

College Of Health Sciences

Department of Medical Laboratory Sciences

Prevalence, Antimicrobial Susceptibility Patterns and Associated Factors of Pathogenic Bacteria Isolated from patients with External Ocular Infection Attending Eye Clinic at Salale University Comprehensive Specialized Hospital, North Shoa Zone, Oromia, Ethiopia.

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A Research Thesis Submitted to the Department of Medical Laboratory Sciences, College of Health Sciences, Salale University in Partial Fulfillment of the Requirements for the Degree of Master of Science in Medical Microbiology

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Approval Sheet

This is to certify that the thesis prepared by Girma Teshome entitled "Prevalence, Antimicrobial Susceptibility Pattern and Associated Factors Pathogenic Bacteria isolated from Patients Suspected with External Ocular Infection at Salale University Comprehensive Specialized Hospital eye clinics, Fitche, Oromia, Ethiopia" and I recommend that to submitted in partial fulfillment of the requirements for the degree of Master of Science in Medical Microbiology complies with regulations of the university and meets the accepted standards concerning originality and quality.

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As mentioned by the Board of Examiners of the MSc thesis open defense was examined. We certified that we have read and evaluated the thesis prepared by ***Girma Teshome*** and examined the candidate. We recommend that the thesis be accepted as fulfilling the thesis requirements for the degree of Master of Science in Medical Microbiology.

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Abbreviations and Acronyms

ALERT……. All Africa Leprosy Rehabilitation and Training Center

AOR…… ……. Adjusted Odds Ration

ART……..Antiretroviral Therapy

ATCC ……American Type Control Culture

AMR……..Antimicrobial Resistance

AST ……. Antimicrobial Susceptibility Test

CI……confidence of interval

CLSI…….. Clinical and Laboratory Standards Institute

EOI……. External ocular infection

ERC……. Ethical Review Committee

MCH……..Mothers and Child Health

MRSA……..... Methicillin-Resistant Staphylococcus aureus

MHA……. …Muller Hinton Agar

MDRO…….. …Multi-Drug Resistant Organism

SOP …….....Standard Operating Procedure

SUCSH……..Salale University Comprehensive Specialized Hospital

TB……..Tuberculosis

VI…….....vision impairment

Abstract

Background: - External ocular infection (EOI) is an inflammation of the outer part of the eye that is responsible for causing blindness and vision impairment. Both gram-positive and gram-negative bacteria are the major causative agents of External ocular infections.

Objective: To assess the prevalence, antimicrobial susceptibility patterns, and associated factors among patients suspected of external ocular infection at Salale University Comprehensive Specialized Hospital eye clinic from June to August 2024.

Methods: The hospital-based cross-sectional study was conducted among 295 study participants suspected of external ocular infection at Salale University comprehensive specialized Hospital eye clinics from June to August 2024 by using a convenience sampling technique. Isolation and identification of pathogenic bacteria were performed using cultures such as Mac Conkey, blood agars, and Mannitol salt agar for Gram-positive and Gram-negative bacteria. Kirby Bauer disc diffusion method was used to test antimicrobial susceptibility patterns. Data were coded and entered EpiData version 4.6.0.6 and analyzed by SPSS version 25. Logistic regression analysis was performed. The data were presented in tables and figures by using frequencies and percentages. The association between independent variables and outcome variables was tested at 95% CI and P value < 0.05 was considered statistically significant.

Results: A total of the 295 study participants suspected external ocular infection, 180 (61%) with a 95% CI (55, 67%) of the participants had culture confirmed bacterial EOIs isolated. Of the 180(61%) bacterial species assessed, 145 (80.6%) were Gram-positive bacteria. The most frequent pathogens isolated were *S. aureus* 86 (47.8%) followed by *Coagulase negative Staphylococci* (CoNS) 45 (25%). Most of the bacteria isolated showed high resistance to Penicillin G and ampicillin with 91.6% and 70.6%, respectively while imipenem, 94%, was the most effective antibiotic against Gram-positive and Gram-negative bacteria. Overall, 135 (75%) of the isolates were multidrug-resistant (MDR). Sex (p-value 0.044) and previous use of antibiotics (p-value 0.040) were statistically significantly associated with External ocular infections.

Conclusion: The prevalence of bacterial pathogens among patients with external ocular infection was high (61%). Most of the isolate's bacteria were resistant to penicillin-G and ampicillin. Sex and previous use of antibiotics were statistically significantly associated with External ocular infections.

Keywords: -Antibiotics, Antimicrobial Resistance, External Ocular Infection MDR, Pathogenic bacteria.

1. Introduction

1.1. Background

Anatomically, the eye is divided into three tunics: the outer (conjunctiva, sclera, and cornea), middle (lens, ciliary body, and iris), and inner (retina) coats. The eyes have external parts that serve as a biome for pathogenic and non-pathogenic organisms from the eye. And tears films have antimicrobial constituents that prevent opportunistic microbes from causing any External ocular infection to the eyes. Associated factors such as trauma, contact lens wear, surgery, use of topical antibiotics, and reduced systemic immunity are the factor that dysregulation of the homeostatic balance and result of predispose the eyes to opportunistic pathogens such as bacteria, fungi, viruses, and protozoa (1,2).

Even though they are less common viral and Acanthamoeba keratitis causes for corneal blindness of eye infection in developing countries. Different types of factors such as contact lens wear, trauma, ocular surface diseases, lid diseases, and post-ocular surgery are the associated factors for external ocular infection. The current mainstay of treatment for external ocular infection is broad-spectrum topical antimicrobial treatment. But its effectiveness is being challenged by the emergence of antimicrobial resistance, including multidrug resistance, in some parts of the world (3).

External ocular infection (EOI) is the infections that affect the outer part of the eye such as the lids, conjunctiva or cornea. The most common types of EOI are conjunctivitis, blepharitis, and keratitis, dacrocytes. These EOI are mostly caused by bacteria. This bacterial infection causes visual impairment if not correctly identified and treated. The using broad-spectrum antibiotics before concluding culture and susceptibility testing, along with casual prescriptions, ineffective dosing, and poor patient compliance have increased bacterial resistance to commonly used topical antibiotics (4–6)

The external part of the eyes shows a series of signs and symptoms such as the formation of pus, conjunctival hyperemia, lid edema, and visual impairment when infected by bacteria. The bacteria cause EOI may come from the outside environment or endogenously transported by blood or normal flora can cause infection when they enter the aqueous humor or vitreous fluid (7,8).

Many microorganisms cause external ocular infection. But bacteria are major causative agents of external ocular infection. The most prevalent bacteria associated with external ocular infection are *Staphylococcus aureus (S. aureus), Streptococcus pneumoniae (S. pneumoniae), Bacillus, Pseudomonas aeruginosa (P. aeruginosa), Enterobacteriaceae, Neisseria gonorrhoeae, Morraxella* spp*., and Haemophilus influenza* (9).

External ocular infection can be caused by single microbial or mixed microbials. It is associated *with socio-demographic and clinical* factors such as contact lenses, trauma, surgery, age, occupation, residence, education, frequency of face washing and the occurrence of systemic disease are predisposing factors for external ocular infections (10–12).

Most Gram-positive isolates had shown greater resistance ($R > 62.1\%$) to ampicillin, amoxicillin-clavulanic acid, cefoxitin, erythromycin, and oxacillin. but had a higher rate of antibiotic sensitivity ($S > 62.1\%$) to ceftaroline, daptomycin, linezolid, moxifloxacin, rifampicin, teicoplanin, tigecycline, mupirocin, vancomycin, trimethoprim-sulfamethoxazole, and nitrofurantoin (13,14).

Among gram-negative bacteria, E. *coli* was >50% resistant to amoxicillin and tetracycline but the resistance pattern to the rest of the antibiotics was < 30%. Ampicillin, ciprofloxacin, gentamycin, and ceftriaxone were resistant to *Klebsiella* species. *Pseudomonas* species were susceptible to ampicillin, ciprofloxacin, gentamycin, and ceftriaxone > 75%.*H. influenzae* were sensitive to ceftriaxone, and 90% were also susceptible to ampicillin, ciprofloxacin, gentamycin, and nalidic acid (15).

1.2. Statement of problem

Globally, external ocular infections are affecting the eyes and leading to vision loss (16). World Health Organization (WHO) report shows that 285 million people are visually impaired worldwide. Out of that, 39 million people were blinded by the year 2010. The other report by WHO shows that more than 90% of the world's visually impaired people live in developing countries, and 82% of visual impairment, including blindness, is preventable(17). This eye infection's leading causes of blindness and low vision in the United States are primarily age-related eye diseases such as age-related macular degeneration, cataracts, diabetic retinopathy, and glaucoma. Other common eye disorders include amblyopia and strabismus (18). In Africa, approximately 2.2 million people were blinded due to ocular infection (19). In 2015 report in

Sudan showed that bacterial external ocular infections are significantly prevalent among the pediatric population and cause more than 65% of morbidity in all cases (20).

The study conducted in South India on the Prevalence of bacterial pathogens causing external ocular infection indicated that total patients with bacterial external ocular infection were analyzed, of which (61%) eyes had an adnexal bacterial infection, (28.7%) had corneal infection, (0.8%) had scleral involvement and the remaining (9.39%) eyes had infection of the intra-ocular tissues. The predominant bacterial species isolated was *S. aureus* (25%) followed by *S. pneumoniae* (21.78%) and coagulase-negative staphylococci (18.3%). The largest number of gram-positive isolates were susceptible to cefazolin (87.34%), chloramphenicol (83.65%) and grateful oxacin (81.89%) and gram-negative isolates were to amikacin (93.38%), gatifloxacin (91.91%) and ofloxacin (87.5%), while aerobic actinomycetes were to amikacin (100%), gatifl oxacin (5%), chloramphenicol (87.5%) and ofloxacin (81.25%) (21).

A study conducted at the University Hospital of San Giovanni di Dio e Ruggi d'Aragona on patients with bacterial conjunctivitis indicates that the prevalence of the bacteria isolates, 81.1% was Gram-positive. The most prevalent bacteria *CoNS (Staphylococcus epidermidis, Staphylococcus haemolyticus, and Staphylococcus hominis)* were the main species isolated, followed by *S. aureus* (33%). Also, *Pseudomonas spp*. (26%) and *Escherichia coli* (14%) were the major species isolated among Gram-negative bacteria (13).

The study conducted on external ocular infection in Adult Patients in Iraq Bagdad showed that external ocular infection affects the age groups between 20-49 years, and most patients were males 65 (61.9%). And also that the most common eye infection was conjunctivitis 45.7% , followed by blepharitis at 21.9%; blepharoconjunctivitis, which 14.3%; dacryocystitis,12.4%; and keratitis, which affected 5.7% of patients (22).

Another study conducted in Africa among Sudanese children at Khartoum state on bacterial external ocular infection based on the clinical categorization, the predominant bacterial external ocular infection were conjunctivitis (59.2%) followed by keratitis (20.7%), blepharitis (7.7%), keratoconjunctivitis (3.1%), dacryocystitis (3.1%), eye abscess (3.1%) and blepharoconjunctivitis (3.1%). The proportions of bacteria isolated regardless of their Gram staining were coagulase-positive *S. aureus* 30.0% followed by *S. pneumonia* 23.8%, *H.*

influenzae 16.9%, *P. aeruginosa* 10%, *N. gonorrhoeae* 7.7%, *CoNS (coagulase-negative staphylococci)* 3.9%, *Klebsiella* 3.1%, *M. catarrhalis* 3.1% and last of all *E. coli* 1.5% (20) .

A study conducted at Southwest Ethiopia on invitro Biofilm Formation and Antibiotic Susceptibility Patterns of Bacteria from Suspected external ocular Infected Patients Attending Ophthalmology Clinic shows that the prevalence of bacterial pathogens was 46.1%, with the most prevalent bacterial isolates being coagulase-negative staphylococcus (CoNS) (27.7%) followed by *Staphylococcus aureus* (19.7%) gram-positive bacteria. Among Gram-negative bacteria isolated *Pseudomonas aeruginosa* (6.8%) was the leading one and its showed antimicrobial resistance (64%) for tetracycline, (66.7%) for erythromycin, and (77.1%) for penicillin. But amoxicillin-clavulanic acid, ciprofloxacin, and gentamicin were the most effective drugs for external eye ocular infection due to susceptibility ranging from 70 to 100% among both Gram-negative and Gram-positive groups. Methicillin-resistant *S. aureus* (MRSA) accounted for 13.8%. Multidrug resistance (MDR) accounted for 68.7%. The highest biofilm formation rate of pathogens bacterial ocular infection was 66.1%, where *P. aeruginosa* (40%), *CoNS (*34.1%), and *S. aureus* (31%) formed a strong biofilm phenotype (23) .

Facility-based cross-sectional study conducted at Menelik II Referral Hospital in Addis Ababa, Ethiopia on Antibiotic Susceptibility Patterns and Bacterial Spectrum among Patients with External Eye ocular infection shows that the prevalence of isolated bacteria was (54.5%). The types of external ocular infection based on clinical diagnosis were blepharitis (37.8%), followed by conjunctivitis (22.6%), and keratitis (17.6%). The gram-positive bacteria isolated were (92.9%) with the most prevalence *CoNS* with a frequency of (41.3%) was the most common bacterial isolates, followed by *S.aureus* (36.4%), *Viridians streptococcus* (8.7%), and *Klebsiella species* (3.3%).

Different types of studies conducted on the prevalence, antimicrobial susceptibility, and associated factors of pathogenic bacteria causing infection show different types of results, especially on the prevalence and associated factors. Also, there is no study conducted on this topic at this study site. So, this study helped to know the current status of bacterial infection, antimicrobial patterns, and its associated factors, particularly in the study area.

