



St. Paul's Hospital Millennium Medical College

Department of Microbiology, Immunology and Parasitology

Detection of Bacterial Etiological Agents, Antibiotic Susceptibility and Associated Risk Factors from Patients with Ear Infections at St. Paul's Hospital Millennium Medical College

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Approval by the board of examination

As a member of the examining board of the final MSc open defence, we hereby certify that we have read and evaluated the thesis entitled Detection of Bacterial Etiological Agents, Antibiotic Susceptibility and Associated Risk Factors from Patients with Ear Infections at St. Paul's Hospital Millennium Medical College presented by Semegn Desalegn. The thesis has been examined and approved in its present form as fulfilling the requirements for the degree of Master of Science in Medical Microbiology

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Supervisors Approval

This is to certify that the thesis prepared by Semegn Desalegn the thesis entitled Detection of Bacterial Etiological Agents, Antibiotic Susceptibility and Associated Risk Factors from Patients with Ear Infections at St. Paul's Hospital Millennium Medical College and submitted in fulfillment of the requirement for the Degree of Master of Science (MSC) in Medical Microbiology Complies with the regulation of the College and meets the accepted standard with respect to originality and quality.

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Declaration

I Semegn Desalegn, hereby declare that the thesis entitled The detection of bacterial etiological agent, antibiotic susceptibility and associated risk factors from ear infection patients at St. Paul's Hospital Millennium Medical College is my original work and has not been previously used for any academic award. I confirm that all source used have been duly acknowledged and cited according to the required academic convention.

Investigator: - Semegn Desalegn

Signature _____

Date _____

Abstract

Background

Ear infection refers to inflammation of the ear and represents a significant health problem affecting both adults and children. Ear discharge is among the most common clinical manifestations of ear infections. Otitis media, in particular, is a leading cause of healthcare visits and antibiotic prescriptions. Its complications and long-term sequelae constitute major, yet preventable, causes of hearing loss, especially in developing countries. In resource-limited settings, antibiotic therapy is frequently prescribed empirically without guidance from culture and antibiotic susceptibility testing (AST). Consequently, inappropriate use and overuse of antimicrobial agents contribute to the emergence and spread of antimicrobial resistance. Therefore, identifying the predominant bacterial etiologies of ear infections, determining their antimicrobial susceptibility patterns, and assessing associated risk factors are essential for mitigating the multidimensional impact of ear diseases.

Objective: Detection of Bacterial Etiological Agents, Antibiotic Susceptibility and Associated Risk Factors from Patients with Ear Infections at St. Paul's Hospital Millennium Medical College.

Method: A prospective cross-sectional study was conducted at St. Paul's Hospital Millennium Medical College Ear, Nose and Throat Outpatient department. Information with regard to risk factors and clinical characteristics was gathered by well-structured pre tested questionnaire and ear swab samples were collected from all 224 participants by attending physician, nurse and the investigator (laboratory technologist). The samples were cultured on MacConkey agar, Blood agar and chocolate agar simultaneously. Positive growths were sub cultured and biochemical test were performed with standard microbiological technique. Quality control and standard operating procedures were followed for quality and reliability of the result. The data was entered into Excel sheet and analyzed using SPSS version 27 software computer packages. Descriptive statistics, including frequency, mean and percentages, was computed. To analyze the risk factors,

univariable and multivariable logistic regression models was employed. A P-value of less than 0.05 was considered statistically significant.

Results: - Of 224 ear discharge samples, Bacterial culture positivity rate was 126(56.3%) of which 31(13.85%) were with mixed isolates and 95(42.45%) with single isolate. So there was a total of 157 bacterial isolate from 31 mixed bacteria which is 62 isolate and 95 single isolate. Out of a total of 157 bacterial isolate, 122(77.7%) were gram negative bacteria and 35(22.3%) gram positive bacterial isolates. The predominant bacterial etiological agents detected were *Staphylococcus aureus* 30(19.1%), *Pseudomonas species* 24(15.3%), *Klebsiella oxytoca* 20(12.7%), *Citrobacter species* 17(10.8%) and *E.coli* 16(10.2%). Fungal elements of 8(3.6%) was observed microscopically from gram stain. The most Effective antibiotics for GPB was Cefoxitin, Gentamycin and Trimetoprim-Sulphamethoxazole and resistant to Macrolide class (erythromycin), Tetracycline's class, Lincomycin class (clindamycin) and Ciprofloxacin. The most Effective antibiotics for GNB was Carbapenems class (meropenem), Cephalosporin class (cefepime and ceftazidim), Aminoglycosides class (Gentamycin and amikacin) and Fluoroquinolones class (Ciprofloxacin) and resistant to Penicillin's class (Ampicillin and Augmentin) and Tetracycline's class (Tetracycline). Among the socio demographic factors and clinical characteristics, Age group, number of rooms that the patient reside and history of head trauma was significantly associated with bacterial ear. Overall MDR rate among ear discharge bacterial isolates was 52(33.1%) of which 35(28.70%) were GNB and 17(48.60%) GPB.

Conclusion:- *S.aureus*, *Pseudomonas species*, *Klebsiella oxytoca*, *Citrobacter species* and *E.coli* were the leading cause of ear infection. Most of the ear discharge isolate were resistant to antibiotics classes of Penicilline, Tetracycline, Macrolide and Lincomycin class. However, the antibiotics like Aminoglycosides, cephalosporins, Fluoroquinolones, and Carbapenems were the most effective in treating ear discharge bacterial isolates. These result help as a baseline for effective treatment and guide the empirical treatment. Age group, history of head trauma and small number of room was significantly associated with ear infection. Identification of these specific risk factors will adds a public health relevance. The presence of high rate resistant strains calls for periodic and

continuous follow-up of antimicrobial drug susceptibility testing and update of empirical treatment guidelines.

Key Words: Otitis Media, Antimicrobial resistance, Multidrug resistance and Risk factors.

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Acronyms and Abbreviations

AOM	Acute Otitis Media
AST	Antimicrobial Susceptibility Testing
COM	Chronic Otitis Media
ENT	Ear Nose Throat
GPB	Gram Positive Bacteria
GNB	Gram Negative Bacteria
MDR	Multidrug Resistance
OM	Otitis Media
OME	Otitis Media with Effusion
OPD	Outpatient Department
SPHMMC	St. Paul's Hospital Millennium Medical College
SOP	Standard Operating Procedure
WHO	World Health Organization

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1, Introduction

1.1, Background

Ear infection refers to an inflammatory disorder affecting the external ear (otitis externa), middle ear (otitis media), or inner ear (otitis interna), and is most commonly caused by bacterial, viral, or fungal pathogens. Otitis externa involves infection of the external auditory canal and is frequently associated with moisture exposure and local trauma. Otitis media is characterized by infection of the middle ear and often occurs as a complication of upper respiratory tract infections. Otitis interna, also known as labyrinthitis, affects the inner ear structures and may result in disturbances of hearing and balance ¹.

Otitis media (OM) or middle ear inflammation is a spectrum of diseases, including acute otitis media (AOM), otitis media with effusion (OME) and chronic suppurative otitis media (CSOM). Acute otitis media (AOM) is the presence of fluid along with a rapid onset of signs and symptoms of inflammation in the middle ear. Otitis media with effusion (OME) is the presence of ear effusion in the absence of acute infection. Whereas; COM is defined when ear effusion (fluid) remains in the middle ear for a long time ².

Otitis media (OM) is among the most common childhood illnesses globally, particularly affecting young children. Although many cases of OM resolve spontaneously without complications, the condition may be associated with hearing impairment and long-term sequelae. In developing countries, chronic suppurative otitis media (CSOM) remains a leading cause of preventable hearing loss. Otitis media may be of viral or bacterial etiology; during upper respiratory tract infections, viral pathogens may ascend through the Eustachian tube to the middle ear, facilitating secondary infection by bacterial otopathogens colonizing the nasopharynx. The diagnosis of otitis media is based on characteristic clinical signs and symptoms. Acute otitis media (AOM) is typically characterized by acute ear pain and bulging of the tympanic membrane, while otitis media with effusion (OME) is commonly associated with hearing impairment. Diagnostic evaluation includes pneumatic otoscopy, which remains the cornerstone of AOM

diagnosis and involves assessment of tympanic membrane color, opacity, position, and integrity. Additional diagnostic modalities include tympanometry, which objectively evaluates tympanic membrane mobility and middle ear function, and audiometry, which assesses hearing thresholds. The management of AOM primarily focuses on symptomatic treatment of ear pain and fever, with antibiotic therapy reserved for children with severe, persistent, or recurrent infections. In contrast, the management of OME generally involves a period of watchful waiting, with the placement of ventilation (tympanostomy) tubes considered mainly for children with chronic middle ear effusion accompanied by hearing loss, developmental delay, or learning difficulties ².

Infection involving the middle ear that is accompanied by persistent ear discharge (otorrhea) through a perforated tympanic membrane is most commonly classified as chronic suppurative otitis media (CSOM). Otorrhea refers to the drainage of fluid from the ear and typically signifies infection of the external or middle ear. In cases of chronic middle ear disease, the discharge is usually purulent or mucopurulent, persistent or recurrent, and frequently occurs through a tympanic membrane perforation. Persistent otorrhea reflects ongoing inflammation and infection of the middle ear mucosa and is strongly associated with hearing impairment ³.

Chronic suppurative otitis media is defined as a chronic inflammatory condition of the middle ear and mastoid cavity, characterized by persistent or recurrent ear discharge through a permanent tympanic membrane perforation, generally lasting two weeks or longer and often extending for much longer periods. CSOM commonly arises as a complication of inadequately treated or recurrent acute otitis media and is particularly prevalent in low- and middle-income countries. Clinically, patients typically present with chronic ear discharge, conductive hearing loss, and occasionally a sensation of ear fullness or discomfort. CSOM is broadly classified into tubotympanic (safe) and atticotympanic (unsafe) types, with the latter being associated with cholesteatoma and an increased risk of serious complications. CSOM represents a significant public health concern and remains one of the leading preventable causes of hearing loss, particularly among children. Early identification and appropriate management of ear discharge are

essential to prevent long-term auditory deficits and potentially life-threatening intracranial complications ³.

Recent studies from Canada, the United States, the Netherlands and the UK suggest a decline in OM incidence since the mid-1990s. This decline is attributed to the introduction of clinical guidelines that recommend stricter diagnostic criteria and the judicious use of antibiotics in OM as well as the introduction of pneumococcal conjugate vaccination. By contrast, studies from developing countries and indigenous populations continue to demonstrate a heavy burden of OM, particularly CSOM and its complications ².

In low-income countries, antibiotic prescribing is frequently performed without reference to culture and antibiotic susceptibility testing (AST) results. This practice contributes to the inappropriate use of antimicrobial agents, thereby promoting the emergence and spread of antimicrobial resistance. Furthermore, many healthcare professionals in these settings lack adequate awareness of local antimicrobial resistance patterns within their health facilities. As a result, the provision of optimal patient care remains challenging unless clinicians adhere to evidence-based antibiotic prescribing guided by AST results and actively minimize the misuse and overuse of antimicrobial agents to curb the continued development of resistant bacterial strains ⁴.

The etiologies of ear infections can be bacteria, viruses, or fungi. However, bacteria such as *Pseudomonas aeruginosa* (*P. aeruginosa*), *Staphylococcus aureus* (*S. aureus*), *Streptococcus pneumoniae* (*S. pneumoniae*), *Streptococcus pyogenes* (*S. pyogenes*), *Haemophilus influenzae* (*H.influenzae*), and *Proteus* species are predominantly responsible for the infection ⁵.

Upper respiratory tract infections, a prior history of acute otitis media (AOM), low socioeconomic status, exposure to passive smoking, poor nutritional status, and other related factors are recognized risk factors for the development of otitis media (OM). If left untreated, AOM may progress to chronic otitis media (COM) and lead to serious complications, including tympanic membrane perforation, facial nerve paralysis, meningitis, and mastoiditis. Otitis media is a common condition in childhood and is

frequently associated with concurrent infections of the upper or lower respiratory tract. One of the major clinical consequences of otitis media is conductive hearing loss ^{2,6}.

Challenges in ear and hearing care extend to the health system level, where the capacity to effectively integrate such services is often limited. Despite the substantial and well-documented need for ear and hearing care, available data from many countries indicate inadequate service provision across all levels of the health system. Although interventions for the prevention, early detection, and management of ear diseases and hearing loss should be implemented at the community and primary healthcare levels, these services remain largely unavailable in low- and middle-income countries due to shortages of essential equipment, specialized personnel, and infrastructure. Furthermore, the availability of ear and hearing care services at secondary and tertiary levels varies considerably by region and income level. Globally, only approximately 38% of the population has access to newborn hearing screening services, and recent studies indicate that nearly one-third of countries have minimal or no newborn hearing screening programs ⁷.

This study will clearly identify the prevention methods, identification and management of ear disease. Help to reduce the infection severity, prevent complications, improves patients(communities) knowledge and behavior regarding ear infections to avoid harmful practices and seek treatment earlier and better treatment.

1.2, Statement of the problem

WHO report of 2021, Over 1.5 billion people currently experience some degree of hearing loss. The prevalence and incidence rate ear infection varies across regions and countries from 3.64% in central Europe to more than 43% in parts of sub-Saharan Africa. Globally, more than 3 in 1000 people have hearing loss due to otitis media of varying severity. It is estimated that each year 21,000 people die as a result of otitis media complications, such as mastoiditis, meningitis and brain abscess ⁷.

If Acute otitis media left untreated, otitis media may progress to purulent infection, frequently accompanied by tympanic membrane perforation and subsequent complications, including recurrent acute otitis media and persistent middle ear effusion ⁸. Otitis media constitutes a significant public health concern affecting individuals of all sexes and age groups, particularly in low–socioeconomic settings where delayed access to medical care contributes to an increased risk of complications ⁸.

Bacterial pathogens represent the most frequent cause of ear infections, with a growing number of cases attributable to multidrug-resistant organisms. This situation underscores the need to critically review current approaches to the management of ear infections and to ensure the rational selection of effective antimicrobial agents ⁹.

In many cases, clinicians manage ear infections empirically or follow standard treatment guidelines (STGs) without incorporating laboratory findings or antimicrobial susceptibility testing (AST) results. This practice creates a gap in effective management, increasing the risk of infections caused by multidrug-resistant bacteria. Consequently, it contributes to a higher financial burden and adversely affects patients' quality of life ¹⁰.

Ethiopia experiences a high incidence of otitis media, necessitating prompt and effective intervention. Inappropriate antibiotic regimens or inadequate treatment may result in irreversible damage, highlighting the importance of understanding the disease's prevalence, etiological profile, and associated risk factors ¹¹.

Early diagnosis and appropriate management of otitis media are critical for patient health, particularly among children, as the condition can lead to complications ranging from tympanic membrane perforation to neurological impairments. However, the implementation of timely and effective interventions remains challenging in sub-Saharan Africa due to limited access to advanced medical facilities ¹².

The etiology, prevalence, and antimicrobial resistance patterns of ear infections vary across geographic regions and are influenced by climatic conditions, local antibiotic

prescribing practices, and the prevalence of resistant bacterial strains ¹³. The increasing and irrational use of broad-spectrum antibiotics has accelerated the development and dissemination of antimicrobial resistance among bacterial isolates, representing a major global public health threat ¹⁴. Furthermore, the risk factors associated with ear infections are multifactorial and include cultural, socioeconomic, environmental, genetic, nutritional, and medical determinants ¹⁵⁻¹⁷.

