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Zimam, Ayehubizu

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By: Zimam Ayehubizu (MSc candidate)

Advisor: Dr. Fantahun Biadglegne (PhD, Post Dr. Research Fellow, AvH)

Mr. Wondemagegn Mulu (MSc, Associate Professor)

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By:-Zimam Ayehubizu

Phone number: 0912838065

Email: tsedy98@gmail.com

This is to certify that the thesis entitled **"Bacterial isolates and antibiotic resistance profiles of external ocular infections and its associated factors among patients at Felege Hiwot Referral Hospital, Northwest Ethiopia"**

submitted by Zimam Ayehubizu the Master's degree in Medical Microbiology was carried out under our supervision and the thesis has not been previously submitted in part or full for any degree or diploma of this or any other University.

Advisor(s)	Signature	Date
1. Dr. Fantahun Biadglegne (PhD, Post Dr. Research Fellow, AvH)		
2. Mr. Wondemagegn Mulu (MSc, Associate. Pro)	_____	_____

Examiner(s)	Signature	Date
1_____	_____	_____
2._____	_____	_____

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List of abbreviations

AST …… Antimicrobial Susceptibility Testing

AMR ……...Antimicrobial Resistance

ATCC …….American Type Culture Collection

BA ……. Blood Agar

CA…….. Chocolate Agar

CLS I…….. Clinical Laboratory Standard Institution

CoNS .....Coagulase-negative Staphylococci

EOIs…….External ocular infections

FHRH …….Felege Hiwot Referral Hospital

MAC ……...MacConkey Agar

MDR ……...Multi-Drug Resistance

MHA …….. Mueller Hinton Agar

MRSA……. Methicillin resistance *S. aureus*

SOP …… Standard Operating Procedure

WHO ……..World Health Organization

Abstract

Background:- Despite bacterial ocular infection is a major public health problem in Ethiopia and empirical therapy with topical ophthalmic broad spectrum antibiotic formulations is a prevailing practice , there is dearth of data on the bacterial agents and antimicrobial resistance profiles and associated factors of eye infections in the study area..

Objective:-The aim of this study was to determine the bacterial isolates and antibiotic resistance profiles of patients with external ocular infections and identify its associated factors at Felege Hiwot Referral Hospital, Northwest Ethiopia.

Methods:- A hospital based cross - sectional study was conducted. A total of 360 patients with external ocular infection were consecutively recruited from1 February to 30 April, 2019. Data were collected using structured questionnaire by face-to-face interview and patient card review. Conjunctival, eyelid margin and lacrimal sac swabs were collected. Bacterial species were identified using standard bacteriological techniques. Antibiotic sensitivity testing was done using Kirby-Bauer disk diffusion technique. The data was analyzed using SPSS version 23 and binary logistic regression analysis was calculated to identify the factors associated with external ocular infections. P. value < 0.05 was considered as statistical significant.

Results:- A total of 360 patients were enrolled in this study and majority of them were males (64.7%). The median age of the study participants was 59.5 years and most of them were from 55 - 64 years (32.8%). The overall prevalence of culture confirmed bacterial external ocular infections was 208 (57.8%) (95% CI= 52.6 - 62.8%). The most common eye infection was conjunctivitis (60.4%) and blepharitis (55.8%). Having ocular trauma (AOR = 9.97, CI = 4.54 - 21.9), previous ocular disease (AOR = 3.53, CI = 2.18 - 21.9) and having eye allergy (AOR= 4.71, CI = 1.19 - 18.59) were the associated factors for external ocular infection. *S. aureus* was the most frequent isolate (37%) followed by CoNS (23.1%) and *K. pneumoniae* (13.5%). Most of the bacterial isolates showed higher rate of resistance to penicillin (86.9), ampicillin (83.1%) and tetracycline (47.6%). Overall, 45.2% of the isolates were MDR.

Conclusions: - Bacterial external ocular infection linked with high levels of resistance against penicillin groups and multiple drug resistant *K. pneumoniae* isolates is high. Therefore treatment of eye infections in the study area needs to be guided by drug-susceptibility testing of isolates.

Keywords:-Bacterial isolates, External ocular infections, antibiotic resistance, FHRH

1. Introduction

1.1. Background

Damage to the structure of the eye as a result of ocular infections is responsible for increased incidence of morbidity and blindness in the world. Infection and inflammation of the ocular regions may also lead to blindness if prompt and appropriate therapy is not instituted (Wang *et al.*, 2015). Gram positive and Gram negative bacteria are major causative agents of ocular infections (Ubani, 2009). External ocular infections (EOIs) frequently involve the eye lid, conjunctiva and corneal parts of the eye. The most common external ocular infections include conjunctivitis, blerpharitis, canaliculitis, keratitis and dacryocystitis (Shiferaw *et al.*, 2015; Musfer *et al.*, 2018).

Conjunctivitis (red eye) is inflammation of the conjunctiva and most commonly characterized by conjunctival hyperemia and mucopurulent discharge (Silvester *et al.*, 2016). Blepharitis is inflammation of the eyelids which could be characterized by redness, itching and greasy or crusty eyelashes (Theresea & Madhavan, 2015). Dacryocystitis is an inflammation of the lacrimal sac, which often occur due to acquired or congenital obstruction of nasolacrimal duct (Bremond *et al.*, 2011; Assefa *et al.*, 2015). Keratitis or corneal ulcer can cause corneal opacity and perforation and is the second most common cause for monocular blindness in developing countries after cataract (Duan *et al.*, 2016). Endophthalmitis is a potentially sight-threatening infection which is characterized by marked inflammation of intraocular tissues and fluids (Theresea & Madhavan, 2015).

External microbial infections of the eye are usually centralized in one place but may frequently distributed to other tissues. The conjunctiva and eyelid have a normal microbial flora controlled by its own mechanism and by the host modification of this normal flora contributes to different ocular infections (Bharathi *et al.*, 2010). The most frequent bacteria associated with ocular infections are *Staphylococcus aureus (S. aureus),* coagulase negative staphylococci *(*CoNS*), Streptococcus pneumoniae (S. pneumoniae), Bacillus, Pseudomonas aeruginosa (P. aeruginosa), Neisseria gonorrhoeae, Morraxella species, Haemophilus influenzae* and *Enterobacteriaceae* (Miller, 2017; Duan *et al.*, 2016).

Even-though the eye is hard and protected by the continuous flow of tear which contains antibacterial compounds, inflammation and scarring once occurred may not be easily resolved and requires immediate management. If ocular infections are left untreated, it can damage the structures of the eye leading to visual impairments and blindness (Harbarth & Samore, 2005).

Virulence nature of the pathogen organism, poor personal hygiene, poor living conditions, poor socio-economic status, decreased immune status, trauma, use of contact lenses, surgery, chronic nasolacrimal duct obstruction, and systemic diseases are documented determinants for the occurrence of ocular disease (Tesfaye *et al.*, 2013; Ubani, 2009; Muluye *et al.*, 2014).

Treatment for most ocular bacterial infections is primarily empiric with broad-spectrum antibiotics. However, widespread and misuse of broad-spectrum antibiotics for bacterial and viral infections or prophylactics has resulted in emerging global increase of antibiotic resistance among Gram-positive and Gram-negative bacteria (Bertino, 2009; Ubani, 2009). In Ethiopia, empirical treatment of bacterial ocular infections with broad-spectrum antibiotics are routinely practiced (Tesfaye *et al.*, 2013). These could be responsible for emerging antibiotic resistance problems over time (Belyhun *et al.*, 2018).

Multi-drug resistant (MDR) bacterial isolates like Methicillin-resistant *S. aureus* (MRSA) are emerging pathogens and becoming very serious problem. In Ethiopia, the prevalence of bacterial infections and development of MDR are becoming difficult in the treatment of external ocular infections where the diagnosis is without laboratory confirmation (Aklilu *et al.*, 2018). Therefore, the aim of this study was to determine the bacterial isolates and antimicrobial resistance (AMR) profile of patients with external ocular infections at Felege Hiwot Referral Hospital, North west Ethiopia.

1.2. Statement of the problem

Bacterial agents are known to cause external ocular infections such as conjunctivitis, keratitis, blepharitis, hordeolum, dacryocystitis which are responsible for increased incidence of morbidity and blindness worldwide (Hemavathi *et al.*, 2014; Aweke *et al.*, 2014).There are 1.4 million blind children estimated worldwide, of whom about 320,000 live in Sub-Saharan Africa (Nigatu, 2004).

The World Health Organization (WHO) estimated that 285 million individuals are visually impaired globally. Among them, 90% are from low-income countries. In the case of sub-Saharan Africa, an estimated 26 million individuals live with visual impairment, of whom 5.9 million individuals are classified blind (Schaftenaar *et al.*, 2014).

According to previous studies in other parts of Ethiopia, 48.8% - 74.4% of patients had culture confirmed bacterial external ocular infections and higher incidence of drug resistance. In Ethiopia, 1.6% prevalence of blindness was reported and it was estimated that 87.4 % of the cases were due to lack of prompt treatment of microbial infections (Teweldemedhin *et al.*, 2017; Belyhun *et al.*, 2018; Shiferaw *et al.*, 2015; Amisalu *et al* 2015).

According to a study in 2013, the cost of treating bacterial conjunctivitis alone was estimated to be $377 to $857 million per year in United States (Azari & Barney, 2013). On the other hand mobility was constrained among 83% of people with blindness compared to 49% for visually impaired and only 13% for sighted individuals in sub-Saharan Africa (Schaftenaar *et al.*, 2014).

The emergence of resistant bacterial strains towards the routinely used antibiotics in the hospitals is a worldwide problem. In Ethiopia, use of antibiotics without prescription is a common practice, which leads to misuse of antibiotics. Hence, the empirical choice of an effective treatment is becoming more difficult as ocular pathogens are increasingly becoming resistant to commonly used antibiotics. Although effective management of external ocular infections demands knowledge of the specific etiology and its antimicrobial resistance profile to commonly used antibiotics, in Ethiopia eye infections are mostly managed empirically and little is known about the specific bacterial etiologies (Teweldemedhin *et al.*, 2017; Amisalu *et al.*, 2015).

Bacterial etiologic agents of ocular infections, their antibiotic susceptibility, and resistance profiles vary according to geographical and regional location. The susceptibility profiles of bacterial isolates to various antibiotics also vary from hospital to hospital and in the same hospital from time to time (Musa *et al.*, 2014; Bharathi *et al.*, 2010; Tesfaye *et al.*, 2013). The information on the clinical importance of external eye infections has been reported in Ethiopia by clinical observation only. Thus, data on the microbiologic studies with culture and drug sensitivity test that showed the magnitude of the problem. This might be due to lack of access to microbiology laboratory, high cost and long time for diagnosis (Amisalu et al., 2015).

In Felege Hiwot Referral Hospital, there is limitation of published data on the bacterial isolates and antibiotic resistance profiles and associated factors of external ocular infections. Thus, this study identified the bacterial pathogens, determined their resistance profile to the commonly used antibiotics and identified factors associated with external ocular infections among patients in Ophthalmology unit of Felege Hiwot Referral Hospital, Bahir Dar Ethiopia.

1.3. Significance of the study

- Data generated from this study will be used as baseline for further similar studies.
- Information on the bacterial etiologic agents causing external ocular infections and their resistance profile to the commonly prescribed antibiotics is essential for optimal management of the cases and preserve local knowledge.
- Information from this study will be an input to document a multi-centered data at the national level to formulate policy for treatment and prevention of external ocular infection
- Date from this study will provide insights to revise and or develop guidelines for empirical therapy
- Data will be used as an input for concerned bodies in planning and managing of drug resistance that further reduce the morbidity and mortality of patients

2. Literature review

2.1. Epidemiology of bacterial external ocular infection

The external ocular surface acquires a microbial flora at birth and some of the commensal flora may become resident in the conjunctiva and eyelids with a potential to become pathogenic. Moreover, all microorganisms derived from the environment can also transiently colonize the eye and when given the opportunity, can invade the ocular tissues and cause infection (Bremond *et al.*, 2011). Generally Gram positive cocci are the most common opportunistic pathogens as a primary cause of bacterial eye infections and are responsible for 60% to 80% of acute infections (Mshangila *et al.*, 2013). Among them, *Staphylococcal* species and *S. pneumoniae* are most frequently isolated (Asbell &Decory, 2018).

According to the 2009 and 2013, Antibiotic Resistance Monitoring in Ocular Microorganisms (ARMOR) (2009 and 2013) and the Tracking Resistance in the United States Today (TRUST) report, *S. aureus*, with a high percentage of MRSA is the most worldwide infectious agent in external ocular infection (Asbell *et al.*, 2015).

A retrospective review in United Kingdom, in 2016 from a total of 8209 conjunctival swabs processed, 1300 (15.8%) were culture positive, of which 977 (75.2%) were Gram positive. In this study, *S. aureus* was the most prevalent organism identified. Resistance of all bacterial isolates to chloramphenicol was 8.4% varying from 3.0% to 16.4% while that for ciprofloxacin and gentamicin was 16.4% and 14%, respectively. Proportion of methicillin resistance among *S. aureus* isolates was 8.3% (Silvester *et al.*, 2016).

Another retrospective study conducted on ocular infection in south India revealed that a total of 4417 ocular samples were submitted for microbiological evaluation from 2002 to 2007, of which 2599 (58.8%) had bacterial growth. The rate of culture-positivity was 88% in eyelids infection and 70% in Conjunctival infections. The most common bacterial species isolated were *S. aureus* (26.69%) and *S. pneumoniae* (22.14%). *S. aureus* was more prevalent in eyelid infections (51.2%), *S. pneumoniae* in lacrimal apparatus and corneal infections (64.19%), *Corynebacterium* species., in blepharitis and conjunctivitis (71%), *P. aeruginosa* in keratitis and Dacryocystitis (66.5%), *Haemophilus* spp., in dacryocystitis and conjunctivitis (66.7%). In this study, the largest numbers of Gram positive isolates were susceptible to moxifloxacin (98.7%) and

vancomycin (97.9%) and Gram-negative isolates to amikacin (93.5%) and gatifloxacin (92.7%) (Bharathi *et al.*, 2010).

