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# Bacterial Isolates Recovered From Clinically Suspected External Ocular Infections: Antimicrobial Susceptibility Profile And Extended-Spectrum $\beta$ - Lactamase Production In Bacteria Northwest Ethiopia

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**BAHIR DAR UNIVERSITY**



**COLLEGE OF MEDICINE AND HEALTH SCIENCES**

**SCHOOL OF MEDICAL LABORATORY SCIENCE**

**DEPARTMENT OF MEDICAL MICROBIOLOGY**

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LACTAMASE PRODUCTION IN BAHIR DAR, NORTHWEST ETHIOPIA**

**BY: ABEBE ASCHALE (MSc CANDIDATE)**

**BAHIR DAR, ETHIOPIA**

**SEPTEMBER, 2026**

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**A THESIS SUBMITTED TO THE DEPARTMENT OF MEDICAL MICROBIOLOGY,  
SCHOOL OF MEDICAL LABORATORY SCIENCE, COLLEGE OF MEDICINE AND  
HEALTH SCIENCES, BAHIR DAR UNIVERSITY, IN PARTIAL FULFILLMENT OF  
THE REQUIREMENTS FOR THE DEGREE OF MASTERS OF SCIENCE IN  
MEDICAL MICROBIOLOGY.**

**BAHIR DAR, ETHIOPIA**

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**COLLEGE OF MEDICINE AND HEALTH SCIENCES**  
**SCHOOL OF MEDICAL LABORATORY SCIENCE**  
**DEPARTMENT OF MEDICAL MICROBIOLOGY**

**Approval of thesis for defense**

We hereby certify that we have supervised, read, and evaluated this thesis entitled "*Bacterial isolates recovered from clinically suspected external ocular infections: Antimicrobial susceptibility profile and extended-spectrum  $\beta$ -lactamase production in Bahir dar, Northwest Ethiopia*" by Abebe Aschale prepared under our guidance. we recommend the thesis to be submitted for the Department of Medical Microbiology.

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**Approval of Thesis Defense Result**

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## ABBREVIATIONS AND ACRONYMS

|                       |                                                |
|-----------------------|------------------------------------------------|
| ATCC.....             | American Type Culture Collection               |
| BDU.....              | BahirDar University                            |
| BHI.....              | Brain Heart Infusion                           |
| CI.....               | Confidence Interval                            |
| CMHS.....             | College of Medicine and Health Sciences        |
| CLSI.....             | Clinicaland Laboratory Standard Institute      |
| CoNS.....             | Coagulase-Negative Staphylococci               |
| DMLS.....             | Department of Medical Laboratory Science       |
| EOIs.....             | External Ocular Infections                     |
| ESBLs.....            | Extended-Spectrum $\beta$ -Lactamases          |
| FHCSH.....            | Felege Hiwot ComprehensiveSpecialized Hospital |
| H <sub>2</sub> S..... | HydrogenSulfide                                |
| IgA.....              | Immunoglobulin A                               |
| IQR.....              | Interquartile Range                            |
| IRB.....              | Institutional Review Board                     |
| MDR.....              | Multidrug-Resistance                           |
| MRS.....              | Methicillin-resistant staphylococci            |
| SOPs.....             | StandardOperatingProcedures                    |
| SPSS.....             | Statistical Package for the Social Sciences    |
| TGSTH.....            | Tibebe Ghion Specialized Teaching Hospital     |
| WHO.....              | World Health Organization                      |

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## ABSTRACT

**Background:** External ocular infections are a significant cause of preventable visual impairment worldwide. The rise of extended-spectrum  $\beta$ -lactamase (ESBL) producing bacteria poses a serious threat to effective treatment.

**Objective:** The aim of this study was to investigate the bacterial profile, antimicrobial susceptibility profiles and extended-spectrum  $\beta$ -lactamase production among bacterial isolates from patients with external ocular infections at Bahir Dar, Northwest Ethiopia.

**Methods:** A hospital-based cross-sectional study was conducted among 377 clinically suspected patients from February 9 to May 8, 2026. Data were collected using a structured questionnaire through face-to-face interviews. External ocular samples were taken by an experienced ophthalmologist and optometrist, and processed using standard microbiological techniques. Antimicrobial susceptibility testing (AST) and phenotypic confirmation of ESBL production were performed according to CLSI guideline. Bivariate and multivariate logistic regression was used to investigate the association between predictors and outcome variables. P-values  $<0.05$  with a 95% confidence interval were considered statistically significant.

**Results:** Of the 377 cases, 219 (58.1%) were tested positive for at least one bacteria. Most of the isolates were Gram-positive bacteria ( $n=141$ ) with the most common being *CoNS* ( $n=78$ ) and *S. aureus* ( $n=63$ ). The most common Gram-negative isolates ( $n=78$ ) were *E. coli* ( $n=24$ ) and *K. pneumoniae* ( $n=23$ ). Among the staphylococcal isolates, penicillin resistance was very high ( $\geq 98.4\%$ ) and 33.3% of *S. aureus* isolates were methicillin-resistant. One hundred two Gram-positive and thirty-seven Gram-negative isolates demonstrated multi-drug resistance. ESBL producers were detected in 20.8% (5/24) of *E. coli* and 30.4% (7/23) *K. pneumoniae* isolates. Age  $\geq 50$  (AOR: 3.56), Ocular trauma (AOR: 7.52), ocular allergy (AOR: 7.01), previous ocular infection (AOR: 3.76), and previous ocular surgery (AOR: 3.98) were associated with external ocular infections.

**Conclusion:** The high prevalence of bacterial external ocular infections with a significant burden of MDR and ESBL producing strains limits the use of conventional empiric therapy.

**Keywords:** External ocular infection, Antimicrobial Susceptibility Test, Extended-spectrum  $\beta$ -lactamase production

# 1. INTRODUCTION

## 1.1. Background

External ocular infections are the most common eye diseases encountered in ophthalmology clinics and involve the conjunctiva, eyelids, cornea, and lacrimal apparatus (1). These infections, including conjunctivitis, keratitis, dacryocystitis, blepharo-conjunctivitis and blepharitis, are major causes of ocular morbidity worldwide, particularly in developing countries where access to eye care services is limited (2, 3). Furthermore, if left untreated, external ocular infections may lead to serious complications such as corneal opacity, visual impairment, and blindness (4).

These terms refer to distinct clinical entities affecting different anatomical structures of the external eye. Conjunctivitis is inflammation of the conjunctiva, presenting with redness, discharge, and irritation (5). Blepharitis is inflammation of the eyelid margins, marked by redness, itching, and crusting (6). Keratitis is inflammation of the cornea, presenting with pain, photophobia, and blurred vision, and can progress to corneal ulceration if untreated (7). Dacryocystitis is inflammation or infection of the lacrimal sac, usually due to nasolacrimal duct obstruction, causing pain, redness, and swelling near the medial canthus with mucopurulent discharge (8).

Anatomically, the eye has two compartments, internal and external. The internal compartment is sterile, which is physically isolated from the immune system (9). In contrast, the external compartment is inhabited by several microorganisms due to constant exposure to the external environment and is colonized by a variety of microorganisms (10). Clinically, external ocular infections are characterized by a range of symptoms and signs, such as the formation of pus, conjunctival hyperemia, itching, lid edema, falling lashes, and visual impairment (11). These infections may be caused by various infectious agents, such as parasites, bacteria, viruses, and fungi (9). Among these pathogens, bacteria account for 74% of ocular infections globally (12).

Both Gram-positive and Gram-negative bacteria are important causative agents of external ocular infections. Studies have reported that *Staphylococci* are the leading causes of external ocular infections worldwide (13) among Gram-positive bacteria, while *Pseudomonas aeruginosa* (*P. aeruginosa*), *Klebsiella pneumoniae* (*K. pneumoniae*), *Enterobacter* spp., and

*Escherichia coli*(*E.coli*)are the major Gram-negative bacteria isolated from external ocular infections.Other bacteria associated with external ocular infections include*Streptococcus pneumoniae*,*Bacillus*,*N. gonorrhoeae*, *Moraxella* spp.,and *Haemophilus influenzae*(12, 14, 15).

External ocular infections are frequently facilitated by predisposing factors that compromise the eye's natural defense mechanisms, including ocular trauma which directly breaks the epithelial barrier, poor hygiene, use of traditional medicine, contact lens wear especially with improper care, pre-existing ocular surface diseases like dry eye which destabilize the tear film, systemic immunosuppression from conditions like diabetes, previous ocular surgery or infection, and demographic factors like extreme age or agricultural occupations are the major factors which increase the risk of external ocular infections(12, 16, 17).

The management of bacterial external ocular infections commonly involves treatment with broad-spectrum antibiotics(18). Frequently prescribed antimicrobial classes include beta-lactams, aminoglycosides, fluoroquinolones, sulfonamides, and tetracyclines. However, resistance to these antibiotics has increased noticeably in recent years(19).The widespread use of topical ophthalmic antibiotics, together with the inappropriate and excessive use of antibiotics for systemic infections, has accelerated the emergence and development of antibiotic resistance among bacterial isolates(15).As a result,antimicrobial resistance may increase the risk of treatment failure and may result in poor clinical outcomes (20).

One of the major drivers of antimicrobial resistance(AMR) is the rise of bacteria that produceextended-spectrum  $\beta$ -lactamases (ESBLs)(21).ESBLs are mutant, plasmid-mediated, clavulanate-susceptible enzymes that can hydrolyze penicillin, expanded-spectrum cephalosporins, and monobactams, while remaining ineffective against cephamycins and carbapenems(15). Members of the family Enterobacteriaceae, particularly *K. pneumoniae* and *E. coli*are among the most common ESBL-producing organisms(22).

The epidemiology of ESBL-producing bacteria varies considerably across geographical regions, countries, and between hospitals(22). Therefore, this study aims to address this gap by generating up-to-date evidence on the bacterial profile, antibiotic susceptibility, and ESBL production among Gram-negative isolates from patients with external ocular infections in Bahir Dar city hospitals, informing targeted antimicrobial use.

## 1.2. Statement of the problem

External ocular infections affect either the ocular surface or surrounding structures of the eye and range from self-limiting to sight-threatening conditions (23). According to the World Health Organization (WHO), 2.2 billion people are affected by visual impairment worldwide, and over half of these cases—including blindness—are preventable with timely treatment (24). In Africa, approximately 26.3 million people have visual impairment, with 5.9 million of them being blind, representing 15.3% of the world's blind population, with ocular trauma and related infections accounting for up to 5% of all blindness worldwide (17). In Ethiopia, the prevalence of blindness is 1.6%, with 87.4% of cases being caused by preventable factors, like bacterial infections (20).

In Ethiopia, ocular infections are among the leading causes of outpatient ophthalmic visits. Despite this high burden, empirical treatment is practiced without microbiological confirmation (12). Previous studies conducted in Ethiopia have reported a high prevalence of multidrug-resistance and ESBL production among ocular isolates, highlighting an important public health concern (17). Broad-spectrum antibiotics are widely prescribed for bacterial ocular infections, including in Bahir Dar. However, the indiscriminate use of these agents contributes to the emergence and spread of antimicrobial resistance, thereby reducing treatment effectiveness (25).

In Northwest Ethiopia, particularly at Felege Hiwot Comprehensive Specialized Hospital in Bahir Dar, a previous study highlighted that a high burden of external ocular infections, with *S. aureus* as the dominant pathogen, followed by *K. pneumonia* and *Proteus* species. The study also documented high levels of resistance to commonly prescribed antibiotics, including penicillin, ampicillin, and tetracycline, while ciprofloxacin and gentamicin remain effective, their efficacy may be compromised by the emergence of multidrug-resistant strains (MDR) (25).

Although previous study has described the bacterial profile and antimicrobial susceptibility patterns of bacterial isolates, information regarding the prevalence of ESBL-producing Gram-negative bacteria in Bahir Dar remains limited. Furthermore, updated local evidence on bacterial pathogens and their current antimicrobial susceptibility patterns is lacking. Therefore, this study was conducted to identify the bacterial etiologic agents, their susceptibility profile to the commonly used antibiotics, and detect ESBL production among bacterial isolates from patients

with external ocular infections attending in the ophthalmology units of hospitals in Bahir Dar, Ethiopia.

### **1.3. Significance of the study**

External ocular infections are often managed empirically without microbiological confirmation, which increases the risk of treatment failure when the causative organism is resistant. By identifying the bacterial etiologic agents in Bahir Dar and their current antimicrobial susceptibility patterns, including the previously unquantified prevalence of ESBL-producing Gram-negative isolates, this study gives clinicians locally relevant evidence to guide empiric therapy rather than relying on outdated or extrapolated data. This can shorten the time to effective treatment, reduce unnecessary antibiotic use, and lower the risk of complications such as corneal opacity, visual impairment, and blindness from inadequately treated infections.

Beyond individual patient care, the findings contribute to local antimicrobial resistance surveillance and fill a documented gap in local knowledge, since ESBL prevalence and up-to-date susceptibility data were previously unavailable for this setting. Health policymakers, regional and zonal health bureaus, and hospital administrations can use this evidence to update treatment guidelines, prioritize antibiotics shown to remain effective locally, and design targeted stewardship or infection-prevention interventions suited to local resistance patterns. The results also establish baseline data that can support future research, including resistance monitoring and molecular characterization of resistance mechanisms in this and other regions of Ethiopia.

## 2. LITERATURE REVIEW

Bacterial eye infections remain a major problem of ocular morbidity worldwide and can rapidly compromise vision if not treated appropriately (26). Globally, the microbiology of eye infections is dominated by Gram-positive cocci (*S. aureus* and CoNS species were the major causes) for many external ocular infections, while Gram-negative bacteria (*P. aeruginosa* and Enterobacteriaceae species) are common in contact-lens-associated keratitis and severe sight-threatening infections (27). In Ethiopia, several cross-sectional hospital-based studies across the region (including the Northwest of the region) consistently reported that external ocular infections are frequent and that both Gram-positive and Gram-negative bacteria are recovered. Many studies from Ethiopia found a high rate of culture positivity where *S. aureus* and CoNS species commonly isolated alongside *P. aeruginosa* and species in the family Enterobacteriaceae (17, 25, 26).

### 2.1. Bacterial profile of external ocular infections

A study conducted in Japan at Saga Prefectural Hospital from January 2006 to December 2009, which recruited 242 patients with various ocular infections, found that microorganisms were isolated from 78 eyes. Among these, infectious corneal ulcer was the most common clinical presentation with 103 patients (42.6%), followed by endophthalmitis in 52 patients (21.5%), and conjunctivitis in 39 patients (16.1%) (28). Similarly, a retrospective laboratory-based observational study at an eye care hospital in Banlagaro, India, involving 235 patients, reported a bacterial prevalence of 81 (34.5%), with *Staphylococcus* species 36 (39.9%), *Pseudomonas* species 20 (22.2%), and *Escherichia coli* 12 (13.3%) as the predominant isolate. This study recommends that all ocular specimens should undergo microbiological culture and perform antibiotic susceptibility tests to guide therapy (29).

Another retrospective cross-sectional study conducted in South China, obtained 737 culture-positive isolates from 3040 clinical samples. *Staphylococcus epidermidis* was the dominant pathogen isolated in external ocular infections. The organisms cultured from the ocular surface (cornea) accounted for the majority of the isolates (82.77%, n = 610) (30).

In European settings, long-term surveillance data provide valuable insights into the stability of these microbial patterns. A 30-year monocentric cross-sectional retrospective study conducted in Italy, at Turin Ophthalmic Hospital, identified 15,517 culture-positive isolates from ocular infections. Gram-positive bacteria were responsible for 73.5% of cases, led by *S. aureus* (30.1%), and followed by  $\alpha$ -hemolytic *Streptococci* (24.5%), *S. pneumoniae* (10.7%), and CoNS (5.5%). Gram-negative bacteria were responsible for 26.53% of infections, led by *Pseudomonas* spp. (6.4%), followed by *Moraxella* spp. (4.8%), and *Pasteurella* spp. (4.1%). The study noted a limitation in the available epidemiological data, as it did not store the antimicrobial susceptibility information for methicillin and vancomycin for Gram-positive isolates (31).

Similarly, a more recent five-year retrospective cross-sectional study at the University Hospital San Giovanni di Dio e Ruggi d'Aragona, Italy (January 2015-December 2019) analyzed 1364 conjunctival swabs and found bacterial growth in 285 samples (21%). The majority of positive samples were obtained from patients aged 61-69 years (45.9%) and from males (54.7%). Among the bacterial isolates, 81.1% were Gram-positive, with CoNS as the main species isolated, followed by *S. aureus* (33%). In contrast, *Pseudomonas* spp. (26%) and *Escherichia coli* (14%) were the major species isolated among Gram-negative bacteria (19).

Within the African context, external ocular profiles showed both similarities to and differences from global trends. In Nigeria, a cross-sectional study done in Owerri, Imo State in 2021-2022 examined bacterial pathogens associated with ocular infections among 50 outpatients, from whom 50 conjunctival swabs were collected. All samples yielded positive bacterial growth, resulting in 57 bacterial isolates. From these, Gram-positive bacteria accounted for 35 (61.4%) of isolates, with *Bacillus* species as the predominant species 17 (29.8%). Gram-negative bacteria accounted for 22 (38.6%) of the total isolates including, *Haemophilus* spp. (12.3%) and *Klebsiella* spp. (3.5%) (32).

Another study in Ghana showed that 98 (95.1%) tested positive for culture from 103 ocular samples. Gram-negative bacteria were predominated (58.2%), with *P. aeruginosa* (38.8%) and *P. putida* (11.2%) were the two most common species, while *S. aureus* (26.7%) and Coagulase-negative staphylococci (CONS) (13.3%) were the leading Gram-positive pathogens. These bacterial pathogens were isolated from conjunctivitis, preseptal cellulitis, and keratitis (33). In another African setting cross-sectional study conducted in Sudan in 2016, aged 1 day to 15 years,

included 204 ocular samples, reported bacterial growth in 130 (63.7%) cases. *Staphylococcus aureus* 39 (30%) and *Streptococcus pneumoniae* 31 (23.8%) were the most common Gram-positive, while *Haemophilus influenzae* 22 (16.9%), and *Pseudomonas aeruginosa* 13 (10%) were the most common Gram-negative bacteria isolated(34).