1.3. Significance of the study

The various pathogenic bacteria isolated from patients with external ocular infection were identified, and their antibiotic susceptibility patterns were studied. This information was a key indicator in determining the proper course of treatment for patients with EOI and directing pathogen-specific therapy for clinicians. In line with this, the study assists healthcare providers in choosing the right and specific antibiotic by revealing patterns of drug resistance in common bacteria identified in EOI patients.

The finding of this research might be an input for current questions about antimicrobial resistance (AMR) at the national level in general and at the regional level in particular. On the other hand, the results of this study were used as the baseline for Planning and Policymakers, Fitche Public Health Research and Referral Laboratory, Salale University Comprehensive Specialized Hospital, Salale University, and other stakeholders. Finally, the results of this study were a base and might be applied at various levels by students and other researchers.

2. Literature reviews

2.1. Prevalence of bacteria profile

The study conducted at US Oklahoma City on Ocular Bacterial External ocular infection by review of Causative Organisms isolated with a designated anatomical location, 55.81% were from the cornea, 7.86% from the eyelid, 5.33% from the conjunctiva, 2.55% from the vitreous, 0.44% from the aqueous humor, and 0.22% from the anterior chamber. And found in anatomical locations such as the lacrimal gland (0.89%) and sac (0.44%), canaliculus (0.22%), and from contact lens (0.44%). Among the isolated bacteria, 79.51% were Gram-positive, and 20.38% were Gram-negative. Among the Gram-positive isolates, *Staphylococcus* and *Streptococcus* were the most isolated genera (49.39% and 17.39%, respectively). *Pseudomonas* was the most frequently isolated Gram-negative bacteria (10.08%)(24).

The study conducted in Italy on the prevalence and antibiotic resistance patterns of ocular bacterial strains isolated from Pediatric Patients in the University Hospital of Campania "Luigi Vanvitelli," Naples, indicate that the prevalence of pediatric patients with external ocular infection shows (32%) tested positive for bacterial infection. From this the highest prevalence bacterium is gram-positive bacteria with a prevalence of 85%, while 15% were Gram-negative bacteria. Among the isolated bacteria, the most frequently isolated gram-positive bacteria were coagulase-negative *Staphylococci* (60.4%), followed by *Staphylococcus aureus* (16.4%) (14).

A cross-sectional study conducted at Jeddah Saudi Arabia; a tertiary care hospital indicated that the prevalence of infection conducted on the bacterial spectrum of External ocular infection is 76%. Among that gram-negative organisms represented 71% of all culture reports with *H. influenza* 26%, followed by *S. aureus* (12%) *and P.aeruginosa* (10%)(4).

Another study conducted in Ghana on external bacterial ocular and periocular and their antimicrobial treatment patterns among a Ghanaian ophthalmic population indicate that ninety-five (95.1%) of the isolated sample were culture positive, and single colony cultured was identified. Gram-negative bacteria isolated was 58.2% with *Pseudomonas aeruginosa* (38.8%) and *Pseudomonas putida* (11.2%) being the predominant species. The gram-positive bacteria isolated was 41.8%, with a predominant of bacteria pathogens being *Staphylococcus aureus* (27.6%) and *Coagulase-negative staphylococci, CONS* (13.3%). The bacteria pathogens isolated from conjunctivitis were *Pseudomonas aeruginosa* (40.0%), *Staphylococcus aureus* (21.8%) and

Pseudomonas putida (16.4%). *Pseudomonas aeruginosa* (41.7%) and *Staphylococcus aureus* (33.3%) were most prevalent identified in cases of keratoconjunctivitis. *Staphylococcus aureus* (100.0%) was the sole organism implicated in cases of presential cellulitis, whereas most cases of keratitis was caused by *Pseudomonas aeruginosa* (75.0%)(25)

A cross-sectional study conducted in Asmara Eritrea to assess the incidence of bacterial keratitis among suspected keratitis patients who visited Berhan Aini hospital and community hospitals of Godaif and Biet Mekae indicated that total prevalence 66.66% of cases were culture positive. The most common isolated bacteria was *Staphylococcus aureus*, 50%; followed by Coagulase-negative *Staphylococcus aureus CoNS*, 30%; *Streptococcus pneumoniae*, 15%; and *Streptococcus viridans* 5%(26)**.**

The Cross-sectional study conducted in Ethiopia in Jimma University on bacterial profile and antimicrobial susceptibility pattern of infection indicates that total prevalence of ocular samples were collected for microbiological evaluation, of which (74.7%) had bacterial growth. Among these, the gram-positive cocci holds 52.0% and the predominant isolate was *S. aureus* (28.4%).Also gram-negative bacteria accounted for 48.0% and the predominant isolate pathogen bacteria was *P. aeruginosa* (20.9%) (27).

Another study conducted in Ethiopia in Jinka General Hospital ophthalmic clinic, Southern Ethiopia on external ocular infected patients attending indicate that overall prevalence of Gram-positive bacterial pathogens among samples was (34.3%). *S. aureus* (47.39%), followed by coagulase-negative *Staphylococcus spp*. (31.9%), and *S. pneumoniae* (10.9%) were predominantly isolated. (28).

The hospital based cross-sectional study conducted at Shashamane Compressive Specialized Hospital Oromia, Ethiopia on bacterial etiology of ocular and periocular among patients attending eye indicate that 60% were culture positive. Among the bacteria isolated gram-positive (68.2%) and gram-negative (31.8%). From Gram-positive bacteria, *Staphylococcus aureus* were predominant. Among Gram-negative bacteria, *Escherichia coli* were predominant (29).

The study conducted in Ethiopia Amhara region in Dabre Markos Comprehensive Specialized Hospital, northwest Ethiopia on external ocular infection among patients attending eye clinic shows that the overall prevalence of culture-confirmed bacterial isolates was 62.8%. Among the bacterial isolates, mixed bacteria were not found in this study. Among the isolates, 78.5% were

Gram-positive. *S. aureus* was the predominant Gram-positive bacteria, accounting for 58.8% followed by CoNS with 26.5% prevalence and *S. pneumoniae* with a proportion of 7.8% (8/102). Among gram-negative bacteria isolated, *E. coli (32.1%)* was the predominant bacterial isolated, followed by *P. mirabilis* (21.4%). The least isolated bacteria were *S. pyogenes* from Gram-positive (0.98%) and *P. aeruginosa* from Gram-negative (3.6 %,)(11).

2.2. Antimicrobial Susceptibility Patterns

A study conducted at the Regional Ophthalmic Institute in India on antimicrobial susceptibility pattern of External ocular infection shows that *S. aureus* isolates, maximum sensitivity was seen for Vancomycin (100%) followed by Chloramphenicol (78%) in this study. Rifampicin was also observed as a sensitive *antistaphylococcal* agent. Chloramphenicol, Rifampicin, and Cefazolin showed moderate sensitivity. Resistance was seen for Norfloxacin (77%) and Ciprofloxacin (66%), respectively. Among *CONS,*100% sensitivity (100%) to vancomycin and chloramphenicol followed by erythromycin (80%) and methicillin (80%). Among the Gram-negative isolates, E. coli was most isolated and Sensitivity (100%) to moxifloxacin, gentamycin, tobramycin, ceftazidime, and ciprofloxacin and 83.3% sensitivity to cefazolin, erythromycin and netilmycin. With ofloxacin, maximum resistance (66.7%) was seen *Klebsiella* showed sensitivity to moxifloxacin, gentamycin, and tobramycin in 100% of cases. And 50% sensitivity in cefazolin, cetazidime, ciprofloxacin, erythromycin and netlimycin. Resistance of Klebsiella was seen with ofloxacin in 100% of cases. Pseudomonas aeruginosa, an important causative agent of a variety of infectious diseases leading to blindness and serious eye consequences was isolated in 3 cases. And also showed sensitivity for cefazolin, moxifloxacin, and ceftazidime in 100% of cases. With gentamycin, 100% resistance was seen. Ofloxacin, tobramycin, and netilmicin showed resistance in 2 out of 3 cases (66%). Ciprofloxacin and erythromycin showed resistance in 1 out of 3 cases (33.3%) (30).

The study conducted in Riyadh on bacterial profile and antibiotic Susceptibility pattern of bacterial keratitis at a Tertiary Hospital indicates that all gram-positive cases were Vancomycin sensitive. *Staphylococcus epidermidis* showed only 4 resistant cases to chloramphenicol (7.27%), and resistance to penicillin and ampicillin was found in (90%) and (89.83%) cases, respectively. But *Streptococcus pneumoniae* were resistant to gentamicin (100%) and *Staphylococcus aureus* showed high sensitivity for erythromycin (100%) (31).

The cross-sectional study conducted on EOI patients at Borumeda Hospital Northeast of Ethiopia indicates that antimicrobial susceptibility patterns of bacterial isolates from ophthalmic patients showed that all the gram-positive bacteria were sensitive to vancomycin, but a largest number of bacterial isolates were resistant to one or more than one antimicrobials. Among the gram-positive bacteria showed that 93.3 % and 100 % of the isolates were sensitive to cefoxitin and vancomycin respectively. However, more than half of the Gram-positive isolates (53.9 %) and the other (34.7 %) showed resistance against amoxicillin and ampicillin, respectively. Cefoxitin resistance was observed among *CoNS* (7.8 %). According to the study, there was no drug-resistant isolated strain among the *Viridian streptococci* and *Streptococcus agalactiae* isolates. The drug susceptibility patterns of the Gram-negative bacterial isolates showed that (83.3 %) were susceptible to gentamicin. However, the majority of Gram-negative bacteria isolates (66.7 %) were resistant to tetracycline, norfloxacin, ceftriaxone, and ciprofloxacin(10).

According to the cross-sectional study conducted at ALERT, Addis Ababa, Ethiopia on EOI patients indicate that he overall prevalence of bacterial pathogens among samples was (59.4%). Among the isolated bacteria, gram-positive bacteria were the most common isolates with a 70.2% prevalence. The most isolated gram-positive bacteria were *Staphylococcus aureus* with 36.8% of the cases. Among the isolated bacteria, 91.6% of them showed high resistance to Penicillin and Tetracycline (70.4%). But 94% Gentamicin was the most effective antibiotic against gram-positive and gram-negative bacteria isolated. The overall prevalence of multiple drug resistance was (93%): gram-positive (97.5%) and gram-negative (82%) (5).

Another cross-sectional study conducted at Felege Hiwot Referral Hospital Northwest Ethiopia indicate that the AMS gram-positive bacteria isolates were resistant to penicillin (87%), ampicillin (86.2%) and tetracycline (52.2%). *S. aureus* isolates revealed 96.1% of resistance to both ampicillin and penicillin. *S. pneumoniae* isolates revealed high (66.7%) percentage of resistance to sulphamethoxazole-trimethoprim. Gram-positive isolate revealed low percentage of resistance to ciprofloxacin (9.6%), clindamycin (7.5%) and gentamycin (10,4,6).

2.3. Associated Factors for Infection

The study conducted at the community hospital of Asmara, Eritrea shows that the risk factors predisposing to ocular conditions keratitis are trauma, foreign bodies, wearing contact lenses, ocular and eyelid surgery, other than these ocular surface diseases, corneal epithelial abnormalities and systemic diseases have a contributing factor. Several factors such as personal

hygiene, living conditions, sociodemographic or economic status, frequency of face washing, and cigarette smoking were also considered as associated factors for bacterial-based ocular External ocular infection(26)

The other study conducted at in Jijiga,Somalia Karamara Hospital, Eastern Ethiopia indicated that using soap for washing the face (AOR = 0.43; 95% CI: 0.29, 0.95), having diabetes mellitus (AOR = 3.11; 95% CI: 1.45, 6.75), and history of hospitalization (AOR = 2.82; 95% CI: 1.44, 5.54) were significantly associated with infection (12).and also study conducted at Felege Hiwot General Hospital indicate that Ocular trauma ($P < 0.001$), ocular disease ($P < 0.001$) and having eye allergy ($P = 0.027$) were significantly associated with EOIs (32) .

The study conducted on Ocular bacterial External ocular infection at Quiha Ophthalmic Hospital, Northern Ethiopia indicates that Ocular surface disease, ocular trauma, hospitalization, and cosmetic application practices were significantly associated with the occurrence of external ocular infection of bacteria (33). Another's study conducted in Ethiopia at Dabre Markos comprehensive specialized hospital showed that the prevalence of culture-confirmed bacteria was significantly associated with age groups 15–24 (AOR: 9.18, 95%CI: 1.01–82.80; P = 0.049) and 25–64 (AOR: 7.47, 95%CI: 1.06–52.31; P = 0.043) being farmer (AOR: 5.33, 95% CI:1.04–37.33; P = 0.045), previous history of eye surgery (AOR: 5.39, 95% CI: 1.66–17.48; P= 0.005), less frequency of face washing (AOR: 5.32, 95% CI: 1.31–7.23; P = 0.010) and face washing once a day (AOR: 3.07, 95% CI: 1.13–25.13; P = 0.035) were also significantly associated with the prevalence of culture-confirmed bacteria (11).

The study conduct on the bacterial profile of external ocular infections, its associated factors, and antimicrobial susceptibility pattern among patients attending the ophthalmology and optometry department at Hawassa University Comprehensive Specialized Hospital, Sidama, Ethiopia showed that Less frequent face washing, systemic disease, and a history of eye surgery were significantly associated with culture-confirmed external ocular infections (EOIs) (34).