In a study conducted in Egypt, 70 out of 89 ocular specimens (78.7%) were positive. *S. aureus* isolates (19.6%) were the most predominant bacteria in mixed growth followed by CoNS (16.4%) and *B. subtilis* (15.3%). The rate of isolation was higher among university clinic workers 61(22.2%) and children 59 (21.5%) than the faculty members, personnel's and students. Besides to the above isolates, *S. pneumoniae, S. pyogenes, K. pneumoniae, Micrococcus roseus, E. coli, P. aeruginosa* and *E. aerogenes* have been documented .Ceftriaxone was effective against 74.9% of the isolates. Gram positive isolates were more susceptible to erythromycin (83.4%) and ceftriaxone (82.2%). Gram negative isolates were more susceptible to gentamicin (90.2%) and chloramphenicol (82.9%) **(**Shahaby *et al.,* 2015).

A cross - sectional study conducted on external ocular infection in Uganda in 2013 showed that from the eyelid margin and conjunctival samples processed, 59.5% and 45.8% were culture positive, respectively. The most common organisms identified were CoNS (65.9%) and *S. aureus* (21.0%). CoNS showed the highest resistance to tetracycline (58.2%), and erythromycin (38.5%), whereas in *S. aureus*, resistance rate to tetracycline and erythromycin were 55.2% and 31.0%, respectively. MRSA were also found in 27.6% of isolates. However, CoNS, *S. aureus* and other bacterial isolates revealed low rate of resistance to ciprofloxacin (11.1% - 24.2%), gentamicin (5.6-31.0%) and tobramycin (17.2% -25.3%) (Mshangila *et al.*, 2013).

A study conducted on external ocular infection in Saudi Arabia, reported that Gram negative organisms represented 71% of all culture reports (218). The most frequent isolate were *H. influenzae* (26%), *S. aureus* (12%) *and P. aeruginosa* (10%). Gram negative isolates showed the highest susceptibility to amikacin, colistin and ceropenem. Moreover, Gram-positive isolates were susceptible to vancomycin. Resistant to multiple antibiotic classes were seen in 39% of cultures (Musfer *et al.*, 2018).

A study conducted in Nigeria in 2010, documented that 74.9% of isolates were *S. aureus*, 10.2% were CoNS, 6.4% were *P. aeruginosa*, 3.2% were *E. coli*, 2.1% were *Klebsiella* spp., Moreover, 1.5% of the isolates were *S.pneumoniae*, 1.2% were *H. influenzae*, 0.3% were *P. mirabilis* and 0.3% were *N. gonorrhoeae*. The prevalence of conjunctivitis was 26.3%. The pathogens

demonstrated susceptibilities to erythromycin (57%) and ceftriaxone (67%) but susceptibilities to the remaining antibiotics were rather poor, 31.3% to amoxicillin, 42.7% to amoxicillin-clavulanic acid, 39.2% to chloramphenicol, 38.6% to gentamicin, 29.5% to ofloxacin and 32.2% to cloxacillin (Okesola & Salako,2010).

A study conducted on bacterial blepharitis in Libya in 2014 from 22 anterior blepharitis and 34 seborrheic blepharitis cases, *S. aureus* (25%), *S. epidermidis* (25%), *Klebsiella* spp., (18%) and *P. aeruginosa* (9%) were the common isolates. High level of resistance rates were observed among gram negative bacteria against ampicillin, trimethoprim-sulphametoxazole, and cephalosporin (Musa *et al*., 2014).

Different similar studies have been carried out in other parts of Ethiopia. In a study conducted in Gondar from September 2004 to August 2008, among the 236 eye swabs cultured, 54.2% were positive for different types of bacterial pathogens. Gram negative bacteria accounted for 44.5% and the predominant isolate was *E. coli* (14.8%). The Gram positive bacteria comprised (55.5%) and the predominant isolate was *S. aureus* (21.1%). MDR were observed in 77.3% of bacterial isolates to the commonly prescribed antibiotics (Anagaw *et al*., 2011).

A similar study conducted on external ocular infection in Hawassa documented that among 281 ocular specimens processed, 48.8% were culture positive. Gram positive cocci accounted for 61.5% of bacterial isolates. The most frequent isolates were *S. aureus* (21.0%) followed by CoNS (18.2%) and *S. pneumoniae* (14.0%). Most Gram positive isolates were susceptible to amoxicillin-clavulanic acid (95.5%) and vancomycin (96.6%) and Gram negative isolates were susceptible to ciprofloxacin (89.1%) and gentamicin (83.7%). From this finding, ciprofloxacin was effective against 86.7% of isolated pathogen. MDR was observed in 69.9% of the bacterial isolates. Gram positive isolates were more susceptible to amoxicillin-clavulanic acid and vancomycin, while Gram negative isolates were more susceptible to ciprofloxacin and gentamicin. Relatively, ciprofloxacin is effective against most isolated pathogen (Aweke *et al*., 2015).

In another study conducted on ocular infections in Borumeda, 2015 reported 59.4% of bacterial isolation from a total of 160 external ocular samples. The majority of the isolates (93.7 %) were Gram positive. The proportion of CoNS among the Gram positive bacterial isolates was 53.7 %. All Gram positive isolates were susceptible for vancomycin but 67.4 % of them were resistance against amoxicillin (Shiferaw *et al.*, 2015).

Another study conducted on external ocular infection in Gondar, Ethiopia from 312 processed samples, 58.3% were bacterial culture positive. The proportions of Gram positive bacterial pathogens were 88% and *S. aureus* (50.3%) was the predominantly isolated pathogen, followed by CoNS (33.5%) and *Klebsiella* spp., (4.7%). Conjunctivitis was the dominant clinical feature, but high positive results for bacterial pathogens were observed among patients with dacryocystitis cases. Moreover, Gram positive bacterial isolates were susceptible to ciprofloxacin, chloramphenicol, amoxicillin-clavulanic acid and ceftriaxone. However, 65% of these Gram positive bacterial pathogens showed resistant to penicillin, ampicillin and amoxicillin. The proportion of MRSA infection was 24% and MDR was observed in 87% of the isolated bacteria (Getahun *et al.*, 2017).

A retrospective study conducted by Muluye *et al.*, in Gondar University Hospital, Ethiopia from 2009 to2012 showed that a total of 102 eye discharges were submitted for microbiological evaluation, of which 60.8% had bacterial growth. The most frequent bacterial pathogens were Gram positive bacteria (74.2%). The predominant isolate was CoNS (27.4%) followed by *S. aureus* (21%). Most of the bacterial isolates were resistance to ampicillin (71%), amoxicillin (62.9%), erythromycin (43.5%), gentamycin (45.2%), penicillin (71%), trimethoprim-sulphamethoxazole (58.1%), and tetracycline (64.6%) while ceftriaxone and ciprofloxacin showed 75.8% and 80% susceptibility, respectively. From the total bacterial isolates, 87.1% were showed MDR (Muluye *et al.*, 2014).

Study conducted on external ocular infections in Addis Ababa, Ethiopia by Nigatu *et al.*, in 2004, the most common etiologic agents isolated were *S. aureus (*24.3%), followed by *S. pneumoniae* (21%), CoNS (10.6%), *H. influenzae* (9.4%), *Psuedomonas* spp., (8.5%), *H. aegyptius* (5.1%) and *K. pneumoniae* (4.7%). So, Gram positive bacteria constituted 57.9% of the total bacterial isolates. All strains from Federal Police and Minillik II Memorial Hospitals were susceptible to

ciprofloxacin. In general rates of susceptibilities to all antibiotics tested for Gram positives were lower as compared to Gram negatives. More than 75% of the *Pseudomonas* spp., isolates from this study was resistance to almost all antibiotics tested except for ciprofloxacin, gentamicin and norfloxacin (Nigatu, 2004).

Another study conducted on external ocular infection among 210 patients in Gondar in 2018, isolated 131 (62.4%) pathogenic bacteria from external ocular infections. The proportion of conjunctivitis, dacryocystitis and blepharitis were (32.8%), (23.7%) and (16%), respectively. The most prevalent isolates were CoNS (27.5%), *S. aureus* (26.7%), *Pseudomonas* spp., (10.7%) and *E. coli* (7.6%). Tetracycline, amoxicillin, chloramphenicol, ampicillin, and nalidic acid showed resistance to bacterial isolates with a respective rate of 35.9%, 32.1%, 26.2%, 25.2% and 23.7%. Their MDR pattern to the commonly prescribed antibiotics tested were (20.6%), (18.3%), (17.6%), (5.3%) and (4.6%) to two, three, four, five and six antibiotics, respectively. Overall, the MDR prevalence rates were (66.4%) (Belyhun *et al.*, 2018).

2.2. Factors associated with external ocular infections

Different factors associated with external ocular infections have been documented so far. Major risk factors for bacterial ocular infections with external sources are surgical and nonsurgical trauma and use of contact lenses. According to the study done in western India, use of lenses was found to be the most common predisposing factor for corneal infection caused by *P. aeruginosa* (Kumar *et al.*, 2011).

In particular, increased susceptibility to ocular infections, more severe clinical presentation and higher recurrence rates are associated with advanced immunosuppression due to HIV infection with reflected in a low CD4 count. Many patients present with advanced stages of immunodeficiency and already suffer from opportunistic infections that may affect the eye at the time of HIV diagnosis (Schaftenaar *et al.*, 2014).

Use of self-administered eye drops for ophthalmic conditions is a common practice in rural populations. The use of self-administered therapy in cases of ophthalmic disease can delay institution of effective therapy and negatively impact visual outcome. The indiscriminate use of traditional eye medicines (TEM) in developing countries is responsible for increased occurrence of corneal infections and ulceration (Maregesi *et al.*, 2016).

There have been an increasing number of MRSA cases reported in postsurgical and external ocular infections, with known risk factors for MRSA colonization, such as admission to a hospital, surgery and contact with a MRSA colonized patient, intravenous drug use, or previous antibiotic exposure and with more than half demonstrating both multidrug resistance and resistance to ophthalmic antibiotics (Hesje *et al.*, 2011).

In Ethiopia, it is in common practice that antibiotics can be purchased without prescription, which leads to misuse of antibiotics. This may contribute to the emergence and spread of antimicrobial resistance. Other factors may include availability of the suboptimal quality or substandard antimicrobial drugs, increased usage of a particular antimicrobial agent, poor sanitation, contaminated food and cross-contamination from humans or animals (Teweldemedhin *et al.*, 2017; Amisalu *et al.*, 2015). Therefore, this study carried out to determine the profile and associated factors of bacterial isolates and their antimicrobial resistance among patients with external ocular infections at Felege Hiwot Referral Hospital.

3. Objective of the study

3.1. General objective

- ❖ To assess the bacterial isolate and antimicrobial resistance profiles of external ocular infections and its associated factors among patients attending at Felege hiwot Referral Hospital, Bahir Dar, North West Ethiopia

3.2. Specific objectives

- To identify the bacterial pathogens from patients with external ocular infections
- To determine the resistance profiles of the bacterial isolates to the commonly prescribed antibiotics
- To identify factors associated with aerobic bacterial external ocular infections

4. Material and methods

4.1. Study area

The study was conducted at Felege Hiwot Referral Hospital (FHRH), Bahir Dar. Bahir Dar is 565km away from Addis Ababa, the capital city of Ethiopia. FHRH is one of the biggest tertiary level referral Hospitals in the Region visited by around 7 million peoples from the surrounding Zones and nearby regions both for inpatient and outpatient treatment. The hospital officially commenced its function in 1963 and currently it delivers health care services with medical, surgical, gynecological, orthopedic, intensive care units, pediatrics and Ophthalmology unit with a total of 400 beds and 561 staffs (FHRH, 2018). On average, 100 patients attend daily at the ophthalmology unit for different ophthalmic cases to get secondary eye care unit, refraction examination, minor and major ocular surgery and other common examinations and treatment services. During data collection, the Ophthalmology unit has two ophthalmologist, four optometrists, five ophthalmic officers, seven ophthalmic and BSC nurses and one cataract surgeon.

4.2. Study design and period

A cross-sectional study design was employed from February to April, 2019

4.3. Population

4.3.1. Source population

All patients who were attending at Ophthalmology unit during the study period.

4.3.2. Study population

All patients with external ocular infection that attended at Felege Hiwot Referral Hospital Ophthalmology unit during the study period.

4.4. Inclusion and exclusion criteria

4.4.1. Inclusion criteria

Patients who had eye of red, discharging, mucoid or mucopurulent secretion and/or conjunctival thickening were included to the study.

4.4.2. Exclusion criteria

Patients who received antibiotic treatment for the past two weeks

Patients who had case of keratitis were excluded

4.5. Variables of study

4.5.1. Dependent variable

Bacteria profile of external ocular infections

4.5.2. Independent variables

Demographic variables (age, sex, marital status, educational and occupational status and residence), types of ocular infections (conjunctivitis, blepharitis, blepharo-conjunctivitis and dacryocystitis), previous ocular infection, previous eye surgery, previous ocular trauma, previous eye allergy and history of self-medication were independent variables.

4.6. Sample size determination and sampling technique

The sample size was determined using single population proportion formula ($N=z^2XP$ (1-p)/ $(d)^{2)}$where: N = the number of ophthalmic patients involved in this study; Z = Standard normal distribution value at 95% CI, which was 1.96; d = margin of error taken as 5%; P= the prevalence of bacterial ocular infections reported in Gondar Teaching Hospital, Ethiopia which was 62.4% (Belyhun *et al.*, 2018).N = $\underline{(1.96)^2 \text{ X } 62.4\% \text{ (1-}}$ $\underline{62.4\%)}/(5\%)^2$= 360. Thus, a total of 360 patients included in the study. Convenient sampling technique was used to include the study participants from the study population attending at Ophthalmology unit.

4.7. Data Collection

4.7.1. Demographic and clinical data

Demographic characteristics and clinical data such as history of ocular infection, previous ocular trauma, previous eye surgery, previous eye allergy and history of self-medication of the study participant were collected from each participant by a trained ophthalmic nurse with face-to-face interview and patient card review using a pretested structured questionnaire.