In Ethiopia, several cross-sectional studies conducted across different region reports high rates of external ocular infections and both Gram-positive and Gram-negative bacteria play a significant role in external ocular infections(35). In Borumeda Hospital, a hospital-based cross-sectional study on 160 patients reported that the overall prevalence of bacterial pathogens among external ocular samples was 95 (59.4 %), of which (93.7 %; 89/95) were Gram-positive and the other 6.3 % (6/95) were Gram-negative bacteria. CoNS alone accounted 53.7 % (n = 51/95) (20).

Similarly, a cross-sectional study conducted at Karamara Hospital in Jijiga from May 1 to June 30, 2020, involving 288 patients, identified bacterial infection in 179(62.2%) cases. Gram-positive cocci constitute 87.7% of the isolate with *S. aureus* (53.1%), and CoNS(32.4%) were the leading species. 12.3% of isolates were Gram-negative bacteria, predominantly *E. coli* spp.(6.2%). In addition, *Klebsiella pneumoniae* (3.3%) were also isolated(36).

Studies in northwest Ethiopia further reinforce these findings and provide a direct rationale for the current investigation. A cross-sectional study was conducted at Debre Markos Comprehensive Specialized Hospital between January 1 and June 30, 2021, on 207 patients showed that 130 (62.8%) bacterial growth cases. Gram-positive bacteria accounted for 78.5% (102/130) of the isolates, and dominated by *S. aureus* with 46.2% (60/130) prevalence. *E. coli* was the predominant bacterial isolate from Gram-negative with a prevalence of 32.1% (9/28), followed by *P. mirabilis* (21.4%, 6/28)(12).

Another comparable result was also reported from a prospective cross-sectional study at Gondar Teaching Hospital, which included 210 patients, found pathogenic bacteria in 131 (62.4%) cases, with CoNS (27.5%), *S. aureus* (26.7%), and *Pseudomonas* species (10.7%) as the most common isolates(26). Most relevant to the present proposal, a hospital-based cross-sectional study at Felege Hiwot Referral Hospital in Bahir Dar from February 1 to April 30, 2019, involving 360 suspected patients, documented bacterial growth in 208 (57.8%) cases, with *S. aureus* as the predominant pathogen. A comparable study in Gondar from January to April 2016, which included 312 participants, reported a bacterial prevalence of 58.3%(25, 37).

A summary of selected studies on bacterial profiles of external ocular infections from global, African, and Ethiopian contexts is presented in Table 1, illustrating geographic variations in prevalence and predominant pathogens. It also highlights the consistent role of *S. aureus* and CoNS as leading isolates from Gram-positive, while *E. coli*, *Klebsiella* species, and *Pseudomonas* species are among the Gram-negative bacteria.

**Table 1: Summary of selected studies on the bacterial profile of external ocular infections**

| <b>Authors &amp; Year</b>             | <b>Study area</b>       | <b>Sample Size</b> | <b>Proportion/<br/>Isolates</b> | <b>Predominant Bacterial Isolates</b>                                                             |
|---------------------------------------|-------------------------|--------------------|---------------------------------|---------------------------------------------------------------------------------------------------|
| Shimizu <i>et al.</i> ,<br>2013(28)   | Japan                   | 242 patients       | 32.2% (78)                      | <i>Staphylococcus</i> spp., <i>Pseudomonas</i> spp.                                               |
| Hemavathi <i>et al.</i> ,<br>2014(29) | India                   | 235 patients       | 34.5% (81)                      | <i>Staphylococcus</i> spp. (39.9%),<br><i>Pseudomonas</i> spp. (22.2%), <i>E. coli</i><br>(13.3%) |
| Manente <i>et al.</i> ,<br>2022(19)   | Italy                   | 1364 swabs         | 21% (285)                       | CoNS (81.1% of Gram+),<br><i>Pseudomonas</i> spp. (26% of Gram–)                                  |
| Ogu <i>et al.</i> ,<br>2022(32)       | Nigeria                 | 50 patients        | 100% (57)                       | <i>Bacillus</i> spp. (29.8%), <i>Haemophilus</i><br>spp. (12.3%)                                  |
| Junior <i>et al.</i> ,<br>2022(33)    | Ghana                   | 103 samples        | 95.1% (98)                      | <i>P. aeruginosa</i> (38.8%), <i>S. aureus</i><br>(26.7%)                                         |
| Shiferaw <i>et al.</i> ,<br>2015(18)  | Borumeda<br>Hospital    | 160 patients       | 59.4% (95)                      | CoNS (53.7%), Gram-negatives<br>(6.3%)                                                            |
| Haile <i>et al.</i> ,<br>2022(12)     | DebreMarkos<br>Hospital | 207 patients       | 62.8% (130)                     | <i>S. aureus</i> (46.2%), <i>E. coli</i> (32.1% of<br>Gram–)                                      |
| Ayehubizu <i>et al.</i> ,<br>2021(25) | Bahir Dar               | 360 patients       | 57.8% (208)                     | <i>S. aureus</i> , <i>Klebsiella</i> spp.                                                         |

## 2.2. Antimicrobial Susceptibility Profile and Extended-Spectrum $\beta$ -Lactamase Production

External ocular infections are often treated empirically with broad-spectrum antibiotics because therapy is often initiated before microbiological confirmation (17). Although this approach enables prompt treatment, the inappropriate and excessive use of antibiotics has accelerated the emergence of antimicrobial resistance among external ocular bacterial pathogens (25). In addition to conventional resistance mechanisms, extended-spectrum  $\beta$ -lactamase (ESBL)-producing bacteria have become an important concern because these enzymes hydrolyze penicillins, expanded-spectrum cephalosporins and monobactams while remaining inactive against cephamycins and carbapenems (38). Given these challenges, studies from different countries have investigated antimicrobial susceptibility patterns and the prevalence of ESBL-producing organisms in ocular infections (15, 17).

A three-year prospective study in India (Feb 2011- Jan 2014) analyzed 252 ocular Gram-negative isolates from 221 specimens. ESBL production was detected in 17% (42/252) of isolates, most commonly in *P. aeruginosa* (38%) and *E. coli*(17%). ESBL-producing Enterobacteriaceae showed the highest susceptibility to chloramphenicol (92%) and amikacin (85%), but high resistance (up to 77%) to cefazolin, cefotaxime, ceftazidime, and ciprofloxacin. Non-ESBL Enterobacteriaceae remained largely susceptible to tobramycin (99%)and amikacin (95%)(15).

A five-year retrospective study conducted in East China identified that 530 bacterial strains from 2727 patients and a total of 506 bacterial strains underwent antimicrobial susceptibility testing (AST). Approximately 95.4% of *S. aureus* and *S. epidermidis*were resistant to penicillin G. 66.97% and 50.91% of *Staphylococcus epidermidis* strains were resistant to erythromycin and ciprofloxacin, respectively. *Staphylococcus aureus* showed 56.72% and 50.75% resistance to erythromycin and clindamycin, respectively. 42.49% (215/506) were MDR in this study (23).

A hospital-based cross-sectional study (Jan 1-June 10, 2020) at Arba Minch General Hospital examined 259 suspected external ocular infections. All Gram-positive isolates were penicillin-resistantbut fully susceptible to clindamycin, with ciprofloxacin also highly effective (93%).*S. aureus* (24.8%) showed high resistance to tetracycline (92.3%) and had a 61.5% MRSA rate; most were susceptible to gentamicin (84.6%) and chloramphenicol (76.7%). CoNS isolates showed high resistance to tetracycline (73.9%) and erythromycin (69.6%), with 65.2%

methicillin resistance. Among Gram-negative Enterobacteriaceae, all were resistant to ampicillin and amoxicillin-clavulanate, but highly susceptible to gentamicin (100%) and ciprofloxacin (92.4%). *P. aeruginosa* showed notable resistance to cefepime (66.7%) and moderate resistance to piperacillin (52.4%), yet remained highly susceptible to gentamicin, norfloxacin, ciprofloxacin, and amikacin (80.9-100%). *E. coli* showed high resistance to ceftriaxone (66.7%) and moderate resistance to amikacin and meropenem. Overall MDR prevalence was 71.3%, with 46% of MDR isolates being Gram-positive. Among Gram-negative isolates, 22.6% were ESBL producers, mainly *K. pneumoniae* (66.6%) and *E. coli* (40%)(17).

### **2.3. Factors associated with ocular infections**

A retrospective study carried out in Nottingham, on 283 patients with suspected bacterial keratitis showed that ocular surface diseases, contact lens wear, and systemic immunosuppression were highly associated with bacterial keratitis (39).

A cross-sectional study conducted in Tigray, from September to December 2015, on 271 patients showed that ocular surface disease, ocular trauma, hospitalization, and cosmetic application practice were significantly associated with ocular bacterial infections (16). Similarly, ocular trauma has been repeatedly shown to predispose individuals to infections by providing a direct portal of entry for pathogens (25, 40). Likewise, a cross-sectional study conducted in Debre Markos on patients with external ocular infections identified that, aged 15-64 years, being a farmer, previous eye surgery, and infrequent face-washing were significant risk factors for bacterial infection(12).

A study conducted in Arba Minch identified that ocular surface disease, ocular trauma, and hygiene-related exposures were the important factors influencing the occurrence of external ocular infections (17). Another, similar cross-sectional study conducted in Shashemene on 332 patients with ocular and periocular infections found that ocular trauma and poor hygiene were the important contributors to the occurrence of bacterial ocular infections (41).

In general, studies from Ethiopia and other countries consistently show that ocular surface disease, ocular trauma, and poor hygiene or exposure-related practices are the most common risk factors for bacterial external ocular infections. Other contributing factors reported include previous eye surgery, hospitalization, cosmetic use, contact lens wear, systemic immunosuppression, age, and occupation.

### **3. OBJECTIVES**

#### **3.1. General objective**

- ✓ To determine the bacterial profile, antimicrobial susceptibility patterns, and extended-spectrum  $\beta$ -lactamase production among bacterial isolates from patients with external ocular infections at Bahir Dar, Northwest Ethiopia, from February 9 to May 8, 2026.

#### **3.2. Specific objectives**

- ✓ To identify the bacterial species isolated from patients with external ocular infections.
- ✓ To determine the antimicrobial susceptibility profiles of bacterial isolates from patients with external ocular infections.
- ✓ To determine ESBL-producing bacteria among Gram-negative isolates.
- ✓ To identify the risk factors associated with bacterial external ocular infections

## 4. METHODS AND MATERIALS

### 4.1. Study setting

The study was conducted at three governmental hospitals in Bahir Dar, the capital of Amhara Regional State, located 565 km from Addis Ababa: Felege Hiwot Comprehensive Specialized Hospital (FHCSH), Addisalem Primary Hospital (APH), and Tibebe Gihon Specialized Teaching Hospital (TGSTH), and it has a population of approximately 455,901: 228,189 males (42, 43).

FHCSH, established in 1969 and upgraded to a comprehensive specialized hospital in 2017, is one of the largest tertiary-level institutions in Northwest Ethiopia, with 560 inpatient beds and over 900 health professionals. Its ophthalmology unit has 16 beds and serves about 2,000 patients monthly, staffed by 2 ophthalmologists, 1 cataract surgeon, 3 optometrists, and 13 nurses(44).

TGSTH is another hospital located in Bahir Dar. It was established in 2019 GC. It is a tertiary-level teaching hospital under Bahir Dar University and serves a population of over 7 million. It is a modern tertiary-level facility equipped to deliver comprehensive clinical, surgical, and diagnostic services. The hospital operates 7 major inpatient wards-including a fully functional ophthalmology ward-with a capacity of over 500 beds, including 20 Intensive Care Unit (ICU) beds and dedicated High-Dependency Units for critically ill patients. Its infrastructure includes 126+ clinical service rooms and 14 fully equipped operating theaters, supporting a wide spectrum of elective and emergency surgical procedures. Additionally, it houses around 1,000 healthcare professionals(45). The ophthalmology unit is a key clinical department, comprising a 10-bed ward dedicated to inpatient eye care and a busy outpatient clinic. It is staffed by 4 ophthalmologists, 3 optometrists, and 5 ophthalmic nurses. This team supports a high patient volume, with an average of 600 outpatient visits per month. The unit is equipped to diagnose and manage a broad spectrum of ocular conditions, including infections, trauma, cataracts, glaucoma, and refractive errors, and performs routine outpatient and emergency ophthalmic procedures.

Addisalem Primary Hospital was established on July 17, 2015 GC, in Bahir Dar city at Atse Tewodros sub-city (45). The hospital has 52 beds for inpatients and delivery service. In the hospital, a total of 4 specialist physicians and 17 general practitioners served an average of 104,700 attendants annually (46). It is a key public health institution, staffed by a total of 365 professionals who provide both clinical and administrative services to the surrounding

population. The hospital operates 5 inpatient wards and delivers essential medical, surgical, maternal, and emergency care services to the community. The ophthalmology unit is an integral part of the hospital's service structure, with an average monthly patient flow of approximately 400 visits. The unit is staffed by 2 optometrists and functions with a dedicated examination room equipped to support routine diagnostic and therapeutic eye care. This setup enables the hospital to address common ocular conditions and contribute significantly to the early detection and management of preventable eye diseases in the area.

## **4.2. Study design and period**

A hospital-based cross-sectional study was conducted from February 9 to May 8, 2026, in Bahir Dar, Northwest Ethiopia.

## **4.3. Population**

### **4.3.1. Source population**

- All patients who visited FHCSH, TGSTH, and APH in the ophthalmology department during the study period.

### **4.3.2. Study population**

- Patients who were clinically suspected with external ocular infections in FHCSH, TGSTH, and APH during the study period.

## **4.4. Eligibility criteria**

### **4.4.1. Inclusion criteria**

Patients presenting with clinical signs of external ocular infection were included:

- Keratitis: corneal opacity, ulceration, or infiltrate observed via slit-lamp examination
- Conjunctivitis: conjunctival injection with mucoid or purulent discharge
- Blepharitis: eyelid margin inflammation with scaling or crusting
- Dacryocystitis: tenderness and swelling over the lacrimal sac area

### **4.4.2. Exclusion criteria**

- Patients who received antibiotic treatment for the past 2 weeks
- Patients who underwent recent ocular surgery

## 4.5. Variables

### 4.5.1. Dependent variables

- Bacterial external ocular infections

### 4.5.2. Independent variables

- Age
- Sex
- Residence
- Level of education
- Occupation
- Previous ocular infections
- Previous ocular surgery
- Trauma
- Eye allergy
- Systemic disease
- Contact lens wearing
- Frequency of face washing
- Hospitalization
- Self-medication

## 4.6. Operational definition

**More frequent face washing:** Face washing two or more times per day with soap and water.

**Frequent face washing:** Face washing once a day with soap and water.

**Less frequent face washing:** Face washing less than once per day (e.g., every other day using soap and water).

## 4.7. Sample size determination

The sample size for this study was calculated using the single population proportion formula, assuming a 95% confidence level, a 5% margin of error, and prevalence of external ocular infection (62.8%), based on findings from a nearby study conducted at Debre Markos Referral Hospital, and prevalence of ESBL production (22.6%) at Arba Minch General Hospital, Ethiopia, then take the larger sample size (12, 17).

### A. Using prevalence of external ocular infection (62.8%) from Debre Markos Referral Hospital

$$n = Z^2 \cdot p(1-p) / d^2$$

Where:

✓ n = required sample size

- ✓  $Z = Z\text{-score for } 95\% \text{ confidence level} = 1.96$
- ✓  $p = \text{estimated prevalence of external ocular infections} = 0.628$
- ✓  $d = \text{margin of error} = 0.05$

$$n = (1.96)^2 \cdot (0.628) \cdot (1-0.628) / (0.05)^2$$

$$n = 0.897 / 0.0025$$

$$n = 358.8$$

Thus, the initial sample size is approximately 359

To account for a 5% non-response rate, the sample size is adjusted as follows:

$$f_{\text{inal}} = 359 + (5\% \times 359) = 359 + 18 = 377$$

Final sample size = 377 participants

#### **B. Using prevalence of ESBL producer (22.6%) from Arba Minch General Hospital**

$$n = Z^2 \cdot p(1-p) / d^2$$

Where:

- ✓  $n = \text{required sample size}$
- ✓  $Z = Z\text{-score for } 95\% \text{ confidence level} = 1.96$
- ✓  $p = \text{estimated prevalence of external ocular infections} = 0.226$
- ✓  $d = \text{margin of error} = 0.05$

$$n = (1.96)^2 \cdot (0.226) \cdot (1-0.226) / (0.05)^2$$

$$n = 0.67 / 0.0025$$

$$n = 268$$

To account for a 5% non-response rate, the sample size is adjusted as follows:

$$f_{\text{inal}} = 268 + (5\% \times 268) = 268 + 13 = 281$$

Final sample size = 281 participants

The larger sample size (377 participants) was used to ensure sufficient power.

### **Sample allocation**

The total sample size of 377 was proportionally allocated to the three study hospitals based on their average monthly ophthalmology patient flow to ensure representativeness.

### **Estimated monthly ophthalmology patient flow:**

FHCSH: 2,000 patients, TGSTH: 600 patients, and APH: 400 patients.

Total estimated monthly flow:  $2,000 + 600 + 400 = 3,000$  patients/month.

### **Proportional allocation formula:**

$$\text{Hospital Sample} = \frac{\text{Hospital Patient Flow}}{\text{Total Patient Flow}} \times \text{Total Sample Size}$$

$$\text{FHCSH} = 2000 / 3000 \times 377$$

$$\text{FHCSH} = 251.3 \text{ round to } \mathbf{251}$$

$$\text{TGSTH} = 600 / 3000 \times 377$$

$$\text{TGSTH} = 75.4 \text{ round to } \mathbf{75}$$

$$\text{APH} = 400 / 3000 \times 377$$

$$\text{APH} = 50.2 \text{ round to } \mathbf{51}$$

**Table2: Summary table for sample size allocation**

| <b>Hospitals</b> | <b>Monthly patient flow</b> | <b>Proportion</b> | <b>Sample size (n)</b> |
|------------------|-----------------------------|-------------------|------------------------|
| <b>FHCSH</b>     | 2000                        | 66.67%            | 251                    |
| <b>TGSTH</b>     | 600                         | 20.00%            | 75                     |
| <b>APH</b>       | 400                         | 13.33%            | 51                     |
| <b>Total</b>     | 3000                        | 100%              | 377                    |

#### **4.8. Sampling technique and procedure**

A convenient sampling technique was used. All patients who fulfilled the inclusion criteria during the study period were enrolled in the study consecutively until the predetermined sample size for each hospital was reached.