2.4. Conceptual framework

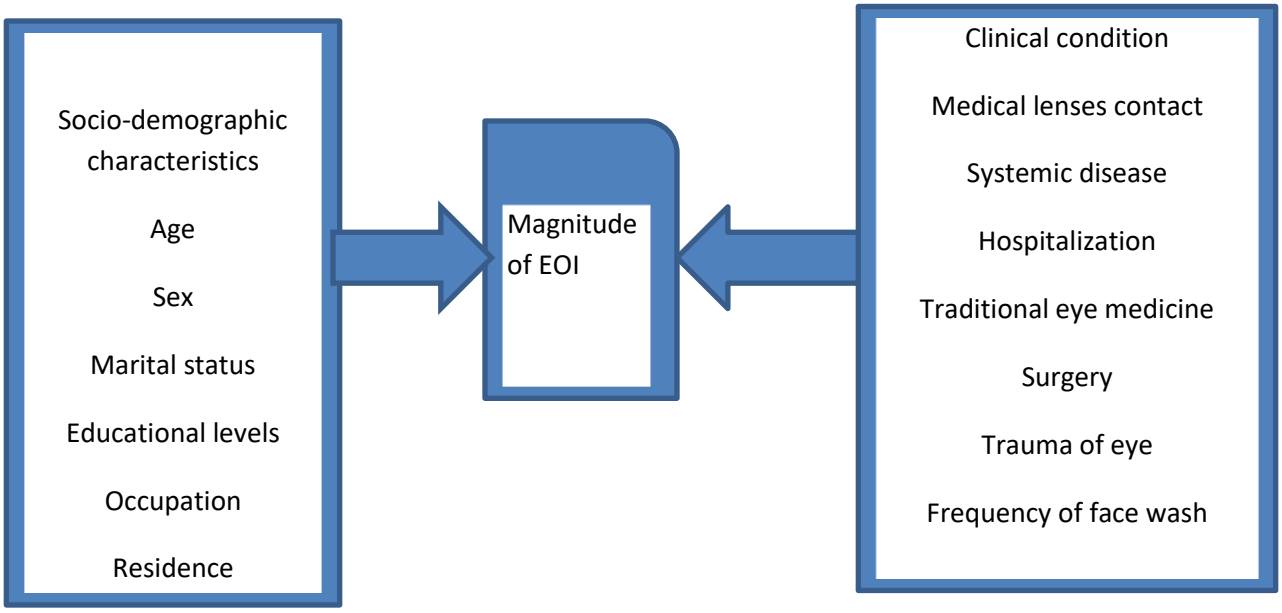

Figure 1:- Conceptual framework show the relationship between associated factors and the outcome

3. Objectives

3.1. General objective

- To determine the prevalence, antimicrobial susceptibility patterns and associated factors of pathogenic bacteria isolated from patients with external ocular infection attending eye clinic at Salale University Comprehensive Specialized Hospital, North Shoa Zone, Oromia, Ethiopia, from June to August 2024.G.C.

3.2. Specific objectives

- To determine the prevalence of pathogenic bacteria isolated from study participant with external ocular infection.
- To identify antimicrobial susceptibility patterns of pathogenic bacteria isolated from study participants with external ocular infection.
- To assess associated factors among study participants with external ocular infection.

4. Methods and Materials

4.1. Study area and study period

The study was conducted at Salale University Comprehensive Specialized Hospital (SLUCSH), Oromia region, Central Ethiopia from June to August 2024. It is in Fitche town and approximately 114 Km far from Addis Ababa. The hospital is the only Comprehensive Specialized Hospital found in the North Shoa zone and gives services to approximately for 1.8 million catchment area populations. This hospital has about 189 health professionals and 140 administrative staff. It gives services such as emergency, outpatient, inpatient, MCH, TB clinic, ART clinic, laboratory service, pharmacy service, radiology service, ophthalmology, dermatology, psychiatric service, dental clinic and so on. The hospital eye clinics giving service as outpatient services and average daily flow rate of EOI in the eye clinic of the hospital was 25 and the study was conducted for 3 months with 22 working days each month making a total of 1650 patients visiting the eye clinic.

4.2. Study design

A Hospital-based cross-sectional study was conducted.

4.3. Population

4.3.1 Source population

All patients who visited eye clinic of SLUCSH during the study period.

4.3.2. Study population

All patients suspected of EOI visiting the eye clinic of SLUCSH during the study period were the study populations.

4.4. Eligibility criteria

4.4.1 Inclusion criteria

All age group patients suspected of EOI during the study periods and who are volunteers to participate in the study were included.

4.4.2. Exclusion criteria

- Those Patients who taking and took antibiotics last two weeks of the study period.
- Patients with acute physical eye injury were excluded from the study.

4.5. Study Variables

4.5.1. Dependent variables:

- Bacteria profile of external ocular infection
- Antimicrobial susceptibility pattern

4.5.2. Independent variables:

- Demographic variables such as: -Age**,** Sex, Occupation, residence, socioeconomic status
- Other variables such as Previous antibacterial therapy, use of contact lenses, Repeated External ocular infection, Duration of stay in hospital, Trauma, Surgery, Systemic diseases, etc.

4.6. Sample Size Determination and Sampling Technique

4.6.1. Sample Size Determination

The sample size was determined by using a single population proportion formula considering 95% CI, 5% marginal error, and a 62.8% prevalence of EOI from a previous study conducted at Debre Markos Comprehensive Specialized Hospital (DMCSH), Northwest Ethiopia in 2022(11) .

$$n = pq\left(\frac{z\frac{\alpha}{2}}{d}\right)^2$$

Where n = sample size, p = 62.8%, q = 1-p = 0.372, d = margin of error that can be tolerated, 5% (0.05), Z = level of 95% confidence interval (1.96). Using the above formula, the sample size was calculated to be 359. The average daily flow rate of EOI in the eye clinic of the hospital was 25 and the study was conducted for 3 months with 22 working days each month making a total of 1650 patients visit the eye clinic. Because of the total population size 1650 was below 10000, so we applied a sample size correction formula. Considering this, the final sample size was calculated as follows; N final = n/ (1+n-1/N) =295.

4.6.2. Sampling technique

A Non-probability convenience sampling technique was used to recruit the study participants, and a consecutive sample was collected from patients with the order of their arrival until the achievement of the expected sample size.

4.7. Data and sample collection procedures

4.7.1. Socio-Demographic characteristics and external ocular infections related information.

One doctor, two BSc nurses and two laboratories were identified and trained for one day on how to sample and collect data by semi-structured questionnaires by using local language. The sample collection method was by interview using questionnaires. Each eligible patient was provided a swab of eye specimen once during the study period. Socio-demographic data and related information about external ocular infection were collected.

4.7.2. Specimen collection

The specimen was collected from each patient by gently swabbing the eye, using sterile cotton swabs moistened by saline. Sample collection was continued until the expected sample size was achieved. Following collection, the swabs were placed into the sterile test tube with a screw cup and transported to the Microbiology Laboratory of fitche regional laboratory and public health institute within 30 minutes by a data collector or principal investigator**.** Then the collected specimens were processed immediately. If there is delay in processing, the specimen was placed in tube with normal saline and refrigerated at Amies transport media for sample processing.

4.8. Laboratory testing procedures

4.8.1. Culture identification

In the microbiology laboratory at Fitche Regional Laboratory and Public Health Institute, all the external ocular infection swab samples were cultured on differential and selective media for bacterial cultivation by two laboratory personnel with principal investigator. The swabs were inoculated onto Mac Conkey agar, MSA and Blood agar plates (BAP). The plates were incubated at 35°C-37°C for 24-48 hours and examined for bacterial growth. When growth observed, colonies was examined morphologically for size, shape, and odor. Further identification was done by sub-culturing the isolate from a single colony of pure culture. Definitive diagnosis was done based on colony characteristic appearance on their respective media, gram staining reaction and biochemical tests.

4.8.2. Biochemical tests

Biochemical tests were performed on colonies from primary cultures for final identification of the isolates, Gram-negative bacteria was tested for catalase, motility, indole, citrate and oxidase,

triple sugar iron, urease test while Gram positive bacteria was tested for catalase, coagulase, MSA, optochin and bacitracin sensitivity tests. The result of the biochemical tests was compared with standard identification chart.

4.8.3. Antimicrobial susceptibility test (AST)

The Kirby Bauer disk diffusion method on Mueller-Hinton agar (MHA) was done to test antimicrobial susceptibility patterns according to Clinical Laboratory Standards Institute (CLSI, 2023) guidelines. Antibiotics regularly available and frequently prescribed in the study area may be considered for the study. According to the CLSI guideline 2023, the following antibiotics were tested against gram-positive bacterial isolates. Penicillin (10µg), Erythromycin (15µg), Cotrimoxazole (1.25/23.75µg), Cefoxitin (30µg), Gentamicin (10µg), Ciprofloxacin (5µg), Tobramycin (10µg), Chloramphenicol (30g), imipenem (30µg), Ampicillin (10µg), erythromycin and augmentin. Similarly, the following antibiotics were tested against gram-negative isolates, Erythromycin (15µg), Cotrimoxazole (1.25/23.75µg), Cefoxitin (30µg), Gentamicin (10µg), Ciprofloxacin (5µg), Tobramycin (10µg), Chloramphenicol (30g), imipenem (30µg), Ampicillin (10µg), erythromycin and Augmentin. Bacterial isolates that are resistant for at least one antibiotic from three or more classes of antibiotics were considered as multidrug-resistant (MDR). About 3-5 pure colonies of isolated bacterial organisms were picked with a sterile wire loop and added to normal saline then mixed thoroughly to adjust by comparing with 0.5McFarland standards for susceptibility testing. After 15 minutes, sterile swab was dip into the prepared suspension of the isolate in broth and speeded uniformly over a Muller-Hinton agar (MHA) plate, and then the above-mentioned antibiotics were placed on MHA of pathogenic bacteria by using sterile forceps placing each disk within equal distances from each other disks. After that, the medium was incubated at 37℃ for 16-18 hours. After incubation, the plates were examined for the zone of inhibition, and the diameter of each inhibition zone was measured with a pair of calipers or a ruler, and recorded in mm. The results were then interpreted as sensitive (S), intermediate (I), or resistance (R) according to CLSI guidelines(35,36).

4.9. Operational Definitions

Ocular infection: Eye infection occurs when harmful microorganisms invade any part of the eyeball or the surrounding area.

External ocular infection: Eye infection occurring at the outer part of the eye including lids, conjunctiva, cornea, and lacrimal apparatus infections.

Prevalence of culture-confirmed bacteria: This indicates the prevalence of bacteria isolated from EOI suspected patients using the routine culture method.

Acute physical eye injury: A physical eye injury that occurs within 24 hours.

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

Face washing once a day: Face washing once a day with soap and water.

Less frequent face washing: Face washing occasionally less than once a day is using soap and water.

multi-drug resistance (MDR): Bacteria that resist more than one drug in three or more classes of antimicrobial drugs.

Systemic disease: The diseases of the eye that directly or indirectly result from a disease process originating from another system of the body.

4.10. Data Management and Quality Assurance

A 5% pre-testing of the questionnaires was done at Kuyu General Hospital to assess the problem of the checklist, proper training was given to data collectors before the start of data collection, and supervision was done during data collection by the principal investigator.

4.10.1. Pre-analytical

Before the actual data collection, data collectors were trained, and study participants was informed about the study and asked for their consent and assent. The swab collection containers were labeled with patient identifier codes and date of collection accordingly. The specimens were collected and transported by the data collector or principal investigator as quickly as possible to the laboratory and stored according to SOPs. All patient information collected during the study period was checked for clarity and completeness regularly. Each lot of the culture medium was checked for expiration dates prior to use as part of quality control.

4.10.2. Analytical

Two well-trained BSc laboratory personnel were performing all the laboratory tests alongside with the investigator. All samples brought to the testing bench were analyzed as soon as possible.

The performance of the media and antibiotic discs was evaluated by using positive controls; i.e. American Type Culture Collection, *S.aureus,* ATCC 25923 and *E. coli* ATCC 25922 were used.

4.10.3. Post-Analytical

All laboratory results were recorded on a Laboratory result form and logbook during the study period. Each specimen with culture growth was stored in deep freezer for 3 year any kind of checkup and future use.

4.11. Data processing and analysis

Data was entered into Epi data version 4.6 and export to SPSS version 25.0 for analysis. Descriptive statistics like frequency and percentage, were used to describe the study participants. Results were presented by text, figures, and tables. Logistic regression analysis was performed to determine the association of factors associated and pathogenic bacteria. The P-value of below 0.05 is considered as statistically significant.

4.12. Ethical Considerations

This study was conducted after obtaining full approval from the Institutional Research Ethics Review Committee (ERC) with the number **SLU-IREC-169/2016** of Salale University. In addition, the supportive letter was obtained from Salale University, College of Health Sciences. Then, permission was obtained from the Salale University Comprehensive Specialized Hospital Medical director to conduct the study. Accordingly, informed consent or assent was obtained from the study participants or guardians Participants were informed about the purpose of the study, the benefits they get from the study, risks associated with the study, the confidentiality of personal data, and their right when take part in the study. Study participants were recruited after they provided informed written consent and/or assent. Laboratory results of patients were communicated with their respective physicians for better management.

4.13. Dissemination of the Result findings

The study result was presented to Salale University College of Health Sciences, Department of Medical Laboratory Science as MSc thesis. In addition, the findings of this the study was submitted to SUCSH and other concerned bodies. Moreover, the result of this study will be presented in scientific conferences and published in reputable journals.

5. Results

5.1. Socio-demographic characteristics of study participants suspected of EOI.

A total of 295 study participants suspected of external ocular infection were included in the study. Among them, 142 (48.1%) were female and 153 (51.9%) were male. The mean age was 31.01 with the SD±14.29 and range from 1-70. About 60.3 % of them were rural residents. Most of the study participants 169 (57.3%) were in the age group between 25-64 years. Two hundred thirty-four (79.3%) of the respondents were able to read and write while 61 (20.7%) cannot read and write. 168 (56.9%) of the respondents were married and 81(27.5%) were government employers (Table 1).