4.7.2. Ocular sample collection and transportation

The presences of external ocular infections were clinically assessed with thoroughly examination of all patients using a slit lamp microscope by ophthalmologist (Sharma, 2012; Miller *et al*., 2018). Conjunctival and eyelid swabs were collected using sterile cotton swab pre-moistened with sterile physiological saline (Therese & Madhavan, 2015). Pus from lacrimal sac was collected using dry sterile cotton swab by applying pressure over the lacrimal sac and allowing the purulent material to reflux through the lacrimal punctum. In cases of acute lacrimal abscess or chronic dacryocystitis, pus was drain and taken on a dry sterile cotton swab (Miller *et al*., 2018). All swabs were transferred into a tube that had 2ml brain heart infusion broth (BHIB) (Oxoid, Basingstoke, UK) (Baron *et al*., 2013). All samples were labeled and transported to Microbiology Laboratory of Felege Hiwot Referral Hospital with the minimum delay possible (30 minutes). All ocular samples were collected by the ophthalmologist and ophthalmic nurse.

4.8. Culture and identification of bacterial isolates

The collected eye swab samples were inoculated on Blood Agar (BA), Chocolate Agar (CA) (CA) and MacConkey Agar (MAC) plates (Oxoid, Ltd Basingstoke, Hampshire, UK) using sterile wire loops. All the agar plates were incubated at 37 °C for 24 hours. CA and BA were incubated within a candle-jar to facilitate CO2 tension. After 24 hours of incubation, all plates were examined for bacterial pathogen growth. Identification of bacterial pathogens were made initially by colony morphology and Gram staining followed by using different enzymatic and biochemical tests. Catalase, coagulase, Optochin (5μg) and bacitracin(0.04μg) tests were applied to identify and differentiate gram positive cocci, while biochemical tests, such as triple sugar iron agar (TSI), citrate utilization, oxidase test, lysine decarboxylase agar (LDC), urease and indole tests were used to identify gram negative bacterial pathogens (Sharma, 2012; Cheesbourgh, 2006).

4.8.2. Antimicrobial susceptibility testing

Antimicrobial susceptibility testing for all the isolated bacterial species was done on Mueller Hinton agar (MHA) (Oxoid, UK) by Kirby-Bauer disk diffusion technique as per Clinical and Laboratory Standard Institute (CLSI) guideline (CLSI, 2018;). To standardize the inoculums density, 3-5 bacterial colonies of the test organism were picked and emulsified in 5 ml of normal saline and mixed gently then compared with 0.5 McFarland standard solutions. MHA and MHA

with 5% sheep blood was inoculated by streaking the swab over the entire agar surface and after few minutes, the antibiotic disks dispensed (using sterile forceps on the agar surface, no closer than about 24 mm from disc to disc, and 10 discs were applied on 150 mm petri-dishes and incubated at 37 °C for 18-24 hours. The presence or absence of zone of inhibition and its diameter was determined by measuring the diameter using caliper. The zone of diameters were interpreted as susceptible (S) intermediate (I) and resistant (R), the list of antibiotics tested for the bacterial isolates were Penicillin (10µg), Erythromycin (15µg), Clindamycin (2µg), Cefoxitin (30µg), Chloramphenicol (30µg), Amoxicillin-clavulanic acid (30µg), Tobramycin (10µg), Ampicillin (10µg), Gentamycin (10µg) Ciprofloxacin (5µg), Tetracycline (30µg), Trimethoprim sulphamethoxazole (1.25/23.75µg), Piperacilin (100µg), Ceftazidine (30/20 µg), Tobramycin (10µg) (Oxoid UK). Antibiotics were selected based on the isolate type, group of locally available antimicrobials, and local prescribing pattern in Ophthalmology unit. Bacterial isolates that are resistant to three or more antibiotic classes were considered as MDR (CLSI, 2018).

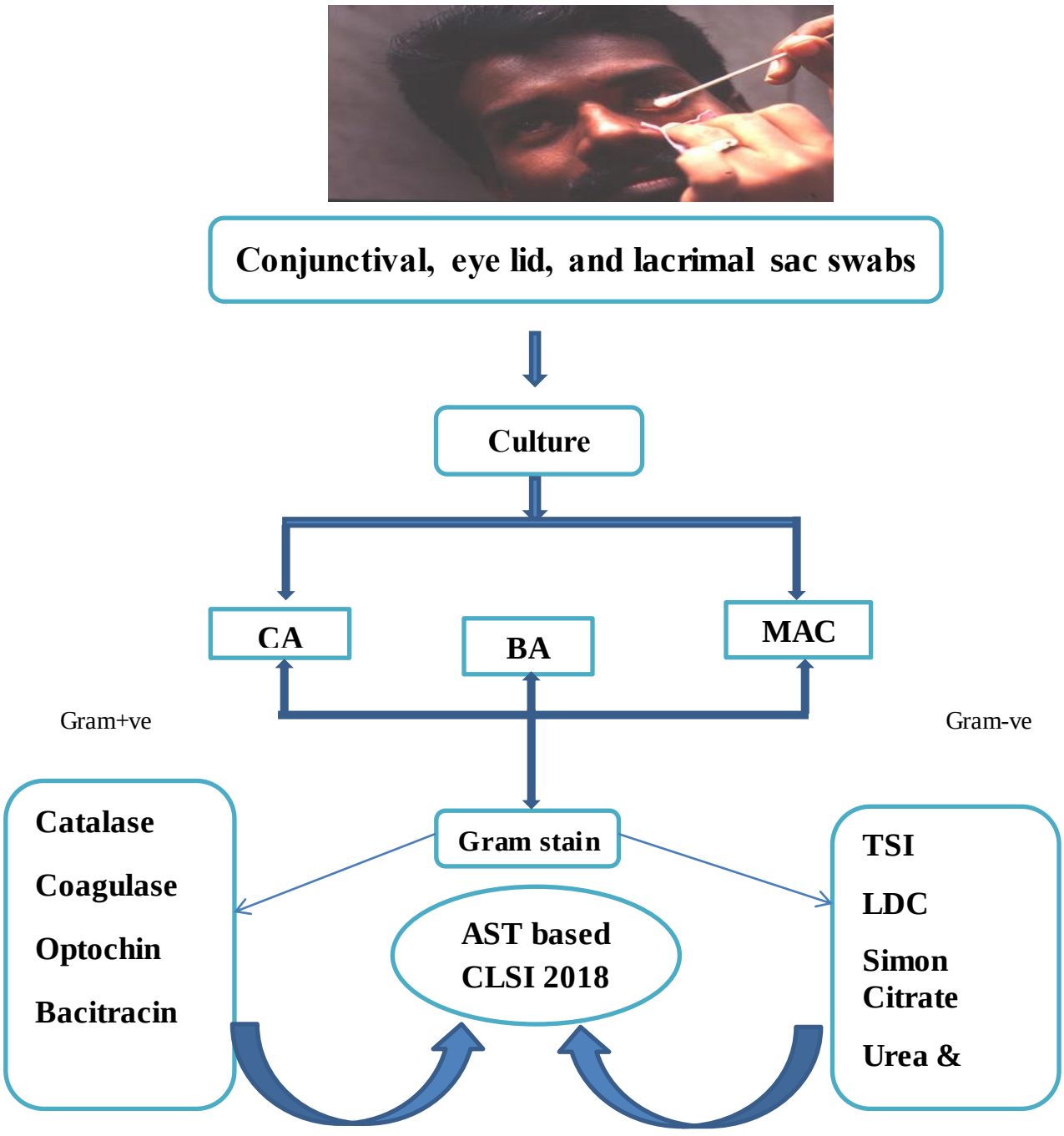

Figure 1: Work flow of eye swab samples collection and processing

N.B.: TSI= Triple sugar iron agar, LDC= lysine decarboxylase test, BA= Blood agar, CA=Chocolate agar, MAC=MacConkey agar, MHA =Muller Hinton agar, and AST= Antimicrobial susceptibility test

4.9. Quality control

All data quality control tools (pre-analytical, analytical and post-analytical stages) of quality assurance that was incorporate in standard operating procedures (SOPs) of the microbiology laboratory were strictly followed. Adequate specimen was collected using appropriate equipment and methods. All the equipment was checked for their functionality.

4.9.1. Quality control during data collection

Data on demographic characteristics and eye related medical history were collected by trained ophthalmic nurse. Structured questionnaires were used to collect the data. The questionnaires were pretested prior to data collection. Supervision of the data collection was made regularly on daily basis and the collected data checked for completeness and accuracy.

4.9.2. Quality control during sample processing

All specimens were collected following SOPs for external ocular specimen collection. The sterility of culture media was checked by incubating 5% of each batch of the prepared media for overnight and observed for the presence of any growth. The performances of all the prepared culture media and biochemical tests were checked by inoculating standard strains, such as *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) and *Staphylococcus aureus* (ATCC 25923). The strains were used as a quality control throughout the study for culture, Gram staining and antimicrobial susceptibly testing.

4.10. Data analysis

Data was checked for completeness, coded, and first entered in to EpiData and then rechecked and transferred to the Statistical Package for Social Science (SPSS) version 23 for analysis. Descriptive statistics such as: median, frequency, range and cross tabulations were used to describe demographic, bacterial isolates and antibiotic sensitivity profiles. Bivariate analysis, Chi-square test and fisher's exact test was used to see the association between dependent and independent variables. To determine independent predictors of bacterial external ocular infection, multivariable logistic regression analysis was done by taking variables whose p-value was < 0.21 in the binary logistic regression model. P-value of < 0.05 was considered statistically significant.

4.11. Ethical consideration

This proposal was reviewed and approved by College of Medicine and Health Science Institutional Review Board (IRB). Permission to conduct the study was obtained from Felege Hiwot Referral Hospital. Detail information including the objective of the study was given to each study participants. Informed written consent was obtained from each study participants and for children assent was obtained from parents before they are asked to give data and sample. Participants were notified about their right to refuse to participate in the study and confidentiality of the information kept. Positive findings were reported to the health professionals who are working at Felege Hiwot Referral Hospital Ophthalmology unit.

5. Results

5.1. Demographic Characteristics

A total of 360 patients with external ocular infections were enrolled in the study. The median age of the participants was 59.5 years and majorities (32.8%) of the participant's age were from 55 - 64 years old. Two hundred seventy five (76.4%) of study participants were rural residents and 233(64.7%) were males. Two hundred sixty-four (73.3%) of the study groups were illiterate and 248 (68.8%) and 48(13.3%) of them were married and single, respectively (Table 1).

Table 1:- Demographic characteristics of patients with EOIs at Felege Hiwot Referral Hospital, Northwest Ethiopia, February to April, 2019.

Variables	Number	Percent
Age (years)*		
≤ 5	3	0.8
5-14	4	1.1
15-24	2	0.6
25-34	7	1.9
35-44	35	9.7
45-54	83	23.1
55-64	118	32.8
≥65	108	30.0
Sex		
Male	233	64.7
Female	127	35.3
Residence		
Rural	275	76.4
Urban	85	23.6
Marital status		
Married	248	68.9
Single	48	13.3
Others*	64	17.5
Religion		
Orthodox	326	90.6
Muslim	34	9.4
Occupation		
Farmer	270	75
Civil servant	11	3.1
Housewife	53	14.7
No job	6	1.7
Business man	15	4.4
Pre-school	5	1.4
Education		

Not read and write	264	73.3
Read and write only	77	21.4
Elementary completed	5	1.4
High school completed	4	1.1
College and above	10	2.8
Total	**360**	**100**

*WHO age classification standard (WHO, 2019); *others: divorced, widowed and under age

5.2. Magnitude of external ocular infections

From a total of 360 patients with external ocular infections, 208 (57.8%) had pathogenic bacteria. The proportion of culture confirmed conjunctivitis, blepharitis and dacrocystitis was 125 (60.4%), 67 (55.8%) and 5 (45.5%), respectively. However, the difference was not statistical significant (p= 0.578) (Figure 2).

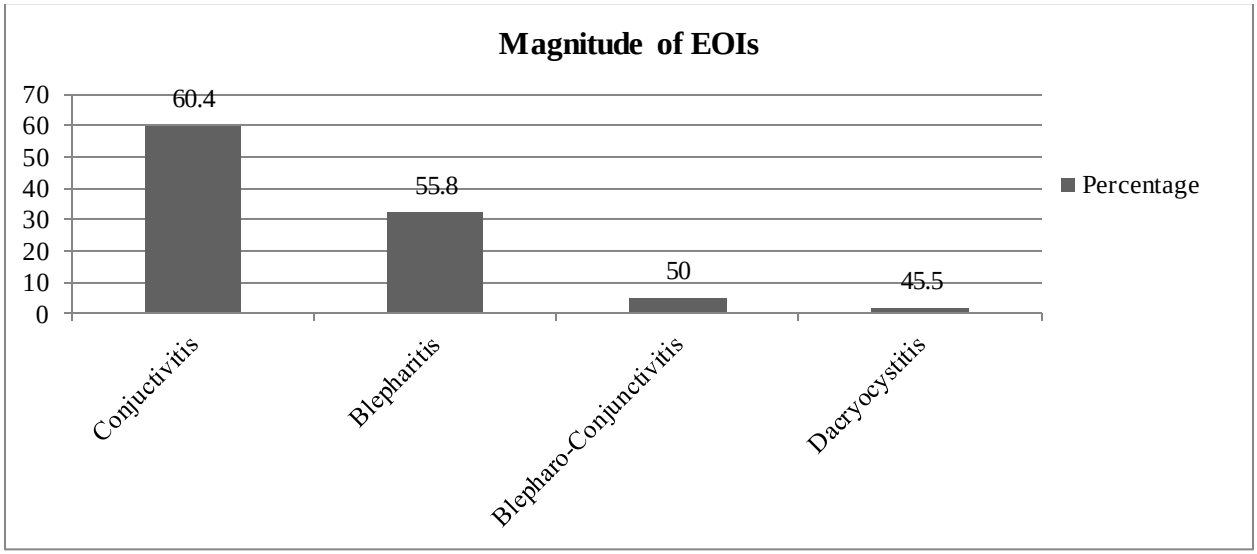

Figure 2: Distribution of culture confirmed bacterial EOIs among patients at Ophthalmology unit of FHRH, February to April, 2019.

