ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

DEPARTMENT OF MEDICAL LABORATORY SCIENCES

Prevalence of pathogenic bacteria and antimicrobial susceptibility patterns among patients with ear infection at Arsho Advanced Medical Laboratory, Addis Ababa Ethiopia.

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A thesis submitted to the Department of Medical Laboratory Science, College of Health Science, Addis Ababa University Addis Ababa, Ethiopia, in partial fulfillment of the requirements degree of masters of postgraduate program in clinical laboratory science (diagnostic and public health microbiology specialty track).

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Addis Ababa University

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This is to certify that the thesis prepared by Melesse Desse, entitled: Distribution of Bacterial Isolates And their Drug Susceptibility of Ear Discharge among Patients with Ear Infection Referred to Arsho Advanced Medical Laboratory and submitted in partial fulfillment of the requirements for Master of Science degree of postgraduate program in clinical laboratory science (diagnostic and public health microbiology). Complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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Abbreviations

AAML	Arsho Advanced Medical Laboratory
AMR	Antimicrobial Resistance
AOM	Acute Otitis media
AST	Antimicrobial susceptibility testing
ATCC	American Type Culture Collection
COM	Chronic otitis media
CSOM	Chronic suppurative otitis media
ESBLS	Extended Spectrum Beta-Lactamases
EU	European Union
GN	Gram negative
GP	Gram positive
MEF	Middle ear fluid
MIC	Minimum inhibitory concentration
NP	Nasopharyngeal
OM	Otitis Media
OP	Oropharyngeal
SOP	Standard operating procedure
TASH	Tikur Anbessa Specialized Hospital
V2C	Vitek 2 compact
µl	Micro liter

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Abstract:

Background: Otitis media is a major health problem and occurs with a high incidence and prevalence in both developed and developing countries. The burden and population demographics of otitis media differ greatly between developed and developing regions. Sub Saharan Africa account for most deaths from complications arising from otitis media.

Objective: The objective of this study was to determine the bacterial isolates and their drug susceptibility patterns from patients who had ear infection at Arsho Advanced Medical Laboratory.

Methods: Cross-sectional study was conducted at Arsho medical laboratory, Addis Ababa, Ethiopia from January to April 2019. By using an aseptic technique ear discharge samples were collected by a sterile swab and inoculated on Blood Agar, Chocolate Agar and Mac Conkey's media. Isolated bacterial species were identified and drug susceptibility testing was performed by using the Vitek 2 system (bio-Mérieux). Data were entered and analyzed by using SPSS version 20 software and P-value of < 0.05 was considered statistically significant.

Results: Out of 422 samples processed, bacterial pathogens were isolated from 19.5% (82) samples. From the total bacterial isolated 78% were gram negative bacteria and the predominant isolate was *Pseudomonas species* and *Proteus spps* 21.9 % each followed by *Klebsiella spps* 20.3%. Among gram negative bacteria isolates 46.9 % had multiple antibiotic resistant patterns. *Pseudomonas aeruginosa* 92.4% and *proteus vulgaris had* 80 % were resistant against the commonly used antibiotics Ampicillin & Amoxicillin.

Conclusion: Majority of the bacterial isolates were multidrug resistant, hence, efforts to isolate microorganisms and determine the susceptibility pattern should be strengthened to improve the treatment outcome of otitis media instead of the usual trend of empirical treatment.

Keywords: Ear infection, Multidrug resistance and Antimicrobial susceptibility.

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1. Introduction

1.1 Background

Ear infection /Otitis media/ is a spectrum of disease, which includes, Acute Otitis Media without perforation, Acute Otitis Media with perforation, Otitis Media with effusion and Chronic Suppurative Otitis Media. Acute Otitis Media without perforation is presented as Bulging tympanic membrane with or without ear pain. Acute otitis media with perforation is having recent discharge through perforated tympanic membrane with or without ear pain (1, 2).

The world health organization (WHO) defined Chronic Otitis Media (COM) as "Otorrhoeas'' through a perforated tympanic membrane present for at least two weeks (3). Ear infection is common in children because their eustachian tubes are shorter, narrower and more horizontal than the adult ear (4). Children are prone to developing Acute Otitis media (AOM) due to anatomical and immunological immaturity, whereas adult ear infections are typically chronic otitis media in nature (COM), this causes hearing loss, has an impact on speech and language development and also affects school performance and social interaction (5).

About 65-330 million people suffer from ear infection worldwide and 60% of them had suffer in significant hearing impairment Over 90% of the burden is borne by countries in the South-east Asia and Western Pacific regions and Africa (6). The health-economic burden of ear infection is also severe especially in Africa and other developing nations where the disease prevalence is estimated as high as 11%. The etiologies and prevalence of ear infection is different indifferent geographical areas (7).

The World Health Organization (WHO) has classified the prevalence of chronic suppurative otitis media (CSOM) in Africa among children and adults as high, estimated to be between 3.0% and 6.0%. Ethiopia falls within this category (8).Predominantly both gram positive and gram negative organisms and fungi (*Aspergillus or Candida*) are responsible for infection of Otitis media. But also viruses such as Herpes virus may also effect perforations in the ear drum (2, 9). The Common causative organism aerobic (e.g. *Pseudomonas aeruginosa, Escherichia coli,Staphylococcus aureus, Streptococcus pyogenes, Proteus mirabilis, Klebsiella species)* (10).

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The rapid and global spread of antimicrobial-resistant organisms in recent years has been recorded. Although resistant gram-positive infections have been concerning to clinicians, the increasing incidence of antibiotic-resistant gram-negative infections has become the most critical issue in bacterial resistance (11) According to data from 2009, 25,000 patients in the European Union (EU) die annually as a result of infections caused by resistant bacteria. The costs incurred by antimicrobial resistance amount to an estimated EUR 1.5 billion annually, due to loss of productivity and an increase in healthcare expenditure costs (12).

Studies conducted in many developing countries including Africa, have indicated high antibiotic resistance among Gram-negative bacteria to commonly used antibiotics, leading to a loss of efficacy for treatment of common infections (13).Due to the limited laboratory diagnosis in developing countries, physicians are often forced to syndromatic diagnosis and prescription of broad spectrum antibiotics for most infections that led to emergence of drug resistant bacterial strains especially for multidrug-resistant gram negative bacteria which produce extended spectrum beta-lactamases (ESBLs)(14).

Rapid bacterial identification and susceptibility testing improves patient therapy and decreases emergence of resistance. There is a need to provide rapid, efficient and accurate system for identification and antimicrobial susceptibility testing of pathogens (15). In this regard the automated identification or Antimicrobial susceptibility testing (AST) systems aid in rapid diagnosis and treatment of bacterial pathogens.

The Vitek 2 automated system (bioMe ´rieux), a second generation of Vitek ,fully automated, offers a more sophisticated model of data analysis and one of the most widely used instruments in clinical microbiology laboratories for the identification and evaluation of the susceptibility profiles (16,17). Vitek 2 compact system utilizes 64 biochemical and substrates to cover a total of 115 Gram-positive and 135 Gram-negative spps in an approximate turnaround time of 10 hours (18).

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1.2 Statement of the Problem

Ear infection especially chronic ottits media is a persistent and potentially dangerous disease because of its various complications. It is still a significant health problem especially in developing countries where not many institutions are able/have facilities for microbiology which can attribute to blind treatment.

Ottits media is a common childhood disease that can cause conductive hearing loss which may lead to delayed development of speech, language and cognitive skills in children (4, 19).

Antibiotic resistance is now a major issue confronting healthcare providers and their patients. Changing antibiotic resistance patterns, rising antibiotic costs and the introduction of new antibiotics have made selecting optimal antibiotic regimens more difficult now than ever before (20). These bacteria are able to cause serious disease and this is a major public health problem. Antibiotic resistant bacteria can also be passed from person to person within the community due to a number of reasons mostly poor hygiene (21).

The etiologies of ear infections differ in geographical area. Moreover antimicrobial resistance profile of bacteria varies among populations because of difference in geography local antimicrobial prescribing practices and prevalence of resistant bacterial strains. Thus up to date information on microbial resistance needs to be available at national and local levels to guide the rational use of the existing antimicrobials (22).

The aim of this study was isolation of ear infection causing bacteria and their drug susceptibility test from ear discharge swab samples patients attended at Arsho Advanced Medical Laboratory by employing the fully automated Vitek 2 compact system.

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1.3 Significance of the study

Ear infections due to bacterial pathogens are a major cause of morbidity and mortality in Ethiopia and other developing countries. Most patients are treated empirically based on their clinical symptoms. Therefore, to know and understand the etiology of major pathogens causing ear infections may play a positive role in better healthcare management. For these reasons, surveillance of ear infections from ear discharge cultures and their antibiotic resistance patterns are vital to the care of patients and prevention of ear infection. The results of this study could help to provide information on the magnitude of multidrug resistance of ear infection causing organisms, helps the physician in the selection of better antibiotic treatment and helps to initiate further large scale epidemiological study on MDR ear infection causing organisms

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2.Literature Review

Studies have been conducted on microbiology of ear infection and have showed different results from region to region. Microbiological cultures in some studies showed frequently multiple organisms and these vary depending on climate, patient population, collection and processing techniques of specimens and prior use of antibiotics (4).

A study from Gujarat India in 2015, Evaluation of bacteriological profile and antibiotic susceptibility pattern of patients with otorrhea in a tertiary care teaching hospital conducted by *Panchal PD et al*, A total of 100 patients' samples visiting ENT OPD were taken who complaining of ear discharge. The most common organism isolated was *Pseudomonas aeruginosa* (25.88%) and *Staphylococcus aureus* (21.17%), highly sensitive to Aminoglycosides (100%). they conclude that always advisable to test culture and sensitivity, whenever facilities are available, for better management of otitis media. Patients should be advised to take the complete antibiotic treatment course to avoid development of resistance (23).

In 2015 a retrospective data from public healthcare sector in Pakistan by Ayub et al. Nearly the otitis media is the most common type of ear infection (50%). The common pathogen causing ear infection was *Pseudomonas aeruginosa* (13%), other *Pseudomonas species* (7%), *Staphylococcus aureus* (5%), *Proteus miabilis* and *Klebshiella pneumonia* (2%).Trough careful consideration it was seen that *Pseudomonas aerogenosa* is the very most common pathogen which was highly sensitive (93%) to a wide variety of antibiotics. The (27%) of pathogens are resistant to antibiotic therapy while (38%) of the pathogens are sensitive towards various antibiotics. They concluded proper diagnosis and treatment is required in these cases especially for pediatrics because they are more susceptible to those infections (24).