Due to limited awareness and inadequate microbiology laboratory capacity, clinicians often prescribe antibiotics empirically, without guidance from culture and antimicrobial susceptibility testing, when managing presumed cases of otitis media. This practice may contribute to the emergence and spread of antimicrobial resistance. Therefore, the present study aims to identify the bacterial etiological agents responsible for ear infections, which is essential for guiding the selection of effective antimicrobial therapy, monitoring bacterial distribution patterns, and assessing variations in antimicrobial susceptibility. In addition, the study will analyze associated risk factors in order to strengthen evidence-based prevention and control strategies.

1.3, Significance of the study

The result of the study will help as a baseline for effective treatment of the ear infection. The disease will be managed timely and appropriately. The finding of this study will strengthen the prevention and control measures of ear infection. Implementation of this and other studies reduces the complication associated with ear infection. Furthermore, prescribing antibiotics based on antibiogram results generated from this and related studies within the country provides a rational strategy to optimize treatment and mitigate the emergence of antimicrobial resistance.

2, Literature Review.

2.1 overview of ear infection

Ear infections represent a common health problem affecting both children and adults. The etiology, prevalence, and antimicrobial resistance patterns of ear infections vary across different geographic regions and are influenced by climatic conditions, local antibiotic prescribing practices, and the prevalence of resistant bacterial strains ¹⁸.

Otitis media constitutes a significant global health problem, with high incidence and prevalence in both developed and developing countries. The health and economic burden associated with ear infections is particularly substantial in Africa and other low- and middle-income regions. In children, ear infections represent a major public health concern and are frequently associated with adverse outcomes, including hearing impairment, delayed speech and language development, and impaired academic and educational performance ^{19,20}.

Acute otitis media is the most frequently diagnosed form of ear infection and is characterized by inflammation and infection of the middle ear. Chronic ear infections may adversely affect a child's learning ability and can have long-term consequences on quality of life and overall development ²¹.

Common ear conditions, including otitis media, can be effectively managed through medical and surgical interventions, which reduce associated morbidity and mortality and may prevent or reverse hearing loss related to these conditions. It is estimated that approximately 60% of childhood hearing loss is attributable to preventable causes, including vaccine-preventable diseases, ear infections, perinatal factors, and exposure to ototoxic medications ²².

Vaccination against common bacterial and viral pathogens associated with otitis media plays an important role in reducing disease incidence. The primary objective of these vaccines is to reduce or eliminate nasopharyngeal colonization by key otopathogens, including *Streptococcus pneumoniae*, non-typeable *Haemophilus influenzae*, and *Moraxella catarrhalis*. The seven-valent pneumococcal conjugate vaccine (PCV7),

introduced in the United States and several European countries in 2000, was associated with a 29% reduction in acute otitis media (AOM) caused by pneumococcal serotypes included in the vaccine, a 6–7% overall reduction in AOM incidence, and a 20% decrease in the use of ventilation tube placement for chronic or recurrent otitis media. Subsequently, the thirteen-valent pneumococcal conjugate vaccine (PCV13), introduced approximately a decade later, has been associated with further reductions in the incidence of AOM, mastoiditis, and the need for ventilation tube insertions ²³.

Early identification and appropriate management of otitis media are essential for preventing the onset or progression of hearing loss. As chronic suppurative otitis media (CSOM) frequently develops as a consequence of inadequately treated acute otitis media (AOM), emphasis should be placed on the timely diagnosis and effective management of AOM to prevent recurrence and progression to chronic ear disease. Furthermore, comprehensive evaluation and appropriate medical and surgical management of individuals with CSOM and nonsuppurative otitis media (NSOM) can prevent or reverse auditory impairment and reduce the risk of recurrent infections ²⁴.

Otorrhea and the associated hearing impairment can and should be effectively addressed through access to high-quality ear care services. The primary objectives of managing discharging ears are to achieve a dry, infection-free ear and to restore or improve hearing function. While medical therapy may be sufficient in some cases to control ear discharge and enhance hearing, surgical intervention is often required to achieve definitive eradication of infection and sustained improvement in hearing outcomes. One of the most commonly employed management approaches is ear cleaning, or aural toileting, which involves the removal of discharge, pus, and debris from the ear using various techniques. These procedures may be performed by the affected individual, family members, trained community health workers, or primary healthcare providers. Common techniques include wicking, suctioning, and ear irrigation. Aural toileting offers the advantage of being repeatable and requiring minimal equipment and technical expertise. However, it should not be regarded as a standalone therapeutic intervention and must be integrated with appropriate medical or surgical management for optimal outcomes ².

The incidence of acute otitis media (AOM) is highest among infants aged 6 to 11 months. In Europe, prospective birth cohort studies conducted in countries such as Germany and the Netherlands have reported a cumulative prevalence of approximately 35% by the age of two years. By three years of age, an estimated 50–85% of children experience at least one episode of AOM. Similarly, a retrospective cohort study conducted among pediatric populations in Spain indicated that approximately 60% of children had experienced AOM by the age of four years ²⁵.

The judicious use of antibiotics is essential to reduce the development and spread of antimicrobial resistance. Clinical practice guidelines vary across countries with regard to antibiotic use for acute otitis media. Guidelines from the United Kingdom, the United States, and France recommend antibiotic therapy for all children younger than two years of age. In contrast, guidelines from countries such as the Netherlands and Scotland advocate for antibiotic use only in cases presenting with severe symptoms, persistent clinical signs, or associated comorbidities. Furthermore, French and Italian guidelines recommend antibiotic therapy for children older than two years only when severe symptoms are present, with initial observation and reassessment within 48–72 hours if antibiotics are withheld at the first clinical encounter ²⁵.

Acute otitis media (AOM) is the most prevalent pediatric infection, with an incidence of 60–80% during the first six years of life and a substantial risk of recurrence. Recurrent AOM, defined as four or more episodes within a year, is common and affects up to 20% of children under one year of age, while up to 40% of older children experience six or more episodes over time. Chronic otitis media (COM) is characterized by persistent inflammation of the middle ear cleft and predominantly affects younger age groups. Without timely and appropriate management, COM can lead to destructive and irreversible complications, representing the leading cause of childhood hearing impairment in developing countries ²⁶.

Chronic suppurative otitis media (CSOM) represents the greatest clinical burden for otologists in Nigeria, with a prevalence of 11.1% reported in the study population. The majority of patients presenting with CSOM reside in rural areas, highlighting the

contribution of socioeconomic factors to disease prevalence. Most rural inhabitants in Nigeria have limited education, low income, and inadequate access to healthcare services, conditions that contribute to a high incidence of complications, including subperiosteal mastoid abscess and intracranial suppuration. Delays in seeking appropriate medical care for ear discharge are common, and initial treatment is often obtained from unlicensed and untrained vendors. The indiscriminate sale and use of systemic antibiotics by such sources further promote the development of bacterial antibiotic resistance^{20,27}.

Although ear infections primarily affect infants and young children, adults can also be affected. Children are more susceptible due to the anatomical configuration of the Eustachian tube, which allows easier passage of pathogens from the nasopharynx^{6,28}. In developing countries, untreated otitis media often progresses to purulent infection, frequently accompanied by tympanic membrane perforation and subsequent complications. These may include recurrent acute otitis media, persistent middle ear effusion, hearing impairment, mastoiditis, meningitis, chronic otitis media, brain abscess, and sepsis^{6,20,29}.

2.2 Epidemiology of ear infection and drug resistance pattern of bacterial isolate across the world

According to a very recent systematic review (2025) of community-based studies worldwide, Chronic Suppurative Otitis Media (CSOM) is a long-lasting middle ear infection often associated with ear discharge and perforated eardrum affecting about 3.8% of the global population. That corresponds to roughly 297 million people worldwide. Of those with CSOM globally, $\approx 21.5\%$ have bilateral disease (i.e. both ears affected). Hearing loss is common among those with CSOM in the pooled data, 62% had "disabling hearing loss" (defined as $> 25\text{--}30$ dB hearing threshold) according to the review³⁰.

In addition to CSOM, broader analyses (including acute ear infections and other forms) show a substantial global burden: according to a 2024 study of upper respiratory infections (URIs) and ear infections, there were 391 million new episodes of Otitis Media globally in 2021, across all age groups. The incidence (new cases per population) is

highest in early childhood, particularly children under 2 years old, and peaks in children aged 2–4 years old ³¹.

The global economic burden is also substantial, because ear infections (particularly chronic or recurrent) lead to frequent health-care visits, treatments, and when leading to hearing loss result long-term disability with consequences for education, employment, and quality of life ³².

Major bacterial causes of acute otitis media (AOM) and discharging ear disease worldwide are *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis* (AOM) and *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Proteus spp.*, *Klebsiella spp.* (chronic/discharging ears). There is widespread and rising resistance to many β -lactams (penicillins, some cephalosporins) in some regions; macrolide and trimethoprim-sulfamethoxazole resistance varies by country. However, susceptibility to ciprofloxacin, gentamicin and some topical agents is often higher for discharging/chronic ear infections. (Findings come from global systematic reviews and narrative reviews of AOM/CSOM) ³⁰.

2.3 Epidemiology of ear infection and drug resistance pattern of bacterial isolate in Africa

Meta-analysis focused on Otitis Media with Effusion (OME) in Africa (i.e. fluid in the middle ear without acute infection but potential for hearing problems) estimated an overall prevalence of 6% (95% CI: 5–7%) across Africa. The prevalence differed by age: about 8% in children (95% CI: 7–9%) and about 2% in adolescents/adults (95% CI: 0.1–3%) in the pooled data. However, in that meta-analysis, regional variation within Africa was substantial: East Africa (which includes Ethiopia) had one of the lower reported estimates for OME ($\approx$ 2%, 95% CI 1–3%) ³³.

Because many of the affected live in low- and middle-income countries (LMICs), and risk factors (malnutrition, overcrowding, limited access to health care, poor hygiene, low immunization coverage) are more frequent, Africa especially Sub-Saharan Africa bears a disproportionate share of the global burden of ear disease and hearing loss³⁰.

P. aeruginosa and *S. aureus* are the dominant isolates in many Sub-Saharan Africa studies, with frequent isolation of *Proteus*, *Klebsiella* and other Gram-negatives. MDR is high resistance to first-line drugs: Systematic reviews/meta-analyses of Sub-Saharan Africa report high levels of resistance to commonly used antibiotics (ampicillin/amoxicillin, cotrimoxazole) often >50% and in many pooled estimates >70% for older agents and variable resistance to cephalosporins. This means empiric therapy with older oral agents is frequently unreliable in many Sub Saharan Africa settings. Some agents (e.g., ciprofloxacin, gentamicin) still show better activity in parts of Sub Saharan Africa but susceptibility varies widely by site and over time so local lab data matter ¹³.

2.4 Epidemiology of ear infection and drug resistance pattern of bacterial isolate in Ethiopia

Data from Ethiopia suggest ear infections and their sequelae are common, especially among children and in clinical settings. A recent (2024) systematic review and meta-analysis of bacterial ear infections in Ethiopia found a pooled prevalence of bacterial ear infections of 73.4% (95% CI: 65.1–81.6) among patients suspected of ear infection in healthcare settings. Meta-analysis noted substantial variation between studies (prevalence in individual studies ranged from ~ 28.2% to 98.2%), likely reflecting differences in study populations (age, hospital vs community), methodology, and regional variation ³⁴.

In Ethiopia the prevalence of ear infection is high estimating from 28.5%-98.2% in different parts of Ethiopia study^{5,6,15,35}. According to a 2023 national hearing survey in Ethiopia (age 5+), overall prevalence of hearing loss was 18.4%. Factors strongly associated with hearing loss included ear discharge, wax impaction, and abnormal tympanic membrane (which may reflect previous or ongoing ear disease) ³⁶.

The 2024 meta-analysis also warned about high rates of antimicrobial resistance in bacterial ear infections and complicating treatment. A 2024 systematic review & meta-analysis of Ethiopian studies found a pooled MDR prevalence of 72.5% among bacterial ear isolates (based on 14 studies and 3,667 isolates). That indicates extremely high levels of multidrug resistance among clinical ear isolates in Ethiopia³⁴. Multidrug resistance rate

is also high from studies conducted in Ethiopia 45.72% at Gondor³⁷ and 43% in Bahirdar³⁸.

Leading bacteria are *Staphylococcus aureus*, *Proteus spp.*, *Pseudomonas aeruginosa*, and *Escherichia coli* with Gram-negative organisms frequently predominant in discharge/CSOM samples. Typical Ethiopian resistance patterns show frequent high resistance to Ampicillin/Amoxicillin, Tetracyclines, and Trimethoprim-sulfamethoxazole; many isolates meet MDR definitions (resistance to ≥ 3 classes). ESBL-producing *Enterobacteriales* and other multi-mechanism resistant strains have been reported in ear isolates³⁴.

Key drivers of MDR in ear infections are:- Frequent empirical antibiotic use (often without culture), Limited microbiology/lab capacity in many settings, few culture and AST results to guide therapy, Recurrent/chronic infections and biofilms (biofilm-associated infections such as CSOM are harder to eradicate and facilitate MDR), Poor access to care, overcrowding, and delayed treatment result more severe/chronic disease that selects for resistant strains.

Worldwide, there is an increase population of MDR bacteria, which is becoming a serious concern for healthcare professionals and the population at large. Due to different economic and social related factors, the burden of AMR is greater in developing countries, where lack an antimicrobial stewardship program, limited diagnostic facilities and inadequate patient education^{22,23}.

According to reports of many studies, *P. aeruginosa*, *S. aureus*, *P. mirabilis*, *K. pneumoniae* and *E.coli* are the common organisms isolated from cases of ear infection. *S. pneumoniae* and *H. influenzae*, in particular the non-typable strains (NTHi), are responsible for up to 80% of bacterial AOM³⁹.

Increased antimicrobial resistant bacteria in ear infection can led to the development of complications such as meningitis and brain abscess. In addition, ear infection may leads to tumor in the middle ear, post aural swelling and aural sinus complications^{6,29,37}.

Up-to-date information on microbial resistance needs to be available at national and local level to guide the rational use of the existing antimicrobials.

3, Objective

3.1. General objectives

To determine bacterial etiological agent, their antibiotic susceptibility and associated risk factors from ear infection patients at St. Paul's Hospital Millennium Medical College.

3.2. Specific objective

- To determine bacterial etiological agents of ear infection among patients at SPHMMC
- To determine the antimicrobial susceptibility profiles of bacterial isolates from ear infection patients at SPHMMC
- To analyze the associated risk factors of ear infection at SPHMMC.

4, Method

4.1. Study area

This study was conducted at St. Paul's Hospital Millennium Medical College (SPHMMC) ENT OPD. SPHMMC is found in Ethiopia, Addis Ababa Gulele Sub-city. It was established through a decree of the council of ministers in 2010 although the medical school was opened in 2007 and the hospital was established in 1968 by the late emperor Hailesilase, it is governed by board under the federal ministry of health.