5.3. Type of bacterial isolates

A total of 208 bacterial isolates were identified of which 138 (66.3%) were Gram positives. *S. aureus* 77 (37%) was the most predominant isolates followed by CoNS 48 (23.1%) and *K. pneumoniae* 28 (13.5%). From cases of conjunctivitis, *S. aureus* was the predominant isolate (26.6%). Among cases of blepharitis, *S. aureus* (15%) was also the most frequent isolate followed by *K. pneumoniae* (10.8%). Among patients suffering from blepharo- conjunctivitis, *S. aureus* and *K. pneumoniae* accounted for 13.6% each (Table 2).

Table 2: Isolation rates of individual bacterial isolates from patients with external ocular infections in patients at FHRH, February to April, 2019.

Bacterial species	**Conjunctivitis (n=207)**	**Blepharitis (n=120) N (%)**	**Blephari-conjunctivitis (n=22) N (%)**	**Dacryocystitis (n=11) N (%)**	**Total N (%)**
S. aureus	55 (26.6)	18 (15)	3 (13.6)	1(9.1)	77(37)
CoNS	32 (15.5)	12 (10)	2 (9.1)	2 (18.2)	48 (23.1)
K. pneumoniae	12 (5.8)	13 (10.8)	3 (13.6)	0	28 (13.5)
Proteus spp.,	5 (2.4)	10 (8.3)	0	0	15 (7.2)
S. pneumoniae	5 (2.4)	4 (3.3)	2 (9.1)	0	9 (4.3)
Citrobacter spp.,	4 (2.9)	4 (3.3)	0	1 (9.1)	9 (4.3)
P. aeruginosa	4(1.9)	1 (0.8)	0	1 (9.1)	6 (2.9)
Entrobacter spp.,	3 (1.4)	1 (9.2)	1 (4.5)	0	5 (2.4)
E. coil	3 (1.4)	2 (1.7)	0	0	5 (2.4)
S. pyogenes	2 (1)	0	2 (9.1)		4 (1.9)
K. rinoscleromatis	0	2 (1.7)	0	0	2 (1)
Total	**125 (60.4)**	**67 (55.8)**	**11 (50)**	**5 (45.5)**	**208**

5.4. Antimicrobial sensitivity profile of Gram positive isolates

Out of 138 Gram positive bacterial isolates, 92.7%, 90.4%, 89.6%, 81.1% and 76.1% were susceptible to clindamycin, ciprofloxacin, gentamicin, erythromycin and trimethoprim-sulphamethoxazole, respectively. However, they were highly resistance for penicillin (86.9%), ampicillin (86.3%) and tetracycline (42.1%). *S. aureus* isolates revealed 96.1% rate of resistance against ampicillin and penicillin each. The proportion of MRSA among the total *S. aureus* isolates was (16.9%). *S. pneumoniae* isolates revealed high (66.7%) rate of resistance to trimethoprim-sulphamethoxazole. All isolate of *S. pneumoniae* and *S. pyogenes* were susceptible to ampicillin, penicillin and erythromycin (Table 3).

Table 3: - Antimicrobial sensitivity profile of Gram positive isolates from external ocular infections at Felege Hiwot Referral Hospital Ophthalmology unit, February to April, 2019.

Organism isolates	Profile	Antibiotics tested N (%)									
		AMP	CFX	SXT	CAF	ERY	PE	GEN	TTC	CIP	DA
S. aureus	S	3(3.9)	69(83.1)	61(79.2)	57(74.0)	59(76.6)	3(3.9)	70(90.9)	42(54.5)	74(96.1)	72(93.5)
(N=77)	I	0	0	2(2.6)	1(1.3)	0	0	0	10(13)	1(1.3)	2(2.3)
	R	74(96.1)	8(16.9)	14(18.2)	19(24.7)	18(23.4))	74(96.1)	7(9.1)	25(32.5)	2(2.6)	3(3.9)
CoNS	S	3(6.3)	33(68.7)	38(79.1)	32(66.7)	40(87.5)	2(4.2)	42(87.5)	16(33.3)	39(81.3)	45(93.7)
(N=48)	I	0	7(14.6)	0	0	0	0	0	4(8.3)		0
	R	45(93.7)	9(18.7)	10(20.9)	16(33.3)	8(12.5)	46(94.8)	6(12.5)	28(58.3)	9(18.7)	3(6.3)
S. pneumoniae	S	9(100)	NA	3(33.3)	6(66.7)	9(100)	9(100)	NA	4(44.4)	NA	7(77.8)
(N=9)	I	0	NA	0	0	0	0	NA	2(22.2)	NA	1(11.1)
	R	0	NA	6(66.7)	3(33.3)	0	0	NA	3(33.3)	NA	1(11.1)
S. pyogenes	S	4(00)	NA	3(75)	4(100)	4(100)	4(100)	NA	2(50)	NA	4(100)
(N=4)	I	0	NA	0	0	0	0	NA	0	NA	0
	R	0	NA	1(25)	0	0	0	NA	2(50)	NA	0
Total isolates	S	19(13.7)	102(81.6)	105(76.2)	99(71.7)	112(81.1)	18(13.1)	112(89.6)	64(46.4)	113(90.4)	128(92.7)
(n=138)	R	19(86.3)	16(12.8)	2(22.5)	38(27.5)	2(18.9)	20(86.9)	14(10.4)	58(42.1)	11(8.8)	7(5.1)

AMP- Ampicillin, CFX - Cefoxitin, SXT - Trimethoprim-Sulphamethoxazole, CAF- Chloramphenicol, ERY- Erythromycin, PE- Penicillin, GEN- Gentamycin, TTC-Tetracycline, CIP- Ciprofloxacin, DA- Clindamycin, NA- Not applicable, S= Susceptible, I=Intermediate R= Resistance

5.5. Antimicrobial sensitivity profile of Gram negative isolates

Majority of Gram negative bacterial isolates revealed resistance against ampicillin (87.5%) and tetracycline (53.1%). but, 88.5%, 84.3% and 73.4% of Gram negative isolates were susceptible to ciprofloxacin, gentamicin and amoxicillin-clavulanic acid, respectively. *K. pneumoniae* isolates revealed 100% and 60.7% rate of resistance against ampicillin and tetracycline. *Proteus* spp., also revealed 93.3% rate of resistance against ampicillin. On the other hand, isolates of *P.aeruginosa* revealed 83.3% rate of resistance to piperacillin. Moreover, all isolates of *E. coli*, *Enterobacter* spp., and *Citrobacter* spp., were resistant for tetracycline, amoxacillin-clavulanic acid and ampicillin, respectively (Table 4).

Table 4:- Antimicrobial sensitivity profile of Gram negative isolates from external ocular infections at FHRH Ophthalmology unit, February to April, 2019.

Organism isolated	**Profile**	**Antibiotics tested N (%)**								
		CIP	SXT	Amp	AMC	TTC	GEN	CAZ	PIP	TOR
K. pneumoniae (N=28)	S	25(89.2)	15(53.5)	0	25(89.2)	11(39.3)	24(85.7)	NA	NA	NA
	I	0		0	0	0	0	-	-	-
	R	3(10.8)	13(46.5)	28(100)	3(12.1)	17(60.7)	4(14.3)	-	-	-
Proteus spp., (N=15)	S	14(93.3)	10(66.7)	1(6.7)	8(53.3)	11(73.4)	14(93.4)	-	-	-
	I	0	0	0	0	0	0	-	-	-
	R	1(6.7)	5(33.3)	14(93.3)	7(46.7)	4(26.6)	1(6.6)	-	-	-
P. aeruginosa (N=6)	S	4(66.7)	NA	NA	NA	NA	3(50)	4(66.7)	1(16.7)	3(50)
	I	0	NA	NA	NA	NA	0	1(33.3)	0	0
	R	2(33.3)	NA	NA	NA	NA	3(50)	1(16.7)	5(83.3	3(50)
Enterobacter spp., (N=5)	S	4(80)	5(100)	1(20)	0	3(60)	5(80)	NA	NA	NA
	I	1(20)	0	0	0	0	1(20)	-	-	-
	R	0	0	4(80)	5(100)	2(40)	0	-	-	-
E. coil (N=5)	S	5(100)	4(80)	5(100)	4(80)	0	4(80)	-	-	-
	I	0	0	0	0	0	0	-	-	-
	R	0	1(20)	0	1(20)	5(100)	1(20)	-	-	-
Citrobacter spp., (N=9)	S	8(88.9)	9(100)	1(11.1)	8(88.9)	3(33.3)	7(77.8)	-	-	-
	I	0	0	0	1(11.1)	2(22.2)	0	-	-	-
	R	1(11.2)	0	8(88.9)	1(11.2)	4(44.5)	2(22.2)	-	-	-
K.rinoscleromatis (N=2)	S	2(100)	1(50)	0	2(100)	0	2(100)	-	-	-
	I	0	0	0	0	0	0	-	-	-
	R	0	1(50)	2(100)	0	2(100)	0	-	-	-
Total=70	S	62(88.5)	44(68.7)	8(12.5)	47(73.4)	28(43.7)	59(84.3)	4(66.7)	1(16.7)	3(50)
	R	7(7.1)	20(31.3)	56(87.5)	17(26.6)	34(53.1)	9(12.8)	1(16.7)	5(83.3)	3(50)

CIP-Ciprofloxacin GEN-Gentamycin, SXT-Trimethoprim-Sulphamethoxazole TTC-Tetracycline, TOR- Tobramycin, PEP- Piperacillin, AMP- Ampicillin, AMC- Amoxicillin-Clavulanic acid, CAZ- Ceftazidime, NA- Not applicable S= Susceptible, I- Intermediate R= Resistance

5.6. Multi-drug resistance profile of the bacterial isolates

From the total isolated bacterial species, 94(45.2%) were MDR. Only 4 (1.9%) bacterial isolates were susceptible for all antibiotics tested. The MDR rate of *Enterobacter* spp., *K. pneumoniae*, *S. aureus* and *Proteus* spp., were 4(80%), 18(64.3%), 35(45.5%) and 5(33.3%), respectively (Table 5)

Table 5:- Antibiogram of external ocular infection bacterial isolates at Ophthalmology unit of FHRH, February to April, 2019.

Isolates	**Ro**	**R1**	**R2**	**R3**	**R4**	**R5**	**R6**	**MDR ≥3**
S. aureus (n=77)	0	14(18.2)	28(36.4)	23(29.9)	10(12.9)	2(2.5)	0	35 (45.5)
CoNS (n=48)	2(4.1)	10(25)	10(20.8)	11(22.9)	5(10.4)	6(12.5)	4(8.3)	26(54.2)
S. pneumoniae (n=9	0	3(33.3)	5(55.6)	1(11.1)	0	0	0	1(11.1)
S. pyogenes (n=4)	0	4 (100)	0	0	0	0	0	0
K. pneumoniae (n=28)	0	7(25)	3(10.7)	10(35.7)	6(21.4)	2(7.1)	0	18(64.3)
K. rinoscleromatis (n=2)	0	0	0	2(100)	0	0	0	2 (100)
Enterobacter spp., (n=5)	0	0	1(20)	1(20)	3(60)	0	0	4(80)
Proteus spp., (n=15)	1(6.7)	2(13.3)	7(46.6)	4(26.6)	0	1(6.7)	0	5(33.3)
Citrobacter spp., (n=9)	1(11.1)	4(44.4)	3(33.3)	1(11.1)	0	0	0	1(11.1)
E. coil (n=5)	0	3(60)	1(20)	1(20)	0	0	0	1(20)
P. aeruginosa (n=6)	0	0	5(83.3)	1(16.7)	0	0	0	1(16.7)
Total	**4 (1.9)**	**47 (24)**	**63 (30.3)**	**55(26.4)**	**24 (11.5)**	**11 (5.3)**	**4 (1.9)**	**94 (45.2)**

CoNS* = Coagulase negative Staphylococci; Ro = susceptible to all antimicrobials tested; R1, R2, R3, R4, R5, R6: Resistance to one, two, three, four, five and six antibiotics, respectively.

5.7. Bivariable and multivariable analysis of factors associated with external ocular infections

On multivariate analysis, external ocular infection was significantly associated with previous ocular disease (AOR=3.531, CI=2.175-21.9), eye allergy (AOR= 4.71, CI=1.191 - 18.59) and trauma (AOR=9.97, CI=4.543 - 21.9). Participants who had history of ocular disease were 3.5 times more likely to have bacterial external ocular infection compared to the counters. Likewise, participants who had previous eye allergy were 4.7 times more likely to have bacterial external ocular infection compared to those who had no eye allergy. Participants who had trauma were 10 times more likely to become culture positive for external ocular infection as compared to their counter parts (Table 6).

Table 6:- Bivariable and multivariable analysis of factors associated with bacterial external ocular infection at Felege Hiwot Referral Hospital, February to April, 2019.