#### **4.9. Data collection**

##### **4.9.1. Socio-demographic and clinical data**

The clinical pictures of external ocular infections of all patients were examined using a slit-lamp bio-microscope and diagnosed by an ophthalmologist or optometric nurse. Subsequently, socio-demographic and clinical data were collected from each participant by a trained ophthalmic nurse through face-to-face interviews using a pretested structured questionnaire.

##### **4.9.2. Laboratory data collection**

###### **4.9.2.1. Collection, handling, and transport of external ocular specimens**

Ocular specimens were collected from the eyes of patients with external ocular infections after obtaining informed consent. Specimens from the external part of the eye, such as the conjunctiva, eyelid, lacrimal sac, and cornea were collected by an ophthalmologist and ophthalmic nurse. Two separate specimens were collected from each infected ocular site. The first swab or scraping was dedicated to preparing a direct Gram stain smear for immediate microscopic examination, while a second, independently collected specimen from the same site was used for culture inoculation. Conjunctival specimens were collected using a sterile saline-moistened cotton swab, applied by gently passing the swab over the lower tarsal and fornix conjunctiva twice. In cases of dacryocystitis, specimens were taken by puncture and aspiration of the lacrimal sac. An antiseptic was first applied to the area of puncture, and then the lacrimal sac was punctured in the area below the medial canthal ligament. Specimens from corneal ulcers or keratitis were collected with the help of the slit lamp microscope. Briefly, a local topical anesthetic, proparacaine was applied (37).

The edge of the ulcer was firmly scraped using an insulin syringe, and several scrapings were collected. In the case of blepharitis, discharge from the margin of the eyelid was collected using cotton swabs and placed into a sterile tube. All the swabs were finally immersed in Amies transport medium and corneal scraping were immersed in a tube that has 3 ml of Brain Heart Infusion (BHI) broth (37). Collected specimens were labeled accurately with patient ID, age, sex,

hospital name, date, and site of specimen collection, and specimens collected at FHCSH and APH were transported to TGSTH microbiology laboratory for analysis within two hours of collection. Transport followed standard cold chain procedures, maintaining specimens at 2-8°C, using iceboxes, to minimize overgrowth of contaminants or death of pathogens.

#### **4.9.2.2. Direct examination**

External ocular specimens collected from patients were stained with a Gram staining technique and examined under microscopy for the preliminary differentiation of Gram-positive and Gram-negative bacteria (26).

#### **4.9.2.3. Culture and identification**

The collected eye specimens were inoculated to blood agar, MacConkey agar, and mannitol salt agar to support the growth of a wide range of bacterial external ocular pathogens. The inoculated media were incubated aerobically at 35-37°C for 24-48 hours. Cultures were examined daily, specimens showing no growth after 48 hours were considered sterile (47).

Gram-positive cocci were differentiated using standard biochemical tests including catalase, and coagulase. Gram-negative isolates were characterized using a combination of biochemical profiles from triple sugar iron (TSI) agar reactions, and supplemental tests for indole production, citrate utilization, urease activity, oxidase, and motility. Final bacterial identification was established by correlating microscopic appearance, growth characteristics, and biochemical profiles with standard bacteriological references, ensuring accurate species-level classification for all significant isolates (12).

#### **4.9.2.4. Antimicrobial susceptibility testing**

Antimicrobial susceptibility testing was performed using the agar disk diffusion technique on Muller-Hinton agar (MHA) according to the 2025 Clinical and Laboratory Standard Institute (CLSI) guidelines (48). The sensitivity/resistance of isolated strains was tested with commonly used antibiotics. The following antibiotic disks were used for Gram-positive bacteria: Penicillin (10µg), Erythromycin (15µg), Cotrimoxazole (1.25/23.75µg), Cefoxitin (30µg), Ciprofloxacin (5µg), Gentamicin (10µg), Chloramphenicol (30µg), Tetracycline (30µg), and Clindamycin (2µg). For Gram-negative bacteria: Ampicillin (10µg), Ceftriaxone (30µg), Ceftazidime (10µg), Gentamicin (10µg), Ciprofloxacin (5µg), Cotrimoxazole (1.25/23.75µg), Meropenem (10µg), Amikacin (30µg), and Tetracycline (30µg) were used. Similarly, the

following antibiotics were used to test against *P.aeruginosa*: Cefotaxime (10µg), Ciprofloxacin (5µg), Meropenem (10µg), and amikacin (30µg ) (48).

Three to five bacterial colonies of the test organism were picked and emulsified in 5 ml of normal saline and mixed gently. To standardize the inoculum density for a susceptibility test, a 0.5 McFarland standard solution was used. Within 15 min after adjusting the turbidity of the inoculum's suspension, a sterile cotton swab was dipped into the adjusted suspension. The plates were then inoculated by streaking the swab over the entire agar surface (MHA), and the antimicrobial-impregnated disks were placed using sterile forceps on the agar surface, incubated at 37°C for 24h, and the zone of inhibition was determined. The zone diameters were interpreted according to the CLSI, 2025 guideline as susceptible (S), intermediate (I), or resistant (R). Reference strains of *S. aureus* (ATCC 25923), *P. aeruginosa* (ATCC 27853), and *E. coli* (ATCC 25922), obtained from the Amhara Public Health Institute, were used as quality control organisms and were tested in parallel with the study isolates during each batch of susceptibility testing to verify the reliability of the antimicrobial disks, media, and testing conditions(48).

**Table3: Summary table for the selected drugs, their classes, reasons for selection, role in AST, and cross-resistance**

| <b>Antibiotic class</b>                              | <b>Selected antibiotic</b>                      | <b>Reason for selection</b>                                           | <b>Role of AST</b>                                    | <b>Cross-resistance</b>                                                      |
|------------------------------------------------------|-------------------------------------------------|-----------------------------------------------------------------------|-------------------------------------------------------|------------------------------------------------------------------------------|
| Penicillin's                                         | Penicillin, Ampicillin                          | Baseline resistance monitoring.                                       | Routine primary testing                               | Cross-resistance is occurred between ampicillin and amoxicillin              |
| Cephalosporins                                       | Cefoxitin, Ceftriaxone, Ceftazidime, Cefotaxime | Used in severe infections and ESBL screening agents                   | Routine primary testing and screening for ESBL        |                                                                              |
| Carbapenems                                          | Meropenem                                       | Last-resort for MDR Gram-negatives                                    | Confirmatory for resistant isolates                   |                                                                              |
| Aminoglycosides                                      | Gentamicin, Amikacin,                           | Broad-spectrum; used in ocular infections                             | Confirmatory for Gram-negative and some Gram-positive |                                                                              |
| Fluoroquinolones                                     | Ciprofloxacin                                   | Commonly prescribe for ocular infection locally                       | Routine AST test                                      | Cross-resistance is common between Ciprofloxacin and levofloxacin            |
| Tetracyclines                                        | Tetracycline                                    | Commonly prescribe for ocular infection locally                       | Routine AST test                                      | Cross-resistance common among tetracyclines (doxycycline, minocycline)       |
| Macrolides                                           | Erythromycin                                    | Used for Gram-positive infections                                     | Routine AST test                                      | Cross-resistance with other macrolides (azithromycin, clarithromycin)        |
| Lincosamides                                         | Clindamycin                                     | Monitors inducible resistance                                         | Routine AST test                                      |                                                                              |
| Sulfonamides                                         | Cotrimoxazole                                   | Broad-spectrum used in empirical therapy                              | Routine AST test                                      |                                                                              |
| Extended-Spectrum Beta-Lactamase (ESBL) Confirmatory | Ceftazidime/Clavulanate                         | Phenotypic confirmation of ESBL production in Gram-negative isolates. | Confirmatory for ESBL detection                       | ESBL producers often resistant to penicillins, cephalosporins, and aztreonam |

#### **4.9.2.5. Screening of extended- spectrum beta- lactamase producing strains**

According to the 2025 CLSI guidelines, the screening of ESBL-producing Gram-negative isolates in this study was performed using a panel of disc on MHA. The screening antibiotic was ceftazidime (30 µg). Using CLSI screening breakpoints, any isolate showing a zone diameter of  $\leq 22$  mm for ceftazidime was flagged as a potential ESBL producer and preceded to a phenotypic confirmation. All screening tests were conducted concurrently with routine antimicrobial susceptibility testing using the same 0.5 McFarland standardized inoculum and incubation conditions (35-37°C for 16-18 hours) (48).

#### **4.9.2.6. Confirmatory test for extended- spectrum beta- lactamase production**

For the phenotypic confirmation of ESBL production, the study adhered to the CLSI recommended combined disk diffusion method. Each bacterial isolate identified as a potential ESBL producer during initial screening was standardized to a 0.5 McFarland suspension and inoculated as a confluent lawn onto MHA plates. On each plate, two antibiotic disks were placed at a standardized center-to-center distance of 24 mm: ceftazidime (30 µg) and ceftazidime/clavulanate (30/10 µg). Following aerobic incubation at 37°C for 16-18 hours, the zones of inhibition were measured. ESBL production was confirmed phenotypically if the inhibition zone around the ceftazidime/clavulanate disk was at least 5 mm larger than that around ceftazidime alone. This confirmation step was controlled using reference strains *K. pneumoniae* ATCC 700603 (ESBL-positive) and *E.coli* ATCC 25922 (ESBL-negative) in each run to ensure test accuracy and reliability (48). The overall laboratory workflow is depicted in figure 2.

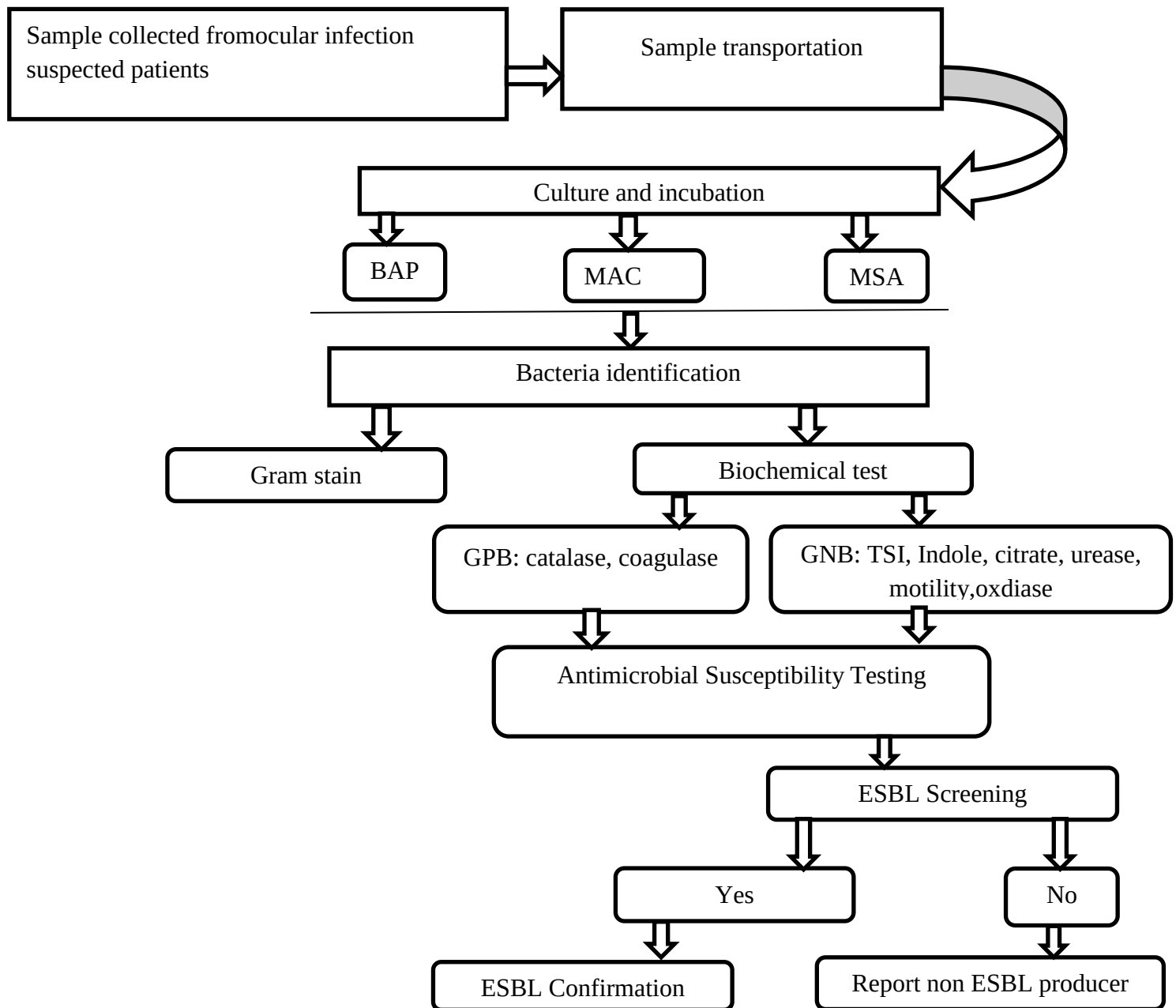


Figure 1: Flow chart for external ocular samples processing, identification of bacteria, antimicrobial susceptibility tests, and determination of ESBL production

#### **4.10. Data Quality Control**

To ensure the reliability and validity of the study findings, rigorous data quality control measures were implemented throughout the research process. Standard operating procedures (SOPs) were strictly followed during sample collection, transport, and laboratory processing to avoid contamination and preserve sample integrity. Structured questionnaires were used to collect the data. The clarity, comprehensibility, and feasibility of the questionnaire were assessed through a pilot study. The questionnaire was pretested using 5% of the sample size at FHCSH eye unit before running the actual data.

Supervision of data collection was conducted regularly on a daily basis and the collected data was checked for completeness and accuracy by the principal investigator. All ocular specimens (conjunctival swabs, eyelid swabs, and corneal scraping) were collected following standard operating procedures. Culture media were prepared aseptically by autoclaving, and 5% of each batch was checked for sterility through overnight incubation at 37°C. The performances of all the prepared culture media and biochemical tests were checked by using American Type Culture Collection (ATCC) standard reference strains (*E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *K. pneumoniae* ATCC 700603 and *S. aureus* ATCC 25923). The strains were used as a quality control throughout the study for culture, Gram staining, and antimicrobial susceptibility testing.

#### **4.11. Data processing and analysis**

Data were checked for completeness and coded, and entered to Statistical Package for Social Science (SPSS) version 25 for analysis. Descriptive statistics such as frequency, percentage, and median were used to summarize socio-demographic characteristics, bacterial isolates, antimicrobial susceptibility patterns, and ESBL production. Bivariate logistic regression was applied to determine the association between predictors and outcome variables with a 95% confidence interval. Variables with a P-value < 0.25 in the crude analysis were subjected to adjusted analysis through multivariate logistic regression with a 95% confidence interval to control confounding factors. P-values < 0.05 were considered statistically significant, and results were presented in the form of text, figures and tables depending on the data.

#### **4.12. Ethical considerations**

The study was conducted after being ethically approved by the Institutional Review Board (IRB) of the College of Medicine and Health Sciences (CMHS), Bahir Dar University (BDU) (Protocol Number: 6022/2026). A support letter was obtained from the Department of Medical Microbiology (DMM), CMHS-BDU. Further support letters and permission were obtained from the Amhara Public Health Institute (APHI), and the administrators of the study hospitals. Written assent of caregivers/guardians (for participants below 18 years old) and consent (for adults) were obtained before data collection. All the information obtained from the study participants was coded to keep confidentiality. Finally, the bacteriological culture results were communicated for attending physicians.

#### **4.13. Dissemination of results**

The final report of the study will be submitted to Department of Medical Microbiology, School of Medical Laboratory Science, College of Medicine and Health Sciences at Bahir Dar University. The finding of the study will also be submitted to the Bahir Dar city health Department and other concerned institutions. Besides, it also presented in local and/or international scientific meetings and conferences. Finally, the finding of the study will be disseminated through publication in reputable local or international journals.

## 5. RESULTS

### 5.1. Socio-demographic and clinical characteristics of the study participants

A total of 377 study participants clinically diagnosed with external ocular infections (EOI) were enrolled in the study. Of the study participants, 54.6% (206/377) were males. The ages of the study participants ranged from 4-84 years with a median age of 51 years (IQR: 32-67 years). Participants aged  $\geq 50$  years accounted for 51.5% (194/377) of the study population. About 67.6 % (255/377) of the study participants were rural residents, 46.9% (177/377) were farmers, 65.5 % (247/377) were married, and 54.6 % (206/377) were unable to read and write (Table 6).

Of the culture-confirmed bacterial isolates, the prevalence of external ocular infection was highest among male patients 118/206 (57.3%), patients aged  $\geq 50$  years 124/194 (63.9%), and rural residents 158/255 (62%). High prevalence was also observed among participants who were unable to read and write 131/206 (63.6%), and working as a farmer 105/177 (59.3%) (Table 4).