ENT OPD is an active department that provides specialized care for head and neck. There are many equipment and materials for diagnosis of ear these are otoscope, ear specula, cotton-tipped swabs, Jobson Hornes probe, Tuning fork, Audiometry, Endoscopes/Video otoscopes and etc. For the patients with ear complaint of ear discharge antibiotics are prescribed empirically (general adapt to local antibiogram). Ear discharge samples were sent for microbiology Laboratory for the cases that are CSOM with persistent otorrhea, treatment failure, or prior antibiotics.

4.2. Study period

This study was conducted from March 2024 to November 2025.

4.3. Study design

A hospital-based prospective cross-sectional study design was conducted at SPHMMC ENT OPD and study participants were recruited purposefully from those who were coming with complaint of ear infection until the sample size is achieved. Information with regard to risk factors was gathered by well-structured pre tested questionnaire by attending physician or nurse.

4.4. Source population

The source population was all patients attending SPHMMC of ENT OPD with Complaint of ear problems and have ear discharge during the study period.

4.5. Study population

Clinically suspected patients of ear infection and who had ear discharge and came for treatment at SPHMMC ENT OPD during the study period.

4.6. Exclusion and Inclusion criteria

4.6.1. Inclusion criteria

Patients clinically suspected of ear infection attending SPHMMC ENT OPD and had a symptom of ear discharge and willing to be included in the study.

4.6.2. Exclusion criteria

Ear infection suspected patients who were on antimicrobial treatment within two weeks prior at the time of sample collection and those who were under otitis media surgery were excluded.

4.7. Study size

Single Population Proportion for cross sectional study was used.

By using 95% confidence interval, Z value is 1.96 with 5 % margin of error (W=5%)

$\sqrt{P}$ = estimated prevalence rate =80.4% (4) taken from study conducted at Bahir Dar ⁶.

$$Z_{\alpha/2}=1.96$$

$$P \text{ value}=0.05$$

$$n= Z(\alpha/2)^2 p(1-p) / w^2,$$

$$n= \frac{(1.96)^2 \times 0.804(0.164)}{(0.05)^2}$$

$$=203 + (10\% \text{ Non-response rate}) =203+21= 224$$

The total number of clinically suspected ear infection patients who participate in the study was 224 from April-July 2024.

4.8. Sampling procedure

Purposive sampling procedure was used during the study period.

4.9. Study measurements

4.9.1 Study variable

The dependent variables were otitis externa; otitis media and hearing loss (otitis interna). The independent variables are age, gender, residence, monthly income, educational status, Number of rooms, Over all knowledge, History of exposure to loud sound, history of habit of introducing foreign bodies in to the ear, history of head trauma, history of chronic disease, habit of ear wash, history of smoking, duration of breast feed ,history of hospitalization, history of upper respiratory tract infection, treatment option and vaccination status.

4.9.2 Instruments/materials

The instruments that was used in this study were WHO standard check list (questionnaire), Specimen collection tools (sterile cotton swab, sterile container), Triple package, Culture medias like:- MacConkey agar, Blood agar and Chocolate agar media for bacterial growth support. biochemical test like:- TSI, Citrate, LYSIN test, Oxidase test, Indole, Catalase, coagulase and Urease test for bacterial identification. Antibiotic drugs like Ampicillin, amoxicillin, Gentamicin, ciprofloxacin, amoxicillin-clavulanic acid, chloramphenicol, tetracycline, Meropenem, Cefepime, Tobramycin, Ceftazidime, Amikacin, ceftriaxone, piperacillin was used for GNB and Penicillin, Cefoxitin, Vancomycin, chloramphenicol, tetracycline, erythromycin, ciprofloxacin, clindamycin, Trimetoprim- sulphamethoxazole and ceftriaxone was used for GPB.

4.10. Data and specimen collection

4.10.1. Questioners

Questionnaires' was developed based on WHO guideline and literature review to capture all potential risk factors associated with ear infection and antibiotics resistance for possible isolates. The questionnaire was written in English and translated in to Amharic. Each participant was interviewed.

4.10.2. Sample collection and transportation

Qualified (Resident doctors) and experienced professionals (Nurse and The Investigator) collected ear swab sample, Ear discharge samples were collected aseptically from the external auditory canal under direct visualization, avoiding contact with the tympanic membrane from ear infection suspected participant. The sample was transported with amies transport media to the college of microbiology unit.

4.10.3.1 Isolation and Identification of Bacteria

All the specimens were aseptically inoculated onto a series of culture media separately, which comprise 5% sheep blood agar, chocolate agar and MaConkey agar. The MaConkey agar plates were incubated aerobically at 37°C for 24 hours. The chocolate agar, as well as blood agar plates were kept in a candle jar under a microaerophilic atmosphere (5–10% O₂ and 10% CO₂) and incubated at the same temperature for 48 hours. The identification of isolates were performed as per the published standard bacteriological procedures ⁴⁰.

4.10.3.2 Antibiotic Susceptibility Testing

The antimicrobial susceptibility tests was done by the Kirby-Bauer disc diffusion ⁴¹. method and the results were interpreted as per the Clinical and Laboratory Standards Institute (CLSI) guidelines 2022) ⁴⁰. The selection of antibiotics was based on standard treatment guidelines followed by hospitals and also as per the CLSI guidelines 2022. The following antibiotic discs (Oxoid, Basingstoke, Hampshire, UK) was used for Gram-negative bacteria (GNB), such as ampicillin (AMP, 10µg), amoxicillin (AMX, 30µg), amoxicillin-clavulanic acid (AMC, 30µg), piperacillin (PIP, 100µg), ceftriaxone (CRO, 30µg), cefotaxime (CTX, 30µg), cefepime (FEP, 30µg) and meropenem (MEM, 10µg). European committee on antimicrobial susceptibility testing guidelines was used for antibiotic drugs like chloramphenicol (CHL, 30µg), gentamicin (GEN, 10µg), amikacin (AMK, 10µg), tetracycline (TET, 30µg), ciprofloxacin (CIP, 5µg), and sulfamethoxazole-trimethoprim or co-trimoxazole (SXT, 25µg).

In the case of Gram-positive bacteria (GPB), penicillin, amoxicillin-clavulanic acid, cefoxitin (FOX 30µg), vancomycin (VAN, 30µg), chloramphenicol, tetracycline, erythromycin (ERY, 15µg), ciprofloxacin, co-trimoxazole, and clindamycin (CLN, 300 µg) were used.

To conduct the antimicrobial assay, standard inoculums of each bacterial isolate was prepared at 0.5 McFarland, and lawn cultures was performed on Mueller–Hinton agar plates. The minimum and maximum number of antibiotics discs placed on a plate was 7 and was 12 respectively and then plates were incubated at 35– 37°C for 24 hours.

4.11. Data quality and management

A structured questionnaire was developed for face-to-face interview. The questionnaire was developed in English and translated into Amharic. The questionnaire was pre-tested on 5 patients who were not included in the study.

Two qualified professionals on data collection from study site was selected. Their duties and responsibilities was explained well. One senior laboratory technologist (The principal investigator) was on the duty of data collection, sample collection and processing.

The principal investigator was also part of maintaining the validity of the data during data collection and was monitoring the overall activities of data collection. There was strict supervision during data collection and the collected data was checked for completeness, accuracy, clarity, and consistency by the principal investigator.

A standard operating procedure for specimen collection and processing was used to maintain a good quality data. Standard bacteriological method was followed to maintain correct laboratory test results. Standard reference strains *S.aureus* (25923), *P. aeruginosa*(27853), *Moraxella*(25238) and *E.coli*(25922) was used to control quality of culture and susceptibility testing.

Quality control tests was done or checked by a senior laboratory technologist and microbiologist before starting any test to follow quality assurance. The samples were tested in accordance with the laboratory's Standard operating procedure (SOP) after the

quality control pass has been confirmed. The data were double entered in to excel sheet computer and exported analyzed using SPSS version 27 software statistical packages.

4.12. Operational definition:

Allergy: - When an immune system overreaction to a normally harmless substance or allergen. When experience allergy symptoms, your nasal passageways, sinuses and even Eustachian tubes become inflamed. This inflammation causes the Eustachian tubes to become blocked and fluid to become trapped in the middle ear. This can then become a breeding ground for bacteria and results otitis media.

Asthma: - is a chronic lung disease causing airways to become inflamed and narrowed leading to symptoms like wheezing, coughing, chest tightness and shortness of breath.

Upper respiratory tract infection: - an illness caused by a viral or bacterial infection of the nose, sinuses, pharynx and larynx or trachea.

Chronic disease: - is a health condition or disease that persistent or long-lasting in its effects that can last for more than three months. Those diseases are like diabetes, heart disease, cancer, asthma and hypertension.

Otitis externa: - Otitis externa is a condition that causes inflammation (redness and swelling) of the external ear canal.

Otitis media: - Otitis media is inflammation or infection located in the middle ear.

Otitis interna: - Otitis interna is an inflammation that is located in your inner ear. This condition is more commonly known as labyrinthitis or vestibular neuritis.

Tonsillopharyngitis:- is an infection and inflammation of the tonsils and pharynx, commonly known as sore throat, which can be caused by viruses or bacteria.

4.13. Data Analysis and procedure

Data were entered in to Excel sheet and analyzed using SPSS version27 software. Descriptive statistics including frequency, mean and percentage was computed and the results were presented using Tables. to analyse the relationship between the study variables, univariate and multivariate logistic regression model was used P-value < 0.05 is considered as a statistically significant.

4.14. Ethical consideration

Ethical approval was obtained from St. Paul's Hospital Millennium Medical College Ethical Review board (IRB). Full explanation about the purpose of the study was clearly explained to department of ENT and other concerned bodies prior to the actual data collection time. Study participants was recruited after they are informed about the objectives and purpose of the study and a written informed consent were obtained. The respondents were informed of their right to refuse or agree to be part of the study, or discontinue their participation whenever they need. Confidentiality of the data was maintained during data collection.

5, Results

5.1 Socio-demographic, clinical and risk behavioral characteristics of study participant.

During the study period, 224 ear discharge samples were collected at St. Paul's Millennium Medical College of ENT department and then tested for the presence of bacterial pathogens at SPHMMC Microbiology Laboratory. Among study participant female to male ratio was 1.26:1 with 125(55.8) female patient and 99(44.2) male patients. The minimum age being 1 years old and the maximum being 75 years old. The median age of the participant was 27 years (IQR 18-39 years) and the mean age was 29.4 years (SD 16.5 years) whereas residence wise 163(72.8%) were from urban areas whereas 61(27.2) were from Rural areas, were more complaint with ear infection were from urban or here in Addis Ababa. Income wise, most of the ear compliant patients were under low economic status with monthly income of below 5000 (**Table 1**).

The overall knowledge regarding ear infection was assessed and 137(61.2) were non knowledgeable and 87(38.8) were knowledgeable regarding the cause, prevention and consequence of ear infection, which showed most of the ear complaints were non knowledgeable regarding ear infection (**Table 1**).

On the other hand, 129(57.6%) of them had no habit of introducing foreign bodies in the ear and the remaining 95(42.4%) have the habit of doing so; and 43(19.2%) have history of exposure to loud sound were 181(80.8%) had no exposure to loud sound. One important factor assessed was history of head trauma, were 36(16.1) had history of head trauma and 188(83.9%) had not have history of head trauma (**Table 1**). Likewise, 113(50.4%) have habit of ear wash, 111(49.6) not had habit of ear wash. 72(32.1%) were smokers and 142(67.9) had no history of smoking and 50(22.2%) had history of upper respiratory infection were as 174(77.7) not had history of upper respiratory infection (**Table 1**).

The presence of chronic disease was also assessed and most of the ear compliant have no history of chronic disease with 200(83.9%) and most ear complaint had history of taking medicine 166(74.1%). Furthermore, 38(17%) have a history of not being treated,

16(7.1%) use traditional medicine and 4(1.8%) used medicine from pharmacy without being diagnosed. In contrast, 36(16.1%) had history of hospitalization last 3 months, 188(83.9%) had no history of hospitalization last 3 months and finally 112(50%) were vaccinated and the rest 112(50%) were not vaccinated (**Table 1**).

Table 1 The distribution of ear infection with sociodemographic and clinical characteristics in relation to bacterial infection at St. Paul's Millennium Medical College, Ethiopia, 2025 .N=224: Total sample, n=126: Bacterial culture positive, n= 95 bacterial culture negative.

Characteristics			Bacterial infection	
		Frequency (%)	Yes N(%)	No N(%)
Age group	Under 2 years	9 (4)	4 (3.2)	5 (5.1)
	3-11year	17 (7.6)	10 (7.9)	7 (7.1)
	12-18year	31 (13.8)	17 (13.5)	14 (14.3)
	19-45	135 (60.3)	75 (59.5)	60 (61.2)
	46-65	25 (11.2)	14 (11.1)	11 (11.2)
	65 and above	7 (3.1)	6 (4.8)	1 (1)
Sex	Female	125 (55.8)	75 (59.5)	50 (51)
	Male	99 (44.2)	51 (40.5)	48 (49)
Residence	Urban	163 (72.8)	92 (73)	71 (72.4)
	Rural	61 (27.2)	34 (27)	27 (27.6)
Education status	Illiterate	40 (17.9)	25 (19.8)	15 (15.3)
	Primary	74 (33)	42 (33.3)	32 (32.7)
	Secondary	42 (18.8)	20 (15.9)	22 (22.4)
	Preparatory	24 (10.7)	12 (9.5)	12 (12.2)
	University	44 (19.6)	27 (21.4)	17 (17.3)
Monthly income	low below 5000	174 (77.7)	99 (78.6)	75 (76.5)
	middle 10000	33 (14.7)	18 (14.3)	15 (15.3)
	above 10000	17 (7.6)	9 (7.1)	8 (8.2)
Overall knowledge	Yes	87 (38.8)	51 (40.5)	36 (36.7)
	No	137 (61.2)	75 (59.5)	62 (63.3)
The habit of introducing a foreign body into the ear	Yes	95 (42.4)	51 (40.5)	44 (44.9)
	No	129 (57.6)	75 (59.5)	54 (55.1)
History of exposure to loud noise	Yes	43 (19.2)	28 (22.2)	15 (15.3)
	No	181 (80.8)	98 (77.8)	83 (84.7)
History of head trauma	Yes	36 (16.1)	15 (11.9)	21 (21.4)
	No	188 (83.9)	111 (88.1)	77 (78.6)

Habit of ear washing	Yes	113 (50.4)	66 (52.4)	47 (48)
	No	111 (49.6)	60 (47.6)	51 (52)
History of smoking	Yes	72 (32.1)	41 (32.5)	31 (31.6)
	No	152 (67.9)	85 (67.5)	67 (68.4)
History of upper respiratory infection	Allergic rhinitis	17 (7.6)	11 (8.7)	6 (6.1)
	Allergic rhinitis and Tonsillopharyngitis	5 (2.2)	3 (2.4)	2 (2)
	Tonsillopharyngitis	20 (8.9)	11 (8.7)	9 (9.2)
	Adenoid	3 (1.3)	1 (0.8)	2 (2)
	Asthma	5 (2.2)	3 (2.4)	2 (2)
	None	174 (77.7)	97 (77)	77 (78.6)
Breast feed	Six months	11 (4.9)	5 (4)	6 (6.1)
	One year	99 (44.2)	52 (41.3)	47 (48)
	Two year	114 (50.9)	69 (54.8)	45 (45.9)
Chronic disease	Hypertension	13 (5.8)	8 (6.3)	5 (5.1)
	Hypertension and Diabetic Mellitus	2 (0.9)	1 (0.8)	1 (1)
	Diabetic Mellitus	2 (0.9)	1 (0.8)	1 (1)
	Diabetic Mellitus and Hypertension	2 (0.9)	2 (1.6)	0 (0)
	HIV	4 (1.8)	1 (0.8)	3 (3.1)
	HIV and Diabetic Mellitus	1 (0.4)	1 (0.8)	0 (0)
	None	200 (89.3)	112 (88.9)	88 (89.8)
Treatment	Prescription medicine	166 (74.1)	97 (77.2)	69 (70.4)
	Traditional medicine	16 (7.1)	11 (8.7)	5 (5.1)
	Self-medicated from the pharmacy	4 (1.8)	3 (2.4)	1 (1)
	Not treated	38 (17)	15 (11.9)	23 (23.5)
History of hospitalization (last 3 months)	Yes	36 (16.1)	21 (16.7)	15 (15.3)
	No	188 (83.9)	105 (83.3)	83 (84.7)
Vaccination	Yes	112 (50)	69 (54.8)	43 (43.9)
	No	112 (50)	57 (45.2)	55 (56.1)
Total		N=224 (100)	n= 126 (100)	n=98 (100)

5. 2. Frequency and distribution of etiologic agents

From a total of 224 samples, 126(56.3%) of the ear discharge sample were bacterial culture positive,

31(13.85%) of them had mixed isolate and 95(42.45) with single isolates. So there was a total of 157 bacterial isolate of which 31 mixed bacteria had 62 isolate and 95 with single isolate . Out of the total of 157 bacterial isolate, 122(77.7%) were gram negative bacteria and 35 (22.3%) were gram positive bacteria. The predominant bacteria were *S. aureus* 30(19.1%) *Pseudomonas species* 24(15.3), *Klebsiella oxytoca* 20(12.7), *Citrobactor species* 17(10.8%) and *E. coli* 16(10.2) respectively, (**Figure 1**). A fungal element of 8(3.6%) observed on gram stain microscopically from a total 224 samples.