Risk factors	External Ocular infection		COR (95% CI)	P-value	AOR (95% CI)	P-value
	Infected	Non-infected				
Sex						
Male	133 (57.1%)	100 (42.1%)				
Female	75 (59.1%)	52 (40.9%)	0.92 (0.59 - 1.43)	0.72	NA	NA
Residence						
Rural	167(60.7%)	108(39.3%)	1.659 (1.017 - 2.707)	0.043	0.683 (0.392 - 1.190)	0.178
Urban	41 (50.6%)	44 (49.4%)				
Hospitalized						
Yes	5 (55.5%)	4 (44.5%)				
No	203 (57.8%)	148 (42.2%)	1.1 (0.290 - 4.156)	0.891	NA	NA
Previous ocular disease						
Yes	135 (74.2%)	47(25.8%)	4.13 (2.64 - 6.46)	<0.01	3.531 (2.175 - 5.731)	< 0.01
No	73 (41%)	105 (59%)				
Previous eye surgery						
Yes	39 (81.3%)	9 (18.53)	0.272 (0.128 - 0.582)	0.001	1.633 (0.680 - 3.921)	0.272
No	169 (54.2%)	143 (45.8%)				
Previous ocular trauma						
Yes	84 (91.3%)	8 (8.7%)	12.19 (5.68 - 26.176)	<0.01	9.974 (4.543 - 21.900)	< 0.01
No	124 (46.3%)	144 (53.7%)				
Presence of eye allergy						
Yes	205 (59%)	142 (41%)				
No	3 (23%)	10 (77%)	4.81 (1.3 - 17.79)	0.019	4.705 (1.191 - 18.585)	0.027
History of self-medication						
Yes	5 (33.3)	10 (66.7%)				
No	203 (58.8%)	142 (41.2%)	2.86 (0.96 - 8.54)	0.06	0.61 (0.16 – 2.29)	0.46

Statistically significant association (P-value < 0.05), COR- Crude Odds Ratio, AOR- Adjusted Odds Ratio, CI-Confidence interval, NA- Not applicable

Discussion

Bacterial causes of external ocular infection is a serious health problem and is highly associated with resistance to antibiotics especially in developing countries like Ethiopia (Asbell *et al*., 2015; Belyhun *et al*., 2018). External ocular infections are responsible for increased incidence of morbidity and blindness worldwide and their morbidity varies from self-limiting light to life threatening infections (Tesfaye *et al*., 2013).

In this study, the overall prevalence of bacterial external ocular infection (57.8%) was comparable with previous studies in Gondar (58.3%) (Getahun *et al*., 2017), Borumeda (59.4%) (Shiferaw *et al.*, 2015), Ethiopia and India (58.8%) (Bharathi., *et al* 2010). But it is lower than previous studies in Jimma (74.7%), Ethiopia (Tesfaye *et al*., 2013), Nigeria (74.9%) (Okesola & Salako, 2010), China (82.78%) (Wang *et al.*, 2015) and Egypt (78.7%) (Shahaby *et al.*, 2015). However, this finding showed higher prevalence than the study conducted in Hawassa, Ethiopia (48.8%) (Aweke *et al*., 2015), Addis Ababa (47.4%) (Nigatu, 2004), Ethiopia and Bangalore (34.5%) (Hemavathi & Shenoy, 2014). The variations in the magnitude of external ocular infections could be due to differences in study period and design, geographical location, socioeconomic status and infection prevention practice in diverse settings.

In this study, the most common types of external ocular infection was conjunctivitis followed by Blepharitis. This pattern was similar with studies reported in Gondar (Getahun *et al*., 2017), Hawassa (Aweke *et al*., 2014, Amisalu *et al*., 2015), and Nigeria,(Okesola & Salako,2010). But in another studies done in Gondar and Borumeda, Ethiopia, blepharitis was the most common ocular infection followed by conjunctivitis (Shiferaw *et al*., 2015; Belayhun *et al* 2018).

In this study, Gram positive cocci bacteria accounted for 66.3% of external ocular infection. This is in line with reports from Hawassa (61.5%) (Amisalu *et al*., 2013) and Jimma (52%) (Tesfaye *et al*., 2013), Ethiopia. Moreover, similar findings have been documented in Egypt (58.9%) (Shahaby *et al*., 2015) and China (78.4%) (Wang *et al*., 2015). The increased rate of Gram positive cocci might be due to contamination of the eye from skin flora as a result of touching eyes with hands. Moreover, anatomical disruption such as cataract extraction and lens implantation might be a good opportunity for *Staphylococci* to elicit infection.

In this study, *S. aureus* was the most frequent etiology of external ocular infections. This is supported by studies conducted in Gondar (Getahun *et al.*, 2017), Jimma (Tesfaye *et al.*, 2013) and United Kingdom (Silvester *et al.*, 2016). However, studies in Uganda (Mshangila *et al.*, 2013), Borumeda, (Shiferaw *et al.*, 2015) and Gondar, Ethiopia (Belayhun *et al.*, 2018) reported that CoNS was the commonest isolate. Similarly, *S. aureus* was the leading bacterial isolate in conjunctivitis, blerpharitis, and blepharo-conjunctivitis in the present study. This is consistent with similar studies conducted in Jimma (Tesfaye *et al.*, 2013), India (Bharathi *et al.*, 2010) and Nigeria (Ubani, 2009). Conversely, CoNS isolates were more frequent in dacryocystitis.

Gram negative bacterial accounted 33.3% of external ocular infections in the present study. This is consistent with studies reported in Hawassa (38.5%), Ethiopia (Aweke *et al.*, 2014) and India (35%) (Bharathi *et al.*, 2010). However, it was higher than studies in Borumeda (Shiferaw *et al.*, 2017 and Gondar (Getahun *et al.*, 2017), Ethiopia which documented 6.3% and 12% of Gram negative bacterial external ocular infection, respectively.

Among Gram negative bacteria isolates, *K. pneumoniae* was the predominant etiology of external ocular infections in the present study. This is supported by studies conducted in Hawassa (Amisalu *et al.*, 2015), Gondar (Getahun *et al.*, 2017), Ethiopia, Egypt **(**Shahaby *et al.*, 2015) and Libya (Musa, Nazeerullah & Sarite, 2014). But other studies in Nigeria (Okesola & Salako, 2010), Saudi Arabia (Musfer *et al.*, 2018), Jimma (Tesfaye *et al.*, 2013) and Gondar (Belyhun *et al.*, 2018), Ethiopia reported that *P. aeruginosa* as the dominant Gram negative bacterial isolate. In contrast to this study, *E. coli* were reported as dominant bacteria from Gondar (Anagaw *et al.*, 2011).

In this study, all of the bacterial isolates have shown high rate of resistance to ampicillin (86.9%) and tetracycline (47.6%). Similar findings have been documented in Gondar (Muluye *et al.*, 2014), Hawassa and (Aweke *et al.*, 2015), Jimma (Tesfaye *et al.*, 2013) where 69.9 - 72.7% and 34.3 - 64.6% rate of resistance were documented to ampicillin and tetracycline, respectively and Uganda, resistance to tetracycline was 55.2% (Mshangila *et al.*, 2013).

In this study Gram positive bacterial isolates were highly resistant to ampicillin (86.3%), penicillin (86.9%) and tetracycline (42.1%). Comparable rate of resistance have been reported in Jimma, for ampicillin (37.5%), penicillin (100%) and tetracycline (61.4%) ((Tesfaye *et al.*, 2013)

and Addis Ababa for penicillin (73.9%), ampicillin (81.5%) and tetracycline (47.7%) (Akililu *et al*., 2018) Ethiopia. This might be due to earlier exposure of the isolates to these drugs (allocated as first line drugs). Moreover, these drugs are very common and patients can access them easily with low price and often can be purchased without prescription over the counter in different pharmacies. Similarly, *S. aureus* isolates were highly resistant to ampicillin and penicillin, 96.1% each. This finding is parallel with a study conducted in Addis Ababa, Ethiopia where 96.9% of the isolates were resistant to penicillin (Akililu *et al*., 2018). The highest level of resistance of *S. aureus* isolates to penicillin and ampicillin in the present study might be due to a fact that most strains of *S. aureus* are currently resistant to penicillin through beta-lactamase production and alteration of the penicillin binding proteins (Deyno, Fekadu & Astatkie, 2017)

In the present study, the proportion of MRSA among the total *S. aureus* isolates was (16.9%). This is higher than study in United Kingdom reported 8.3% of MRSA. This is lower as compared with study in Uganda reported relatively higher percentage 31.9% of MRSA. A review paper in United States reported that from 3% to 64% of ocular staphylococcal infections were due to methicillin-resistant *S. aureus*. And this condition is becoming more common and the organisms are resistant to many antibiotics (Shanmuganathan *et al*., 2005).

In this study majority (66.6%) of *S. pneumoniae* isolates were resistant to trimethoprim-sulphamethoxazole. This is in agreement with previous works in Hawassa, Ethiopia (65%) (Tesfaye *et al*., 2013) and Nigeria (75%) (Ubani, 2009). This might be due to the fact that Mutations in the dihydrofolate reductase and dihydropteroate synthetase genes (El Moujaber *et al*., 2017).

This study showed that gram negative bacterial isolates were most resistant for ampicillin (87.5%) and tetracycline (53.1%). Similarly, studies conducted in Gondar and Addis Ababa documented 56.5 - 81.5% and 33.3 - 34.8% rate of resistance against ampicillin and tetracycline, respectively (Getahun *et al*., 2017; Akililu *et al*., 2018). In the present study, all isolates of *K. pneumoniae* were resistant to ampicillin (100%). This is in agreement with previous works in Gondar, Ethiopia (100%) (Getahun *et al*., 2017) and Egypt (100%) (Musfer *et al*., 2018). This might be due to the fact that *K. pneumoniae* possesses beta-lactamase giving it resistant to ampicillin and many strains have acquired an extended-spectrum beta-lactamase with additional resistance to amoxicillin and ceftazidine (Bush, 2018).

In the present study, *Proteus* spp., were resistant to ampicillin (93.3%) and tetracycline (46.7%). This finding supported by a study conducted in Hawassa, Ethiopia ampicilin (100%) and tetracycline (40%) (Amisalu *et al.*, 2015). Similarly, *Cirobactor* spp., were resistant to ampicillin (88.9%) and tetracycline (44.5%). This is comparable with studies conducted in Addis Ababa, Ethiopia, ampicillin (100%) and tetracycline (50%) (Akililu *et al.*, 2018).

In the present study, *Enterobacter* spp., isolates were resistant to amoxicillin-clavulanic acid (100%) and ampicilin (80%). This finding is higher than a study reported in Gondar where isolates of *Enterobacter* spp., showed 33.3 % rate of resistance for amoxicillin-clavulanic acid and ampicillin each (Getahun *et al.*, 2017). The highest level of resistance of *Enterobacter* spp., isolates to amoxicillin-clavulanic acid and ampicillin might be due to the acquisition of plasmid gene encoding B-lactamases enzyme, efflux pumps and several metabolic pathway which ultimately produces altered bacterial cell walls lacking the binding site of the antimicrobial agent (Nirbhavane *et al.*, 2017).

In this study, *E. coli* isolates were 100% resistant to tetracycline. This is in agreement with previous works in Borumeda and Gondar, Ethiopia, where all *E. coli* were resistant for tetracycline (Shiferaw *et al.*, 2015; Assefa *et al.*, 2015). This might be due to multiple tetracycline efflux pumps and genetic exchange of resistance determinants among various environmental, commensal, and clinical bacteria (Markley & Wencewicz, 2018).

In general, the reason for the observed resistance to different antibiotics of isolates might be the empirical prescription of broad spectrum antibiotics to treat bacterial infections without definite diagnosis. In the community side, irrational use of antibiotics is also a common practice. In Ethiopia, use of antibiotic without prescription, is common which leads to misuse of antibiotics. This might contribute to the emergence and spread of antibiotic resistant isolates.

In this study, the overall rate of MDR isolates (45.2%) was similar with findings from Tigray, Ethiopia (53.9%) (Teweldemedhin *et al.*, 2017) and Saudi Arabia (39%) (Musfer *et al.*, 2018). But, it was higher than the study conducted in China (12.1%) (Wang *et al.*, 2015). Conversely, higher rate of MDR was reported in Gondar, Ethiopia (66.4 and 87.1%) (Belyhun *et al.*, 2018; Muluye *et al.*, 2014). These variations might be due to the difference in the type and generation

of antibiotics that we used for susceptibility testing. Moreover, other studies calculated the MDR profile for resistance to two or more antibiotics.

The proportion of MDR isolates of *S. aureus* in the present study (45.5%*)* is consistent with a study reported in Gondar, Ethiopia (64.6%) (Getahun *et al.*, 2017). The proportion of MDR rate of *K. pneumoniae* (64.3%) in the present study is also similar with other studies conducted in Gondar (62.5 and 77.7%) (Belayhun *et al.*, 20118; Getahun *et al.*, 2017). However, it was higher than studies from Hawassa (33.3%) (Amisalu *et al.*, 2015). *Enterobacter* spp., MDR rate in this study (80%) was also in agreement with previous works in Tigray, Ethiopia (70%) (Teweldemedhin *et al.*, 2017). In general, the major Gram negative bacterial isolates revealed higher rate of MDR profile which might be associated with biofilm formation, higher resistance gene plasmid transfer, modified target genes, decreased antibiotic penetration and efflux and metabolic pathway that allows for resistance to antimicrobials (Munita & Arias, 2016).

History of ocular trauma was a predictor variable for external ocular infection in this study. This finding is consistent with previous studies in Addis Ababa (Akililu *et al.*, 2018), Tigray (Teweldemedhin *et al.*, 2017), Ethiopia, Nepal (Gautam *et al.*, 2018), India (Chidambaram *et al.*, 2018) and Iran (Eghtedari *et al.*, 2018). This might be due to normal flora of the eye causes infection following in mechanical trauma of the conjunctiva or cornea when epithelium or stroma layers of eye is disrupted by direct tissue damage through the virulence factors of exotoxin A. This causes tissue necrosis corneal ulcer.

In the present study, previous ocular disease is also another predictor variable for the occurrence of external ocular infection. This is supported by studies conducted in Tigray (Teweldemedhin *et al.*, 2017), Ethiopia, Iran (Eghtedari *et al.*, 2018) and Nepal (Gautam *et al.*, 2018). The reason might be due to the inflammatory reaction and anatomical disruption which might create a good opportunity for some normal floras such as members of the *Staphylococci* to elicit infection (Chirinos *et al.*, 2013). Presence of eye allergy also strongly associated with external bacterial ocular infection in the present study. In most conjunctivitis infection, allergy is one of the major predisposing factors. Itching symptom of allergy might be an opportunity for skin floras to inoculums in eye and cause chronic bacterial ocular infection.

6. Limitation of the study

This study was not without limitations thus anaerobic bacteria and *Chlamydia trachomatis* were not isolated due to the limitations of laboratory setups. Cases of keratitis which requires special training and experience for corneal scraping were not included.