A retrospective study was conducted from Bangladesh carried out on Bacterial isolates and drug susceptibility patterns of ear discharge from patients with ear infection in 2015, from a total of 115 ear discharge samples were tested for bacterial isolation and 86 (74.78%) cases were found positive; 49 (56.98%) were gram negative bacteria and the predominant isolate were

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S. aureus 21 (24.42%) followed by *Pseudomonas aeruginosa* 20 (23.26%). under five children were more affected by ear infection. The prevalence of ear infection was higher in females than males (64.35% vs 35.65%) (P=0.879). The prevalence of ear infection was very high in the study area.Majority of the bacterial isolates were resistant to multiple antibiotics. Hence antibiotics susceptibility test is mandatory before prescribing any antibiotics (25).

Findings in 2016 showed that in the University of Teaching Hospital and Beit Cure Hospital in Lusaka, Zambia by Harrison Phiri and his colloquies , out of the 169 microbiological isolates, the most frequent isolates were *Proteus mirabilis* 49(29.0%), *Pseudomonas aeruginosa*, 32(18.9%), coagulase negative S*taphylococcus* 18(10.7%) and *Klebsiella Pneumoniae* 17(10.1%). High sensitivity rates were revealed to Gentamycin (64-100%), meropenem (68-100%), ceftazidime (85-100%), ceftriaxone (64-80%), and ciprofloxacin (66-88%). High resistance rates were recorded to Amoxicillin-Clavulanate (as high as 100%), Ampicillin (as high as 100%), tetracycline (as high as 91.2%) and Cotrimoxazole (as high as 100%) and Penicillin (as high as 100%). they concluded from their study *Proteus mirabilis* was the most dominant microbiological isolate followed by *Pseudomonas aeruginosa*. The isolated microorganisms had high susceptibility rates to Gentamycin, Ceftazidime, Ceftriaxone and Ciprofloxacin. There were high resistance rates to Amoxicillin-Clavulanate, Ampicillin, tetracycline, Cotrimoxazole and Penicillin (26).

Another prospective study conducted by Jonathan M, Mustapha A, Musa I, Garba Lawal G & Abimbola O, in Nigeria 2016, on Antibacterial susceptibility spectrum of some gram negative bacteria from suspected Otitis media patients, A total of Fifty four (54) sample and out of this 84 bacterial isolated. The study reveals highest frequency of *Pseudomonas aeruginosa* 60 (71.43 %) followed by *Proteus mirabilis* 14 (16.67), *Klebsiella pnuemoniae* 6 (7.14) and *Escherichia coli* 4 (4.76). *P. aeruginosa* had the highest prevalence among patients < 30 years, where as *K.Pnuemoniae* and *E. coli* had the least isolated across all age groups. Antimicrobial susceptibility test showed highest frequency of resistance among all isolates to Amoxicillin, Cotrimoxazole, Nitrofurantoin and Nalidixic acid. However, Gentamicin, floxacin, Augmentin and Tetracycline were effective against *Pseudomonas aeruginosa* but ineffective against other isolates. Although antibiotics are the most preferred and prescribed drugs in incidents of otitis media, it is clear from this study that antimicrobial resistance still remains a persistent among bacterial pathogens of otitis media (27).

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A prospective study showed that bacteria isolates and antibiotic susceptibility of ear infections in Abeokuta, Nigeria conducted by Motayo in 2012, A total of 91 ear swab samples were processed 78(85.7%) isolates were recovered consisting 57(73%) GNB, 20(25.6%) GPB,

Pseudomonas aeruginosa 38(48.7%) was the most predominant bacteria species isolated from ear infection. This was followed by *Staphylococcus aureus* 20(25.6%), *Proteus spp.* 16(20.5%) and *Escherichia coli* 2(2.5%). *Klebsiella spp.* 1(1.3%) and *Candida albicans* 1(1.3%) was the least prevalent. A total of 78 isolates were recovered from samples, age group 1-14 years of age gave a frequency of 60(76.9%). There is a need for increased surveillance of conditions such as otitis media in order to prevent emergence of multi-drug resistant opportunistic pathogens capable of complicating an otherwise simple infection (28).

Retrospective Study showed that Pathogenic bacteria profile and antimicrobial susceptibility patterns of ear infection at Bahir Dar Regional Health Research Laboratory Center in 2016, Ethiopia Of the total 368 ear swab samples processed, 296 (80.4 %) were culture positive. The proportion of ear infection was higher in males (92.7 %) than females (65 %) (P = 0.014). The frequency of ear infection below 21 years of age was 65.2 %. The predominant isolate was *Pseudomonas aeruginosa* (29.7 %) followed by *Staphylococcus aureus* (26.3 %) & *Proteus* spp. (21.9 %). High level of antimicrobial resistance rates were observed for amoxicillin/clavulanic acid, ampicillin and penicillin whereas ciprofloxacin, ceftriaxone, chloramphenicol, cotrimoxazole, gentamicin and amikacin were found effective against the isolated bacteria. Aerobic bacterial otitis media linked with high levels of resistance against amoxicillin/clavulanic acid and ampicillin is major health problem in the study area. Therefore, they suggested that treatment of otitis media in the study area needs to be guided by antibiotic susceptibility testing of isolates (29).

A cross-sectional study was conducted by Deyno S, and his colloquies in University of Hawassa comprehensive specialized hospital Ethiopia, in 2017.Out of 117 patient specimens the prevalence of *S.aureus* infection was 28.2%. *S.aureus* isolates revealed up to 97.0% level of resistance pattern to the antimicrobials tested. In the determination of the susceptibility of *S. aureus* on nine selected antibiotics, 97.0% of the isolates were resistant to Cloxacilin and 74.2% showed resistance to Vancomycin. The overall rate of multi drug resistance was 100%, all of the isolates were found to

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be resistant to more than two tested antimicrobials. They concluded that *S.aureus* has gotten shockingly resistant to many of common antimicrobials (30).

Another study conducted on Dessie Regional Health Research Laboratory, Ethiopia in 2011 showed that out of 897 ear discharge samples, the most frequent isolates were *Proteus mirabilis and P. vulgaris accounted* 26.5%, *S. aureus* 24.6%, *P.aeroginosanosa* 18% and E. coli 17.7%. The antimicrobial susceptibility testing showed that Norfloxacin, ciprofloxacin and Gentamicin revealed high level of sensitivity whereas high resistance rates were observed for Amoxycillin, Tetracycline and Erythromycin. Antibiograms of isolates showed that 72.6% of isolates were resistant to two and more antimicrobials. And they concluded that Otitis media linked with high levels of multiple antibiotic resistant bacteria is a major health concern in all age groups of the study population. There is a need for culture and susceptibility test facilities for appropriate antimicrobial therapy of otitis media and antimicrobial resistant infections (22).

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3. Objectives

3.1. General objective

To determine the Prevalence of pathogenic bacteria and antimicrobial susceptibility patterns among patients with ear infection referred to at Arsho Advanced Medical Laboratory, Addis Ababa Ethiopia

3.2 . Specific objectives

- To determine the Prevalence of ear infection causing bacteria.
- To assess the overall antimicrobial susceptibility profile of bacteria.
- To analyze the distribution of bacteria on sex and different age group.

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4. Hypothesis

The Prevalence bacterial pathogens causing ear infection and multi drug resistance bacteria isolated in our laboratory is the same with previous studies.

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5. Materials and methods

5.1. Study area

The study was conducted on Arsho Advanced Medical laboratory. AAML is a private diagnostic laboratory found in Addis Ababa Ethiopia with Ten branches two of them are Medium clinics and located Addis Ababa and one Brach out of Addis Ababa in Jigjiga Ethiopia. It has many clients from different health facilities (hospitals, health centers, clinics, institution). Currently the microbiology laboratory runs about 50 microbiological samples on average per day. On average 3 to 5 ear discharge samples were processed.

5.2. Study design and period

Cross-sectional study was conducted at Arsho Medical Laboratory from January to April 2019 Addis Ababa, Ethiopia.

5.3. Population

5.3.1. Source population

All patients referred to Arsho Advanced medical laboratory for culture testing was the study subjects.

5.3.2. Study Population

All patients referred to Arsho Advanced Medical laboratory for Ear discharge culture.

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5.4. Inclusion and exclusion criteria

5.4.1. Inclusion criteria

All patients with actively draining ear discharge who had consent or whose guardians consented to participate in the study.

5.4.2. Exclusion criteria

- ✓ Patients on antibiotic treatment (ear drops/systemic) within the previous 2 weeks
- ✓ Patients who had refused consent to participate in the study.

5.5. Study variables

5.5.1. Dependent variables

- ➢ Prevalence of ear infection causing bacteria.
- ➢ Antimicrobial susceptibility profile of bacteria

5.5.2. Independent variables

- ✓ Age
- ✓ Sex
- ✓ Referred health institutions /Hospitals, Clinics…/
- ✓ Duration of ear discharge

5.6.Measurement and Data collection

5.6.1. Sample size calculation and sampling method

The sample size was calculated based on single population proportion. Since there is Limited published data available on prevalence of antimicrobial susceptibility pattern from Ear discharge samples in Ethiopia from the instrument Vitek 2 compact. The value of P taken as 50% (0.50). Considering 95% confidence interval, 5% margin of error, and the sample size is calculated using the following standard formula.

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The sample size $n = z (\alpha/2)^2 p (1-p)/d^2$

Where

n = Sample size

α = level of significance

z = at 95% confidence interval Z value (a = 0.05) =>Z α/2=1.96

p = prevalence

d = Margin of error at (5%) (0.05)

$n = (1.96)^2 0.5(1-0.5)/ (0.05)^2$

n=384

To minimize errors arising from the likelihood of non compliance, ten percent (10%) of the sample size added to the normal sample. Accordingly the required sample sizes were 422.

5.6.2. Data collection procedure

Data was collected using structured data collection form to obtain information on socio demographic status previous antibiotic usage. Detailed clinical history regarding age, gender, duration of discharge and antibiotic therapy was taken. Patients of any age group, both gender, unilateral or bilateral draining ears due to ear infection of more than two weeks (WHO- definition) were included in the study. Informed consent was taken from each patient and verbal informed consent was taken on behalf of children from their parents or guardians (see more on annex 1-8).

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5.7. Laboratory analysis

5.7.1. Sample collection and processing

Study participants were recruited into the study as they came to the AAML until the required number was obtained with strict application of the inclusion/exclusion criteria. Ears were first inspected; pus from the outer part of the ear canal was cleaned by suction. Ear discharge samples were collected using a sterile swab stick & transported by Amies transport media and taken to the Microbiology unit of the laboratory for processing. Upon receipt of the samples was inoculated on Blood agar, Chocolate agar & Mac Conkey agar were incubated at 37 ^{0}c aerobically ,use 5% to 6% Co2 incubator for Blood agar and Chocolate agar. Suspected bacterial pathogen were preliminarily characterized by colony morphology and done Gram-stain techniques. Antimicrobial susceptibility testing of bacterial isolates was determined with automated Vitech 2 compact system using the instruction of the manufacturer.