Table 4: Socio-demographic characteristics and culture positivity among study participants with external ocular infections in Bahir Dar governmental hospitals, Northwest Ethiopia, 2026 (N=377)

| Variables          | Category                 | Frequency N (%) | Culture positive N (%) |
|--------------------|--------------------------|-----------------|------------------------|
| Sex                | Male                     | 206 (54.6)      | 118 (57.3%)            |
|                    | Female                   | 171 (45.4)      | 101 (59.1%)            |
| Age                | < 18 years               | 26 (6.9%)       | 11 (42.3%)             |
|                    | 18-49                    | 157 (41.6%)     | 84 (53.5%)             |
|                    | $\geq 50$ years          | 194 (51.5%)     | 124 (63.9%)            |
| Residence          | Urban                    | 122 (32.4%)     | 61 (50%)               |
|                    | Rural                    | 255 (67.6%)     | 158 (62%)              |
| Occupations        | Housewife                | 63 (16.7%)      | 36 (57.1%)             |
|                    | Farmer                   | 177 (46.9%)     | 105 (59.3%)            |
|                    | Civil servant            | 20 (5.3%)       | 12 (60%)               |
|                    | Merchant                 | 51 (13.5%)      | 29 (56.9%)             |
|                    | Daily laborer            | 29 (7.7%)       | 18 (62.1%)             |
|                    | Others                   | 37 (9.8%)       | 19 (51.4%)             |
| Educational status | Unable to read and write | 206 (54.6%)     | 131 (63.6%)            |
|                    | Able to read and write   | 74 (19.6%)      | 42 (56.8%)             |
|                    | Elementary school        | 53 (14.1%)      | 27 (50.9%)             |
|                    | High school              | 20 (5.3%)       | 8 (40%)                |
|                    | College and above        | 24 (6.4%)       | 11 (45.8%)             |

|                |               |             |             |
|----------------|---------------|-------------|-------------|
| Marital status | Never married | 83 (22%)    | 41 (49.4%)  |
|                | Married       | 247 (65.5%) | 153 (61.9%) |
|                | Divorced      | 27 (7.2%)   | 14 (51.9%)  |
|                | Widowed       | 20 (5.3)    | 11 (55%)    |

Regarding clinical and behavioral predisposing factors, 116 (30.8%) participants had a history of eye trauma, while 85 (22.5%) participants reported a previous history of eye surgery. Contact lens use was reported by 22 (5.8%) participants. In addition, 112 (29.7%) participants had a history of previous ocular infections, and 97 (25.7%) participants reported a history of eye allergy. Less frequent face washing practice was observed among 94 (24.9%) participants. Presence of chronic disease was reported by 77 (20.4%) participants (Table 5).

Table 5: Clinical and related characteristics, and bacterial culture positivity among study participants with external ocular infections in Bahir Dar governmental hospitals, Northwest Ethiopia, 2026 (N=377)

| <b>Variables</b>             | <b>Category</b> | <b>Frequency N (%)</b> | <b>Culture positive N (%)</b> |
|------------------------------|-----------------|------------------------|-------------------------------|
| History of Eye Trauma        | Yes             | 116 (30.8%)            | 96 (82.8%)                    |
|                              | No              | 261 (69.2%)            | 123 (47.1%)                   |
| History of eye surgery       | Yes             | 85 (22.5%)             | 66 (77.6%)                    |
|                              | No              | 292 (77.5%)            | 153 (52.4%)                   |
| Contact lens use             | Yes             | 22 (5.8%)              | 12 (54.5%)                    |
|                              | No              | 355 (94.2%)            | 207 (58.3%)                   |
| History of ocular infection  | Yes             | 112 (29.7%)            | 86 (76.8%)                    |
|                              | No              | 265 (70.3%)            | 133 (50.2%)                   |
| History of eye allergy       | Yes             | 97 (25.7%)             | 80 (82.5%)                    |
|                              | No              | 280 (74.3%)            | 139 (49.6%)                   |
| Frequency of face washing    | More frequently | 114 (30.2%)            | 70 (61.4%)                    |
|                              | Frequently      | 169 (44.8%)            | 94 (55.6%)                    |
|                              | Less frequently | 94 (24.9%)             | 55 (58.5%)                    |
|                              |                 |                        |                               |
| Presence of chronic disease* | Yes             | 77 (20.4%)             | 45 (58.4%)                    |
|                              | No              | 300 (79.6%)            | 174 (58%)                     |
| History of hospitalization   | Yes             | 52 (13.8%)             | 31 (59.6%)                    |
|                              | No              | 325 (86.2%)            | 188 (57.8%)                   |
| History of self-medication   | Yes             | 117 (31%)              | 69 (59%)                      |
|                              | No              | 260 (69%)              | 150 (57.7%)                   |

\*Chronic diseases such as Diabetes mellitus, Hypertension, and HIV

## 5.2. Clinical presentation of external ocular infections

From a total of 377 patients with external ocular infections, conjunctivitis was the most common clinical presentation, affecting 160/377 (42.4%) patients, followed by blepharitis 113/377 (30%). Keratitis and blepharo-conjunctivitis were reported in 44/377 (11.7%) and 42/377 (11.2%) patients, respectively. Dacryocystitis was the least common clinical presentation, accounting for less than one-tenth of cases 18/377 (4.8%) (Figure 3).

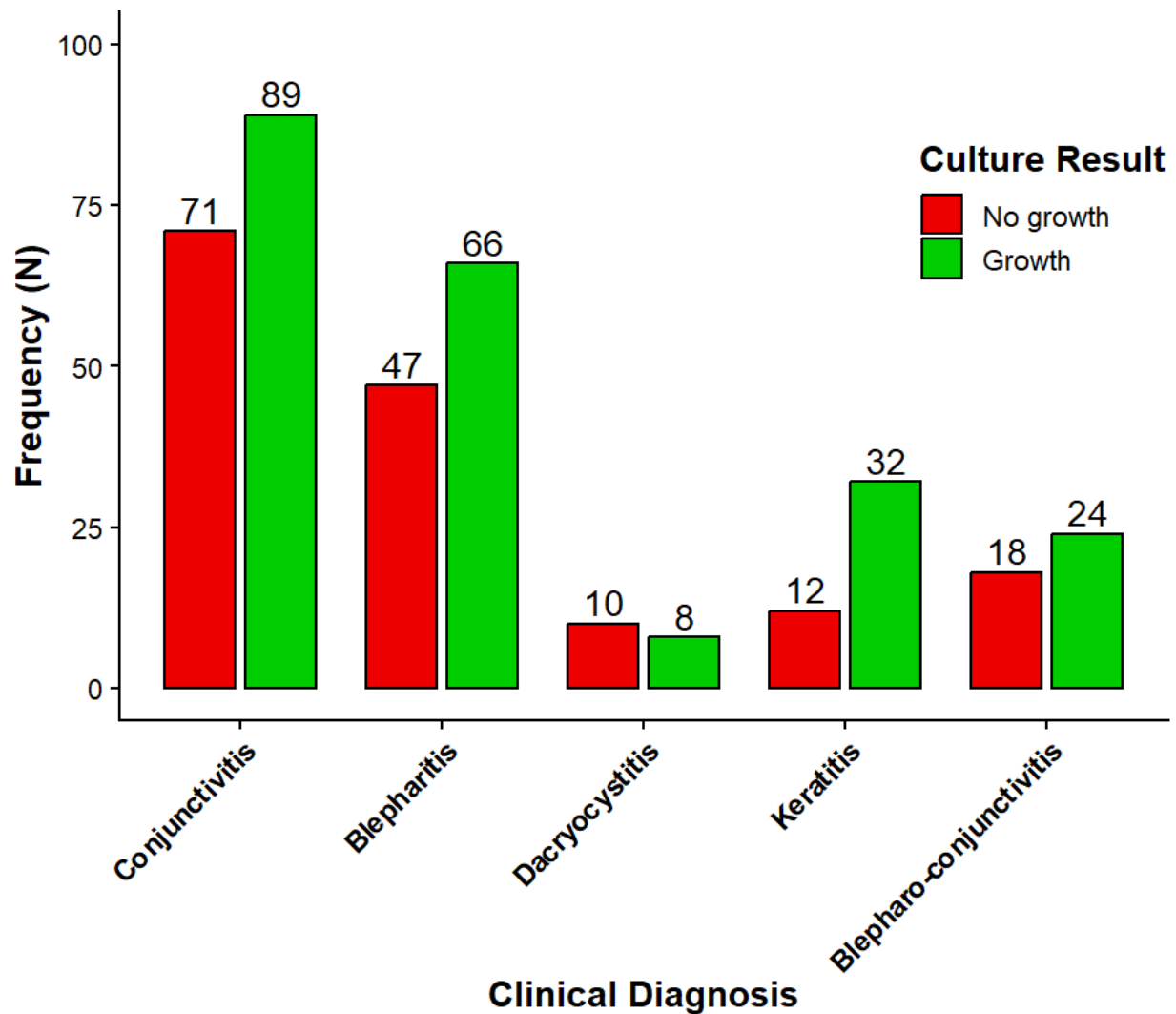


Figure2: Distribution of the clinical diagnosis by culture result in external ocular infected patients in Bahir Dar governmental hospitals, Northwest Ethiopia, 2026 (N=377)

### 5.3. Bacterial isolate and clinical diagnosis

The overall prevalence of bacterial isolates was 219/377 (58.1%) with a 95% CI of 53%-63%. Mixed bacterial isolates were not found from a single sample of the current study. Among the isolates, 64.4% (141/219) were Gram-positive. CoNS was the predominant Gram-positive bacteria accounting for 55.3% (78/141) followed by *S. aureus* with 44.7% (63/141). Among Gram-negative isolate, *E. coli* was the predominant bacterial isolate with a prevalence of 30.8% (24/78) followed by *K. pneumoniae* 29.5% (23/78) (Figure 4).

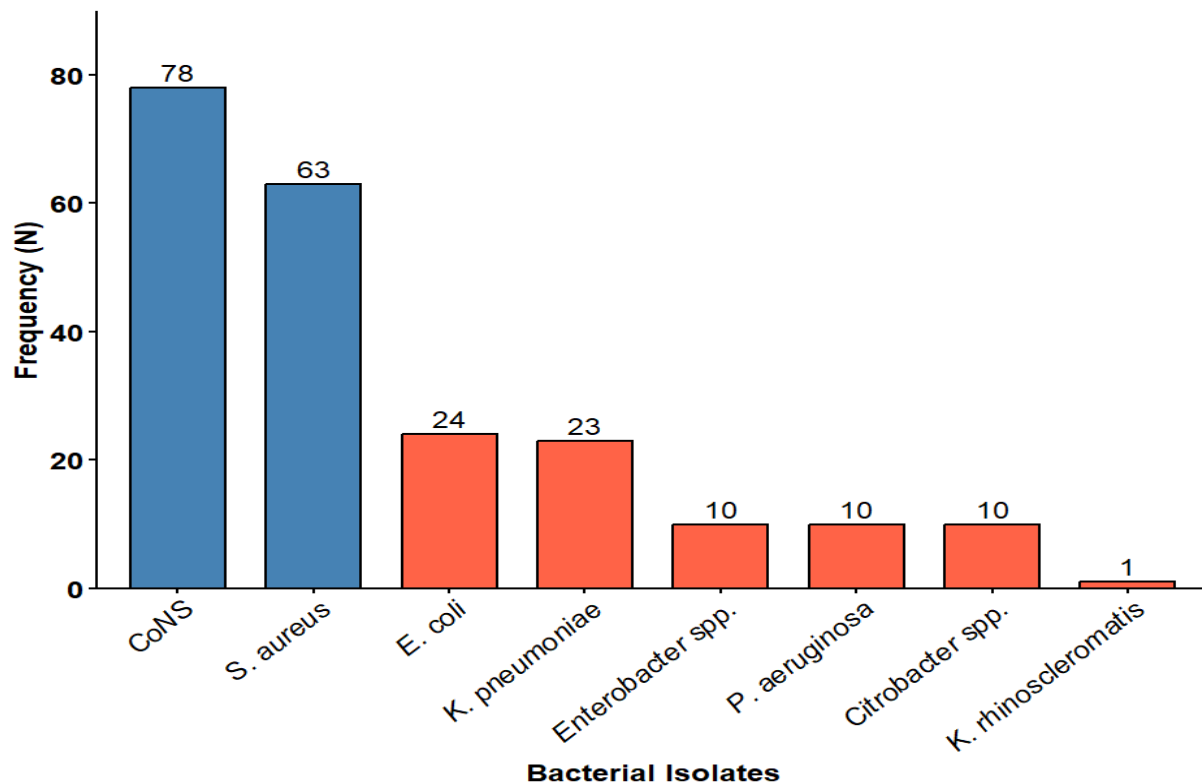


Figure 3: Frequency distribution of bacterial isolates recovered from external ocular infections in Bahir Dar governmental hospitals, Northwest Ethiopia, 2026 (N=377)

Among the 219 bacterial isolates recovered, CoNS were the predominant pathogens across the majority of the clinical conditions, accounted for 32/89 (36%) of isolates from conjunctivitis, 27/66 (40.9%) from blepharitis, and 10/24 (41.7%) from blepharo-conjunctivitis. This was followed by *S. aureus* 23/89 (25.8%), 22/66 (33.3%), 5/24 (20.8%) from conjunctivitis, blepharitis, and blepharo-conjunctivitis, respectively. Among the Gram-negative isolates, *E. coli* was recovered from 13/89 (14.6%) cases of conjunctivitis, and 4/32 (12.5%) cases of keratitis.

Similarly, *K. pneumoniae* was identified in 11/89(12.4%) isolates from conjunctivitis, and 5/24(20.8%) isolates from blepharo-conjunctivitis (Table 6).

Table 6: Bacterial profiles among different clinical features of external ocular infection in Bahir Dar governmental hospitals, Northwest Ethiopia, 2026 (N=377)

| Bacterial species          | Clinical diagnosis     |                     |                 |                                |                       | Total            |
|----------------------------|------------------------|---------------------|-----------------|--------------------------------|-----------------------|------------------|
|                            | Conjunctivitis (n=160) | Blepharitis (n=113) | Keratitis(n=44) | Blepharo-conjunctivitis (n=42) | Dacryocystitis (n=18) |                  |
| CoNS                       | 32(36%)                | 27(40.9%)           | 7(21.9%)        | 10(41.7%)                      | 2(25%)                | 78(35.6%)        |
| <i>S. aureus</i>           | 23(25.8%)              | 22(33.3%)           | 10(31.3%)       | 5(20.8%)                       | 3(37.5%)              | 63(28.8%)        |
| <i>E. coli</i>             | 13(14.6%)              | 5(7.6%)             | 4(12.5%)        | 1(4.2%)                        | 1(12.5%)              | 24(11%)          |
| <i>K. pneumoniae</i>       | 11 (12.4%)             | 3(4.5%)             | 4(12.5%)        | 5(20.8%)                       | 0(0%)                 | 23(10.5%)        |
| <i>Enterobacter</i> spp.   | 4(4.5%)                | 3(4.5%)             | 1(3.1%)         | 1(4.2%)                        | 1(12.5%)              | 10(4.6%)         |
| <i>P. aeruginosa</i>       | 3(3.4%)                | 2(3%)               | 5(15.6%)        | 0(0%)                          | 0(0%)                 | 10(4.6%)         |
| <i>Citrobacter</i> spp.    | 3(3.4%)                | 3(4.5%)             | 1(3.1%)         | 2(8.3%)                        | 1(12.5%)              | 10(4.6%)         |
| <i>K. rhinoscleromatis</i> | 0 (0%)                 | 1(1.5%)             | 0 (0%)          | 0 (0%)                         | 0 (0%)                | 1(0.5%)          |
| <b>Total</b>               | 89(40.6%)              | 66(30.1%)           | 32(14.6%)       | 24(11%)                        | 8(3.7%)               | <b>219(100%)</b> |

#### 5.4. Antimicrobial susceptibility patterns of Gram-positive bacterial isolates

Antimicrobial susceptibility test was performed for all recovered Gram-positive bacterial isolates (n=141) against a panel of nine selected antibiotics. Overall, the Gram-positive isolates showed high rate of resistance to penicillin 99.3% (n=140/141), tetracycline 66% (n=93/141), and erythromycin 63.8% (n=90/141). Conversely, the highest susceptibility rates among Gram-positive isolates were recorded for clindamycin, gentamicin, and chloramphenicol with 91.5% (129/141), 87.2% (123/141), and 80.9% (114/141) rate of susceptibility respectively. Among the 141 bacterial isolates recovered, 35 were identified as methicillin-resistant staphylococci (MRS), yielding an overall prevalence of 24.8% (35/141).

Among the Gram-positive isolates, *S. aureus* showed high susceptibility to clindamycin 90.5% (n=57), gentamicin 87.3% (n=55), chloramphenicol 79.4% (n=50), and ciprofloxacin 77.8% (n=49). Nevertheless, it showed high resistance to penicillin 98.4% (n=62), tetracycline 68.3% (n=43), and erythromycin 68.3% (n=43). The proportion of methicillin-resistant *S. aureus* among

the total *S. aureus* isolates was 33.3% (n=21). Similarly, CoNS isolates were highly susceptible to clindamycin 92.3% (n=72), gentamicin 87.2% (n=68), ceftazidime 82.1% (n=64), and chloramphenicol 82.1% (n=64). In contrast, high resistance was observed to penicillin 100% (n=78), tetracycline 64.1% (n=50), and erythromycin 60.3% (n=47) (Table 7).