7. Conclusions

The prevalence of culture confirmed bacterial external ocular infection and isolates resistance to three or more antibiotics from different classes is prevalent in Felege Hiwot Referral Hospital Previous ocular diseases, presence of eye allergy and previous ocular trauma were predictor factors for the occurrence of external bacterial ocular infection. Conjunctivitis and blepharitis were the most prevalent type of external ocular infections. The predominant bacterial species was *S. aureus* and it was resistant to ampicillin, penicillin and tetracycline. Among Gram negatives, *K. pneumoniae* was the dominant isolate and were highly resistant to ampicillin, trimethoprim- sulphamethoxazole and tetracycline. Both Gram positive and Gram negative isolates were susceptible for ciprofloxacin and gentamycin. Isolates of *K. pneumoniae* and *Enterobactor* spp., were the leading MDR bacteria.

8. Recommendations

- Treatment of external ocular infections should be guided by culture and antibiotic susceptibility testing at FHRH.
- Interventions to external ocular infection should integrate with the previous ocular disease, trauma history and presence of eye allergy in the study site.
- Additional, continuous large scale studies should be considered for further characterization of external ocular infection bacterial profile and AMR.
- Further studies including Keratitis and intraocular infections of eye is required using molecular techniques.
- The prevalence of extended- spectrum beta lactamase and carbapenemase production in external ocular infections is recommended.

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10. ANNEXS

Annex I Participant information sheet (English and Amharic version)

Introduction

Hello, how are you?

My name is Zimam Ayehubizu and I am MSc student of Bahir Dar University, College of Medicine and Health Science, Department of Medical laboratory Sciences. I am doing a research entitled "Profile and associated factors of Bacterial isolates and Antimicrobial resistance among patients with External Ocular Infections'' at Felege Hiwot Referral Hospital, North west Ethiopia

Purpose of the study

The objective of this research is to determine profile of bacterial isolates and antibiotic resistance on external ocular surface infections

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

Risk associated with the specimen collection:

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

Procedure of the study

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

Confidentiality

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

Benefit

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

Withdrawal rights

Your participation in this study is purely voluntary, and you may stop the participation at any

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

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

1. Bahir Dar University, College of Medicine and Health Sciences, Department of Medical Laboratory Sciences

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

2. Principal Investigator: **Zimam Ayehubizu**

Tel: +251-912838065

Email: tsedy98@gmail.com

Amharic version participant information sheet

ባህር ዳር ዩኒቨርስቲ ህክምናና ጤና ሳይንስ ኮሌጅ የህክምና ላቦራቶሪ ሳይንስ ትምህርት ቤት ከፈለገ ህይወት ሪፈራል ሆስፒታል ጋር በመተባበር በአማርኛ የተዘጋጀ የተሳታፊዎች መረጃ ለማወቅ፡፡

መገቢያ፡

ሰላም እንደምን አሉ?

ስሜ ዝማም አየሁብዙ እባላለሁ፡፡

የባህር ዳር ዩኒቨርስቲ የላቦራቶሪ የተምህርት ክፍል የማስተርስ ድግሪ ተማሪ ነኝ በአሁኑ ሰዓት የዉጨ‹ኛውን ክፍል ኢንፈፌክሽን ህመም የሚያመጣውን ተህዋስያን ማወቅ እና የተህዋስያኑ መድሃኒት የመቋቋም ያለዉን የስርጭት መጠን በፈለገ ህይወት ሪፍራል ሆስፒታል ለማወቅ ጥናት እያካሄድኩ ነው፡፡

የጥናቱ ዋና አላማ፡

የጥናቱ አላማ የዉጪኛው የአይን ኢንፈክሽን ተህዋስያን የሚያመጣውን የተህዋስያን አይነትን የተህዋስያኑን መድሃኒት የመቋቋም ያለዉን ስርትጭት እና የህመሙን መንስኤዎች አይን ህሙማን ላይ ምን ያህል እንደ ሆነ ለማወቅ ነው፡፡

የጥናቱ ጊዜ፡ክትትል በሚያደርጉ የአይን ታካሚዎች ብዛት የሚወሰን ሲሆን 2 ወር እና ከዛም በላይ ሊወስድ ይችላል፡፡

ሊከሰቱ ስለሚችሉ ስጋቶችና የምቾት መንደሎች፡ለጥናቱ በሚወሰደዉ ናሙና ምክንያት የተለ የችግር አይከሰትም፡፡የሚያሰጋ ምንም ነገር የለዉም ምክንያቱም የጥናቱ ናሙና አወሳሰድ ከወትሮዉ በሽተኛዉ ለራሱ ብሎ ከሚሰጠዉ የተለየ አይደለም፡፡ ናሙና በሚወሰድበት ሂደት ከትንሽ የህመም ስሜት ውጪ ይሄነው የሚባል ችግር የሚያስከትል ወይም የሚያሰጋ አይደለም፡፡

የጥናቱ ሂደት፡

እርስዎ በጥናቱ ለመሳተፍ ፈቃደኛ ከሆኑ ከአይኖት ላይ ልምድ ባላቸዉ ኦፕቶሞሎጂስት ዶክተር ወይም ነርስ ናሙና ይሰጣሉ፡፡

የጥናቱ ሚስጢራዊነቱ፡

የሚሰጡት መረጃ ሚስጥራዊነቱ የተጠበቀ ነው፡፡በስም አይጻፍም የዚህ ኮድ መፍቻ በፋይል ተቆልፎ የሚቀመጥ ሲሆን የተፈቀደለት ሰው ብቻ ፋይሉን ማየት ይችላል፡፡ከዚህ ጥናት በሚወጡ ዘገባዎች ወይንም የህትመት ውጤቶች ላይ ስምም ወይም ሌላ የእርስዎን ማንነት

የሚገልጽ መረጃ አይኖርም።ከምርመራ የሚገኘውም ውጤት ወይም ሌላ መረጃ ለሚመለከታቸው አካላት ለምሳሌ፤እርስዎን የሚንከባከቡ የህክምና ባለሙዎች እና ጥናቱን ለሚያካሄዱት ባለሙያዎች እንዲሁም ጥናቱ ስነምግባርን ጠብቆ መከናወኑን ለሚከተሉት የኮሚቴ አባላት ብቻ ይገለፃል።ከምፒውተር ላይ ያሉ መርጃዎች ምስጢራዊነታቸው የተጠበቀ ሲሆን በወረቀት ያሉ መረጃዎችም ደህንነቱ በሚጠበቅ ቦታ የሚቆለፉና የተፈቀደለት ሰው ብቻ ሊያያቸው እንዲችል ተደርጎ ይጠበቃሉ።

የሚያስገኘው ጥቅም፡

በጥናቱ በመሳተፈዎ ይረዳዎታል።ስለሆነም ከጥናቱ በሚገኘው እውቀት የአይን ኢንፌክሽን ተህዋስያን ባክቴሪያ አማካኝነት የሚመጣውን በሽታ በተሻለ ደረጃ ለመቆጣጠርና ለበሽታው ትክክለኛዉን ፀረ-ባክቴሪያ ለመምረጥ ሀኪሞችን ይረዳል።

ከጥናቱ ስለማቋረጥ፡

በጥናቱ የሚሳተፉት ፈቃደኛ ከሆኑ ጥቅም ሆነ ጉዳት አይኖረውም።ጊዜዎትን መሰዋት አድርገው ስለተባበሩኝ ከልብ አመሰግናለሁ።

ስለጥናቱ ሕጋዊነት ለመጠየቅ ከፈለጉ፡

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

1. የባህርዳር ዩኒቨርስቲ፣የህክምናና ጤናሳይንስ ኮሌጅ፣የህክምና ላቦራቶሪ ሳይንስ ትምህርት ክፍል
2. የጥናቱ አስከያጅ፡ዝማም አየሁብዙ

ስ.ቁ፡-0912838065

ኢሜል: Email: tsedy98@gmail.com

Annex II consent form (English and Amharic version)

I have been requested to participate in this study, which plans to determine 'Profile and associated factors of Bacterial isolates and Antimicrobial resistance among patients with External Ocular Infections' among patients with external ocular infectionattend at Felege Hiwot Referral Hospital, North west Ethiopia' in which I will be benefited from study. I have been informed this study which involves collecting swab from conjunctiva and eyelid specimen.

During collection of the specimen I have been told that there is no harm except little discomfort and I have also read the information sheet or it has been read to me. I have been also informed that all information contained within the questionnaire is to be kept confidential. Moreover, I have also been well informed of my right to keep hold of information, decline to cooperate and drop out of the study if I want and that none of my actions will have any bearing at all on my overall health care and hospital access.

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

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

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

_____ _____ ____/____/___

Name of study participant Signature Day/month/year

_____ _____ ____/____/___

Witness (Illiterate) Signature Day/month/year

_____ _____ ____/____/___

Name of the researcher Signature Day/month/year

Consent form in Amharic

በዚህ ጥናት ለሚዳሰሱ ጥናቱች ሀሳባቸዉን መግለጽ ለሚችሉ

እኔ----------በፊለገ ህይወት ሪፍራል ሆስፒታል በተመላላሽ የአይን ታካሚዎች መሀከል የዉጪኛው የአይን ኢንፌክሽን ተህዋስያን የሚያመጣውን የተህዋስያን አይነትን የተህዋስያኑን መድሃኒት የመቋቋም ያለዉን ስርትጭት እና የህመሙን መንስኤዎች አይንህሙማን ላይ ምን ያህል እንደሆነ ለማውቅ ነው የተዘጋጀ ጥናት ላይ እድሳተፍ ተጠይቂስለጉዳዩምለመረዳት በቂ መረጃ አግኝቻለሁ።

ስለሆነም ናሙና የሚሰበሰበው ከታችኛው የአይን ሽፋን ዉስጥ እና ከኮንጃቲቫ መሆኑን ስለተርዳሁኝ ናሙና ወስዶ መመርመር አስፈላጊ ስለሆነ ናሙናዉን በመስጠት ልተባበር ሙሉ ፈቃደኛ መሆኔን ገልጫለሁ። ናሙና በሚወስድበት ወቅት ከትንሽ የህመም ስሜት ውጪ ምንም አይነት ጉዳት እንደሌለው ተነግሮኛል እንዲሁም ከመጠይቁ አንብቢያለሁ ወይም ተነቦልኛል። ከምርመሩ መሳተፍ ወይም አለመሳተፍ መብቴ የተጠበቀ መሆኑን እና ላለመሳተፍ ብወስን በፊለገ ህይወት ሪፍራል ሆስፒታል በሚደረግልኝ ህክምና ላይ ምንም ተፅዕኖ እንደማይኖረዉ ተረድቻለሁ።ስለዚህ የጥናቱን ጠቃሚነት አምኜበት የስምምነት ቃሌን የሰጠሁት በፍፁም ፈቃደኝነት ነዉ። በመጨሻም እኔ ከጥናቱ ዉጤት ተጠቃሚ ልሆን እንደሚችል ተገልፆልኝ በመሳተፌና በመተባበሬ ወገኖቼን ልረዳ በመቻሌ ደስተኛ መሆኔን ገልጬ፤ ግለፅ ያልሆኑ ጥያቄዎች ላይ ማብራርያ እንዲሰጠኝ ጠይቄ መልስ ተሰጥቶኛል። እንዲሁም በጥናቱ ሂደት

እንድሳተፍ ፍቃደኝነቴን በፊርማዬ አረጋግጠለሁ።

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

Annex III Parental consent form (English and Amharicversion)

I_____ parent, after being fully informed about the purpose of thisstudy, study title: "n at Felege hiwot referral Hospital, NorthwestEthiopia

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

Study.

_____ _____ ____/____/___

Name of study participant Signature Day/month/year

_____ _____ ____/____/___

Witness (Illiterate) Signature Day/month/year

_____ _____ ____/____/___

Name of the researcher Signature Day/month/year

Parental consent form Amharic version

የስስምምነት መጠየቂያ ቅጽ

እኔ----------የልጄ አስታማሚ ስሆን የዚህን ጥናት አላማ በዉል ተረድቻለሁ። የጥናቱርዕስ በፈለገ ህይወት ሪፈራል ሆስፒታል የአይን ታካሚዎች መሀከል የዉጪኛው የአይን ኢንፌክሽን ተህዋስያን የሚያመጣውን የተህዋስያን አይነትን እና የተህዋስያኑን መድሃኒት የመቋቋም ያለዉን ስርትጭት እና የህመሙን መንስኤዎች በአይን ህሙማን ለይ ምን ያህል እንደሆነለማውቅ በጥናቱ ልጄ እንዲሳተፍ ምርጫው የእኔ መሆኑን ነግረውኛል። ናሙና ሲወሰድ ከትንሽ የህመም ስሜት ውጪምንም አይነት ጉዳት ልጄ ላይ እንደሌለዉ ተነግሮኛል። በጥናቱ ወቅትም የልጄ መረጀዎች በሚስጥር ስለሚያዝ በሌላሰዉ ዘንድ እንደማይታወቅ ተረድቻለሁ። በውጤቱ ከሚገኘዉ የህክምና አገልግሎት በቀር ሌላ ልጄ በግሉ የሚያገኘዉጥቅም እንደሌለ ተረድቻለሁ። ጥያቄ እንድጠይቅ ዕድል ተሰጥቶኝ ለጥያቄዎቼም በቂ ምላሽ አግኝቻለሁ። የልጄ በጥናቱመሳተፍ በእኔ ፍላጎት ብቻ እንደሆነ እና በጥናቱም አለመሳተፍ ምንም አይነት ተፅዕኖ በልጄ ላይ እንደማያስከትልተረድቻለሁ። በከዚህ ባሻገር የልጄ በጥናቱ ውስጥ ለመካተት የእኔ የወላጁ አሳዳጊ ፈቃድ እንደሚያስፈልግተረድቻለሁ። በእኔ ፍቃደኝነት ልጄ በጥናቱ እንደሚሳተፍ ከዚህ በታች በፊርማዩ አረጋግጣለሁ።

