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Phenotypic Antimicrobial Resistance Profiles of *Klebsiella pneumoniae* Isolated from Patients at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia

By: Tewachew Awoke (BSc, MSc Candidate)

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Principal Investigator:

Tewachew Awoke (BSc, MSc Candidate)

Advisors:

Dr. Tamrat Abebe (PhD, Assistant Professor)

Dr. Adane Mihret (DVM, PhD, Associate Professor)

Dr. Abraham Aseffa (MD, PhD, Senior Scientist)

Mr. Brhanu Teka (MSc, PhD Fellow)

Mr. Biruk Yeshitela (MSc, PhD Fellow)

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LIST OF ABBREVIATIONS/ACRONYMS

AAU	Addis Ababa University
AHRI	Armauer Hansen Research Institute
ALERT	All Africa Leprosy, Tuberculosis and Rehabilitation Training Centre
AST	Antimicrobial Susceptibility Test
ATCC	American Type Culture Collection
CDC	Centers for Disease Control and Prevention
CDT	Combined Disc Test
CLSI	Clinical Laboratory Standards Institute
DMIP	Department of Medical Microbiology, Immunology and Parasitology
DNA	Deoxyribonucleic Acid
ECDC	European Centers for Disease Control and Prevention
ESBL	Extended Spectrum β-lactamase
ESKAPE	*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, Enterobacter* species
HV	Hypervirulent
ICUs	Intensive Care Units
LPS	Lipopolysaccharide
mCIM	modified Carbapenem Inactivation Method
MDR	Multidrug Resistant
OPD	Outpatient Department
SPSS	Statistical Package for Social Sciences
TSAH	Tikur Anbessa Specialized Hospital
WHO	World Health Organization

ABSTRACT

Background: *Klebsiella pneumoniae* is a cause of mild to life threatening infections. The bacterium poses an urgent public health threat because it has the potential to become resistant to virtually all categories of antimicrobials available. Multidrug resistant *K. pneumoniae* has caused nosocomial outbreaks in different continents. Little is known concerning the antimicrobial resistance profiles of *K. pneumoniae* in Ethiopia.

Objective: The aim of this study was to determine the phenotypic antimicrobial resistance profiles of *K. pneumoniae* isolated from patients at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia.

Methodology: A cross-sectional study was conducted on antimicrobial resistance profiles of 132 *K. pneumoniae,* isolated from patients from September 2018 to February 2019. Identification of *K. pneumoniae* was done by examining gram stain, colony characterstics on MacConkey agar, 5% sheep blood agar and biochemical tests. Antimicrobial susceptibility testing was done by Kirby-Bauer disc diffusion technique. Extended spectrum β-lactamase (ESBL) and carbapenemase production was confirmed by combined disc test and modified carbapenemase inactivation method, respectively. Data was double entered using Epidata 3.1 and exported to SPSS version 25 software for analysis. Binary logistic regression and Chi-Square or Fisher's exact test were used to measure the association. P-value less than 0.05 was considered as statistically significant.

Results: Among the total *K. pneumoniae* isolates high frequency of resistance was observed to cefotaxime and ceftriaxone 128 (97%), trimethoprim-sulfamethoxazole 124 (93.9%) and cefepime 111 (84.1%). Most frequent susceptibility was observed to amikacin 123 (93.2%), imipenem 107 (81.1%), meropenem 96 (72.7%) and ertapenem 93 (70.5%). Majority 130 (98.5%) of the isolates were multidrug resistant (MDR). Two isolates showed complete non-susceptibility to all antimicrobial agents tested. The magnitude of ESBL and carbapenemase production was 77.3% and 21.2%, respectively. Of the total (n=30) non-ESBL producers, 73.3% were carbapenemase producers. Non-ESBL producers showed high resistance to imipenem (53.3%), meropenem (80%) and ertapenem (83.3%). Higher proportion of ESBL production was observed in those aged below 5 years than those 18 to 45 years and among those admitted to the intensive care units and pediatric wards than those in medical wards. Previous use of carbapenems was associated with carbapenemase production ($P<0.001$).

Conclusion: All *K. pneumoniae* isolates showed appreciably high frequency of resistance to most of the tested antimicrobials. The magnitude of ESBL and carbapenemase production as well as MDR *K. pneumoniae* was very alarming in the study area. Of particular concern is that the majority of non-ESBL producers were carbapenemase producers. Therefore, strengthening antimicrobial stewardship programs together with effective infection control and antimicrobial surveillance practices is strongly recommended in Tikur Anbessa Specialized Hospital.

Key words: *Klebsiella pneumoniae,* extended spectrum β-lactamase, carbapenemase, multidrug resistance

1. INTRODUCTION

1.1. Background

Klebsiella pneumoniae is a Gram-negative, rod shaped bacteria that belongs to the family *Enterobacteriaceae*. The bacterium is primarily opportunistic pathogen that attacks immune-compromised individuals who are hospitalized and suffer from severe underlying diseases. In these patients it results in hospital-acquired urinary tract infections, blood stream infections, wound infections and respiratory tract infections (Podschun and Ullmann, 1998). In addition, *K. pneumoniae* has emerged as a cause of severe community acquired infections such as community acquired pneumonia, pyogenic liver abscess, metastatic infections such as meningitis (Keynan and Rubinstein, 2007;De Jesus *et al.*, 2015). It accounts for about one third of all Gram-negative infections overall (Navon-Venezia *et al.*, 2017).

K. pneumoniae is intrinsically resistant to penicillins such as ampicillin (Magiorakos *et al.*, 2012) but aminoglycosides, fluoroquinolones and third generation cephalosporins such as ceftriaxone had been commonly used antimicrobials to treat infection caused by *K. pneumoniae* in hospitals around the world especially in many African hospitals (Henson *et al.*, 2017). However, the extensive use of these and other antimicrobial compounds both in hospitalized patients and in agriculture has led to the development of multidrug-resistance (MDR) (Levy and Marshall, 2004). Nowadays, *K. pneumoniae* strains are recognized as urgent threat to human health, because of the emergence of MDR strains associated with hospital outbreaks and Hypervirulent (HV) strains associated with severe community-acquired infections (Holt *et al.*, 2015). Resistance rates in *K. pneumoniae* steadily increase over the years and the bacterium is becoming resistant to virtually all aminoglycosides, quinolones and β-lactams (Pistella and Santini, 2016).

