**ADDIS ABABA UNIVERSITY**

**COLLEGE OF HEALTH SCIENCES**

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# Multidrug resistance among fermentative and non-fermentative gram-negative bacilli isolated from clinical specimens at Arsho Advanced Medical Laboratory, Addis Ababa, Ethiopia


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This is to certify that the thesis prepared by **Yasin Desalegn**, entitled: "**Multidrug resistance among fermentative and non-fermentative gram-negative bacilli isolated from clinical specimens at Arsho Advanced Medical Laboratory, Addis Ababa, Ethiopia"** and submitted in partial fulfillment of the requirements for Master of Science degree in Clinical Laboratory Sciences (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

| | |
|---|---|
| AAU | Addis Ababa University |
| AST | Antimicrobial Susceptibility Testing |
| CFU | Colony Forming Unit |
| CLSI | Clinical and Laboratory Standards Institute |
| DNA | Deoxyribonucleic Acid |
| ESBLs | Extended Spectrum β-Lactamase |
| FGNB | Fermentative Gram-negative Bacilli |
| MDR | Multidrug Resistance |
| MIC | Minimum Inhibitory Concentration |
| NFGNB | Non-Fermentative Gram-negative Bacilli |
| PCR | Polymerase Chain Reaction |
| RERC | Research Ethics Review Committee |
| SB | Significant Bacteriuria |
| SOPs | Standard Operating Procedures |
| Spp | Species |
| SPSS | Statistical Package for the Social Sciences |
| WHO | World Health Organization |
| XDR | Extensive drug resistance |

## Operational definition

- **Multidrug resistant (MDR)**: the bacterium that was simultaneously resistant for three or more antimicrobials belonging to different classes of antibiotics tested.

## Abstract

**Background:** Antimicrobial resistance has recently been identified as one of the three most important problems facing human health by the World Health Organization. Gram negative bacteria are major producers of extended-spectrum beta-lactamase and carbapenemase enzymes which made them multidrug resistant.

**Objective:** This study aimed to determine magnitude of multidrug resistant fermentative and non-fermentative gram-negative bacilli isolated from clinical specimens at Arsho Advanced Medical Laboratory, Addis Ababa, Ethiopia.

**Methods:** A cross-sectional study was conducted from January 1 to March 30, 2019. A total of 873 different clinical samples were collected and inoculated to MacConkey agar, Blood Agar and Chocolate agar. Species identification, antimicrobial susceptibility testing and extended spectrum beta-lactamase screening was performed using VITEK®2 automation. Extended spectrum beta-lactamase production was confirmed with combined disc method. Carbapenem resistance was performed on Muller Hinton agar and carbapenemase producers were confirmed by modified carbapenem inactivation method as per Clinical and Laboratory Standards Institute guideline. Data was entered and analyzed using SPSS version 23**.**

**Results:** From 873 various clinical samples processed, 175 gram negative bacteria were isolated of which 88% (154/175) were fermenters and 12% (21/175) were non-fermenters. Majority of the isolates were from urine (56%) followed by wound (21.7%) specimen. The predominant isolates were *Escherichia coli*, 58.3% (102/175) from fermenters and *Pseudomonas aeruginosa*, 5.1% (9/175) from non-fermenters. Highest resistance level was seen in ampicillin (86%) and cefalotin (73.2%), and lowest resistance against meropenem (9.8%) and tobramycin (18.9%). Overall Multidrug resistance was 80.5%, extended spectrum beta-lactamase production was 28% and carbapenemase production was 5.4%.

**Conclusion**： Prevalence of multidrug resistance in gram negative bacteria was high. Meropenem (91.8%) was the most active antibiotic against extended spectrum beta-lactamase producers while gentamicin (55.6%) for carbapenemase producers.

**Key words:** Gram negative bacteria, Multidrug resistance, ESBLs, Carbapenemase.

# 1. Introduction

## 1.1 Background

Gram negative bacilli can be either fermentative or non-fermentative. Fermentative Gram-negative Bacilli (FGNB) are important causes of urinary tract infections (UTIs), bloodstream infections, hospital and healthcare-associated pneumonias. Among those *Escherichia coli (E. coli)* is a frequent cause of UTIs, *Klebsiella spp* and *Enterobacter spp* are important causes of pneumonia, and all of the FGNB have been implicated in bloodstream infections [1].

Non-Fermentative Gram-Negative bacilli (NFGNB) are diverse group of bacteria in taxonomy which includes organisms like *Pseudomonas spp, Acinetobacter* spp, *Alkaligenes* spp, *Stenotrophomonas maltophilia and Burkholderia cepacia* complex. They have been considered as commensals for long times and most of them found almost universally, commonly distributed in soil, water, sewage, human, foods and animals. However, numerous recent studies shown that NFGNB are important cause of different types of nosocomial infections including ventilator associated pneumonia, septicemia, urinary tract infection, and surgical site infection [2-8]. Infections from NFGNB accounted nearly 15% from all Gram-negative bacilli [9-10]. Immunosuppression, neutropenia, mechanical ventilation, cystic fibrosis, indwelling catheters, invasive diagnostic and therapeutic techniques have been identified as major risk factors for gram negative bacterial infections [7-13].

For the clinicians engaged in hospital practice in the late 20$^{th}$ century, choosing therapy for significant infections caused by Enterobacteriaceae was simple. Whether targeted or empirical, cephalosporins were seen as reliable antibiotic. Unfortunately, this reliability has been challenged in the early 21$^{st}$ century because of the emergence of resistance strains for cephalosporins, which was primarily mediated by Extended Spectrum β- lactamase (ESBLs) production, has increased [14].

Antimicrobial resistance has recently been identified as one of the three most important problems facing human health by the World Health Organization (WHO) [15]. Frequent isolation of multi-drug resistant (MDR) pathogens in both nosocomial and community-acquired infections more expanded the problem of antimicrobial resistance [16]. Among FGNB, *E. coli and*

*Klebsiella pneumonia (K. pneumonia),* and from *NFGNB Acinetobacter baumannii (A. baumannii),* and *Pseudomonas aeruginosa (P. aeruginosa)* have been recognized as the most common and serious MDR pathogens [15, 17].

An increase in the incidence of nosocomial infection and the prevalence of MDR in both FGNB and NFGNB, have significantly increased the profile of the pathogens [3, 18, 19]. Both FGNB and NFGNB are also incriminated as the major producers of extended spectrum β- lactamases (ESBLs) and carbapenemase enzymes which break down antibiotics to be ineffective.

Therefore, current and updated information of the magnitude of both enzymes in GNB is important to understand their epidemiology and to implement health care settings infection control strategy to prevent the spread of MDR GNB. Even though MDR GNB causes significant public health problems, little is known about the magnitude of their ESBLs and carbapenemase production in Ethiopia. This might be due to only few studies have been conducted in Ethiopia for ESBLs and carbapenemase detection as most of our microbiology laboratories does not provide those tests because of poor laboratory equipment and supplies.

The VITEK 2 COMPACT automated system (bio Mérieux, France) is one of the most commonly used instruments in clinical microbiology laboratories for the identification and assessment of the susceptibility profiles of Fermentative and Non-Fermentative GNB including their ESBL production [20,21]. Against this background, the aim of this study was to determine the prevalence and magnitude of MDR profile of FGNB and NFGNB among different sample types using the fully automated VITEK 2 compact system, and to determine the magnitude of ESBL and carbapenemase in both FGNB and NFGNB using conventional CLSI recommended methods.

## 1.2. Statement of the Problem

The worldwide emergence and spreading of antibiotic-resistant bacteria have been a great challenge to treat common infectious diseases and resulted chronic disease, disability, and death. Drug resistant bacterial infections are responsible for 500,000 deaths each year worldwide. This phenomenon is happening in every region of the world and has the potential to affect everyone, in any age and in any country. If the world fails to act to control resistance, our globe will cost 10 million dollars each year starting from 2050 [22, 23].

In developed nations the burden of infectious diseases is relatively low and resistance to first-line antibacterial agents usually overcome by second and third-line agents. But in low income countries, where there is high burden of infectious diseases, patients may not be able to obtain or afford costs of effective second-line treatments. In sub-Saharan Africa (SSA), the situation is aggravated by poor hygiene, poor water supplies, civil conflicts, and high number of immunocompromised people, which facilitate both the evolution of resistant pathogens and their rapid spread in the community. The burden of illnesses due to treatable bacterial infections, their specific etiologies, and the awareness of antibacterial resistance are not well-established in most of SSA countries because of limited capacity for disease detection and surveillance [24].

Antibiotic resistance in FGNB and NFGNB are complicating the treatment of serious nosocomial infection in health care settings and threatening to create pan drug resistant strains, spp resistant to all currently available agents. Approximately 20% of *K. Pneumoniae* infections and 31% of *Enterobacter spp* infections in intensive care units involve resistant strains to third generation cephalosporins worldwide. In India 71.31 % *Acinetobacter* isolates, Ghana 89.5% of all GNB, Ethiopia 96% of GNB was reported as MDR [25, 26, 27].

Resistance in *K. pneumoniae* to third-generation cephalosporins is typically caused by the acquisition of plasmid containing genes that encode for ESBLs. It cause a serious antibiotic management problem as the enzyme-encoding genes can be transferred easily from one organism to the other via plasmids and these plasmids often carry other resistance genes as well. ESBLs-producing *K. pneumoniae* and *E. coli* are now relatively common in healthcare settings and often

exhibit MDR. ESBLs and carbapenemase producing Enterobacteriaceae have now emerged in the community as well [28]. The carbapenemase of Enterobacteriaceae differs from their ESBLs in that, ESBLs hydrolyze the beta-lactams and cephalosporins not carbapenems but most carbapenemases hydrolyze beta-lactams, cephalosporins, monobactams and also carbapenems, so that no beta-lactam can remain effective [29].

For the past 30 years, strains of *A. baumannii* have acquired resistance to newly developed antimicrobial drugs and became MDR. It became prevalent in many hospitals all over the world and has been recently recognized there as a leading nosocomial pathogen [30]. In Singapore about 71% of *A. baumannii* and 25% of other *Acinetobacter* spp and in Sudan 50% of the isolates were carbapenem resistant, in Ethiopia 51.5% of GNB were resistant to cefepime which is among fourth generation cephalosporins [31, 32, 33]. But prevalence of ESBLs and specially carbapenemase production, and resistance for carbapenems and advanced generations of cephalosporins in comparison with different clinical samples among NFGNB and FGNB has not yet been reported adequately from Ethiopia and the study will therefore take up to close this gap in our knowledge.

## 1.3. Significance of the study

- The current information generated from this study can be used by different stakeholders such as microbiologists, infectious disease specialists, and public health professionals to prevent or slow the emergence and spread of ESBLs and carbapenemase producing MDR strains in the healthcare setting around the study area and also for the country.
- The result can be used to recommend the implementation of ESBLs and carbapenemase screening and confirmatory tests in health facilities, because early detection of those bacteria is a key to prevent treatment failure and to control both nosocomial and community infections and outbreaks.
- Most of microbiology laboratories in Ethiopia have only limited biochemical tests which are difficult for proper identification of gram negative bacteria that are rare to be isolated. However Vitec-2 compact GN cards have more than 45 biochemical tests to provide more accurate identification and antibiotic susceptibility tests of GNB so that the correct antibiotic will be selected accordingly which minimizes drug resistance.
- The detection of ESBLs and carbapenemase producing gram negative bacteria provides updated information to plan for infection control and surveillance.

# 2. Literature Review

## 2.1 Cephalosporins and Carbapenems

Cephalosporins and carbapenems are beta-lactam antibiotics, which are among the most frequently prescribed antibiotics worldwide [34]. Beta lactam antibiotics have a highly strained and reactive cyclic amide called β-lactam ring. These groups of antibiotics include penicillins, cephalosporins, carbapenems, and monobactams [35].

### 2.1.1 Cephalosporins

In 1945, the mold *Cephalosporium acremonium* was isolated by Giuseppe Brotzu from sewage outflow and was found to produce substances that inhibited both gram-negative and gram-positive bacteria. Over the next decade cephalosporin C, the parent compound of current cephalosporins, was identified [36].

Traditionally, cephalosporins divided into 1st-generation, 2nd-generation, 3rd-generation, 4th generation, and 5th-generation, according to their antibacterial activity. They differ in their antimicrobial spectrum, β-lactamase stability, absorption, metabolism, and side effects. Generally, first generation members have narrower activity than third-generations, fourth-generations, or fifth-generations that have broader spectrum cephalosporins [35].

**Table 2.1 Major groups of cephalosporins according to their antimicrobial activity**

| First-generation | Second-generation | Third- generation | Fourth-generation | Fifth- generation |
|---|---|---|---|---|
| Cephalothin | Cefamandole | Cefotaxime | Cefepime | Ceftobiprole |
| Cephapirin | Cefuroxime | Ceftizoxime | Cefpirome | Ceftaroline |
| Cefazolin | Cefonicid | Ceftriaxone | | |
| Cephalexin[a] | Ceforanid | Ceftazidime | | |
| Cephradine[a] | Cefoxitin[b] | Cefoperazone | | |
| Cefadroxil[a] | Cefmetazole[b] | Cefixime[a] | | |
| | Cefminox[b] | Ceftibuten[a] | | |
| | Cefotetan[b] | Cefdinir[a] | | |

[a]Oral cephalosporins; all the others are parental cephalosporins. [b] besides being cephamycins (chemical classification), they are usually included in the microbiological classification as second-generation cephems [35].

### 2.1.2 Carbapenems

Carbapenems are derivatives of thenamycin, which is a product of a soil fungus called *Streptomyces cattleya* [36]. The term "carbapenem" is defined as the 4:5 fused ring lactam of penicillins with a double bond between C-2 and C-3 but with the substitution of carbon for sulfur at C-1. They are a group of broad-spectrum beta-lactam antibiotic agents which possess the broadest spectrum of activity with greatest potency against both Gram positive and Gram-negative bacteria. As a result, they are often used as "last-line agents" or "antibiotics of last resort" when patients become seriously ill or suspected for harboring resistant bacteria [37, 38].
Like all β-lactam antimicrobial agents, carbapenems act by inhibiting bacterial cell wall synthesis by binding to and inactivating penicillin binding proteins (PBPs). Carbapenems are stable for most β-lactamases and extended-spectrum β-lactamases [39].

**Table 2.2 Status of carbapenems** [39]

| **Compound name** | **US approval date or current status** |
|---|---|
| Imipenem | 1987 |
| Meropenem | 1996 |
| Ertapenem | 2001 |
| Doripenem | Phase III clinical trials |
| Tebipenem | Phase II clinical trials (Japan) |
| Panipenem | Marketed in Japan, China and Korea |
| Biapenem | Marketed in Japan |

## 2.2 Extended Spectrum β-lactamase and Carbapenemase Enzymes

Extended Spectrum β-lactamases are a group of enzymes that break down antibiotics belonging to the penicillin and cephalosporin groups and render them ineffective. They hydrolyze the beta-lactam ring of antibiotics by adding a water molecule to the common beta-lactam bond, and this inactivates early cephalosporins, and monobactams, but not carbapenems so that carbapenems are widely regarded as the drugs of choice for the treatment of severe infections caused by ESBLs-producing gram negative bacteria. But currently there is an emergence of the carbapenem-resistance among those bacteria [40-42].

Carbapenemases are also enzymes that can hydrolyze beta-lactams of not only penicillins and cephalosporins, but also monobactams and carbapenems, so that no beta-lactam antibiotics can remain effective. Even though carbapenem drugs are widely regarded as the drugs of choice for the treatment of severe infections caused by ESBL-producing gram negative bacteria, there is an emergence of carbapenemase mediated resistance for those drugs. To overcome this problem few novel antibiotics have been added to the pool of existing drugs after passing through various phases of clinical trials. The initiative of Infectious Disease Society of America to develop ten new antibiotics against GNB by 2020 is a step to fill the gap [29, 40, 41].

## 2.3 Targets of antibiotics

Generally there are three proven targets for the main antibacterial drugs:

1. **Bacterial cell-wall biosynthesis**; the layer of the bacterial cell wall that confers strength is the peptidoglycan, a meshwork of strands of peptide and glycan that can be covalently cross-linked. The larger the fraction of adjacent peptide strands that are connected in amide linkage by action of a family of trans-peptidases, the higher the mechanical strength to osmotic lysis. Trans-peptidase and trans-glycosylase domains are the target sites for the killing of bacteria by the β-lactams which act as pseudo-substrates and acylate the active sites of penicillin-binding proteins (PBPs). Then the ring opened and the drug will occupy the enzyme active sites to prevent normal crosslinking of peptide chains in the peptidoglycan which leads it mechanically weak and susceptible to lysis due to changes in osmotic pressure.
2. **Bacterial protein synthesis;** since the RNA and protein machinery of the prokaryotic ribosomes is sufficiently distinct from the eukaryotic machinery, there are many inhibitors of protein synthesis targeting different steps in ribosome action without affecting the host protein synthesis.
3. **Bacterial DNA replication and repair;** fluoroquinolones, such as ciprofloxacin, are synthetic antibiotic structures that kill bacteria by targeting the enzyme DNA-gyrase, which is responsible for uncoiling the intertwined circles of double-stranded bacterial DNA that arise after each round of DNA replication. After the drug is complexed to the

enzyme, it cannot relegate the cleaved DNA and finally leads to bacterial cell death [35, 43].

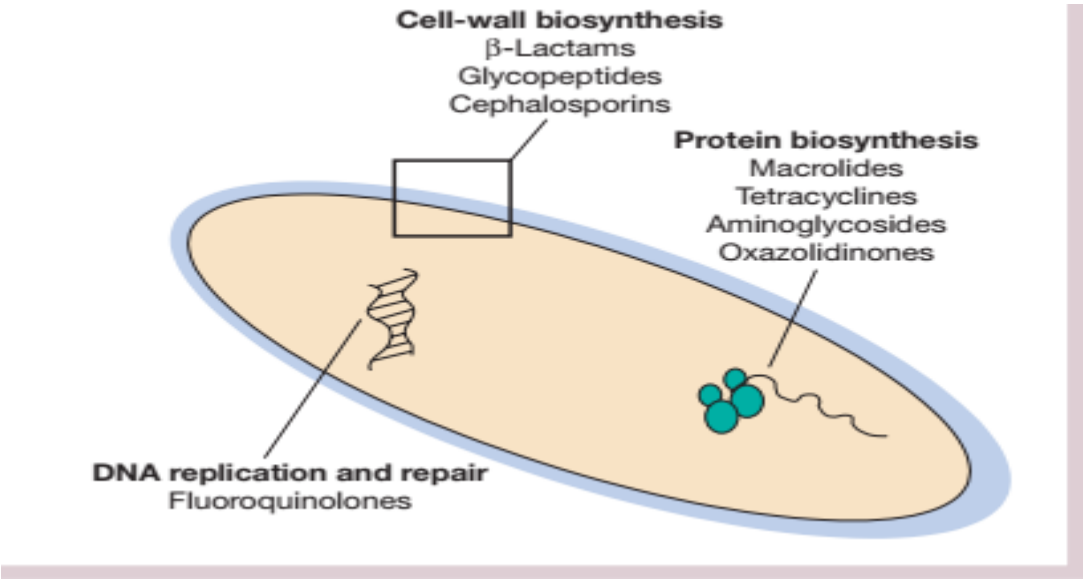



**Figure 2.1 Proven targets for antibacterial drugs** [43]

On the other hand, bacteria have different mechanisms to escape from the actions of antibiotics. Those mechanisms could be expressed intrinsically by a given spp or acquired from others [44].