5.7.2 Principle of Vitek 2 compact system

Vitek 2 compact system is an automated microbiology bacterial identification and antimicrobial susceptibility system. Which uses advanced colorimetric technology to determine individual biochemical reactions contained microbe identification cards. After inoculation with a standardized suspension of the unknown organism, each card are incubated and read by the instruments internal optics. Comparison of results to known species specific reactions in the Vitek 2 database yields organism identifications. The absorbance optical system allows interpretation of test reactions using different wavelengths in the visible spectrum. During incubation, each test reaction is read every 15 minutes to measure either turbidity or colored products of substrate metabolism. In addition, a special algorithm is used to eliminate false readings due to small bubbles that may be present (31).

5.7.3. Suspension Preparation for ID card and AST card

Suspension preparation for ID card and AST card was done by suspending 3 ml of saline Aqueous 0.45% NaCl, pH 4.5 to 7.0 in a 12 x 75 mm clear plastic (polystyrene) test tube to achieve a turbidity equivalent to that of a McFarland 0.50 standard (McFarland is adjusted only for ID), as measured by the Densi Chek (bioMe´rieux) turbidity meter. After a specified McFarland

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turbidity achieved 145µl & 280µl is transferred to the next tube having 3 ml 0.45 NaCl for Gram negative & Gram positive respectively (31).

5.7.4. Inoculation

Identification cards GP and GN were inoculated or filled with microorganism suspensions using an integrated vacuum apparatus. Each card has a pre-inserted transfer tube used for inoculation and has bar code label that contain information on product type, lot number, expiration date, and unique identifier that can be linked to the sample. A test tube containing the microorganism suspension was placed into a special rack cassette to which the identification card were placed in the neighboring slot while inserting the transfer tube into the corresponding suspension tube. The cassettes can accommodate 10 tests tubes. The cassette then placed manually (Viteck 2 compact) into a vacuum chamber station. From the screen menu fill 'command key pressed. As a result air was air was re-introduced into the station, the organism suspension was forced through the transfer tube into micro-channels that fill all the test wells this filling processes lasts for about 70 seconds (32).

5.7.5. Card sealing, loading and incubation

Inoculated cards are passed by a mechanism, which cuts off the transfer tube and seals the card prior to loading into the carousel incubator. The carousel incubator can accommodate up to 30cards. All card types were incubated on-line at 35.5 + 1.0ºC. Each card removed from the carousel incubator once every 15 minutes, transported to the optical system for reaction readings based on their wave length, and then returned to the incubator until the next read time. Then data collected at 15-minute intervals during the entire incubation period are analyzed by advanced expert system of the device (31).

5.7.6. Bacterial identification

Identification and antimicrobial susceptibility testing of isolated bacteria were determined by automated Vitek 2 compact system using bacterial isolation and identification cards based on standardized ID schemes & CLIS GL application. The Vitek 2 compact system is an integrated modular system that consists of a filling-sealer unit, a reader-incubator, a computer control module, a data terminal, and a multi copy printer. The system detects bacterial growth and

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metabolic changes in the micro-wells of thin plastic cards by using a spectrophotometer technology. The reagent cards have 64 wells that can each contain an individual test substrate. Substrates measure various metabolic activities such as acidification, alkalinization, enzyme hydrolysis, and growth in the presence of inhibitory substances. Substrates and biochemical tests used for identification of gram negative bacteria were Ala-PhePro-Arylamidase, Adonitol, L-Pyrrolydonyl-Arylamidase, L-Arabitol, D-Cellobiose, BetaGalactosidase, H2S production, Beta-N-Acetyl Glucosaminidase, GlutamylArylamidasepNA, D-Glucose,Gamma-Glutamyl-Transferase, Fermentation/ Glucose, Beta-Glucosidase, D-Maltose, D-Mannitol, D-Mannose, Beta-Xylosidase, Beta-Alanine arylamidase, etc and Substrates and biochemical tests used for identification of gram positive bacteria were D-Amygdalin, Phaspatidiylinstol phospholipase, D-xylose, Urease, Ala-Phe-Pro Arylamidase, Bgalactosidase, Alphaglucosidase, cyclodextrine, Optochin resistance, Bacitracin resisistance, L-lactate alkalization, etc (32).

5.7.7. Drug susceptibility testing

Antimicrobial Susceptibility testing with the Vitek -2 compact system was performed using an AST-GP 71 and AST GN-72 card. The cards were filled with inoculums in filling chambers .The Vitek -2 systems automatically processes the antimicrobial susceptibility cards until Minimum inhibitory concentration (MIC's) are obtained. The Vitek -2 compact system subsequently corrects, where necessary for MIC's or clinical category in accordance with the internal database of possible phenotypes for microorganism antimicrobial agent combinations .Preparation of inoculums were done by transferring 280 µl of culture suspension from the 0.5 McFarland culture suspension for gram positives and145 µl for gram negative for filling the identification cards (30,31).

Antibiotics with their different concentration used for determination of drug susceptibility profile in this investigation were Quinopristin /Dalfopristin, Cefoxitin Screen, Benzyl Penicillin, Oxacillin, Gentamicin, Ciprofloxacin, Levofloxacin, Inducible Clindamycin Resistance, Erythromycin, Clindamycin,Vancomycin and Tetracycline for Gram positives.Trimethoprim/ Sulfamethoxaole, Ampicillin, Amoxicillin/ ClavulanicAcid, Piperaciln/Tazobactam, Cefalotin, Cefazolin, Cefuroxime, Cefoxitin, Cefpodoxime,Ceftazidime, Ceftriaxone, Cefepime,

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Gentamycine,Toberamycin, Ciprofloxacin, Levofloxacin, Teteracyclin for gram negatives bacteria(31, 33).

5.8. Data Quality Assurance

All laboratory assays was done by maintaining the quality control procedures. Standard Operating Procedures (SOPs) will be strictly followed verifying that media meet expiration date and quality control parameters per CLSI guideline. Visual inspections of cracks in media or plates, unequal fill, hemolysis, evidence of freezing, bubbles, and contamination were performed. Culture media was tested for sterility and performance using reference strains of *S. aureus* (ATCC 25923) for gram positives; *E. coli* (ATCC 25922) and *P. aeruginosa* (ATCC 27853) for gram negative run simultaneously with the test organisms. The performance of VITEK 2-COMPACT for both isolation and AST were tested with *E. coli* (ATCC 25922) and *P. aeruginosa* (ATCC 27853) performance of equipments were monitored by using standard procedure.

5.8.1 Pre analytical phase

Socio-demographic characteristics of patients were collected using structure data collection sheets after getting informed consent. All ear swab culture specimens were collected by well trained laboratory personnel by following standard operational Procedure. When specimens reach the laboratory, it was checked to ensure that the correct specimen had been sent and the name on the specimen is the same as that on data collection form. To avoid sample contamination leak proof and sterile sample container was used.

5.8.2. Analytical phase

All materials, equipment and Procedures were adequately controlled. All stains and reagents were clearly labeled, dated, and stored correctly. Gram stain preparation and reporting of smears as detail in the SOPs of the microbiology laboratory of AAML was strictly follow. At regular intervals and whenever a new batch of gram stain is prepared, control smears of appropriate organisms were stained to ensure correct staining reactions. For each item of equipment there is clear operating and cleaning instructions, and service sheets. The temperature of a refrigerator, incubator, and water-bath was monitor and documented Culture media was tested for Performance and sterility.

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5.8.3. Post Analytical Phase

Post-analytical phase the results were recorded with the patients' identification number. The terminology and format used in reporting was standardized. All reports were concise and clearly presented. Before leaving the microbiology laboratory, all reports were double checked for correctness. Purified bacterial cultures will be stored in nutrient broth with 20% glycerol at –81°C by sub culturing every month. These cultures may be stocked in this condition for 5-10 years.

5.9. Data analysis and interpretation

All data collected in the study was sorted, coded and entered in a computer using SPSS software (Statistical Package for social sciences statistical software version 20). Data was cross checked against the data files for any inconsistencies and obvious data entry errors. Central tendencies measures like the mean were computed; cross tabulations were done to establish relationships between variables. Data from bacterial isolation was analyzed using qualitative methods. The study finding was presented using words and tables, Conclusions and recommendations were made based on the results.

5.10. Ethical considerations

All ethical considerations and obligations were fully addressed and the study was conducted after the approval of the Internal Review Board of Arsho Advanced Medical Laboratory private limited company and Departmental Ethics and Research committee of the Department of Medical Laboratory Sciences, Collage Health Science, and School of Allied Health Science of Addis Ababa University. Written informed consent was obtained from the participants before data collection. Each respondent was given the right to refuse to take part in the study and to withdraw at any time during the study period. All the information obtained from the study subjects were coded to maintain confidentially. When the participant was found to be positive for bacterial pathogen, they were informed and receive proper treatment. Both consent and assent was used for above 12 years age. Consent form was completed and signed by a family member and/or adult guardian for participants under the age of 12 years.

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5.11. Dissemination of the result

After the completion of the study the research will be presented to Department of Medical Laboratory Sciences, College of Health Science, and Addis Ababa University. It will also be submitted for scientific publication.

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5.12. Operational definitions

Multi Drug Resistance:

Multi Drug Resistance is defined as non-susceptibility to at least one agent in three or more antimicrobial categories (34).

Antimicrobial resistance:

Antimicrobial resistance is the ability of microbes to resist the effects of drugs that is, germs are not killed, and their growth is not stopped. It happens when microorganisms such as bacteria change when they are exposed to antimicrobial drugs (35)

Susceptible:

A bacterial isolate was considered susceptible to an antimicrobial agent when inhibited by usually achievable concentrations of antimicrobial agent when the dosage recommended to treat the site of infection is used (36, 37).

Intermediate:

A category that implies that an infection due to the isolate may be appropriately treated in body sites where the drugs are physiologically concentrated or when a high dosage of drug can be used (37).

Resistant:

A bacterial Isolate was considered non susceptible to an antimicrobial agent when not inhibited by the usually achievable concentrations of the agent with normal dosage (36).

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6. Results

6.1 Demographic characteristics of the study population in relation to ear infection

A total of 422 patients attending at Arsho laboratory Microbiology Laboratory unit with ear infection from January to April 2019 were recruited in this study. Out of 422 ear discharged participants 88.9% (375) had unilateral, 11.1 % (47) had bilateral ear sample swabs. From the study participants Majority of the study participants were females 258 Vs 164 (61.1% Vs 38.9%). The mean age of the study participants was 24 years with the minimum and maximum age of 1 year and 70 years old respectively. Majority of the study participants 76.8 (324 %) were between 25 and 44 age groups as shown in Table 1.