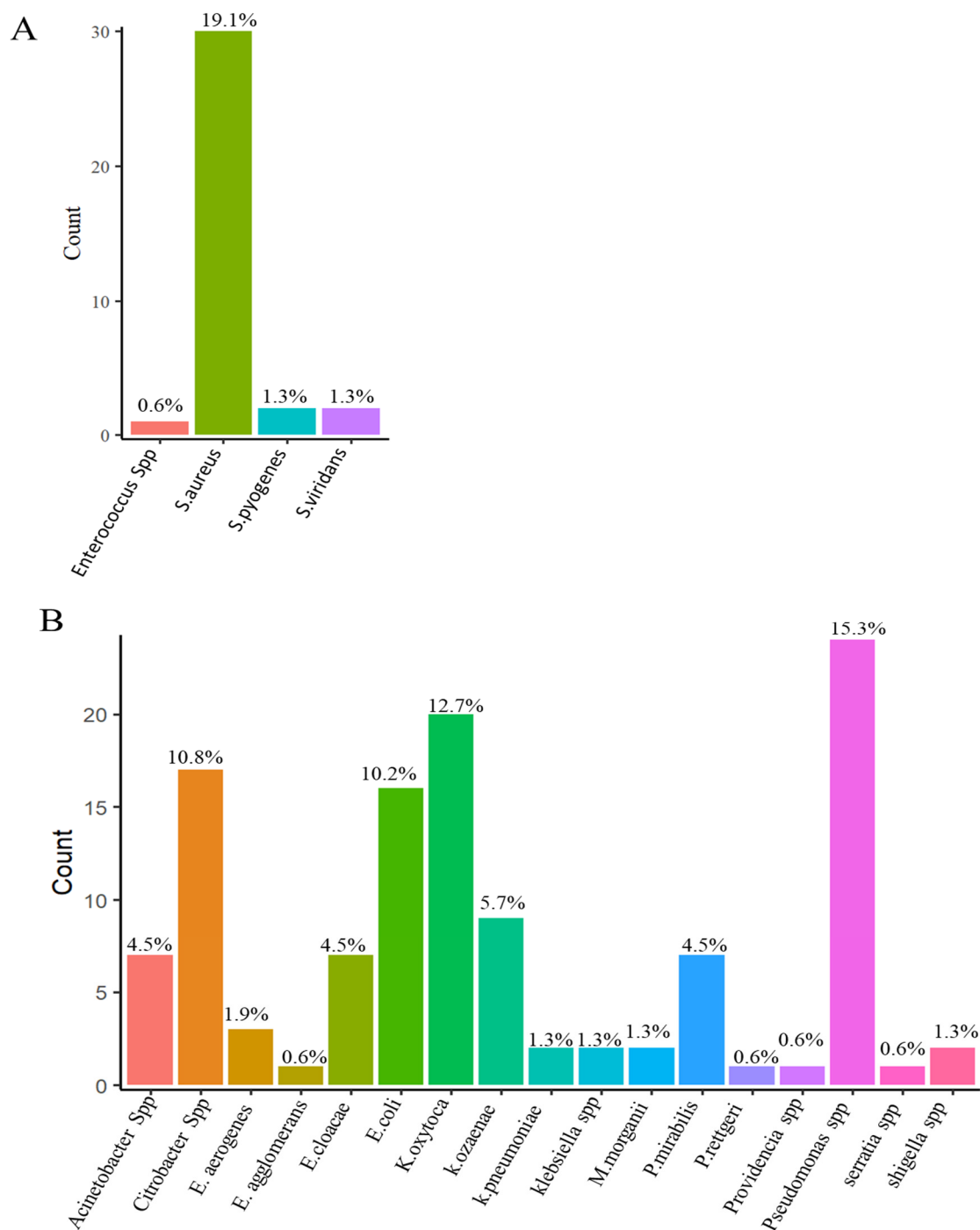


Figure 1 :-Bacterial isolates from patients with ear infection:- (A) Gram positive bacterial with a total of 35 (22.3%) isolate. (B) Gram negative bacterial with a total of 122(77.7%) isolate.

5.3. Associated Risk factors

When conducting univariate and multivariate analysis to assess the predictive role of different variables, we found that Residency, educational status, monthly income, presence of chronic disease, history of smoking, and recent history of hospitalization (within the last 3 months) don't show any association, and the P-values were greater than 0.25 and were not included for further multivariate analysis. Whereas at the level of univariate analysis, having few number of rooms showed a significant association with having an ear infection. The odds of having an ear infection were twice as great in individuals living in one room compared to those individuals living in houses with greater than 3 rooms (COR 2.4, 95%CI 1.1-5.6, P-Value =0.04). similarly, those individuals living in two-room houses also showed increased prevalence of ear infection compared to those living to those individuals living in houses with greater than 3 rooms (COR 2.7, 95%CI 1.2-6.2, P-Value =0.015) (**Table 2**). Likewise, history of head trauma also showed an association at univariate analysis with marginal level of significance (P=0.057), where individuals who had a history of head trauma showed an increased odds of ear infection (COR 2, 95%CI 0.97 - 4.2, P-Value =0.057) (**Table 2**). However, multivariate analysis showed that a significant odds of ear infection was observed among children under the age of 2, (AOR 14, 95%CI 1.1 - 192, P-Value =0.047) and Individuals living in a two-room house (AOR 2.6, 95%CI 1.1 - 6, P-Value =0.02) compared to their respective reference group (**Table 2**). Thus, our result showed that age group and the number of rooms that the patient resides in are predictive variables associated with ear infection.

Table 2. The sociodemographic and clinical characteristics of ear infection at SPHMMC

Variables	Bacterial infection		COR(95%CI)	P-value	AOR(95%CI)	P-value
	Yes	No				
Gender						
Female	75 (60)	50(40)	0.7 (0.41- 1.2)	0.2	0.74 (0.42 - 1.3)	0.32
Male	51(51.5)	48(48.5)				
Age Group						
Under 2 year	4 (44.4)	5 (55.6)	7.5(0.63- 90)	0.11	14 (1.1 -192)	0.047
3-11 years	10 (58.8)	7 (41.2)	4.2 (0.41 - 43)	0.22	6 (0.54 -66)	0.14
12-18 years	17 (54.8)	14(45.2)	4.9 (0.53 -46)	0.16	7.5(0.75 - 76)	0.86
19-45 years	75 (55.6)	60(44.4)	4.8 (0.56 - 40)	0.15	5 (0.56 -46)	0.15
46-65 years	14 (56)	11 (44)	4.7(0.49 - 45)	0.17	4(0.41 -40)	0.22
65 and above	6(85.7)	1(14.3)				
Family member						
less than 4	60 (50)	60 (50)	0.6(0.11 - 4.1)	0.66	0.38 (0.05 - 2.6)	0.33
5 to 10 member	64(64.6)	35(35.4)	0.3(0.06 - 2.3)	0.28	0.25 (0.04 - 1.7)	0.15
>10 member	2(40)	3(60)				
Number of room						
One room	34(53)	30(47)	2.4(1.1-5.6)	0.042	2(0.9 -5)	0.08
Two room	41(50)	41(50)	2.7(1.2-6.2)	0.015	2.6(1.1 -6)	0.02
Three room	21(56.8)	16(43.2)	2.1(0.81 -5.4)	0.13	2 (0.7 -5)	0.16
> 3 room	30(73.2)	11(26.8)				
History of otitis media						
Yes	93(59)	65(41)	0.69 (0.39-1.3)	0.22	0.8 (0.45 -1.6)	0.58
No	33(50)	33(50)				
History of Head Truma						
Yes	15 (41.7)	21(58.3)	2(0.97 - 4.2)	0.057	2 (0.9 - 4.1)	0.08
No	111(59)	77(41)				
Vaccination						
Yes	69(61.6)	43(38.4)	0.6(0.38 - 1.1)	0.11	0.69 (0.4 -1.2)	0.2
No	57(51)	55(49)				
History of URT infection						
Allergic rhinitis	14(63.6)	8(36.4)	0.7 (0.28 -1.8)	0.48	0.8 (0.31 -2.2)	0.73
Tonsillo pharyngitis	11(55)	9(45)	1 (0.4 - 2.6)	0.94	0.85 (0.3 - 2.4)	0.76
Adenoid	1(33.3)	2(66.7)	2.5 (0.2 - 28)	0.25	1.4 (0.11 -17)	0.78
Asthma	3(60)	2(40)	0.8 (0.14 - 5.2)	0.85	1.3 (0.17 - 10.4)	0.76
None	97(55.7)	77(44.3)				

5. 4. Antimicrobial susceptibility pattern of bacterial isolates

Antimicrobial susceptibility showed high resistance to different drugs among gram negative isolates. Antimicrobial susceptibility pattern of *Pseudomonas spp* showed sensitive to Ceftazidime 24(100%), Meropenem 22(91.7%), Piperacillin 19(79.2%), Amikacin 17(70.8%), Cefepime 16(66.7%) and Tobramycin 16(66.7%). Ciprofloxacin resistant 10(41.7%) and intermediate 1(4.20%).

Klebsiella oxytoca showed resistance to Ampicillin 15(75.7%) and Ceftriaxone 10(50%). and Highly sensitive to Chloramphenicol 19(95%) Meropenem 18(90%), Piperacillin 18(90%) and Gentamicin 17(85%). Less sensitive to Cefepime 14(70%) Ciprofloxacin 12(60%) ,Trimethoprim sulfamethoxazole 12(60%), Tetracycline 11(55%), Amikacin 11(55%) and Augmentin 11(55%).

Citrobactor spp showed resistance to Ampicillin 9(52.9%) and sensitive to Meropenem 17 (100%), Chloramphenicol 17(100%), Piperacillin 15(88.2%), Amikacin 15(88.2%), Cefepime 14(82.4%), Ceftriaxone 14(82.4%), Gentamicine 13(76.50%), Trimethoprim sulfamethoxazole 13(76.50%), ciprofloxacin 13(76.50%), Augmentin 13(76.50%) and Tetracycline 11(64.70%).

E.coli showed resistance to ampicillin 13(81.3%), Tetracycline 10(62.5%) ciprofloxacin 8(50%), Ceftriaxone 8(50%), Tetracycline 10(62.5%), Trimetoprime sulphamethoxazole 10(62.5%), Augmentin resistant 9(56.3%) and 5(31.3%) intermediate, Piperacillin resistant 8(50%) and 7(43.8%) was intermediate. whereas sensitive to Meropenem 16(100%), Chloramphenicol 12(75%) gentamicin10(62.5%) and Amikacin 10 (62.5%), Trimethoprim sulfaaxozazole 10(62.5%) and Cefepime 10(62.5%).(Table 3 and Table 4)

Table 3 Antimicrobial susceptibility result of gram negative bacteria clinically suspected ear infections at SPHMMC