## 2.4. Mechanism of microbial drug resistance

Bacterial drug resistance has close association with the improper use of antimicrobial agents in clinical practice in which prolonged therapy with antibiotics can lead to the development of resistance in a microorganism that initially is sensitive to antibiotics, but later it can adapt gradually and develop resistance. Drug resistance mechanisms allow bacteria that harbor these mechanisms to survive or actively grow in the presence of a given anti-microbial agent. The main mechanisms employed by gram-negative bacteria include enzymatic barrier, membrane barrier, antibiotic target modification, efflux pumps, and mutations that alter the expression and/or function of porins. Combinations of these mechanisms can cause high levels of resistance to carbapenems in certain bacterial spp, such as *K. pneumoniae, P. aeruginosa*, and *A.*

*baumannii*. Many gram negative bacteria have genes that enable organisms to resist antibiotics by those mechanisms, which could be intrinsic or acquired [38, 45, 46].

### 2.4.1. Intrinsic Resistance Mechanisms

Intrinsic resistance comprises those mechanisms that exist irrespective of antibiotic exposure which are specified by naturally occurring gene found on host chromosome. The cell envelope of many gram negative bacteria is a major barrier for antibiotics and the molecular mechanism of antibiotics resistance consists of the plasma membrane, the periplasm and the outer membrane. The outer membrane of GNB constitutes a semipermeable barrier to the uptake of antibiotics and substrate molecules. Because uptake of small hydrophilic molecules such as β-lactams antibiotics is restricted to a small portion of the outer membrane, the movement of such molecules into the cell will be limited intrinsically [45, 46].

### 2.4.2. Acquired Resistance Mechanisms

Acquired mechanisms are resistance mechanisms caused by changes in bacterial genome through mutation or horizontal gene acquisition which leads to a change in the nature of proteins expressed by the organism. Mutation is a spontaneous change in the DNA sequence within the gene that may lead to a change in the trait that it codes for, whereas horizontal gene transfer is a transfer of resistance genes from one bacterium to another. The main mechanisms of resistance gene transfer in a bacterium are plasmid transfer, transfer by viral delivery, and transfer of free DNA. Horizontal gene transfer may occur via transformation, transduction or conjugation [45].

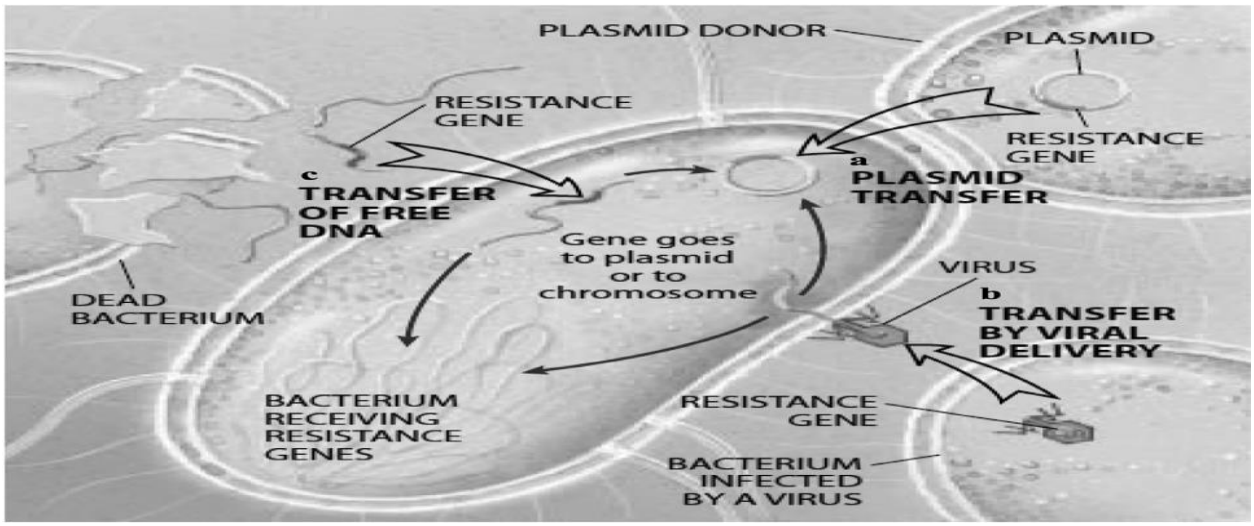



**Figure 2.2 main mechanisms of resistance gene transfer in a bacterium, (a), plasmid transfer; ( b), transfer by viral delivery; (c), transfer of free DNA** [47].

Either it is intrinsic or acquired; resistance for antibiotics is accomplished by different methods in different microbes. Among those the following are some.

### I. Resistance by reduced permeability or uptake

Drug molecules can be transferred to a cell by diffusion through porins (a membrane protein filled with water), diffusion through the bilayer, and by self-promoted uptake. The outer membrane in gram-negative bacteria contains an inner layer that has phospholipids and an outer layer that has the lipid A. Such composition reduces drug uptake and transfer through the outer membrane. Clinically important bacterial pathogens such as*, K. pneumonia* and *P. aeruginosa* used this reduced drug uptake system to resist anti-microbials such as the β-lactams, fluoroquinolones, aminoglycosides, as well as chloramphenicol [45, 47].

### II. Circumstantial Resistance

It is the difference between the in-vitro and in-vivo effects of an antimicrobial agent. Agents that appear to be active in the laboratory may not be the same in vivo because of failure to reach the site of infection, such as the inability of first generation cephalosporins to cross the blood-brain

barrier. On the other hand, drugs such as aminoglycosides may be inactivated by in vivo antagonist [45].

### III. Resistance by enhanced efflux

Drug resistant bacteria harbor energy-driven drug efflux pumps which extrude antimicrobial agents and reduce their intracellular concentrations to non-inhibitory levels. It is most common drug resistance mechanisms of bacteria. Bacterial efflux pumps actively transport many antibiotics out of the cell and are major contributors to the intrinsic resistance of Gram-negative bacteria to many of the drugs that can be used to treat Gram-positive bacterial infections. When over expressed, efflux pumps can also give high levels of resistance to previously clinically useful antibiotics [45, 48].

### IV. Resistance by enzymatic inactivation

Enzymatic inactivation either by hydrolysis or by modification is a major mechanism of resistance to antibiotic in pathogenic bacteria. Some bacteria produce modifying enzymes that reside within or near the cell surface, which selectively target and inactivate the drug. Most commonly, the protective enzymes produced by the bacterial cell will add an acetyl or phosphate group to a specific site on the antibiotic, which will reduce its ability to bind to the bacterial ribosomes. Extended spectrum Beta-Lactamases which hydrolyze penicillins and cephalosporins; and carbapenemases which hydrolyze penicillins, cephalosporins monobactams, and carbapenems are among major enzymes of GNB [45].

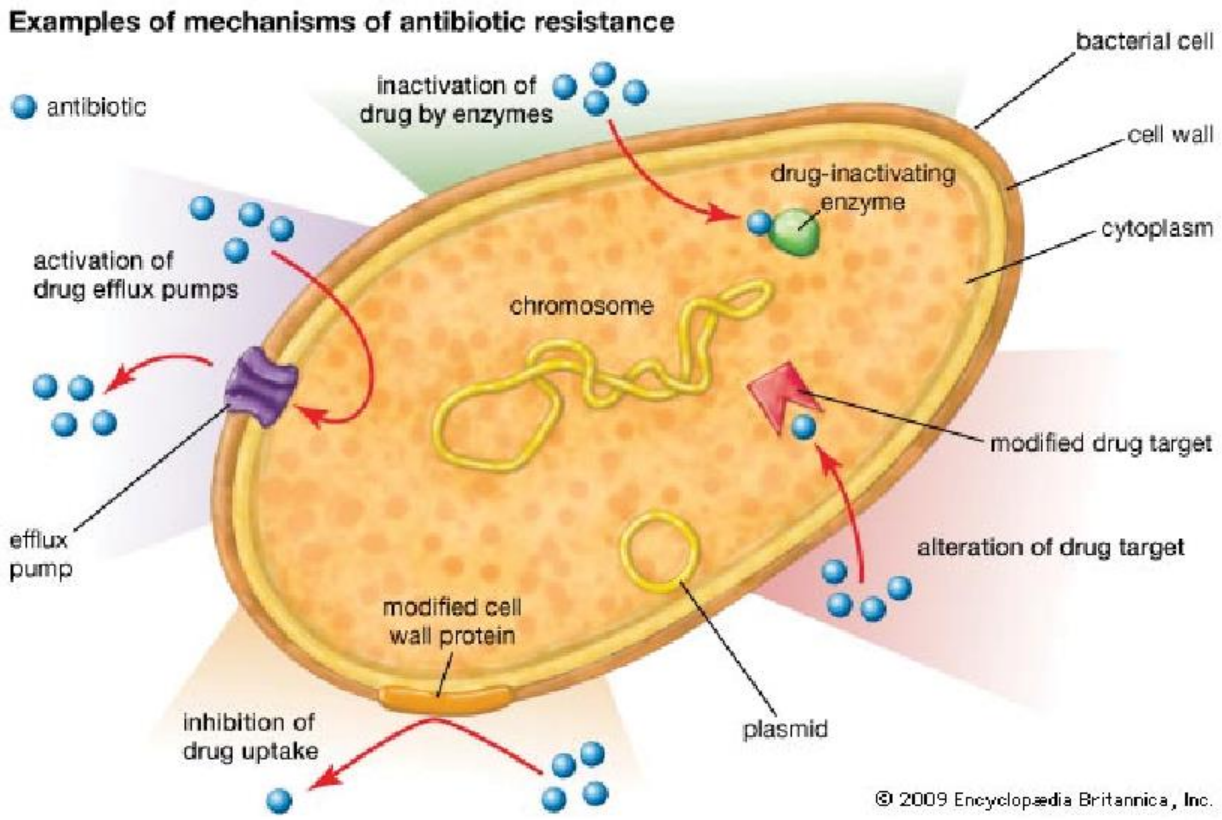



**Figure 2.3 Antibiotic resistance mechanisms of bacteria showing modifications in drug-target sites, impermeable cell walls, active efflux and drug-inactivating enzymes** [49].

## 2.5. Prevalence of gram negative bacteria, MDR profiles, magnitude of ESBLs and Carbapenemase production in different countries

In a study conducted in United States of America from 1993 to 2004, antimicrobial susceptibility profiles of 74,394 GNB isolates recovered. The isolates were *P. aeruginosa* (22.2%), *E. coli* (18.8%), *K. pneumonia* (14.2%), *Enterobacter cloacae* (9.1%), *Acinetobacter spp* (6.2%), *Serratia marcescens* (5.5%), *Enterobacter aerogenes* (4.4%), *Proteus mirabilis* (4.0%), *Klebsiella oxytoca* (2.7%) and *Citrobacter freundii* (2.0%). The sources of 84% isolates were respiratory tract (52.1%), urine (17.3%), and blood cultures (14.2%). *P. aeruginosa* was the organism most frequently isolated in the respiratory tract (26.9%), while *E. coli* was most frequently isolated from both urine (42.4%) and blood (23.9%). About 88% of *Acinetobacter spp* 82% of *P. aeruginosa*, and 98% Enterobacteriaceae family were susceptible to imipenem whereas 95% of isolates of Enterobacteriaceae was susceptible for ertapenem. Among *Acinetobacter spp* isolated, 77.2% were susceptible to ceftazidime and 71.1% were susceptible to amikacin. Ceftazidime, ceftriaxone, cefepime, piperacillin-tazobactam, imipenem, ertapenem, aztreonam, tobramycin, and amikacin were very active against *E. coli*, with mean resistance rates below 5%. Piperacillin (10.5%) and ciprofloxacin (15%) were the least active of the agents tested against P. *mirabilis*. Piperacillin had higher resistance rates with *K. pneumonia, K. oxytoca* and *Acinetobacter* spp, which had higher resistance rates to all of the β-lactam class antibiotics tested except ceftazidime, compared to that of ampicillin-sulbactam [50].

In a large global surveillance study in 11 Asia–Pacific region countries in hospitalized patients with intra-abdominal infections (IAIs) (n=3052) and urinary tract infections (UTIs) (n=1088) from 2013 to 2015, they found that amikacin (98.3, 96.4 %), imipenem (97.1, 95.5 %) and ertapenem (95.3, 93.2 %) had the highest rates of susceptibility for isolates of *K. pneumoniae* from IAIs and UTIs, respectively, whereas susceptibility to advanced-generation cephalosporins was less than 84% and less than 71 %, respectively. *K. pneumoniae* with ESBLs positive phenotype were more common in UTIs (27.1 %) than IAIs (16.2 %). Imipenem and amikacin were the most active agents against ESBLs positive *K. pneumoniae* from IAIs (95.1, 91.8 %) and UTIs (94.9, 92.3 %), respectively, whereas less than 54% were susceptible to piperacillin–tazobactam. Against *Enterobacter* spp and *P. aeruginosa*, amikacin demonstrated the highest

rates of susceptibility for isolates from IAIs (99.7, 95.5 %) and UTIs (90.9, 91.5 %), respectively.

*K. pneumoniae*, *Enterobacter* spp and *P. aeruginosa* from urine demonstrated lower susceptibility to levofloxacin (74.1, 81.8 and 73.8 %) than from IAI (87.6, 91.8 and 85.4 %). For *A. baumannii*, rates of susceptibility to all agents tested were <43 % [51].

In a study conducted in Singapore 746 GNB were collected for testing in 2008. Resistance to extended-spectrum cephalosporins was present in a 33.3% of Enterobacteriaceae isolates, and ESBLs carriage was present in 19.6% of *E. coli* and 30.1% of *K. pneumonia*. All Enterobacteriaceae were susceptible to imipenem and meropenem. The most active antibiotics against *P. aeruginosa* were amikacin, meropenem and piperacillin-tazobactam. Carbapenem resistance was 70.5% in *A. baumannii* and 25.0% for other *Acinetobacter* spp [32].

In a large surveillance conducted in Korea 2015, the resistance rates of *E. coli* to ampicillin (72%), ampicillin-sulbactam (45%), cefotaxime (35%), fluoroquinolone (48%), gentamycin (29%), and cotrimoxazole (39%) were high, but for piperacillin-tazobactam (5%), cefoxitin (9%), imipenem (< 0.1%), and amikacin (1%) were low. For *K. pneumoniae*, the resistance rates to third generation cephalosporins were similar to those of *E. coli*; while the resistance rates to piperacillin-tazobactam (21%) and amikacin (5%) were higher, and for gentamicin (19%) and fluoroquinolone (34%) were lower than for *E. coli*. Resistance rates of *A. baumannii* were very high for piperacillin (86%), piperacillin-tazobactam (82%), cefotaxime (84%), cefepime (83%), imipenem (85%), amikacin (60%), gentamycin (75%), ciprofloxacin (87%), and cotrimoxazole (64%). For *P. aeruginosa*, the degree of resistance to piperacillin (27%), piperacillin-tazobactam (25%), ceftazidime (19%), cefepime (18%), aztreonam (23%), imipenem (35%), amikacin (13%), gentamycin (18%), and ciprofloxacin (34%) was less than that for *A. baumannii*. They also reported that resistance rate of *A. baumannii* to imipenem increased to 85% [52].

In other study conducted in India, Kanpur, A total of 121 blood culture samples were analyzed in 2015. From that 27(22.3%) developed septicemia with the positive blood culture. Of the 27 positive culture, 24(88.9%) showed bacterial growth, 16(59.3%) of them were gram negative

bacilli with *E.coli* (22%), *K. pneumoniae (22%), P. aeruginosa (11%), K. oxytoca* (4%), and *Enterococcus faecalis* (4%). Resistance to imipenem was seen approximately in 66% in *E.coli, K. pneumoniae* and *Pseudomonas spp* ESBLs producers among the *E.coli* and *Klebsiella* isolates were 50% and 66.6% respectively. Maximum isolated strains showed high resistance towards penicillins, cephalosporins and fluoroquinolones but tigecycline and polymyxin-B were the most effective antimicrobial agents [53].

In a prospective study conducted in India 2017, 152 MDR GNB isolates were obtained from 100 patients. Majority of the isolates were *E. coli* (39%), *K. pneumoniae* (24%), *P. aeruginosa* (19%), *Acinetobacter spp* (7%), and *Enterobacter* spp (5%). Maximum number of ESBL was seen among enteric gram negative bacilli (36%), metalo-beta lactamase was seen among *P. aeruginosa* and *Acinetobacter spp* (55% each). Amikacin and meropenem were the most common antibiotics given as specific therapy [54].

In south India 2013, they processed 7182 clinical samples from various sources, pus (n=1870), blood (n=1131), urine (n=3286), sputum (n=622) and endotracheal aspirates (n=273). A total of 122 *Acinetobacter* spp were isolated and tested against ampicillin, amoxicillin-clavulanic acid, ceftazidime, ciprofloxacin, amikacin, cotrimoxazole, piperacillin-tazobactam, imipenem, colistin, and polymyxin B. Pus samples showed the greatest isolation rate of 38.52 %, followed by endotracheal aspirate at 20.49 %. Out of 122 *Acinetobacter* isolates, 87 (71.31 %) were multi-drug resistant. Among those 15 (17.24 %) were pan-drug resistant. More than 90 % of isolates displayed resistance to ampicillin, amoxicillin-clavulanic acid, ceftazidime, and amikacin. Least resistance was seen to piperacillin-tazobactam and imipenem [25].