Table 7: Antimicrobial susceptibility patterns of Gram-positive bacterial isolates recovered from external ocular infections in Bahir Dar governmental hospitals, Northwest Ethiopia, 2026

| Gram-positive isolates (n=141) | Antimicrobial susceptibility pattern |             |            |            |             |              |             |              |              |              |
|--------------------------------|--------------------------------------|-------------|------------|------------|-------------|--------------|-------------|--------------|--------------|--------------|
|                                |                                      | PG<br>n (%) | E<br>n (%) | T<br>n (%) | CD<br>n (%) | CAF<br>n (%) | GM<br>n (%) | CIP<br>n (%) | SXT<br>n (%) | FOX<br>n (%) |
| CoNS<br>(n=78)                 | S                                    | 0(0.0)      | 31(39.7)   | 27(34.6)   | 72(92.3)    | 64(82.1)     | 68(87.2)    | 58(74.4)     | 40(51.3)     | 64(82.1)     |
|                                | I                                    | 0(0.0)      | 0(0.0)     | 1(1.3)     | 0(0.0)      | 0(0.0)       | 0(0.0)      | 1(1.3)       | 0(0.0)       | 0(0.0)       |
|                                | R                                    | 78(100)     | 47(60.3)   | 50(64.1)   | 6(7.7)      | 14(17.9)     | 10(12.8)    | 19(24.4)     | 38(48.7)     | 14(17.9)     |
| <i>S. aureus</i><br>(n=63)     | S                                    | 1(1.6)      | 20(31.7)   | 20(31.7)   | 57(90.5)    | 50(79.4)     | 55 (87.3)   | 49(77.8)     | 28(44.4)     | 42(66.7)     |
|                                | I                                    | 0(0.0)      | 0(0.0)     | 0(0.0)     | 1(1.6)      | 0(0.0)       | 0(0.0)      | 1(1.6)       | 0(0.0)       | 0(0.0)       |
|                                | R                                    | 62(98.4)    | 43(68.3)   | 43(68.3)   | 5(7.9)      | 13(20.6)     | 8(12.7)     | 13(20.6)     | 35(55.6)     | 21(33.3)     |

**Key:** -CoNS = *Coagulase-negative staphylococci*, S = Sensitive, I = Intermediate, R = Resistance, PG= Penicillin, E = Erythromycin, T = Tetracycline, CD = Clindamycin, CAF = Chloramphenicol, GM= Gentamicin, CIP = Ciprofloxacin, SXT = Trimethoprim/sulfamethoxazole, FOX = Cefoxitin

### 5.5. Antimicrobial susceptibility patterns of Gram-negative bacterial isolates

The antimicrobial susceptibility pattern of Enterobacteriaceae isolates showed high susceptibility to amikacin 66/68(97.1%), and meropenem 63/68(92.6%), followed by gentamicin 54/68(79.4%), ciprofloxacin 53/68(77.9%), chloramphenicol 49/68(72.1%), and Trimethoprim/sulfamethoxazole 44/68 (64.7%). In contrast high resistance rates were observed against ampicillin 64/68(94.1%) and amoxicillin-clavulanic acid 60/68(88.2%), while more than half of the isolates were resistant to tetracycline 38/68(55.9%) and ceftazidime 36/68(52.9%). Among those, *E. coli* isolates were showed high susceptibility rate for amikacin 23/24(95.8%), and meropenem 22/24(91.7%), whereas high resistance was recorded against ampicillin 21/24(87.5%), amoxicillin-clavulanic acid 17/24(70.8%), tetracycline 14/24(58.3%), and

ceftazidime 12/24(50%). Similarly, *K. pneumoniae* showed high susceptibility to amikacin 22/23(95.7%), and meropenem 21/23(91.3%), but complete resistance was detected to ampicillin and amoxicillin-clavulanic acid 23/23(100% each), with additional high resistance to tetracycline and ceftazidime 15/23(65.2% each), and ceftriaxone 13/23 (56.5%)(Table 8).

Table 8: Antimicrobial susceptibility patterns of Gram-negative bacterial isolates recovered from external ocular infections in Bahir Dar governmental hospitals, Northwest Ethiopia, 2026

| Isolates (n=78)                 | Antimicrobial susceptibility pattern |          |          |          |          |          |          |          |           |           |          |          |
|---------------------------------|--------------------------------------|----------|----------|----------|----------|----------|----------|----------|-----------|-----------|----------|----------|
|                                 |                                      | T n(%)   | CAF n(%) | GM n(%)  | CIP n(%) | SXT n(%) | AK n(%)  | CAZ n(%) | CRO n (%) | MEM n (%) | AMP n(%) | AMC n(%) |
| <i>E. coli</i> (n=24)           | S                                    | 8(33.3)  | 17(70.8) | 18(75)   | 17(70.8) | 12(50)   | 23(95.8) | 11(45.8) | 12(50)    | 22(91.7)  | 2(8.3)   | 7(29.2)  |
|                                 | I                                    | 2(8.3)   | 2(8.3)   | 2(8.3)   | 1(4.2)   | 0(0.0)   | 0(0.0)   | 1(4.2)   | 1(4.2)    | 0(0.0)    | 1(4.2)   | 0(0.0)   |
|                                 | R                                    | 14(58.3) | 5(20.8)  | 4(16.7)  | 6(25)    | 12(50)   | 1(4.2)   | 12(50)   | 11(45.8)  | 2(8.3)    | 21(87.5) | 17(70.8) |
| <i>K. pneumoniae</i> (n=23)     | S                                    | 7(30.4)  | 16(69.6) | 18(78.3) | 18(78.3) | 16(69.6) | 22(95.7) | 8(34.8)  | 10(43.5)  | 21(91.3)  | 0(0.0)   | 0(0.0)   |
|                                 | I                                    | 1(4.3)   | 0(0.0)   | 0(0.0)   | 0(0.0)   | 0(0.0)   | 0(0.0)   | 0(0.0)   | 0(0.0)    | 0(0.0)    | 0(0.0)   | 0(0.0)   |
|                                 | R                                    | 15(65.2) | 7(30.4)  | 5(21.7)  | 5(21.7)  | 7(30.4)  | 1(4.3)   | 15(65.2) | 13(56.5)  | 2(8.7)    | 23(100)  | 23(100)  |
| <i>P. aeruginosa</i> (n=10)     | S                                    | NT       | NT       | NT       | 6(60)    | NT       | 9(90)    | 8(80)    | NT        | 10(100)   | NT       | NT       |
|                                 | I                                    |          |          |          | 2(20)    |          | 0(0.0)   | 1(10)    |           | 0(0.0)    |          |          |
|                                 | R                                    |          |          |          | 2(20)    |          | 1(10)    | 1(10)    |           | 0(0.0)    |          |          |
| <i>Citrobacter</i> spp. (n=10)  | S                                    | 7(70)    | 8(80)    | 9(90)    | 9(90)    | 7(70)    | 10(100)  | 7(70)    | 6(60)     | 9(90)     | 0(0.0)   | 0(0.0)   |
|                                 | I                                    | 0(0.0)   | 0(0.0)   | 0(0.0)   | 0(0.0)   | 0(0.0)   | 0(0.0)   | 0(0.0)   | 0(0.0)    | 0(0.0)    | 0(0.0)   | 0(0.0)   |
|                                 | R                                    | 3(30)    | 2(20)    | 1(10)    | 1(10)    | 3(30)    | 0(0.0)   | 3(30)    | 4(40)     | 1(10)     | 10(100)  | 10(100)  |
| <i>Enterobacter</i> spp. (n=10) | S                                    | 4(40)    | 7(70)    | 8(80)    | 8(80)    | 8(80)    | 10(100)  | 4(40)    | 4(40)     | 10(100)   | 0(0.0)   | 0(0.0)   |
|                                 | I                                    | 0(0.0)   | 0(0.0)   | 0(0.0)   | 0(0.0)   | 0(0.0)   | 0(0.0)   | 1(10)    | 0(0.0)    | 0(0.0)    | 1(10)    | 0(0.0)   |
|                                 | R                                    | 6(60)    | 3(30)    | 2(20)    | 2(20)    | 2(20)    | 0(0.0)   | 5(50)    | 6(60)     | 0(0.0)    | 9(90)    | 10(100)  |

**Key:** S = Sensitive, I = Intermediate, R = Resistance, T = Tetracycline, CAF = Chloramphenicol, GM= Gentamicin, CIP = Ciprofloxacin, SXT = Trimethoprim/sulfamethoxazole, AK= Amikacin, CAZ= Ceftazidime, CRO= ceftriaxone, MEM= Meropenem, AMP= Ampicillin, AMC= Amoxicillin-clavulanic acid, NT= Not tested

## 5.6. Multi-drug resistance pattern of bacterial isolates

Of the 219 bacterial isolates recovered from external ocular infections, the prevalence of MDR bacteria was 63.5%(139/219). Gram-positive isolates showed higher MDR rates of 72.3% (102/141) compared to the Gram-negative isolates, of which 47.4% (37/78) were MDR. Among Gram-positive isolates, *S. aureus* showed the highest MDR prevalence which is 79.4% (50/63), followed by CoNS which is 66.7% (52/78). Among Gram-negative isolates, *K. pneumoniae* was

the most frequently MDR, with 60.9% (14/23), followed by *Enterobacter* spp., at 60% (6/10) and *E. coli* at 54.2% (13/24) (Figure 5)

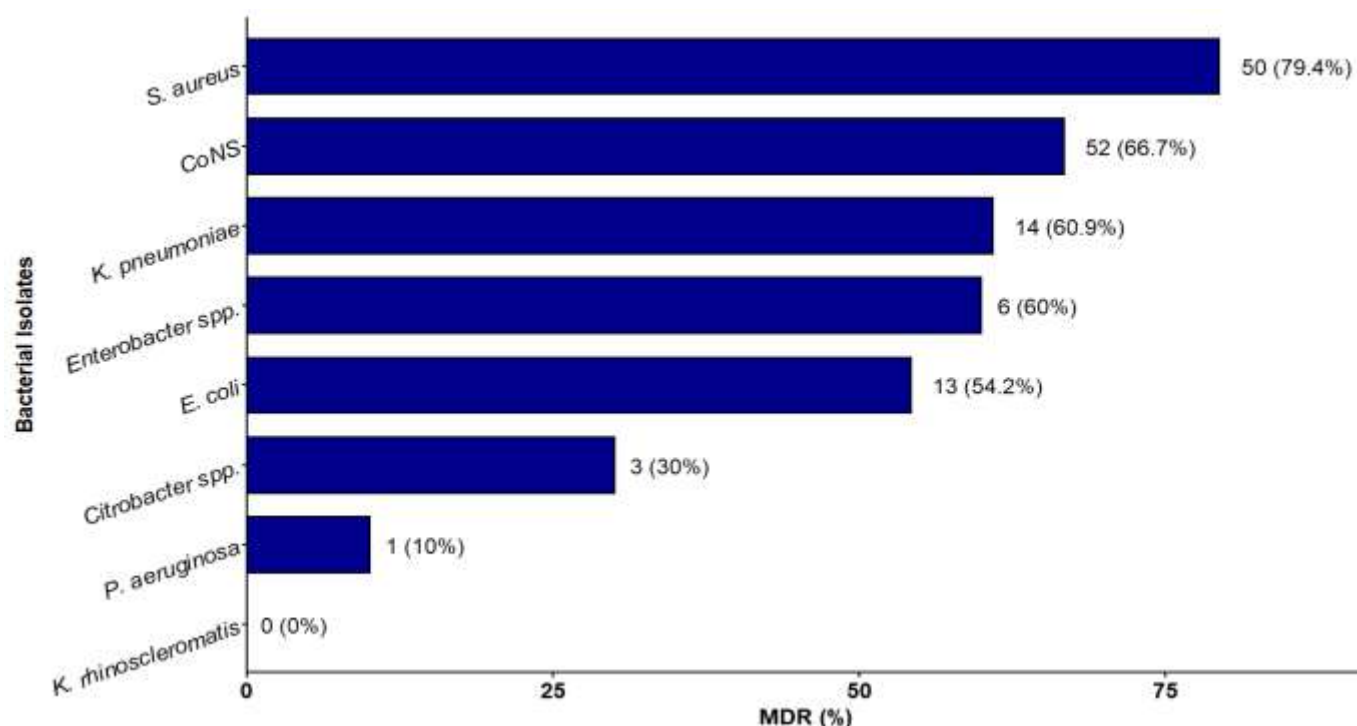


Figure4: Multi-drug resistance prevalence of bacterial isolates recovered from external ocular infections in Bahir Dar governmental hospitals, Northwest Ethiopia, 2026 (N=377)

From the total bacterial isolates, 11 (5%) were susceptible to all antimicrobial classes tested, while 24 (11%) resistant to one antimicrobial class and 45 (20.5%) were resistant to two antimicrobial classes (Table 9).

Table 9: Multi-drug resistance patterns of bacterial isolates from external ocular infection at Bahir Dar governmental hospitals, Northwest Ethiopia, 2026

| Isolates                       | MDR patterns |             |             |                              |
|--------------------------------|--------------|-------------|-------------|------------------------------|
|                                | R0<br>n (%)  | R1<br>n (%) | R2<br>n (%) | ≥ R3 (Total<br>MDR)<br>n (%) |
| CoNS(n=78)                     | 0(0.0)       | 11(14.1)    | 15(19.2)    | 52(66.7)                     |
| <i>S. aureus</i> (n=63)        | 1(1.6)       | 0(0.0)      | 12(19)      | 50(79.4)                     |
| <i>E. coli</i> (n=24)          | 2(8.3)       | 2(8.3)      | 7(29.2)     | 13(54.2)                     |
| <i>K. pneumoniae</i><br>(n=23) | 0(0.0)       | 4(17.4)     | 5(21.7)     | 14(60.9)                     |

|                                            |        |        |          |           |
|--------------------------------------------|--------|--------|----------|-----------|
| <i>Enterobacter</i><br>spp.(n=10)          | 0(0.0) | 2(20)  | 2(20)    | 6(60)     |
| <i>P.</i><br><i>aeruginosa</i> (n=10)      | 8(80)  | 1(10)  | 0(0.0)   | 1(10)     |
| <i>Citrobacter</i><br>spp.(n=10)           | 0(0.0) | 4(40)  | 3(30)    | 3(30)     |
| <i>K.</i><br><i>rhinoscleromatis</i> (n=1) | 0(0.0) | 0(0.0) | 1(100)   | 0(0.0)    |
| <b>Total (n=219)</b>                       | 11(5)  | 24(11) | 45(20.5) | 139(63.5) |

**Key:** **CONS**=Coagulase-negative staphylococci, **Ro**= Bacterial isolate sensitive to all antibiotics, **R1**= Bacterial isolate resistance to one antibiotic, **R2**= Bacterial isolate resistance to two antibiotics of different classes, **R3**= Bacterial isolate resistance to three or more antibiotics of different classes, **MDR**= Multi-drug resistance

### 5.7. Extended-spectrum $\beta$ -lactamase producers

Among the 47 Gram-negative isolates (*E. coli* and *K. pneumoniae*) recovered from patients with external ocular infections, 12 (25.5%) were found to be extended-spectrum  $\beta$ -lactamase (ESBL) producers in the confirmatory test. Species specific analysis of the isolates tested for ESBL production showed that, *E. coli* constituted 24 isolates. Of which 5 (20.8%) isolates were ESBL producers. Similarly, among *K. pneumoniae* isolates tested, 7 (30.4%) isolates were identified as ESBL producers. The proportion of ESBL production was comparatively higher in *K. pneumoniae* isolates than *E. coli* isolates (Table 10).

Table 10: Distribution of ESBL production among Gram-negative bacterial isolates recovered from external ocular infections at Bahir Dar governmental hospitals, Northwest Ethiopia, 2026

| Bacterial isolates          | ESBL producers |           |
|-----------------------------|----------------|-----------|
|                             | Yes, n (%)     | No, n (%) |
| <i>E. coli</i> (n=24)       | 5(20.8)        | 19(79.2)  |
| <i>K. pneumoniae</i> (n=23) | 7(30.4)        | 16(69.6)  |
| Total (n=47)                | 12(25.5)       | 35(74.5)  |

### 5.8. Factors associated with external ocular infections

Various socio-demographic and clinical characteristics of the study participants were assessed for their possible association with the prevalence of bacterial external ocular infections. Prior to the analysis, multi-collinearity analysis was carried out among the independent variables to identify potential correlation and ensure the validity of the regression model. Bivariate and multivariable logistic regression analyses were performed to identify factors associated with bacterial external ocular infections, and adjusted odds ratios (AOR) with 95% confidence intervals (CI) were used to determine the strength of the association. Variables with a p-value less than 0.25 in the bivariate logistic regression were entered in to the multivariable logistic regression model.

In bivariate logistic regression, age group  $\geq 50$  years ( $p=0.038$ ), rural residence ( $p=0.028$ ), unable to read and write ( $p=0.095$ ), history of eye trauma ( $p=0.001$ ), history of eye surgery ( $p=0.001$ ), presence of previous ocular infections ( $p=0.001$ ), and presence of eye allergy ( $p=0.001$ ) showed statistically significant association with external ocular infections. After adjustment for potential confounders, age group  $\geq 50$  years, history of eye trauma, history of eye surgery, presence of previous ocular infections, and presence of eye allergy were significantly associated with external ocular infections.

Patients with age group  $\geq 50$  (AOR: 3.56, CI: 1.11-11.63;  $P=0.04$ ) years had 3.56 times higher odds of the outcome compared with age group  $< 18$  years. Patients with eye allergy were 7.01 times more likely to have the outcome compared with those without eye allergy (AOR- 7.01, CI: 3.60-13.67;  $P<0.001$ ). Similarly, patients with eye trauma had 7.52 times higher odds of the outcome compared with those without eye trauma (AOR-7.52, CI: 4.07-13.92;  $P<0.001$ ). History of eye surgery was also significantly associated with the outcome. Patients who had undergone eye surgery were approximately four times more likely to have the outcome compared with those who had no history of eye surgery (AOR = 3.98, CI: 2.07-7.66,  $p<0.001$ ). Likewise, patients with a history of previous ocular infection had 3.76 times higher odds of developing the outcome than those without a history of ocular infection (AOR = 3.76, CI: 2.10-6.74,  $p<0.001$ ) (Table 11).