Based on the cultured swab a total of 422 samples of ear discharge samples were cultured no growth showed 80.5% (340). The overall prevalence of bacterial isolates was 19.5% (82), from the total bacterial isolated were showed 78 % (64) Gram negative bacteria while 22 % (18) showed Gram positive bacteria. Majority 39 % (32) of the bacterial isolated were found in 1 -14 years age groups followed by 35.4 % (29) were 25-44 years age groups.

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Table1. Demographics of the study population in relation to prevalence of ear discharge infection, patients visited AML January to April 2019.

Variables	Category	Total Ear swab Samples	No Growth No (%)	Growth of Gram Negative No (%)	Growth of Gram Positive No (%)
Gender	**Female**	258(61.1 %)	214(63%)	34(53.1%)	10(55.6 %)
	Male	164 (38.9%)	126(37 %)	30(46.9%)	8(44.4 %)
	Total	422(100%)	340(80.5%)	64(15.2%)	18(4.3%)
Age group	<1	5(1.2%)	5	0	0
	1-14	104(24.6%)	72(21.2%)	22(34.5%)	10(55.5%)
	15-24	105(24.9%)	88 (25.9%)	14(21.9%)	3(16.7%)
	25-44	162(38.4%)	133(39.1%)	26(40.6%)	3(16.7%)
	45-64	40(9.5%)	37(10.9%)	1(1.5%)	2(11.1%)
	>65	6(1.4%)	5 (1.4%)	1(1.5%)	0
	Total	**422(100)**	**340(80.5%)**	**64(15.2%)**	**18(4.3%)**

Age classification based on WHO Age classification for health (38)

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6.2. Prevalence of Gram positive and Gram negative bacteria

Among the isolated pathogens 78% (64) were gram Negative & 22 % (18) gram positive bacteria respectively as shown Table 2. From gram positive bacteria predominant bacterial isolates were *S.*aureus 12 (66.6%), followed *CoNs 6(33.4%)*. while gram Negative bacterial isolated the most predominant bacteria was *pseudomonas* spp. and proteus spps, 14 (21.9 % each) followed by *Klebsella* spps.13(20.4%) , *Enterobacter* species 10 (15.6 %) and *Providencia* spp 4 (6.3%) respectively, other bacteria's were least accounted for 9(14%).The frequency of positive ear discharge cultures was higher in the age group at 1-14 years 32(39%) followed by the age group at 25-44years (35.4 %) as shown Table 1. *S. aureus, Proteoues spp, P. aeruginosa,, Kelebsela spp, H.influnzae and E.coli w*ere the dominant bacterial isolated on the above mentioned age groups.

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Table 2. Distribution of Gram positive and Gram negative bacterial isolated from ear discharge among different age groups of patients visiting Arsho Medical Laboratory from January to April 2019.

Age group	Gram Positive Bacteria	S.auers 12(66.6%)	Gram Negative Bacteria	P.aeroginosa 13(20.3%)	P.fluorescens 1(1.6%)	P. mirabilils 9(14.1%)	P. vulgaris 5(7.8%)	K.pneumoniae 6(9.4%)	K.oxytoca 6(9.4)	K.ozonae 1(1.6%)	E.coli 5(7.8%)	E.cloacae 5(7.8%)	Providencia rettgerii 3(4.7%)	providentia stuartii 1(1.6%)	Salmonella group A 1(1.6%)	H. influenzae 1(1.6%)	Aeromonas caviae 1(1.6%)	Aeromonas hydrophila 1(1.6%)	Entrobacter amnigenus 1(1.6%)	Acinetobacter lwoffii 1(1.6%)	Cupriavidus pauculus 1(1.6%)	Bukholderia cepacia 1(1.6%)	Citrobacter freundii 1(1.6%)
1-14		8		2	0	6	1	2	3	0	3	1	1	0	0	1	1	0	0	0	0	0	1
15-24		2		4	1	1	1	1	0	0	0	1	0	1	1	0	0	0	0	1	1	1	0
25-44		1		6	0	2	3	3	2	1	2	3	2	0	0	0	0	1	1	0	0	0	0
45-64		1		1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
≥65		0		0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0

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6.3. Antimicrobial susceptibility pattern of gram positive bacteria.

The overall drug susceptibility profile of Gram positive bacteria against antimicrobial drugs tested were summarized under Table 3. The antibiotic susceptibility of gram positive was done of 12 *S.aureus* isolates high levels of susceptibility exhibited on Levofloxacin (91.7%), Moxifloxacin, (100%),*Quinupristin* (100%), *Dalfopristin* (100%), Linezolid (100%), Daptomycin(100%), Vancomycin (100%), Minocycline (100%), Tigecycline (100%) & Rifampicin (100%) . However resistance rates were seen ranging from (16.7 - 75%) for Ciprofloxacin (16.7%), Tetracycline (25%), Clindamycin &Erythromycin (33.3%),Gentamicin (41.6%), Benzylpenicilline (50%) and Oxacillin (75%) (Based on cefoxitin screening)

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Table 3: Percentage of antibacterial susceptibility pattern of gram positive bacteria from ear discharge samples at AAML from January to April 2019 (N=18).

organizims	*Pattern*	*PEN*	*OXA*	*GEN*	*CIP*	*LEV*	*MXF*	*ERY*	*CL*	*QDA*	*LNZ*	*DAP*	*VAN*	*MNO*	*TET*	*TGC*	*RIF*	*TMP*
S.aureus (N=12) %	*I*	*0*	*0*	*0*	*8.3*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*
	R	*50*	*75*	*41.6*	*16.7*	*8.3*	*0*	*33.3*	*33.3*	*0*	*0*	*0*	*0*	*0*	*25*	*0*	*0*	*33.3*
	S	*50*	*25*	*58.4*	*75.0*	*91.7*	*100*	*66.7*	*66.7*	*100*	*100*	*100*	*100*	*100*	*75*	*100*	*100*	*66.7*

Abrevations:- PEN=Benzylpenicillin, OXA=Oxacillin,GEN= Gentamicin,CIP= Ciprofloxacin,LEV= Levofloxacin,MXF= Moxifloxacin,ERY= Erythromycin,CL= Clindamycin,QDA= Quinupristin,Dalfopristin,LNZ= Linezolid,DAP= Daptomycin,VAN= Vancomycin,MNO= Minocycline,TET= Tetracycline,TGC= Tigecycline,RIF= Rifampicin,TMP= trimethoprime/sulfamethoxazole.,

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6.4. Antimicrobial susceptibility patterns for Gram Negative bacteria.

A total of 13 different gram negative species of bacteria were isolated and identified, in which 64 (78 %) patients had the presence of bacterial associated with ear infection/OM, as shown table 5). The overall antimicrobial susceptibility among gram negatives were Ampicilin shows high resistance (85.9 %) followed by Cefazolin (70.3%) and Tetracycline, Cefalotin (65.7% each) respectively. High level of sensitivity is shown by Levofloxacin (95.3%),Tobramycin (92.2%), followed by Piperacillin/Tazobactam, Ciprofloxacin (86 % each) and Gentamycin (81.2%).

Among the isolated gram negative bacteria *P.aeroginosa* (N=13) were the most predominant isolate, *P.aeroginosa* were 100 % resistant to Ampicillin, Amoxicillin clavulanicacid, Cefalotin and Tetracycline. (92.3%) to Ceftazidime, Cefoxitin , Cefpodoxime and (84.6 %) were resistance to Ceftriaxone, Cefuroxime and CefuroximeAxetil. The lest resistance was observed to (7.7%), Piperacillin/Tazobactam (15.4%) & Cefepime(23.1%). The most active agent for *P.aeroginosa* was seen Gentamycin, Levofloxacin & Tobramycin (100%).While *P.fluorescens* (100%) susceptible for all 18 antimicrobial drugs.

The second most predominant isolate were *P.mirabilis (N=9)* and it was resistance both tetracycline and Cefazoline 77.8 %. It was High sensitivity were seen on Levofloxacin, Piperacillin/Tazobactam and Tobramycin. On the other hand *P. vulgaris 100% resistance to Ampicillin, Cefazolin, Cefuroxime, Cefalotin and Tetracycline were observed but it was 100% sensitive to* Ciprofloxacin & Levofloxacin.

K.oxytoca & K.pneumoniae were high level of susceptibility 100% seen to Ciprofloxacin, *Levofloxacin and Tobramycin. K.ozonae high* susceptible 100% for all 16 listed drugs except Ampicillin and Amoxicillin Clavulanic acid it was 100% resistance.

Forth commonly isolated gram negative bacteria E.coli and *E.clocae* 80% to 100% resistance was observed on Amoxicillin clavulanic acid and Ampicillin. *E.clocae* was *100 %* susceptible *on Cefepime, Ceftazidime, Ceftriaxone, Ciprofloxacin, Gentamycin, Levofloxacin, Piperacillin/ Tazobactam, Tetracycline, Tobramycin, and Cotrimoxazol.*

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Isolated *Providential Rettgrii* and *Providential stuartii* showed highly resistance to Ampicillin, Amoxicillin clavulanic, *Cefazolin, Gentamycine, and Tetracycline (100%). Salmonella group A except for and H.influenza was* effective against most of listed antimicrobial drugs.

Isolted E.aerogens, Citrobacter freundii, Acinetobacter lwoffii,Cupriavidus pauculs, Bukholderia Cepacia and Aeromonas spps are the least isolated gram negative bacteria. *Aeromonas hydrophila* showed high susceptibility rate (100%) for all drugs and *Aeromonas caviae1* showed (100%) resistance for Ampicilline, Amoxicillin Clavulanic acid and cephazolin. Trimethoprime/ Sulfamethoxazole, *Tobramycin, Tetracyclin, Piperacillin/Tazobactm, Levofloxacin* and Gentamycin were the most effective against *E. amnigenus*. *Citrobacter freundii* showed High susceptibility on Piperacillin/Tazobactam & Gentamycin. *Acinetobacter lwoffii* were effective against most drugs except for Cefazolin and Cefalotin were 100% resistance documented.

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Table4: Percentage of antibacterial susceptibility pattern of Gram Negative bacteria isolated from ear discharge culture sample at AAML from January to April 2019 (N=64).