In Eastern India, 2017, 411 non-fermenters were isolated from 3116 culture positive clinical samples (isolation rate of 13.19%). Urine was the most common specimen (29.44%), followed by pus (27.49%), blood (15.57%), sputum (12.90%), tracheal aspirate (8.27%) and 6.33% other samples. *A. baumannii* was the predominant isolate, 211 (51.34%), followed by *P. aeruginosa* 173 (42.09%). Isolation of non-fermenting GNB was maximum from urine sample (29.44%) followed by, pus (27.49%), blood (15.57%), sputum (12.90%) and then endotracheal tube (8.27%). *A. baumannii* showed (59.24%) highest sensitivity to gentamicin and (23.22%) least

sensitivity to ceftriaxone whereas *P. aeruginosa* was mostly sensitive to amikacin (83.24%) but least sensitive to ceftriaxone (29.48%) [55].

In Saudi Arabia a total 8908 non-fermenters were examined from blood, urine, wound swabs, sputum, and other body fluids. Most strains (n=6497, 72.9%), Were *P. aeruginosa* followed by *A. baumannii* (n=2252, 25.3%). Resistance rates of *P. aeruginosa* were: polymyxin B, 2.2%; imipenem, 15.9%; ciprofloxacin, 22.0%; amikacin, 22.9%; and gentamicin, 31.2%. Resistance rates among *A. baumannii* were: imipenem, 5.4%; polymyxin B, 13.2%; ciprofloxacin, 64.0%;trimethoprim/sulfamethoxazole, 73.8%; amikacin, 76.9%; and gentamicin, 77.8% [56].

In Ghana, 2014, 67 wound swab samples were collected and 189 spp isolated, 72 (38.1%) were Enterobacteriaceae and 48 (25.4%) were non-fermenters. Among *E. coli* (n = 19), *K. pneumoniae* (n = 13), *Enterobacter cloacae complex* (n = 10), *P. aeruginosa* (n = 20) and *A. baumannii* (n = 8) which were tested for meropenem and imipenem, only 5% of *P. aeruginosa* were resistant for meropenem and others were 100% sensitive for both carbapenems. But they showed different response for cephalosporins [57]. In 2015 again , of the total 200 resistant Gram-negative bacterial isolates obtained, *E. coli* was most frequent pathogen 49 (24.5%), followed by *P. aeruginosa* 39 (19.5%), *K. pneumoniae* 38 (19.0%), *Enterobacter spp* 12 (6.0%), and *Acinetobacter spp* 8 (4.0%). The antibiotic susceptibility profile showed that the isolates were most resistant to ampicillin (94.4%), trimethoprim/sulfamethoxazole (84.5%), cefuroxime/Axetile (80.0%), cefuroxime (79.0%), cefotaxime (71.3%), cefoxitin (57.5%) but least resistant to ertapenem (1.5%). Multidrug resistance was reported in 89.5% of the bacterial isolates, ranging from 53.8% in *Enterobacter spp* to 100% in *Acinetobacter spp* and *P. aeruginosa* [26].

In other study conducted in Rwanda 2014, they evaluated the AST patterns of 154 bacterial isolates cultured from urine (55.2%); blood (25.3%), sputum (16.2%), and wound swab (3.3%) specimens. *E. coli* (56.4%) was the most frequent pathogen isolated from urine specimens followed by *Klebsiella spp* (32.9%). *Klebsiella spp* was the predominant pathogen among isolates from blood (28.2%), sputum (60%), and wound swabs (28%). Remarkably, *Klebsiella spp* featured prominently across all types of clinical specimens. From total specimens evaluated,

31.4% of *E. coli* and 58.7% of Klebsiella isolates were resistant to at least one of the third generation cephalosporins and 8% of *E. coli* isolates were resistant to imipenem [58].

In Sudan, Khartoum, 2016, 149 Gram-negative bacilli, *E. coli* (n=81), *Klebsiella* spp (n=44), *Proteus* spp (n=17), *Pseudomonas* spp (n=6), and *Enterobacter* spp (n=1) were isolated from 147 different clinical specimens. The specimens were urine (n=106), wound swab (n=25), blood (n=10), sputum (n=3), eye swab (n=1) and others (n=4). The most predominant GNB isolates was *E. coli* 84(54.4%), followed by *Klebsiella* spp 44(29.5%) from total specimens. Major isolates by specimen site were *E. coli* (67 from urine, 12 from wound swab), *Klebsiella* spp (24 from urine, 6 from wound swab, 10 from blood, 3 from sputum) and *Pseudomonas* spp (2 from urine, 3 from wound swab). More than 50% of the isolates were carbapenem resistant. Urine samples had the maximum number of carbapenem resistant isolates [33].

In a study conducted in Ethiopia at Debre Markos Referral Hospital 2014, A total of 575 clinical samples processed from wound (n = 238), ear swab (n = 58), urine (n = 108), stool(n = 58), blood (n = 41), urethral discharge (n = 28), nasal/throat swab (n = 12), CSF (n = 32). Two hundred and eighty (48.7%) were culture positive and from those 98(35%) isolates were Enterobacteriaceae and 30(10.7%) were *P. aeruginosa* with a total gram negative bacterial prevalence of 22.3% from 575 clinical samples. The overall multidrug-resistant Gram negative bacterial isolates were 96 (72.2%) majority of which were resistant to cotrimoxazole (53.1%), amoxicillin (58.8%), ampicillin (70.4%) and gentamicin (76.9%). *E. coli* was the leading isolate followed by *Klebsiella* spp from urinary tract infection and *P. aeruginosa* was the most frequent gram negative isolate from ear infection [59].

In Arba Minch, Ethiopia, 2015, 129 urine samples were collected from study participants clinically suspected to have UTI. From isolated bacteria 66.7% (n=32) were gram negatives (*E. coli* (20), *K. pneumoniae* (8), *P. mirabilis* 3, and *Citrobacter* spp (1)). Among GNB 81.62% were resistant to ampicillin, penicillin and amoxicillin, and 68.75% were resistant to erythromycin. Among the most prevalent Gram negative bacilli, 85% of *E. coli* isolates were resistant against ampicillin, penicillin and amoxicillin whereas 70% exhibited resistant to erythromycin and 60% for tetracycline. In contrast, low rank of resistance was observed to ciprofloxacin (20%),

gentamicin (25%), trimethoprim-sulfamethoxazole (30%) and chloramphenicol (30%). Nearly 62.5% of *K. pneumoniae* isolates were resistant to erythromycin and trimethoprim-sulfamethoxazole [60].

In other study from sputum samples at Arba Minch Hospital, Southern Ethiopia, 2013, one hundred and seventy sputum samples were collected from adult patients in 2013. From 73 (42.9%) positive cultures, *P. aeruginosa* 12 (7.1%), *K. pneumoniae* 11 (6.5%), *E. coli* 5 (2.9%), were gram negative bacteria isolated. *P. aeruginosa* showed 50% resistance to gentamycin. Antimicrobial resistance of *K. pneumoniae* were (100%) to tetracycline, ampicillin and trimethoprim-sulfamethoxazole. *E. coli* isolates showed resistance to tetracycline 5(100%), chloramphenicol 5(100%), doxycycline 5(100%), gentamycin 1(20%), ampicillin 5(100%) and trimethoprim-sulfamethoxazole 5(100%) [61].

In a cross-sectional study conducted in Dil Chora Referral Hospital, Dire Dawa, Ethiopia 2015, 186 urine specimens was analyzed and the prevalence of significant bacteriuria was 14%. Gram-negative bacteria were more prevalent 19(73%) than gram positives. Among gram negatives 9(34.6%) were *E. coli*, 4 (15.4%) *P. aeruginosa*, 3 (11.5%) *Klebsiella* spp and 1 (3.9%) were *Citrobacter* spp. Most of them were resistant against ampicillin (89.5%), amoxicillin (73.7%), tetracycline (73.7%), trimethoprim–sulphamethoxazole (42.1%), and chloramphenicol (31.6%). Majority of the bacterial isolates were sensitive to ciprofloxacin (84.2%), ceftriaxone (94.7%), and gentamicin (89.5%) [62].

In other study conducted in Addis Ababa, Ethiopia in 2014, a total of 107 wound swabs collected and 90 (84.1%) were culture positive. Among those 24 (23.1%) was *E. coli*, 23 (22.1%) *Acinetobacter spp*, 10 (9.6%) *K. pneumonia*, 6(5.8%) *P. aeruginosa*, 3 (2.9%) *K. ozaenae* and 2 (1.9%) were *Citrobacter* spp. More than 58 (75%) of the gram negative isolates showed MDR (resistance $\geq$ 5 drugs). Pan-antibiotic resistance was 8 (34.8%) in *Acinetobacter* spp and 3 (12.5%) in *E. coli* [63].

In other study in Ethiopia, Addis Ababa Regional Laboratory, a total of 500 blood culture results reviewed in 2016, 164 (32.8%) was culture positive. Out of a total of 164 isolates, 37 (22.6%)

were gram-negative bacteria, *K. pneumoniae* 23 (14.02%), *E. coli* 6 (3.6%), *A. baumannii* 4 (2.4%) and *P. aeruginosa* 2 (1.2%). Higher percentage of drug resistant gram-negative isolates were seen to ampicillin (88.5%), amoxicillin-clavulanic acid (80%), trimethoprim-sulphamethoxazole (80%), and ceftriaxone (77.1%) and least resistant to cefepime (51.5%) [31].

In other study from body fluids at Tikur Anbesa Specialized Hospital, Addis Ababa, Ethiopia, 2016, 14.1% (54/384) of the body fluids had bacterial growth. Among gram negative bacterial isolates *K. pneumoniae* were 16.7% (9/54), *Pseudomonas spp* 11.1% (6/54), *Acinetobacter spp* 9.3% (5/54), *E. coli* 5.6 % (3/54), *K. oxytoca* 3.7 % (2/54).The highest resistance rate was recorded for gentamycin 65.6%, ampicillin 62.5%, ciprofloxacin 53.1%, ceftriaxone 50% and tobramycin 50%. The level of MDR for gram-negative isolates was found to be 96% (29/32) and *K. pneumoniae* were the dominant isolate 90% (8/9) [27].

# 3. Objectives of the study

## 3.1. General objective

- To determine magnitude of multidrug resistant fermentative and non-fermentative gram-negative bacilli isolated from clinical specimens at Arsho Advanced Medical Laboratory, Addis Ababa, Ethiopia, from January 1 to March 30, 2019.

## 3.2. Specific objectives

- To determine the prevalence of gram negative bacilli
- To determine the overall drug resistance profile of gram negative bacilli
- To determine the prevalence of ESBLs and carbapenemase in gram negative bacilli

# 4. Hypothesis

The spectrum of GNB and their MDR profiles, magnitude of ESBLs and their carbapenemase production in this study are the same with previous studies conducted in Ethiopia.

# 5. Materials and methods

## 5.1. Study area

This study was conducted in the Department of Microbiology at Arsho Advanced Medical Laboratory Addis Ababa, Ethiopia. Arsho is among the oldest and the largest private Medical Laboratory where patients are referred to culture and antibiotic sensitivity testing. The laboratory has eight case teams including the Microbiology Laboratory. The main microbiology comprised of Microbiology specialists, Laboratory Technologists and Technicians. On average about 30 patients per day were referred to the department during my study period.

## 5.2. Study design and period

A cross sectional study was conducted from January 1 to March 30, 2019 at Arsho Advanced Medical Laboratory Addis Ababa, Ethiopia.

## 5.3. Population

5.3.1. **Source population:** The source population comprised of all patients attended Arsho Advanced Microbiology Laboratory, Addis Ababa, during the study period.

5.3.2. **Study Population:** patients attended Arsho Advanced Microbiology Laboratory during the study period that fulfilled the inclusion criteria.

## 5.4. Inclusion and exclusion criteria

5.4.1. **Inclusion criteria:** patients who visited Arsho Microbiology Laboratory during the study period and gave assent/consent to participate in the study were included.

5.4.2. **Exclusion criteria**

- Patients who had history of antibiotics treatment with in two weeks before giving sample.

## 5.5. Study variables

### 5.5.1. Dependent variables

- Prevalence of gram-negative bacterial isolates
- Magnitude of MDR gram-negative bacteria
- Magnitude of ESBLs producing gram-negative bacteria
- Magnitude of carbapenemase producing gram-negative bacteria

### 5.5.2. Independent variables

- Age group
- Gender
- Types of specimens

## 5.6. Measurement and Data collection

### 5.6.1. Sample size determination

Sample size was calculated based on a single population proportion formula. The expected prevalence, p=68% (p=0.68) taken from Teklu D *et al* study in Addis Ababa [67].

At 95% confidence interval,

Margin of error tolerated is 5 % (0.05)

$$\text{Study participants: } n = \frac{Z^2 P (1- P)}{d^2}$$

Where n = sample size

z = 95% statistic for level of confidence (1.96)

P = population proportion (68%)

d = margin of error (degree of accuracy desired

(d=0.05) and 95% level of confidence (z=1.96).

The sample size was estimated to be: $= \frac{(1.96)^2 \times 0.68\ (1 - 0.68)}{(0.05)^2} = 336$

Therefore, by adding 10% contingency, a minimum of **370** participants should be included in the study. We included 873 different participants in order to get more representative findings.

### 5.6.2. Sampling Method

A convenient sampling was used to enrolled consecutive patients attending the laboratory during the study period who fulfilled the entry criteria.

## 5.7. Data collection procedure

### 5.7.1. Socio-demographic and Clinical data

Socio-demographic status including age, gender and history of antibiotics was collected from the laboratory request.

## 5.8. Sample Collection and Laboratory analysis

### 5.8.1. Sample Collection

For urine sample collection, study participants were given pre-labeled (date, time, identification code, age), leak proof, wide mouth, sterile, screw-capped plastic container to collect the mid-stream urine (MSU) after appropriate collection instructions. Blood specimens drown from arm while patients were being at hypo or hyper thermic considering the ages of the patients. CSF and other body fluids were collected by physicians and transported as soon as possible with sterile bottles which were leak proof and tightly capped. Swab samples were also collected by physicians and transported with Amies transport medium (Amies with charcoal). All samples which were in need of transportation were transported with triple-packaging accordingly with standard operating procedures.

### 5.8.2. Bacterial identification and Antimicrobial susceptibility testing

#### 5.8.2.1. Identification

The clinical samples were inoculated onto primary isolation culture media following standard conventional procedures. The primary isolation culture Medias used were blood agar base (Oxoid, Basingstoke, and Hampshire, UK) to which 5% sheep blood is incorporated, MacConkey agar (Oxoid, Basingstoke, and Hampshire, UK) and Chocolate agar (Oxoid, Basingstoke, and Hampshire, UK) by using inoculating loop. Clean-catch midstream urine collected from patients was inoculated onto Blood Agar and MacConkey agar by using a 1µl calibrated loop. Colony counts yielding bacterial growth of $\geq 10^5$/ml of urine (≥100,000 colonies) were regarded as significant for bacteriuria. Blood was transferred to blood culture bottles containing Brain Heart infusion broth (Oxoid, Basingstoke, and Hampshire, UK). All specimens other than urine were inoculated in to Blood agar, Chocolate agar and MacConkey agar with a calibrated loop of 10µl. Inoculated plates were incubated at $37^0$C for 18-24 hours aerobically, but Blood agar, Blood culture bottles, and Chocolate agar were incubated in 5 % $CO_2$ incubator. Pure isolates of bacterial pathogen was preliminary characterized by colony characteristics and Gram-stain. Species characterization and antimicrobial susceptibility testing were performed with the VITEK®2-COMPACT using the GN and AST-GN72 cards, in accordance with the manufacturer's instructions.

The VITEK 2 instrument automatically filled, sealed, and incubated the individual test cards with the prepared quality control bacteria and pure cultures of bacterial isolates after suspending in 3 ml of sterile saline in a 12 × 75 mm clear plastic (polystyrene) test tube to achieve a turbidity equivalent to that of a McFarland 0.50 standard (range, 0.50 to 0.63), as measured by the Densi Chek (bioMe'rieux) turbidity meter. The suspensions were used for the inoculation of GN72 identification cards while AST cards were inoculated after bacterial suspensions further was diluted following the instruction of the manufacture.

VITEK 2 Compact system accommodates very small plastic colorimetric reagent cards which are designed to contain microliters of biochemical tests and selective growth media for detection and identification of organism. With its colorimetric reagent cards, and associated hardware and

software advances, the VITEK 2 offers a state-of-the-art technology platform for phenotypic identification methods.

The reagent cards have 64 wells that can each contain an individual test which measures various metabolic activities such as acidification, alkalization, enzyme hydrolysis, and growth in the presence of inhibitory substances. An optically clear film present on both sides of the card allows for the appropriate level of oxygen transmission while maintaining a sealed vessel that prevents contact with the organism-substrate admixtures. Each card has a pre-inserted transfer tube used for inoculation. Cards have bar codes that contain information on product type, lot number, expiration date, and a unique identifier that can be linked to the sample either before or after loading the card onto the system. Identification cards were inoculated with microorganism suspensions using an integrated vacuum apparatus.

All card types were incubated on-line at 35.5 ± 1.0ºC. Each card was removed from the carousel incubator once every 15 minutes, transported to the optical system for reaction readings, and then returned to the incubator until the next read time.

The GN72 card was used for the automated identification of 135 taxa of the most significant fermenting and non-fermenting gram-negative bacilli. The GN card is based on established biochemical methods and newly developed substrates measuring carbon source utilization, enzymatic activities, and resistance. There were 47 biochemical tests and one negative control well [64].

#### 5.8.2.2. Antimicrobial susceptibility testing (AST)

The antimicrobial susceptibility testing card comprised of various antibiotics with their different concentration, ampicillin (4,8,32), amoxicillin/clavulanic acid (4/2,16/8,32/16), cefalotin (2,8,32), cefazoline (4, 16, 64), cefepime (2,8,16,32), cefoxitin (8,16,32), cefpodoxime (0.5, 1, 4), ceftazidime (1,2,8,32), ceftriaxone (1,2,8,32), cefuroxime (2,8,32), ciprofloxacin (0.5,2,4), gentamicin (4,16,32), levofloxacin (0.25,0.5,2,8), nitrofurantoin (16,32,64), piperacillin/tazobactam (2/4,8/4,24/4,32/4,32/8), tetracycline (2,4,8), tobramycin (8,16,64), and trimethoprim/sulfamethoxazole (1/19,4/76,16/304). Based on that reading, AST profile established and interpreted according to a specific algorithm. Final results analyzed using version

6.01software, an Advanced Expert System (AES) specifically designed to evaluate the results generated by the VITEK 2 system. Final identification and AST results were available in approximately 10 hours or less [64]. We used the definition of Magiorakos *et al* [65] for categorizing bacterial isolates as MDR.

