚያዊ መረጃ የተሳታፊው መለያ ቁጥር፡ ----------			
ተ.ቁ	የግለሰቡ አካላዊማህበራዊ እና ኢኮኖሚያዊ መጠይቅ ዝርዝር	የመልስ አማራጮች	ይለፍ
1	እድሜ?	…….. አመት	
2	ጾታ?	ı. ወንድ 2. ሴት	
3	የመኖሪያ አድራሻ?	…… 1. ገጠር 2. ከተማ	
4	ልጅዎ ትምህርት ይከታተላል?		
5	የትምህርት ደረጃው/ዋ ሁኔታ?	1. አይማርም 2. ለትምህርት ያለደረሰ /ች/ 3. የመጀመሪያ ደረጃ ተማሪ	
6	በአማካይ የቤተሰቦ ወርሃዊ ገቢ ስንት ነው?	1. ≤600 2. 600-1500 3. 1500-4500 4. >4500	
ክፍል- 2:ከጤና ጋር የተያያዙ መረጃዎች			
1	የዉጨኛዉ የአይን ህመም አይነት	ı. ኮንጀክቲቫይቲስ 2. ዳክሮሳይስቲቲስ 3. ብሊፋርቲስ 4. ኬራቲቲስ 5. ብሊፌሮኮንጀክቲቫይቲስ 6. ሌላከ ሆነ ይጠቀስ……	
2	ከዚህ በፊት የአይን ቀዶ ህክምና ተደርጎለት/ላት ያዉቃል?	ı. አዎ 2. አላደረግሁም	
3	በህክምና የተረጋግጠ የህመም አይነት	ı. አዎ	

	አለበት/ባት?	2. የለም	
4	በተራቁጥር 3 ጥያቄ አዎ ከሆነ መልስዎ	ነ. ስኳርበሽታ 2. የኤዲስበሽታ 3. ሌልከሆነይጠቀስ……..	
5	ባለፈዉ ሁለት ሳምንት ዉስጥ የህመም መድሃኒት ተጠቅሞ/ማ ነበር?	ነ. አዎ 2. አይ	
6	አይኑ/ኗ አካባቢ ድንገተኛ ጉዳት ገጥሞት/ሚት ያዉቃል?	1. አዎ 2. አይ	
7	ከዚህ ቀደም የአይን መነፀር ተጠቅሞ/ማ ነበር?	1. አዎ 2. አይ	
8	ፊቱን/ቷን በቀን ለምን ያህል ጊዜ ይታጠል/ትታጠባለች?	3. አልፎአልፎ 4. አንድጊዜ 5. ሁለትጊዜ 6. በተደጋጋሚ	

Table 13: Questionnaire for adolescences /12-17 years old/ (English version)

Part I: Socio-demographic characteristics			
Identification code ----------			
No.	Questionnaire on identification of the respondents	Alternative choice for responses	Remark
1	Age	_____ years	
2	Sex	1. Male 2. Female	
3	Address (Residence area)	…….. 1. Urban 2. Rural	
4	What is your occupation?	1. Farmer 2. Student 3. Laborer 4. Other……..	
5	What is your educational status?	1. Illiterate 2. Primary school 3. Secondary school level 4. College and above	
6	How much monthly income in ETB do you have?	1. ≤600 2. 600-1500 3. 1500-4500 4. >4500	
Part II: Clinical data			
1	Types of external ocular infection presentation	1. Conjunctivitis 2. Blephartis 3. Dacrocystitis 4. Keratitis 5. Blepheroconjuctivitis 6. Other----------	

2	Do you have history of previous eye surgery?	1. Yes 2. No	
3	Do you have systemic disease?	1. Yes 2. No	
4	In Question number (3) If yes what?	1. Diabetes mellitus 2. HIV 3. Others , Specify----------	
5	Have you used antibiotic in the last 2 weeks?	1. Yes 2. No	
6	Did you have ever any type of trauma?	1. Yes 2. No	
7	Do you have used contact eye lenses?	1. Yes 2. No	
8	How many times do you wash your face within a day?	1. Rarely 2. One times 3 Two times 4 More Than two times per day	

Table 14 የወጣቶች መጠይቅ (Amharic version questionnaire for 12 to 17 years participants)