Table 1:- Socio-demographic characteristics of study participants suspected with external ocular infections attending the eye clinic at SUCSH, from June to August 2024.

Variables	**Category**	**Frequency (No) (%)**
Age	<15	45(15.3)
	15-24	75(25.4)
	25-64	169(57.3)
	>64	6(2)
Sex	Females	142(48.1)
	Males	153(51.9)
Residence	Urban	178(60.3)
	Rural	117(39.7)
Marital status	Marriage	168(56.9)
	Single	79(26.8)
	Windowed	1(0.34)
	Divorced	6(2)
	Underage	41(13.9)
Educational status	unable to read and write	61(20.7)
	able to read and write	79(26.8)

	Secondary	31(10.5)
	college and above	124(42)
Occupational status	government employer	81(27.5)
	Farmer	47(15.9)
	Merchant	42(14.2)
	Student	65(22)
	Housewife	40(13.6)
	daily labor	6(2)
	Others	14(4.7)

5.2. Clinical characteristics of study participants suspected EOI.

Two hundred sixty-three (89.2%) of participants were not used medical contact lenses. Only 24 (8%) of the participants have systemic disease and the rest 270(91.5) have no systemic disease. Of the patients who stayed in the hospital for less than one week were 17(5.8%) and about 278(94.2%) of the participants were visited the hospital within one day of the external ocular infection happened.

The patients use traditional medicine and modern eye treatment for their illness with a frequency of 6 (2%) and 119 (40.3%), respectively and only 15 (5.1%) of them made eye surgery due to different cases. However, most of the participants 294 (99.7%) used soap for washing their face and among them, 168 (57%) washed their face twice a day (Table 2).

Table 2: Summary of clinical characteristics of external ocular infection among the study participant at SUCSH, from June to August 2024

Variables	**Category**	**Frequency (No) (%)**
Types of ocular External ocular infection	Conjunctivitis	118(40)
	Blephritis	94(32)
	Keratitis	50(17)
	Dacrocysitis	33(11)
Using of medical contact lenses	No	263(89.2)
	Yes	32(10.8)

Presence of different types of Systemic disease among patients	Rheumatoid arthritis	3(1)
	Diabetes	6(2)
	Hypertension	15(5.1)
	Don't have systemic disease	270(91.5)
	Others	1(0.3)
Hospitalized for long period of times	No	278(94.2)
	Yes	17(5.8)
Utilization of traditional eye medicine	No	289(98)
	Yes	6(2)
Utilization of modern eye treatment	No	176(59.7)
	Yes	119(40.3)
patients who have made eye surgery	No	280(94.9)
	Yes	15(5.1)
Patient who are using soap to wash their face	No	1(0.3)
	Yes	294(99.7)
frequency of face washing	once a day	85(28.8)
	twice a day	168(56.9)
	three times a day	42(14.2)

5.3. Prevalence of pathogenic bacteria and its distribution across socio-demographic and other clinical characteristics of study participants

Out of 295 patients with external ocular infection only 180(61%) with a 95% CI (55-67%) were culture positives while the remaining were culture negatives. Most of the External ocular infections were isolated from female patients 95(52.8%) and the age group between 25-64, 108(64%). Concerning occupation, residency, and educational level, government employers were 51(28.3%), urban 103(57.2%), and those college and above 77 (42.8%) were the most bacterial infected patients with external ocular infections. Bacterial isolated were 106(59%) among conjunctivitis patients and 156(86.7%) among the patients that don't use medical contact lenses the patients with systemic disease of hypertension 11(6.1) higher than the other patients with systemic disease. The bacteria isolated among the study Participants admitted for less than one week were 12(6.7%) and 168 (93.3%) among those who came to the hospital within one day of the occurrence of the external ocular infection (Table 3).

Bacterial growth was observed in 180(61%) of the participant patients and yielding a total of 180 bacterial isolates. Among culture positive, 145(80.6%) were gram positive and 35(19.4%) were gram negative, *S. aureus* 68(29.2%) and *E. coli* 15(5.1%) were the dominant isolates (Figure 2). Single bacterial isolates were recovered from 180/180(100%) patients and no mixed bacterial cultures were identified.

Table 3: Prevalence of pathogenic bacterial infection among study participant with respect to Socio-demographic and clinical characteristics at SUCSH, from June to August 2024

Variables	**Category**	**Positive**	**Negative**	**Total**
		No (%)	**No (%)**	**No (%)**
Age	<15	26(14)	19(16.5)	45(15.3)
	15-24	40(22.2)	35(30.4)	75(25.4)
	25-64	108(60)	61(51.3)	169(57.3)
	>64	6(3.3)	0	6(2)
Sex	Male	85(47.2)	68(59.1)	153(51.9)
	Female	95(52.8)	47(40.9)	142(48.1)
Residence	Urban	103(57.2)	75(65.2)	178(60.3)
	Rural	77(42.8)	40(34.8)	117(39.7)
Marital status	Marriage	108(60)	60(52.2)	168(57)
	Single	45(25)	34(29.6)	79(26.8)
	Windowed	0	1(0.9)	1(0.3)
	Divorced	4(2.2)	2(1.7)	6(2)
	Underage	23(12.8)	18(15.7)	41(13.9)
Educational status	unable to read and write	40(22.2)	21(18.3)	61(20.8)
	able to read	52(28.9)	27(23.5)	79(26.8)

	and write			
	Secondary	11(6.1)	20(17.4)	31(10.5)
	college and above	77(42.8)	47(40.9)	124(42)
Occupational status	government employer	51(28.3)	30(22.6)	81(27.5)
	Farmer	32(17.8)	15(13)	47(16)
	Merchant	20(11.1)	22(19.1)	42(14.2)
	Student	40(22.2)	25(21.7)	65(22
	Housewife	26(14,4)	14(12.2)	40(13.6)
	daily labor	2(1.1)	4(3.5)	6(2)
	Others	9(5)	5(4.3)	14(4.7)
Using of medical contact lenses	No	156(86.7)	107(93)	263(89.2)
	Yes	24(13.3)	8(7)	32(10.8)
Systemic disease of patients	rheumatoid and arthritis	3(1.7)	0	3(1)
	Diabetes	4(2.2)	2(1.7)	6(2)
	Hypertensive	11(6.1)	4(3.5)	15(5.1)
	don't have systemic disease	161(89.4)	109(94.8)	270(91.5)
	Others	1(0.6)	0	1(0.3)
Hospitalized for long period of times	Yes	12(6.7)	5(4.3)	17(5.8)
	No	168(93.3)	110(95.7)	278(94.2)
patient used traditional eye medicine	Yes	6(3.3)	0	6(2)
	No	174(96.7)	115(100)	289(98)
patient used medicinal	Yes	81(45)	38(33)	119(40.3)

eye treatments	No	99(55)	77(67)	176(59.7)
patients made eye surgery	Yes	11(6)	4(3.5)	15(5.1)
	No	169(94)	111(96.5)	280(94.9)
patient use soap for face wash	Yes	179(99.4)	115(100)	294(99.7)
	No	1(0.6)	0	1(0.3)
frequency of face washing	once a day	63(35)	22(19.1)	85(28.8)
	twice a day	85(47.2)	83(72.2)	168(57)
	three times a day	32(17.8)	10(8.7)	42(14.2)

Out of 295 patients with External ocular infection suspected study participant, 118 (40%) were conjunctivitis, 94(32%) were blepharitis, 50(17%) were keratitis and 33(11%) were dacrocystis among external ocular infections case participated.

Among culture positive EOI conjunctivitis, Blepharitis, Dacrocystis, and keratitis gram-positive bacteria were constituted 98/106 (92.5%), 17/34 (50%), 11/14 (78.6%) and 19/26 (73.1%), respectively. Whereas of gram-negative bacterial isolates were obtained from conjunctivitis 8/106 (7.5%), Blepharitis 9/34 (26.5%) and keratitis 15/26 (57.7%). Among patients positive for bacterial isolates and clinically categorized as conjunctivitis was 98(67.6%), *S.aureus* was the most common isolates in 53/98 (54%) isolates followed by *CoNS* 32/98 (32.7%) isolates. And among the Gram-negative bacterial isolates were 8 (23%); with *E. coli* 4(50%), were the most commonly gram-negative isolates bacterial among conjunctivitis.

Table 4:- prevalence of culture-confirmed EOI and frequency of bacterial isolated among the study participant at SUCSH, from June to August 2024

Bacterial species	Types of external ocular infections			
	Conjunctivitis, n (%)	Keratitis, n (%)	Blepharitis, n (%)	Dacryocystitis, n (%)
Gram positive	98(67.6)	19(13.1)	17(11.7)	11(7.6)

S. aureus	53(54)	15(79)	10(58.8)	89(72.7)
CoNS	32(32.7)	4(21)	6(35.3)	3(27.3)
S. pneumonia	13(13.3)	0	1(5.9)	0
Gram-negative	8(23)	15(43)	9(26)	3(9)
E. coli	4(50)	8(53.3)	3(33.3)	0
K. pneumonia	1(12.5)	4(26.7)	4(44.4)	1(33.3)
P.aeruginosa	1(12.5)	2(13.3)	0	1(33.3)
Proteus species	0	1(6.7)	1(11.1)	1(33.3)
Citrobacter	0	0	1(11.1)	0
Acetinobacter spp	2(25)	0	0	0
Total	106(59)	34(14.4)	26(19)	14(8)

5.3.1 Prevalence of pathogenic bacteria isolated among the study participants.

In this study among the total 295 samples cultured, 180(61%) with a 95% CI of 55-67% were culture positive. From this the 145(80.6%) was gram-positive, while 35(19.4%) was gram-negative bacterial isolated. Among the isolated gram-positive bacteria *S.aureus* 86(48%), *CoNS* 45(25%) and *S.pneumoniae* 4(8%)and also the gram negative isolated *E.coli* 15(8.3%),*K.pneumoniae* 10(5.6%), *P.aureginosa* 4(2.2%),*Proteus spps* 3(1.7%), *Acetinobacter* 2(1.1%) and *Citrobacter* 1(0.6%). Among the isolated bacteria *S.aureus* 86(59.3%) and *E.coli* 15(42.9%) were the frequently isolated gram positive and gram negative bacteria respectively.

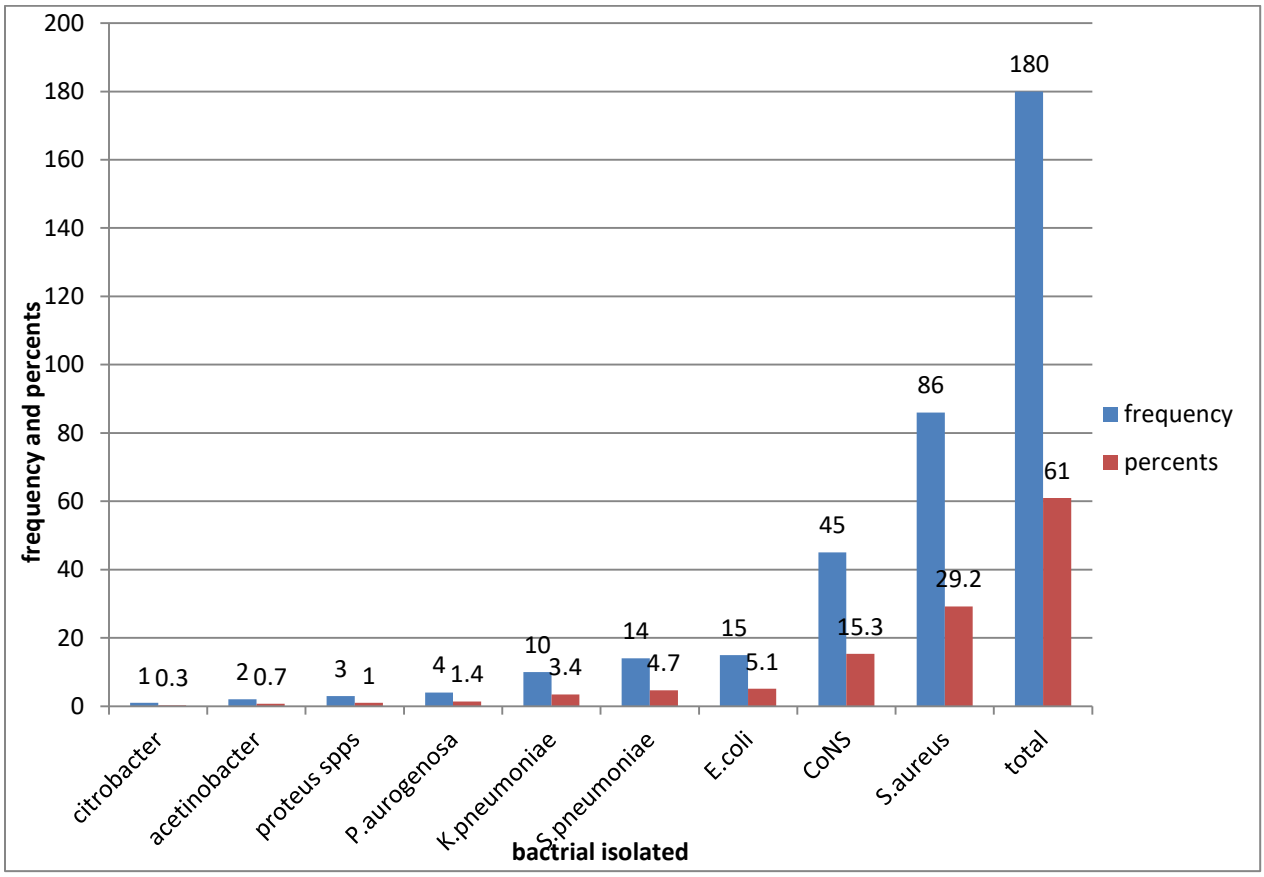

Figure 2:- prevalence of bacteria isolated among the study participant suspected with external ocular infection at SUCSH eye clinic, 2024

5.4. Antimicrobial susceptibility patterns of pathogenic bacterial isolated.

Gram-positive isolates were susceptible to 83.7% of cefoxitin and tobramycin, 82.6% of imipenem and cotrimoxazole, 73.3% of ciprofloxacin, 68.6% of chloramphenicol, and 67.4% of erythromycin. *S. aureus* isolates revealed (86%) and (50%) percentage of resistance to penicillin G and ampicillin, respectively whereas they are susceptible to Augmentin 72.1% and gentamycin 60%. *CoNS* isolates showed that 86.4% and 67.4% of resistance to penicillin G and erythromycin, respectively but they were sensitive to Imipenem 97.7%, tobramycin 95.5%, Augmentin 93.2%, ciprofloxacin 91%, Chloramphenicol 86.4%, gentamycin 88.6%, and cotrimoxazole 68.2%.