### 5.8.3. Isolate Collection and Handling

Isolates were collected using Tryptic Soy Broth (TSB) (Oxoid LTD, Basingstoke, Hampshire, UK) containing 20% Glycerol and stored at -81°C until transported to EPHI using triple packaging with cold box and ice. We performed ESBLs phenotypic confirmation, carbapenem resistance and carbapenemase detection in EPHI microbiology laboratory. For future reference or analysis, the isolates are again stored at -81°C.

### 5.8.4. Culturing

The isolates preserved at -81°C were recovered by inoculating them in 5% sheep blood agar and incubated for 18-24 hours at 37 °C. Fresh and pure colonies obtained from the blood agar plate were used for ESBLs phenotypic confirmatory test, carbapenem resistance and carbapenemase detection.

### 5.8.5. Test for ESBLs production

#### 5.8.5.1. ESBLs detection with VITEK 2 compact automation

The ESBLs screening was with the VITEK ESBLs test panel with six wells containing three cephalosporins, (cefepime, cefotaxime and ceftazidime) alone and in combination with clavulanic acid. Growth in wells having cephalosporin plus clavulanic acid compared with those containing the cephalosporin alone was assessed by means of an optical scanner. Final results analyzed using version 4.0 software advanced expert system (AES), designed to assess the results produced by the VITEK. The relative reduction in growth in wells having cephalosporin plus clavulanic acid compared with those containing the cephalosporin alone was screened as positive for ESBLs production [64]. All the strains which were screened as positive for ESBLs production by VITEK were subjected to ESBLs phenotypic confirmatory test.

#### 5.8.5.2. ESBLs confirmation with combined disc method

ESBLs production was phenotypically confirmed by combined disc method in which discs of ceftazidime (30μg) and cefotaxime (30μg) alone and in combination with clavulanic acid (10μg) were placed onto Mueller-Hinton agar plate (HiMEDIA Laboratories Pvt. Ltd, Mumbai, India) seeded with a turbidity suspension of an isolate equivalent to that of a 0.5 McFarland turbidity standard. A difference of ≥5 mm between the zones of inhibition of a ceftazidime (30μg) alone and ceftazidime with clavulanic acid (30μg/10μg) or cefotaxime (30μg) and cefotaxime with clavulanic acid (30μg/10μg) was considered as positive for an ESBL production based on CLSI guide line. Quality control strains, *E. coli* ATTC 25922 (ESBLs negative) and *K. pneumoniae* 700603 (ESBLs positive) were included in each run [66].

### 5.8.6. Tests for carbapenemase production

Bacterial isolates which were not susceptible to meropenem (MEM 10μg) based on CLSI break points were subjected for confirmation for carbapenemase production [66]. Confirmation for carbapenemase production in Enterobacteriaceae and *P. aeruginosa* were done by Modified Carbapenem Inactivation Method (mCIM), where a 1-μL loop-full of bacteria for Enterobacteriaceae or 10-μL loop-full of bacteria for *P. aeruginosa* were emulsified from an overnight incubated blood agar plate in 2 mL sterile triptic soya broth and 10-μg meropenem disk was added to each suspension and incubated at 37°C in ambient air for 4 hours ± 15 minutes. The meropenem in the disk was hydrolyzed/ inactivated by test isolates that produced carbapenemase but there was no hydrolysis/ inactivation of meropenem by isolates that did not produce carbapenemase. Each meropenem disc was then removed from the suspension and placed on the Muller Hinton Agar plate inoculated with the meropenem-susceptible *E. coli* ATCC®25922 indicator strain in order to know whether the meropenem was hydrolyzed or not. The meropenem from carbapenemase producer isolates did not inhibit the growth of the meropenem-susceptible *E. coli* or there were only limited growth inhibition (Zone diameter of 6–15 mm or presence of pinpoint colonies within a 16–18 mm zone). But the meropenem from carbapenemase negative test isolates inhibited the growth of the meropenem-susceptible *E. coli* [66].

For carbapenemase producer Enterobacteriaceae, a second 2-ml tube with TSB was labeled for the EDTA Modified Carbapenem Inactivation Method (eCIM) test to differentiate Metallo-β-lactamase from serine carbapenemase. Twenty micro liter of 0.5 molar mass of Ethylene-di-amine tetra acetic acid (0.5 M EDTA) was added to the 2-ml TSB tube and processed in parallel with the mCIM with the same procedure of mCIM. Then the meropenem disks from the mCIM and eCIM tubes were placed on the same MHA plate inoculated with the meropenem-susceptible *E.coli* ATCC® 25922 indicator strain. A ≥ 5-mm increase in zone diameter for eCIM versus zone diameter for mCIM indicated positive for Metallo-β-lactamase but for serine carbapenemase producers, the activity of the carbapenemase was not affected by the presence of EDTA and there was no or marginal (≤ 4 mm) increase in zone diameter in the presence of EDTA compared to the mCIM zone diameter [66].

### 5.8.7. Quality Control

All laboratory assays were done by maintaining the quality control procedures. Standard Operating Procedures (SOPs) were 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. aurous* (ATCC 25923); *E. coli* (ATCC 25922) and *P. aeruginosa* (ATCC 27853). The performance of VITEK 2-COMPACT were also tested with *E. coli* (ATCC 25922) and *P. aeruginosa* (ATCC 27853). The performance of equipments was monitored by using standard procedure.

### 5.8.8. Data Quality Control

The data from data collection format was checked for its completeness and test results were recorded carefully. The results of Culture, antibiotics susceptibility test, ESBLs and carbapenemase test were documented carefully before entry to SPSS version 23.

### 5.9. Data analysis and interpretation

The data was entered and analyzed using Statistical Package for Social Sciences (SPSS) version 23.0. Descriptive statistics was computed and presented using figures and tables. Binary logistic regression was used to see the relation between dependent variable and independent variables. Moreover, a multivariate analysis was computed to identify factors that independently influence the occurrence of dependent variables. The strength of association was presented by odds ratio. P-value less than 0.05 were considered significant in all analysis.

### 5.10. Ethical considerations

All ethical considerations and obligations were duly addressed, and the study was conducted after the approval of the Research Ethics Review Committee (RERC) of the department of Medical Laboratory Sciences. Written informed assent/consent was obtained from the participants before data collection and respondent was given the right to refuse to take part in the study or to withdraw at any time during the study period. All the information obtained from the study subjects were coded to maintain confidentiality. When the participants were found to be positive for bacterial pathogen, they were informed by the hospital clinician for proper treatment.

### 5.11. Dissemination of the result

The result of this study was submitted to the department of Medical Laboratory Sciences, Addis Ababa University. It will also be disseminated to Arsho Advanced Medical Laboratory, hospital, zonal and regional health officers according to the university's and other ethical regulations. The results of the study will also be presented in national and international conferences and manuscript will be prepared and submitted for publication.

# 6. Results

## 6.1. Socio-demographic characteristics

### 6.1.1. Distribution of processed clinical samples against demographic characteristics and specimen types

A total of 873 various clinical samples were processed during the study period, out of which 66.7% (582) were collected from females and 33.3% (292) from males. Among processed clinical samples the highest number were urine, 64.2% (561), followed by blood 12.3% (107), and wound 10.4% (91). Majority of clinical samples were collected from 25-44 years old patients which accounts 43.9% (383) and the lowest samples were from under one year children, 0.7% (6), (Table 6.1.1).

**Table 6.1.1 Distribution of processed clinical samples against demographic characteristics and specimen types at Arsho Advanced Microbiology Laboratory, Addis Ababa, Ethiopia from January 1 to March 30, 2019.**

| Gender | | | Types of Sample n (%) | | | | | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Urine | blood | wound | CSF | Ear swab | Body fluids | Nasal swabs | |
| Male | Age | <1 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 2 (0.2) |
| | | 1-14 | 13 | 3 | 5 | 1 | 2 | 2 | 2 | 28 (3.2) |
| | | 15-24 | 6 | 4 | 3 | 3 | 2 | 1 | 2 | 21 (2.4) |
| | | 25-44 | 70 | 24 | 20 | 7 | 7 | 2 | 0 | 130 (14.9) |
| | | 45-64 | 27 | 14 | 5 | 5 | 5 | 3 | 0 | 59 (6.8) |
| | | ≥65 | 35 | 4 | 8 | 2 | 1 | 1 | 0 | 51 (5.8) |
| | **Male Total** | | 153 (17.5) | 49 (5.6) | 41 (4.7) | 18 (2) | 17 (1.9) | 9 (1) | 4 (0.5) | 291 (33.3) |
| Female | Age | <1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 4 (0.5) |
| | | 1-14 | 37 | 4 | 10 | 7 | 2 | 1 | 4 | 65 (7.4) |
| | | 15-24 | 32 | 11 | 4 | 5 | 5 | 1 | 2 | 60 (6.9) |
| | | 25-44 | 180 | 28 | 23 | 11 | 6 | 5 | 0 | 253 (29) |
| | | 45-64 | 88 | 7 | 8 | 4 | 4 | 1 | 0 | 112 (12.8) |
| | | ≥65 | 70 | 7 | 4 | 4 | 0 | 3 | 0 | 88 (10) |
| | **Female Total** | | 408 (46.7) | 58 (6.6) | 50 (5.7) | 32 (3.7) | 17 (1.9) | 11 (1.3) | 6 (0.7) | 582 (66.7) |
| Total | Age | <1 | 3 | 1 | 1 | 1 | 0 | 0 | 0 | 6 (0.7) |
| | | 1-14 | 50 | 7 | 15 | 8 | 4 | 3 | 6 | 93 (10.6) |
| | | 15-24 | 38 | 15 | 7 | 8 | 7 | 2 | 4 | 81 (9.3) |
| | | 25-44 | 250 | 52 | 43 | 18 | 13 | 7 | 0 | 383 (43.9) |
| | | 45-64 | 115 | 21 | 13 | 9 | 9 | 4 | 0 | 171 (19.6) |
| | | ≥65 | 105 | 11 | 12 | 6 | 1 | 4 | 0 | 139 (15.9) |
| | **Grand Total** | | 561 (64.2) | 107 (12.3) | 91 (10.4) | 50 (5.8) | 34 (3.8) | 20 (2.3) | 10 (1.2) | 873 (100) |

### 6.1.2. Distribution of isolated GNB against demographic characteristics and specimen types

Among all clinical samples collected, only 175 yielded gram negative bacteria of which 62.9% (110) were from female and 37.1% (65) from males. The isolates were obtained from patients ranged from one day old to 92 years old of age with the mean age of 43 years, mod 30 years, median 39 years and standard deviation of 20.7. Majority of the isolates, 42.9% (75/ 175), were found from the age group of 25-44 years old patients followed by 45-64 years old patients which were 22.3% (39/175), (Table 6.1.2).

Urine samples yielded most of the isolates, 56% (98/175) followed by wound, 21.7% (38/175). *E. coli* 61.8% (68/110) and *K. pneumoniae* 20% (22/110) were the most frequently isolated bacteria from females and also from males 52.3% (34/65) and 12.3% (8/65) respectively (Table 6.1.2).

**Table 6.1.2 Distribution of GNB against demographic characteristics and specimen types at Arsho Advanced Microbiology Laboratory Addis Ababa, Ethiopia from January 1 to March 30, 2019.**

| Variables (numbre) | | Gram Negative bacterial Isolates, n(%) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Fermentative GNB | | | | | | | Non-Fermentative GNB | | | | |
| | | *E.coli* | *K. pneumoniea* | *K. oxytoca* | *P.mirabilis* | *E. cloacae* | *C. koseri* | *C. barakkii* | *P.aeruginosa* | *A. baumannii* | *A. lowffi* | *S. paucimobilis* | Others |
| Gender | male (65) | 34 (52.3) | 8 (12.2) | 3 (4.6) | 0 (0) | 2 (3.1) | 1 (1.5) | 1 (1.5)2 | 4 (6.2) | 2 (3.1) | 2 (3.1) | 2 (3.1) | 4 (5.3) |
| | femal(110) | 68 (61.8) | 22 (20) | 1 (0.09) | 3 (2.7) | 0(0) | 1 (0.1) | 1 (0.09) | 5 (4.5) | 4 (3.6) | 0 (0) | 0 (0) | 5 (4.5) |
| Age Group | < 1 (3) | 1 (33.3) | 2 (66.7) | 0(0) | 0 (0) | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) |
| | 1-14 (11) | 7(63.6) | 2(18.2) | 0(0) | 1(9.1) | 0(0) | 0(0) | 1(9.1) | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) |
| | 15-24 (13) | 6 (46.2) | 2 (15.4) | 0(0) | 0(0) | 1 (7.7) | 0(0) | 0(0) | 2 (15.4) | 0(0) | 1 (7.7) | 1 (7.7) | 1 (7.7) |
| | 25-44 (75) | 40 (53.3) | 14 (18.7) | 3 (4) | 2 (2.7) | 1 (1.3) | 2(2.7) | 1 (1.3) | 4 (5.3) | 4 (5.3) | 0(0) | 1 (7.7) | 3 (4.0) |
| | 45-64 (39) | 26 (66.7) | 5 (12.8) | 0(0) | 1 (2.6) | 0(0) | 0(0) | 0(0) | 2 (5.1) | 0(0) | 1 (2.6) | 0(0) | 3 (7.7) |
| | >65(34) | 22(62.9) | 7(20) | 1(2.9) | 0(0) | 0(0) | 0(0) | 0(0) | 1(2.9) | 2(5.7) | 0(0) | 0(0) | 2(5.7) |
| Tpes of Specimen | Urine (98) | 79 (80.6) | 11 (11.2) | 2 (2.04) | 0(0) | 1 (1.0) | 1 (1.0) | 1 (1.02) | 1 (1.02) | 0(0) | 0(0) | 0(0) | 2 (2.04) |
| | Wond (38) | 14 (36.8) | 8 (21.05) | 1 (2.6) | 2 (5.3) | 1 (2.6) | 1 (2.6) | 1 (2.6) | 5 (13.2) | 3 (7.9) | 0(0) | 0(0) | 2 (5.3) |
| | Blood (20) | 4 (20) | 11 (55) | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) | 1 (5) | 0(0) | 1 (5) | 3 (20) |
| | Ear (8) | 1 (12.5) | 0(0) | 1 (12.5) | 1 (12.5) | 0(0) | 0(0) | 0(0) | 3 (37.5) | 0(0) | 0(0) | 0(0) | 2 (25) |
| | CSF (4) | 1 (25) | 2 (50) | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) | 1 (25) | 0(0) | 0(0) |
| | B. Fluid (5 | 3 (60) | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) | 2 (40) | 0(0) | 0(0) | 0(0) |
| | Nasal(2) | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) | 1(50) | 1(50) | 0(0) |
| TOTAL | 175 (100) | 102 (58.3) | 32 (18.3) | 4 (2.3) | 3 (1.7) | 2 (1.1) | 2 (1.1) | 2 (1.1) | 9 (5.1) | 6 (3.4) | 2 (1.1) | 2 (1.1) | 9(5.1) |

**Note:** Other isolates were *Providencia rettgeri*, *Morganella morganii*, *Shigella dysenteriae*, *Raoultella planticola*, *Raoultella ornithinolytica*, *Serratia fonticola*, *Burkholderia cepacia and Ralstonia Pickettii*.

## 6.2. Frequency of GNB isolates

A total of 175 gram negative bacterial isolates belonging to 19 species were isolated, and among them 88%(154) were fermenters and 12%(21) were non-fermenters. From fermenters *E. coli* accounts the highest percentage, 58.3% (102/175) followed by *K. pneumoniae* with 18.3% (32/175). On the other hand *P. aeruginosa*, 5.1% (9/175) was the most frequent isolate followed by *A. baumannii* 3.4% (6/175) from non-fermenter GNB. More than three-forth, 77.5% (79/102) of *E. coli* were isolated from urine specimens whereas 55.6% (5/9) of *P. aeruginosa* were from wound (Table 6.1.2).

## 6.3. Antibiotics resistance profile of gram negative bacilli

The antibiotics resistance profile of all gram negative isolates against 20 antibiotics is presented in table 6.2. The highest resistance level was seen against ampicillin (86%), followed by cefalotin (73.2%), trimethoprim/sulfamethoxazole (68.9%), cefazoline (67.1%), and tetracycline (66.5%) whereas the lowest resistance were recorded against meropenem (9.8%), tobramycin (18.9%) and nitrofurantoin (19.5%), (Table 6.2).

Fermentative GNB totally showed higher resistance to ampicillin (86.9%), cefalotin (72.4%) and tetracycline (68.3%) but lower resistance for meropenem (5.5%), nitrofurantoin (12.4%) and cefoxitin (14%). From this group *E. coli* showed high resistance for ampicillin (81.4%) trimethoprim/sulfamethoxazole (66.9%), tetracycline (69.6%), and cefalotin (65.7%) but lowest resistance for meropenem (4.9%) followed by cefoxitin(12%). *K. pneumoniae* which is the second frequently isolated next to *E. coli*, showed highest resistance to ampicillin (100%) followed by cefalotin (90.6%) and least resistance for meropenem and cefoxitin (9.4% each), (Table 6.2).

Non-fermenters showed 100% resistance for ampicillin, amoxicillin/clavulanic acid, cefalotin, cefazoline, cefuroxime, cefuroxime-axetile, ceftriaxone, and cefoxitin. Least resistance for non-fermenters was recorded to gentamicin and tobramycin 20% each. *P. aeruginosa* and *A. baumannii* are frequently isolated from non-fermenters in which *P. aeruginosa* showed 100% resistance for ampicillin, amoxicillin-clavulanic acid, cefalotin, cefazoline, cefuroxime,

cefuroxime-axetile, ceftriaxone, cefpodoxime, cefoxitin, tetracycline, trimethoprim-sulfamethoxazole and nitrofurantoin but least resistance was also recorded against ciprofloxacin (0%), gentamicin (11%) and tobramycin (11.1%). *A. baumannii* showed 100% resistance for ampicillin, amoxicillin-clavulanic acid, cefalotin, cefazoline, cefuroxime, cefuroxime-axetile, ceftriaxone and cefoxitin but lower resistance against tetracycline (16.7%), gentamicin and tobramycin (33.3% each), (Table 6.2).