K. pneumoniae acquires resistance against existing antimicrobials by multiple mechanisms resulting in increased MDR of *K. pneumoniae.* These mechanisms include the acquisition of novel antibiotic-inactivating enzymes, modification of antibiotic target sites, change in membrane permeability, activation of efflux pump system and alteration of metabolic pathways (De Jesus *et al.*, 2015).

There are many enzymes produced by *K. pneumoniae* including extended-spectrum β-lactamases (ESBLs), metallo-β-lactamases, oxacillinases, and carbapenemases which can degrade newer β-lactam antibiotics and worsen its challenge in public health (Navon-Venezia *et al.*, 2017). Additionally, aminoglycoside modifying enzymes and 16S rRNA methylase makes aminoglycoside non-functional (Magalhães and Blanchard, 2009).

Moreover, under the widespread use of antimicrobials which provides a selective pressure, resistance is disseminated due to clonal expansion of antibiotic-resistant clones and transmissibility of resistance determinants mediated by plasmids, transposons and gene cassettes in integrons (Lee *et al.*, 2016). While *K. pneumoniae* has many plasmids that harbor most of antibiotic resistant genes including ESBLs and carbapenemases, it serves as a worldwide source for inter- and intra-species spread of multidrug resistance via horizontal gene transfer (Navon-Venezia *et al.*, 2017;Wyres and Holt, 2018). The bacteria caused hospital outbreaks in different countries, requiring early detection of carbapenemase and ESBLs in infected patients and/or carriers to prevent development of outbreaks (Nordmann *et al.*, 2011).

Generally, *K. pneumoniae* has been observed to develop resistance to antimicrobials more easily than most bacteria. As new resistance mechanisms develop, fewer and fewer treatments are available for infections by MDR *K. pneumoniae* (Navon-Venezia *et al.*, 2017). Although some treatments still remain, they have more side effects and not accessible in developing countries (WHO, 2014), thus the best option is to control the development and spread of antimicrobial resistance. It is obvious that, to better understand the extent of drug resistance in different settings and design better control strategies research in the area is crucial. Therefore, this study was planned to determine the phenotypic antimicrobial resistance profiles of *K. pneumoniae* at Tikur Anbessa Specialized Hospital (TASH), Addis Ababa, Ethiopia.

1.2. Statement of the problem

Antimicrobial resistance is a growing problem in modern healthcare around the world. In the United States alone, the Center for Disease Control and Prevention (CDC) estimated that more than 2 million people contract antibiotic-resistant infections every year, resulting in more than 23, 000 deaths (CDC, 2013). It is estimated that antimicrobial resistance related deaths each year will raise from currently 700,000 to 10 million and costs 100 trillion dollar to global economic output by 2050 if containment of antimicrobial resistance at a global level is not effectively implemented (O'Neill, 2018).

MDR bacterial infections that pose a serious risk to patients are increasing worldwide (Levy and Marshall, 2004;WHO, 2014). One of the most common species of bacteria that cause problems in healthcare today is *K. pneumoniae* (Holt *et al.*, 2015), which together with other highly important MDR pathogens, comprises the ESKAPE group that stands for *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter* species to emphasis these pathogens effectively "escape" the effects of antibacterial drugs (Pendleton *et al.*, 2013).

K. pneumoniae has gained attention worldwide especially in developed countries due to its high drug resistance. Its contribution to the antimicrobial resistance crisis is impossible to quantify, it is unique amongst the Gram-negative ESKAPE pathogens because of its high diversity of acquired antimicrobial resistance genes, high plasmid load, significantly more varied DNA composition reflecting diverse horizontal gene transfer partners and broad ecological range (Navon-Venezia *et al.*, 2017;Wyres and Holt, 2018). Continuous global dissemination of multidrug resistance and extremely drug resistance *K. pneumoniae* with superior ability to cause multi-continent outbreaks has been noted (Holt *et al.*, 2015;Navon-Venezia *et al.*, 2017). However, to the best of our knowledge, studies emphasizing the degree of drug resistance in *K. pneumoniae* in Africa particularly in Ethiopia are still limited. Moreover, information on ESBLs and carbapenemase producing bacteria is still scarce in Ethiopia not only in *K. pneumoniae* but also in other Gram-negative bacteria.

The rapid increase in the frequency of drug resistance among *K. pneumoniae* isolates, especially the frequency of ESBL- and carbapenemases producing strains (Nordmann *et al.*, 2011;WHO, 2014;Padmini *et al.*, 2017), requires close monitoring of susceptibility patterns of these bacteria among patients in tertiary care facilities such as Tikur Anbessa Specialized Hospital. Therefore, the current study aimed to describe the resistance patterns using wide range of antimicrobial as well as to determine the magnitude of ESBL production, carbapenemase production and multidrug resistance in *K. pneumoniae*. The findings will lead to actions that will save many lives through evidence based interventions and serve as recent information to monitor further progress in infection control and antimicrobial stewardship programs in the hospital.

1.3. Significance of the study

The study had significance in the better management of study participants infected with ESBL and carbapenemase producing *K. pneumoniae* isolates. While resistance spectrum varies from country to country even in different regions, examining the local resistance pattern is essential to illustrate antimicrobials that should be used for treatment of infections caused by microorganisms in that setting. Thus, our study examined the resistance spectrum of *K. pneumoniae* with emphasis on determining appropriate antimicrobials to be used for treatment of infections caused by *K. pneumoniae* in the study area.

Knowing the magnitude of ESBL and carbapenemase production as well as multidrug resistance rate in bacteria like *K. pneumoniae* has an important role in controlling the spread of resistance by designing specific control strategies (Nordmann *et al.*, 2011). The current study will serve as an input to policy makers and hospital managers in planning, implementing and evaluating various interventions to reduce the spread of resistance.

Additionally, the study enabled us to compare with similar studies done in other parts of the world, which have different access to antimicrobials. Furthermore, the finding of this study will be used as recent information for those who need to conduct further investigation in the area.