ክፍል **1:** የግለሰቡ ማህበራዊ እና ኢኮኖሚያዊ መረጃ የተሳታፊው መለያ ቁጥር፡ ----------			
ተ.ቁ	የግለሰቡ አካላዊ ማህበራዊ እና ኢኮኖሚያዊ መጠይቅ ዝርዝር	የመልስ አማራጮች	ይለፍ
1	እድሜ?	…….. አመት	
2	ጾታ?	1. ወንድ 2. ሴት	
3	የመኖሪያ አድራሻ?	…… 1. ከተማ 2. ገጠር	
4	የስራ ድርሻዎት ምንድን ነዉ?	1. ገበሬ 2. ተማሪ 3. የጉልበት ሰራተኛ 4. ሌላ……	
5	የትምህርት ደረጃ/ዎት/ ሁኔታ?	1. ያልተማረ 2. ለትምህርት ያለደረሰ /ች/ 3. የመጀመሪያ ደረጃ ት/ት 4. የሁለተኛ ደረጃ ት/ት 5. ኮሌጅ እና ከዚያ በላይ	
6	በአማካይ የቤተሰቦ ወርሃዊ ገቢ ስንት ነው?	1. ≤600 2. 600-1500 3. 1500-4500 4. >4500	
ክፍል- 2: ከጤና ጋር የተያያዙ መረጃዎች			
1	የዉጨኛዉ የአይን ህመም አይነት	1. ኮንጀክቲቫይቲስ 2. ዳክሮሳይስቲቲስ 3. ብሊፋርቲስ 4. ኬራቲቲስ 5. ብሊፌሮኮንጀክቲቫይቲስ 6. ሌላ ከሆነ ይጠቀስ……	

2	ከዚህ በፊት የአይን ቀዶ ህክምና አድርገዉ ያዉቃሉ?	1. አዎ 2. አላደረግሁም	
3	በህክምና የተረጋግጠ የህመም አይነት አለብዎት?	1. አዎ 2. የለብኝም	
4	በተራ ቁጥር 3 ጥያቄ አዎ ከሆነ መልስዎ	1. ስኳር በሽታ 2. የኤዲስ በሽታ 3. ሌል ከሆነ ይጠቀስ……..	
5	ባለፈዉ ሁለት ሳምንት ዉስጥ የህመም መድሃኒት ተጠቅመዉ ነበር?	1. አዎ 2. አይ፤ አለተጠቀመሁም	
6	አይንዎ አካባቢ ድንገተኛ ጉዳት ገጥምዎት ያዉቃል?	1. አዎ 2. አለገጠመኝም	
7	ከዚህ ቀደም የአይን መነፀር ተጠቅመዉ ያውቃል?	1. አዎ 2. አላውቅም	
8	ፊትዎን በቀን ለምን ያህል ጊዜ ይታጠባሉ?	1. አልፎ አልፎ 2. አንድ ጊዜ 3. ሁለት ጊዜ 4. በተደጋጋሚ	

Annex XVI: Chartsof standard operating procedures for external ocular infections bacterial identification