**Table 6.2 Antimicrobial resistance profile of gram negative isolates from different clinical specimens at Arsho Advanced Microbiology Laboratory Addis Ababa, Ethiopia, from January 1 to March 30, 2019**

| Species (number) | | Tested Antibiotics , n (%) | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | AMP | AMC | TZP | CFA | CFZ | CFU | CFXA | FOX | CPD | CAZ | CRO | CFP | GM | TBM | CIP | LEV | TEC | NFT | SXT | MEM |
| ***E.coli* (102)** | **n** | 83 | 25 | 14 | 67 | 58 | 54 | 54 | 12 | 50 | 50 | 50 | 49 | 25 | 14 | 53 | 53 | 71 | 4 | 71 | 5 |
| | **%** | 81.4 | 24.5 | 13.7 | 65.7 | 56.9 | 52.9 | 52.9 | 12 | 49 | 49 | 49 | 48 | 25 | 13.7 | 52 | 52 | 69.6 | 3.9 | 69.6 | 4.9 |
| ***K. pneumoniea* (32)** | **n** | 32 | 16 | 10 | 29 | 28 | 28 | 28 | 3 | 26 | 26 | 26 | 26 | 23 | 10 | 13 | 5 | 20 | 10 | 23 | 3 |
| | **%** | 100 | 50 | 31.3 | 90.6 | 87.5 | 87.5 | 87.5 | 9.4 | 81.3 | 81.3 | 81.3 | 81.3 | 72 | 31.3 | 40.6 | 15.6 | 62.5 | 31.3 | 72 | 9.4 |
| ***K. oxytoca* (4)** | **n** | 4 | 2 | 1 | 3 | 3 | 3 | 3 | 1 | 3 | 3 | 3 | 3 | 3 | 3 | 1 | 1 | 3 | 1 | 3 | 0 |
| | **%** | 100 | 50 | 25 | 75 | 75 | 75 | 75 | 25 | 75 | 75 | 75 | 75 | 75 | 75 | 25 | 25 | 75 | 25 | 75 | 0 |
| ***P. aeruginosa* (9)** | **n** | 9 | 9 | 4 | 9 | 9 | 9 | 9 | 9 | 9 | 4 | 9 | 3 | 1 | 1 | 0 | 2 | 9 | 9 | 9 | 3 |
| | **%** | 100 | 100 | 44.4 | 100 | 100 | 100 | 100 | 100 | 100 | 44.4 | 100 | 33.3 | 11 | 11.1 | 0 | 22.2 | 100 | 100 | 100 | 33.3 |
| ***A. baumannii* (6)** | **n** | 6 | 6 | 4 | 6 | 6 | 6 | 6 | 6 | 6 | 4 | 4 | 4 | 2 | 2 | 4 | 2 | 1 | 5 | 4 | 5 |
| | **%** | 100 | 100 | 66.7 | 100 | 100 | 100 | 100 | 100 | 100 | 66.7 | 66.7 | 66.7 | 33 | 33.3 | 66.7 | 33.3 | 16.7 | 83.3 | 66.7 | 83.3 |
| ***A. lowffi* (2)** | **n** | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | **%** | 0 | 0 | 0 | 50 | 50 | 0 | 0 | 50 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| ***P.mirabilis* (3)** | **n** | 2 | 0 | 0 | 1 | 2 | 2 | 2 | 0 | 1 | 2 | 1 | 1 | 0 | 1 | 1 | 1 | 3 | 2 | 2 | 0 |
| | **%** | 66.7 | 0 | 0 | 33.3 | 66.7 | 66.7 | 66.7 | 0 | 33.3 | 66.7 | 33.3 | 33.3 | 0 | 33.3 | 33.3 | 33.3 | 100 | 66.7 | 66.7 | 0 |
| ***E. cloacae* (2)** | **n** | 2 | 2 | 0 | 2 | 2 | 1 | 2 | 2 | 2 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 0 |
| | **%** | 100 | 100 | 0 | 100 | 100 | 50 | 100 | 100 | 100 | 0 | 50 | 0 | 50 | 0 | 0 | 0 | 50 | 50 | 50 | 0 |
| ***C. koseri* (2)** | **n** | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| | **%** | 50 | 0 | 0 | 50 | 50 | 50 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 50 | 0 | 0 | |
| ***C. brakii* (2)** | **n** | 2 | 1 | 0 | 2 | 1 | 1 | 2 | 2 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | **%** | 100 | 50 | 0 | 100 | 50 | 50 | 100 | 100 | 50 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Total** | **n** | **141** | **61** | **33** | **120** | **110** | **105** | **106** | **35** | **98** | **89** | **94** | **86** | **55** | **31** | **72** | **64** | **109** | **32** | **113** | **16** |
| | **%** | **86** | **37** | **20** | **73.2** | **67** | **64** | **65** | **21** | **59.8** | **54** | **57.3** | **52.4** | **34** | **18.9** | **43.9** | **39** | **66.5** | **19.5** | **69** | **9.8** |

**Note:** AMP = ampicillin, AMC = amox/clavulanic acid, TZP = piperacillin /tazobactam, CFA = cefalotin, CFZ = cefazoline, CFU = cefuroxime, CFXA = cefuroxime-Axetile, FOX = cefoxitin, CPD = cefpodoxime, CAZ = ceftazidime, CRO = ceftriaxone, CFP = cefepime, GM = gentamicin, TBM = tobramycin, CIP = ciprofloxacin, LEV = levofloxacin, TEC = tetracycline, NFT = nitrofurantoin, SXT = trimethoprim/sulfamethoxazole and MEM = meropenem

### 6.4. Multi-drug resistance profile of gram negative bacilli

Over all 80.5% (132/164) of gram negative isolates were MDR. As summarized in Figure 6.1, all GNB isolated from body fluids were MDR followed by blood (93.8%) and wound (88.9%).

From isolated species, 100% MDR were recorded in *A. baumannii*, *P. mirabilis*, *C. brakii* and *E. cloacae* whereas the least resistance, 33.3%, was recorded against *P. aeruginosa*. Only 7.3% (12/164) gram negative isolates were susceptible for all tested antibiotics (Table 6.3).

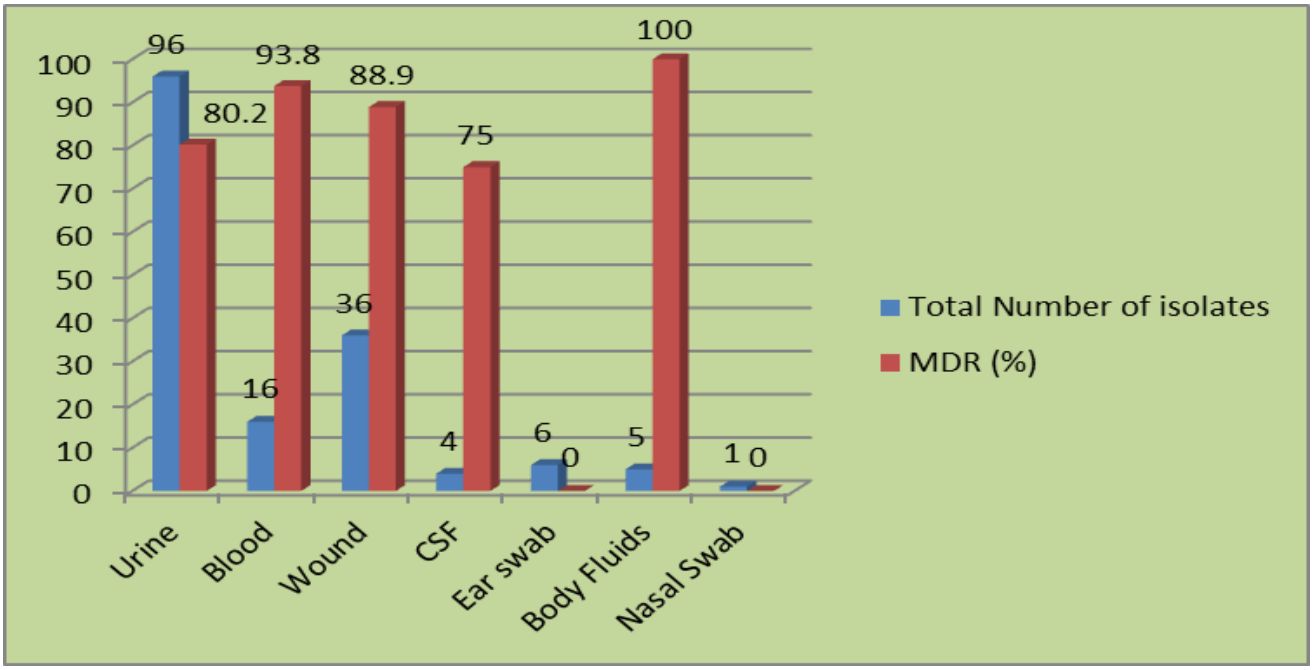



**Figure 6.1: Distribution of MDR GNB in different clinical specimens at Arsho Advanced Microbiology Laboratory Addis Ababa, Ethiopia, from January 1 to March 30, 2019.**

**Table 6.3: Multidrug resistance level of gram negative bacteria isolated from different clinical specimen at Arsho Advanced Microbiology Laboratory, Addis Ababa, Ethiopia, from January1 to March 30, 2019.**

| Isolates(number) | Level of antibiotics resistance n (%) | | | | | | | | Total MDR isolates( ≥ R3) |
|---|---|---|---|---|---|---|---|---|---|
| | R0 | R1 | R2 | R3 | R4 | R5 | R6 | ≥R7 | |
| *E. coli* (102) | 6(5.9) | 8 (7.8) | 5 (4.9) | 10 (9.8) | 8 (7.8) | 14 (13.7) | 14 (13.7) | 37 (36.3) | **83 (81.4)** |
| *K. pneumoniae* (32) | 0 (0) | 1 (3.1) | 2 (6.2) | 2 (6.2) | 0 (0) | 2 (6.2) | 2 (6.2) | 23 (71.9) | **29 (90.6)** |
| *K. oxytoca* (4) | 0 (0) | 0 (0) | 1 (25) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 3 (75) | **3 (75)** |
| *P. aeruginosa* (9) | 4 (44.4) | 0 (0) | 2 (22.2) | 2 (22.2) | 0 (0) | 1 (11.1) | NA | NA | **3 (33.3)** |
| *A. baumannii* (6) | 0 (0) | 0 (0) | 0 (0) | 2 (33.3) | 0 (0) | 1 (16.7) | 1 (16.7) | 2 (33.3) | **6 (100)** |
| *A. lowffi* (2) | 2 (100) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | **0(0)** |
| *P. mirabilis* (3) | 0 (0) | 0 (0) | 0 (0) | 1 (33.3) | 0 (0) | 1 (33.3) | 0 (0) | 1 (33.3) | **3(100%)** |
| *E. cloacae* (2) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 1 (50%) | 0 (0) | 1 (50%) | **2(100%)** |
| *C. koseri* (2) | 0 (0) | 1 (50%) | 0 (0) | 0 (0) | 1 (50%) | 0 (0) | 0 (0) | 0 (0) | **1 (50%)** |
| *C. brakii* (2) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 2(100%) | 0 (0) | 0 (0) | **2(100%)** |
| Total (164) | **12 (7.3)** | **10 (6.1)** | **10 (6.1)** | **17 (10.4)** | **9 (5.5)** | **22 (13.4)** | **17 (10.4)** | **67 (40.9)** | **132 (80.5%)** |

**Note:** R0: resistance to no antibiotics, R1-7: resistance to 1, 2, 3, 4, 5, 6, and 7 groups of antibiotics; ≥R3: resistance to 3 or more antibiotics from different classes. NA: not applicable (because only 5 different groups of anti-*Pseudomonas* antibiotics were tested during the study).

### 6.5. Magnitude of ESBLs Producing gram negative bacilli

Among 175 GNB isolated from different clinical samples, 28% (49/175) were ESBLs producers. Among those 36.7% (18/49) were from male and 63.3% (31/49) were from female patients. About 43% (21/49) were from age group of 25-44 followed by 22.4% (11/49) from 65 and above years old patients (Figure 6.2).

There was intra-species variation in ESBLs production in which the highest percentage was recorded among *K. pneumoniae,* 50% (16/32) followed by *E. coli*, 29.4% (30/102) and the lowest production was observed in *P. aeruginosa* with 11.1% (1/9) proportion (Table 6.4).

From the total 49 ESBLs producer GNB 61.2% (30/49) were *E. coli* and 32.7% (16/49) were *K. pneumoniae*. Regarding ESBLs production across different clinical samples processed, blood had highest proportion, 55% (11/20), followed by wound 26.3% (10/38) and urine 23.5% (23/98) (Table 6.4).

All ESBLs positive *E. coli* and *K. pneumoniae* were MDR but from non-ESBLs producers only 73.6% of *E. coli* and 81.3% of *K. pneumoniae* were multidrug resistant.

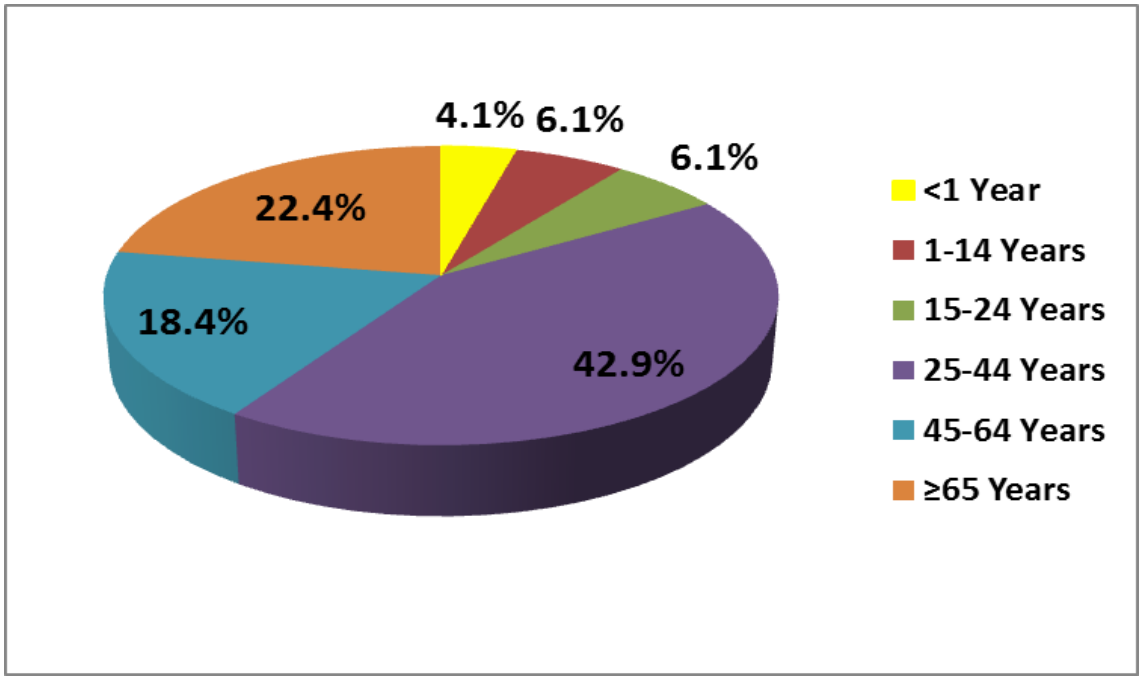



**Figure 6.2, Distribution of ESBLs producing GNB against age category at Arsho Advanced Microbiology Laboratory Addis Ababa, Ethiopia, from January 1 to March 30, 2019.**

**Table 6.4 Distribution of ESBLs producing GNB in different isolated species and clinical specimens at Arsho Advanced Microbiology Laboratory, Addis Ababa, Ethiopia, from January 1 to March 30, 2019.**

| Isolated Spp | Clinical Specimens | | | | | | | Total ESBLs |
|---|---|---|---|---|---|---|---|---|
| | Urine (n=98) | Blood (n=20) | Wond (n=38) | CSF(n=4) | Ear Swab (n=8) | Body fluids (n=5) | Nasal swab(n=2) | |
| *E. coli (n=102)* | 18 (18.4%) | 2 (10%) | 7 (18.4%) | 1 (25%) | 0 (0) | 2 (40%) | 0 (0) | **30 (29.4%)** |
| *K. pnamoniae* (n=32) | 4 (4.1%) | 9 (45%) | 2 (5.3%) | 1 (25%) | 0 (0) | 0 (0) | 0 (0) | **16 (50%)** |
| *K. oxytoka* (n=4) | 1 (1%) | 0 (0) | 1 (2.6%) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | **2 (50%)** |
| *P. aerugenasa* (n=9) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 1 (12.5%) | 0 (0) | 0 (0) | **1 (11.1%)** |
| **Total ESBLs** | **23 (23.5%)** | **11 (55%)** | **10 (26.3%)** | **2 (50%)** | **1 (12.5%)** | **2 (40%)** | **0 (0)** | **49 (28%)** |

## 6.6. Association of independent variables with magnitude of ESBLs producing gram negative bacilli

Using binary logistic regression analysis, magnitude of ESBLs production had statistically significant association with type of specimen. The chances of getting ESBLs positive among GNB isolated from blood specimen were 3.99 (95% CI = 1.47 – 10.80, p = 0.006) times higher than GNB isolated from urine specimen.

In multinomial logistic regression analysis also magnitude of ESBLs production had statistically significant association with specimen type (P< 0.05) in which bacterial isolates from blood specimen were 3.68 (95% CI = 1.55 – 12.89, p = 0.007) folds higher than GNB isolated from urine specimen. However, other variables (gender and age group) did not have statistically significant association with magnitude of ESBLs production in our study (Table 6.5).