2. LITRATURE REVIEW

2.1. Microbiology and characteristics of *K. pneumoniae*

Friedlander, in 1882, described an encapsulated bacillus isolated from the lungs of patients who died of pneumonia. It was initially named Friedlander's bacillus but was changed to *Klebsiella pneumoniae* later (Shon *et al.*, 2013). *K. pneumoniae* is a Gram-negative, rod shaped bacteria that belongs to the family *Enterobacteriaceae*. It is a close relative of many familiar genera, such as *Citrobacter*, *Escherichia*, *Enterobacter* and *Serratia* collectively refereed as coliforms. Unlike most other members of the *Enterobacteriaceae* family, it is non motile and has polysaccharide capsule which gives moist and mucoid colony characteristics on artificial media (De Jesus *et al.*, 2015). Therefore, *K. pneumoniae* should be suspected when large colonies with a mucoid consistency are recovered on primary isolation plates such as MacConkey agar. The colonies on MacConkey agar typically appear large, mucoid, and pink, indicating fermentation of lactose. *K. pneumoniae* produces indole from tryptophan and hydrolyze urea slowly, producing a light pink color in the slant of Christensen's urea agar (Procop *et al.*, 2017).

K. pneumoniae is a frequently encountered hospital-acquired opportunistic pathogen that typically infects immunocompromised individuals (Podschun and Ullmann, 1998;Wu and Li, 2015). Over the last two decades, there has been a concerning rise in the acquisition of resistance to a wide range of antimicrobials by *K. pneumoniae (*Navon-Venezia et al., 2017). As a consequence of this antibiotic resistance, simple infections such as urinary tract infections (UTIs) have become recalcitrant to treatment, and more serious infections such as pneumonias and bacteremia have become increasingly life-threatening (Paczosa and Mecsas, 2016).

Since the 1980s, strains of *K. pneumoniae* that can cause serious infections in healthy individuals have also gained traction in the human population. These strains are considered HV compared to classical *K. pneumoniae* strains due to their ability to infect both healthy and immunocompromised individuals and because of the increased tendency of these infections to be invasive. This additional virulence correlates with the acquisition of a 200 to 220kb plasmid containing genes that enhance capsule production as well as encode siderophores (Paczosa and Mecsas, 2016). Because of the higher production of capsule, the colonies grown on an agar plate appear hypermucoviscous (Shon *et al.*, 2013).

The carriage and expression of drug resistance do not enhance the virulence of *K. pneumoniae* strains, despite making them more difficult to treat (Paczosa and Mecsas, 2016). However, its flexible accessory genome resulted, the emergence of strains with both antibiotic-resistant and HV, leading to untreatable severe disease (Shon *et al.*, 2013).

2.2. Epidemiology of *K. pneumoniae*

K. pneumoniae is ubiquitous in nature (Podschun and Ullmann, 1998). Classical strains of *K. pneumoniae* typically cause nosocomial infections and can be found worldwide. In contrast to the majority of infections caused by classical *K. pneumoniae* strains, many HV *K. pneumoniae* infections originate in the community (Paczosa and Mecsas, 2016). Although HV strains initially dominated in the Asian Pacific Rim (e.g., Taiwan, Korea, Vietnam and Japan), an increasing number of cases are being reported from other continents (Shon *et al.*, 2013).

In Ethiopia *K. pneumoniae* is one of the frequently isolated bacterium in studies on Gram-negative bacteria. For instance, Ethiopian annual antimicrobial surveillance report showed that *K. pneumoniae* was the second most common pathogen isolated from urine and pus (Ethiopian Public Health Institute, 2018). A recent study in Addis Ababa on *Enterobacteriaceae* clinical isolates noted *K. pneumoniae* as the second most frequent bacteria (Teklu *et al.*, 2019). According to Desta *et al*. (2016), on gastrointestinal colonization of ESBL-producing *Enterobacteriaceae* among admitted patients at Tikur Anbessa Specialized Hospital, Addis Ababa, *K. pneumoniae* was the second most frequently isolated bacteria next to *E. coli*. A study in Bahir Dar reported *K. pneumoniae* as the most common pathogen accounting for 52.4% of all Gram-negatives isolated from different clinical specimens (Moges *et al.*, 2019). In a study from Gondar *K. pneumoniae* was the second most common *Enterobacteriaceae* causing urinary tract infection (Eshetie *et al.*, 2015).

2.3. Habitat, risk factors and transmission of *K. pneumoniae*

The bacterium is a member of the normal microbiota which is found in the mucosal surfaces of mammals. In humans, *K. pneumoniae* is present as a saprophyte in the nasopharynx and in the intestinal tract as a resident flora and also in the skin as a transient colonizer (De Jesus *et al.*, 2015). Furthermore, the bacteria can occur as free-living in the soil, water, and on plants as well as in various hospital environment sites such as medical equipment, sink drains and cleaning equipment, where their source is believed be contamination (Podschun and Ullmann, 1998).

People with underlying forms of immunodeficiency are at a much greater risk for infections with classical *K. pneumoniae* strains including the antimicrobial resistance ones particularly in the health care settings than the general population. These immunodeficiency conditions include malignancy, diabetes, solid organ transplantation, treatment with corticosteroids, chronic liver disease and renal failure. Particularly, patients who undergo procedures with reused scopes or with inserted medical devices have an open highway for *K. pneumoniae* entry (Paczosa and Mecsas, 2016).

Neonates, elderly and those in the intensive care unit (ICU) have more risk of infection with *K. pneumoniae* due to the fact that their immunity is not well developed and/or compromised (Paczosa and Mecsas, 2016). During travels, acquisition can occur in the absence of healthcare contact or along with leisure and medical tourism (De Jesus *et al.*, 2015). For instance, travel to the Asian Pacific Rim (e.g., Taiwan, Korea, Vietnam and Japan) or exposure to people from that area, where HV strains were established is a risk for HV *K. pneumoniae* infection (Shon *et al.*, 2013). Moreover, previous antimicrobial therapy particularly with broad spectrum antimicrobials can lead to acquisition of *K. pneumoniae* including multidrug resistant isolates (Podschun and Ullmann, 1998).

The spread of nosocomial infections including the resistance ones occur from patient to patient, healthcare workers to patients and vice versa as well as through contaminated hospital environment and equipment (De Jesus *et al.*, 2015). Most importantly, *K. pneumoniae* liver abscess arise from the endogenous spread of the bacteria from the gastrointestinal tract (Paczosa and Mecsas, 2016). The other way of transmission of *K. pneumoniae*, including the resistant ones especially among hospitals in developing world, is through cockroach infestation, which complicates nosocomial infections (Donkor, 2019**)**.