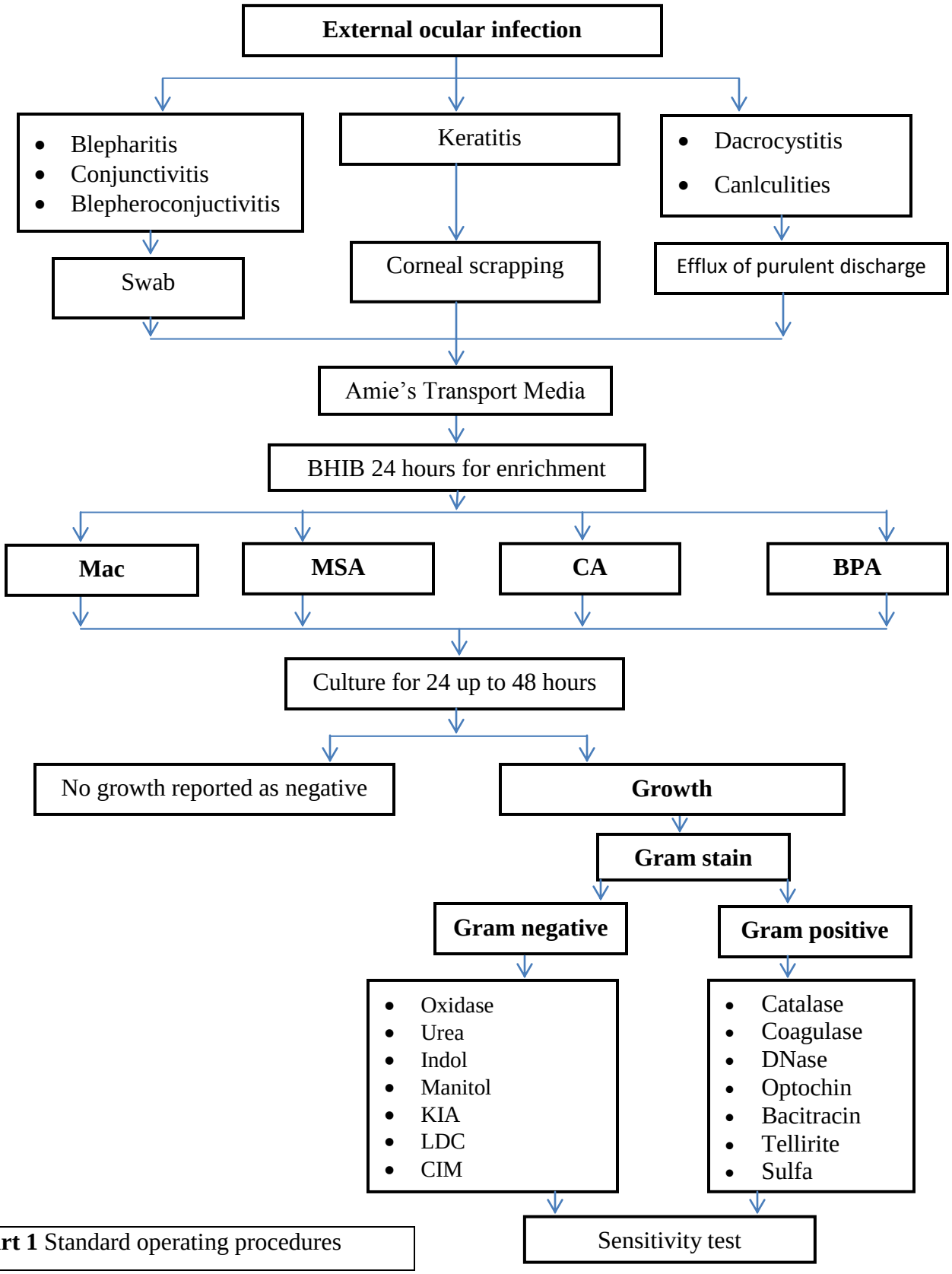

Chart 1 Standard operating procedures

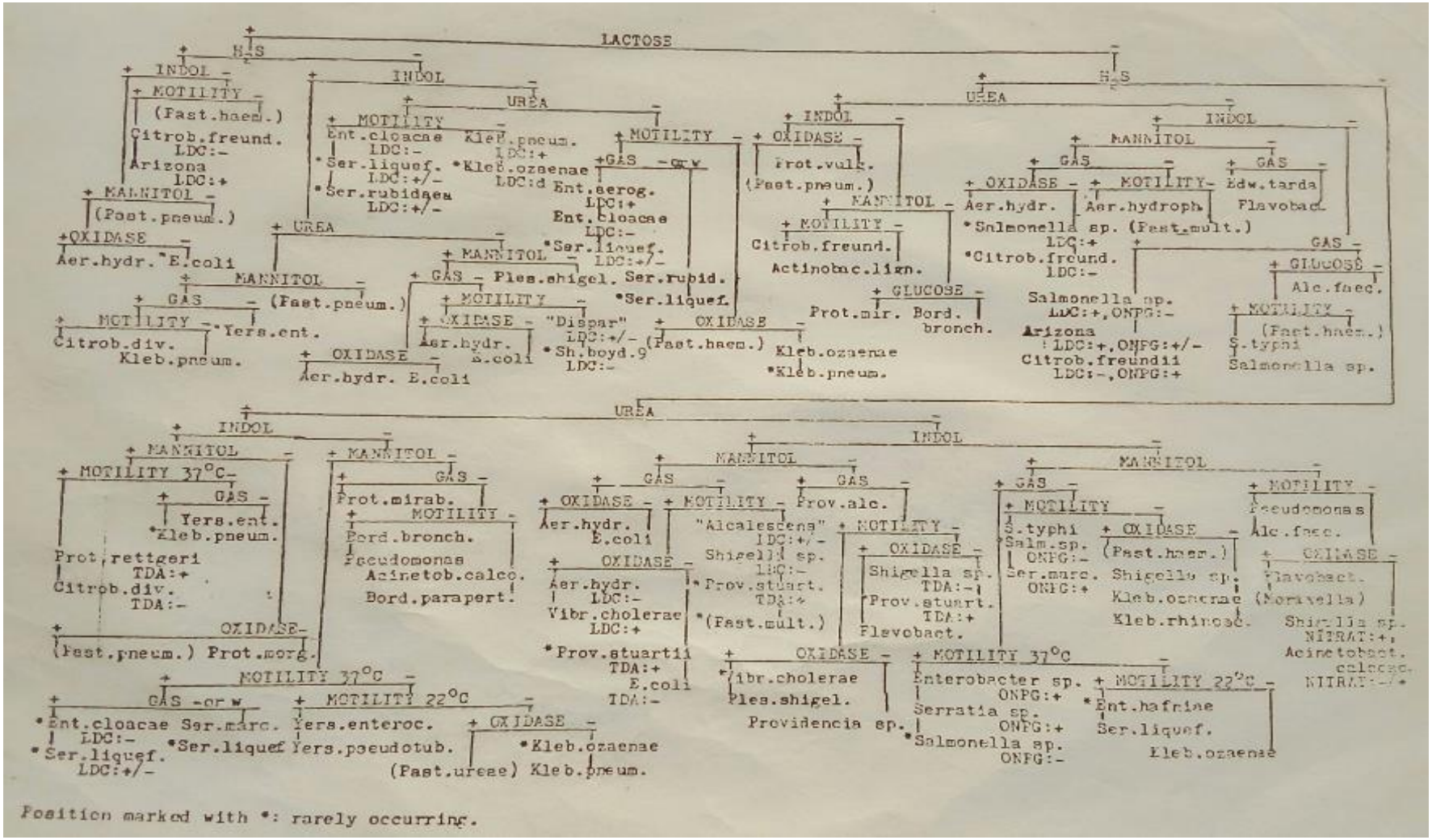

Chart. 2 Biochemical test of gram negative rods identfication charts[Yikatit 12 Hospital Medical college Microbiology Laboratory, 2019]

Directors of antibiotic susceptibility analysis

1. Antibiotics of gram positive bacterial isolates for Sensitivety testing

	Pen	Ery	Gen	Cot	Cpr	Cxt	Tob	Chl	Tet	Amp	Cft	Van
S												
I												
R												

Pen, Penicillin; Ery, Erythromycin; Gen, Gentamicin; Cot, cotrimoxazole; Cpr, Ciprofloxacin; Cxt, Cefoxitin; Tob, Tobramycin; Chl, Chloramphenicol; Tet, Tetracycline; Amp, Ampicillin; Cft, Ceftriaxone; Van, Vancomycin; S, Sensitive; R, resistant and I, Intermediate

2. Antibiotics of gram negative bacterial isolates for sensitivety testing

	Amk	Amp	Gen	Cot	Cpr	Cxt	Caz	Cft	Mer	Tob	Chl
S											
I											
R											

Amk, Amikacin; Amp, Ampicillin; Gen, Gentamicin; Cot, cotrimoxazole; Cpr, Ciprofloxacin; Caz, ceftazidime; Cft, Ceftriaxone; Mer, Meropenum; Tob,Tobramycin; Chl, Chloramphenicol; S, Sensitive; R, resistant and I, Intermediate

3. Multidrug resistance patterns of bacterial isolates from external ocular infection

Bacterial Isolates	Drug Resistance status of bacterial isolates									Total n
	R_0 n(%)	R_1n(%)	R_2 n(%)	R_3 n(%)	R_4 n(%)	R_5 n(%)	R_6 n(%)	R_7 n(%)	R_8 n(%)	
Gram positive isolates										
Gram negative isolates										