**Table 6.5; Association of gender, age and types of specimens with magnitude of extended spectrum β-lactamases producing gram negative bacilli at Arsho Advanced Microbiology Laboratory Addis Ababa, Ethiopia, from January 1 to March 30, 2019.**

| Variable (n) | ESBLs Positive n(%) | Bi-variable | | Multi-variable | |
|---|---|---|---|---|---|
| | | COR (CI) | P-value | AOR (95% CI) | P- value |
| **Gender** | | | | | |
| Female (113) | 31 (27.4) | **Ref*** | 0.822 | **Ref*** | 0.999 |
| Male (62) | 18 (29) | 1.08 (0.54-2.15) | 0.820 | 1.02 (0.49, 2.13) | 0.960 |
| **Age (years)** | | | | | |
| <18 (16) | 5 (45.5) | Ref* | 0.773 | **Ref*** | 0.910 |
| 19-36 (66) | 17 (25.8) | 0.76 (0.23-2.52) | 0.567 | 0.69 (0.20, 2.42) | 0.657 |
| 37-56 (48) | 15 (31.3) | 1.00 (0.925-3.389) | 0.927 | 1.06 (0.29, 3.82) | 0.998 |
| >56 (45) | 12 (26.7) | 0.80 (0.23-2.78) | 0.988 | 1.00 (0.27, 3.80) | 0.726 |
| **Specimen type** | | | | | |
| Urine (98) | 23 (23.5) | **Ref*** | 0.045** | **Ref*** | 0.048** |
| Blood (20) | 11 (55) | 3.99 (1.47, 10.80) | 0.006** | 3.68 (1.55, 12.89) | 0.007** |
| Wound (38) | 10 (26.3) | 1.17 (0.493, 2.752) | 0.728 | 1.10(0.48, 2.87) | 0.737 |
| Others (19) | 5 (26.3) | 1.17 (0.379, 3.58) | 0.709 | 1.14 (0.39, 3.97) | 0.718 |

****** = Statistical significant association between the variables and magnitude of ESBLs producing gram negative bacteria

**Ref*** =Reference

Others = CSF (4), body fluids (5), Nasal swab (2) and ear swab (8)

COR = Crud odds ratio, AOR = Adjusted odds ratio, CI = Confidence Interval

### 6.7. Magnitude of carbapenemase producing GNB

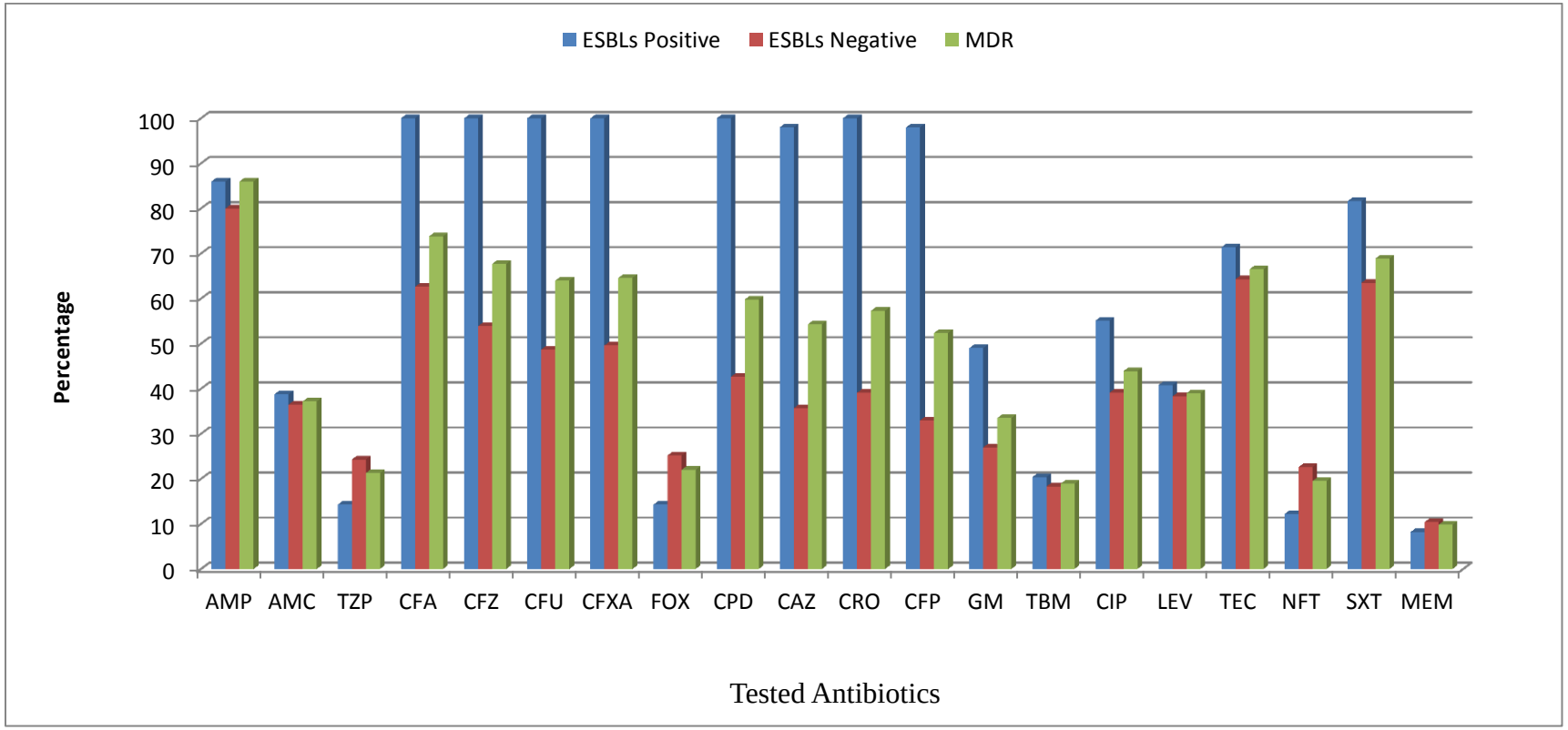
Out of the total 175 gram negative bacilli isolated from different clinical samples, 16 isolates were resistant for meropenem. Out of 16 meropenem resistance isolates 11 were tested for carbapenemase production by Modified Carbapenem Inactivation Method (mCIM) according to CLSI guideline. Five meropenem resistance isolates were from *A. baumannii* which was the species not recommended by CLSI guideline to perform Modified Carbapenem Inactivation Method (mCIM) so that only 11 isolates were tested. Nine out of 11 were carbapenemase producer of which five were from male and four from female patients. Five out of nine were metallo-β-lactamase and four were serine carbapenemase producers. The overall prevalence of carbapenemase producing GNB was 5.4% (9/167), since 8 *Acinetobacter* spp were excluded from the test. Distribution of carbapenemase producer GNB by isolated species and specimen types are presented by table 6.6.

**Table 6.6 Distribution of carbapenemase producing gram negative bacteria against isolated species and specimen types at Arsho Advanced Microbiology Laboratory Addis Ababa, Ethiopia, from January 1 to March 30, 2019**

| Carbapenemase producer GNB, (n) | | | | |
|---|---|---|---|---|
| Isolated Spp | Types of specimen | | | |
| | Wound | Urine | Body Fluid | Total |
| *E. coli* | 0 | 2 | 1 | 3 |
| *K. pneumoniae* | 3 | 0 | 0 | 3 |
| *P. aeruginosa* | 2 | 1 | 0 | 3 |
| Total | 5 | 3 | 1 | 9 |

## 6.8. Magnitude of antibiotic resistance in ESBLs, Non-ESBLs and MDR Gram negative bacilli against different classes of antibiotics

Generally ESBLs producers showed higher resistance for majority of the antibiotics tested but non-ESBLs producers were more resistant for piperacillin/ tazobactam, cefoxitin, nitrofurantoin and meropenem. Multidrug resistant GNB, for most of the antibiotics, showed higher resistance than non-ESBLs producers and lower than ESBLs producers (Figure 6.3).




Note: AMP = ampicillin, AMC = amox/clavulanic acid, TZP = piperacillin /tazobactam, CFA = cefalotin, CFZ= cefazoline, CFU = cefuroxime, CFXA = cefuroxime-Axetile, FOX = cefoxitin, CPD = cefpodoxime, CAZ= ceftazidime, CRO = ceftriaxone, CFP = cefepime, GM = gentamicin, TBM = tobramycin, CIP =ciprofloxacin, LEV = levofloxacin, TEC = tetracycline, NFT = nitrofurantoin, SXT = trimethoprim/sulfamethoxazole and MEM = meropenem.

**Figure 6.3 Antibiotics resistance pattern of ESBLs positive, ESBLs negative and MDR GNB against different classes of antibiotics at Arsho Advanced Microbiology Laboratory Addis Ababa, Ethiopia, from January 1 to March 30, 2019.**

## 6.9. Effective antibiotics against ESBLs and carbapenemase producing gram negative bacilli

In our study, the most active drugs for ESBLs producing GNB were meropenem, piperacillin/tazobactam and cefoxitin with sensitivity of 91.8%, 83.7% and 71.4% respectively. Levofloxacin and nitrofurantoin also showed moderate activity with 57.1% sensitivity each.

In the present study, ninety percent (18/20) of the antibiotics tested had less than 25% activity against carbapenemase producer GNB. However, relatively higher activity was seen against gentamicin with 55.6% sensitivity and tobramycin 33.3% sensitivity (Table 6.7).

**Table 6.7 Antibiotic susceptibility patterns of ESBLs and carbapenemase producing gram negative bacilli at Arsho Advanced Microbiology Laboratory Addis Ababa, Ethiopia, from January 1 to March 30, 2019.**

| Tested Antibiotics | ESBLs producer GNB | | Carbapenemase producer GNB | |
|---|---|---|---|---|
| | Total No. | Susceptible, n (%) | Total No. | Susceptible, n (%) |
| Ampicillin | 49 | 0 (0) | 9 | 0 (0) |
| Amox/clavulanic acid | 49 | 15 (30.6) | 9 | 0 (0) |
| Piperacillin /tazobactam | 49 | 41 (83.7) | 9 | 1 (11.1) |
| Cefalotin | 49 | 0 (0) | 9 | 0 (0) |
| Cefazoline | 49 | 0 (0) | 9 | 0 (0) |
| Cefuroxime | 49 | 0 (0) | 9 | 0 (0) |
| Cefuroxime-Axetile | 49 | 0 (0) | 9 | 0 (0) |
| Cefoxitin | 49 | 35 (71.4) | 9 | 2 (22.2) |
| Cefpodoxime | 49 | 0 (0) | 9 | 0 (0) |
| Ceftazidime | 49 | 1 (2) | 9 | 1 (11.1) |
| Ceftriaxone | 49 | 0 (0) | 9 | 0 (0) |
| Cefepime | 49 | 1 (2) | 9 | 2 (22.2) |
| Gentamicin, | 49 | 25 (51) | 9 | 5 (55.6) |
| Tobramycin | 49 | 22 (44.9) | 9 | 3 (33.3) |
| Ciprofloxacin | 49 | 20 (40.8) | 9 | 2 (22.2) |
| Levofloxacin | 49 | 28 (57.1) | 9 | 2 (22.2) |
| Tetracycline | 49 | 14 (28.6) | 9 | 1 (11.1) |
| Nitrofurantoin | 49 | 28 (57.1) | 9 | 2 (22.2) |
| Trimethoprim/sulfamethoxazole | 49 | 9 (18.4) | 9 | 1 (11.1) |
| Meropenem | 49 | 45 (91.8) | 9 | 0 (0) |

# 7. Discussion

Gram-negative bacteria are important causes of urinary tract infections, bloodstream infections, ventilator associated pneumonia, septicemia, and surgical site infection [1-3]. The worldwide emergence and spreading of antibiotic resistant gram negative bacteria have been a great challenge to treat common infectious diseases [22]. ESBLs and carbapenemases are the common hydrolyzing enzymes produced by GNB and give the ability to resist antibiotics which leads to multi-drug resistance [26].

## 7.1. Magnitude of gram negative bacilli

In our study the overall prevalence of GNB was 20%. Among those *E. coli* with 58.3% was the most frequent isolates followed by *K. pneumoniae* with 18.3% from all GNB. This finding was in line with the previous studies reported in Ethiopia: Addis Ababa; *E. coli* 53.5% and *K. pneumoniae* 24.1% [67], Arba Minch, *E.coli* 41.6% and *K. pneumoniae* 16.6% [60], Dire dawa; *E. coli* 34.6% and *Klebsiella* spp 11.5% [62]. The same findings were also reported from other countries; Sudan; *E. coli* 54.4% and *Klebsiella* spp 29.5% [33], India; *E. coli* 39% and *K. pneumoniae* 24% [54]: Iran; *E. coli* 42.8%, and *Klebsiella* spp 16.5% [68].

The present study indicated that *P. aeruginosa* with 5.1% was the most frequent isolate from non-fermenter GNB. This finding was comparable with previous studies done in Addis Ababa 11.1% [27], Debre Markos 10.7% [59], India11% [53] and Iran 14.2% [68] but lower than the study done in USA, 22.2% [50]. All of the above reports indicated that fermentative GNB are the major causes for most of gram negative bacterial infections than non-fermentative.

## 7.2. Antibiotics resistance profile of gram negative bacilli

In the present study from all GNB, highest resistance level was seen in ampicillin (86%), followed by cefalotin (73.2%), trimethoprim/sulfamethoxazole (68.9%) and tetracycline (66.5%). The lowest resistance levels were also recorded against meropenem (9.8%). Comparable results were reported in Ethiopia like in Addis Ababa; ampicillin 88.5%, trimethoprim/sulfamethoxazole 80.0%, tetracycline 62.8% [31], meropenem 5.2% [67], Dire Dawa; ampicillin 80%, trimethoprim/sulfamethoxazole 42.1% [62], Arba Minch; ampicillin

81.6%, trimethoprim/sulfamethoxazole 46.8%, tetracycline 50% [60], Debre Markos; ampicillin 70.4% and tetra 75.9% [59]. There are also equivalent reports in other countries like in Ghana; ampicillin 94.4%, trimethoprim/sulfamethoxazole 84.5%, tetracycline 30.5%, and meropenem 2.5% [26], Rwanda; ampicillin 98%, cefalotin 67%, trimethoprim/sulfamethoxazole 75.7%, and tetracycline 80% [58]. This indicated the widespread of resistance for commonly prescribed antibiotics which might be resulted from poor antibiotic stewardship in developing countries.

The most predominant isolate from fermentative GNB was *E. coli* in our study, which was highly resistance to ampicillin 81.4%, tetracycline 69.6%, trimethoprim/sulfamethoxazole 69.6%, and cefalotin 65.7%. There were also significant level of resistance to ciprofloxacin 52%, ceftriaxone 49%, and cefepime 48% which was almost in agreement with study conducted in Addis Ababa; ampicillin 95.8%, tetracycline 83.3%, ceftriaxone 83.3% and ciprofloxacin 66.7% [63], Debre-Markos; tetracycline 75% [59], Arba-Minch; ampicillin 85%, tetracycline 60% [60], Singapore; trimethoprim/sulfamethoxazole 51.3% [32], Ghana; trimethoprim/sulfamethoxazole 69.2% and ampicillin 94.7%[57], Rwanda; ampicillin 96%, trimethoprim/sulfamethoxazole 76%, ciprofloxacin 66% and ceftriaxone 30% [58].

In *K. pneumoniae* the highest level of resistance was observed against ampicillin 100%, cefalotin 90.6%, cefuroxime 87.5%, cefpodoxime 81.3%, ceftazidime 81.3%, ceftriaxone 81.3%, cefepime 81.3%, gentamicin 72%, and tetracycline 62.5%, which was comparable with reports from Addis Ababa; ampicillin 100% and ceftriaxone 90% [63], Ghana; ampicillin 100%, gentamicin 72% [57], Rwanda; ampicillin 100%, cefuroxime 68%, cefalotin 67 %, ceftriaxone 55% and gentamicin 53% [58]. However, our result was higher than the reports from Singapore; ceftriaxone 30.8%, cefepime 30.8% [32], Czech Republic; ampicillin 24%, cefuroxime 21% and gentamicin 12% [69]. The difference was due to that the bacterium was non-ESBLs producer in the reports of those countries.

From non-fermentative GNB *P. aeruginosa* showed 44.4% resistance for piperacillin /tazobactam and ceftazidime each, 33.3% for cefepime and meropenem each, but lower resistance for tobramycin and gentamicin 11% each. This finding was almost in line with study in Rwanda; piperacillin /tazobactam 33%, cefepime 33%, and gentamycin 25% [58], India;

piperacillin /tazobactam 61.8%, ceftazidime 69.4%, cefepime 65.4%, meropenem 34.7%, and gentamycin 24.3% [55], Singapore; ceftazidime 23.4% and gentamycin 17.6% [32]. However, it was higher than reports from Asian pacific countries; piperacillin /tazobactam 11.6%, ceftazidime 19.7%, cefepime 12.9%, [51] and Singapore; piperacillin /tazobactam 11.7%, cefepime 11.2%, and meropenem 9% [32]. The difference might be resulted from practicing good infection prevention in those countries around their health facilities. Since serious infections with *P. aeruginosa* are predominantly nosocomial, they are more likely to be drug resistant than community acquired infections [70].

The second predominant non-fermenter GNB isolates in our study was *A. baumannii* which showed 66.7% resistance for piperacillin /tazobactam, ceftriaxone, ceftazidime, cefepime, ciprofloxacin, and trimethoprim/sulfamethoxazole each. This result was in agreement with a study conducted in Asian Pacific countries; piperacillin /tazobactam 84.6%, ceftriaxone 84.6%, ceftazidime 83.1%, and cefepime 84.6%[51], Singapore; piperacillin /tazobactam 79.9 %, ceftazidime 71.9%, cefepime 79.1%, and ciprofloxacin 75.5% [32], India; piperacillin /tazobactam 69.7%, ceftriaxone 76.8%, ceftazidime 76.3%, cefepime 67.8%, and ciprofloxacin 42.2% [55], Rwanda; ceftriaxone 40%, ceftazidime 33%, ciprofloxacin 25%, and trimethoprim/sulfamethoxazole 67% [58].

### 7.3. Multidrug resistance profile of gram negative bacilli

In our study, the overall magnitude of MDR among all GNB isolate was 80.5%. There were also comparable findings from other studies conducted in Addis Ababa 76.3% [63], Debre Markos 76.1% [59] and Ghana 89.5% [26]. However, it was lower than studies conducted in Dire Dawa 100% [62] and Nepal 96.8% [71]. The difference might be due to sample difference, patient condition, and definition for MDR. Our result was higher than reports from Addis Ababa 68.3% [67], Bahr Dar 54.3% [72] and Nepal 33.14% [73]. The difference might be resulted from proper infection prevention, proper selection and utilization of antibiotics in those areas, since bacterial drug resistance has close association with the over-and miss-use of antimicrobial agents [38, 45, 46].

The present study showed that from major isolates *A. baumannii* with 100% and *K. pneumoniae* with 90.6% was the major MDR GNB followed by *E. coli* 81.4% and *P. aeruginosa* 33.3% which were comparable with reports from Addis Ababa; *A. baumannii* 95.7%, *E. coli* 83.3%, *P. aeruginosa* 66.7% [63] and *K. pneumoniae* 90% [27], Debre Markos; *K. pneumoniae* 81.8%, *E. coli* 61.5% and *P. aeruginosa* 73.3% [59], *Ghana; A. baumannii* 100%, *K. pneumoniae* 94.7%, and *E. coli* 89.9% [26], India*; A. baumannii 87%* [25], Nepal; *Klebsiella* spss.100% and *E. coli* 95.5% [71]. A 100% MDR *A. baumannii* in the present study strengthens the report of Sekyere J. *et al* which reported *A. baumannii* as the famous extra-drug-resistant pathogen with resistance to almost all antimicrobials being reported [49]. High level of MDR in *E. coli* and *K. pneumoniae* might be due to extended-spectrum β-lactamases production [47], as they were the first and second among ESBLs producing GNB in the current study.