Table 11: Bivariate and multivariate analysis of factors associated with external ocular infections at Bahir Dar governmental hospitals, Northwest Ethiopia, 2026

| Variables                   | Categories               | External ocular infections |     | Odds ratio      |          |                  |          |
|-----------------------------|--------------------------|----------------------------|-----|-----------------|----------|------------------|----------|
|                             |                          | Yes                        | No  | COR (95%CI)     | P-value  | AOR (95%CI)      | P-value  |
| Sex                         | Male                     | 118                        | 88  | 0.93(0.62-1.40) | 0.73     | -                | -        |
|                             | Female                   | 101                        | 70  |                 | 1        |                  |          |
| Residence                   | Urban                    | 61                         | 61  |                 | 1        |                  |          |
|                             | Rural                    | 158                        | 97  | 1.63(1.05-2.52) | 0.03*    | 0.73(0.38-1.41)  | 0.35     |
| Age group                   | < 18 years               | 11                         | 15  |                 | 1        |                  |          |
|                             | 18-49 years              | 84                         | 73  | 1.57(0.68-3.63) | 0.29     | -                | -        |
|                             | ≥ 50 years               | 124                        | 70  | 2.43(1.05-5.55) | 0.04*    | 3.56(1.11-11.63) | 0.04**   |
| Educational level           | Unable to read and write | 131                        | 75  | 2.06(0.88-4.84) | 0.1*     | 1.33(0.43-4.16)  | 0.62     |
|                             | Read and write           | 42                         | 32  | 1.55(0.62-3.91) | 0.35     | -                | -        |
|                             | Elementary school        | 27                         | 26  | 1.23(0.47-3.23) | 0.68     | -                | -        |
|                             | High school              | 8                          | 12  | 0.79(0.24-2.62) | 0.70     | -                | -        |
|                             | College and above        | 11                         | 13  |                 | 1        |                  |          |
| Occupation                  | Civil servant            | 12                         | 8   |                 | 1        |                  |          |
|                             | Farmer                   | 105                        | 72  | 0.97(0.38-2.50) | 0.95     | -                | -        |
|                             | Housewife                | 36                         | 27  | 0.89(0.32-2.48) | 0.82     | -                | -        |
|                             | Merchant                 | 29                         | 22  | 0.88(0.31-2.52) | 0.81     | -                | -        |
|                             | Daily laborer            | 18                         | 11  | 1.09(0.34-3.52) | 0.88     | -                | -        |
|                             | Others                   | 19                         | 18  | 0.70(0.23-2.12) | 0.53     | -                | -        |
| History of eye trauma       | Yes                      | 96                         | 20  | 5.34(3.14-9.24) | < 0.001* | 7.52(4.07-13.92) | <0.001** |
|                             | No                       | 123                        | 138 |                 | 1        |                  |          |
| History of eye surgery      | Yes                      | 66                         | 19  | 3.12(1.80-5.52) | < 0.001* | 3.98(2.07-7.66)  | <0.001** |
|                             | No                       | 153                        | 139 |                 | 1        |                  |          |
| Presence of chronic disease | Yes                      | 45                         | 32  | 1.02(0.61-1.69) | 0.94     | -                | -        |
|                             | No                       | 174                        | 126 |                 | 1        |                  |          |
| Contact lens use            | Yes                      | 12                         | 10  | 0.68(0.36-2.04) | 0.73     | -                | -        |
|                             | No                       | 207                        | 148 |                 | 1        |                  |          |
| Previous ocular infections  | Yes                      | 86                         | 26  | 3.28(2.00-5.41) | < 0.001* | 3.76(2.10-6.74)  | <0.001** |
|                             | No                       | 133                        | 132 |                 | 1        |                  |          |
| Eye allergy                 | Yes                      | 80                         | 17  | 4.78(2.70-8.47) | <0.001*  | 7.01(3.60-13.67) | <0.001** |
|                             | No                       | 139                        | 141 |                 | 1        |                  |          |
| Face washing frequency      | Less frequent            | 55                         | 39  | 0.89(0.51-1.55) | 0.67     | -                | -        |
|                             | Frequent                 | 94                         | 75  | 0.79(0.49-1.28) | 0.33     | -                | -        |
|                             | More frequent            | 70                         | 44  |                 | 1        |                  |          |

|                 |     |     |     |                 |      |   |   |
|-----------------|-----|-----|-----|-----------------|------|---|---|
| Hospitalization | Yes | 31  | 21  | 1.08(0.59-1.95) | 0.81 | - | - |
|                 | No  | 188 | 137 |                 | 1    |   |   |
| Self-medication | Yes | 69  | 48  | 1.05(0.68-1.64) | 0.82 | - | - |
|                 | No  | 150 | 110 |                 | 1    |   |   |

Key: \* Variables with P-value of  $\leq 0.25$  were eligible for multivariable logistic regression; \*\* statistically significant at P-value of  $< 0.05$ ; AOR: Adjusted odd ratio; COR: Crude odds ratio, 1: Reference group, CI: Confidence interval

## 6. DISCUSSION

Bacterial external ocular infections remain as a significant public health concern due to their potential cause of severe visual impairment and permanent blindness if diagnosed or treated improperly (17). The current study was designed to assess the overall proportion of bacterial isolates, their antimicrobial susceptibility patterns, ESBL production, and factors associated with external ocular infections among patients attending the ophthalmology clinics at governmental hospital in Bahir Dar, Ethiopia.

The overall proportion of culture confirmed bacterial isolates among patients with external ocular infections in this study was 219/377 (58.1%, 95% CI: 53%-63%). This finding shows that bacterial pathogens remain as an important cause of external ocular infections in the study area and highlights the burden of bacterial ocular disease among patients attending hospitals in Bahir Dar. This finding is comparable with different studies done in Ethiopia like, Bahir Dar (57.8%), Gondar (58.3%, 62.4%), Borumeda (59.4%), Debre Markos (62.8%), and Jijiga (62.2%) (12, 20, 25, 26, 36, 37). The observed similarity can be explained by the fact that these studies included patients with similar clinical conditions, conducted in comparable hospital-based settings, and conducted in similar culture based microbiological diagnostic methods.

However, the proportion in the current study was lower than that reported from Tigray (66.7%) (36) and study from Ghana (95.1%) (49). This may be due to the inclusion of both external and intra-ocular or periocular conditions, while the present study focused specifically on external ocular infections. On the other hand, the proportion was higher than reports from Hawassa (50.5%), Jimma (46.1%), Italy (21%), Japan (32.2%) and India (34.5%) (19, 28, 50-52). This variation can be explained by the difference in access to health care, socioeconomic conditions, hygiene practices, and geographical locations. The results indicated that bacterial external ocular infections are still highly prevalent in Ethiopia and other developing countries. This highlights the need to improve routine bacteriological diagnosis, and evidence-based treatment practices.

In the current study, Gram-positive cocci were the most common culture-confirmed isolates recovered from external ocular infections with a proportion of 64.4%. This finding indicates that Gram-positive cocci remain the predominant etiologic agents of external ocular infections in the study area. This predominance may be explained by the fact that Gram-positive cocci

(staphylococci) are common commensals of the skin, eyelid margins and conjunctival sac, and can readily gain access to ocular tissues through direct hand-to-eye contact, ocular trauma, surgical procedures, or disruption of normal ocular defense mechanisms.

This finding is in line with studies conducted in Bahir Dar (66.3%) (25), Shashemene (68.2%) (41), and Hawassa (61.5%) (53). However, it is lower than findings reported from previous studies in Ethiopia; Gondar (88%) (37), Dilla (70.6%) (54), Debre Markos (78.5%) (12), Jijiga (87.7%) (36), Borumeda (93.7%) (20), and studies conducted abroad; Nigeria (86.5%) (55), and Italy (73.5%, 81.1%) (19, 31). But, the proportion observed in the current study is higher than Jimma (52%) (56). The observed differences among the studies may be due to variation in geographic location, sample size differences, and diagnostic scope and case mix.

The proportion of culture-confirmed Gram-negative bacteria in the current study was 35.6%, which is in line with previous reports from Ethiopia (33.3%, 29.8%, 29.4%, 31.8%) (25, 41, 54, 57). The observed similarity may be attributed to comparable study setting, patient population characteristics, and bacteriological diagnostic methods. However, the proportion was higher than studies conducted in Gondar, Borumeda, Debre Markos, and Jimma, which reported a respective prevalence of 12%, 6.3%, 21.5%, and 11.5% (12, 20, 37, 56). These variations may be due to poor personal hygiene practices and sanitation which may increase the likelihood of fecal contamination of the ocular surface and autoinoculation of enteric Gram-negative bacteria.

In the current study, Gram-positive bacterial isolates showed a high level of antimicrobial resistance to penicillin (99.3%), tetracycline (66%), and erythromycin (63.8%), while showing relatively high susceptibility to clindamycin (91.5%), gentamicin (87.2%), and chloramphenicol (80.9%). These findings suggest that commonly prescribed and readily available antibiotics are becoming increasingly ineffective against the ocular Gram-positive pathogens, whereas clindamycin and gentamicin are remaining valuable treatment options.

However, some important ocular pathogens, including *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Neisseria gonorrhoeae*, were not isolated in the present study. The absence of these organisms may partly explain the differences in the observed bacterial spectrum, particularly the predominance of Gram-positive isolates (64.4%) over Gram-negative isolates (35.6%). Their non-isolation may also be related to specific growth requirements and the culture conditions used in this study.

The high penicillin resistance observed in this study is consistent with a study from East China (23) where 95.4% of *S. aureus* and *CoNS* isolates were resistant to penicillin, and also consistent with a study from Arba Minch, Ethiopia (100%)(17) where all the isolates were resistance to penicillin. Wide spread resistance to penicillin has also been reported among ocular isolates in Bahir Dar(96%)(25), Gondar(96.1%)(37), Jijiga (97.9%)(3), and other Ethiopian studies (4, 20). The consistently high level of penicillin resistance may be attributed to the widespread of  $\beta$ -lactamase enzyme production, prolonged empiric use of penicillin, easy availability of antibiotics without prescription, and inappropriate antibiotics use.

In the current study, Gram-positive isolates showed high resistance to tetracycline (66%). The resistance to tetracycline was comparable with reports from Sudan (62.7%)(34), This may be explained by similarities in antibiotic accessibility, and prescribing culture between settings. Tetracycline is an inexpensive, widely available antibiotic that is commonly accessed without prescription in resource-limited settings. But, lower than findings from ALERT hospital(79.2%)(57), Arba Minch (85.5%)(17), and Shashemene (79.3%)(4). This may be related to increased previous antibiotic exposure in those settings where selection of more resistant strains occurs.

However, the finding was higher than reports from Gondar (29.4%) (37), Jijiga (29.4%)(3), and Italy (43.6%)(58). The lower resistance in Italy is probably because of more strict policies for antibiotic dispensing and more well-established antimicrobial stewardship programs in high income settings that restrict unregulated use of tetracycline. The high resistance rates observed in Gram-positive isolates have important clinical and public health implications. Because these organisms are among the most common cause of external ocular infections, the reduced susceptibility to penicillin, and tetracycline limits its usefulness in empiric therapy. Therefore, treatment decisions should preferably be guided by antimicrobial susceptibility testing.

On the other hand, the current study showed high susceptibility to clindamycin, gentamicin, and chloramphenicol, which is in agreement with reports from Arba Minch (17), Bahir Dar (25), and Gondar (37), where these drugs were effective against most of the ocular pathogens. Methicillin-resistant *S. aureus* (MRSA) was detected from 33.3% of *S. aureus* isolates. The prevalence of MRSA (33.3%) suggests the burden of antimicrobial resistance among the external ocular

pathogens in this study area. The emergency of MRSA is of clinical importance because it frequently associated with multiple classes of antibiotics.

In the present study, the prevalence of MRSA was higher than reports from Ethiopian studies; Bahir Dar, Gondar, Debre Markos, Borumeda with respective prevalence of 16.9%, 24%, 20%, 4.8% (12, 20, 25, 37), and abroad, India 8.8%(59), and Japan 9.7% (28). These variations may be due to differences in antibiotic use, health care settings and infection prevention measures. The high prevalence of MRSA in the present study emphasizes the growing problem of antimicrobial resistance in the ocular bacterial pathogens. However, the prevalence was lower than finding reported from Arba Minch, 61.5% (17). The lower prevalence than Arba Minch may reflect differences in antimicrobial stewardship practices.

In the current study, Gram-negative Enterobacteriaceae bacterial isolates showed high susceptibility to amikacin (97.1%), meropenem (92.6%), gentamicin (79.4%), and ciprofloxacin (77.9%). These results indicate that aminoglycosides, fluoroquinolones, and carbapenems class of antibiotics are remaining effective therapeutic options against Gram-negative ocular pathogens in the study area. In contrast, high resistance was observed against ampicillin (94.1%) and amoxicillin-clavulanic acid (88.2%). These results indicate commonly used  $\beta$ -lactam antibiotics have become increasingly ineffective due to the widespread use of those antibiotics in the community and spread of resistant strains.

The susceptibility of Gram-negative Enterobacteriaceae to meropenem and amikacin was higher than susceptibility reported from Arba Minch with a respective prevalence of 59.2 % and 69.4% (17). The lower resistance might be due to the limited clinical use of these antibiotics in the study area, and restricted access of last-resort agents results in less selection pressure for resistance. On the other hand, ampicillin resistance (94.1%) is comparable to studies at Bahir Dar (87.5%) (25), and Arba Minch (100%) (17), indicating the common problem of ampicillin resistance among Gram-negative ocular isolates in different parts of Ethiopia. Clinically, the results suggest that ampicillin and amoxicillin-clavulanic acid should be avoided as empirical therapy, whereas meropenem and amikacin should be preserved and used with strict antimicrobial stewardship.

The overall proportion of MDR bacterial isolates were 63.5%. This finding shows the increasing challenge of antimicrobial resistance. The high burden of MDR isolates indicates limited option for treatment and increased risk of therapeutic failure among patients with external ocular

infections. The MDR proportion observed in this study is comparable with studies conducted from Debre Markos (59.2%)(12) and Gondar (66.4%)(26). However, it is higher than findings reported from Bahir Dar (45.2%) (25) and Tigray (53.9%). In contrast, the current MDR rate is lower than studies reported from Arba Minch (71.3%) (17), Hawassa (70%), Gondar (87%) (37), and Jijiga (87.7%) (3). The observed MDR pattern of culture-confirmed bacteria to different antibiotics could be linked to prescription of broad-spectrum antibiotics, lack of regular screening of antimicrobial resistance patterns before prescription, self-medication practice, and misuse of antibiotics(12).

In the current study, 25.5% of Gram-negative isolates identified as extended-spectrum  $\beta$ -lactamase producer. These findings indicate that Gram-negative pathogens causing external ocular infections have acquired the ability to hydrolyze extended-spectrum cephalosporins, thereby limiting the effectiveness of commonly prescribed  $\beta$ -lactam antibiotics. The proportion of ESBL producing isolates in this study is comparable with study reported in Arba Minch (17), where 22.6% of Gram-negative isolates were identified as ESBL producers. The close agreement, observed in the same national and clinical context, supports the consistency of ESBL circulation among Gram-negative ocular pathogens in different regions of Ethiopia. In contrast, the present finding is slightly higher than the 17% ESBL prevalence reported from India(15). This may be due to sample size and time period difference.

The detection of ESBL-producing bacteria among ocular isolates has important clinical implications. ESBL producers are commonly resistant to penicillin and cephalosporins, which severely narrows therapeutic options and increases the likelihood of treatment failure when empirical therapy is used. The presence of ESBL-producing *E. coli* and *K. pneumoniae* in external ocular infections suggests the need for routine culture and antimicrobial susceptibility testing before initiating treatment, particularly in recurrent or severe external ocular infections.

In the current study, age group  $\geq 50$  showed association with the external ocular infections. This could be explained by age-related changes in ocular defense mechanisms, as well as decreased tear production. In the present study, ocular trauma was significantly associated with external ocular infections. This finding is in agreement with studies done in Bahir Dar, Shashemene, Arba Minch, Tigray (4, 16, 17, 25), and study conducted in Mexico city (60) which reported this variable as a significant predictor of external ocular infections. The results may be explained by

ocular trauma disrupts the integrity of the corneal and conjunctival epithelium, which are the eye's principal barrier for microbial invasion. These injuries lead to the entry of foreign material and pathogenic microorganisms directly into ocular tissues.

Eye allergy was also a significant predictor of bacterial external ocular infections in this study. This finding was consistent with studies conducted in Bahir Dar and Gondar (25, 40). Chronic inflammation and irritation with frequent rubbing of the eyes may predispose to infection, as the conjunctival epithelium can be damaged and bacterial entry facilitated. Allergic conditions may change the function of the tear film and local immune responses providing a good environment for bacterial growth.

This study found that bacterial external ocular infections are very common in Bahir Dar (58.1%) with Gram-positive cocci as the major pathogens. High burden of antimicrobial resistance was evident including MDR (63.5%), MRSA (33.3%) and ESBL-producing Gram-negative bacteria (25.5%) which limited effective empirical treatment options. Ocular trauma and eye allergy were found to be significant risk factors. In conclusion, the data emphasize the importance of routine culture and susceptibility testing, rational antibiotic use and improved antimicrobial stewardship.

## **7. LIMITATION OF THE STUDY**

This study has some limitations. Since participants were selected using a convenience sampling technique, the findings may not be representative of the wider community. Furthermore, this study did not use chocolate agar, which are required for the optimal recovery of fastidious organisms such as, *Haemophilus influenzae*, and *Neisseria gonorrhoeae*. As a result, these organisms - despite being recognized causes of external ocular infections in other studies - may not have been detected even if present, which could have underestimated the true bacterial etiological spectrum in this study.

## 8. CONCLUSION

The prevalence of culture-confirmed external ocular infections was high in the present study. CoNS and *S. aureus* were the predominated isolates among Gram-positive, and *E. coli* and *K. pneumoniae* were the predominated among the Gram-negative isolates. The study found a high burden of antimicrobial resistance both in Gram-positive and Gram-negative, including MRS, and ESBL producing Enterobacteriaceae. High resistance was observed against routinely used antibiotics such as penicillin, ampicillin, amoxicillin-clavulanic acid, tetracycline and erythromycin. Clindamycin, gentamicin, chloramphenicol, ciprofloxacin, amikacin and meropenem were found to be highly effective against most isolate.

The detection of ESBL-producing organisms shows the emergence of resistant Gram-negative pathogens that may compromise the effectiveness of commonly used  $\beta$ -lactam antibiotics. Overall, these results indicate the growing challenge of antimicrobial resistance in external ocular infections and highlight the need for routine bacteriological diagnosis, antimicrobial susceptibility testing, ESBL screening, and continuous antimicrobial resistance surveillance to guide evidence-based treatment and improve patient outcomes.

## 9. RECOMMENDATIONS

- Rational use of antibiotics and implementation of antimicrobial stewardship program should be strengthened
- Clindamycin, gentamicin, and chloramphenicol (for Gram-positive isolates) and amikacin, meropenem, and ciprofloxacin (for Gram-negative isolates) showed high in vitro effectiveness in this study and should be retained as preferred empiric options
- Regular monitoring of antimicrobial resistance trends
- Broaden the etiological scope to include non-bacterial pathogens
- Further studies involving molecular characterization of resistant isolates are recommended to better understand resistance mechanisms and guide future treatment strategies

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## **ANNEXES**

### **Annex I: Information sheet** (English version)

Information sheet for the participants of the study entitled Bacterial isolates recovered from clinically suspected external ocular infections: Antimicrobial susceptibility profile and extended-spectrum  $\beta$ -lactamase production in Bahir dar, Northwest Ethiopia.

**Name of the Organization:** Bahir Dar University, College of Medicine and Health Sciences, Department of Medical Laboratory Sciences.