2.4. Virulence factors, pathogenesis and clinical manifestation of *K. pneumoniae*

Presumably acquisition resulting in colonization is the first step necessary for subsequent endogenous *K. pneumoniae* infection. Entry into an extra-intestinal organ or site is the next critical step in pathogenesis. Mechanisms for *K. pneumoniae* entry include ascension into the bladder from the perineum, disruption of the bowel enabling entry of gastrointestinal tract colonizers into the peritoneal cavity, aspiration of oropharyngeal colonizers into the respiratory tract or disruption of the skin barrier (Shon *et al.*, 2013).

The following virulence factors facilitate the adhesion and further infection by *K. pneumoniae*. The bacteria express numerous adherence factors such as fimbriae capable of recognizing varied receptors, thus facilitating the binding of the bacteria to several target cells and invasion of host tissue (Shon *et al.*, 2013;De Jesus *et al.*, 2015). The expression of the various fimbriae could have disadvantage to the bacterium due to the heightened host immune response that may be triggered, thus outlying the opportunistic nature of *K. pneumoniae* (De Jesus *et al.*, 2015). The presence of a thick capsule at cell surface protects *K. pneumoniae* from opsonization and phagocytosis (Podschun and Ullmann, 1998;Wu and Li, 2015;Paczosa and Mecsas, 2016). In addition, capsules inhibit dendritic cell maturation (Li *et al.*, 2014), possibly have a neutralizing effect against antibodies through the release of excessive capsular material and can play an important role outside the human host by offering some protection against desiccation in the environment (De Jesus *et al.*, 2015).

Lipopolysaccharide (LPS) is the primary means of protection against complement-mediated killing. Lack of components of LPS has observed making *K. pneumoniae* incapable of causing urinary tract infections, pneumonia, and sepsis (Paczosa and Mecsas, 2016). Outer membrane proteins such as OmpK36 contribute to not only resistance to antimicrobials but also to phagocytosis by host cells (Li *et al.*, 2014). Cellular components such as siderophores can contribute to virulence via chelating mechanisms that remove iron from host iron-binding proteins. In addition, urease, a nickel-containing enzyme, hydrolyses urea to ammonia and carbamate, thereby exacerbating urinary tract infection. Biofilms, which may assist *K. pneumoniae* in weathering harsh host environments, are often found in the respiratory or urinary tracts of chronically infected patients (Wu and Li, 2015).

Classical strains of *K. pneumoniae* cause serious infections, such as pneumonia, bacteremia, or meningitis, when infecting immunocompromised individuals (Paczosa and Mecsas, 2016). Moreover, infections involving the urinary tract, abdominal cavity, surgical sites, and soft tissues are the most common clinical manifestations (Shon *et al.*, 2013). However, HV *K. pneumoniae* can affect healthy persons, causing life-threatening infections, such as pyogenic liver abscess, meningitis, necrotizing fasciitis, endophthalmitis and severe pneumonia. Most of these infections are resulted from metastatic spread of the bacteria from liver to other organs which is a characteristic of HV *K. pneumoniae* and is uncommon for enteric Gram-negative bacilli in the presence of host immune defense (Li *et al.*, 2014).

2.5. Treatment of infections caused by *K. pneumoniae*

The four major antimicrobial categories for treating *K. pneumoniae* infections are cephalosporins, carbapenems, aminoglycosides and quinolones (Navon-Venezia *et al.*, 2017). Other antimicrobials in categories such as β-lactam/β-lactamase inhibitors, cephamycins, monobactams and folate pathway inhibitors can be used for treatment of infections caused by *Enterobacteriaceae* including *K. pneumoniae* (Rodriguez-Bano *et al.*, 2018). However, resistance of *K. pneumoniae* to these important categories is becoming common. For instance, at the Europe level, more than one third (34.1%) of *K. pneumoniae* isolates reported to European antimicrobial resistance surveillance network for 2017 were resistant to at least one of the antimicrobial groups under regular surveillance, i.e. fluoroquinolones, third-generation cephalosporins, aminoglycosides and carbapenems (ECDC, 2017).

2.6. Antimicrobials mechanism of action and resistance

There are five major modes of antimicrobial mechanisms of activity. These are interference with cell wall synthesis, inhibition of protein synthesis, interference with nucleic acid synthesis, inhibition of a metabolic pathway and disorganizing of the cell membrane permeability (Levy and Marshall, 2004;Shaikh *et al.*, 2015). Resistance mechanisms of *K. pneumoniae* against different classes of antimicrobials include release of antibiotic-inactivating enzymes, modification of antibiotic target sites, change in membrane permeability, activation of efflux pump systems, and alteration of metabolic pathways (Kim *et al.*, 2016). The detailed mechanism of action of and resistance to each major antimicrobial category used for the treatment of infections caused by *K. pneumoniae* is described below.

2.6.1. Aminoglycosides

Aminoglycosides are routinely used in clinical settings as antimicrobial agents for the treatment of infections due to *K. pneumoniae* (Garneau-Tsodikova and Labby, 2016). The antimicrobial agents in the aminoglycosides category include but not limited to gentamicin, tobramycin, amikacin and netilmicin (Magiorakos *et al.*, 2012;CLSI, 2018). The antibacterial action of aminoglycosides is inhibition of protein synthesis by binding to the bacterial ribosome (Magalhães and Blanchard, 2009;Garneau-Tsodikova and Labby, 2016).

Mechanism of bacterial resistance to aminoglycosides includes efflux pumps, mutations in the ribosomal target, inactivation of the drug by modifying enzymes and ribosomal modification. The most common and widespread mechanism of resistance is inactivation of aminoglycoside by modifying enzymes, causing them to bind poorly to ribosomes allowing the bacteria to survive (Magalhães and Blanchard, 2009;Garneau-Tsodikova and Labby, 2016). This includes acetylation of the drug via aminoglycoside acetyltransferase, adenylation via aminoglycoside nucleotdyltransferase or phosphorylation via aminoglycoside phosphotransferase (Vetting *et al.*, 2003). However, any one of these enzymes alone cannot confer resistance to all aminoglycosides because of their narrower substrate specificities. Conversely, when greater than one of these enzymes are produced in single organisms, they could readily become resistant to multiple aminoglycosides (Magalhães and Blanchard, 2009).