### 7.4. Magnitude of ESBLs producing gram negative bacilli

The overall magnitude of ESBLs producing GNB in the present study was 28%, which was in agreement with study reported in Adama 25% [74], Harer 33.3% [108], Czech republic 24.2% [69], Nepal 26.8% [71], Saudi Arabia 36% [75] and Israel 42.5% [76]. Our result was lower than other results reported from Addis Ababa 57.7% [67], Bahr Dar 57.6% [77], Uganda 62% [78], and Nigeria 58% [79]. However it was higher than studies conducted in Cameroon 12% [80], Italy 6.3% [81] and France 3.2% [82].The difference in magnitude might be resulted from differences in infection control strategy of the countries, variation in drug management policies, method difference to conduct the study and study participant conditions.

In the present study 50% of *K. pneumoniae* and 29.4% of *E. coli* were the major ESBLs producing GNB which are comparable with previous studies done in Addis Ababa; *K. pneumoniae* 78.6% and *E. coli* 52.2% [67], Bahr Dar; *K. pneumoniae* 69.4% and *E. coli* 58.2% [77], Uganda; *K. pneumoniae* 72.7% and *E. coli* 58.1% [78], Nigeria; *K. pneumoniae* 62% and E.*coli* 54.2% [79], India; *K. pneumoniae* 66.6% and *E. coli* 50% [53], North-West India again by Saroj H. *et al*; *K. pneumoniae* 56.5% [83] Nepal; 26.87% *E. coli* [71], Saudi Arabia; *K. pneumoniae* 65.6% and *E. coli* 29%, [75].

Our result was higher in magnitude than studies reported in Adama; *K. pneumoniae* 25% and *E. coli* 25% [74], Cameroon; *K. pneumoniae* 18.8% and *E. coli* 14.3% [80], and Singapore; *K. pneumoniae* 30.3% and *E.coli* 19.6% [32]. This might be the result of proper infection prevention practices in that area of study, difference in antibiotic stewardship and method difference.

### 7.5. Distribution of ESBLs producing gram negative bacilli in different specimens

In our study, the highest number of ESBLs producer GNB was found from blood specimens 55% (11/20) (AOR = 3.68, 95% CI= 1.55-12.89, P = 0.007) followed by wound 26.3% (10/38) and urine 23.5% (23/98). Other researchers also reported blood as major clinical specimen for ESBLs producing GNB: in Addis Ababa 84.4% [67], United Arab Emirates 58% [84], and Iran 87.8% [85]. This indicated that systemic bacterial infections by ESBLs producing GNB became the major problem for treatment by routine antibiotics which are susceptible for ESBLs enzymes.

However, different researchers reported other clinical samples as major source of ESBLs producer GNB like in Adama 53% from wound [74], Bahr Dar 48.3% from urine [77], Cameroon 48.4% from urine [80], and Nepal 75% from urine [86]. The difference might be resulted from study participants.

### 7.6. Magnitude of carbapenemase producing gram negative bacilli

Out of 11 meropenem resistant GNB in our study, 81.8% (9/11) showed carbapenemase production which implied that carbapenemase production is a main mechanism of carbapenem resistance even though it could also be resulted by other mechanisms like production of β-lactamases together with porins loss [87].

In the present study, the overall prevalence of carbapenemase producing GNB was 5.4% (9/167) which was in agreement with other reports from Gondar 2.73% [88], Sudan 6% [89], Saudi Arabia 5.8% [90], and Jordan 2.8% [91].This indicated that carbapenemase production is emerging in different part of the world.

However, the prevalence of carbapenemase producing GNB in the present study was lower compared to the studies conducted in Addis Ababa 12.12% [92], Nigeria 12.4% [93], Nepal 20% [94] and Sudan 56% [44]. The difference might be due to method difference, the patient condition, poor infection control in health care settings, misuse and availability of non-prescribed antibiotics that could accelerate drugs resistant in those countries [87].

The present study revealed that, among carbapenemases produced 55.6% were Metallo β-lactamases (MBL) and 44.4% were serine carbapenemase. This was comparable with results from Nigeria; MBL 55.5% and serine 44.5% [93], Nepal MBL 55% and serine 45% [94]. This indicated that Metallo β-lactamases are more prevalent than serine carbapenemases in different parts of the world.

### 7.7. Antibiotics susceptibility profile of ESBLs & Carbapenemase producing gram negative bacilli

In our study, the highest susceptibility of ESBLs producing isolates was found against meropenem and piperacillin/tazobactam with sensitivity of 91.8% and 83.7% respectively. The result was in line with studies conducted in Ghana; meropenem 100% [95], India; meropenem 87.5% and piperacillin/tazobactam 89.28% [96], other study in North-west India by Saroj H. et *al*; meropenem 97%, Nepal; piperacillin/tazobactam 93.4% [97] and Czech Republic meropenem 98% [69].

The present study showed that ESBLs producer GNB had 100% resistance for ampicillin, cefalotin, cefazoline, cefuroxime, cefuroxime/Axetile, cefpodoxime, and ceftriaxone. Only ceftazidime and cefepime showed 2% activity from first to fourth generation cephalosporins tested in our study. Relatively similar resistance were reported from Harer; ceftriaxone 94.7%, ceftriaxone 94.7% [98], Ghana; ampicillin 100%, cefuroxime 100%, ceftazidime 90% [95], India; ampicillin 96%, ceftriaxone 94.9% [99], Nepal; ampicillin 100% [86], ceftazidime 100%, ceftriaxone 100% [71]. This indicated that drug resistance, particularly against cephalosporins among gram-negative bacteria is an important challenge which is further aggravated by the limited treatment options against these ESBLs producers [40].

The current study indicated that more than 90% of tested antibiotics had less than 25% activity against carbapenemase producer GNB. Among those a 100% resistance was recorded against ampicillin, amox/clavulanic acid, cefalotin, cefazoline, cefuroxime, cefuroxime/Axetile, cefpodoxime, ceftriaxone and meropenem. Furthermore, cefepime, cefoxitin, ciprofloxacin, levofloxacin and nitrofurantoin had 77.8% resistance each. Our report was comparable with other reports like in Gondar; ampicillin 100%, amox/clavulanic acid 100%, cefpodoxime 100% [88], Sudan; ceftriaxone 100%, amox/clavulanic acid 69% [89], Jordan; amox/clavulanic acid 100%, piperacillin/tazobactam 100%, cefepime 100% [91], Nepal; ampicillin 100%, cefalotin 100%, cefpodoxime 100%,ceftriaxone 100%, and meropenem 100% [94]. This indicated that MDR in gram negative bacteria become a major global health problem so that improvement in antibiotic stewardship and infection control policies are needed before it is too late [46, 50].

Carbapenemase producer GNB in our study showed maximum susceptibility for gentamycin 55.6% and tobramycin 33.3% from all tested antibiotics. Similar susceptibility for gentamycin was reported in a study conducted in Gondar 20% [88], Sudan 53% [89], Saudi Arabia 57.3% [90], and Nepal 16% [94]. This indicated how treatment options for carbapenemase producing GNB are limited and infections caused by these organisms may result high morbidity and mortality in the near future unless we continue searching for other highly effective novel antibiotics [48, 87].

# 8. Strength and Limitation of the study

## 8.1. Strength of the study

- VITEC 2 COMPACT automation for screening and CLSI recommended conventional methods for confirmation of ESBLs producing GNB were used.
- We have tried to characterize carbapenemase phenotypically to show the dominant type at the study site.
- AST for 20 antibiotics from different classes including β-lactams and β-lactamase inhibitors like piperacillin/tazobactam were tested.

## 8.2. Limitation of the study

- Unable to confirm carbapenemase production in *Acinetobacter* spp because the method we used did not recommend it.
- ESBLs and carbapenemase detection was only performed phenotypically.
- Since the study was conducted in a single laboratory, the results may not represent the entire city/country.

# 9. Conclusion and Recommendations

## 9.1. Conclusion

Our study demonstrated significant prevalence of GNB with ESBLs and carbapenemase production and high percentage of MDR isolates. The highest ESBLs production was found primarily from blood specimens. *E. coli* and *K. pneumoniae* were the most frequently isolated fermentative GNB while *P. aeruginosa* and *A. baumannii* from non-fermenters. The highest proportion of *K. pneumoniae* was ESBLs producer followed by *E. coli*. Carbapenemase production was detected from three species, *E. coli*, *K. pneumoniae* and *P. aeruginosa*. The production of those hydrolyzing enzymes made the treatment option narrower and difficult for that group of bacteria. Especially the production of carbapenemase made carbapenem drugs which are currently the last resort of antibiotics for life treating infections, ineffective. Meropenem was the most effective antibiotic against ESBLs producer MDR-GNB, while gentamicin was relatively active against carbapenemase producers.

## 9.2. Recommendations

- Increasing the number of microbiology laboratories which detect ESBLs and carbapenemase production is vital for proper and timely patient isolation.
- Proper isolation of admitted patients infected with ESBLs and carbapenemase producing GNB is required to reduce transmission to others.
- Supporting drug resistance surveillances and prospective studies on drug resistance is a key to select effective antibiotic for treatment of infections caused by ESBLs and carbapenemase producing gram negative bacteria.
- Molecular analysis is recommended for the presence of encoding genes of enzymatic production.

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# Annexes

## Annex I: Sample collection procedures

### ❖ Urine

Midstream urine (MSU) for microbiological examination is collected as follows:

- Label the container with the date, the name and number of the patient, and the time of collection.
- Give the patient a sterile, dry, wide-necked, leak proof container and request a 10–20 ml.

  ❖Important: Explain to the patient the need to collect the urine with as little contamination as possible, i.e. a clean-catch specimen.
- Wash the hands. Cleanse the area around the urethral opening with clean water, dry the area with a sterile gauze pad, and collect the urine with the labia held apart.
- As soon as possible, deliver the specimen with a request form to the laboratory. When immediate delivery to the laboratory is not possible, refrigerate the urine at 4–8 °C. When a delay in delivery of more than 2 hours is anticipated, add boric acid preservative.

### ❖ Pus

- Using a sterile technique aspirate or collect from a drainage tube up to 5 ml of pus. Transfer to a leak-proof sterile container. When pus is not being discharged, use a sterile cotton-wool swab to collect a sample from the infected site. Immerse the swab in a container of Amies transport medium.
- Label the specimen and as soon as possible deliver it with a completed request form to the laboratory.

❖ ***CSF***

- Take a sterile, dry, screw-capped container and label.
- Collect about 1 ml of CSF in container.
- Immediately deliver the samples with a request form to the laboratory.

❖ **Blood**

- Using a pressure cuff, locate a suitable vein in the arm. Deflate the cuff while disinfecting the puncture site.
- Wearing gloves thoroughly disinfect the puncture site using 70% ethanol alcohol.
- Lift back the tape or remove the protective cover from the top of the culture bottle(s). Wipe the top of the bottle using an alcohol swab.
- Using a sterile syringe and needle, withdraw about 20 ml of blood from an adult or about 2 ml from a young child.
- Insert the needle through the rubber liner of the bottle cap and dispense 10–12 ml of blood into the culture medium bottle containing 25 ml of broth.
- Using a fresh alcohol swab, wipe the top of each culture bottle and replace the tape or protective cover(s). Without delay, mix the blood with the broth.
- Clearly label each bottle with the name and number of the patient, and the date and time of collection.
- As soon as possible, incubate the inoculated media. Protect the cultures from direct sunlight until they are incubated.

## Annex II. Gram staining technique

**Method:**

1. After making a smear, leave the slide in a safe place for the smear to air-dry then fixed by heat, alcohol, or occasionally by other chemicals.
2. Cover the fixed smear with crystal violet stain for 30–60 seconds.
3. Rapidly wash off the stain with clean water. *Note*: When the tap water is not clean, use filtered water or clean boiled rainwater.
4. Tip off all the water, and cover the smear with Lugol's iodine for 30–60 seconds.
5. Wash off the iodine with clean water.
6. Decolorize rapidly (few seconds) with acetone–alcohol. Wash immediately with clean water.

   ***Caution***: Acetone–alcohol is highly flammable; therefore use it well away from an open flame.
7. Cover the smear with neutral red stain for 2 minutes.
8. Wash off the stain with clean water.
9. Wipe the back of the slide clean, and place it in a draining rack for the smear to air-dry.
10. Examine the smear microscopically, first with the 40x objective to check the staining and to see the distribution of material, and then with the oil immersion objective to report the bacteria and cells.

## Annex III: Media preparations

### 1. MacConkey agar

It is prepared from ready to use dehydrated powder purchased from suppliers. The medium contains Peptone, lactose, bile salts, sodium chloride, neutral red and agar.

**Procedure:**

1. Prepare as instructed by the manufacturer. Sterilize by autoclaving at 121 ºC for 15 minutes.

2. When the medium has cooled to 50–55 ºC, mix well and dispense aseptically in sterile petri dishes. Label the petri dishes with date of preparation, expiry date and a batch number.

3. Store the plates at 2–8 ºC preferably in plastic bags to prevent loss of moisture

- The pH of medium should be pH 7.2–7.6 at room temperature.

## 2. Blood Agar

**Composition of Blood Agar:**

- Pancreatic digest of casein
- Papaic digest of soy meal
- NaCl
- Agar
- Distilled water

**Procedure for the preparation of Blood Agar**

1. Prepare the blood agar base as instructed by the manufacturer. Sterilize by autoclaving at 121°C for 15 minutes.
2. Transfer thus prepared blood agar base to 45-50°C incubator.
3. When the agar base is cooled to (45-50°C), add appropriate amount of sterile blood aseptically and mix well gently. Avoid the formation of air bubbles. You must have warmed the blood to room temperature at the time of dispensing to molten agar base.
4. Dispense it to sterile petri plates aseptically
5. Label the medium with the date of preparation, expiry date and give it a batch number.
6. Store the plates at 2-8°C, preferably in sealed plastic bags to prevent loss of moisture.

### ✓ Quality control for Blood Agar medium

1. The pH of the blood agar range from 7.2 to 7.6 at room temperature.
2. Inoculate the plates with 5-hour broth cultures of *Streptococcus pyogenes* and *S. pneumoniae.* Inoculate also a plate with *H. influenzae* and streak with *S. aureus ( Satellitism Test).*
3. Incubate the plates in a carbon dioxide enriched atmosphere at 35-37°C overnight.

4. Check for the growth characteristics of each spp:
    - *S. pyogenes*: Beta-hemolysis
    - *S. pneumoniae*: Alpha-hemolysis
    - Satellitism *of H. influenzae*

## 3. Chocolate agar plate (CAP)

CAP is a medium that supports the special growth requirements needed for the isolation of fastidious organisms, such as *H. influenzae*, when incubated at 35-37°C in a 5% CO2 atmosphere.

### Procedure:

1. Heat-lyse a volume of horse or sheep blood that is 5% of the total volume of media being prepared very slowly to 56°C in a water bath.

2. Dispense 20 ml into 15x100 mm Petri dishes. Allow the media to solidify and condensation to dry.

3. Place the plates in sterile plastic bags and store at 4ºC until use.
4. As a sterility test, incubate un-inoculated plate for 48 hours at 35-37°C with ~5% $CO_2$ (or in a candle-jar).

## 4. Tryptic Soy Broth (TSB) with 20%Glycerol

It is used for long term storage of bacterial isolates. The preparation starts with weighing commercially available dehydrated medium as manufacturer instruction and dissolving it into distilled water. Then heat to homogenize, dispense in to appropriate container and autoclave at 15 psi at 121ºC for 20 minutes. Its contents are pancreatic digest of casein, Papaic digest of soya-bean meal, Sodium chloride, Di-potassium phosphate and Dextrose.

### Procedure:

1. Suspend 30 gram (as manufacturer instruction) of powder in 1 liter of distilled water.

2. Adjust pH 7.3+0.2.

3. Warm gently to dissolve.

4. Dispense and autoclave at 121°C for 15 minutes.

5. Then to make 20% glycerol TSB, mix 800ml TSB with 200ml glycerol.

## 5. Preparation of Mueller Hinton Agar (MHA)

It used for antibiotic susceptibility testing. To prepare MHA, dissolve the recommended amount of commercially available dehydrated medium in distilled water, heat to dissolve, and autoclave at 15 psi at 121ºC for 20 minutes. Its contents are Beef extract, Acid digest of casein, Starch.

## Procedure:

1. Weigh, dissolve by heat the medium as instructed by the manufacturer.

2. Adjust the pH of the medium to 7.2–7.4.

3. Autoclave at 121°C at 15 psi for 20 minutes. Do not over heat.

4. Pour into 150 mm or 100 mm diameter sterile petri dishes to a depth of 4 mm (about 25 ml per plate).

It should be dispensed into plates on a level surface so that the depth of the medium is uniform because too thin medium may give falsely large inhibition zones and too thick medium may give falsely small zones.

### Annex IV. Application of Antimicrobial Discs into MHA plates

Application the discs onto MHA plate should be done within 15 minutes of inoculation. The selected antimicrobial discs will be dispensed evenly using forceps/sterile needle/surgical blade. Each disc must be pressed down gently to ensure complete contact with the agar surface.

1. A maximum of 12 discs on a 150 mm plate or 5 discs on a 100 mm plate should be applied, keeping at least a distance of 24 mm between discs. Since some of the drugs could diffuse instantaneously, a disc should not be relocated once it has come in contact with the agar surface.

2. Place discs that give predictably small zones like aminoglycosides, next to those discs that give larger zones like cephalosporins.

3. Disc containers should be removed from the refrigerator or freezer one to two hours before use, so they may equilibrate to room temperature before opening. This procedure minimizes the amount of condensation that occurs when warm air contacts cold disks.

4. Incubation should be within 15 minutes after discs are applied, the plates will be inverted and incubated at 35º ± 2ºC in ambient air.

Each plate was examined after overnight incubation (16-18 hours), for confluent growth and circular zones of inhibition according to the recommendation of CLSI 2018.

## Annex V: VITEK 2 COMPACT system guidelines

**Principle:** Very small plastic reagent cards were designed to contain microliters of biochemical tests, selective growth media and different antibiotics for detection, identification and AST of organism.

**Procedure:** The VITEC 2 COMPACT inoculum is automatically introduced via a filling tube into a miniaturized plastic wells closed card containing specific concentration of antibiotic (ASTGN) and microliters of biochemical tests and selective growth media (GN72). Cards are incubated in a temperature controlled compartment. The antibiotic is rehydrated when the organism suspension is introduced in to the card during the automated filling process. Optical readings are performed every 15 minutes to measure the amount of light transmitted through each well including a growth control well. Algorithmic analysis of the growth kinetics in each well is performed by the system's soft wares to drive the MIC data. The MIC data is validated with the advanced expert system (AES) software a category interpretation is assigned and the organism antimicrobial patterns are reported.