Specie	Pattern	AMP	AMC	CFZ	CEFP	CEFX	CFPD	CEFZ	CFXN	CEFRO	CEFAX	CFALO	CIP	GEN	LEV	PIP	TETR	TOB	TMP
	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
P.aeroginosa	R	100	100	92.3	23.1	92.3	92.3	76.9	84.6	84.6	84.6	100	7.7	0	0	15.4	100	0	84.6
(N=13)%	S	0	0	7.7	76.9	7.7	7.7	23.1	15.4	15.4	15.4	0	92.3	100	100	84.6	0	100	7.4
	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
P.fluorescens	R	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
(N=1) %	S	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
P. mirabilils	I	11.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
(N=9) %	R	66.7	55.6	77.8	55.6	22.2	55.6	55.6	55.6	66.7	66.7	55.6	11.1	22.2	0	0	77.8	0	22.2
	S	22.2	44.4	22.2	44.4	77.8	44.4	44.4	44.4	33.3	33.3	44.4	88.9	77.8	100	100	22.2	100	77.8
P. vulgaris	I	0	0	0	0	0	0	20	0	0	0	0	0	0	0	0	0	0	0
(N=5) %	R	100	40	100	40	20	20	20	20	100	80	100	0	40	0	20	100	20	60
	S	0	60	0	60	80	80	60	80	0	20	0	100	60	100	80	0	80	40
	I	0	0	0	0	0	0	0	0	0	16.7	0	0	0	0	0	0	0	0
K.pneumoniae	R	83.3	50	33.3	16.7	33.3	16.7	16.7	16.7	16.7	16.7	33.3	0	16.7	0	16.7	33.3	0	33.3
(N=6) %	S	16.7	50	66.7	83.3	66.7	83.3	83.3	83.3	83.3	66.6	66.7	100	83.3	100	83.3	66.7	100	66.7

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K.oxytoca	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
(N=6)%	R	83.3	16.7	33.3	16.7	0	16.7	16.7	16.7	16.7	33.3	16.7	0	16.7	0	0	50	0	66.7
	S	16.7	83.3	66.7	83.3	100	83.3	83.3	83.3	83.3	66.7	83.3	100	83.3	100	100	50	100	33.3
K.ozonae	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
(N=1) %	R	100	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	S	0	0	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
E.coli	I	0	0	0	0	20	0	0	0	0	0	20	0	0	0	20	0	20	0
(N=5) %	R	100	80	60	60	20	60	60	60	60	60	60	60	40	40	20	80	0	80
	S	0	20	40	40	60	40	40	40	40	40	20	40	60	60	60	20	80	20
E.cloacae	I	0	0	0	0	0	0	0	0	40	0	0	0	0	0	0	0	0	0
(N=5) %	R	100	80	80	0	80	80	0	0	20	60	80	0	0	0	0	0	0	0
	S	0	20	20	100	20	20	100	100	40	40	20	100	100	100	100	100	100	100
Providencia	I	0	0	0	66.7	0	0	66.7	66.7	0	0	0	66.7	0	0	0	0	0	0
rettgerii	R	100	100	100	0	66.7	66.7	0	0	66.7	100	100	0	100	0	33.3	100	100	66.7
(N=3)%	S	0	0	0	33.3	33.3	33.3	33.3	33.3	33.3	0	0	33.3	0	100	66.7	0	0	33.3
Providentia	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
stuartii	R	100	100	100	0	0	0	0	0	100	100	100	0	100	0	0	100	0	0
(N=1) %	S	0	0	0	100	100	100	100	100	0	0	0	100	0	100	100	0	100	100

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Salmonella group A (N=1) %	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	R	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	0	0
	S	0	100	100	100	100	100	100	100	100	100	100	100	100	100	100	0	100	100
H.influenzae (N=1)%	I	0	0	0	0	0	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
	S	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Aeromonas caviae1 (N=1)%	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	R	100	100	100	0	0	0	0	0	0	0	100	0	0	0	0	0	0	0
	S	0	0	0	100	100	100	100	100	100	100	0	100	100	100	100	100	100	100
Aeromonas hydrophila (N=1)%	I	0	0	0	0	0	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
	S	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Entrobacter aerogens (N=1)%	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	0	0	0
	R	100	100	100	100	100	100	100	100	100	100	100	100	0	0	0	0	0	0
	S	0	0	0	0	0	0	0	0	0	0	0	0	100	100	0	100	100	100
Citrobacter freundii (N=1)%	I	0	0	0	0	0	0	100	0	0	0	0	0	0	0	0	0	0	0
	R	100	100	100	100	100	100	0	100	100	100	100	100	0	100	0	100	100	100
	S	0	0	0	0	0	0	0	0	0	0	0	0	100	0	100	0	0	0
Acinetobacter lwoffii(N=1)%	I	0	0	0	0	0	100	0	0	0	0	0	0	0	0	0	0	0	0
	R	0	0	100	0	0	0	0	0	0	0	100	0	0	0	0	0	0	0
	S	100	100	0	100	100	0	100	100	100	100	0	100	100	100	100	100	100	100

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Cupriavidus	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
pauculs	R	0	0	100	0	0	0	0	0	0	0	100	0	0	0	0	100	0	100
(N=1)%	S	100	100	0	100	100	100	100	100	100	100	0	100	100	100	100	0	100	0
Burkholderia	I	0	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cepacia(N=1)	R	100	0	100	0	0	100	0	0	100	100	0	0	0	0	0	100	0	0
%	S	0	100	0	100	100	0	100	100	0	0	100	100	100	100	100	0	100	100
Resistance	I	1.6	0	0	0	1.5	1.5	6.2	3.0	3.1	56.3	1.5	3.1	0	0	3.1	0	0	0
rate (%)	R	85.9	62.5	70.3	26.6	40.6	50.0	36	37.6	53.1	1.5	65.7	10.9	18.8	4.7	10.9	65.7	7.8	46.9
Total 64	S	12.5	37.5	29.7	73.4	57.9	48.5	57.8	59.4	43.8	42.2	32.8	86	81.2	95.3	86	34.3	92.2	53.1

***Abrevations**: AMP=Ampicillin,AMC=*_Amoxicillin clavulanic acid *CFZ=Cefazolin,CEFP=Cefepime,CEFX= Cefoxitin,CFPD= Cefpodoxime, CEFZ =Ceftazidime CFXN= Ceftriaxone,CEFRO =Cefuroxime CEFAX =Cefuroxime Axetil ,CEFALO =Cefalotin ,CIP= Ciprofloxacin ,GEN= Gentamycin ,LEV= Levofloxacin ,PIP =Piperacillin/Tazobactam ,TETR =Tetracycline ,TOB =Tobramycin ,TMP=* trimethoprime/sulfamethoxazole.

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6.4. Multidrug resistance patterns for the isolated gram negative bacteria.

From 17 antibiotics which have been tested against each bacterial isolates 46.9% with an overall gram negative multi drug resistance (resistant for ≥ four different classes of antibiotics). *P.aruginosa (92.4%)*, *P. vulgaris* (80.%) ,*E.cloli 60% and P.mirabilis 44.5%* had multidrug resistance observed as shown Table 5.

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Table 5: Multidrug resistance pattern of Gram negative bacterial isolates from ear discharge samples at Arsho Advanced Medical Laboratory from JanuarytoApril2019

Organisms	R_0	R_1	R_2	R_3	≥R4
P.eruginosa (N=13)	0	0	0	7.6%(1)	92.4% (12)
P. fluorescens(N=1)	100%(1)	0	0	0	0
Proteus mirabilils (N=9)	11.1%(1)	11.1%(1)	11.1%(1)	22.2%(2)	44.5%(4)
Proteus vulgaris (N=5)	0	0	0	20%(1)	80%(4)
Klebsiella pneumoniae (N=6)	0	66.8%(4)	0	16.6%(1)	16.6%(1)
Klebsiella oxytoca (N=6)	16.6%(1)	16.7%(1)	0	50%(3)	16.7%(1)
K.ozonae(N=1) %	0	100%(1)			
E.coli (N=5)	0	0	20%(1)	20%(1)	60%(3)
E. cloacae (N=5)	0	20%(1)	80%(4)	0	0
Providencia rettgerii (N=3)	0	0	0	0	100%(3)
Providencia stuartii (N=1)	0	0	0	0	100%(1)
Salmonella group A(N=1)	0	0	100%(1)	0	0
H. influenzae(N=1)	100%(1)	0	0	0	0
Aeromonas caviae (N=1)	0	0	100%(1)	0	0
Aeromonas hydrophila(N=1)	100%(1)	0	0	0	0
Entrobacter aerogens (N=1)	0	0	100%(1)	0	0
Citrobacter freundii(N=1)	0	0	0	0	100%(1)
Acinetobacter lwoffii(N=1)	0	100%(1)	0	0	0
Cupriavidus pauculs(N=1)	0	0	0	100%(1)	0
Bukholderia Cepacia(N=1)	0	0	0	100%(1)	0
Total (64)	0.02%(5)	14.5%(9)	14%(9)	17%(11)	46.9%(30)

R_0- no resistant for any antibiotic, R_1-resistant for 1 class of antibiotic, R_2- resistant for 2 classes of antibiotics, R_3- resistant for 3 classes of antibiotics, R_4- resistant for 4 and more than 4 classes of antibiotics. Classification Based on CLSI 2017 category (33).

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7. Discussion

Ear infection is a more frequent treatable health care problem worldwide, yet if left untreated; it can cause a serious complication such as a speech development disorder, hearing loss, distress in patients and their family quality of life, and economic burden on the health care system. The burden and prevalence of ear infection are more intense in developing countries due to the poor living standard and hygienic conditions along with lack of proper nutrition. Thus, highlighting the etiologic agent of Ear infection and their antibiotic susceptibility pattern will help us to lessen the severity of the infection (37). This study revealed that *Proteus* spp., *S. aureus*, and *Pseudomonas* spp, were the most prevalent multi-drug-resistant pathogenic bacteria isolated suspected patients attending Araho Advanced Medical Laboratory with ear discharges.

We found a prevalence of ear infections of 19.5 %, this studies is beyond to the prevalence of reported by the WHO for Africa 11 % (8).

In the present study the prevalence of bacteria isolated from OM patients was 19.5%. It was lower than study report from Gonder Ethiopia (89.5%) by Muluye et al 2013; from Mekele Ethiopia (98.2%) by Wasihun et'al 2015 and from Hawassa Ethiopia (52.1%)by Worku M et'al 2014 (7,14,44). And also in the lower side from the study conducted by Afolabi et al, 2012 in Nigeria 53.7% but it is in a higher side compared to the study conducted in Kigali Rwanda 5.8% by Kaitesi Batamuliza Mukara et al 2017 (10,19). The geographical variation & climate difference of the study area attributed to this rate of bacterial isolation deference which can be explained by the effect of temperature on the bacterial colonization of otitis media which favors the bacterial to colonize as the temperature increases there by increases the possible isolation rate of the bacteria from the Otities media.

In the present study, the highest percentage of ear infection was found among pediatric patients (39 %) and this agrees with reports from other parts of Ethiopia, in addition, the majority of bacterial isolates were identified in the this age group less than 14 (14, 29). although a study done in Bahir Dar (Ethiopia) reported higher frequency in the age group of 11–18 years (87.9 %) (27).