Antibiotics		<i>Acitinobactor</i> <i>spp</i>		<i>Citrobactor</i> <i>spp</i>		<i>E.coli</i>		<i>Enterobacter</i> <i>aerogens</i>		<i>Enterobacter</i> <i>cloacea</i>		<i>Klebsiella</i> <i>oxytoca</i>		<i>Klebsiella</i> <i>ozenae</i>		<i>Klebsiella</i> <i>pneumonia</i>		<i>Klebsiella</i> <i>rhinoscler</i>		<i>Proteus</i> <i>mirabilis</i>		<i>Pseudomonas</i> <i>spp</i>	
		N	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Ampicillin	R	3	42.9	9	52.9	13	81.3	2	66.7	5	71.4	15	75	5	55.6	1	50	0	0	2	28.6	ND	ND
	S	4	57.1	8	47.1	3	18.8	1	33.3	2	28.6	5	25	4	44.4	1	50	2	100	5	71.4	ND	ND
Augmentin	I	0	0.0	3	17.6	5	31.3	1	33.3	1	14.3	0	0	1	11.1	0	0	0	0	0	0.0	ND	ND
	R	0	0.0	1	5.9	4	25.0	1	33.3	2	28.6	9	45	4	44.4	0	0	0	0	0	0.0	ND	ND
	S	7	100.0	13	76.5	7	43.8	1	33.3	4	57.1	11	55	4	44.4	2	100	2	100	7	100.0	ND	ND
Piperacillin	I	0	0.0	0	0.0	7	43.8	0	0.0	1	14.3	0	0	1	11.1	0	0	0	0	0	0.0	2	8.0
	R	0	0.0	2	11.8	1	6.3	0	0.0	0	0.0	2	10	1	11.1	0	0	0	0	0	0.0	3	13.0
	S	7	100.0	15	88.2	8	50.0	3	100.0	6	85.7	18	90	7	77.8	2	100	2	100	7	100.0	19	79.0
Ceftraxone	I	0	0.0	1	5.9	0	0.0	0	0.0	1	14.3	1	5	0	0.0	1	50	2	100	0	0.0	ND	ND
	R	1	14.3	2	11.8	8	50.0	2	66.7	2	28.6	9	45	5	55.6	0	0	0	0	0	0.0	ND	ND
	S	6	85.7	14	82.4	8	50.0	1	33.3	4	57.1	10	50	4	44.4	1	50	0	0	7	100.0	ND	ND
Cefepime	I	1	14.3	3	17.6	5	31.3	0	0.0	3	42.9	1	5	1	11.1	0	0	0	0	0	0.0	5	21.0
	R	0	0.0	0	0.0	1	6.3	0	0.0	0	0.0	5	25	2	22.2	0	0	0	0	0	0.0	3	13.0
	S	6	85.7	14	82.4	10	62.5	3	100.0	4	57.1	14	70	6	66.7	2	100	2	100	7	100.0	16	67.0
Meropenem	I	1	14.3	0	0.0	0	0.0	0	0.0	0	0.0	2	10	0	0.0	0	0	0	0	0	0.0	0	0.0
	R	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0	0	0.0	0	0	0	0	0	0.0	2	8.0
	S	6	85.7	17	100.0	16	100.0	3	100.0	7	100.0	18	90	9	100.0	2	100	2	100	7	100.0	22	92.0
Chloramphenicol	R	2	28.6	0	0.0	4	25.0	1	33.3	2	28.6	1	5	2	22.2	0	0	1	50	2	28.6	ND	ND
	S	5	71.4	17	100.0	12	75.0	2	66.7	5	71.4	19	95	7	77.8	2	100	1	50	5	71.4	ND	ND
Gentamycine	I	0	0.0	0	0.0	1	6.3	0	0.0	0	0.0	2	10	1	11.1	0	0	0	0	0	0.0	ND	ND
	R	0	0.0	4	23.5	5	31.3	1	33.3	2	28.6	1	5	3	33.3	0	0	0	0	0	0.0	ND	ND
	S	7	100.0	13	76.5	10	62.5	2	66.7	5	71.4	17	85	5	55.6	2	100	2	100	7	100.0	ND	ND
Amikacine	I	0	0.0	2	11.8	4	25.0	2	66.7	0	0.0	9	45	2	22.2	0	0	0	0	3	42.9	4	17.0
	R	0	0.0	0	0.0	2	12.5	0	0.0	0	0.0	0	0	0	0.0	0	0	0	0	1	14.3	2	8.0
	S	7	100.0	15	88.2	10	62.5	1	33.3	7	100.0	11	55	7	77.8	2	100	2	100	3	42.9	17	71.0
Tetracycline	R	3	42.9	6	35.3	10	62.5	0	0.0	4	57.1	9	45	5	55.6	1	50	1	50	5	71.4	ND	ND
	S	4	57.1	11	64.7	6	37.5	3	100.0	3	42.9	11	55	4	44.4	1	50	1	50	2	28.6	ND	ND
Ciprofloxacin	I	0	0.0	2	11.8	0	0.0	0	0.0	2	28.6	3	15	1	11.1	0	0	1	50	0	0.0	1	4.0
	R	2	28.6	2	11.8	8	50.0	0	0.0	1	14.3	5	25	4	44.4	1	50	1	50	1	14.3	10	42.0
	S	5	71.4	13	76.5	8	50.0	3	100.0	4	57.1	12	60	4	44.4	1	50	0	0	6	85.7	13	54.0
Trimethoprim sulfahamethoxazole	I	3	42.9	0	0.0	1	6.3	0	0.0	1	14.3	0	0	0	0.0	0	0	0	0	0	0.0	ND	ND
	R	1	14.3	4	23.5	10	62.5	0	0.0	1	14.3	8	40	5	55.6	1	50	1	50	0	0.0	ND	ND
	S	3	42.9	13	76.5	5	31.3	3	100.0	5	71.4	12	60	4	44.4	1	50	1	50	7	100.0	ND	ND

Table 4: Antimicrobial susceptibility result of other gram negative bacteria clinically suspected ear infections at SPHMMC

Antibiotics		<i>Enterobacter agglomerals</i>		<i>Morganella morgana</i>		<i>Providencis rettgeri</i>		<i>Serratia</i>		<i>Shigella spp</i>		<i>Shigella dysentriae</i>	
		n	%	n	%	n	%	n	%	n	%	n	%
Ampicillin	R	1	100.0	1	50.0	1	50.0	0	0.0	0	0.0	1	100.0
	S	0	0.0	1	50.0	1	50.0	1	100.0	1	100.0	0	0.0
Augmentin	I	0	0.0	1	50.0	1	50.0	0	0.0	0	0.0	0	0.0
	R	1	100.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	S	0	0.0	1	50.0	1	50.0	1	100.0	1	100.0	1	100.0
Piperacillin	I	0	0.0	1	50.0	0	0.0	0	0.0	0	0.0	0	0.0
	R	0	0.0	0	0.0	1	50.0	0	0.0	0	0.0	0	0.0
	S	1	100.0	1	50.0	1	50.0	1	100.0	1	100.0	1	100.0
Ceftriaxone	I	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	R	1	100.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	S	0	0.0	2	100.0	2	100.0	1	100.0	1	100.0	1	100.0
Cefepime	I	0	0.0	0	0.0	0	0.0	1	100.0	0	0.0	0	0.0
	S	1	100.0	2	100.0	2	100.0	0	0.0	1	100.0	1	100.0
Meropenem	I	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	S	1	100.0	2	100.0	2	100.0	1	100.0	1	100.0	1	100.0
Chloranphenicol	R	0	0.0	1	50.0	0	0.0	1	100.0	0	0.0	0	0.0
	S	1	100.0	1	50.0	2	100.0	0	0.0	1	100.0	1	100.0
Gentamicin	I	0	0.0	0	0.0	1	50.0	0	0.0	0	0.0	0	0.0
	R	0	0.0	1	50.0	0	0.0	0	0.0	0	0.0	0	0.0
	S	1	100.0	1	50.0	1	50.0	1	100.0	1	100.0	1	100.0
Amikacin	I	0	0.0	1	50.0	1	50.0	0	0.0	0	0.0	0	0.0
	R	0	0.0	1	50.0	0	0.0	0	0.0	0	0.0	0	0.0
	S	1	100.0	0	0.0	1	50.0	1	100.0	1	100.0	1	100.0
Tetracycline	R	0	0.0	0	0.0	1	50.0	1	100.0	1	100.0	1	100.0
	S	1	100.0	2	100.0	1	50.0	0	0.0	0	0.0	0	0.0
Ciprofloxacin	I	0	0.0	1	50.0	0	0.0	0	0.0	0	0.0	0	0.0
	S	1	100.0	1	50.0	2	100.0	1	100.0	1	100.0	1	100.0
Trimetoprim sulphamethoxazole	I	1	100.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	S	0	0.0	2	100.0	2	100.0	0	0.0	1	100.0	1	100.0

Similarly the level of resistance among gram positive isolate was also high. Antimicrobial susceptibility pattern *S.aureus* showed resistant to Erytromycine 21(70%), Clindamycin 16(53.3) and Tetracyclin 15(50%). intermediate to Clindamycin 2(6.70%), Ciprofloxacin 24(80%) And sensitive to Gentamicin 30(100%), Trimetroprime-sulphamethoxazole 28(93.3%), cefoxitine 27(90%) (**Table 5**).

Table 5. Antimicrobial susceptibility test result of gram positive bacterial Isolates.

Antibiotics		<i>Entrococus</i>		<i>S.auras</i>		<i>Streptococcus pyogen</i>		<i>Streptococcus viridians</i>	
		Count	Percent	Count	Percent	Count	Percent	Count	Percent
Cefoxitin	R	ND	ND	3	10.0	ND	ND	ND	ND
	S	ND	ND	27	90.0	ND	ND	ND	ND
Clindamycin	I	ND	ND	2	7.0	0	0.0	0	0.0
	R	ND	ND	16	53.0	2	100.0	2	100.0
Erytromycin	S	ND	ND	12	40.0	0	0.0	0	0.0
	I	ND	ND	1	3.0	0	0.0	0	0.0
Penicillin	R	ND	ND	21	70.0	0	0.0	2	100.0
	S	ND	ND	8	27.0	2	100.0	0	0.0
Vancomycin	S	1	100.0	ND	ND	2	100.0	ND	ND
Tetracyclin	R	1	100.0	ND	ND	ND	ND	0	0.0
	S	0	0.0	ND	ND	ND	ND	2	100.0
Gentamicin	R	1	100.0	15	50.0	2	100.0	2	100.0
	S	0	0.0	15	50.0	0	0.0	0	0.0
Ciprofloxacin	S	ND	ND	30	100.0	ND	ND	ND	ND
	I	0	0.0	24	80.0	ND	ND	ND	ND
Trimetoprim sulphamethoxazole	R	1	100.0	4	13.0	ND	ND	ND	ND
	S	0	0.0	2	7.0	ND	ND	ND	ND
Ampicillin	R	ND	ND	2	7.0	ND	ND	ND	ND
	S	ND	ND	28	93.0	ND	ND	ND	ND
Chloramphenicol	S	1	100.0	ND	ND	ND	ND	ND	ND
Ceftriaxone	R	1	100.0	ND	ND	ND	ND	ND	ND
	R	ND	ND	ND	ND	2	100.0	2	100.0

5.5. Multidrug resistance

Six antibiotic class was used for Gram negative bacteria to analyse MDR . these antibiotics classes are 1, Beta lactams:- a, Penicillins(Ampicillin, Piperacillin, and Augmentin). b, Cephalosporins (Ceftriaxone 3rd generation cephalosporin, ceftazidime 3rd generation cephalosporin and Cefepime 4th generation cephalosporin). c, Carbapenem (Meropenem), 2. Aminoglycosides (Gentamicin, Amikacin and Tobramycin), 3. Fluoroquinolones (Ciprofloxacin), 4. Tetracyclines (Tetracyclines), 5. Folate pathway inhibitors(Trimethoprim sulphamethoxazole) and 6. Amphenicols (chloramphenicol).

In case of Gram positive nine antibiotic classes was used to analyse MDR these are 1,Beta lactams (Penicillin, Ampicillin ,Cefoxitin 2nd generation cephalosporin, and Ceftriaxone 3rd generation cephalosporin), 2. Aminoglycosides (Gentamicin), 3. Fluoroquinolones (Ciprofloxacin), 4. Folate pathway inhibitors(Sulfonamides) (Trimethoprim-Sulfamethoxazole), 5. Macrolides (Erythromycin), 6. Lincosamides (Clindamycin), 7. Glycopeptides(Vancomycin), 8. Tetracyclines (Tetracycline) and 9. Amphenicols(Chloramphenicol).

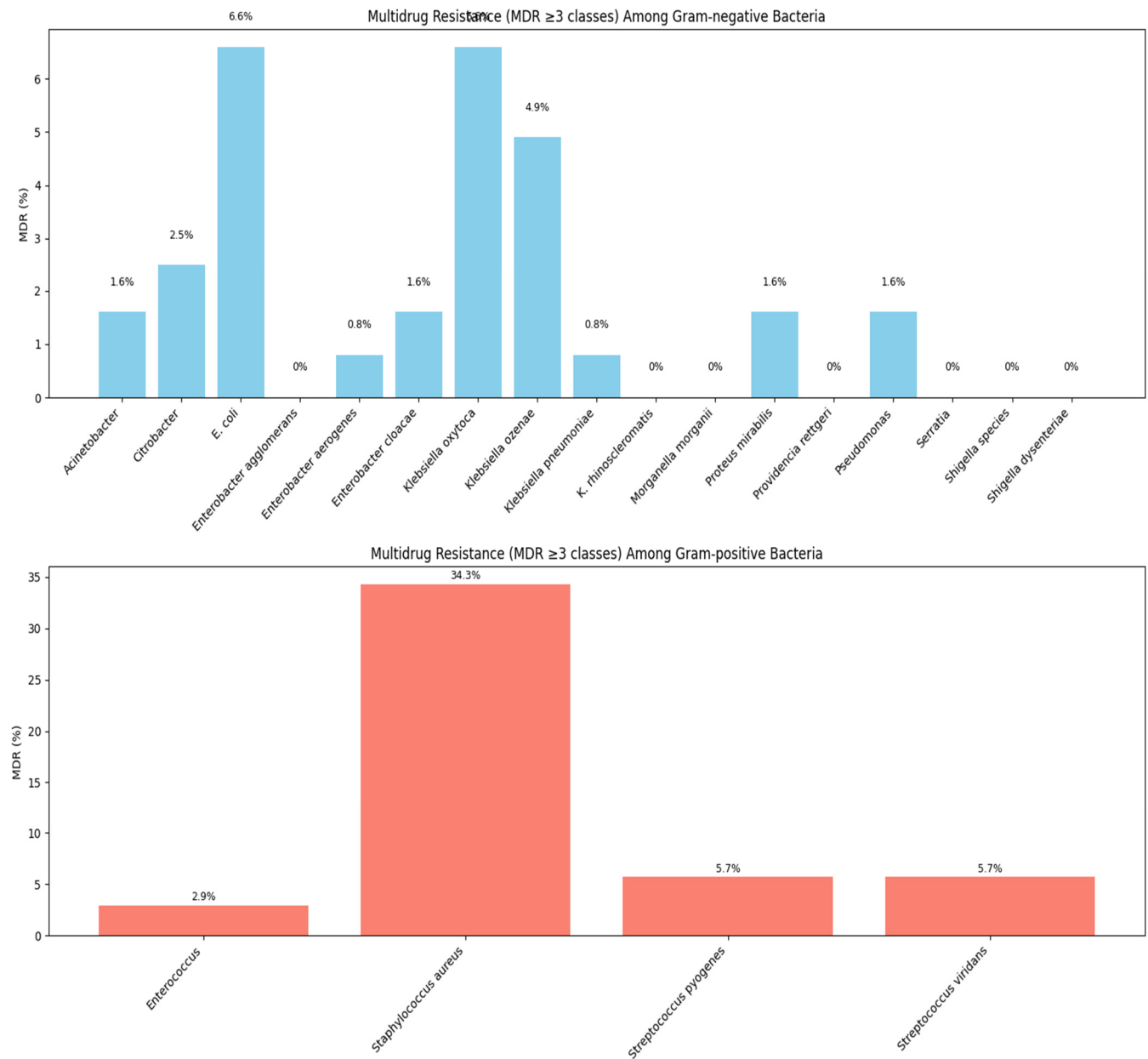
In this study from a total of 122 gram negative bacteria 35(28.70%) isolates were MDR and from a total of 35 of Gram positive bacteria 17(48.60%) isolates were MDR. Out of 17(48.60%) of gram positive isolate of MDR *S. aureus*, *Streptococcus pyogenes* and *S. viridian*, show MDR 35(28.70%) ,2(5.7%) and 2(5.7%) respectively. Gram negative isolate of MDR *Klebsiella Oxytoca*, *E.coli*, and *Proteus mirabilis* show MDR 8(6.6%), 8(6.6), and 2(1.6%) respectively. (**Figure 2**). Overall MDR rate among ear discharge bacterial isolates was 52(33.1%). (**Table 6**).

Table 6 Multidrug Resistance Patterns of Bacteria isolated from Patients with ear discharge at SPHMMC ENT OPD.