_____ _____ ____/____/___

የተሳታፊው ሥም ፊርማ ቀን /ወር/ዓ.ም

_____ _____ ____/____/___

ምስክር (ማንበብና መፃፍ ለማይችሉ) ፊርማ ቀን /ወር/ዓ.ም

_____ _____ ____/____/___

የተመራማሪውስምፊርማቀን/ወር/ዓ.ም

Annex IV Questionnaire (English and Amharic version)

Questionnaires: Administered for investigation "Profile and associated factors of Bacterial isolates and Antimicrobial resistance among patients with External Ocular Infections'at Felege Hiwot Referral Hospital, North west Ethiopia'

Part I. Background information

Section I :-Sociodemographic characteristic Date of collection _____	
1. Patients MRN _____	Choice/Answer
2. Sex	1. Male 2. Female
3. Age	_____in Year
4. Address	1. Urban 2. Rural
5. Marital status	1. Single 2. Married 3. Widowed 4. Divorced 5. Under age
6. Religion	1. Orthodox 2. Muslim 3. Catholic 4. Protestant 5. Others _____
7. Ethinicity	1. Amhara 2. Oromo 3. Tigray 4. Others specify

8. Occupation ?	1. Farmer 2. Civil servants 3. Housewives 4. No job 5. Under age 6. Business man
9. Educational status	1. Not read and write 2. Read and write 3. Elemetary completed 4. High school completed 5. College and above
Section II. Clinical data	
10. Clinical manifestation of external ocular eye infection	1. Conjunctivitis 2. Blepharitis 3. Dacryocystitis 4. Blepharoconjunctivitis
11. Do you use medical contact lenses?	1. No 2. Yes
12. Did you use traditional eye medicine?	1. No 2. Yes
2. Did you have eye surgery before?	1. No 2. Yes If yes When?_____
3. Didyou have eye trauma before?	1. No 2. Yes
15. Hospital patient setting ----------	1. Inpatient 2. Out patient
16. Have you been hospitalized for the last oneyears?	2. No 2. Yes
17. previous ocular disease	1. No 2. Yes
18. Previous history of self-medication with	1. No 2.Yes

antibiotics	Name of antibiotic you taken ----------
19. Which systemic diseases do you have? Do you take antibiotic for this disease	1. Rheumatoid and Arthritis 2. Diabetes 3. Blood pressure 4.Other's _____ 1. No 2. Yes
20. Did you have eye allergy before?	1. No 2. Yes

Name of Participial investigator_____

Signature_____ Date_____

Questionnaire Amharic version

በፈለገ ህይወት ሪፍራል ሆስፒታል የአይን ታካሚዎች መሀከል የዉጪኛው የአይን ኢንፌክሽን ተህዋስያን የሚያመጣውን የተህዋስያን አይነትን የተህዋስያኑን መድሃኒት የመቋቋም ያለዉን ስርጭት እና የህመሙን መንስኤዎች በአይን ህሙማን ላይ ምን ያህል እንደሆነ ለማውቅ የተዘጋጀ ጥናት በአማርኛ የተዘጋጀ ቃለ መጠየቂያ

ክፍል 1 አጠቃላይ ግላዊ መረጃ	
1. የታካሚዉ ህክምና ቁጥር	……..
2. ፆታ	1. ወንድ 2. ሴት
3 እድሜ	---------- አመት
4 አድራሻ	1. ከተማ 2. ገጠር
5. የጋብቻ ሁኔታ	1.ያላገባ 4. የሞተበት/የሞትባት 2.ያገባ 5. ለጋብቻ ያልደረሰ 3.የፈታ
6. ሀይማኖት	1. ኦርቶዶክስ ተዋህዶ 4. ካቶሊክ 2. ሙስሊም 5. ሌላ (ይገለፅ)----- 3. ፕሮቴስታንት
7. ብሄር	1. አማራ 3. ትግራይ 2. ኦሮሞ 4. ሌላ (ይገለፅ----
8. የስራ ሁኔታ	1. ገበሬ 4. ስራ ፈላጊ 2. የመንግስት ሰራተኛ 5. ተማሪ 3. የቤት እመቤት 6. የግል ስራ
9. የትምህርት ደረጃ	1. ማንበብና መፃፍ የማይችል 2. ማንበብና መፃፍ ብቻ የሚችል 3. አንደኛ ደረጃ ት/ት ያጠናቀቀ 4. ሁለተኛ ደረጃ ት/ት ያጠናቀቀ

	5. ኮሌጅና ከዛ በላይ
ክፍል 2 የጤናመረጃ	
10. የአይን ኢንፌክሽን አይነት	1. ኮንጃቸቲቫትስ 2. የዉጨኛዉ የአይን ክዳን ኢንፌክሽን 3. ዳክሪዮሲስታይትስ
11. የህክምና ለእይታ ሚረዳ መነፅር ይጠቀማሉ?	1. የለም 2. . አዎ
12. የባህል የአይን መድሃኒት ታክመዉ ያዉቃሉ?	1.የለም 2. አዎ መልስዎ አዎ ከሆነ መቼ----------
13. ባይንዎት ላይ ላይ አደጋ ደርሶብዎ ያዉቃል?	1. የለም 2. አዎ
14. የአይን ቀዶ ህክምና አድርገዉ ያዉቃሉ?	1.የለም 2. አዎ መልስዎ አዎ ከሆነ መቼ----------
15. የታካሚዉ ሁኔታ	1. ተኝቶ ሚታከም 2.ከዉጭ ሆኖ ሚታከም
16. ባለፈዉ አንድ አመት ዉስጥ ሆስፒታል ትኝተዉ ያዉቃሉ?	1. የለም 2. አዎ
17. ካሁን በፊት የአይን በሽታ ነበረብዎ?	1. የለም 2.አዎ
18. ከአሁን በፊት ያለ ሀኪም ትዛዝ መድሃኒት ገዝተዉ ወስደዉ ያዉቃሉ?	1. የለም 2. አዎ
19. የትኛዉ በሽታ በዉስጥዎ አለብዎ	1. መገጣጠሚያን የሚቆረጣጥም 2. ስኳር 3. የደምግፊት 4. ሌላ ይጠቀስ
እነዚህነ በሽታዎች ለማከም መድሃኒት ይወስደዋል?	1. የለም 2. . አዎ
ከአሁን በፊት አላርገ,ጅክ ነበረብዎ?	

የተመራማሪው ስም _____ _____ _

ፊርማ ----------ቀን --------/------/----------ዓ.ም

Annex V Laboratory procedure (Standard Operative Procedures)

1. Sample Collection

Objective and Scope:

To describe the specimen collection instructions and subsequent handling of specimens by Researcher (Laboratory Technologist) for culture of bacteria. This document contains procedure for clinical specimens containing bacteria from the lower eye lid, conjunctival swabs, dacryyosystitis and traumatized eyes for processing at Felege Hiwot referral Hospital microbiology laboratory.

A. Conjunctiva and lid margin swab for bacterial culture

Sample will be collected at bedside by an ophthalmologist prior to administering antibiotics or topical medications. After detailed ocular examinations, external ocular sample were collected by swabbing the purulent conjunctivitis. Patient was requested to look up, lower eyelid was pulled down and then samples were collected. The sample collector holds the palpebra apart and gently collects discharge from the surface of the eye using sterile cotton swab that has been premoistened with sterile saline. The sterile normal saline moistened swab was rubbed over the lower Conjunctival sac from medial to lateral side and back again.

1) Roll a sterile, pre-moistened cotton swab, using a new swab for each of the following body sites:

2) Inoculate the following media.

a) blood agar

b) Chocolate agar plate

c) MacConkey's agar

B. Dacryocystitis

1) Cleanse skin with alcohol and tincture of iodine or iodophor

2) Collect a specimen of purulent discharge by using a swab like conjunctivitis collection

3) Do not perform a needle aspiration of the lacrimal gland.

2. Microscopy

Gram stain

Gram reaction principle

Differences in Gram reaction between bacteria is thought to be due to differences in the permeability of the cell wall of Gram positive and Gram negative organisms during the staining

process. Following staining with a triphenyl methane basic dye such as crystal violet and treatment with iodine, the dye–iodine complex is easily removed from the more permeable cell wall of Gram negative bacteria but not from the less permeable cell wall of Gram positive bacteria. Retention of crystal violet by Gram positive organisms may also be due in part to the more acidic protoplasm of these organisms binding to the basic dye (helped by the iodine).

Procedure

1. Prepare smear on clean slide then air-dry and heat-fix specimen using a Bunsen burner or spirit lamp
2. Allow slide to cool on staining rack
3. Flood slide with crystal violet; leave for 1 minute
4. Rinse slide in clean running water
5. Flood slide with Gram's iodine; leave for 1 minute
6. Rinse slide in clean running water
7. Apply acetone and rinse immediately under running water (exposure to acetone 5 seconds)
8. Counter-stain with carbol fuschin/safranine for 1minute
9. Rinse in clean running water then dry with blotting paper
10. View specimen with 10x objective
11. Place a drop of immersion oil on the slide and view with 100x oil-immersion objective

- **General protocol of Culture media preparation**

  1. Weighing and dissolving of culture media
  2. Sterilization
  3. Addition of heat sensitive ingredients
  4. p_H testing of culture media
  5. Dispensing of the culture media
  6. Sterility testing
  7. Quality assurance of culture media
  8. Storage of culture media

  Prepare media made from dehydrated products in as damp-free an environment as possible. To prevent the risk of inhaling fine particles of dehydrated media, wear a dust mask while handling dehydrated media, powder or use granulated media

  ¬ Wash the hands immediately after preparing media.

¬ Once the ingredients are weighed, do not delay in making up the medium. Follow exactly the manufacturer's instructions.

¬ Use completely clean glassware, plastic or stainless steel equipment that has been rinsed in pure water. The container in which the medium is prepared should have a capacity of at least twice the volume of the medium being prepared.

¬ Use distilled water from a glass still. Deionized water can also be used providing the exchange resins do not contain substances inhibitory to bacteria. Water containing chlorine, lead, copper, or detergents must not be used. Besides containing substances harmful to bacteria, impure water can alter the pH of a medium or cause a precipitate to form.

¬ Add the powdered or granular ingredients to the water and stir to dissolve. Do not shake a medium but mix by stirring or by rotating the container.

¬ When heating is required to dissolve the medium, stir while heating and control the heat to prevent boiling and foaming which can be dangerous and damage the medium, Overheating a medium can alter its nutritional and gelling properties, and also its pH.

¬ Autoclave a medium only when the ingredients are completely dissolved. Always autoclave at the correct temperature and for the time specified.

¬ Dispense medium in bottles or tubes in amounts convenient for use. Know the length of time prepared media can be stored without deteriorating (take into account storage temperature).

❖ **Dispensing sterile media into petri dishes**

1. Lay out the sterile petri dishes on a level surface.

2. Mix the medium gently by rotating the flask or bottle. Avoid forming air bubbles. Flame sterilize the neck of the flask or bottle and pour 15–20 ml of medium into each dish (90–100 mm diameter). air bubbles enter while pouring, rapidly flame the surface of the medium before gelling occurs. Rotate the dish on the surface of the bench to ensure an even layer of agar.

3. When the medium has gelled and cooled, stack the plates and seal them in plastic bags to prevent loss of moisture and reduce the risk of contamination. Do not leave the plates exposed to bright light especially sunlight.

4. Store at 2–8 ^{o}C.

Note: Agar plates should be of an even depth (not less than 4 mm) and of a firm gel. The surface of the medium should be smooth and free from bubbles.

4. Each Media Preparation

I). Preparation of 5%Solid Blood Agar

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

Formula / Liter Supplements

To make about 35 blood agar plates:

Blood agar base……. 40 g

Distilled water……1000 ml

Defibrinated blood . . . . . ……..50 ml

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

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

3. Avoid forming air bubbles.

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

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

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

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

II. Chocolate (Heated Blood) Agar

When blood agar is heated, the red cells are lyzed and the medium becomes brown in colour. It is referred to as chocolate agar and supplies the factors required for the growth of *H. influenzae*. It is also used to culture nutritionally demanding pathogens such as *N. meningitidis* and *S. pneumoniae*.

1 Prepare as described for blood agar except after adding the blood, heat the medium in a 70 $_{o}$C water bath until it becomes brown in colour. This takes about 10–15 minutes during which time the medium should be mixed gently several times.

2 Allow the medium to cool to about 45$_{o}$C, remix and dispense in sterile petri dishes as described for blood agar.

Important: Care must be taken not to overheat or prolong the heating of the medium because this

will cause it to become granular and unfit for use.

3 Date the medium and give it a batch number. Store the plates as described for blood agar.

III. MacConkey Agar

Intended Use

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

Principles of the Procedure

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

Formula / Liter

Enzymatic Digest of Gelatin ..... 17 g

Enzymatic Digest of Casein ..... 1.5 g

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

Lactose..... 10 g

Bile Salts Mixture .....1.5 g

Sodium Chloride.....5 g

Neutral Red..... 0.03 g

Crystal Violet..... 0.001 g

Agar..... 13.5 g

C°Final pH: 7.1 ± 0.2 at 25

Precaution: i. for Laboratory Use.

ii. Irritant

Directions

1. Suspend 50 g of the medium in one liter of purified water.

2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.

3. Autoclave at 121C for 15 minutes.

IV). Mueller Hinton Agar

Intended Use

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

Principles of the Procedure

Beef Extract and Acid Hydro lysate of Casein provide nitrogen, vitamins, carbon, and amino acids in Mueller Hinton Agar. Starch is added to absorb any toxic metabolites produced. Agar is the solidifying agent. A suitable medium is essential for testing the susceptibility of microorganisms to sulfonamides and trimethoprim. Antagonism to sulfonamide activity is demonstrated by para-amino benzoic acid (PABA) and its analogs. Reduced activity of trimethoprim, resulting in smaller growth inhibition zones and inner zonal growth, is demonstrated on medium possessing high levels of thymide. The PABA and thymine/thymidine content of Mueller Hinton Agar are reduced to a minimum, reducing the inactivation of sulfonamides and trimethoprim.