Recently, dissemination of resistance caused by ribosomal modification through methylation is becoming a growing concern as the genes are located on mobile genetic elements such as plasmids (Garneau-Tsodikova and Labby, 2016). These ribosomal modifying enzymes confer high-level resistance against most clinically important aminoglycosides (Magalhães and Blanchard, 2009). They can occur concomitantly with other resistance genes (Li *et al.*, 2012;El Salabi *et al.*, 2013;Padmini *et al.*, 2017).

2.6.2. Quinolones

Quinolones are synthetic drugs active against both Gram-negative and Gram-positive bacteria. This class of antimicrobials is important in human and veterinary medicine and used for treatment of various bacterial infections (Sárközy, 2001). The clinically available quinolones are classified into several generations based on their spectrum of activity and date of their marketing.

Nalidixic acid is a first generation quinolones. Second generation quinolones, including ciprofloxacin which contain a fluorine atom, due to which they are named as fluoroquinolones. There are also new generation quinolones such as levofloxacin and trovafloxacin (Cattoir and Nordmann, 2009). Ciprofloxacin and levofloxacin are recommended drugs for treatment of infections caused by *Enterobacteriaceae* according to CLSI (2018). They are the only classes that inhibit bacterial DNA synthesis by interfering with the action of two essential bacterial DNA topoisomerase II enzymes namely DNA gyrase and topoisomerase IV (Sárközy, 2001).

The most common cause of quinolone resistance in *Enterobacteriaceae* including *K. pneumoniae* is due to chromosomal encoded point mutations in the quinolone resistance-determining regions of the DNA gyrase and topoisomerase IV genes. Additional resistance mechanism is decreased intracellular drug accumulation by over expression of chromosomally encoded multidrug efflux pumps or decreased penetration of fluoroquinolones through diminishing expression of outer membrane porins (Cattoir and Nordmann, 2009). Apart from chromosomally encoded quinolone resistance, plasmid mediated resistance that was first found in strains of *K. pneumoniae* in United States in 1998 (Martínez-Martínez *et al.*, 1998) has gained more attention recently. Plasmid mediated resistance genes confer resistance through protection of DNA gyrase and type IV topoisomerase from quinolone inhibition, acetylation of fluoroquinolones and extruding fluoroquinolones via efflux pump. The genes are often carried by mobile genetic elements with other resistance determinants especially ESBL and carbapenemases (Cattoir and Nordmann, 2009).

2.6.3. Cephalosporins

Cephalosporins are divided to 1st, 2nd, 3rd, 4^{th} and 5th generations. Third generation cephalosporins which includes cefotaxime, ceftazidime, and ceftriaxone are among the most widely used subclass of antimicrobials (El Salabi *et al.*, 2013). Cephalosporins together with penicillins, monobactams and carbapenems that share common beta-lactam ring are commonly known as beta-lactams. They exhibit bactericidal activity by binding to and inactivating the penicillin-binding proteins which are essential for bacterial cell wall formation (Meletis, 2016).

Resistance to β-lactam antimicrobial agents in Gram-negative bacilli is primarily mediated by β-lactamases. The persistent exposure of bacterial strains to a multitude of β-lactams has induced dynamic and continuous production and mutation of β-lactamases in these bacteria, expanding their activity even against the newly developed β-lactam antibiotics. These enzymes are known as ESBLs (Shaikh *et al.*, 2015). ESBLs are β-lactamases capable of hydrolyzing penicillins, first, second, and third-generation cephalosporins, and aztreonam (but not the cephamycins or carbapenems) and inhibited by β-lactamase inhibitors such as clavulanic acid (Harada *et al.*, 2008).

Antibiotic choices for infections caused by ESBL-producing organisms are limited. β-lactam/ β-lactamase inhibitor combinations can be used for low to moderate severity infections. Carbapenems have the most consistent activity against ESBL-producing organisms (Tamma and Rodriguez-Baňo, 2017).

2.6.4. Carbapenems

Carbapenems have a carbapenem together with the beta-lactam ring which makes them more stable against most β-lactamaseses (Meletis, 2016). They are the most effective against Gram-positive and Gram-negatives. According to CLSI (2018), meropenem, imipenem, ertapenem and doripenem are recommended treatments for infections caused by *Enterobacteriaceae*. Their effectiveness, stability and fewer adverse effects compared to other last-line drugs such as polymyxins makes them most reliable last-resort treatments for bacterial infections (Meletis, 2016). They are widely considered as the drugs of choice for the treatment of severe infections caused by ESBL-producing *Enterobacteriaceae* (Harada *et al.*, 2008). However, in recent years, carbapenem resistance *Enterobacteriaceae* particularly *K. pneumoniae* is rising alarmingly (Logan and Weinstein, 2017;Pitout *et al.*, 2015).

Resistance against carbapenems is mainly through carbapenemase enzyme production**.** Production of other enzymes which have weak carbapenemase activity such as ESBLs and AmpC β-lactamases together with porin alteration, drug efflux pumps and alterations in penicillin-binding proteins are also mentioned as additional resistance mechanisms (Logan and Weinstein, 2017).

Carbapenemase-producing Gram-negatives in particular are resistant to all or almost all beta-lactams, fluoroquinolones and/or aminoglycosides concomitantly. Therefore, older agents, such as polymyxins and fosfomycin, which were rarely implemented in the past because of efficacy and toxicity concerns, together with the newer tigecycline, have become last-line choices (Meletis, 2016). However, these drugs are not only less effective, more toxic, and widely unavailable (WHO, 2014) but also recent resistance reports are on rise (Lee *et al.*, 2016), intensifying the magnitude of the problem. Therefore, the world seems in the cusp of post antibiotic era left with very few treatment options and relying on prevention and control strategies.