The isolation rate of GN bacteria was 78% which is comparable with the report from the study conducted at Dessie in Ethiopia (74.2 %) by Abera B, et,al, 2011; A similar report also

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communicated from other countries like in Lusaka, Zambia (73.0%) by Phiri1 H et;al , Dhaka Bangladesh (74.7 %) by Akter *et al* 2015, Abeokuta, Nigeria ((73%) by Motayo B et'al 2012(25,26). In the other way, the present study report is in high side from the study report in in Addis Ababa (60.5 %) by Ferede et al. 2001 and from out of Ethiopia by Gujarat India (63.5%) by *Panchal PD et al, 2015 (23,39)*. This variation might be due to differences in the etiological agent distribution of OM in accordance to climatic conditions.

The occurrence of *S. aureus 66.6 %* of current study was comparable with findings with Addis Ababa ,Ethiopia which is (57.4 %) by Tadesse et al. 2018 and in Chitwan, Nepal 78 % by Basnet R et'al 2017. But higher than the report from studies conducted in Gonder Ethiopa (26.5%) by Muluye et'al 2013. Probable explanation to this deference in isolation rate of isolates might be related to the effects of climate and geographical variation (7, 42, 43).

The predominate isolated GN bacteria from the specimens in our study were Pseudomonas aeruginosa 20.3% which is comparable from the study report from Dessie Ethiopia 18% by Bayeh Abera and Mulugeta Kibret 2011 and from Wollo Ethiopia 17 % by Denboba et al and which is lower than the finding from *Bahirdar Ethiopia 29.7% by* Hailu *et al* 2016, and from Nigeria 28.3% by Osazuwa F, et 'al; 2011. (22, 29, 36, 40). Similarly this may be also due to the difference in climate and geographical variations in different Study area.

The knowledge of the bacteriology of an Ear infection and the laboratory susceptibility testing of micro-organism implicated could make drug selection in antimicrobial chemotherapy more rational (30).Therefore, this study tried to address antibacterial susceptibility and resistance pattern of the commonly isolated bacteria from ear infection. Based on the Table 4 &5, *Moxifloxacin*, *Quinupristin* , *Daptomycin* ,*Linezolid* ,Glycopeptides/*Vancomycin/*, *Minocycline, Tigecycline & Rifampicin,* revealed high level of susceptible (100 %) of all identified GP bacteria (*S.aureus and CoNs)* but the resistance rate was high in *Oxacillin (83.3%),Gentamicin (50%) and Erythromycin (33.3%)* . These results were almost comparable with previous study done in Hawassa Ethiopia by Deyno et al 2017 and Dessie Ethiopia by Bayeh Abera1and Mulugeta Kibret 2011 (22, 30). On the other hand, the total resistance rate for the identified GN bacterial were highly resistance to Ampicilin (85.9 %) followed by Cefazolin (70.3%) and *Tetracycline, Cefalotin* (65.7%).

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Pseudomonas Spps , Proteus spps and Klebsiella spp showed high resistance to Ampiciline and Amoxicillin clavulanic acid with 50% to 100%. But, it was highly susceptible to *Ciprofloxacin, Gentamycin ,Levofloxacin , Piperacillin/Tazobactam and Tobramycin (80% to 100%)*. Similar finding were also reported in Gondar Ethiopia by Muluye et al 2013, Dessie Ethiopia by Bayeh Abera1and Mulugeta Kibret 2011 and Lusaka Zambia by Phiri H, et'al. The Prescription of antibiotics without laboratory diagnosis and over sales of antibiotics without clinicians prescription and also unable to take the full dose of the prescribed drugs in the public side might be attributed to the high emergency of the drug resistance bacteria (7,22, 26).

Multi-drug-Resistant Pathogenic bacteria isolated from Ear discharges almost all the isolated bacteria were found to be resistant to one and more than one commonly used antibiotics (Table 6). Among the total isolated *P*.eruginosa 92.4 % and *P.vulgaris* 80 % developed MDR to four and more than four class of antibiotics in clinical use. Similarly *E.coli* 80 % isolates were able to resist two and more classes of antibiotics.Similar findings were also reported in Wollo Area, Northeastern Ethiopia by Denboba eta'l 2016 (41).

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8. Strength and limitation of the study

8.1. Strength

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- The present study able to generate information regarding to which is the predominate isolated pathogens from OM and also communicated information for clinician how much the isolated bacteria is resistance to one or more common Antibiotics which are commonly utilized by the clinician to treat their patients suffering from OM infectious.
- It makes the reports of culture results faster, which has the potential to improve patient care, and reduce health care costs

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8.2. Limitation of the study

- The present study could not incorporate or generate information regarding to the *Streptococcus spps* since the study was limited with a specific reagent card for Vitak -2 compact for identification & susceptibility study of the isolated bacteria.
- Being the study as a single laboratory based it may lack representativeness

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9. Conclusion

In conclusion, based on WHO classification the overall prevalence of bacterial isolates was high (19.5%) and majority of the isolates were GN bacteria 78%. The predominant isolates were *P.aergenosa* and *Proteus species* (21.1%) followed by *S.aureus* 66%. The bacteria which have been isolated from OM have shown high level of antibiotics resistance in the study area. Majority of the bacterial isolates had multiple antibiotic resistant patterns. Knowledge of the pathogenic agents responsible for OM and choice of effective antibiotics according to susceptibility pattern will guide the treatment. It also helps in reducing complications of the disease and decreasing emergence of resistance to antibiotics. Hence antibiotics susceptibility test is mandatory before prescribing any antibiotics.

Our study revealed almost all the isolated pathogenic bacteria were considerably susceptible to ciprofloxacin and gentamicin. Particularly, Ciprofloxacin was shown to be highly effective for the three leading isolated pathogenic bacteria associated with middle Ear infection in this study: *Proteus* spp., *S. aureus*, and *Pseudomonas* spp. moreover , with a slight variation several other studies have shown a similar high efficiency of ciprofloxacin against these bacterial species.

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10. Recommendations

It is recommended that the government/ stakeholders should improvise mechanisms of Constant provision of appropriate medication to treat the common Ear infection causing microorganisms (*Proteus mirabilis, Pseudomonas aureginosa, Staphylococcus aureus*,) at all levels of health delivery. Constant provision of information regarding the common pathogens responsible for Ear infection as it is essential in the selection of the most appropriate treatment regimen and formulation of treatment protocols that will minimize complications.

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Annexes

Annex I: Participants information sheet [English version].

Principal Investigator: Melesse Desse, Addis Ababa University school of Allied Health Sciences.

Purpose: The purpose of this study is to assess the prevalence of AST profiles of bacteria, multi drug resistance of isolated bacteria from Ear swab culture.

Procedures to be carried on: you are invited to participate in the study after giving your consent and by giving the requested sample for investigation.

Risks associated with the study: There is no risk and serious invasive procedure at the beginning as well as at the end of the study and there is no additional time required from you to stay during study.

Benefits of the study: There will be no financial benefit to you. But the result of the study will be used for to develop antibiogram that helps the patients avoiding empirical treatment.

Confidentiality of your information: The results of the laboratory findings will be kept confidential and could only be accessed by the researcher and the responsible physician. There will be no personal information to be attached to your data.

Termination of the study: We will respect your decision if you later on change your mind and you can refuse to participate or withdraw from the study at any time. Refusal to participate will not result in loss of medical care provided or any other benefits. You can get your results of the analysis.

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Annex II. Informed consent [English version]

I undersigned individual, am oriented about the objective of the study. I have informed that all of my information will be kept confidential and used only for this study. Your signature below indicates that you have read /or listened, and understand the information provided for you about the study.

Before you sign, please understand purpose of the study, procedure, risks and benefits of participation, right to refuse or withdraw, confidentiality and privacy, and who to contact if you have any question.

I have read /or listened to the description of the study and I understand what procedures are and what will happen to me in the study.

Based on the above information I agree to participate in the research

Signature: _____ Date: _____

Name of Data collector _____ Signature _____

if you have any question you can ask the principal investigator

Principal investigator Mr Melesse Desse [BSc, Msc candidate]

Mobile 0913194837

E-mail.melessedessie@yahoo.com

Annex III: Participant's information sheet [Amharic version]

ጥናቱን የታሚያጠናው፤

ሚላሰ ደሴ በአዲስ አበባ ዩኒቨርሲቲ ጠፍ ሳይንስ ኮሌጅ የህክምና ላቦራቶሪ ሳይንስ ዲፓርትመንት

የጥናቱአላማ፤

የጥናቱ ዓላማ መድሃኒት የተለማመዱ በደም ወስጥ የሚኙ ህዋስ /ባክቴሪያዎችን/ ስርጭት በአርሾ አደባንስድ ሚዲካል ላቦራቶሪ ከጆሮ ላይ ከሚወሰድ ናማ ወስጥ በሚላየት የፀረ-ባክቴሪያ መድሃኒት የመቋቋም አቅማቸውን ማወቅ፤ አሁን ያሉበትን ደረጃ ማለየት እና የመፍትሄ አቅጥጫ ማስቀመጥ፡፡

ለጥናቱ ተሳታፊዎች ያለው ልዩ ጥቅም፤

በጥናቱ ለሚሳተፉ ፍቃደኛ ተሳታፊዎች ምንም አይነት የገንዘብ ክፍያ የለወም ነገር ግን ከጥናቱ የሚኘው ወጤት ለርስዎ ህክምና ተጨማሪ መረጃ ለማግኘት በተማሳይ ለመድሃኒት ልምምድ ያደረጉትን ካላደረጉት በሚላየት ወጤታማ የሆኑትን መድሃኒቶች ይጠቁማል፡፡

በጥናቱተሳታፊዎች ላይ ያለው ጉዳት

በጥናቱ መጀመሪያም ይሁን መጨረሻ በዚህ ጥናት ላይ በማሳተፍዎ ሊደርስብዎ የሚቻል አንድም ጉዳት አይኖርም፡፡በጥናቱ ምክንያት የሚባክኑት ተጨማሪ ጊዜም አይኖርም፡፡

የሚጃ ሚስጥራዊ አጠባበቅ፣

የሚሰጡት መረጃ በጥናቱ ወቅትም ሆነ ከዚያ በኋላ ባሉት ጊዜያት ማሉ በማሉ ሚስጥራዊነቱ የሚጠበቅና መረጃወም የሚያዘዉ በስም ሳይሆን በማያ ቁጥር ይሆናል፡፡ በጥናቱ ላይ ያለማሳተፍ መበት አለዎት ፡፡ይህ መረጃ በጥንቃቄ የሚያዝ ይሆናል፡፡ በማጨረሻም የጥናቱ ወጤት ለሚሳከተዉ አካል ለጥናቱ አላማ ለህክምና ባለሞያዎች ብቻ የሚለፅ ይሆናል።

ስለዚህ ጥናት ማንኛወም ጥያቄ ካለዎት በማንኛወም ጊዜከዚህ በታች በተጠቀሱት አድራሻዎች ማጠየቅ ይችላሉ;; እኔም የጥናቱ ተሳታፊ ይህንን በመገንዘብ ጥናቱ ላይ ለማሳተፍ ተስማሟቼያለሁ፡፡