Formula / Liter

Beef Extract ..... 2 g

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

Starch ..... 1.5 g

Agar ..... 17 g

Final pH 7.3 ± 0.1 at 25°C

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

Precaution: For Laboratory Use.

Directions

1. Suspend 38 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes. Cool to room temperature.
4. OPTIONAL: Supplement as appropriate. Pour cooled Mueller Hinton Agar into sterile petridishes on a level, horizontal surface to give uniform depth. Allow to cool to room temperature.

5. Check prepared Mueller Hinton Agar to ensure the final pH is 7.3 ±0.1 at 25 °C.

5. How to inoculate culture media

Immediately before inoculating a culture medium check the medium for visual contamination or any change in its appearance which may indicate deterioration of the medium, e.g. darkening in color. When inoculating, or seeding, culture media an aseptic (sterile) technique must be used. This will: – prevent contamination of cultures and specimens,

– prevent infection of the laboratory worker and the environment.

Aseptic techniques

1. Flame sterilizes wire loops, straight wires, and metal forceps before and after use. Whenever possible, use a Bunsen burner with a protective tube, e.g. *Bactiburner* to avoid particles being dispersed when flame sterilizing wire loops

2. Flame the necks of specimen bottles, culture bottles, and tubes after removing and before replacing caps, bungs, or plugs.

3. When inoculating, do not let the tops or caps of bottles and tubes touch an unsterile surface. This can be avoided by holding the top or cap in the hand. Always use racks to hold tubes and bottles containing specimens or culture media.

4. Make slide preparations from specimens after inoculating the culture media.

5. Decontaminate the work bench before starting the day's work and after finishing.

6. Use a safety cabinet when working with hazardous pathogens.

7. Wear protective clothing; wash the hands after handling infected material.

6. Antimicrobial susceptibility tests

Disc diffusion susceptibility tests

Disc diffusion techniques are used by most laboratories to test routinely for antimicrobial susceptibility. A disc of blotting paper is impregnated with a known volume and appropriate concentration of an antimicrobial, and this is placed on a plate of susceptibility testing agar uniformly inoculated with the test organism. The antimicrobial diffuses from the disc into the medium and the growth of the test organism is inhibited at a distance from the disc that is related (among other factors) to the susceptibility of the organism. Strains susceptible to the antimicrobial are inhibited at a distance from the disc whereas resistant strains have smaller zones of inhibition or grow up to edge of the disc. For clinical and surveillance purposes and to promote reproducibility and comparability of results between laboratories, WHO recommends

the (NCCLS) modified Kirby-Bauer disc diffusion technique.

Preparation of turbidity standard

1. Prepare a 1% v/v solution of sulphuric acid by adding 1 ml of concentrated sulphuric acid to 99 ml of water. Mix well.

Caution: Concentrated sulphuric acid is hygroscopic and highly corrosive, therefore do not mouth pipette, and never add the water to the acid.

2. Prepare a 1% w/v solution of barium chloride by dissolving 0.5 g of dehydrate barium chloride ($BaCl2.2H2O$) in 50 ml of distilled water.

3. Add 0.6 ml of the barium chloride solution to 99.4 ml of the sulphuric acid solution, and mix.

4. Transfer a small volume of the turbid solution to a capped tube or screw-cap bottle of the same type as used for preparing the test and control inocula.

Procedure

1. Using a sterile loop, touch 3–5 well-isolated colonies of similar appearance to the test organism and emulsify in 3–4 ml of sterile physiological saline or nutrient broth.

2. In a good light match the turbidity of the suspension to the turbidity standard (mix the standard immediately before use). When comparing turbidities it is easier to view against a printed card or sheet of paper

3. Using a sterile swab inoculate a plate of Mueller Hinton agar. Remove excess fluid by pressing and rotating the swab against the side of the tube above the level of the suspension. Streak the swab evenly over the surface of the medium in three directions, rotating the plate approximately 60_{o} to ensure even distribution.

4. With the Petri-dish lid in place, allow 3–5 minutes (no longer than 15 minutes) for the surface of the agar to dry.

5. Using sterile forceps, needle mounted in a holder, or a multidisc dispenser, place the appropriate antimicrobial discs, evenly distributed on the inoculated plate ensure the discs are correctly placed.

Note: The discs should be about 15 mm from the edge of the plate and no closer than about 25 mm from disc to disc. No more than 6 discs should be applied (90 mm dish). Each disc should be lightly pressed down to ensure its contact with the agar. It should not be moved once in place.

6. Within 30 minutes of applying the discs, invert the plate and incubate it aerobically at $35_{o}C$ for 16–18 h (temperatures over $35_{o}C$ invalidate results for oxacillin).

7. After overnight incubation, examine the control and test plates to ensure the growth is confluent or near confluent. By using a ruler on the underside of the plate measure the diameter of each zone of inhibition in mm. The endpoint of inhibition is where growth starts. The sensitivity and resistance is evaluated based on the WHO standard for each bacteria species [32].

7. Biochemical tests

1. Catalase test

This test is used to differentiate those bacteria that produce the enzyme catalase, such as staphylococci, from non-catalase producing bacteria such as streptococci.

Principle

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

Procedure

1. Pour 2–3 ml of the hydrogen peroxide solution into a test tube.
2. Using a sterile wooden stick or a glass rod (not a nichrome wire loop), remove several colonies of the test organism and immerse in the hydrogen peroxide solution. Important: Care must be taken when testing an organism cultured on a medium containing blood because catalase is present in red cells. If any of the blood agar is removed with the organism, a false positive reaction may occur.
3. Look for immediate bubbling as shown in Plat.

2. Coagulase tests

This test is used to identify *S. aureus* which produces the enzyme coagulase.

Principle

Coagulase causes plasma to clot by converting fibrinogen to fibrin. Two types of coagulase are produced by most strains of *S. aureus*: Free coagulase which converts fibrinogen to fibrin by activating a coagulase-reacting factor present in plasma. Free coagulase is detected by clotting in the tube test._ Bound coagulase (clumping factor) which converts fibrinogen directly to fibrin without requiring a coagulase reacting factor. It can be detected by the clumping of bacterial cells in the rapid slide test.

Slide test method (detects bound coagulase)

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

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

Note: Colonies from a mannitol salt agar culture are not suitable for coagulase testing. The organism must first be cultured on nutrient agar or blood agar.

3 Add a loop full (not more) of plasma to one of the suspensions, and mix gently. Look for clumping of the organisms within 10 seconds. *No* plasma is added to the second suspension. This is used to differentiate any granular appearance of the organism from true coagulase clumping.

Test tube method (detects free coagulase)

1. Take three small test tubes and label:

T _ Test organism (18–24 h broth culture)

Pos _ Positive control (18–24 h *S. aureus* broth culture)

Neg _ Negative control (sterile broth)

*Nutrient broth is suitable. Do not use glucose broth.

2. Pipette 0.2 ml of plasma into each tube.

3. Add 0.8 ml of the test broth culture to tube T. Add 0.8 ml of the *S. aureus* culture to the tube labeled 'Pos'. Add 0.8 ml of sterile broth to the tube labeled 'Neg'.

4. After mixing gently, incubate the three tubes at 35–37$_{o}$C. Examine for clotting after 1 hour. If no clotting has occurred, examine after 3 hours. If the test is still negative, leave the tube at room temperature overnight and examine again.

Note: When looking for clotting, tilt each tube gently.

Results

Clotting of tube contents . . . . . *S. aureus*

No clotting or fibrin clot . . . . . Negative test

Note: There should be no clotting in the negative control tube fibrin clot in tube

3. Indole

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

Principle

The test organism is cultured in a medium which contains tryptophan. Indole production is

detected by Kovac's or Ehrlich's reagent which contains 4 (p) dimethylaminobenzaldehyde. This reacts with the indole to produce a red colored compound. Kovac's reagent is recommended in preference to Ehrlich's reagent for the detection of indole from enterobacteria.

Procedure

Detecting indole using tryptone water

1. Inoculate the test organism in a bijou bottle containing 3 ml of sterile tryptone water.
2. Incubate at 35–37₀C for up to 48 h.
3. Test for indole by adding 0.5 ml of Kovac's reagent. Shake gently. Examine for red color in the surface layer within 10 minutes.

Results

Red surface layer . . . . . Positive indole test

No red surface layer . . . . . Negative indole test

4. Citrate utilization

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

Ways of performing a citrate test

¬ Using a Rosco citrate identification tablet. This is the most economical method when only a few tests are performed. The tablets have a long shelf-life and good stability intropical climates. Using Simmon's citrate agar but the dehydrated medium is only available in 500 g pack size from manufacturers. After being opened the medium does not have good stability in tropical climates.

Citrate utilization using a Simmon's citrate agar

Principle and interpretation

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

Result

Positive: Blue colored growth

Negative: No growth/ no color change

5. Oxidase test

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

Principle

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

Procedure

1 Place a piece of filter paper in a clean petridish and add 2 or 3 drops of *freshly* prepared oxidase reagent.

2 Using a piece of stick or glass rod (not an oxidized wire loop), remove a colony of the test organism and smear it on the filter paper.

3. Look for the development of a blue-purple colour within a few seconds.

6. Urease test

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

Principle

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

Urease test using Christensen's (modified) urea

1 Inoculate heavily the test organism in a sterile Christensen's modified urea

2 Incubate at 35–37 _C for 18 hrs

3 Look for a pink colour in the medium

Results

Pink colour . . . . . Positive urease test

No pink colour . . . . . Negative urease test

7. Motility test medium

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

Principle and interpretation

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

Procedure

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

Result

1) Non motile-- growth restricted to stab line

2) Motile-- Diffused growth or swarm extends as a zone of turbidity from the stab line.

8. Triple sugar iron agar

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

Results

Butt colour Slant colour Interpretation

Yellow Red Glucose only fermented

Yellow Yellow Glucose fermented, also lactose and/or sucrose

Red Red No action on glucose, lactose or sucrose

Bubbles or cracks present: gas production

Black precipitates present: hydrogen sulphide gas production

9. Lysine Iron agar

Principle

Lysine Iron agar is a solid medium useful in the identification of Family *Enterobacteriaceae*. Lysine Iron agar is used in the qualitative determination of lysine decarboxylation, lysine deamination, and hydrogen sulfide production. The medium is not as sensitive as other media in the determination of hydrogen sulfide production. The medium can only be used with organisms

that are capable of glucose fermentation. Lysine Iron agar contains dextrose as fermentable carbohydrate, lysine as an amino acid, bromcresol purple as a pH indicator, and ferric ammonium citrate and sodium thiosulfate as sulfur source and hydrogen sulfide indicator. Initially the organism ferments glucose, causing a production of acid and changing of the pH indicator in the butt to yellow. If an organism produces decarboxylase enzymes, the organism will decarboxylate lysine to produce cadaverine, an alkaline product. The production of cadaverine will cause the pH indicator to change back to purple. If the organism is able to deaminate lysine, the amine converts to alpha-ketocarboxylic acid and the slant turns red. If the organism is not able to deaminate or decarboxylate the lysine the butt will remain yellow, and the slant will remain purple.

Procedure

1. Inoculate the medium using a single well-isolated colony from an 18-24 hour pure culture growing on solid medium.
2. Stab the butt of the agar twice, and streak back and forth on the slant.
3. Incubate tubes, with caps loosened in ambient air at 35-37°C for 18-24 hours.
4. Observe for lysine deamination or decarboxylation and hydrogen sulfide production.

Interpretation of Test

Uninoculated Lysine iron agar appears purple.

Lysine decarboxylation (detected in the butt)

Positive test – purple slant/purple butt (alkaline) K/K

Negative test – purple slant/yellow butt (acid) K/A (fermentation of glucose only)

Lysine deamination (detected in the slant)

Positive test – red slant

Negative slant – no color change (slant remains purple)

10. Bile solubility test

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

Principle

A heavy inoculum of the test organism is emulsified in physiological saline and the bile salt sodium deoxycholate is added. This dissolves *S. pneumoniae* as shown by a clearing of the

turbidity within 10–15 minutes. Viridans and other *streptococci* are not dissolved and therefore there is no clearing of the turbidity.

Tube method

Although the bile solubility test can be performed by testing colonies directly on a culture plate or on a slide a tube technique is recommended because the results are easier to read.

1. Emulsify several colonies of the test organism in a tube containing 2 ml sterile physiological saline, to give a turbid suspension.
2. Add two drops of bile salt sodium deoxycholate
3. Look for clearing of turbidity

7. Quality control

- As quality control, sterility of sheep blood agar, MacConkey agar, mannitol salt agar and Mueller Hinton Agar will be checked by incubating overnight at 35-37 ºC without specimen inoculation.
- The proficiency of catalase reagent (hydrogen peroxide) will be checked by known *S. aureus* (positive control) and *S. pyogenes* (negative control).
- For Gram staining reagents *S. aureus* (Gram positive) and *E. coli* (Gram negative) were used as quality control.
- For bile solubility test Positive Control: *S. pneumonia* negative Control: *S. mitis*.
- Before use of any reagents and culture media any physical change like cracks, excess moisture, color, hemolysis, dehydration, & contamination were assessed and expiration date was also checked. Temperature of incubator and refrigerator was monitored daily. *S. aureus* (ATCC 6538), *S. pneumonia* (NCTC 12977), *S. mitis* (NCTC 10712), *E. coli* (ATCC 8739), *S. aureus* (ATCC 25923) and *S. pyogenes* (ATCC 19615) was used as a quality control throughout the study for culture and antimicrobial susceptibly testing.

Declaration

I, the undersigned, Medical Microbiology student declare that this thesis is my original work and has not been presented in this and any other University and all sources of materials used for this thesis have been duly acknowledged.

Name: Zimam Ayehubizu

Signature: _____

Date: _____

Advisors: Name Signature Date

1. Dr. Fantahun Biadglegne (PhD, Post Dr. Research Fellow, AvH) _____ _____
2. Mr. Wondemagegn Mulu (MSc, Associate Professor) _____ _____

Examiner(s) Signature Date

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