Total isolates										

R_0= sensitive to all antimicrobials; R_1 = resistant to 1antimicrobial; R_2 = resistant to 2 antimicrobials; R_3 = resistant to 3 antimicrobials; R_4 = resistant to 4antimicrobials; R_5 = resistant to 6 antimicrobials; R_7 = resistant to7antimicrobials; R_8 = resistant to 8 antimicrobial

Annex XVIII: Advisors approval sheet

This is to certify that the thesis entitled "Bacterial isolates from external ocular infection and their antibiotic susceptibility status among patients visiting Menelik II Referral Hospital, Addis Ababa, Ethiopia" is submitted in partial fulfillment of the requirements for master's degree with specialization in "Medical Microbiology" from Addis Ababa University, College of Health Science,School of Medicine, Medical microbiology graduate program has been carried out by Asmamaw Nitsuh, ID No: GSR/8441/10 under my supervision. Therefore, I recommend that the student has fulfilled the requirements and hence hereby can submit the thesis to the department.

Name of advisor Signature Date

Dr. Solomon G/Selassie;_____ _____

Dr. Woldaregay Erku; _____ _____

Name of coadvisor Signature Date

Dr. Yonas Tilahun; _____ _____

Declaration

I hereby declare that this MSc thesis is my original work and has not been presented for a degree in any other university and all sources of material used for this thesis have been duly acknowledged.

Name: Asmamaw Nitsuh

Signature: _____

Date: _____