S.pneumoniae isolated shows that 86% imipenem, 85.7% gentamycin, 78.6% erythromycin, 78.6% Augmentin, 78.6%ampicillin and 71.4% chloramphenicol were sensitive but there was resistance to 57% pen-G and 50% cotrimoxazole.

The antimicrobial susceptible patterns of gram-negative isolated pathogenic bacterial showed that *E. coli* was 100% sensitive to ciprofloxacin, gentamycin, and tobramycin. And showed that were sensitive to 93.3% cotrimoxazole, 86.7% to chloramphenicol and Augmentin, and 80% to erythromycin. Ampicillin (53.5%) isolated were resistant to *E. coli*. .*K. pneumoniae* showed 70% ampicillin and 60% cotrimoxazole were resistant.But 100% sensitive to erythromycin, gentamycin, ciprofloxacin, imipenem, Augmentin, and tobramycin.

P.aeruginosa isolates showed 50% resistance to gentamycin and 25% to Ampicillin while it was 100% sensitive to Imipenem, 100% to chloramphenicol, 100% to tobramycin, 100% to erythromycin and cefoxitin. Both *Acetinobacter* spps and *Citrobacter* spps were sensitive to antimicrobial tests, but *Acetinobacter* were resistant to 100% to ampicillin.

Proteus species were 71.4% resistant to ampicillin. But 85.7% were sensitive to Imipenem, 57.1% to chloramphenicol, and 42.9% to Erythromycin and Tobramycin each. In general, Imipenem was the most sensitive to all isolates followed by Chloramphenicol, Erythromycin, and tobramycin.

Table 5: Antimicrobial susceptibility patterns of gram positive and gram negative pathogenic bacterial isolates among external ocular infected study participants at SUCSH eye clinic, from June to August 2024.

Isolated bacteria (180)	Antimicrobial susceptibility patterns, N (%)										
		PE	CHL	ERY	Gen	CPR	Fox	Tob	COT	IMP	AUG	AMP
		No (%)	No (%)	No (%)	No (%)	No (%)	No (%)	No (%)	No (%)	No (%)	No (%)	No (%)
S.aureus	S	11(12.8)	59(68.6)	58(67.4)	52(60)	16(19)	72(83.7)	72(83.7)	62(72.1)	71(82.6)	62(72.1)	27(31.4)
	I	1(1.2)	15(17.4)	16(18.6)	18(21)	63(73.3)	10(11.6)	11(12.8)	13(15.1)	11(12.8)	17(19.8)	16(18.6)
	R	74(86)	12(14)	12(14)	16(19)	12(14)	4(4.7)	3(3.5)	11(12.8)	4(4.7)	7(8.1)	43(50)
CoNS	S	7(15,6)	40(88.9)	39(88.6)	41(91.1)	40(88.9)	40(88.9)	42(93.3)	31(68.9)	43(95.6)	42(93.3)	31(68.9)
	I	0	3(6.7)	3(6.7)	0	2(4.4)	1(2.2)	0	2(4.4)	0	0	2(4.4)
	R	38(84.4)	2(4.4)	3(6.7)	4(8.9)	3(6.7)	4(8.9)	3(6.7)	12(26.7)	2(4,4)	3(6.7)	12(26.7)
S.pneumoniae	S	8(57)	10(71.4)	11(78.6)	12(85.7)	8(57.1)	9(64.3)	11(78.6)	7(50)	12(86)	11(78.6)	11(78.6)
	I	1(7)	1(7.1)	1(7.1)	0	0	2(14.3)	0	2(14)	0	0	0
	R	5(36)	3(21.4)	2(14.3)	2(14.3)	6(42,9)	3(21.4)	3(21.4)	5(36)	2(14)	3(21.4)	3(21.4)
E. coli	S	-	13(86.7)	12(80)	15(100)	15(100)	13(86.7)	15(100)	14(93.3)	15(100)	13(86.7)	7(46.7)
	R	-	2(13.3)	3(20)	0	0	2(13.3)	0	1(6.7)	0	2(13.3)	8(53.5)
K.pneumoase	S	-	10(100)	10(100)	10(100)	10(100)	8(80)	10(100)	14(93.3)	10(100)	10(100)	3(30)
	R	-	0	0	0	0	2(20)	0	1(6.7)	0	0	7(70)
P.aurogi nosa	S	-	4(100)	4(100)	2(50)	4(100)	4(100)	4(100)	3(75)	2(50)	4(100)	3(75)
	R	-	0	0	2(50)	0	0	0	1(25%)	2(50)	0	1(25)
Acetinobacter	S	-	2(100)	2(100)	1(50)	2(100)	2(100)	2(100)	2(100)	2(100)	2(100)	1(50)
	R	-	0	0	1(50)	0	0	0	0	0	0	1(50)
Citrobacter spps	S	-	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)
Proteuis	S	-	3(100)	3(100)	2(66,7)	3(100)	3(100)	3(100)	3(100)	3(100)	3(100)	1(33.3)
	R	-	0	0	1(33.3)	0	0	0	0	0	0	2(66.7)

S=sensitive,I=intermediate,R=resistance,Pen=pencilin,CHL=chloroamphenicol,ERY=erythromycin,Gen=gentamycin,CPR=ciprofloxin,FOX=cefoxitin,TOB=tobramycin,COT=cotrimoxazole,IMP=imipenem,AUG=agumentin,AMP=ampicillin.

5.5. Multidrug resistant patterns of bacterial isolates

In this study the total isolated bacteria were (n=180), multi-drug resistance (MDR) (resistance to three or more antibiotics of different classes) was detected about 135/180 (75%) of the isolates. About 21/35 (60%) of the gram-negative bacterial isolates showed MDR, of which 11/15 (73.3%) were *E. coli* isolates. The gram-positive isolates also showed MDR about 79% (114/145) of the isolates were MDR, of which 70/86 (81.4%) were *S. aureus* and 36/45 (80%) were *CoNS.*

Table 6:- Multidrug resistance profile of bacteria isolated from study participant with external ocular infection at Salale University Comprehensive Specialized Hospital, June to August, 2024

Isolated bacteria	Total bacteria isolated	MDR patterns		
		R3 No with antibiotics	R4 No with antibiotics	R5 and above No with antibiotics
S.aureus	70	53(P, AMP.E)	10(P, AMP,E,GN)	7(P, AMP,E,C,CIP,GN)
CoNS	36	20(P, AMP,GN)	10(P, AMP,CIP,C)	6(P, AMP,E,C,CIP,GN)
S.pneumoniae	8	5(P, CIP.C)	2(P, AMP, CIP, GN)	1(P.CIP.AMP.C.E.COT)
E. coli	11	6(CIP, C, Torb,)	3(AMP, GN,CIP,E)	2(CXT, E,C,CIP,GN,COT)
K.pneumoniae	6	3(AMP, GN,C)	2(AMP, GN,C,CXT)	1(AMP, C,CXT,GN,E)
p.aurogenosa	1	1(AMP, GN,CXT)	0	0
Proteus species	*2*	*2(AMP, GN,E)*	*0*	*0*
Acetinobacter	*1*	*1(GN, COT,IP)*	*0*	*0*
Total	*135*	*91*	*27*	*17*

5.6. Factors associated with the EOI culture-confirmed bacteria isolated.

Socio-demographic and clinical characteristics of the study participants suspected of external ocular infection were evaluated for the associated factor for the culture-positive bacterial isolated

in the study participants with EOI. The socio-demographic and clinical factors assessed for the association factors are age, sex, marital status, occupation, residence, education, previous used of antibiotics, using traditional medicine, history of chronic systemic disease, using medical contact lenses, hospitalization, previous eye surgery, using soap for washing face and frequency of face washing. The variables with a P-value less than 0.25 were further analyzed using multivariate logistic regression, and the multivariable analysis with p<0.05 was considered as significant association. In multivariable analysis, factors like sex and previously used antibiotic p=0.040; AOR=1.673, 95% CI (1.024-2.732) had an association with external ocular infection. The Female study participants (AOR = 0.613; 95% CI = 0.38, 0.987, 24.933, P=0.044) were 0.6 times more likely to develop bacterial EOI than those with Male participants. **Likewise,** the study participants who had previously used antibiotics (AOR = 1.673; CI = 1.024, 2.732, P=0.040) were 1.67 times more likely to develop bacterial EOI than those with counterparts (Table 7).

Table 7:- Bivariate and multivariable analyses of factors associated with external ocular infections among the study participants with external ocular infection at Salale University Comprehensive Specialized Hospital, June to August 2024.

Variables		No of tested	External ocular infection		Bivariate analysis		Multivariate analysis	
			Positive	Negative	COR (95% CI)	P-value	AOR(95% CI)	P-value
Sex	Females	142	95	47	1.617(1.007-2.595)	**0.046***	0.613(0.380-0.987)	**0.044***
	Males	153	85	68				
Marital status	Marriage	168(56.9)	108(60)	60(52.2)	1.409(705-2.817)	.332		
	Single	79(26.8)	45(25)	34(29.6)	1.036(0.484-2.217)	.928		
	Windowed	1(0.34)	0	1(0.9)	.000	1.000		
	Divorced	6(2)	4(2.2)	2(1.7)	1.565(0.257-9.256)	.627		
	Underage	41(13.9)	23(12.8)	18(15.7)	1	1		
Educational levels	unable to read and write	61(20.7)	40(22,2)	21(18.3)	1.163(.613-2.207)	.645		

	able to read and write	79(26.8)	52(28.9)	27(23.5)	1.176(0.652-2.120)	.591		
	Secondary	31(10.5)	11(6.1)	20(17.4)	.336(.148-.762)	.009	0.851(0.458-1.583)	0.610
	college and above	124(42)	77(42.8)	47(40.9)	1	1		
Residence	Urban	178(60.3)	103(57.2)	75(65.2)	1.402(0.864-2.275)	.172	1.251(.648-2.417)	.505
	Rural	117(39.7)	77(42.8)	40(34.8)	1	1		
Occupation	Gov't employer	81(27.5)	51(28.3)	30(22.6)	.944(.289-3.082)	.925		
	Farmer	47(15.9)	32(17.8)	15(13)	1.185(0.338-4.151)	.791		
	Merchant	42(14.2)	20(11.1)	22(19.1)	.505(.145-1.762)	.284		
	Student	65(22)	40(22.2)	25(21.7)	.889(.267-2.957)	.848		
	Housewife	40(13.6)	26(14,4)	14(12.2)	1.032(.289-3.680)	.962		
	daily labor	6(2)	2(1.1)	4(3.5)	.278(.037-2.092)	.214		
	Others	14(4.7)	9(5)	5(4.3)	1	1		
Used medical contact lens	No	263(89.2)	156(86.7)	107(93)	.486(.210-1.122)	.091	.479(194-1.181)	.110
	Yes	32(10.8)	24(13.3)	8(7)	1	1		
Hospitalization	No	278(94.2)	168(93.3)	110(95.7)	.636(.218-1.856)	.408		
	Yes	17(5.8)	12(6.7)	5(4.3)	1	1		
Using modern eye treatment	Yes	119	81	38	0.603(0.371-0.982)	**0.042***	1.673(1.024-2.732)	**0.040***
	No	176	99	77	1	1		
Made eye surgery	No	280(94.9)	169(94)	111(96.5)	0.554(.172-1.782)	.322		

	Yes	15(5.1)	11(6)	4(3.5)				
Frequency of face washing	once a day	85(28.8)	63(35)	22(19.1)	.895(.379-2.115)	.800		
	twice a day	168(56.9)	85(47.2)	83(72.2)	.320(.148-.692)	.004	3.125(1.444-6.761)	0.064
	three times a day	42(14.2)	32(17.8)	10(8.7)		1		

6. Discussion

In this study, the prevalence of pathogenic bacterial external ocular infection was 61% (180/295) with a 95% CI (55, 67%) which is in line with previous studies done in Ethiopia by different authors who reported the prevalence of 59.4% in ALERT (38), 59.4% Boruneda (10), 57.8% in Felege Hiwot hospital (6), 62.8% in Debre Markos hospital (11) and 60% in Shashamane hospital (39).

However, our finding is low compared to a study conducted in Jimma 74.7%,(27) and Kano state, Nigeria 88.6% (39) but it is higher than a study conducted in Minilik 54.5% (28), Jinka 34.5% (40), Baghdad, Iraq (40) and in Italy 32% ((14)). The varied rate of isolation from one place to another might be due to different distribution of bacterial etiology with geographic variation, study period, difference with the study population and infection prevention practices.