GRAM NEGATIVE BACTERIA	R0	R1	R2	R3	R4	R5	R6	MDR (≥3 class)
<i>Acinetobacter species</i>	2(1.6)	2(1.6)	1(0.8)	1(0.8)	1(0.8)	0(0)	0(0)	2(1.6)
<i>Citrobacter species</i>	2(1.6)	9(7.4)	3(2.5)	1(0.8)	1(0.8)	1(0.8)	0(0)	3(2.5)
<i>E. coli</i>	0(0)	2(1.6)	6(4.9)	1(0.8)	2(1.6)	2(1.6)	3(2.5)	8(6.6)
<i>Enterobacter agglomerans</i>	0(0)	1(0.8)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
<i>Enterobacter aerogenes</i>	1(0.8)	1(0.8)	0(0)	1(0.8)	0(0)	0(0)	0(0)	1(0.8)
<i>Enterobacter cloacae</i>	0(0)	4(3.3)	1(0.8)	0(0)	0(0)	2(1.6)	0(0)	2(1.6)
<i>Klebsiella oxytoca</i>	2(1.6)	8(6.6)	2(1.6)	3(2.5)	4(3.3)	1(0.8)	0(0)	8(6.6)
<i>Klebsiella ozenae</i>	1(0.8)	2(1.6)	0(0)	4(3.3)	0(0)	0(0)	2(1.6)	6(4.9)
<i>Klebsiella pneumoniae</i>	0(0)	1(0.8)	0(0)	1(0.8)	0(0)	0(0)	0(0)	1(0.8)
<i>Klebsiella rhinoscleromatis</i>	0(0)	0(0)	2(1.6)	0(0)	0(0)	0(0)	0(0)	0(0)
<i>Morganella morganii</i>	0(0)	1(0.8)	1(0.8)	0(0)	0(0)	0(0)	0(0)	0(0)
<i>Proteus mirabilis</i>	2(1.6)	1(0.8)	2(1.6)	2(1.6)	0(0)	0(0)	0(0)	2(1.6)
<i>Providencia rettgeri</i>	0(0)	2(1.6)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
<i>Pseudomonas species</i>	10(8.2)	8(6.6)	4(3.3)	1(0.8)	1(0.8)	0(0)	0(0)	2(1.6)
<i>Serratia</i>	0(0)	0(0)	1(0.8)	0(0)	0(0)	0(0)	0(0)	0(0)
<i>Shigella species</i>	0(0)	1(0.8)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
<i>Shigella dysenteriae</i>	0(0)	0(0)	1(0.8)	0(0)	0(0)	0(0)	0(0)	0(0)
Total	20(16.40%)	43(35.20%)	24(19.70%)	15(12.30%)	9(7.40%)	6(4.90%)	5(4.10%)	35(28.70%)
GRAM POSITIVE BACTERIA	R0	R1	R2	R3	R4	R5	R6	MDR (≥3 class)
<i>Enterococcus</i>	0(0)	0(0)	0(0)	0(0)	1(2.90)	0(0)	—	1(2.90)
<i>Staphylococcus aureus</i>	8(22.90)	2(5.70)	8(22.90)	6(17.10)	5(14.30)	1(2.90)	—	12(34.30)
<i>Streptococcus pyogenes</i>	0(0)	0(0)	0(0)	2(5.70)	0(0)	0(0)	—	2(5.70)
<i>Streptococcus viridans</i>	0(0)	0(0)	0(0)	0(0)	2(5.70)	0(0)	—	2(5.70)
Total	8(22.90%)	2(5.70%)	8(22.90%)	8(22.90%)	8(22.90%)	1(2.90%)	—	17(48.60%)
Overall	28(39.3%)	45(40.9%)	32(42.6%)	23(35.5%)	17(30.3%)	7(7.8%)	—	52(33.1%)

R0:- No antibiotic resisitant, R1,R2,R3,R4,R5 and R6:- Resistant to 1,2,3,4,5,6 antimicrobial class category respectively.

Figure 2:- Multi drug resistance pattern of gram negative bacteria and gram positive bacteria



Gram negative bacteria MDR rate is 35(28.70%) and MDR of Gram positive bacteria 17(48.60%)

6, Discussion

The prevalence and incidence rate of ear infection varies across regions and countries from 3.64% in central Europe to more than 43% in parts of sub-Saharan Africa ⁷. By 2050 an estimated 2.45 billion people are predicted to develop hearing loss (HL). In approximately 40% of cases, preventable HL is caused by infection. In children under 5 years of age, HL is primary caused by otitis media. HL in children may be reduced by 60% with effective treatment of ear infection and control of vaccine- preventable disease ⁴². The prevalence of HL is higher in developing countries with middle ear disease has the highest incidence. As a result, reporting bacterial etiologies of ear disease, associated risk factors and AST result is vital to prevent the multi-dimensional effect of the ear infection and guide the empirical treatment in low resource areas.

In the present investigation 126(56.25%) of the ear discharge sample were bacterial culture positive out of these 31(13.8%) had mixed isolates and 95(42.45) with single isolates, This result is in consistent with the study conducted in Addis Ababa 48.5% ³⁵. However, it is lower than reports from different studies and other parts of Ethiopia such as Hawasa 75.6% ⁴³, Bahirdar 80.4% ⁶, Dessie 83.6% ⁴⁴, Gondor 89.5% ⁵, Mekelle 98.2% ¹⁵ and Bangladish 70.8% ⁴⁵. The difference in culture positivity rate from different study might be due to the different methodology, sociodemographic characteristics, and high antibiotic usage history which will increase the difficulty of isolating bacteria like fastidious organism like *S. pneumoniae*, *M.catarrhalis* and *H.influenzae*. Fungal isolates were not cultured due to lack of appropriate laboratory facility, this can also be the reason for difference in culture positivity. According to this study 8(3.6%) of fungal element observed microscopically from gram stain.

From the total isolates, 122(77.7%) bacterial isolates were gram negative bacteria and 35 (22.3%) ear discharge sample with gram positive bacterial isolate. which demonstrates the dominant isolates were gram negative and this is in line with the finding in Hawassa 79.5% ⁴⁶, Desse were 74.2% ⁴⁷. Nigeria 75% ⁴⁸, Gondor 56.4% ⁵ and Mekelle 56% ¹⁵. In this study the predominant bacteria from the all gram reaction bacteria was *S.auras*

30(19.1%) followed by *Pseudomonas* species with 24(15.3%), which is similar to the previous studies in Addis Ababa and other parts of Ethiopia ^{5,35,49–54}. The other predominant bacteria of ear infection next to *Pseudomonas* is *klebsiella oxytoca* with 20(12.7%), *Citrobacter spp.* 17(10.8%) and *E.coli* 16(10.2%) respectively. The result aligned with other study that showed *Citrobacter* infections are primarily noted in atticofurcal disease ⁵⁵.

The frequency of *Citrobacter spp* infections in chronic otitis media may vary between 3.6% and 20% in some African countries ^{56,53}. In Gondar the *Citrobacteria* growth was detected in 15(5.58%) ³⁷. Likewise in Dessie *Citrobacter species* were detected in the ear discharge of 10% of patients with chronic otitis media but not in those with acute otitis media. 20 % of *Citrobacter* in Congolese children is reported ⁵³. *Klebsiella species* is another predominant bacteria from previous studies ^{12,34,46,54,57,58}. Similarly, other study showed *E.coli* is although another predominant bacteria isolate from previous study similar to this study ^{12,46,57}.

For this study we have used more than ten antibiotic class for antimicrobial susceptibility test of ear discharge bacterial isolate. The most Effective antibiotics for GPB was Cefoxitin (90%), Gentamicin (100%) and Sulfonamide class (Trimethoprim-sulphamethoxazole (93.3%)). whereas GPB were resistant to Macrolide class (Erythromycin), Tetracycline, Lincosamide class (Clindamycin) and Ciprofloxacin. The most Effective antibiotics for GNB was Carbapenems class (Meropenem), Cephalosporin class (Cefepime and Ceftazidim), Aminoglycosides class (Gentamicin and Amikacin) and Fluoroquinolones class (Ciprofloxacin). Whereas resistant to Penicillin class (Ampicillin and Augmentin) and Tetracycline class (Tetracycline) observed. This result is in concordance with the study conducted in other parts of Ethiopia ^{6,15,44}.

However, carbapenems (Meropenem) are last-line, broad-spectrum antibiotics, they are not recommended for routine treatment of ear infections including AOM, Otitis externa, and most cases of CSOM. they are reserved for severe infection caused by multidrug resistant bacteria and considered in only exceptional situations like life threatening ear infection(example intracranial complications of CSOM), Infection caused by extended-

spectrum Beta-lactamase (ESBL) producing organisms, and failure of all other appropriate antibiotics, guided by culture and susceptibility testing ⁵⁹.

Among the socio demographic factors and associated risk factor, history of head trauma, age group and number of rooms that the patient reside were significantly associated with bacterial ear infection ($P < 0.05$). whereas There was no significant statistical association seen in respect to bacterial infection with sex, resident, educational status , Monthly income, over all knowledge of ear infection, history of Habit of introducing foreign bodies in to the ear, history of exposure to loud sound, habit of ear wash, history of smoking, history of upper respiratory infection, breast feed duration (six month, one year and 2 years), chronic diseases, Treatment option, history of hospitalization last 3 month and vaccination in respect to ear infection with p value of > 0.05 . This was similar to findings of another studies. ⁶⁰. Similarly, gender and Educational status has no significant association with ear infection. Some studies have shown significant association with age groups, sex and Educational status ⁶¹. Whereas another studies have shown significant association with history of smoking and history of exposure to loud sound. ¹². These differences could be difference in the study population and study area.

Similar to this study, different studies have reported Small number of rooms or overcrowding plays a significant role in the development and spread of ear infection. Living in a crowded condition increases transmission of infectious disease through person-to-person and respiratory pathway often leads to swelling of the eustachian tubes, preventing the proper drainage and causing fluid to trap in the ear, which then become infected ⁶². Age group have significant role in the development of ear infection according to this study and is similar to other studies. Children under the age of two are at highest-risk group because of the Eustachian tubes .The Eustachian tubes are shorter, narrower and more horizontal in young children. This makes it more difficult to drain, allowing bacteria to build up and cause infection ¹

History of habit of introducing foreign bodies in to the ear has no statistical significant association with the ear infection according to this study but these practice leads to injuries like perforation and predispose to infection. There was no significant statistical

association seen in respect to bacterial infection with treatment option with p value >0.05 but Treatment options like self-prescription from pharmacy can lead to misdiagnosis of treatment. Un treated acute otitis media can lead to chronic otitis media, hearing loss and mastoidities.

From a total of 122 gram negative bacteria 35(28.70%) isolates were MDR and from a total of 35 of Gram positive bacteria 17(48.60%) isolates were MDR. Overall MDR rate among ear discharge bacterial isolates was 52(33.1%). This finding agrees with the results of studies at Bahirdar and Gondor 43% and 47.3% respectively. ^{38,58}. And this result is lower than studies conducted Dessie 82% Gondor 76.7%, Bahirdar 78.5%, Hawasa 75.6%, and addis ababa 83% ^{6,35,37,43,47}.

According to this study from a total of 126(56.3%) bacterial culture positive 52(33.1%) of bacterial isolate were MDR. Antibigrams pattern of the isolates revealed that multidrug resistance was quite high, which may result in treatment failure and disease complication of Otitis media. This high result drug resistance might reflect the degree of misuse of antibiotics, which is a global problem. Limitation of culture facilities and empirical prescription of these antimicrobial drugs and negligence on patients part might also be the important contributing factors for the development of multidrug resistance.

To conclude, our study has identified *S. aureus* as the common gram positive isolate whereas *Pseudomonas* being the common bacterial isolate. Most of the ear discharge isolate were resistant to antibiotics classes of Penicilline, Tetracycline, Macrolide however the antibiotics like Aminoglycosides, cephalosporins, Fluoroquinolones, and Carbapenems were the most effective in treating ear discharge bacterial isolates. Importantly we find that head trauma, age group and living small number of room were predictive variables associated with ear infection and in this finding there is a high rate of multidrug resistant isolate

7, Strength

All the questionnaire data and the specimen was collected, processed(specimen) and analyzed carefully with close collaborations and engagement of the principal investigator, physician and nurses. Relatively, larger panel of antibiotics were tested both for Gram positive and negative isolates which could use for baseline for effective treatment of the ear infection and can be used to plan future strategies.

8. Limitation

The type of ear infection (Acute Otitis media or chronic otitis media) was not differentiated. The study did not isolate Fungal and viral infection which is also causative agents of otitis media.

9. Conclusion

In this study 126(56.3%) of ear discharge sample were bacterial culture positive this implies that bacterial ear infection is one of a health problem. The predominant bacterial isolates from ear discharge sample were *S. aureus*, *Pseudomonas* spp , *Klebsiella oxytoca*, *Citrobacter* spp and *E.coli* respectively. Most of the ear discharge isolate were resistant to antibiotics classes of Penicilline, Tetracycline, Macrolide and Lincomycin class. However, the antibiotics like Aminoglycosides, cephalosporins, Fluoroquinolones, and Carbapenems were the most effective in treating ear discharge bacterial isolates. However, carbapenems class are last-line drug and they are not recommended for routine treatment of ear infections. These antibiotic susceptibility test result help as a baseline for effective treatment and guide the empirical treatment. Among the socio demographic and clinical characteristics, age group, number of room the patient reside and history of head trauma were significantly associated with bacterial ear infection. Identification of these specific risk factors will adds a public health relevance. The over all MDR rate among ear discharge bacterial isolate was 33.1%. from a total of 56.3% bacterial culture positive. This shows a high multidrug resistance stain. So, There is a need of periodic and continuous follow up of antibiotic usage for successful patients management and prevention of the emergence of MDR bacteria.

10. Recommendation

Due to Increased antimicrobial resistant bacteria in ear infection Up-to-date information on microbial resistance needs to be available. Discouraging the empirical use of broad spectrum agents is essential to prevent the antimicrobial resistant pattern. There is a need to strengthened research and surveillance for establishment of system to monitor patterns of infection, risk factors and drug resistance. Furthermore, emphasizing on the better prevention strategies of ear infection and community awareness is needed. Holestic approach is also needed to reduced the burden of disability like hearing loss, recurrent ear infection and complication to improve quality of life and communication abilities in affected population.