2.6.1. Other categories of antimicrobials

β-lactam/β-lactamase inhibitors such as amoxicillin/clavulanate, ticarcillin/clavulanate, piperacillin/tazobactam, ampicillin/sulbactam, and cefoperazone/sulbactam can be used for treatment of infections caused by *Enterobacteriaceae* (CLSI, 2018;Rodriguez-Bano *et al.*, 2018). For instance, piperacillin/tazobactam is a parenteral, broad spectrum drug combination used in serious infections such as nosocomial pneumonia caused by *Enterobacteriaceae* including *K. pneumoniae* (Padmini *et al.*, 2017). These β-lactamase inhibitors can bind with β-lactamase then inactivate the enzymes irreversibly. Nonetheless, β-lactamase hyper production and coproduction of plasmid-mediated AmpC enzymes can affect inhibitor activity. Moreover, consumption of all β-lactam/β-lactamase inhibitors was also found to be significantly correlated with the rate of resistance to piperacillin/tazobactam (Ryu *et al.*, 2018).

Folate pathway inhibitors like trimethoprim-sulfamethoxazole, monobactams such as aztreonam and cephamycins such as cefoxitin and cefotetan can be used to treat infections due to *Enterobacteriaceae* (Rodriguez-Bano *et al.*, 2018). The action of trimethoprim-sulfamethoxazole is through inhibition of folate pathway synthesis and resistance is through alteration of target. The mechanism of action of cephamycins and mononbactams is cell well synthesis inhibition like cephalosporins (Shaikh *et al.*, 2015). Resistance to cephamycins results from loss of an outer membrane porin protein or production of AmpC β-lactamses (Tamma and Rodriguez-Baño, 2017). Hydrolysis of mononbactams by isolates harboring ESBLs results in resistance (Shaikh *et al.*, 2015).

2.7. Prevalence of antimicrobial resistance in *K. pneumoniae*

Klebsiella pneumoniae is a nosocomial pathogen commonly isolated from the intensive care unit and implicated in hospital outbreaks, with increasingly displaying high drug resistance profiles through β-lactamase production and carbapenem resistance worldwide (Karaiskos and Giamarellou, 2014;Holt *et al.*, 2015).

In Europe and America antimicrobial resistance in *K. pneumoniae* was reported in different studies as well as in surveillance reports. For instance, according to the 2017 European antimicrobial resistance surveillance network, in the continent the rate of resistance to several antimicrobial groups concomitantly was frequent, and ESBL production was common among *K. pneumoniae* and *E. coli*. More than one third (34.1%) of *K. pneumoniae* isolates reported to surveillance network were resistant to at least one of the antimicrobial groups under regular surveillance, i.e. fluoroquinolones, third-generation cephalosporins, aminoglycosides and carbapenems. Resistance percentages were generally higher in *K. pneumoniae* than in *E. coli* (ECDC, 2017). A one-year survey of carbapenemase producing *K. pneumoniae* in Italy confirmed 17.5% of *K. pneumoniae* in nosocomial infections were carbapenemase producers (Corcione *et al.*, 2015).

A ten-year (2005 to 2014) surveillance study of carbapenemase producing *K. pneumoniae* in a Greece reported that 48 % of *K. pneumoniae* were carbapenemase producers. Carbapenemase producers showed high resistance rates (>85 %) to all beta-lactams, amikacin, ciprofloxacin and trimethoprim-sulfamethoxazole (Spyropoulou *et al.*, 2016). A study from Brazil observed high resistance rates of ESBL-producing *K. pneumoniae* nosocomial strains to ceftriaxone (100%), ceftazidime (66.7%), cefepime (47.2%), gentamicin (72.2%), and ciprofloxacin (58.3). However, all isolates were susceptible to amikacin and meropenem (Rocha *et al.*, 2019).

Scholars in Asia also reported antimicrobial resistance profiles of *K. pneumoniae.* Sheemar *et al*. (2016), from India studied a total of 200 *K. pneumoniae* isolates, 58% were ESBL producers. ESBL producing isolates were significantly more resistant to other group of antimicrobials as compared to non-ESBL producers. Carbapenem resistance was observed in 11.21% of ESBL producers and resistance was common to both meropenem and imipenem. According to a study in Bangladesh, resistance of *K. pneumoniae* to ceftriaxone, ciprofloxacin and cotrimoxazole was 50%, 37.5% and 44%, respectively. Antimicrobials that were found most potent against *K.*

pneumoniae were gentamicin and tetracycline (69% in both cases) followed by ciprofloxacin (62.5%). Multidrug resistance was observed among 56% of *K. pneumoniae* isolates and 50% of *K. pneumoniae* isolates were ESBL positive (Chakraborty *et al.*, 2016).

A study in Iran showed that 100% of ESBL producing *K. pneumoniae* isolates were resistant to cefotaxime, 98% to ceftriaxone, 86.3% to trimethoprim/sulfamethoxazole, 80.4% to aztreonam, 76.5% to ceftazidime, 68.6% to tetracycline, 52.9% to gentamicin, 39.2% to nalidixic acid, 37.3% to cefepime, 33.3% to ciprofloxacin, 21.6% to chloramphenicol. Least resistance was observed to amikacin (7.8%) and imipenem (5.9%) (Shahraki-Zahedani *et al.*, 2016). Another study by Babakhani *et al*. (2015) observed that *K. pneumoniae* was resistant to ceftriaxone (94.12%), ciprofloxacin (90.20%), cefotaxime (78.43%), nalidixic acid (58.82%), aztreonam (54.90%), gentamicin (41.18%), piperacillin/tazobactam (19.61%) and amikacin (15.69%). The authors concluded; imipenem (5.88%) and meropenem (3.92%) were the most effective antimicrobials against *K. pneumoniae* with the lowest resistance.

A study from Nepal noted that most of the *K. pneumoniae* were isolated from intensive care unit (25.6%) and 59 % *K. pneumoniae* isolates were MDR (Nepal *et al.*, 2017). ESBL producing *K. pneumoniae* were 100% resistance to cefotaxime and ceftazidime and 86.7% to ceftriaxone. The lowest rates of resistance among ESBL producing *K. pneumoniae* were seen towards imipenem followed by piperacillin/tazobactam, amikacin and cefoperazone/sulbactam.