In this study, Gram-positive cocci are the most common isolates, 80.6% (n=145/180) which is similar to several other studies in Ethiopia, Debre Markos Hospital (11),jijiga, Karama Hospital (12) , Gondar (41) , and in Italy (14). In this study, the frequent bacterial isolates were *S.aureus* 29.2% (n=86/295) followed by *CoNS* 14.9% (n=44/295). There were similarities with previous works conducted in Ethiopia,(6,41,42) and in Uganda (43). In other studies the predominant isolates were *CoNS* (44,45). Because the gram-positive cocci is the normal flora of skin, the contamination of the eye from normal skin flora as a result of touching eyes with hands, cataract extraction, lens implantation, and use of contact lenses the prevalence of Gram-positive cocci may increase (46,47).

The prevalence of Gram-negative bacterial pathogens among patients suffering from external ocular infection in our study was 19.4% (n=35/180) with high prevalence *E.coli* 5.1%. In this study, the low prevalence of Gram-negative enteric bacteria in the present study could be due to effective personal hygiene as the most important mode of transmission for enteric pathogens is fecal-oral contamination of the eye (48). Moreover, there are reports that documented the main cause for Gram-negative bacteria caused infection by eye with the bacteria from another organ of the body contaminated by the bacteria (49). Other Gram-negative bacteria isolated in this study include *K.pneuomoniae, P.auerogenosa, Acetobacter spp, Proteus spp,and Citrobacter* where showed similar isolation of gram -negative bacteria that was studied done in Debre Markos,(11).

In the current study, conjunctivitis was the dominant type of eye infection 40% (n=118/295) followed by blepharitis 32% (n=94/295), keratitis 17% (n=50/295) and dacryocystitis 11% (n=33/295). A similar study done in Felege Hiwot referral hospital which showed patients were suffering from conjunctivitis followed by blepharitis, keratitis and others (6).

According to WHO the misuse and overuse of antimicrobials are the main drivers in the development of drug-resistant pathogens. The development of antimicrobial resistance affects countries at all levels (50). In this study, among the commonly used antibiotics relatively 85% and 90% of all strains were resistant to ampicillin and Penicillin respectively. While Ciprofloxacin was found to be susceptible in 80-100% of all strains except for CoNS which was 74%. These findings were in agreement with the study conducted in hawasa (51) and minilik (11).

In this study, most of bacterial isolates have shown high resistance to penicillin 68.8%, and Ampicillin 73.2%. Similar findings have been reported in Jijiga and Alert respectively, (12,38). However, tobramycin 83.7, imipenem 82.6%, ciproflaxicilin 86%, Cefoxitin 91%, Erythromycin 88.4%, and Chloramphenicol 95% showed susceptibility. In this study, the presence of higher percentage multiple antibiotic resistance patterns was common. The reason for the observed resistance might be the overuse of antibiotics, misuse of antibiotics, spontaneous resistance, and transmitted resistance(50,52)

Socio-demographic data showed that, in this study 40.3% of the external ocular infection suspected patients had a history of antibiotic usage of before they attended salale university comprehensive specialized hospital eye clinic for their infection management. These uncontrolled uses of antibiotics are fuelling the already existing natural antibiotic resistance mechanisms of bacteria and which might be responsible for the relatively higher prevalence rate of resistance to Ampicillin and Penicillin which was similar to the study done in Gondar (53).

The overall prevalence of MDR to two or more of the commonly prescribed antimicrobials were dominated by Gram-positive 79% (n=114/145). Among Gram-positive isolates CoNS 25% (n=36/145), *S. aureus* 48.3% (n=70/145) and *S. pneumoniae* 6% (n=8/145) in the present study and Gram-negative were 60% (n=21/35). The overall of observed was 75% (n=135/180) of the bacterial isolates resistant to multiple drugs in our study, which is lower than the study

conduct at Gondar 87.1% (46) and at Jijiga 87.7% (12) but higher than that study conducted by Debre Markos 59.2% (11) and at Hawassa 69.9% (42).

According to WHO the AMR occurs naturally over time, usually through genetic changes, and is found in people, animals, food, plants, and the environment (in water, soil, and air). They transferred from person to person or between people and animals, including from food of animal origin. The misuse and overuse of antimicrobials; lack of access to clean water, sanitation, and hygiene for both humans and animals; poor infection and disease prevention and control in healthcare facilities and farms; poor access to quality, affordable medicines, vaccines and diagnostics; lack of awareness and knowledge; and lack of enforcement of legislation are the main drivers of antimicrobial resistance (48).

In this study age groups, sex, occupation, residence, educational status, previous eye surgery, previous medicinal eye treatment, hospitalization, using traditional eye medicine, contact lenses, use soap for face washing, frequency of face washing, and the occurrence of systemic disease were used as possible risk and predisposing factors for infection. However, bivariate logistic regression analysis showed that a significant association was found in the female sex (p-value = 0.044) and using medicinal eye treatment (antibiotics) (p-values=0.040), which is similar to the study conducted at Gondar university (43) which shows significance with previous use of antibiotic. Similar findings were found from previous studies conducted in Shashamane (51), Debre markos (11) and Jijiga,karama (12) showed that there is no association between the socio-demographic and clinical characteristics except sex and medicinal treatments.

6.1. Limitations of the study

- ✓ Unable to isolate anaerobic bacterial pathogens like *Chlamydia trachomatis* due to limitations in our laboratory facilities. Anaerobic bacteria require specialized culture media that lack oxygen and may contain reducing agents.
- ✓ This document focuses on bacteria of infection, but other microorganisms such as fungi or viruses that may cause external ocular infection have not been studied and verified.

7. Conclusion and Recommendation

7.1. Conclusion

In the present study, the prevalence of pathogenic bacteria among external ocular infection study participants was 180(61%) with a 95% CI of 55-67%. This study showed that from gram-positive bacteria *Staphylococcus aureus* and *Coagulase negative Staphylococci.* From that of gram-negative bacteria *Escherichia coli* and *K.pnuemoniae* were the most common bacteria isolated from samples of swabs of the external ocular infected patients. The bacteria isolated showed high resistance to penicillin-G and Ampicillin while Imipenem was the best effective antimicrobial for all of the bacterial isolates. Chloramphenicol, Erythromycin, and Tobramycin were moderately effective against most of the isolates, likes *Staphylococcus aureus*, *Escherichia coli*, *Coagulase-negative Staphylococci,* and *Klebsiella pneumoniae.* Socio-demographic and clinical characteristics of the study participant were evaluated However, only the female sex (P=0.046) and modern eye treatment (P=0.042) had a significant association with external ocular infection (P<0.05).

7.2. Recommendation

- ✓ Because of the high prevalence of pathogenic bacteria observed among infected EOI patients in this study, great attention is needed to control external ocular infection.
- ✓ Ampicillin and penicillin-G were no longer effective in treatments of pathogenic bacteria. Since imipenem, tobramycin and ciproflaxicilin were the most effective antimicrobials, the clinicians can use it or in the absence of those, they may use Chloramphenicol, Erythromycin, and gentamycin for the treatment of external ocular infection due to pathogenic bacteria.
- ✓ Correctly using treatment for external ocular infection to prevent drug resistance, therefore
  using of antibiotics according to CLSI guideline is recommended.
- ✓ To increase the target chance for empirical antibiotics surveillance of bacteria profile and their susceptibility pattern on the external ocular infection is recommended.

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9. Annexes

Annex I :- Information sheet, Assent and Consent form

A. Information Sheet for Adult Participant (English Versions)

Name of Organization:- Salale University, College of Health Science Department of Medical Laboratory Science

Principal Investigator:- Girma Teshome

Title:-"Prevalence , antimicrobial-resistant patterns and associated factor of pathogenic bacteria among patients with EOI attending Salale University Comprehensive Specialized Hospital eye clinic."

Introduction

You are invited to participate in a research to be conducted by MSc candidate, from Salale University. Your participation is voluntarily. The research teams include principal investigator, advisors from Salale University, College of Health Science Department of Medical Laboratory Science. Please take as much time as you need to read or listen to the information provided here.

Purpose of the study

The purpose of the study is to determine prevalence, antimicrobial-resistant patterns and associated risk factor of pathogenic bacteria among patients with EOI attending Salale University Comprehensive Specialized Hospital eye clinic.

Procedures and the expected participation: - If you are willing to participate, you need to understand the purpose of the study. You can ask any question that is not clear to you and after that you can give your consent. You will be asked to give a small amount specimen in the sample container that we will be provided to you.

Potential risks and discomforts

swabs collection will have no effect but there might be a little sense while collecting specimens.

Confidentiality

To maintain the confidentiality of the participant's personal information, your name will be kept confidential and samples will be coded. No personal information will be disclosed to a third party or will not appear in any report from this study.

Potential benefits to participants and/or to the society

You will not receive any payment for your participation in this research study as compensation. Most importantly, this study will contribute to providing information or data for future and further nationwide study and to develop health programs for health policy makers.

Participation and withdrawal from the Study

Participation is voluntary and you have the right not to participate in this study. You may withdraw at any time without consequences of any kind. You may also refuse to give any sample. Your response to our request will not affect the service and care that you would normally get from the Hospital.

Contact Information: In case of any questions, problems, unclear ideas, and doubt relating to the study, please direct them to :-**Girma Teshome** (BSc, MSc Student), the Principal Investigator of this study on:-Phone:-+251-915963388. Email:-girmatola@gmail.com

Thank you for your Participation and Cooperation

Information sheet for Adult participant Afaan oromoo version

Waraqaa Odeeffannoo hirmaattota ga'eessotaaf

Maqaa Dhaabbatichaa;-Univarsiitii Salaale, Koollejjii Saayinsii Fayyaa,Dippaartimantii Saayinsii Meedikaal Laaboraatoorii

Maqaa Qorataa:-Girmaa Tashooma

Mata-duree Qorannoo:-**"Prevalence, antimicrobial-resistance patterns and associated factor of pathogenic bacteria among patients with EOI attending Salale University Comprehensive Specialized Hospital eye clinic"**

Seensa

Yuunivarsiitii Salaaletti kaadhimamaan digrii 2ffaa qorannoo adeemsisan irratti akka hirmaattaniif affeeramtaniittu.Hirmaannan keessan fedhii keessan irratti Kan hundaa'edha. Gareen qorattootaa Qorataa muummee,Gorsitoota Univarsiitii Salaale,Koollejjii Saayinsii Fayyaa,Dippaartimantii Saayinsii Meedikaal Laaboraatoorii irraa ramadamani fi ogeessota Hospitaala ispeeshalaayizdii Yuunivaristii Salaalee Waliin ta'uuni. Maaloo! Odeeffannoo waraqaa odeeffannoo irra jiru dubbisuuf ykn dhageeffachuuf yeroo gabaabduu fudhadhaa.

Kaayyoo Qorannichaa

Kaayyoon qorannichaaa, yeroo ammaa kana Hospitaala ispeeshalaayizdii Yuunivaristii Salaalee dhukkubsattoota sababa gara garaan alaa ijaa isaanii dhukkube dhufaniif Baakteeriyaa infeekshinii alaa ijaa fidan adda baasuu ,hamma baakteeriyaa qorichaa dandamachuu fi waantoota isaa fuudhu danda'anii adda baasanii beekudhaafi.

Adeemsa qorannichaa fi hirmaattota ta'uuf waan isin irraa eegamu
Hirmaachuuf heyyamamoo yoo taatan kaayyoo qorannichaa beekun isin barbaachisa. Gaafii siniif hin galle kamiiyyuu gaafachuudhan sana booda heyyamamoo ta'uu ni dandeesu.Meeshaalee,nuti siniif dhiyeessinetti fayyadamuun saamuda/sample/ xiqqoo qorannoof ta'u akka kennitan ni gaafatamtu.
Rakkinoota/balaa/ uumamuu danda'u
Yeroo keessan fudhachuurraa kan hafe,rakkinni isin muudatu hin jiru.

Icciitii Qorannichaa

Icciitii Dhuunfaa hirmaattotaa eeguuf,maqaan icciitin yoo eegamu, saamuda kennitaaf koodiin ni kennamaaf. Icciitin dhuunfaa kamiiyyuu qaama sadaffaaf dabarfammee hin kennamu.Akkasumas qorannoo kanaan ala dhimma biraaf hin oolu.

Qorannichi faayidaan inni hirmaattotaaf/hawaasaf argamsiisu

Qorannoo kana keessatti hirmaachuu keessaniif kanfaltiin isiniif kanfalamu hin jiru. Hundaa olitti qorannoon kun gara fuulduraatti ,qorannoo biyyoolessaaf odeeffannoo dhiyeessuu fi qaamolee poolisii fi sagantaalee fayyaa qopheessaniif bu'aa guddaa qaba.

Haala hirmaannaa/keessa bahuu /qorannichaa

Hirmaannan keessan fedha keessanirratti kan hundaa'e yoo ta'u,hirmaachuu dhiisudhaaf mirga qabdu. Yeroo fi iddoo barbaaddanitti keessaa bahuu ni dandeesu. Akkasumas saamuda hin kennu jechuu ni dandeessu. Deebiin gaafii keenyaf deebistan tajaajilaa fi kunuunsa hospitaalaa argattan kamirrattuu dhiibbaa tokkollee hin qabu.

Odeeffannoo quunnamtii

Waa'ee qorannoo kana gaafilee kamiiyyuu yoo qabaattan akkasumas odeeffannoo dabalataa yoo barbaaddan teessoo qorataa armaan gadii kana fayyadamuun dubbisuu ni dandeessu.

Girmaa Tashoomaa (BSc)- qorataa

Moobaayila:-+251-915963388.email: girmatola@gmail.com

Waan hirmaattaniif baay'ee galatoomaa!