11, References

1. CDC. Ear Infections in Children. *Natl Inst Deaf other Commun Disord*. Published online 2013.
2. Schilder AGM, Chonmaitree T, Cripps AW, et al. Otitis media. *Nat Rev Dis Prim*. 2016;2:1-19. doi:10.1038/nrdp.2016.63
3. WHO. Chronic suppurative otitis media Burden of Illness. *Burd Illn Manag option*. Published online 2004.
4. Van Bonn SM, Schraven SP, Schuldt T, Heimesaat MM, Mlynski R, Warnke PC. Chronic otitis media following infection by non-O1/non-O139 *Vibrio cholerae*: A case report and review of the literature. *Eur J Microbiol Immunol*. 2020;10(3):186-191. doi:10.1556/1886.2020.00013
5. Muluye D, Wondimeneh Y, Moges F, Nega T, Ferede G. Types and drug susceptibility patterns of bacterial isolates from eye discharge samples at Gondar University Hospital, Northwest Ethiopia. *BMC Res Notes*. 2014;7(1):6-10. doi:10.1186/1756-0500-7-292
6. Hailu D, Mekonnen D, Derby A, Mulu W, Abera B. Pathogenic bacteria profile and antimicrobial susceptibility patterns of ear infection at Bahir Dar Regional Health Research Laboratory Center, Ethiopia. *Springerplus*. 2016;5(1). doi:10.1186/s40064-016-2123-7
7. Report W. *WORLD REPORT*.; 2021.
8. Yiengprugsawan V, Hogan A, Strazdins L. Longitudinal analysis of ear infection and hearing impairment: Findings from 6-year prospective cohorts of Australian children. *BMC Pediatr*. 2013;13(1). doi:10.1186/1471-2431-13-28
9. Seid A, Deribe F, Ali K, Kibru G. Bacterial otitis media in all age group of patients seen at Dessie referral hospital, North East Ethiopia. *Egypt J Ear, Nose, Throat Allied Sci*. 2013;14(2):73-78. doi:10.1016/j.ejenta.2013.02.005

10. Shangali A, Kamori D, Massawe W, et al. Aetiology of ear infection and antimicrobial susceptibility pattern among patients attending otorhinolaryngology clinic at a tertiary hospital in Dar es Salaam, Tanzania: A hospital-based cross-sectional study. *BMJ Open*. 2023;13(4):1-8. doi:10.1136/bmjopen-2022-068359
11. Seni J, Mapunjo SG, Wittenauer R, et al. Antimicrobial use across six referral hospitals in Tanzania: A point prevalence survey. *BMJ Open*. 2020;10(12):1-9. doi:10.1136/bmjopen-2020-042819
12. Henok EA, Manilal A, Oumer Y, et al. Factors Associated with Otitis Media Among Pediatrics in Two Government Hospitals in Arba Minch, Southern Ethiopia. *Infect Drug Resist*. 2023;16(September):6405-6426. doi:10.2147/IDR.S424927
13. Tesfa T, Mitiku H, Sisay M, Weldegebreal F, Ataro Z. Bacterial otitis media in sub-Saharan Africa : a systematic review and meta-analysis. Published online 2020:1-12.
14. Alam M, Beig S, Sultan A, Chandra K. Bacteriological and Antibiotic Sensitivity Profile of Cholesteatomatous Otitis Media in Pediatric and Adult Population: Prevailing Scenario in Northern India. *Indian J Otolaryngol Head Neck Surg*. 2022;74(s3):3568-3575. doi:10.1007/s12070-020-01956-0
15. Wasihun AG, Zemene Y. Bacterial profile and antimicrobial susceptibility patterns of otitis media in Ayder Teaching and Referral Hospital, Mekelle University, Northern Ethiopia. *Springerplus*. 2015;4(1):1-9. doi:10.1186/s40064-015-1471-z
16. Hardani AK, Moghimi Esfandabadi F, Delphi M, Ali Samir M, Zamiri Abdollahi F. Risk Factors for Otitis Media in Children Referred to Abuzar Hospital in Ahvaz: A Case-Control Study. *Cureus*. 2020;12(8). doi:10.7759/cureus.9766
17. Ilechukwu G, Ilechukwu C, Ubesie A, et al. Socio-demographic factors associated with otitis media among children in Enugu, South-East Nigeria. *Ann Med Health Sci Res*. 2017;7(3):162-165.

18. Fahad Altaieb F, Mashfi Alshammari O, Mohammed Alanazi H, et al. Pattern and factors associated with cardiovascular diseases among patients attending the cardiac center in Arar City, Northern Saudi Arabia. *Electron Physician*. 2017;9(10):5459-5464. doi:10.19082/5459
19. Mukara KB, Lilford RJ, Tucci DL, Waiswa P. Prevalence of Middle Ear Infections and Associated Risk Factors in Children under 5 Years in Gasabo District of Kigali City, Rwanda. *Int J Pediatr*. 2017;2017:1-8. doi:10.1155/2017/4280583
20. Dabholkar YG, Wadhwa A, Deshmukh A. A study of knowledge, attitude and practices about otitis media in parents in Navi-Mumbai. *J Otol*. 2021;16(2):89-94. doi:10.1016/j.joto.2020.11.002
21. Karunanayake CP, Albritton W, Rennie DC, et al. Ear Infection and Its Associated Risk Factors in First Nations and Rural School-Aged Canadian Children. *Int J Pediatr*. 2016;2016:1-10. doi:10.1155/2016/1523897
22. Ayukekbong JA, Ntemgwa M, Atabe AN. The threat of antimicrobial resistance in developing countries: Causes and control strategies. *Antimicrob Resist Infect Control*. 2017;6(1):1-8. doi:10.1186/s13756-017-0208-x
23. Hosain MZ, Lutful Kabir SM, Kamal MM. Antimicrobial uses for livestock production in developing countries. *Vet World*. 2021;14(1):210-221. doi:10.14202/VETWORLD.2021.210-221
24. Etienne Krug, Alarcos Cieza, Shelly Chadha LS. Childhood Hearing Strategies for Prevention and Care. *World Heal Organ*. Published online 2016:30.
25. Hu T, Podmore B, Barnett R, et al. Incidence of acute otitis media in children < 16 years old in Germany during 2014–2019. *BMC Pediatr*. 2022;22(1):1-11. doi:10.1186/s12887-022-03270-w
26. Hellingman CA, Dunnebier EA. Cochlear implantation in patients with acute or chronic middle ear infectious disease: A review of the literature. *Eur Arch Oto-Rhino-Laryngology*. 2009;266(2):171-176. doi:10.1007/s00405-008-0828-0

27. Akinpelu O V., Amusa YB, Komolafe EO, Adeolu AA, Oladele AO, Ameye SA. Challenges in management of chronic suppurative otitis media in a developing country. *J Laryngol Otol.* 2008;122(1):16-20. doi:10.1017/S0022215107008377
28. Martines F, Salvago P, Ferrara S, et al. Factors influencing the development of otitis media among Sicilian children affected by upper respiratory tract infections. *Braz J Otorhinolaryngol.* 2016;82(2):215-222. doi:10.1016/j.bjorl.2015.04.002
29. Jamal A, Alsabea A, Tarakmeh M. Effect of Ear Infections on Hearing Ability: A Narrative Review on the Complications of Otitis Media. *Cureus.* 2022;14(7). doi:10.7759/cureus.27400
30. Onifade A, Wa H, Siddharth K, Bhutta MF. Epidemiology of Chronic Suppurative Otitis Media : Systematic Review To Estimate Global Prevalence. 2025;9:1-12.
31. Collaborators OM. Global , regional , and national burden of upper respiratory infections and otitis media , 1990 – 2021 : a systematic analysis from the Global Burden of Disease Study 2021. 2025;25(January). doi:10.1016/S1473-3099(24)00430-4
32. Bhatia R, Rana M, Kaur K. Economic Burden of Otitis Media Globally and an Overview of the Current Scenario to Alleviate the Disease Burden : A Systematic Review. Published online 2024:552-558.
33. Choffor-nchinda E, Nansseu JR. Otitis media with effusion in Africa-prevalence and associated factors : A systematic review and meta-analysis. 2020;(October):1205-1216. doi:10.1002/lilo.2.502
34. Tilahun M, Shibabaw A, Alemayehu E, et al. Prevalence of bacterial ear infections and multidrug resistance patterns among ear infection suspected patients in Ethiopia: a systematic review and meta-analysis. *BMC Infect Dis.* 2024;24(1). doi:10.1186/s12879-024-10231-4
35. Hailegiyorgis TT, Sarhie WD, Workie HM. Isolation and antimicrobial drug susceptibility pattern of bacterial pathogens from pediatric patients with otitis

- media in selected health institutions, Addis Ababa, Ethiopia: A prospective cross-sectional study. *BMC Ear, Nose Throat Disord.* 2018;18(1):1-7. doi:10.1186/s12901-018-0056-1
36. Meshesha A, Fröschl U, Kebede M, Biratu TD, Worku Y, Hunduma F. Prevalence of hearing loss and factors associated with hearing loss in Ethiopia : findings from the 2023 National Ethiopia Hearing Survey. Published online 2025:1-8. doi:10.1136/bmjopen-2024-086288
 37. Getaneh A, Ayalew G, Belete D, Jemal M, Biset S. Bacterial etiologies of ear infection and their antimicrobial susceptibility pattern at the university of gondar comprehensive specialized hospital, gondar, northwest ethiopia: A six-year retrospective study. *Infect Drug Resist.* 2021;14:4313-4322. doi:10.2147/IDR.S332348
 38. Endaylalu K, Abera B, Id WM. Extended spectrum beta lactamase producing bacteria among outpatients with ear infection at FelegeHiwot Referral Hospital , North West. 2020;4:1-14. doi:10.1371/journal.pone.0238891
 39. Liese JG, Silfverdal SA, Giaquinto C, et al. Incidence and clinical presentation of acute otitis media in children aged <6 years in European medical practices. *Epidemiol Infect.* 2014;142(8):1778-1788. doi:10.1017/S0950268813002744
 40. Testing S. *M100 Performance Standards for Antimicrobial.*; 2022.
 41. Hudzicki J. Kirby-Bauer Disk Diffusion Susceptibility. *Am Soc Microbiol.* 2009;(December 2009):1-13. <https://www.asm.org/Protocols/Kirby-Bauer-Disk-Diffusion-Susceptibility-Test-Pro>
 42. Zamiri Abdollahi F, Ilesanmi OS, Irvani SSN, Islam SMS, ... Hearing loss prevalence and years lived with disability, 1990–2019: findings from the Global Burden of Disease Study 2019. *Lancet.* 2021;397(10278):996-1009. [https://www.thelancet.com/journals/lancet/article/PIIS0140-6736\(21\)00516-X/fulltext?s2%5C%5Cx3dP1029444002_1683504008768353639](https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(21)00516-X/fulltext?s2%5C%5Cx3dP1029444002_1683504008768353639)

43. Tadesse B, Shimelis T, Worku M. Bacterial profile and antibacterial susceptibility of otitis media among pediatric patients in Hawassa, Southern Ethiopia: Cross-sectional study. *BMC Pediatr.* 2019;19(1):1-8. doi:10.1186/s12887-019-1781-3
44. Argaw-Denboba A, Abejew AA, Mekonnen AG. Antibiotic-resistant bacteria are major threats of otitis media in wollo area, northeastern Ethiopia: A ten-year retrospective analysis. *Int J Microbiol.* 2016;2016. doi:10.1155/2016/8724671
45. Khatun MR, Alam KMF, Naznin M, Salam MA. Microbiology of chronic suppurative Otitis media: An update from a tertiary care hospital in Bangladesh. *Pakistan J Med Sci.* 2021;37(3):821-826. doi:10.12669/pjms.37.3.3942
46. Worku M, Bekele M. Bacterial isolate and antibacterial resistance pattern of ear infection among patients attending at Hawassa university referral Hospital, Hawassa, Ethiopia. *Indian J Otol.* 2014;20(4):155-159. doi:10.4103/0971-7749.146929
47. Abera B, Kibret M. Bacteriology and antimicrobial susceptibility of otitis media at dessie regional health research laboratory, Ethiopia. *Ethiop J Heal Dev.* 2011;25(2):161-167.
48. Iseh KR, Adegbite T. Pattern and Bacteriology of Acute Suppurative Otitis Media in Sokoto, Nigeria. *Ann Afr Med.* 2004;3(4):164-166.
49. Ilechukwu GC, Ilechukwu CA, Ubesie AC, Okoroafor I, Ezeanolue BC, Ojinnaka NC. Bacterial agents of the discharging middle ear among children seen at the university of Nigeria teaching hospital, Enugu. *Pan Afr Med J.* 2017;26:1-8. doi:10.11604/pamj.2017.26.87.9243
50. Nisa ZU, Hassan QU, Hassan SG, Shams S. Prevalence of bacteria and their sensitivity to various antibiotics in chronic suppurative otitis media. *Med Forum Mon.* 2013;24(12):31-33.
51. Bin Mohanna MA, Bahannan AA. Bacterial Profile And Antibigram Of Otitis Media Among Children In Yemen. *J Ayub Med Coll Abbottabad.* 2016;28(3):480-

483.

52. Akter S, Shamsuzzaman SM, Nehar N, Siddiqui I, Jahan F, Islam S. Bacterial isolates and drug susceptibility patterns of ear discharge from patients with ear infection at Shaheed Monsur Ali Medical College. *Bangladesh J Med Microbiol.* 2017;9(2):20-23. doi:10.3329/bjmm.v9i2.31422
53. Nyembue DT, Tshiswaka JM, Sabue MJ, Muyunga CK. Bacteriology of chronic suppurative otitis media in congolese children. *Acta Otorhinolaryngol Belg.* 2003;57(3):205-208.
54. Ahmed KS, Araya M, Kessete Y, et al. Identification of Common Bacterial Etiologic Agents, Antimicrobial Susceptibility Pattern & Associated Risk Factors of Otitis Media Among Pediatric Patients of ENT Center of Orotta National Referral Hospital. *Ethiop Med J.* 2024;62(2):77-86. doi:10.4314/emj.v62i2.2
55. Albert RRA, Job A, Kuruvilla G, Joseph R, Brahmadathan KN, John A. Outcome of bacterial culture from mastoid granulations: Is it relevant in chronic ear disease? *J Laryngol Otol.* 2005;119(10):774-778. doi:10.1258/002221505774481219
56. Tessema G. Otitis media seen in Yekatit 12 Hospital. *Ethiop Med J.* 2001;39(2):113-121.
57. Guteta ET, Abdi FA, Feyisa SG, Kinfu BS, Tafesse TB. Bacterial etiologies, antimicrobial susceptibility profiles and associated factors among patients with otitis media referred to Nekemte Public Health Research and Referral Laboratory Center, Western Ethiopia: A cross-sectional study. *BMC Microbiol.* 2025;25(1). doi:10.1186/s12866-024-03664-z
58. Molla R, Tiruneh M, Abebe W, Moges F. Bacterial profile and antimicrobial susceptibility patterns in chronic suppurative otitis media at the University of Gondar Comprehensive Specialized Hospital, Northwest Ethiopia. *BMC Res Notes.* 2019;12(1):1-6. doi:10.1186/s13104-019-4452-4
59. Medicines E. © World Health Organization 2023.

60. Kohli C, Kadirvelu U, Garg S, Sharma N. Burden of ear morbidities among children in primary care setting in Delhi. *Clin Epidemiol Glob Heal*. 2016;4:S12-S16. doi:10.1016/j.cegh.2016.08.010
61. Shaheen MM, Raquib A, Ahmad SM. Chronic Suppurative Otitis Media and Its Association with Socio-Economic Factors Among Rural Primary School Children of Bangladesh. *Indian J Otolaryngol Head Neck Surg*. 2012;64(1):36-41. doi:10.1007/s12070-011-0150-9
62. Delacy J, Dune T, Macdonald JJ. The social determinants of otitis media in aboriginal children in Australia: are we addressing the primary causes? A systematic content review. Published online 2020:1-9.

12. Annexes

12.1. Patient information sheet form and consent form (English version)

12.1.1, Patient information sheet form (English version)

Title

Detection of Bacteria Etiologic Agents, Their Antibiotic Susceptibility, and Associated Risk Factors from Patients with Ear Infections at St. Paul's Hospital Millennium Medical College.

Principal Investigator:

Semegn Desalegn, phone number 0928641425

Introduction

You are invited to participate in a research study conducted by Semegn Desalegn and the research team at St. Paul's Hospital Millennium Medical College. This study aims to identify bacteria causing ear infections, determine their susceptibility to antibiotics, and investigate associated risk factors.