ፊርማ ----------

መረጃወን የሰበሰበወግለሰብስም---------- ፊርማ ----------

የዋና ተመራማሪ አድራሻ

ሚላሰ ደሴ
አዲስ አበባ ዩኒቨርሲቲ ፣የጠፍሳይንስ ኮሌጅ፣የሕክምናላቦራቶሪ ቴክኖሎጂ ዲፓርትመንት
አዲስአበባ፣ኢትዮጵያ
ኢ-ሜይል፣ melessedessie@yahoo.com
ስ.ቁ +251-913-194837

Annex IV. Informed Consent [Amharic version]

በዚህ ጥናት ለሚሳተፉ ሃሳባችወን መግለጥ ለሚቻሉ የስምምነት መጠየቅያ ቅጽ

እኔ ፊርሜ ከዚህ በታች የተቀመጠው ግለሰብ ከእኔ የሚወሰደዉ ናሙና ለጥናቱ አላማ ብቻ እንደሚወል ተረድቻለሁ፡፡ ሁሉም መረጃዎች እና የናሙና ወጤቱም ሚስጠራዊ መሆኑን ተገንዝቤአለሁ፡፡በጥናቱ ላይ በመሳተፌ ምንም የገንዘብ ክፍያ እንደማላገኝ ተረድቻለሁ፡፡ ከምርመሩ መሳተፍ ወይም አለመሳተፍ መበቴ የተጠበቀ መሆኑን እና ላለመሳተፍ ብወስን በላቦራቶሪው በሚደረግበልኝ ምርመራ ላይ ምንም ተፅዕኖ እንደማይኖረዉ ተረድቻለሁ፡፡ ስለዚህ የጥናቱን ጠቃሚት አምኜበት የስምምነት ቃሌን የሰጠሁት በፍፁም ፈቃደኝነት ነዉ፡፡

ስለዚህ ጥናት ማንኛውም ጥያቄ ካለዎት በማንኛውም ጊዜ ከዚህ በታች በተጠቀሱት አድራሻዎች መጠየቅ ይችላሉ፡፡

እኔም የጥናቱ ተሳታፊ ይህንን በመገንዘብ ጥናቱ ላይ ለመሳተፍ ተስማምቼያለሁ፡፡

የጥናቱ ተሳታፊ ፊርማ ----------ቀን----------

መረጃወን የሰበሰበው ግለሰብ ስም----------

ፊርማ ----------

የዋና ተመራማሪው አድራሻ

መላሰ ደሴ

አዲስ አበባ ዩኒቨርሲቲ፣የጤና ሳይንስ ኮሌጅ፣የሕክምና ላቦራቶሪ ቴክኖሎጂ ዲፓርትመንት

አዲስ አበባ፣ኢትዮጵያ

ኢ-ሜይል፣ E-mail.melessedessie@yahoo.com

ስ.ቁ +251-913194837

Annex V. Parental/Guardian consent form in English

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

Parent/guardian Signature: _____ Date: _____

Name of Data collector _____ Signature _____ if you have any question you can ask the principal investigator

Principal investigator Mr Melesse Desse [Msc candidate]

Mobile 0913194837

E-mail.melessedessie@yahoo.com

Annex VI .Guardian /parental consent form in Amharic

የወላጅ/የአሳዳጊ/ የሞግዚት የስምምነት ማጠየቂያ ቅጽ

እኔ ፊርማዬ ከዚህ በታች የተቀመጠው –የታማሚው ወላጅ/አሳዳጊ/ ሞግዚት ስሆን የዚህን ጥናት አላማ በወል ተረድቻለሁ፡፡

በጥናቱ ወቅትም ታማሚው መረጃዎች በሚስጥር ስለሚዝ በሌላ ሰዉ ዘንድ እንደማይታወቅ ተረድቻለሁ፡፡ በወጩ ከማ ኘዉ የህክምና አገልግሎት በቀር ሌላ ታማሚው በግሉ የሚያገኘዉ ጥቅም እንደሌለ ተረድቻለሁ፡፡ጥያቄ እንድጠይቅ ዕድል ተሰጥቶኝ ለጥያቄዎቼም በቂ ምላሽ አግኝቻለሁ፡፡ የልጄ በጥናቱ መሳተፍ በእኔ ፍላጎት ብቻ እንደሆነ እና በጥናቱም አለመሳተፍ ምንም አይነት ተፅዕኖ ታማሚው ላይ እንደማያስከትል ተረድቻለሁ፡፡ በከዚህ ባሻገር ታማሚው በጥናቱ ወስጥ ለመሳተት የእኔ ወላጅ አሳዳጊ/ሞግዚ ትፈቃድ እንደሚያስፈልግ ተረድቻለሁ፡፡ በእኔ ፍቃደኝነት ታማሚው በጥናቱ እንደሚሳተፍ ከዚህ በታች በፊርማዬ አረጋግጣለሁ፡፡

የጥናቱ ተሳታፊ ወላጅ/አሳዳጊ/ሞግዚት ፊርማ _____

መረጃወን የሰበሰበወግለሰብስም----------

ፊርማ ----------

የዋና ተመራማሪው አድራሻ

መላሰ ደሴ

አዲስ አበባ ዩኒቨርሲቲ፣ የጠፍ ሳይንስ ኮሌጅ፣ የሕክምና ላቦራቶሪ ቴክኖሎጂ ዲፓርትመንት

አዲስ አበባ፣ ኢትዮጵያ

ኢ-ሜይል፣ E-mail.melessedessie@yahoo.com

ስ.ቁ +251-913194837

Annex VII: Assent form for adolescent (12 -17 years old) study participants (English version)

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

Study participant Signature: _____ Date: _____

Name of Data collector _____ Signature _____ if you have any question you can ask the principal investigator

Principal investigator Mr Melesse Desse [Msc candidate]

Mobile 0913194837

E-mail.melessedessie@yahoo.com

Annex VIII: Assent form for adolescent (12-17 years old) study participants (Amharic version)

በአማርኛ የተዘጋጀ ዕድሜያቸዉ ከ12 እስከ 17ዓመት ለሆኑ ታዳጊ ወጣት የጥናት ተሳታፊዎች የተሳትፎ ማረጋጋጫ ቅጽ::

ከዚህ በታች ስሜ የተገለጠው በዚህ ጥናት ወስጥ እንድሳተፍ ፍቃደኝነቴን ተጠይቂያለሁ፡ ፡ ወላጆቼም/አሳዳጊዎቼም በጥናቱ እንድሳተፍ ወይም እንዳልሳተፍ ምርጫው የእኔ መሆኑን ነግረወኛል በጥናቱ ወቅትም የእኔ መረጀዎች በሚስጥር ስለሚያዝ በሌላሰዉ ዘንድ እንደሚይታወቅ ተረድቻለሁ በወጩቱ ከሚኘዉ የህክምና አገልግሎት በቀር ሌላ በግሌ የማኘዉ ጥቅምእንደሌለተረድቻለሁ፡ ፡ ጥያቄ እንድጠይቅ ዕድል ተሰጥቶኝ ለጥያቄዎቼም በቂ ምላሽ አግኝቻለሁ፡ ፡ በጥናቱ መሳተፍ በእኔ ፍላጎት ብቻ እንደሆነ እና በጥናቱም አለመሳተፍ ምንም አይነት ተፅዕኖ በእኔ ላይ እንደሚያስከትል ተረድቻለሁ፡ ፡ በከዚህ ባሻገር የኔ በጥናቱ ወስጥ ለመሳተት የወላጆቸም ወይም የአሳዳጊዎቸ ፈቃድ እንደሚያስፈልግ ተረድቻለሁ፡ ፡ በፍቃደኝነቴ በጥናቱ እንደምሳተፍም ከዚህበታች በፊርማዉ አረጋግጣለሁ፡ ፡

የጥናቱ ተሳታፊ ፊርማ ----------ቀን----------

መረጃወን የሰበሰበወግለሰብስም----------

ፊርማ ----------

የዋና ተመራማሪው አድራሻ

መለሰ ደሴ

አዲስ አበባዩ ኒቨርሲቲ፣የጤና ሳይንስ ኮሌጅ፣የሕክምና ላቦራቶሪ ቴክኖሎጂ ዲፓርትመንት

አዲስአበባ፣ኢትዮጵያ

ኢ-ሜይል፣E-mail.melessedessie@yahoo.com

ስ.ቁ +251-913194837

Annex IX : Laboratory data collection form

1. Patient identification

Sample ID._____

Age (years) _____

Gender ☐ Male ☐ Female

Antibiotic intake before 14 days ☐ yes No ☐

II. Laboratory Data

1. Date of specimen collection_____

2. Specimen type: _____

3. Media used _____

4. Gram stains result _____

5. Biochemical test_____

6. Organism isolated _____

7. Drug susceptibility pattern

7.1. Sensitive to _____

7.2. Intermediate to _____

7.3 Resistance to _____

III. Comments_____

Name of principal investigator _____

Signature _____ Date _____

Annex X . Procedure for Ear Swab sample collection

1. Inspect carefully client ear before sample collection.
2. Clean and disinfect the external area of ear before sample collection alcohol (70%).
3. By using sterile Cotton applicator stick take Ear Swab sample from inner ear until the swab becomes wet
4. Needle aspiration of fluid (if ear drum not ruptured) 1-3ml
5. Take cotton swab and insert in a test tube, add drops of Normal Saline, Transport at room temperature immediately.
6. Process the specimen as soon after receipt as possible. If there is a delay in processing, place the specimen in the refrigerator
7. If samples stored use in Amies transport media for 48 hrs
8. Apply Standard safety precautions for handling of patient specimens when processing these specimens.

   .
9. Check that the patient name and identifiers on the specimen match that on the accompanying

10. Safely dispose of all contaminated materials.

Annex XI . Laboratory procedure for Gram staining technique

1. Labeling the slides clearly with patient code number.

2. Making of smears by spread evenly covering an area about 15-20mm diameter on a slide.

3. Drying of smears after making smears, the slide should be left in a safe place to air-dry, protected from flies and dust.

4. Fix the dried smear by using heat or chemicals (methanol).

5. Cover the fixed smear with crystal violet stain for 30-60 seconds.

6. Rapidly wash off the stain with clean water. If the tap water is not clean, use filtered water or clean boiled rainwater.

7. Tip off all the water, and cover the smear with lugol's iodine for 30-60 seconds.

8. Wash off the iodine with clean water.

9. Decolorize rapidly (few seconds) with acetone alcohol. Wash immediately with clean water.

10. Cover the smear with neutral red or safranine stain for 2 minutes.

11. Wash off the stain with clean water.

12. Wipe the back of the slide clean, and place in a draining rack for the smear to air-dry.

13. Examine the smear microscopically, first with the 40 X objectives to check the staining and to see the distribution of materials and then with the oil-immersion objective to look for bacteria and cells.