B. Consent Form Adult Participant (English Version)

I……, after reading the information sheet and having been explained by Girma Teshome (The Principal Investigator), I do voluntarily agree to take part in this research study on "Prevalence,Antimicrobial-Resistance Patterns and associated factor of pathogenic bacteria Among Patients with EOI Attending Salale University Comprehensive Specialized Hospital". I am aware that results of the study may not benefit me directly, but it might benefit the future effective management of wound patients. For this study sample will be required. The aim and possible risk of the study were explained to me well. I understand that participation in this study is voluntary; the information collected from me will remain confidential, and they will be reported with my approval and the information to be reported is only the results without my personal information. I know there will be no financial benefit to be provided for participating in this study. The interviewer explains for me, as there is no any risk or discomfort, and extra treatment. Moreover, I have been well informed of my right to refuse information, decline to cooperate and drop out of the study if I want and none of my actions will have any bearing at all on my overall health care. Therefore, with full understanding of the information given above, I agreed to give the necessary information for laboratory analysis and participate in this study.

I _____ hereby give my consent for giving of the requested information and specimen for this study.

Participant code: _____ Signature: _____Date: -----/------/--------

Name of the interviewer _____ Signature: ---------- Date: -----/------/------

Thank You for Your Participation and Cooperation

Adult consent form Afaan oromoo version

Guca walii galtee hirmaattotaa

Ani…… waraqaa odeeffannoo ergan dubbisee ykn naaf ibsamee booda heyyama kiyyaan mata duree qorannoo **"Prevalence and antimicrobial-resistance patterns and associated risk factor of pathogenic bacteria patients with EOI attending Salale University Comprehensive Specialized Hospital"**hirmaataa tokko ta'uu koo walii galeera. Akkaataa bu'aa qorannoo kanaatin kallattin kanfaltiin anaaf kanfalamu kan hin jirre ta'uu hubadheera.Haa ta'uu malee kana booda sababa infeekshinii madaatin dhukkubsattoonni miidhaman yaalaa fi kunuunsa gahaa akka argataniif akka gargaaru nan amana. Qorannichaafis saamudni alaa ijaarra akka fudhatamu naaf ibsameera. Saamunni fudhatames rakkina kan narraa hin geenyee fi yaala kiyya irratti dhiibbaa kan hin qabne ta'uusaa hubadheera. Odeeffannoon gaafilee gaafatameef kenne hunduu icciitin isaa kan eeggame ta'uu isaa naaf ibsameera. Qorannicha irratti hirmaachuu dhiisuu fi odeeffannoo kamiiyyuu kennuu dhiisuu fi yeroon barbaadetti qorannicha keessaa of baasuuf mirgi koo eeggamaa akka ta'e naaf ibsameera. Kaayyoo qorannichaas hubadheera. Kanaafuu qorannoo kanaaf odeeffannoo kennuuf,walumaa galatti haala isaa hubadhee jecha walii galtee kiyya kennuuf heyyama kooti. Dabalataanis gaafiileen gaafachuu barbaade naaf heyyamamee ibsi barbaachisaa ta'e naaf kennameera.Hirmaataa qorannoo kana ta'uu koof kanfaltiin argadhu kan hin jirre fi bu'aan qorannoo kanaa qaamolee poolisii baasaniif,poolisii baasan irra deebi'anii akka qoratanii fi fooyyessan akka isaan gargaaru hubadheera.Walumaa galatti ani asii olitti guca wal amanamummaa irratti kan ibsame hunda hubadhee saamuda barbaachisaa fi odeeffannoo gahaa qorannoo Laaboratooriif barbaachisu kennuuf walii galeera.Kanaafuu qorannoo kana keessatti hirmaachuuf heyyamamaa ta'uu koo mallattoo kootin nan mirkaneessa.

Koodii hirmaataa _____ Mallattoo: _____Guyyaa: ------/-----/------

Maqaa gaafataa/qorataa _____Mallattoo: ---------- Guyyaa: ----/-------/-------

Hirmaannaa fi Gargaarsa keessaniif baay'ee galatoomaa!

Information sheet for parents/guardians (English version)

Name of Organization: - Salale University, College of Health Science Department of Medical Laboratory Science

Principal Investigator: -Girma Teshome

Title: - "Prevalence, antimicrobial-resistance patterns and associated factors of pathogenic bacteria among patients with EOI attending Salale University Comprehensive Specialized Hospital"

Introduction

We would like to conduct research entitled "Prevalence, Antimicrobial-Resistance Patterns and Associated Factor of Pathogenic Bacteria Among Patients with EOI Attending Salale University Comprehensive Specialized Hospital" We are requesting you allow your child to voluntarily participate in this study. We are going to inform you about the purpose, responsibility of investigators or data collectors to keep confidentiality and how we are going to use the data. Please take as much time as you need to read or listen to the information provided here.

Purpose of the study

The purpose of the study is to determine Prevalence, Antimicrobial-Resistance Patterns and associated factors of pathogenic bacteria Patients with EOI Attending Salale University Comprehensive Specialized Hospital eye clinic.

Procedures and the expected participation

If you agree for your child to participate in this study and you sign the assent form the following will be done:

- We will ask for a small amount of specimen from your child
- Review your child's medical history

- You will have an interview with us some time about the child medical condition

Potential risks and discomforts

swabs collection will have no effect but there might be a little pain while collecting specimens.

Confidentiality

To maintain the confidentiality of your child's information, your child's name will be kept confidential and samples will be coded. No personal information will be disclosed to a third party or will not appear in any report from this study. Potential benefits to participants and/or to the society. Your child will not receive any payment for the participation in this research study as compensation. Most importantly, this study will contribute to providing information or data for future and further nationwide study and to develop health programs for health policymakers.

Participation and withdrawal from the Study

Your child's participation is voluntary, and you have the right to refuse your child's participation in this study. You can stop your child from participating in the study at any time after giving your consent. You may also refuse to give any sample from your child. Your response to our request will not affect the service and care that your child would normally get from the hospital.

Contact Information: In case of any questions, problems, unclear ideas and doubt relating to the study, please direct them to:

Girma Teshome (BSc, MSc Student), the Principal Investigator of this study: Phone:-+251-915963388. Email:-girmatola@gmail.com

Thank you for your Participation and cooperation.

Information Sheet for Parents/Guardians (Afaan oromoo Version)
Maqaa Dhaabbatichaa;-Univarsiitii Salaale, Koollejjii Saayinsii Fayyaa,Dippaartimantii Saayinsii Meedikaal Laaboraatoorii

Mata-duree Qorannoo**"Prevalence,antimicrobial-resistance patterns and associated factors of pathogenic bacteria among wound infected patients attending Salale University Comprehensive Specialized Hospital "**

Maalummaa qorannichaa: - "Pathogenic bacteria" Jedhamuun baakteeriyoota beekkaman yoo ta'an, baacteeriyaa dhukkuboota garaagaraa fidanii,qorichootan wal baranii haala salphaan baduu hin dandeenye fi waantoota dhukkubicha fidan addaa baasu dha. Haaluma kanaan rakkoon kun yeroo ammaa kana cimaa waan dhufeef rakkina kana hir'isuuf dhukkubsatoota infeekshinii alaa ijaan wal qabatee hospitaala koompireensivi ispeeshaalayzii yuuniversiitii salaaletti yaalamaa jiranirratti qorannoo adeemsisuu yaannerra.
Adeemsa qorannichaa fi hirmaattota ta'uuf wantoota isinirraa eegamu

Qorannoo kana keessatti mucaan keessan/kan guddistan/ akka irratti hirmaatu/ttu yoo walii galtan wantoota armaan gadii kana ni taasifna. mucaan keessan/kan guddistan/ irraa saamuda xiqqoo ni fudhanna.qorannichaaf kan gargaaru raga yaalaa mucaa keessanii ni ilaalla akkasumas isin ni gaafanna

Qorannoo kana keessatti hirmaachun rakkina maalii fida?

Qorannoo kana keessatti hirmaachuu rakkina tokkollee hin fidu,garuu yeroo saamuda fudhannu dhukkubbii xiqqoon mucaa keessanitti dhagahamuu danda'a.
Icciitii qorannichaa

Odeeffannoon waa'ee mucaa keessanii kamiiyyuu akkasumas bu'aan qorannoo laaboraatoorin argame kaayyoo qorannoo kanaatif qofa oola.Saamuda fuudhameef koodiin ni kennamuu fi Odeeffannoo kenname arguu kan danda'u namoota muraasa qorannoo kana adeemsisan qofaadha.

Bu’aalee qorannoo kana keessatti hirmaachuun fidu maali?

Mucaan keessan qorannoo kana keessatti hirmaachuu isaaf/isheef faayidaan kanfaltii argamu hin jiru. Hundaa ol qorannoon kun gara fuulduraatti fi irra caalatti qorannoo biyyoolessaaf odeeffannoo kennuu fi qopheessitoota poolisii fayyaatif sagantaalee fayyaa qopheessuf gahee guddaa gumaacha,

Mirgi qorannoo kana keessatti hirmachuu keessanii maalidha?

Mucaan keessan qorannoo kana keessatti kan hirmaatu/ttu guutumaam guututti heyyama keessan irratti kan hundaa’e waan ta’eef, yeroo fi iddoo barbaaddanitti mucaa keessan qorannoo kana adda kuchisiisuu ni dandeessu,akkasumas sababa adda kuchisiiftaniif tajaalli hospitaala keessatti kennamaa ture kamiiyyuu adda hin jitu.Dabalataanis Qorannicha ilaalchisee gaafii qabdan kamiiyyuu gaafachuu fi deebii argachuuf mirga qabdu. Odeeffannoon isin waa’ee mucaa keessanii nuuf kennitan rakkinichaa hambisuu fi too’achuuf waan fayyaduuf gaafilee siniif dhiyaatef deebii kallattii akka nuuf deebistan kabaja guddaan isin gaafanna.

Odeeffannoo quunnamtii

Qorannoo kana ilaalchisee ykn qorannoo kanaan wal qabatee mucaa keessan balaa/rakkina/quunnamuu danda’u kamiiyyuu ilaalchisee gaafii yoo qabaattan teessoo kanaan fayyadamaa:

Girmaa Tashoomaa (BSc, MSc Student), Principal Investigator Lakk.Bilbilaa: +**251-915963388** email: -**girmatola@gmail.com**

Hirmaannaa fi gargaarsa keessaniif baay’ee galatoomaa!

Assent Form for Children Participant (English Version)

Name of study participant ----------
Name of the participant's family or Guardian ----------
I _____ understand that my parents /guardian have/has given permission for me to take part in a research entitled as **"Prevalence, antimicrobial-resistance patterns and associated factors of pathogenic bacteria among patients with infection attending Salale University Comprehensive Specialized Hospital ."**

The study participant mentioned above who is not able to give informed consent himself because he/she is younger than 18 years not allowed deciding on him/her.

• I agree samples to be collected from me.

• I understand that the information will be confidential.

• I understand that I can stop the study at any time.

• I understand it will not affect my current and future medical services.

• I agree that there is no payment as compensations for me.

It is therefore with full understanding; by taking a full responsibility, I gave my assent voluntarily to the researcher to use the information and specimen for this study. Participant code: _____

Participant's signature/Finger print ---------- Date: ----------/--------/---------
Participant's family/Guardian signature ---------- Date: ----------/--------/---------
Name of the interviewer _____ Signature: ---------- Date: -------/--------/--

Thank you for your Participation and cooperation

Assent form for Children Participant (Afaan oromoo Version)

Maqaa ijoollee hirmaatuu----------

Maqaa maatii/Guddistuu ----------

Ani----------kanan jedhamu maatin/guddistuun koo

“Prevalence,antimicrobial-resistance patterns of pathogenic bacteria among patients infection attending Salale University Comprehensive Specialized Hospital ” Mata duree jedhuun qorannoo adeemsifamu irratti akkan hirmaadhu waliigaluu isaanii hubadheera. Anis saamuda kennuuf walii galeera.

- Odeeffannoon ani kennu icciitiidhan akka qabamu hubadheera.
- Yeroo kamittuu qorannicha adda kutuu akkan danda’u hubadheera.
- Qorannicha keessatti hirmaachuu diduun/adda kutuun/ammas ta’ee gara fuulduraatti tajaajila yaalaa argadhu irratti dhiibbaa akka hin qabne hubadheera.
- Qorannicha keessatti hirmaachun koo kanfaltii tokko malee ta’uusaa hubadheera.
- Walumaagalatti ani guca waliigaltee armaan oliitti eerame kana hubadhee saamudaa fi odeeffannoo gahaa qorannoo laaboraatoorif barbaachisu kennuuf walii galeera. Kanaafuu, qarannoo kana keessatti hirmaachuuf heyyamammaa ta’uu koo mallattoo koon nan mirkaneessa.

Koodii hirtaataa _____ Mallattoo: _____Guyyaa: -----/------/-------
Mallattoo Maatii/guddistuu---------- Guyyaa: ----------/--------/----------
Maqaa Gaafataa/Qorataa _____Mallattoo: ---------- Guyyaa: -----/------/------

Hirmaannaa fi gargaarsa keessaniif baay’ee galatoomaa!

Salale University College of Health Sciences, Department of Medical Laboratory Science, medical microbiology.