In Africa, different studies showed high drug resistance rates in *K. pneumoniae*. South African three years' surveillance of antimicrobial resistance in *K. pneumoniae* depicted that 68.3% of the isolates were ESBL-positive with marked resistance to third- and fourth generation cephalosporins (Perovic *et al.*, 2014). Most isolates (95.5%) were susceptible to carbapenems and 95.3% susceptible to amikacin. The surveillance observed a trend towards a decrease in susceptibility to most antimicrobials. Ibrahim *et al*. (2017) from Nigeria reported that out of 108 *K. pneumoniae* isolates, 68(62.9%) were ESBL producers and 82(75.9%) were carbapenem resistant. From carbapenem resistant isolates, 52 (63.4%) were carbapenemase producers. Combined production of ESBL and carbapenemase was detected in 16(14.8%) *K. Pneumoniae* isolates. A similar study in Uganda showed high magnitude (72.7 %) of ESBL production in *K. pneumoniae* (Kateregga *et al.*, 2015).

A study from Tunisia noted 94.06% of *K. pneumoniae* to be resistant to cotrimoxazole, 87.28% to tobramycin, 82.20% to gentamicin and 57.62% to ciprofloxacin (Alibi *et al.*, 2015). All isolates were susceptible to imipenem and colistin. Besides, lowest rates of resistance were observed to amikacin (3.40%) and fosfomycin (2.55%). These authors isolated most of ESBL-producing *K. pneumoniae* from hospitalized patients, especially in neonatal and pediatric wards. In line with this result, a study from Egypt on *K. pneumoniae* observed 75% susceptibility to imipenem and meropenem (El-Badawy *et al.*, 2017). Resistance to gentamicin, amikacin, and ciprofloxacin was 60%, 26%, 47%, respectively. The study observed highest susceptibility (97%) to tigecycline.

A systematic review in East Africa stated *K. pneumoniae* was the most reported carbapenemase producing isolate across the region and it was the third most common carbapenem resistant bacteria in the region next to *A. baumannii* and *P. aeruginosa* with an average magnitude of 15% (Ssekatawa *et al.*, 2018). An epidemiological study in Kenya observed a significant increase in MDR *K. pneumoniae* isolates over a decade, particularly to third generation cephalosporins. More than half of the isolates were ESBL positive and 79% were MDR (Henson *et al.*, 2017).

In Ethiopia, studies exclusively on *K. pneumoniae* are limited but it was frequently isolated with high drug resistance profiles in studies on Gram-negative bacteria. Ethiopian annual antimicrobial surveillance report showed that *K. pneumoniae* was the second most common pathogen isolated from urine and pus (Ethiopian Public Health Institute, 2018). The majority of the isolates showed resistance to broad-spectrum antimicrobials with the highest resistance to amoxicillin-clavulanate (92%), and trimethoprim-sulfamethoxazole (88%). High level of resistance was also seen to cephalosporins, namely ceftazidime (89%), cefepime (87%), and ceftriaxone (85%). Resistance to gentamicin was (79%), ciprofloxacin (59%), meropenem (26%) and amikacin (26%).

A recent study in Addis Ababa on *Enterobacteriaceae* clinical isolates from different clinical laboratories noted *K. pneumoniae* as the second most common bacteria with highest ESBL production rate (85.4%). The study observed high resistance rates of *K. pneumonia* against cefotaxime (86.4%), cefepime (85.4%), ceftazidime (85.4%), amoxicillin-clavulanic acid (85.4%) and gentamicin (70.0%), whereas resistance level to meropenem (10.7%) and amikacin (21.3%) was low (Teklu *et al.*, 2019).

According to a study by Desta *et al.* (2016), on gastrointestinal colonization among admitted patients at Tikur Anbessa Specialized Hospital, Addis Ababa, all *K. pneumoniae* isolated from neonates, 88% from children and 50% from adults were ESBL positive. Two children were colonized by ESBL and carbapenemase producing *K. pneumoniae*. Another study on similar study area showed 84.2% and 10.5% of *K. pneumoniae* isolates to be ESBL and carbapenemase producers, respectively (Legese *et al.*, 2017).

Similar to this study, a study conducted at Bahir Dar observed *K. pneumoniae* as the most common MDR isolate with a magnitude of 87.6% (Moges *et al.*, 2019). The magnitude of ESBL and carbapenemase production was 53.3% and 10.8%, respectively. The study also indicated 88.2% of *K. pneumoniae* isolates to be resistant to ceftriaxone, 94.1% to cefepime, 97.6% to ceftazidime, 74.1% to amoxicillin-clavulanic acid, 96.5% to cotrimoxazole, 90.6% to tetracycline and 81.2% to gentamicin. A study in Gondar on urinary tract infection observed 95.6% of *K. pneumoniae* as MDR (Eshetie *et al.*, 2015).

Another study in Harar region isolated 57 *Klebsiella* species from patients admitted to four different hospitals (Seid and Asrat, 2005). Of them, 94.7% were *K. pneumoniae* and the rest 5.3% were *K. oxytoca.* The study noted that only *K. pneumoniae* isolates were ESBL producers with the magnitude of 33.3%. Multi-drug resistant isolates were more prevalent among the ESBLs producers (95.0%) than non-producers (53.0%). According to a study in Jimma by Siraj *et al*. (2015) on clinical specimens from inpatients and outpatients, 70.4 % of *K. pneumoniae* isolates were ESBL producers.

3. OBJECTIVES

3.1. General Objective

- To determine the phenotypic antimicrobial resistance profiles of *K. pneumoniae* isolated from patients at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia

3.2. Specific Objectives

- To examine the antimicrobial resistance patterns of *K. pneumoniae* isolates
- To determine the magnitude of extended spectrum β-lactamase production among *K. pneumoniae* isolates
- To determine the magnitude of carbapenemase production among *K. pneumoniae* isolates
- To determine the magnitude of multidrug resistance among *K. pneumoniae* isolates

4. METHODS AND MATERIALS

4.1. Study area

The study was conducted at Tikur Anbessa Specialized Hospital (TASH), Addis Ababa, Ethiopia. Addis Ababa is the capital city of the country at an area of about 540 square kilometers. TASH was established in 1972 and at that time the hospital was the only site for training medical doctors. It is the largest teaching hospital, with over 700 beds and found in the College of Health Sciences of Addis Ababa University. The hospital receives referred patients from all parts of the country and provides local emergency services. The Hospital provides service to both outpatients and inpatients admitted to ICUs, emergency, medical, surgical, obstetric & gynecologic, orthopedic, oncology and pediatric wards.

4.2. Study design and period

A cross-sectional study was conducted from September 2018 to February 2019 at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia.