**Name of Investigator:** Abebe Aschale (BSc, MSc candidate)

### **Introduction**

You are invited to participate in a study to be conducted by an MSc student at Bahir Dar University, College of Medicine and Health Sciences, Department of Medical Laboratory Science. The aim of the study is to determine the spectrum of bacterial isolates, their drug susceptibility patterns, and the detection of ESBL from patients suspected of ocular infections at Felege Hiwot, Tibebe Gion, and Addisalem Hospitals. It is important to know the distribution of the bacteria to control the disease. Please read the following statements and ask any unclear questions before you agree to participate.

Participation in this study is entirely voluntary. If you do not wish to participate, or if you initially agree but later choose to withdraw at any time, there will be no consequences. You will continue to receive all standard eye care services provided by the hospital without any restriction or interruption. If you decide to take part in the study, you will be asked to sign the consent form. You may also request and keep a copy of this information sheet for your records.

### **Study procedure**

Patients presenting with signs and symptoms of ocular infection at selected health facilities in Bahir Dar will be informed about the study. Those who agree to participate will undergo a brief screening interview conducted by a health professional to assess eligibility based on predefined inclusion and exclusion criteria. A trained data collector will administer a structured questionnaire to obtain information on socio-demographic characteristics. An ophthalmologist or optometric nurse will collect an eye discharge sample from the lower eyelid or affected area

using a moistened sterile swab. Proper aseptic procedures will be followed during specimen collection to ensure sample quality and patient safety.

### **Risks**

In this study, the participants will not be caused more discomfort than the routine examination you required during the hospital visit. But discomfort may be faced following sample withdrawal, which will disappear within a short duration. The whole procedure will be carried out by health professionals through standard clinical practice, so participating in this study has no major risks.

### **Benefits**

The laboratory analysis of your eye discharge may help identify the specific bacteria causing your ocular infection. These results may assist your healthcare provider in selecting the most effective treatment.

### **Confidentiality**

All information about the participant will be kept confidential. Logbooks used in the laboratory will have no names but codes. The information sheet that links the coded number to the participant name will be locked inside a box, and it will not be revealed to anyone except your principal investigator.

### **Right to refuse**

You have the full right to withdraw from participating in this study at any time before and after the consent without explaining the reason.

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

- ✓ Please direct any questions/problems you may encounter during this study to Abebe Aschale.
- ✓ Mobile: +251962361025; Email: abebeaschale86@gmail.com
- ✓ For additional information, please contact Bahir Dar University, Medical Faculty Institutional Review Board (IRB) office.

### **Agree to participate?**

☐ Yes ☐ No



**አደጋዎች**

በዚህ ጥናት ውስጥ ተሳታፊዎቹ በሆኑ ፒሳል ጉብኝት ወቅት ከሚፈጸሙት ምርመራዊ በለጠሞች አይሰማቸውም ገርግንና መፍመወጣትን ተከትሎ ምንም ጥያቄም አልነበረም፡፡ ይህም ለጤናማ ጊዜ ውስጥ ይጠፋል፡፡

አጠቃላይ ሂደቱ በጤናማ ዎች በሚከተሉት ካዊልምድባላቸው ይሄደዋል፡፡

በዚህ በዚህ ጥናት ውስጥ መሳተፍ ምንም ስልታዊ አደጋ የለውም፡፡

**ጥቅሞች**

የአይን ምፈሳሽ የላብራቶሪ ታኔ ለዓይን ኢንፌክሽን መንስኤ ሆኑትን ልዩ ባክቴሪያዎች ለመለየት ይረዳል፡፡

እነዚህ ወጣቶች ጤናማ እንደሆኑ በቅርቡ በጥንቃቄ ሆኖ ወንህክ ምናልባትም ዲሞክራሲ ዲሞክራሲያዊነትን ያሳያሉ፡፡

**ሚስጥራዊነት**

በላብራቶሪ ውህሎች ምርመራ ላይ ሚስጥራዊነት ይጠበቃል፡፡ በላብራቶሪ ውስጥ ጥቅም ላይ የሚውሉ ለመገንባት ጥናቶች ምንም ስራም ወምትም፡፡

ኮድ በስተቀር፡፡

ኮድ የተደረገ በትንቁ ጥርክተላት ስምምነት ላይ ገናኘው ሚስጥራዊነትን በላብራቶሪ ውስጥ ጥቅም ላይ የሚውሉ ለመገንባት ጥናቶች ምንም ስራም ወምትም፡፡

**እምቢድ ማለት መብት**

ምክንያቱን ሳይገልጹ ማንኛውም ለሚከተሉት ምክንያቶች ሳይሆን በላብራቶሪ ውስጥ ጥቅም ላይ የሚውሉ ለመገንባት ጥናቶች ምንም ስራም ወምትም፡፡

**ጥያቄ ካለኝ ወይም ግርቢያ ጋር መገናኘት ማድረግ ይቻላል?**

ይህንን ጥናት በተመለከተ ወይም በዚህ ጥናት ጋር በተዛመደ መልኩ ለሚገኝ ጥናታዊ ጥናት አደጋዎች ወይም ጥያቄ ካለዎት

በሚመለከተው ድረ-ገጽ ይጠቀሙ፡ አበበ አስቻለ

የህክምና ላብራቶሪ ሳይንስ ት/ክፍል፡ ባህር ዳርዩ ኒሽር ሲቲ

ጥባይል፡ +251962361025

ኢሜል፡ abebeaschale86@gmail.com

## ለመሳተፍ ይስማማሉ?

☐ እስማማለሁ ☐ አልስማማም

### Annex II: Informed consent (English version)

I have been informed about the study, which is entitled Bacterial isolates recovered from clinically suspected external ocular infections: Antimicrobial susceptibility profile and extended-spectrum  $\beta$ -lactamase production in Bahir dar, Northwest Ethiopia. The objective and the application of the study will be explained to me. I am also informed that all information about the participant will be kept confidential and they have the right to withdraw from the study at any time. I also had the right to ask a question and make a suggestion about the project. I will also be told that the result of the laboratory test will be reported to me if I need it. It is therefore that I voluntarily agreed to the informed consent with full understanding of the research. I voluntarily answered the prepared question and gave my sample for the aforementioned study. I, the undersigned, confirm that, as I give consent to participate in the study, it is with a clear understanding of the objectives and the study conditions.

Participant code: \_\_\_\_\_ Signature: \_\_\_\_\_ Date: \_\_\_\_\_

### በመረጃዎ ተደገፈህም

በባሕር ዳር፣ ሰሜን ምዕራብ ኢትዮጵያ ወስጥ ፀረ-ተህዋሲያን ሱስ ሴፕታልቲክ ፕሮፋይል እና የተራዘሙ ፔክቲረም  $\beta$ -Lactamase

የባክቴሪያ መጠለያ ዓይነትን ኢንፌክሽኖች የማግኘት መብት ስላለዎት ትተን ግሮኛል። የጥናቱ አላማ እና አተገባበር ይብራራል። እንዲሁም ለተሳታፊው መረጃ ሁሉንም ጥርጣሬዎች እና ማንኛውም ሊከሰቱ የሚችሉ ጥያቄዎች መጠየቅ መብት እንደሚጠበቅብዎትን ግሮኛል። ስለፕሮጀክቱ ጥያቄ እና አስተያየትዎ መጠየቅ ቅመብትን በረኝ።

የላብራቶሪ ምርመራ ውጤት ከፈለግኝ እንደሚገኝ ግሮኛል። ስለዚህ እኔ በፈቃደኝነት በመረጃዎ ተመሳሳይ ተመሳሳይ ስምምነትን ከምርመራ ማሳገጥ ቢጋርተስ ማቆም ለሁሉም በፈቃደኝነት የተዘጋጀውን ጥያቄ መላክ ስኬት ከላይለተ ጠቀሰው ማንኛውም ጥያቄዎችን ሰጠሁ። በጥናቱ ላይ መሳተፍ ፍቃድ ሰጥቼ ስር የተፈረመኩት እኔ መሆኔን አረጋግጣለሁ። ዓላማዎችን እና የጥናት ሁኔታዎችን በግልፅ በመረዳት ወ።

የተሳታፊው ፊርማ: \_\_\_\_\_ ቀን: \_\_\_\_\_

Assent\_\_\_\_\_ parent, after being fully informed about the purpose of the research titled: Antimicrobial Susceptibility Profile and Extended-Spectrum  $\beta$ -Lactamase Production of Bacterial Isolates from Ocular Infections in Bahir Dar, Northwest Ethiopia. I, the undersigned, have been told about this research. My child has 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's results, as they are coded with numbers rather than written names. I understand that there may be no benefit to me personally apart from the 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 participate in this research study.

የቤተሰብ፣ ቃድ (በአማርኛ ትርጉም)

የልጅአስታምዝሆንየዚህንጥናትአላማበወልተረድቻለሁ፡፡ የጥናቱርዕስበባህርዳርሆስፒታልየአይንታካማዎችመከልየአይንኢንፋክሽንተህዋስያንየማጭመጣዎንየተህዋስያንአይነትንእናየተህዋስያኑንመድሃኒትየመቋቋምየለወንስርትጭነትአይንህመማንላይምያህልእንደሆነለማወቅበጥናቱልጅእንዲሳተፍምርጫየእኔመሆኑንነግረወኛል፡፡ ናመኖሲወሰድከትንሸየህመማሽጭወጪዎንምአይነትጉዳትልጀላይእንደሌለዉተነግረወኛል፡፡ በጥናቱወቅትምልጀመረጀዎችበሚከፈሉበላማዘበሌላሰወዘንድእንደማይታወቅተረድቻለሁ፡፡ በወጠቱከማኘወያህከምናአገልግልትበቀርሌላለልጀበግልየማገኘዉቅምእንደሌለተረድቻለሁ፡፡ ጥያቄእንደጠይቅዕድልተሰጥቶኝለጥያቄዎቼምበቂምላሸአግኝቻለሁ፡፡ የልጄበጥናቱመሳተፌበእኔፌላጎትብቻእንደሆነእናበጥናቱምበመሳተፌምንምአይነትተፅዕኖበልጀላይእንደማይከሰትልተረድቻለሁ፡፡ በከዚህባሻገርየልጄበጥናቱወስጥለመሳተትየእኔየወላጁአሳዳጊፈቃድእንደማይከፈልግተረድቻለሁ፡፡ በጥናቱእንደሚከተሉትታችበፊርማዬአረጋግጧለሁ፡፡

የተሳታፊዎች: \_\_\_\_\_ ፊርማ: \_\_\_\_\_ ቀን: \_\_\_\_\_

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Questionnaire for the study on ‘Bacterial isolates recovered from clinically suspected external ocular infections: Antimicrobial susceptibility profile and extended-spectrum  $\beta$ -lactamase production in Bahir dar, Northwest Ethiopia.’

### **I. Socio-demographic factors**

|    |                                          |                                                                                                                          |  |
|----|------------------------------------------|--------------------------------------------------------------------------------------------------------------------------|--|
|    | <b>Part I: Socio-demographic factors</b> |                                                                                                                          |  |
|    | Hospital Card No.                        |                                                                                                                          |  |
|    | Telephone                                |                                                                                                                          |  |
|    | Date of interview                        |                                                                                                                          |  |
| 1  | sex                                      | 1. Male<br>2. Female                                                                                                     |  |
| 2  | Age                                      |                                                                                                                          |  |
| 3  | What is your place of residence?         | 1. Urban<br>2. Rural                                                                                                     |  |
| 4  | What is your level of education?         | 1. Unable to read and write<br>2. Able to read and write<br>3. Elementary school<br>4. Highschool<br>5. College or above |  |
| 5  | What is your occupation?                 | 1. Housewife<br>2. Farmer<br>3. Civil servant<br>4. Maechent<br>5. Daily labourer<br>6. Other                            |  |
| 6  | What is your marital status?             | 1. Never married<br>2. Married<br>3. Divorced<br>4. widow/widower                                                        |  |
|    | <b>Part II: Clinical data</b>            |                                                                                                                          |  |
| 7. | Type of ocular infection                 | 1. Conjunctivitis                                                                                                        |  |

|     |                                                                      |                                                                                   |  |
|-----|----------------------------------------------------------------------|-----------------------------------------------------------------------------------|--|
|     |                                                                      | 2. Blepharitis<br>3. Dacryocystitis<br>4. keratitis<br>5. Blepharo-conjunctivitis |  |
| 8.  | Did you have eye trauma before?                                      | 1. Yes<br>2. No                                                                   |  |
| 9.  | Did you have eye surgery before?                                     | 1. Yes<br>2. No                                                                   |  |
| 10. | Which systemic diseases do you have?                                 | 1. Diabetes<br>2. Blood pressure<br>3. Other.....                                 |  |
| 11. | Do you use medical contact lenses?                                   | 1. Yes<br>2. No                                                                   |  |
| 12. | How many times do you wash your face per day?                        | 1. Less frequently<br>2. Frequently<br>3. More frequently                         |  |
| 13. | Have you been hospitalized for the last one year?                    | 1. Yes<br>2. No                                                                   |  |
| 14. | Did you have eye allergies before?                                   | 1. Yes<br>2. No                                                                   |  |
| 15. | Did you have a previous history of self-medication with antibiotics? | 1. Yes<br>2. No                                                                   |  |
| 16. | Did you have previous ocular disease?                                | 1. Yes<br>2. No                                                                   |  |

Filled by:

Date: \_\_\_\_/\_\_\_\_/\_\_\_\_

Checked by: Date: \_\_\_\_/\_\_\_\_/\_\_\_\_

Interviewer's Name: \_\_\_\_\_

Interviewer's Signature: \_\_\_\_\_

**Questionnaire (Amharic version)**

|    |                                        |                                                                                                               |  |
|----|----------------------------------------|---------------------------------------------------------------------------------------------------------------|--|
|    | <b>ክፍል አንድ፡ - ሶሺዮ- ስነ - ሕዝብ ምክንያቶች</b> |                                                                                                               |  |
|    | የ ሆስፒታል ካርድ ቁጥር                        |                                                                                                               |  |
|    | ስልክ ቁጥር                                |                                                                                                               |  |
|    | ቃለ መጠየቅ ቁጥር ተደርጎ በትቀን                  |                                                                                                               |  |
| 1. | ጾታ                                     | 1. ወንድ<br>2. ሴት                                                                                               |  |
| 2. | እድሜ                                    |                                                                                                               |  |
| 3. | አድራሻ                                   | 1. ከተማ<br>2. ገጠር                                                                                              |  |
| 4. | የ ትምህርት ደረጃ ምስክርነት ወ?                  | 1. ማንበብ ምሆኑን መግ ፍለል ችልዎ<br>2. ማንበብና መጻፍ መቻል<br>3. አንደኛ ደረጃ ምጽናት<br>4. ሁለተኛ ደረጃ ምጽናት<br>5. ኮሌጅ/ዩኒቨርሲቲ ደረጃ ምጽናት |  |
| 5. | ስራዎ ምን ድንገት ወ?                         | 1. የቤት እመቤት<br>2. ገበሬ<br>3. የመንግስት ስራተኛ<br>4. ነጋዴ<br>5. የቀንስ ስራተኛ<br>6. ሌላ .....                              |  |
| 6. | የጋብቻ ሁኔታዎስ?                            | 1. ያላገባ/ች                                                                                                     |  |

|     |                                    |                                                                                 |  |
|-----|------------------------------------|---------------------------------------------------------------------------------|--|
|     |                                    | 2.ያገባች<br>3.የፈታች<br>4. የሞተበት/ባት                                                 |  |
|     | ክፍልሁለት፡ -ከሊኒ ካዊሚኛ                  |                                                                                 |  |
| 7.  | የአይንኢንፌክሽን አይነት                    | 1. ኮንጃክቲቫይትስ<br>2. ብሌፋራይትስ<br>3. ዳክሪዮሲስታይትስ<br>4. ኬራታይትስ<br>5. የአይንክዳንኮንጃክቲቫይትስ |  |
| 8.  | ከዚህበፊት የዓይን ጉዳት አጋጥሞትነበር?          | 1. አዎ<br>2. የለም                                                                 |  |
| 9.  | የአይን ቀድሞኑ አድርገው ወያወቃሉ?             | 1. አዎ<br>2. የለም                                                                 |  |
| 10. | የትኛወባሽታብ ወስጥዎአለብዎ                  | 1. ስኳር<br>2. የደምግፊት<br>3.ሌላ .....                                               |  |
| 11. | የህክምና ለአይታሚ ዳመኑ ፅርይጠቀማሉ?           | 1. አዎ<br>2. የለም                                                                 |  |
| 12. | በቀንስንት ጊዜ ይታጠባሉ?                   | 1. አልፎአልፎ<br>2. በተደጋጋሚ<br>3. በጣሞብተደጋጋሚ                                          |  |
| 13. | ባለፈው አንድ አመት ውስጥ ሆስፒታል ትኝተው ወያወቃሉ? | 1. አዎ<br>2. የለም                                                                 |  |
| 14. | ከአሁን በፊት አላርጅክነበረብዎ?               | 1. አዎ<br>2. የለም                                                                 |  |

|     |                                            |                  |  |
|-----|--------------------------------------------|------------------|--|
| 15. | ከአሁን በፊት ያለ ህክምናዊ ዝግጅት ሂደት ዝቅተኛ ደረጃ ወደ ውጭ? | 1. አዎ<br>2. የሌለም |  |
| 16. | ካሁን በፊት የአይን በሽታ በረብሰዎ?                    | 1. አዎ<br>2. የሌለም |  |

የጠየቁበት ምዕራፍ: \_\_\_\_\_

የጠየቁበት ወጪ ምዕራፍ: \_\_\_\_\_

የረገገበት ቀን: \_\_\_\_/\_\_\_\_/\_\_\_\_

### Annex V: Dummy Table

Prevalence of bacterial pathogens across the different clinical features of ocular infections

| Bacterial isolate                                                                                                                                                                                     | Type of eye infection |                |                         |               |           | Total |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|----------------|-------------------------|---------------|-----------|-------|
|                                                                                                                                                                                                       | Blepharitis           | Conjunctivitis | Blepharo-conjunctivitis | Dacrocystitis | Keratitis |       |
| <i>CoNS</i><br><i>S. aureus</i><br><i>S. pneumoniae</i><br><i>S. pyogenes</i><br><i>Enterococci spp.</i><br><i>E. coli</i><br><i>K. pneumoniae</i><br><i>P. mirabilis</i><br><i>Enterobacter spp.</i> |                       |                |                         |               |           |       |

|              |  |  |
|--------------|--|--|
| <b>Total</b> |  |  |
|--------------|--|--|