Annex II:-Questionnaire

Questionnaire format for the assessment of pathogenic bacteria among patients with infection

Date of interview_____ Patient Code No_____			
A Background information			
No	Question	Answer	Skip
1	Sex	Female ☐ male ☐	
2	Age	☐	
3	Marital status	1. Married 2. Single 3. Widowed 4. Divorced 5 underage	
4	Educational level	1. Illiterate 2.Read and write 3. Primary 4. Secondary 5. Collage and above	
5	Residence	1.rural 2.urban	
6	Occupation	1. Government employee 2. Farmer 3. Merchant 4. Student 5. Housewife 6. Daily laborer 7. Others (specify____)	
B .Clinical information about infection			
7	Are you using medical contact lenses?	1. No 2. Yes	
8	Which systemic diseases do you have?	1. Rheumatoid and Arthritis 3. Diabetes 2. Hypertension 4.Other's 5.I don't know	
9	Having been hospitalized for long Period of times?	1. No 2.yes, if yes for how long period?	
10	Have you used traditional eye medicine?	1.no 2.yes	
11	Do you take a medicine to treat eye External ocular infection?	1.no 2.yes,	

12	Have you made surgery of eye?	1.no 2.yes		
13	Are you washed your face using soap?	1.no 2.yes		
14	Frequency of face washing	1 once a day 2.twice a day 3.three times a day 4 no washing		
Name and Signature of the Data Collector/Interviewer_____				
C:- Outcome of laboratory investigation				
14	Name of pathogen isolated	Gram positive	Gram negative	
15	Antibiotics that are effective against the isolate			
16	Antibiotics that are ineffective against the isolate			
Name and signature of the laboratory Technologist/Microbiologist:_____				
Thank You for Your Participation and Cooperation!				

Annex III: - LABORATORY PROCEDURE

A). Sample Collection, Handling and Transport

1. Objective and Scope: To describe the specimen collection instructions and subsequent handling of specimens by the Researcher (Laboratory Technologist) for the culture of bacteria. This document contains the procedure for clinical specimens containing bacteria from the lower eye lid, conjunctival swabs, Blepharitis, Dacryocystitis and Traumatized eyes for processing at Salale University College of Health Science Medical Microbiology Laboratory.

2. Procedure: An adequate specimen was essential for the success for culture of bacteria from surface. Specimens will be collected with the greatest care and transferred to the laboratory skill room promptly.

3. Requirements/Materials:

i). Specimen collection tubes with sterile cotton swabs

ii). Transportation media

4. Specimen Collection

After detailed ocular examinations, samples will be collected by swabbing from eyelid and conjunctiva using sterile cotton-tipped swab moistened with sterile saline.
5. Transport of specimen to salale University College of Health Science Microbiology laboratory:

Specimens will be immediately transported to the Microbiology Laboratory for culture.

B). Specimen Processing.

I. Culture

A. McConkey agar:

Principle

MacConkey agar is used for the isolation of gram-negative enteric bacteria and the differentiation of lactose fermenting from lactose non-fermenting gram-negative bacteria. Pancreatic digest of gelatin and peptones (meat and casein) provide the essential nutrients, vitamins and nitrogenous factors required for growth of microorganisms. Lactose monohydrate is the fermentable source of carbohydrate. The selective action of this medium is attributed to crystal violet and bile salts, which are inhibitory to most species of gram-positive bacteria. Sodium chloride maintains the osmotic balance in the medium. Neutral red is a pH indicator that turns red at a pH below 6.8 and is colorless at any pH greater than 6.8. Agar is the solidifying agent.

Procedure for the Preparation of MacConkey agar

1. Suspend 49.53 grams of dehydrated medium in 1000 ml of distilled water.

2. Heat to boiling to dissolve the medium completely.

3. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

4. Cool to 45°C -50°C.

5. Mix well before pouring into sterile Petri plates and use as necessary

Pink colonies = Lactose fermenters (LF): e.g. *E. coli, Klebsiella*

Pale colonies = non-lactose fermenters (NLF): e.g. *Salmonella, Shigella*, etc

B. Blood Agar (BA)

Principles

It is used to detect and differentiate haemolytic bacteria,especially Streptococcus species. It is also a differential media in allowing the detection of hemolysis (destroying the RBC) by cytolytic toxins secreted by some bacteria, such as certain strains of *Bacillus, Streptococcus, Enterococcus, Staphylococcus, and Aerococcus.*

Procedure for the Preparation of Blood Agar

a.Suspend 40.5 grams in 1000 ml distilled water or deionized water.

b. Heat to boiling to dissolve the medium completely.

c. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

d. Cool to 45-50°C and aseptically add 7% sterile sheep blood.

e. Mix well and pour into sterile Petri plates. Avoid the formation of air bubbles. You must have warmed the blood to room temperature at the time of dispensing to a molten agar base.

f. Dispense 15 ml amounts to sterile Petri plates aseptically.

g. Label the medium with the date of preparation and give it a batch number.

C. Mannitol Salt Agar

Principles

Mannitol Salt Agar is a nutritive medium due to its content of peptones and beef extract, which supply essential growth factors, such as nitrogen, carbon, sulfur and trace nutrients. The 7.5% concentration of sodium chloride results in the partial or complete inhibition of bacterial organisms other than staphylococci. Mannitol fermentation, as indicated by a change in the phenol red indicator, aids in the differentiation of Staphylococcal species. Agar is a solidifying agent.

Procedure

1. Aseptically streak the sample on the plate

2. Incubate for 24-48 hours at 37 0c

3. After the incubation period, observe the agar for bacterial growth

4. Also, observe the plate for a change in the color of the agar. The presence of a distinct yellow color indicates fermentation of the mannitol sugar by S.aureus. Other Staphylococci species will not change the color of the agar. Gram-positive cocci will be identified based on their gram reaction, catalase and Coagulase test results.

II. Gram stain

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

Procedure:

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

4. Place air-dried smears fix with methanol for one minute and colony allows fixing with heat passing 2-3 times; allow drying before staining.

5. Flood the prepared slide with crystal violet for one minute.

6. Rinse the slides gently with tap water.

7. Flood the slide with Gram's iodine for one minute.

8. Rinse the slide gently with tap water.

9. Flood the slide with decolorized for 5 seconds and rinse with tap water Work with one slide at a time.

10. Flood the slide with Safranin for one minute.

11. Rinse the slide gently with tap water.

12. Drain the slide in an upright position. Blot the back of the slide and place on a slide warmer or heating block to completely dry.

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

14. Scan 20-40 fields using oil immersion.

Result interpretation:

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

III. Biochemical tests

1. Catalase test

Catalase test to differentiate Staphylococci which produce the enzyme catalase from Streptococci which are non-catalase producing.

Principle

Catalase acts as a catalyst in the breakdown of hydrogen peroxide to oxygen and water.An organism is tested for catalase production by bringing it into contact with hydrogen peroxide. Bubbles of oxygen are released if the organism is a catalase producer.

Procedure

1. Pour a drop of 3% hydrogen peroxide to a slide

2. using a sterile wooden stick take the test organism and mix with the hydrogen peroxide

Solution

3. Look for immediate bubbling

Interpretation:

Active bubbling . . . . . Positive catalase test: e.g. *Staphylococcus* species

No bubbles . . . . . Negative catalase test: e.g. *Streptococcus* species

2. Coagulase test

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

Principle

Coagulase causes plasma to clot by converting fibrinogen to fibrin.54

Procedure

a. Slide coagulase test

1. Place a drop of physiological saline on two separate slides

2. Emulsify the test organism in each of the drop to make 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

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

No clump formation within 10 secs . . . . . No bound Coagulase (confirm with tube

coagulase test)

b.Tube coagulase test

1. Place 1 ml volumes of the plasma in small tubes.

2. Emulsify several isolated colonies of test organism in 1 ml of rabbit plasma to give a milky suspension.

3. Incubate the tube at 35°C in ambient air or in a water bath for 4 hours.

4. Examine at 1, 2, and 4 hours for clot formation by tilting the tube through 90°.

5. Leave negative tubes at room temperature overnight and re-examine

Coagulase Positive: Clot of any size Coagulase. e.g: *Staphylococcus aureus*

Negative: No clot (plasma remains wholly liquid or shows only a flocculent or ropy precipitate Controls. e.g: *Escherichia coli*

3. Oxidase test

Principle

A piece of filter paper is soaked with a few drops of oxidase reagent. A colony of the test organism is then smeared on the filter paper. Alternatively an oxidase reagent strip can be.

If the organism is oxidase-producing, the phenylenediamine in the reagent will be oxidized to a deep purple colour.

Procedure

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

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

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

Results Blue-purple colour . . . . . Positive oxidase test (within 10 seconds)

No blue-purple colour . . . . . Negative oxidase test (within 10 seconds)

Note: Ignore any blue-purple colour that develops after 10 seconds.

4. Urease Test

Principle

To determine the ability of an organism to produce an enzyme urease which splits urea to ammonia? Ammonia makes the medium alkaline and thus phenol red indicator changes to pink/red in color.

Procedure

The test organism is inoculated on the entire slope of Christensen's medium which contains urea and phenol red indicator in addition to other constituents including agar. It is incubated at 37°C and examined after 4 hours and after overnight incubation. Christensen's urease medium contains: · Peptone water · Urea (20%) · Agar · Phenol red.

Interpretation

Urease positive: Purple-pink color. e.g: *Klebsiella sp., Proteus sp.yersinia enterocolitica*,

Urease negative: Pale yellow color. e.g: *E. coli, Providencia sp., Yersinia pestis*

5. Citrate Utilization

Principle:

This is a test for the ability of an organism to utilize citrate as the sole carbon and energy Source for growth and an ammonium salt as the sole source of nitrogen with resulting alkalinity. Method: Koser's liquid citrate medium or solid Simmons' citrate agar may be used. Simmon's citrate medium contains agar, citrate and bromothymol blue as an indicator. Original color of the medium is green. A part of colony is picked up with a straight wire and inoculated into either of these media. Incubate at 37°C for 96 hours

Interpretation

Simmons' Citrate Medium Positive: Blue color and streak of growth.

Negative: Original green color and no growth.

Koser's Citrate Medium Positive:Turbidity, i.e. growth. e.g : *Klebsiella sp.,*

Enterobacter sp. Serratia spp, Citrobacter s

Negative: No turbidity.e.g*: E.coli, Edwardsiella, Shigella spp, Salmonella typhi*

6. Indole Production

Principle: This test demonstrates the ability of certain bacteria to decompose the amino acid tryptophane to indole, which accumulates in the medium. Tryptophan is decomposed by an enzyme tryptophanase produced by certain bacteria.

Method: Indole production is detected by inoculating the test bacterium into peptone water (tryptophan rich) and incubating it at 37°C for 48–96 hours. Add 0.5 mL Kovac's reagent and shake gently. Tryptophan Tryptptophanase Indole Kovac's reagent consists of:

Paradimethylaminobenzaldehyde 10 g · Amyl or isoamyl alcohol 150 mL · Concentrated hydrochloric acid 50 mL.

Interpretation;- Indole positive: A red color in the alcohol layer.

Indole negative: Yellow colored ring (color of Kovac's reagent) near the surface of the Medium.

IV. Laboratory procedure for Antimicrobial sensitivity testing Procedure

1. Emulsify several colonies of similar appearance of test organism in small volume of Nutrient broth.

2. Match the turbidity of the suspension against the turbidity of 0.5 McFarland standards.

3. With a sterile swab take sample from the suspension (squeeze the swab against the side of the test tube to remove the excess fluid).

4. Spread the inoculum evenly over the Muller Hinton agar plate with the swab.

5. Using a sterile forceps, place the antimicrobial discs on the inoculated plate.

6. Incubate the plate aerobically at 35-37 0c for 16-18 hours.

7. Read the tests after checking that the bacterial growth of the test organism is neither too heavy nor too light.

8. Measure the radius of the inhibition zone. Interpret the reaction of the test organism to each antibiotics used as sensitive, intermediate, or resistance as per the standard

10. Different figures during thesis finding



Media preparation



Dispensing the media



Dispensed biochemical



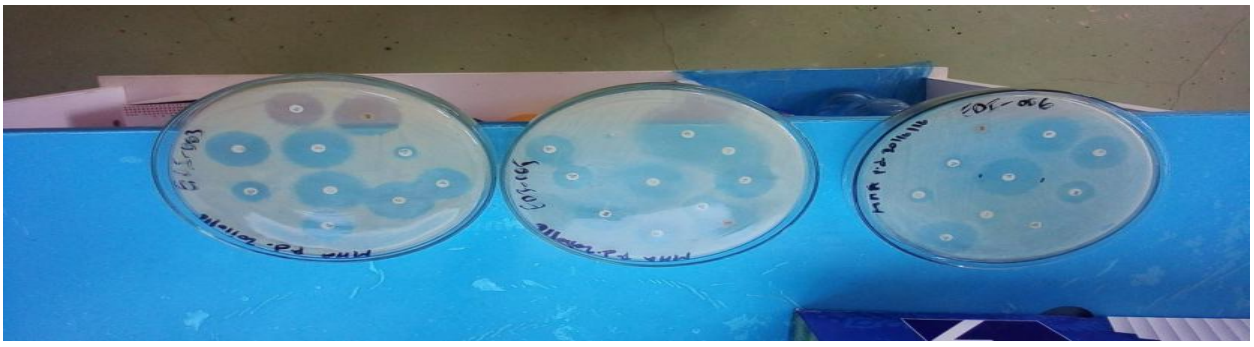
Inoculating specimen on media



Gram stain



Biochemical reading



Different AMS test

Declaration Sheet

Submited by:

Girma Teshome (BSc) _____ 13/4/2017

(MSc candidate) (Signature) (Date: DD/MM/YY)

Approved by:

1. Advisor

Dr. Tadesse Bekele (PhD in Medicinal Chemistry) _____ _____ 22/12/24

(Name) (Signature) (Date: DD/MM/YY)

2. Co-advisor

Mr.Fedasan Alemu (BSc, MSc in Medical Microbiology) 14/04/2017

(Name) (Signature) (Date: DD/MM/YY)