Result

• Gram positive bacteria ----------dark purple

• Gram -negative bacteria ----------pale to dark red

Annex XII. Laboratory procedure for Media Preparation

A. SOP for preparation of Blood agar plate (BAP)

AIM of Blood agar plate: A non-selective medium for the isolation and cultivation of many pathogenic and non-pathogenic microorganisms. The medium is often used to investigate the forms of haemolysis from pathogenic microorganisms from clinical specimen. Blood Agar Base formulation has been used as a base for preparation of blood agar and to support good growth of a wide variety of fastidious microorganisms. Because it is a highly nutritious medium it can also be used as a general purpose growth media without adding blood. Blood Agar Base is suitable to isolate and cultivate a wide range of microorganisms with difficult growth.

Procedure for Preparation to make about 30-35agar plates

- Measure 500ml of distilled water using a measuring cylinder.
- Transfer the distilled water into a 1litre capacity conical flask.
- Weigh 20g of Blood Agar Base II powder using a weighing balance.
- And then add into the 500ml of distilled water and mix thoroughly.
- Boil until completely dissolved
- Autoclave at 121°C for 15 minutes.
- Allow to cool to 45-50°C in a water bath.
- Once the medium has been melted and cooled to 45-50 ºC
- Add 5-10% of defibrinated sterile sheep blood, in this case you can recuperate Haemophylus. Be careful to avoid bubble formation when adding the blood to the cooled medium and rotate the flask or bottle slowly to create a homogeneous solution.
- Aseptically add 25 ml of sterile defibrinated sheep blood with constant shaking.
- When mixing, avoiding froth formation.
- Gently pour 15-20 ml of the ready media on to the plates by using dispenser and allow setting.
- If air bubbles occurred, using a Bunsen burner gently invert and pass the flame over the poured blood agar in the plate to remove air bubbles. Leave standing for thirty minutes to solidify.

- Label on the bottom top of the blood agar plates the batch number & date prepared.
- Store the culture media plates upside down at 2-8^{0}C sealed in plastic bags to reduce chances of contamination. Shelf life: up to sixteen weeks provided there is no change in the appearance of the medium to suggest contamination, haemolysis, or deterioration.

B. Sop for preparation of Chocolate (Heated Blood) Agar

AIM of Chocolate (Heated Blood) Agar: Chocolate agar is a non selective media which supplies the factors X and V required for the proper growth of Haemophilus influenza. It is also used to culture nutritionally demanding pathogens such as Neisseria meningitis and Streptococcus pneumoniae. When Blood agar is heated, the red cells are lysed and the medium becomes brown in colour; it is referred to as chocolate agar. It is appropriate for isolating pathogenic bacteria in sputum, throat swabs, eye swabs, ear swabs, urogenital swabs, cerebrospinal fluid.

Procedure for preparation:

- Prepare as described for Blood agar except after adding blood, heat the medium in a 70^o C water bath until it becomes brown in color. This takes about 10-15 minutes during which time the medium should be mixed gently several times.
- Allow the medium to cool to about 45^{o}C,
- Remix and dispense in sterile petri dishes using a dispenser as described for blood agar.
- Leave standing for thirty minutes to solidify.
- Perform sterility testing as described for blood agar plate.
- Label the bottom of each plate with date of preparation and batch number.
- Store the culture media plates upside down at 2-8^o C sealed in plastic bags to reduce chances of contamination.

Important: Care must be taken not to overheat or prolong the heating of the medium because this will cause it to become granular and unfit for use. Up to sixteen weeks provided there is no change in the appearance of the medium to suggest contamination or deterioration.

C. SOP for preparation of Mac Conkey Agar

AIM of MacconkeyAgar; It is preferable for the isolation and differentiation of clinically important gram negative rods by inhibiting gram positive cocci.

Principle; MacConkey agar is selective and differential medium to distinguish gram negative entrobactericae and lactose fermenting bacteria from non lactose fermenters. MacConkey Agar is a selective and differential medium. It is only slightly selective since the concentration of bile salts, which inhibit gram-positive microorganisms, is low in comparison with other enteric plating media. Crystal violet also is included in the medium to inhibit the growth of gram-positive bacteria, especially enterococci and staphylococci. Differentiation of enteric microorganisms is achieved by the combination of lactose and the neutral red indicator.Colorless or pink to red colonies are produced depending upon the ability of the isolate to ferment the carbohydrate.

Procedure for preparation:

- Prepare as instructed by the manufacturer.
- Suspend 51.1g of powder in 1 liter of distilled or deionized water.
- Heat and boil until completely dissolved with frequent agitation.
- Sterilize in autoclave at 1210C for 15 minutes
- Cool to 45-50 0C
- Mix well and dispense by dispenser (15-20 ml) aseptically into sterile petri dishes.
- Leave standing for thirty minutes to solidify.
- Perform sterility testing as described before.
- Label the bottom of each plate with date of preparation and batch number.
- Store the culture media plates upside down at 2-80C sealed in plastic bags to reduce chances of contamination.
- Test Samples for performance, using stable, typical control cultures.

Annex VIII. SOP of Vitek 2 compact analyzer

Purpose

To describe the procedures for the preparation and identification of test microorganisms (test microbes and Quality Control Organisms) using the VITEK 2 Compact Instrument.

Procedure and Analysis

Follow the operational instructions below strictly for the proper use and required quality control activities on VITEK 2 Compact analyzer.

1. **Initiation of the** Vitek 2 compact analyzer

- The V2C Instrument is always "on"; the instrument will say "Ready" or "Not Ready" on the digital screen. Once the computer is initialized, the instrument will say "Ready."The V2C will not run if it is not on ready mode.
- Select VITEK 2 Compact to initiate the system from the upper left side of the screen.
- After the system is initiated, log onto the system using the appropriate user name and password?
- The system is now initialized and ready for data entry.

2. Preparation of Organisms

A. QC organisms

- If starting from a frozen stock culture, remove the 0.5 mL cry vials from the -80°C freezer. Avoid repeated thawing and freezing of the frozen culture by aseptically removing a small portion (or loop full) of the frozen inoculums, then immediately return cry vials to -80°C freezer.
- Streak isolates the inoculums from a frozen stock culture or other source onto agar plate appropriate for the QC organism.
- Following this streak isolation, a second streak isolation on the appropriate media is recommended.

B. Non-QC organisms

- Use growth on tubes or plates to perform streak isolation on BAP or NA warmed to room temperature. A second streak isolation step is not required unless there is evidence of a mixed culture.

C. For cultures used on BCL and GN cards, incubate cultures for 18-24 h at 36±1°C. For cultures used on GP cards, incubate cultures for 12-48 h at 36±1°C. For cultures used on ANC cards, incubate cultures under anaerobic conditions for 18-24 h (or until sufficient growth is obtained) at 36±1°C. All organisms to be identified must be pure cultures.

3. Perform Gram stain using an isolated colony from a pure culture plate and document the Gram stain reaction.

4. Preparation of Inoculums

Select the appropriate card based on the Gram stain reaction and the organism's microscopic appearance. Allow the card(s) to come to room temperature before opening the package liner.

Aseptically transfer at least 3 mL of sterile saline into a clear polystyrene 12×75 mm testtube. Using sterile cotton swabs, prepare a homogenous organism suspension by transferring several isolated colonies from the plates to the saline tube. Adjust the suspension to the McFarland standard required by the ID reagent using a calibrated V2C DensiCHEK plus Meter, see below table.

Suspension Turbidities Used for Card Inoculation.

Card	McF Range
GN	0.5-0.63
GP	0.5-0.63
ANC	2.7-3.3
BCL	1.8-2.2

Place the prepared suspensions in the cassette (see section 15, Instrument User Manual).

To use the Densi CHEK plus Meter to read samples:

I. Ensure the instrument is ON and set to the PLASTIC tube setting.

II. Blank the Densi CHEK Plus by filling a test tube with sterile saline and inserting the tube into the instrument. Press the "0" key and slowly rotate the test tube. Ensure one full rotation is completed before the reading is displayed. The instrument will display a series of dashes followed by 0.00.

III. To measure a sample, place a well-mixed organism suspension into the instrument and slowly rotate the test tube. Ensure one full rotation has completed before the reading is displayed. The instrument will display a series of dashes followed by a reading.

IV. Remove the test tube after completion of a reading. The instrument will automatically shut off when test tubes are not inserted after one minute.

NOTE: If the instrument flashes 0.00 or 4.00, the suspension is either below 0.0 McF or above4.0 McF and is not within the reading range. Ensure suspensions are within the appropriate reading range to avoid compromised card results. If necessary, re-calibrate the Densi CHEK Plus instrument after processing each cassette.

5. Insert the straw (in the V2C card) into the inoculated suspension tube in the cassette.

NOTE: The age of the suspension must not exceed 30 minutes before inoculating the cards.

6. Proceed to data entry.
7. Filling the Cards
    - Place the cassette in the Filler box on the left side of the V2C unit and hit Start Fill button on the instrument. Filling the cards takes approximately 70 seconds for a cassette regardless of the number of cards in the cassette holder. The V2C instrument will beep when the filling cycle is complete.
    - Discard individual cards that may have been exposed to multiple fill cycles.

    NOTE: The cassette must be placed inside the Loader Door within 10 minutes from the end of the filling cycle to avoid the cards being rejected.

    - When the cards are finished filling, the Load Door is automatically unlocked. Place the cassette in the Load Door. The V2C Instrument will verify the scanned barcodes against the Virtual Cassette (the information scanned in by the analyst). Cards are sealed; straws are cut and the cards are loaded automatically into the carousel. The V2C will beep once all cards are loaded into the cassette.
    - When the cards are loaded, remove the cassette and dispose of the tubes and straws in biohazard container.
    - The Vitek 2 Compact automatically processes the cards once all the cards are loaded.

NOTE: Review the Navigation Tree. If the cassette status description in the Navigation Tree is red, the cassette needs more information to completely process the tests cards. Open up the red colored file and make sure all fields are defined.

8. Results

The VITEK system analyses the data results and determines the identity of the test microbes/QC organism based on colorimetric tests (biochemical reactions).Results are concurrently printed and the data sent to the Results View folder on the left side of the screen also called the Navigation Tree where the information is archived. A red cassette in the Navigation Tree is indicative of an error. If an error occurs during processing, refer to the Software User Manual.

Declaration

I, the undersigned, declare that this M.Sc. thesis is my original work, has not been presented for a degree in this or any other university and that all sources of materials used for the thesis have been duly acknowledged.

Melessse Desse [Msc candidate]

Signature_____ Date of submission_____

This thesis has been submitted with our approval as advisors

Advisor: Dr. Adane Bitew [PhD. Associate Professor]

Signature_____

Date _____

Place: Addis Ababa, Ethiopia September 2019