Antimicrobial sensitivity pattern of Gram-positive bacteria isolated from ophthalmic patients

| Bacterial isolate       | Sensitivity | Antibiotics tested |   |     |     |    |     |    |     |    |  |
|-------------------------|-------------|--------------------|---|-----|-----|----|-----|----|-----|----|--|
|                         |             | P                  | E | COT | CEF | GE | CIP | CL | CHL | TE |  |
| <i>CoNS</i>             | S           |                    |   |     |     |    |     |    |     |    |  |
|                         | I           |                    |   |     |     |    |     |    |     |    |  |
|                         | R           |                    |   |     |     |    |     |    |     |    |  |
| <i>S. aureus</i>        | S           |                    |   |     |     |    |     |    |     |    |  |
|                         | I           |                    |   |     |     |    |     |    |     |    |  |
|                         | R           |                    |   |     |     |    |     |    |     |    |  |
| <i>S. pneumoniae</i>    | S           |                    |   |     |     |    |     |    |     |    |  |
|                         | I           |                    |   |     |     |    |     |    |     |    |  |
|                         | R           |                    |   |     |     |    |     |    |     |    |  |
| <i>S. pyogenes</i>      | S           |                    |   |     |     |    |     |    |     |    |  |
|                         | I           |                    |   |     |     |    |     |    |     |    |  |
|                         | R           |                    |   |     |     |    |     |    |     |    |  |
| <i>Enterococci spp.</i> | S           |                    |   |     |     |    |     |    |     |    |  |
|                         | I           |                    |   |     |     |    |     |    |     |    |  |
|                         | R           |                    |   |     |     |    |     |    |     |    |  |

P- Penicillin, E- Erythromycin, COT- Cotrimoxazole, CEF- Cefoxitin, TE- Tetracycline, GE- Gentamycin, CIP- Ciprofloxacin, CL- Clindamycin, CHL- Chloramphenicol

S- Susceptible

I- Intermediate

R- Resistant

Antimicrobial Sensitivity Pattern of Gram-Negative Bacteria Isolated from Ophthalmic Patients

| Bacterial isolate    | Sensitivity | Antibiotics tested |    |    |     |     |      |    |    |     |     |   |
|----------------------|-------------|--------------------|----|----|-----|-----|------|----|----|-----|-----|---|
|                      |             | AMI                | AM | GE | COT | CIP | CEFT | CE | ME | CHL | AMC | T |
| <i>E. coli</i>       | S<br>I<br>R |                    |    |    |     |     |      |    |    |     |     |   |
| <i>K. pneumoniae</i> | S<br>I<br>R |                    |    |    |     |     |      |    |    |     |     |   |
| <i>P. aeruginosa</i> | S<br>I<br>R |                    |    |    |     |     |      |    |    |     |     |   |
| <i>P. mirabilis</i>  | S<br>R<br>I |                    |    |    |     |     |      |    |    |     |     |   |

AMI- Amikacin, COT- Cotrimoxazole, GE- Gentamicin, CIP- Ciprofloxacin, CHL- Chloramphenicol, AM- Ampicillin, CE- Ceftriaxone, CEFT- Ceftazidime, ME- Meropenem, AMC- Amoxicillin-Clavulanate, T- Tetracycline

#### **ESBL Detection from Ocular Infection Isolates**

| Bacterial isolate    | Combination disk used | ESBL result |
|----------------------|-----------------------|-------------|
| <i>E. coli</i>       |                       |             |
| <i>K. pneumoniae</i> |                       |             |
| <i>P. mirabilis</i>  |                       |             |

#### **Annex VI: SOP for collection and processing of ocular specimens**

## **A. Specimen Collection for external eye**

- ✓ External eye specimen collection is performed to obtain material from superficial ocular structures for the diagnosis of bacterial infections.
- ✓ Common indications include suspected conjunctivitis, blepharitis, dacryocystitis, external ocular cellulitis, and keratitis.
- ✓ Acceptable external eye sampling sites include:
  - Eyelid
  - Lid margin
  - Conjunctiva
  - Lacrimal duct or sac
  - Canaliculus
  - Corneal surface (when appropriate)
- ✓ Specimens are collected using a sterile swab system such as an ESwab® or equivalent transport device.
- ✓ The swab may be lightly moistened with sterile physiological saline prior to sampling to enhance specimen recovery.
- ✓ The swab is gently rotated over the affected area, ensuring full contact with the sampling site while avoiding excessive pressure.
- ✓ After sampling, the swab is placed into the transport tube, the shaft is broken at the designated breakpoint, and the tube is securely sealed.
- ✓ Each specimen must be clearly labeled with patient identifiers, specimen source, laterality (left/right eye), and collection date and time.
- ✓ A separate sterile swab must be used for each anatomical site to prevent cross-contamination and ensure accurate diagnostic interpretation.
- ✓ Specimens should be transported promptly to the microbiology laboratory under conditions that preserve organism viability.
- ✓ Laboratory processing may include Gram staining, slide preparation, and culture plating for aerobic and anaerobic as clinically indicated.
- ✓ Standardized collection procedures help ensure specimen quality and support accurate microbiological diagnosis.

## **B. Corneal Specimen Collection**

- The procedure is clearly explained to the patient.
- The patient is positioned comfortably at the slit lamp.

- The patient must be instructed to keep both eyes open during the procedures, as blinking will only add to discomfort.
- Local anesthetic eye drops are instilled in the affected eye to minimize ocular discomfort and facilitate the corneal scraping procedures.
- A sterile platinum loop is used to scrape the base of the ulcer with care.
- The collected material is plated on the growth media. The area around the material is marked with a permanent marker if a Gram staining test is required.
- At the end of the procedure, the patient is given instruction in appropriate care, i.e., hand washing, lid hygiene, and installation of antibiotic
- All specimens are clearly and correctly labeled before being sent to the microbiology laboratory.

## **Laboratory procedures**

### **Tests for bacterial identification.**

#### **Gram stain for the ocular specimen**

**Purpose:** Used to classify bacteria as gram-positive and gram-negative based on their gram reaction.

**Principle:** Gram's stain is that cells are first fixed to a slide by heat or alcohol and stained with a basic dye (e.g., crystal violet), which is taken up in similar amounts by all bacteria. The slides are then treated with Gram's iodine (iodine KI mixture) to fix (mordant) the crystal violet stain on Gram-positive bacteria, decolorized with acetone or alcohol, and finally counter-stained with safranin.

#### **Procedure**

1. Place the fixed slide on the staining rack.

2. Cover the heat-fixed smear with crystal violet; leave for 10 seconds. Wash off with water and drain well.
3. Cover the smear with Gram's iodine; leave for 10 seconds. Wash off promptly with water and drain.
4. Holding the slide over the sink at a 45-degree angle, run the decolorizing agent (acetone/alcohol) over the slide and watch the excess run off the bottom edge of the slide.
5. Cover the smear with basic fuchsin or safranin (red counterstain); leave for 10 seconds. Wash with water and drain; air-dry in an upright position.
6. Leave until dry and examine under the microscope using 100x oil immersion lens.
7. Report as gram-positive (blue cocci or rods) and gram-negative (red rods or cocci) and others accordingly(61).

### **General protocol of culture media preparation**

1. Weighing and dissolving of culture media
2. pH testing of culture media
3. Sterilization and sterility testing
4. Dispensing of the culture media
5. Storage of culture media
6. Culturing of bacteria
7. Inoculating Techniques
8. Quality control

### **Biochemical testing procedures**

#### **Identification of gram-positive bacteria**

**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 into 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.

**Method:**

1. Pour 2-3 ml of the H<sub>2</sub>O<sub>2</sub> solution into a test tube.
2. Using sterile wooden sticks (swabs), remove several colonies of the test organism and immerse them in the hydrogen peroxide solution.
3. Look for immediate bubbling.

**Coagulase test:** The test is used to differentiate *Staphylococcus aureus* (+ve) from another *staphylococcus* spp. (-ve).

**Principle:** In the presence of the enzyme coagulase, the addition of commercial rabbit plasma produces a clumping reaction. **Procedure**

1. Place a drop of physiological saline on two separate slides.
2. Emulsify the test organism in each of the drops to make a thick suspension.
3. Add one drop of plasma to one of the suspensions and mix gently.

Look for clumping of the organism within 10 seconds.

**Mannitol fermentation test**

Mannitol salt agar is a differential and selective medium. When mannitol is fermented, acid products are produced, and the pH drops. Phenol red is yellow in color below pH 6.8. Thus, mannitol fermenters such as *Staphylococcus aureus* will have a yellow halo around them. Mannitol non-fermenters such as *Staphylococcus epidermidis* will leave the MSA media unaltered (pink).

**Identification of gram-negative bacteria:**

Identification of gram-negative bacteria will be based on their test results through a series of biochemical tests.

**Procedure**

1. Prepare a suspension of the test organism in nutrient broth by adding 3-4 colonies of the test organism to 5 ml of nutrient broth.
2. A loop full of the bacterial suspension is inoculated into indole, citrate agar, triple sugar iron agar, lysine decarboxylase agar, oxidase, urea agar, and motility medium. See them in detail below, one by one.
3. Incubate at 35-37 °C for 18-24 hours.
4. Look for color change (turbidity for motility) in the medium.
5. Identify the test organism by considering the results of biochemical tests.

### **Indole test**

**Principle:** Some bacteria can produce indole from the amino acid tryptophan using the enzyme **tryptophanase**. Production of indole is detected using Ehrlich's reagent or Kovac's reagent. Indole reacts with the aldehyde in the reagent to give it a red color. An alcoholic layer concentrates the red color as a ring at the top.

**Procedure:** The bacteria to be tested is inoculated in peptone water, which contains the amino acid tryptophan, and incubated overnight at 37 °C. Following incubation, a few drops of Kovac's reagent are added. Kovac's reagent consists of para-dimethyl amino benzaldehyde, isoamyl alcohol, and concentrated HCl. Ehrlich's reagent is more sensitive for detecting indole production in anaerobes and non-fermenters. The formation of a red or pink-colored ring at the top is taken as positive.

### **Citrate utilization test:**

**Principle:** This test detects the ability of an organism to utilize citrate as the sole source of carbon and energy. Bacteria are inoculated on a medium containing sodium citrate and a pH indicator, bromothymol blue. The medium also contains inorganic ammonium salts, which are utilized as the sole source of nitrogen. Utilization of citrate involves the enzyme citritase, which breaks down citrate into oxaloacetate and acetate. Oxaloacetate is further broken down into pyruvate and CO<sub>2</sub>. Production of Na<sub>2</sub>CO<sub>3</sub> as well as NH<sub>3</sub> from the utilization of sodium citrate and ammonium salt, respectively, results in alkaline pH. This results in a change in the medium's color from green to blue.

**Procedure:** Bacterial colonies are picked up from a straight wire, inoculated into Simmon's citrate agar, and incubated overnight at 37 °C. If the organism can utilize citrate, the medium changes its color from green to blue.

**Triple sugar iron agar (TSI)** is used to determine if bacteria can ferment glucose and/or lactose and if they can produce hydrogen sulfide or other gases. In addition, TSI detects the ability to ferment sucrose. These characteristics help distinguish various *Enterobacteriaceae*.

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

### **Test for Oxidase Using Filter Paper**

A cytochrome oxidase test can be used to determine whether an organism possesses the enzyme.

1. Submerge a piece of filter paper in the reagent solution.
2. Scrape some new growth from the culture plate and place it on the filter paper using a disposable loop or stick. The edge of the paper can also be used to touch a colony.
3. In 10 seconds, check for the color blue.

**Check for urease by testing:** The enzyme urease, which is produced by certain bacteria, catalyzes the hydrolysis of urea to create carbon dioxide and ammonia. Urea cannot be metabolized by organisms lacking this enzyme. Together with urea, a small amount of yeast extract is also present in urea broth. For organisms unable to metabolize urea, the nutrients required for growth will be inadequate. The hydrolysis of urea will occur. Because ammonia is created during urea hydrolysis, the pH will rise. The pH indicator phenol red turns pink as a result of this pH rise. However, because of the medium's strong buffering, a considerable quantity of ammonia

production is required to cause a color shift. Consequently, the broth will only turn pink when using strong urea hydrolyzers, indicating a successful reaction. This should happen within the next day or two.

### Techniques

1. Remove the two urease broths that were refrigerated.
2. Inoculate one broth using the aseptic technique. Leave the other broth uninoculated, as it will serve as a control.
3. Decide on an appropriate incubation temperature (the one that your organism responds to the best). Incubate for 24 to 48 hours (but don't let it go past 48 hours) for this test.
4. Take out the broths from the incubator and examine their color.

Motility test: To test for motility, select a well-isolated colony and stab the motility medium with a sterile straight wire until it is within 1 cm of the tube's bottom. Make sure the straight wire stays in the same line as it entered the medium when you remove it. Incubate at 35°C for 24 hours or until growth is evident. A red, muddy area that spreads outward from the injection site signifies a successful motility test. A negative test is shown by red development up to the injection line but not past it. Check out the in-depth methods listed below.

### Methods

1. Obtain a motility agar tube from the back shelf.
2. Use an inoculating pick. Straighten the pick as much as possible.
3. Make a stab inoculation (about 2/3 of the way into the agar) from your unknown stock culture. Try to make the stab (in and out) as straight as possible. Straightness is important because you will be evaluating the amount of growth away from the stab. A messy stab will be difficult to evaluate.
4. Incubate at an appropriate temperature for 24 to 48 hours (up to 72 hours).
5. Observe your culture by holding it up to a light source.

### H<sub>2</sub>S production

**Principle:** Hydrogen sulfide (H<sub>2</sub>S) is produced by bacterial anaerobic degradation of the two sulfur-containing amino acids, cysteine and methionine. Hydrogen sulfide is released as a byproduct when carbon and nitrogen atoms in the amino acids are consumed as nutrients by the cells. Under anaerobic conditions the sulfhydryl (-SH) group on cysteine is reduced by cysteine desulfurase. The agar contains high levels of peptones (sources of cysteine and methionine) and ferrous sulfate as an indicator. When H<sub>2</sub>S is produced, the ferrous ion reacts with it to give ferrous sulfide, an insoluble black precipitate.

**Required:** TSI agar slant, inoculating loop, and incubator.

**Procedure:** 1. The triple sugar iron agar slant was inoculated by stabbing the butt and drawing the stick over the surface of the slope.

2. Incubated at 35-37°C for 18 to 24 hours. 3. Looked for black precipitate formed.

### **Result**

- Acid deep (yellow)/alkaline slant (red): - glucose fermented, lactose and/or sucrose not fermented.
- Acid deep (yellow)/acid slant (yellow): - lactose and/or sucrose fermented.
- Alkaline deep and slant (all red): - Glucose, sucrose, and lactose are not fermented.
- Deep split or displaced: - gas production.
- Deep blackened: - H<sub>2</sub>S production.

### **Antimicrobial susceptibility testing:**

#### **Procedure**

1. Prepare a suspension of the test organism by emulsifying several colonies of the organism in a small volume of nutrient broth.
2. Match the turbidity of the suspension with the turbidity standard.
3. With a sterile swab, take a sample from the suspension (squeeze the swab against the side of the test tube to remove the excess fluid).
4. Spread the inoculum evenly over the Muller-Hinton agar plate with the swab.
5. Using sterile forceps or a needle, place the antimicrobial disc on the inoculated plate.

6. Incubate the plate aerobically at 35-37°C for 18-24 hours.
7. Read the test after checking that the bacterial growth is neither heavy nor light.

Measure the radius of the inhibition zone

8. Interpret the reaction of the test organism to each antibiotic.

Sensitive \_\_\_\_\_ zone of radius is wider or equal to the control

Intermediate \_\_\_\_\_ zone of radius is > three mm smaller than the control

Resistance \_\_\_\_\_ no zone of inhibition(48).

### **Procedure for Detection of Extended-Spectrum $\beta$ -Lactamases (ESBLs)**

#### **1. Preparation of Inoculum:**

- ✓ Pick 3-5 well-isolated colonies of the test organism from a fresh culture.
- ✓ Suspend in sterile saline and adjust turbidity to 0.5 McFarland standard ( $1.5 \times 10^8$  CFU/mL).

#### **2. Inoculation of Plates:**

- ✓ Dip a sterile swab into the bacterial suspension.
- ✓ Streak evenly over the Mueller-Hinton agar plate to form a lawn culture.
- ✓ Allow the plate to dry for 3-5 minutes at room temperature.

#### **3. Placement of Antibiotic Discs:**

- ✓ Place discs of cefotaxime, cefotaxime/clavulanic acid, ceftazidime, and ceftazidime/clavulanic acid on the inoculated plate.
- ✓ Maintain a distance of 25-30 mm between discs to avoid overlapping zones.

#### **4. Incubation:**

- ✓ Invert the plates and incubate at 35-37°C for 16-18 hours.

#### **5. Interpretation of Results (Phenotypic Confirmatory Test):**

- ✓ Measure the diameter of the inhibition zones around each disc.

- ✓ ESBL positive: An increase of  $\geq 5$  mm in the zone diameter for the antibiotic + clavulanic acid disc compared to the antibiotic alone.
- ✓ ESBL negative: No significant increase in zone diameter with clavulanic acid.

## DECLARATION

I, Abebe Aschale, declare that this thesis entitled “**Bacterial isolates recovered from clinically suspected external ocular infections: Antimicrobial susceptibility profile and extended-spectrum  $\beta$ -lactamase production in Bahir dar, Northwest Ethiopia**” is my own original work, that it has not been presented for a degree at any other university, and that all sources of materials used for the thesis have been duly acknowledged.

**Name of Principal Investigator:** Abebe Aschale

**Signature:** -----

**Place of Submission:** Bahir Dar University, College of Medicine and Health Sciences, School of Medical Laboratory Science, Department of Medical Microbiology.

**Date of Submission:** -----/-----/-----