Purpose:

The purpose of this study is to enhance our understanding of the bacterial etiology of ear infections, assess the effectiveness of antibiotics against these bacteria, and identify potential risk factors contributing to ear infections in patients.

Procedures

Sample Collection: A swab will be gently inserted into the affected ear(s) to collect samples of bacteria.

Laboratory Analysis:

The collected samples will be analyzed in the laboratory to identify the types of bacteria present and their susceptibility to various antibiotics.

Data Collection: Participants will be asked to provide information regarding their medical history, previous antibiotic use, and any other relevant factors contributing to their ear infection.

Risks and Benefits:

participating in this study has no any risk for you. The information obtained from this study may contribute to the development of more effective treatments for ear infections in the future.

Confidentiality:

All information collected during this study will be kept strictly confidential. Data will be stored securely and accessed only by authorized personnel involved in the research.

Voluntary Participation:

Participation in this study is entirely voluntary. You have the right to refuse to participate or withdraw from the study at any time without penalty or loss of benefits.

Contact Information:

If you have any questions or concerns about the study, please contact Semegn Desalegn, 0928641425.

By signing this consent form, you acknowledge that you have read and understood the information provided above and agree to participate in the study.

12.1.2, Consent form(English version)

I have read the information provided in the Information Sheet for the research study titled "Detection of Bacteria Etiologic Agents, Their Antibiotic Susceptibility, and Associated Risk Factors from Patients with Ear Infections at St. Paul's Hospital Millennium Medical College." I have had the opportunity to ask questions, and any queries have been answered to my satisfaction.

I understand that my participation in this study is voluntary, and I have the right to withdraw at any time without penalty.

I consent to the collection of samples from my ear(s) for the purpose of this study and understand that these samples will be used for laboratory analysis to identify bacteria and assess their antibiotic susceptibility.

I understand that all information collected during this study will be kept confidential and that my identity will not be disclosed in any reports or publications resulting from this research.

I agree to participate in this study and acknowledge that I may contact Semegn Desalegn, Phone number 0928641425. If I have any further questions or concerns.

Participant's Signature: _____

Date: _____

Researcher's Signature: _____

Date: _____

12.2, Patient information sheet form and consent form(Amharic version)

በየመረጃ ወረቀት እና የስምምነት ቅጽ

ርዕስ

በቅዱስ ጳውሎስ ሆስፒታል ሚሊኒየም ሜዲካል ኮሌጅ የጆሮ ኢንፌክሽን ካላቸው ታማሚዎች የባክቴሪያ ኤቲዮሎጂካል ወኪሎች፣ የአንቲባዮቲክ ተጎዲነታቸው እና ተያያዥ የአደጋ ምክንያቶችን ማወቅ

ዋና መረጃ:-

ስመኝ ደሳለኝ, ስልክ ቁጥር 0928641425

መግቢያ

እርስዎም በሰሙኝ ደሳለኝ እና በቅዱስ ጳውሎስ ሆስፒታል ሚሊኒየም ሜዲካል ኮሌጅ የምርምር ቡድን ባደረጉት የምርምር ጥናት ላይ እንዲሳተፉ ተጋብዘዋል። ይህ ጥናት ለጆሮ ኢንፌክሽን የሚዳርጉ ተህዋሲያንን ለመለየት፣ ለአንቲባዮቲክስ ያላቸውን ተጋላጭነት ለመወሰን እና ተያያዥ የአደጋ መንስኤዎችን ለመመርመር ያለመ ነው።

ዓላማ:-

የዚህ ጥናት አላማ የጆሮ ኢንፌክሽንን በባክቴሪያ ስነ-ምህዳር ላይ ያለንን ግንዛቤ ማሳደግ፣ አንቲባዮቲኮችን በእነዚህ ባክቴሪያዎች ላይ ያለውን ውጤታማነት መገምገም እና ለታከሚዎች ጆሮ ኢንፌክሽን አስተዋጽኦ የሚያደርጉ ሊሆኑ የሚችሉ አደጋዎችን መለየት ነው።

ሂደቶች

የናሙና ስብስብ:- የባክቴሪያ ናሙናዎችን ለመሰብሰብ በተጎዳው ጆሮ(ዎች) ውስጥ ስዋብ በቀስታ ይገባል ።

የላቦራቶሪ ትንታኔ:- የተሰበሰቡት ናሙናዎች በቤተ ሙከራ ውስጥ የሚመረመሩት የባክቴሪያ ዓይነቶችን እና ለተለያዩ አንቲባዮቲኮች ተጋላጭነታቸውን ለመለየት ነው።

የመረጃ አሰባሰብ:- ተሳታፊዎች የህክምና ታሪካቸውን፣ የቀድሞ አንቲባዮቲክ አጠቃቀምን እና ሌሎች ለጆሮአቸው ኢንፌክሽን አስተዋጽኦ የሚያደርጉ ሌሎች ተያያዥ ሁኔታዎችን በተመለከተ መረጃ እንዲሰጡ ይጠየቃሉ።

አደጋዎች እና ጥቅሞች:- በዚህ ጥናት ውስጥ መሳተፍ ለእርስዎ ምንም አይነት አደጋ የለውም። ከዚህ ጥናት የተገኘው መረጃ ለወደፊት ለጆሮ ኢንፌክሽኖች የበለጠ ውጤታማ ህክምናዎችን ለማዳበር አስተዋፅኦ ሊያደርግ ይችላል።

ሚስጥራዊነት:- በዚህ ጥናት ውቅት የሚሰበሰቡ መረጃዎች በሙሉ በሚስጥር ይያዛሉ። መረጃው ደህንነቱ በተጠበቀ ሁኔታ ይከማቻል እና በጥናቱ ውስጥ በተሳተፉ የተፈቀደላቸው ሰዎች ብቻ ይደርሳሉ።

በፈቃደኝነት ተሳትፎ:- በዚህ ጥናት ውስጥ መሳተፍ ሙሉ በሙሉ በፈቃደኝነት ላይ የተመሰረተ ነው። በማንኛውም ጊዜ ያለ ቅጣት ወይም ጥቅማጥቅሞች በጥናቱ ለመሳተፍ ወይም ለመተው የመቃወም መብት አልዎት።

የእውቂያ መረጃ፡- ስለ ጥናቱ ማንኛውም አይነት ጥያቄ ወይም ስጋቶች ካሉት ሰመኝ ደሳለኝን 0928641425 ያግኙ።

ይህን የስምምነት ቅጽ በመፈረም፣ ከዚህ በላይ የቀረበውን መረጃ እንዳይነበቡ እና እንደተረዱት እና በጥናቱ ለመሳተፍ እንደተስማሙ እውቅና ይሰጣሉ።

የፍቃድ ፎርም

በቅዱስ ጳውሎስ ሆስፒታል ሚሊኒየም ሜዲካል ኮሌጅ የጆሮ ኢንፌክሽን ለታካሚዎች የባክቴሪያ ኤቲዮሎጂካል ወኪሎች መለየት፣ የአንቲባዮቲክ ተጎጂነታቸው እና ተያያዥ የአደጋ መንስኤዎች በሚል ርዕስ ለምርምር ጥናት በመረጃ ወረቀቱ ላይ የቀረበውን መረጃ አንብቤያለሁ። ጥያቄዎችን ለመጠየቅ እድሉን አግኝቻለሁ፣ እናም ማንኛውም ጥያቄዎች በእኔ እርካታ ምላሽ አግኝተዋል።

በዚህ ጥናት ውስጥ ያለኝ ተሳትፎ በፈቃደኝነት እንደሆነ ተረድቻለሁ፣ እና በማንኛውም ጊዜ ያለ ምንም ቅጣት የማቋረጥ መብት አለኝ።

ለዚህ ጥናት ዓላማ ከጆሮዬ (ዎች) ናሙናዎችን ለመሰብሰብ ተስማምቻለሁ እናም እነዚህ ናሙናዎች ባክቴሪያዎችን ለመለየት እና የአንቲባዮቲክ ተጎጂነታቸውን ለመገምገም ለላቦራቶሪ ምርመራ እንደሚውሉ ተረድቻለሁ።

በዚህ ጥናት ወቅት የሚሰበሰቡት መረጃዎች በሙሉ በሚስጥር እንደሚጠበቁ እና ማንነቴ ከዚህ ጥናት በተገኘ ማንኛውም ዘገባ ወይም ህትመቶች ላይ እንደማይገለጽ ተረድቻለሁ።

በዚህ ጥናት ለመሳተፍ ተስማምቻለሁ እና ተጨማሪ ጥያቄዎች ወይም ስጋቶች ካሉኝ ሰመኝ ደሳለኝን በስልክ ቁጥር 0928641425 ማግኘት እንደምችል አምናለሁ።

የተሳታፊ ፊርማ፡- _____

ቀን፡ _____

የተመራማሪ ፊርማ፡- _____

ቀን፡ _____

የቅጹ አናት

12.3, Assent Form(English version)

Title of study:

Detection of Bacterial Etiologic Agents and Antibiotic Susceptibility in Ear Infections.

Introduction

We invite you to participate in a research study conducted by (Semegn Desalegn/saint Paul's hospital Millennium Medical College

Purpose

The purpose of this study is to understand the bacteria causing ear infections and how they respond to antibiotics.

What you will do

You will undergo a simple examination of your ear by a medical professional.

A small sample will be taken from your ear for laboratory analysis. You will be asked questions about your medical history and symptoms.

Possible Risks and Benefits:

Participating in this study has no any risk for you during the examination or sample collection.

Benefits may include contributing to medical knowledge that could help improve treatments for ear infections in the future.

Your Rights

Your participation is voluntary, and you can withdraw at any time without penalty. Your privacy and confidentiality will be protected.

Contact Information:

If you have any questions or concerns about the study, you can contact Semegn Desalegn at phone number 0928641425.

Assent Statement

I have read and understood the information provided above. I understand that my participation is voluntary, and I agree to take part in this study.

Participant Signature: _____

Date: _____

የየእውቅና ቅጽ

ማረጋገጫ ቅጽ

የጥናት ርዕስ: የባክቴሪያ ኤቲዮሎጂካል ወኪሎችን እና አንቲባዮቲክን በጆሮ ኢንፌክሽን ውስጥ ያለውን ተጋላጭነት ማወቅ

መግቢያ

በ(ሰመኝ ደሳለኝ /የቅዱስ ጳውሎስ ሆስፒታል ሚሊኒየም ሜዲካል ኮሌጅ) ባደረገው የምርምር ጥናት እንድትሳተፉ ጋብዘናል።

ዓላማ

የዚህ ጥናት አላማ ለጆሮ ኢንፌክሽን መንስኤ የሆኑትን ባክቴሪያዎች እና ለአንቲባዮቲክ እንዴት ምላሽ እንደሚሰጡ ለመረዳት ነው።

ምን ታደርጋለህ

በህክምና ባለሙያ የጆሮዎትን ቀላል ምርመራ ያካሂዳሉ።

ለላቦራቶሪ ትንታኔ ትንሽ ናሙና ከጀሮ ወወሰዳል.ስለ ህክምና ታሪክዎ እና ምልክቶችዎ ጥያቄዎች ይጠየቃሉ.

ሊሆኑ የሚችሉ አደጋዎች እና ጥቅሞች:

በዚህ ጥናት ውስጥ መሳተፍ በምርመራው ወቅት ወይም ናሙና በሚሰበሰብበት ጊዜ ለእርስዎ ምንም አይነት አደጋ የለውም.

ጥቅማጥቅሞች ለወደፊቱ የጀሮ ኢንፌክሽን ሕክምናን ለማሻሻል ለሚረዱ የሕክምና እውቀት ማበርከትን ሊያካትት ይችላል።

የእርስዎ መብቶች

የእርስዎ ተሳትፎ በፈቃደኝነት ነው፤ እና በማንኛውም ጊዜ ያለ ቅጣት ማግለል ይችላሉ። ግላዊነትዎ እና ሚስጥራዊነትዎ ይጠበቃል።

የመገኛ አድራሻ:

በጥናቱ ላይ ጥያቄ ወይም ስጋት ካሎት ስመኝ ደሳለኝን በስልክ ቁጥር 0928641425 ማግኘት ይችላሉ።

የድጋፍ መግለጫ

ከላይ የቀረበውን መረጃ አንብቤ ተረድቻለሁ። ተሳትፎዬ በፈቃደኝነት እንደሆነ ተረድቻለሁ፤ እናም በዚህ ጥናት ለመሳተፍ ተስማምቻለሁ።

የተሳታፊ ፊርማ _____

ቀን _____

12.3.2, Procedure for sample collection and processing of ear swab.

Specimen collection

Specimens should be collected using sterile swabs and placed into Amies transport medium (+/-charcoal).

Ear swab samples are processed in a laboratory through a multi-step procedure involving microscopic examination, culturing on specialized media, and potentially antibiotic sensitivity testing.

Specimen transport and storage

Specimens should ideally be stored and transported in sealed plastic bags. Laboratory processing should occur as soon as possible after specimen collection. Specimens should be refrigerated if delays in processing over two hours are unavoidable.

Specimen processing

Reception

The sample, collected in a sterile transport medium, is received in the laboratory, assigned a specimen number,

Microscopic examination

A smear of the specimen is prepared on a clean slide, air-dried, and stained (commonly using a Gram stain.) After inoculating the appropriate agar plates

Culture

The lab technician inoculates various types of agar plates and/or broths with the sample to promote the growth of different microorganisms. and incubate culture media as indicated in Table 1.

Table 1. Culture media, conditions, and target organisms

Standard media	Incubation			Cultures read	Target organism(s)
	Temp (°C)	Atmosphere	Time		
Chocolate agar	35 – 37	5 – 10% CO ₂	40 - 48h	Daily	<i>H. influenzae</i> <i>M catarrhalis</i> <i>S. pneumoniae</i> Other organisms may be significant if pure

					growth
CNA-blood agar	35 – 37	Air	40 - 48h	Daily	β -haemolytic streptococci <i>S. aureus</i> <i>S. pneumoniae</i>
MacConkey agar	35 – 37	Air	40 - 48h	Daily	Enterobacteriaceae Pseudomonads
Sabouraud agar	35 – 37	Air	40 - 48h	Daily	Fungi

Identification and Sensitivity Testing:

Once growth (colonies) is observed, further tests are conducted like biochemical test to identify the specific organism(s). Significant isolates will then undergo antibiotic susceptibility testing to determine which antimicrobial agents will be most effective for treatment (e.g., marked as 'sensitive' (S) or 'resistant' (R)).

Interpretation

Record the semi-quantitative growth of each colony type (i.e. +/- to ++++).

Minimum level of identification in the laboratory

In general significant isolates should be identified as fully as possible (i.e. to species level).

Antimicrobial susceptibility testing

All significant isolates should have antimicrobial susceptibilities determined according to SOP MIC.

Reporting

Gram stain results: organisms that are detected is reported.

Culture: Normal Results (Negative Culture): No significant growth of pathogenic organisms after sufficient incubation (e.g., 3 days) indicates the absence of an infection.

Abnormal Results (Positive Culture): The presence of significant growth of a specific organism(s) indicates an infection. The lab report will provide a descriptive report, including:

Identification of Pathogen: Naming the specific bacteria or fungi, such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, or *Candida*.

Quality assurance

Media and identification tests should be quality controlled according to the relevant SOP.

Limitations

Prior antimicrobial use may result in negative cultures.

Ear swab sample Processing procedure