**Component:**

- ✓ The VITEC 2 COMPACT instrument houses:- Sample processor and reader, Incubator,
- ✓ Computer work station: Allow data entry and analysis, storage, epidemiology reports,
- ✓ Smart carrier station : direct interface between personnel in the bench and the instrument,
- ✓ Barcode scanner: facilitate data entry.

## Annex VI: Data Extraction Format

**Code_____ Age_____ Sex_____ Isolated Spss._____**

**Specimen type_____**

**1. Culture and identification:**

Positive ☐ Negative ☐

**Isolated Bacteria** _____

| 2. **Antimicrobial susceptibility testing** | **S (mm)** | **I (mm)** | **R (mm)** |
|---|---|---|---|
| 1. Ampicillin | _____ | _____ | _____ |
| 2. Amoxicillin-clavulanic acid | _____ | _____ | _____ |
| 3. Piperacillin/tazobactam | _____ | _____ | _____ |
| 4. Cephalothin | _____ | _____ | _____ |
| 5. Cefazoline | _____ | _____ | _____ |
| 6. Cefuroxime | _____ | _____ | _____ |
| 7. Cefuroxime-Axetile | _____ | _____ | _____ |
| 8. Cefoxitin | _____ | _____ | _____ |
| 9. Cefpodoxime | _____ | _____ | _____ |
| 10. Ceftazidime | _____ | _____ | _____ |
| 11. Ceftriaxone | _____ | _____ | _____ |
| 12. Cefepime | _____ | _____ | _____ |
| 13. Gentamicin | _____ | _____ | _____ |
| 14. Tobramycin | _____ | _____ | _____ |
| 15. Ciprofloxacin | _____ | _____ | _____ |
| 16. Levofloxacin | _____ | _____ | _____ |
| 17. Tetracycline | _____ | _____ | _____ |
| 18. Nitrofurantoin | _____ | _____ | _____ |
| 19. Trimethoprim-sulfa. | _____ | _____ | _____ |
| 20. Meropenem (10µg) | _____ | _____ | _____ |

**3. ESBL production:** **Positive** ☐ **Negative** ☐

**4. Carbapenemase production** **Positive** ☐ **Negative** ☐

**COMMENTS:** _____

## Annex VII: Participant Information Sheet

### I. Participant Information Sheet (English version)

**Title of the project:** Multidrug resistance among fermentative and non-fermentative gram-negative bacilli isolated from clinical specimens at Arsho Advanced Medical Laboratory, Addis Ababa, Ethiopia.

**Principal Investigator:** Yasin Desalegn

**Department**: Diagnostic and Public Health Microbiology, College of Health Sciences, Addis Ababa University.

**Introduction:**

My name is Yasin Desalegn and I am Msc student in microbiology at Addis Ababa University, College of Health Sciences, department of Diagnostic and Public Health Microbiology. I am doing a research on multidrug resistance among fermentative and non-fermentative gram-negative bacilli isolated from clinical specimens at Arsho Advanced Medical Laboratory, Addis Ababa, Ethiopia.

**Purpose of the study:**

The purpose of this study is to determine prevalence of multidrug resistance among fermentative and non-fermentative gram-negative bacilli isolated from clinical specimens among patients attending Arsho Advanced Medical laboratory Addis Ababa, Ethiopia. In order to design treatment and preventive strategies, the explanation of the prevalence and antimicrobial resistance of these common bacteria is crucial and particularly since its prevalence in the study area is still remain poorly understood

**Procedure and Participation:** For this study to be successful we need your participation. And I am asking you to participate voluntarily in this study. If you are voluntary to participate in this study, you are expected to understand and sign the informed consent. Then you will give sample for laboratory analysis.

**Confidentiality:** All personal information you give and data obtained from laboratory analysis will be kept confidential.

**Expected benefits:** your participation in this study will benefit for the city and the nation as a whole. If there is any positive finding in laboratory examination the result will be reported to your physician for appropriate treatment and management.

**Risks:** there is no any risk for participating in this study except that you will spend a maximum of 30 minutes to read the consent form and you will give a small amount of sample for laboratory analysis.

**Incentives:** there are no special incentives that you will be given for participating in this research.

**Results Dissemination:**

There will be a report which is written about the finding of the study, either through publication or any other means. The result will not bear any information relevant to your personality in anyway.

**Freedom to withdraw:** You have the right to withdraw or leave the study.

**Person to Contact:**

If you have question or problem related with the present study, you can contact the principal investigator at any time using the following address:

**Principal Investigator:**

Mr. Yasin Desalegn
(Candidate of MSc, Diagnostic and Public Health Microbiology, College of Health Science, Addis Ababa University)
Cell phone: +251913804683
E-mail: desaleyasin89@gmail.com

**Advisor's Name and Address**: Dr. Adane Bitew (PhD, Associate Professor)

Diagnostic and Public Health Microbiology, School of Allied Health Science, College of Health Science, Addis Ababa University, Cell phone: +251911039162

Email: adane.bitew@aau.edu.et

**II. Participant information Sheet (Amharic version)**

**የጥናቱ ተሳታፊዎች የመረጃ ቅጽ**

የጥናቱ ርዕስ፡ በአዲስ አበባ ከተማ፣ በአርሾ የህክምና ላቦራቶሪ ከሚመጡት በሽተኞች ውስጥ የበሽታ አምጭ ባክቴሪያዎችን ስርጭት ማወቅ

**አጠቃሊይ መረጃ፡**

በጥናቱ በመሳተፍዎ ከልብ እያመሰገን ከመወሰንዎ በፊት፡- ይህንን ቅጽ በትክክል ያንብቡ ወይም ሲነበብልዎ በትክክል ያድምጡ፤ እንዱሁም ግልፅ ያልሆነለዎትን ነገር በሙሉ በነፃነት ይጠይቁ።

**መግቢያ፡**

ያሲን ደሳለኝ እባላለሁ፡፡ አዲስ አበባ ዩንቨርሲቲ የህክምና ማይክሮ ባዮሎጅ የ2ኛ ድግሪ ተማሪ ነኝ፡፡ በአዲስ አበባ ከተማ፣ በአርሾ የህክምና ላቦራቶሪ ከሚመጡት በሽተኞች ውስጥ የበሽታ አምጭ ባክቴሪያዎችን ስርጭት ለማወቅ የሚካሄድ ጥናት ነው፡፡

የጥናቱ አላማ፡ በአዲስ አበባ ከተማ፣ በአርሾ የህክምና ላቦራቶሪ ከሚመጡት በሽተኞች ውስጥ የበሽታ አምጭ ባክቴሪያዎችን ስርጭት ለማወቅ፣ በባክቴሪያው ምን ያህሉ ይያዛሉ መድሀኒቱ እንዳይሰራ የሚያደርጉ ኢንዛይሞች ስርጭት እና ፣መድሃኒት የተላመዱ ባክቴሪያ የተባሉትን ረቂቅ ህዋሳት ስርጭታቸውን ለማጥናትና ለህዋሳቱ ተመራጭ የሆኑትን መድሃኒቶች ለመምረጥ ነው፡፡

**ፈቃደኝነት፡**- እርስዎንና ሌሎችንም በጥናቱ በሙሉ ፍቃደኝነት እንዲሳተፉ እየጠየቅን በጥናቱ በመሳተፍ ፍቃደኛ ከሆኑ ለምርመራ ናሙና እንዲሰጡ ይጠየቃሉ።

**ሚስጥራዊነት፡**- የሚሰጡት መረጃ በጥናቱ ወቅትም ሆነ ከዛ በኃላ ባሉት ጊዜያት ሙሉ በሙሉ ሚስጥራዊነቱ የሚጠበቅና መረጃውም የሚያዘዉ በስም ሳይሆን በመለያ ቁጥር ይሆናል፡፡ በጥናቱ ላይ እያሉ በፈለጉት ጊዜ የማቆም ወይም የማቋረጥ መብት አለዎት፡፡

**የሚያገኙት ጥቅም፡**- በጥናቱ ለሚሳተፉ ፍቃደኛ ተሳታፊዎች ምንም አይነት የገንዘብ ክፍያ የለውም። ነገር ግን ዉጤታቸዉ ለሚከታተላቸዉ ሀኪም እንዲደርሰዉ ይደረጋል፡፡
የእርሰዎ በዚህ ጥናት ተሳታፊ መሆን ከተማው እንዲሁም ለሃገር ጠቀሜታ አለው፡፡

**በጥናቱ ተሳታፊዎች ላይ ያለው ጉዳት እና ተዛማጅ ችግር፡**- በዚህ ጥናት ላይ በመሳተፍዎ ሊደርስብዎ የሚችል ጉዳት የለም፡፡

**ውጤቱን ስለመጠቀም፡**- ከዚህ ጥናት በኋላ የበሽታውን ስርጭት በተመለከተ ሪፖርት ይፃፋል፡፡ ሆኖም የእርስዎን ማንነት የሚገልፅ መረጃ የማይካተት ሲሆን ችግሩን ለማሳወቅ ብቻ የሚውል ነው፡፡

**አድራሻ፡**

ማንኛውም ጥያቄ ወይም ጥርጣሬ ካለዎት ይህንን አድራሻ ይጠቀሙ፡

**የዋናው ተመራማሪ አድራሻ**


- ያሲን ደሳለኝ

  ( አዲስ አበባ ዩንቨርሲቲ፣የጤና ሳይንስ ኮላጅ፣ ማይክሮ ባዮሎጅና ትምህርት ክፍል፣ አዲስ አበባ፤ኢትዮጵያ)
- **ስልክ፡-** 09-13-80-46-83
- **ኢ-ሜይል:** desaleyasin89@gmail.com


**ዋና አማካሪ፡**


- **ዶ/ር አዳነ ቢተው**

  ( አዲስ አበባ ዩንቨርሲቲ፣የጤና ሳይንስ ኮላጅ፣ ማይክሮ ባዮሎጅና ትምህርት ክፍል፣ አዲስ አበባ፤ኢትዮጵያ)
- **ስልክ ፡** +251911039162
- **ኢ-ሜይል፡** adane.bitew@aau.edu.et

## Annex VIII: Informed Consent Form for Adults

**I. Informed Consent English version:**

**Title of the project**: **"**Multidrug resistance among fermentative and non-fermentative gram-negative bacilli isolated from clinical specimens at Arsho Advanced Medical Laboratory, Addis Ababa, Ethiopia"

I have been well aware of that this research undertaking is for a partial fulfilment of MSc degree which is fully supported and coordinated by Addis Ababa University, College of Health Sciences, department of Diagnostic and Public Health Microbiology; and the designate principal investigator is Yasin Desalegn. I have been fully informed in the language I understand about the research project objectives that are to determine magnitude of multidrug resistance, ESBLs and carbapenemase producing bacteria among patients attending Arsho Advanced Medical laboratory Addis Ababa, Ethiopia.

I have been informed that all the information I shall provide to the researcher will be kept confidential. I understood that the research do not has any risk and no composition. I also knew that I have the right to withhold information, skip questions to answer or to withdraw from the study any time I have acquainted nobody will impose me to explain the reason of withdrawal. It is also enlighten there would have no effect at all in my health benefit.

I have assured that the right to ask information that is not clear about the research before and or during the research work and to contact

**Principal Investigator's Name**: Yasin Desalegn

Cell phone: +251913804683

E-mail: desaleyasin89@gmail.com

**Advisor's Name and Address**: Dr. Adane Bitew (PhD, Associate Professor)

Diagnostic and Public Health Microbiology, School of Allied Health Science, College of Health Science, Addis Ababa University, Cell phone: +251911039162

Email: adane.bitew@aau.edu.et

I have read this form, or it has been read to me in the language I comprehend and understood the condition stated above, therefore, I am willing and confirm my participation by signing the consent.

Name of the participant _____

Agreed to participate in the study: Yes /No

Signature _____

Name of data collector _____

Signature _____

Date _____

## II. Informed Consent Form (Amharic version)

**የፈቃደኝነት መጠየቂያ ቅፅ፡-**

በአዲስ አበባ ከተማ፣ በአርሾ የህክምና ላቦራቶሪ ከሚመጡት በሽተኞች ውስጥ የበሽታ አምጭ ባክቴሪያዎችን ስርጭት ማወቅ በሚል ርዕስ ላይ ለማጥናት በተመለከተ በሚደረገዉ ጥናት ላይ ለመሳተፍ መሆኑ፤ የጥናቱ ዓላማና ጥቅም ተገልጾልኛል፡፡ የናሙናው ውጤት በሚስጥር እንደሚያዝ ተነግሮኛል፡፡ በተጨማሪም ጥናቱ ዉስጥ አለመሳተፍ መብቴ እደሆነና በማንኛዉም ጊዜ ከጥናቱ በራሴ ዉሳኔ መዉጣት እንደምችልና በዚህም ምክንያት ምንም አይነት መጉላላት እንደማይደርስብኝ በሚገባ ተረድቻለሁ፡፡ ስለሆነም ሁኔታዉን በሚገባ በማጤን በፈቃደኝነት በምርምሩ ላይ ለመሳተፍ ለተመራማሪዉ ፈቃደኝነቴን ሰጥቻለሁ፡፡ በተጨማሪም የምሰጠዉ ናሙና ለባክቴሪያዎች ምርመራ ብቻ እንደሚዉል ተነግሮኝ ተስማምቻለሁ፡፡ ማንኛውንም ያልገባኝን ነገር የመጠየቅ ዕድል ተሰጥቶኝ በሚገባኝ ቋንቋ መልስ አግኝቻለሁ፡፡

ይህን ሁለ በማገናዘብ ምርምሩ ላይ ስለኔ መረጃ እና ናሙና ለመስጠት ተስማምቻለሁ፣ ይህንንም በፊርማየ አረጋግጣለሁ፡፡

የተሳታፊው ስም፡ _____ ፊርማ _____ ቀን _____

መረጃውን ያስረዳው አካል _____ ፊርማ _____ ቀን _____

**Annex VIIII: English Versions of Consent form (for mothers/guardians)**

**Code number----------**

**Name of mother/guardian for child study subject----------**

I have been informed about the study which is aimed to assess **"**Multidrug resistance among fermentative and non-fermentative gram-negative bacilli isolated from clinical specimens at Arsho Advanced Medical Laboratory, Addis Ababa, Ethiopia" For this study sample will be collected from my child which is taken for the child's own diagnostics test and the left over sample will be taken for the research after the requested test is done. The aims of the study were well explained to me.

I am also informed that all the information contained within the questionnaire is to be kept confidential. Moreover I have been well informed of my right to keep hold of information, decline to cooperate and make my child withdraw from the study.

It is therefore with full understanding of the situation that I gave the informed consent voluntarily to the researcher to use the sample taken from my child for the investigation. In addition, I have had the opportunity to ask questions about it and received clarification to my satisfaction. I have also been informed that the benefit of participation is to get the results of analysis from my child sample measured for free via the counselor.

I_____ hereby give my consent for providing the requested information and specimens as the doctors find best for me.

Participant's mother/guardian signature /finger print ----------

Name of deponent ----------signature----------

(For mothers unable to read)

Name of counselor nurse --------signature----------

Date----------

**II: Amharic Versions of Consent form (for mothers/guardians)**

**የተሳታፊወች ስምምነት ማረጋገጫ ቅጽ**

የሚስጥር ቁጥር ––––––––––––––––––––

የተሳታፊዉ ልጅ እናት/ አሳዳጊ ስም ––––––––––––––––––––

እኔ ስሜ ከላይ የተጠቀሰው ተሳታፊ በአዲስ አበባ ከተማ፣ በአርሾ የህክምና ላቦራቶሪ ከሚመጡት በሽተኞች ውስጥ የበሽታ አምጭ ባክቴሪያዎችን ስርጭት ለማወቅ ናሙና ተወስዶ ስለሚሰራው ጥናት በቂ ገለጻ ተደርጎልኛል፡፡ ለጥናቱም ከልጆች የተወሰደ ናሙና ከተወሰደ የታዘዘለትን የላቦራቶሪ ምርመራ ተሰርቶ ሲያልቅ የተረፈዉ ናሙና እንደሚያስፈልግ ተገልጾልኛል፡፡ የጥናቱን አላማዎችም ተረድቻለሁ፡፡ በመጠይቁ ላይ የገለጽኳቸው መረጃዎች በሙሉ በሚስጥር የተጠበቁ እንደሚሆኑ ተነግሮኛል ፡፡ በጥናቱ ላይ ያለመሳተፍና ማንኛውንም መረጃ ያለመስጠት እንዲሁም በማንኛውም ጊዜ ከጥናቱ ራሴን የማግለል መብቴ የተጠበቀ እንደሆነ ተገልጾልኛል፡፡

ስለዚህ ለዚህ ጥናት መረጃና የስምምነት ቃሌን የሰጠሁት በአጠቃላይ ሁኔታውን በመረዳትና በፍጹም ፍቃደኝነት ነው፡፡ ናሙናው ለምርምር እንደሚውልም ተረድቻለው ፡፡በተጨማሪም ጥያቄ ለመጠየቅ ተፈቅዶልኝ ለማወቅ የፈለኩትን ያህል ማብራሪያ አግኝቻለሁ ፡፡የዚህ ጥናት ተሳታፊ በመሆኔ የማገኘው ጥቅም የሁሉንም ምርመራ ውጤት በነጻ ማግኘት እንደሆነ ተረድቻለሁ፡፡ በአጠቃላይ እኔ ከላይ በመተማመኛ ቅፅ የተጠቀሱትን ሁሉ በሚገባና በተረጋጋ መንፈስ አንብቤዋለሁኝ/ ተረድቻላዉ፡፡ ስለዚህ በዚህ ጥናት ለመሳተፍ ፈቃደኛ መሆኔን በፊርማዬ አረጋግጣለሁ፡፡

የተሳታፊዉ ወላጅ ወይም አሳዳጊ ፊርማ / የጣት አሻራ ––––––––––––––––––––

የምስክር ስም ––––––––––––––––––––––––––––––––––––––––– ፊርማ--––––

(የስምምነት ቅጹን ማንበብ ለማይችሉ ተሳታፊዎች)

የአማካሪ ነርስ ስም ––––––––––––––––––––––––––––––––––– ፊርማ ––––––––––

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## Annex X. 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.

**M.Sc. candidate: Yasin Desalegn (BSc)**

Signature: _____

Date of submission: _____

This thesis has been submitted with our approval as advisors.

| | |
|---|---|
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