4.3. Population

4.3.1. Source population

All bacterial species isolated from patients who were admitted or attended TASH

4.3.2. Study population

All *K. pneumoniae* isolated from patients who were admitted or attended TASH

4.4. Sample size and sampling procedure

4.4.1. Sample size

Sample size was determined using single population proportion formula

$$\mathbf{n} = \frac{(Z\alpha/_2)^2 \, P \, (1- P)}{d^2}$$

Where n = sample size

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

p = proportion

d= margin of error (degree of accuracy desired)

Considering 95.6% estimated previous proportion (P) of MDR *K. pneumoniae* at Gondar Referral Hospital, Northwest Ethiopia (Eshetie *et al.*, 2015) and d= 3.5%

The sample size was estimated to be:

$$n = \frac{(1.96)^2 \times 0.956\ (1 - 0.956)}{(0.035)^2} = 132$$ non-repetitive *K. pneumoniae* isolates

4.4.2. Sampling technique

Convenience sampling technique was used

4.5. Inclusion and Exclusion criteria

4.5.1. Inclusion criteria

All *K. pneumoniae* isolated from different types of samples (urine, blood, sputum, CSF, wound and other body fluids) that were collected from patients during routine bacteriological analysis at the time of the study

4.5.2. Exclusion criteria

K. pneumoniae isolated from patients who didn't give informed consent/assent were excluded

4.6. Study variables

4.6.1. Dependent variables

- Antimicrobial resistance patterns
- ESBL production
- Carbapenemase production

4.6.2. Independent variables

- Age
- Sex
- Specimen type
- Patient setting
- Ward type
- Previous antimicrobial medication

4.7. Operational Definitions

Multidrug-resistant (MDR): - non-susceptibility to at least one agent in three or more antimicrobial categories (Magiorakos *et al.*, 2012), excluding ampicillin, as *K. pneumoniae* is intrinsically resistant to ampicillin.

Non-susceptible:- resistance and intermediate antimicrobial susceptibility test result.

4.8. Data collection tools and procedures

4.8.1. Data collection

Scio-demographic characteristics and clinical information was obtained from *K. pneumoniae* culture-positive patients using well-designed questionnaires and from their medical records by health workers.

4.8.2. Bacterial Isolation and Identification

Inoculation of clinical specimens on appropriate culture media was done regularly, when the specimens come to the microbiology laboratory of TASH. Briefly, blood specimens were collected on BacT/ALERT® 3D culture bottles and incubated in automatic BacT/ALERT® 3D at 37^{o}C of 5% CO_2 for 5 days. The microbial growth that could be detected by flag and audible sound of the instrument was subsequently sub-cultured on MacConkey agar (Oxoid, UK), 5% sheep blood agar (Oxoid, UK) and chocolate agar (Oxoid, UK) plates. A negative result was checked by examining the flag, doing gram stain and subculturing at the end of the 5^{th} day prior to discarding as negative (Thorpe *et al.*, 1990). Inoculation of wound specimens, sputum and body fluids was done on MacConkey agar, 5% sheep blood agar and chocolate agar, while inoculation of urine was done only on MacConkey agar and 5% sheep blood agar.

After incubation of all inoculated culture plates at 35-37°C for 18-24 hours, preliminary identification of *K. pneumoniae* was done through examining colony characteristics on MacConkey agar and blood agar. *K. pneumoniae* colonies are large, mucoid and pink (due to fermentation of lactose) on MacConkey agar and large, mucoid, greyish-white colonies on 5% sheep blood agar plates (Cheesbrough, 2006;Procop *et al.*, 2017). Further identification was done using Gram stain and the biochemical characteristics of the bacteria. *K. pneumoniae* is Gram-negative and rod shaped, indole negative, gas and acid producer, hydrogen sulfide negative, citrate positive, mannitol fermenter, malonate positive, lysine decarboxylase positive, urea slow producing and non-motile (De Jesus *et al.*, 2015;Procop *et al.*, 2017). After identification, the isolates were sub-cultured on 5% sheep blood agar plate and incubated over night; to get fresh colonies for Antimicrobial Susceptibility Testing (AST) and for storage in 10% skim milk medium at -80°C for further molecular analysis (Cody *et al.*, 2008).

4.8.3. Antimicrobial Susceptibility Testing

Using a sterile wire loop, 3–5 pure colonies were picked from blood agar and emulsified in 3–4 ml normal saline to prepare a 0.5 McFarland standard using McFarland Densitometer. From the standard, we inoculated onto Muller-Hinton agar (Oxoid, UK) using sterile swab for the AST (Cheesbrough, 2006). The AST was performed using the Kirby–Bauer disc diffusion method by using the following antimicrobial discs, Tetracycline (30 µg), Gentamicin (10 µg), Amikacin (30 µg), Tobramycin (10 µg), Ciprofloxacin (5 µg), Amoxicillin-clavulanate (20/10 µg), Piperacillin-tazobactam(100/10 µg), Trimethoprim-sulfamethoxazole (1.25/23.75 µg), Aztreonam (30 µg), Chloramphenicol (30 µg), Cefoxitin (30 µg), Cefazolin (30 µg), Cefuroxime (30 µg), Ceftriaxone (30 µg), Ceftazidime (30 µg), Cefotaxime (30 µg), Cefepime(30 µg), Imipenem(10 µg), Ertapenem (10 µg) and Meropenem(10 µg) (Oxoid, UK) and (BD, USA). The results were read and interpreted after 16-18 hours of incubation at 35± 2°C according to CLSI (2018).

4.8.4. Screening test for extended spectrum β-lactamase (ESBL)

The ESBL screening test was performed by the standard disc diffusion method using Ceftazidime (30 µg), Cefotaxime (30 µg), Ceftriaxone (30 µg) and Aztreonam (30 µg) (BD, USA) antimicrobial discs. Four antimicrobial discs were used for screening to improve the sensitivity of ESBLs detection, as recommended by CLSI guidelines. Isolates that showed an inhibition zone size of ≤ 22 mm for Ceftazidime (30 µg), ≤ 25mm for Ceftriaxone (30 µg), ≤27 mm for Cefotaxime (30 µg) and/or ≤ 27 mm for Aztreonam (30 µg) were considered as potential ESBL producers and selected for confirmation for ESBLs production using Combined Disc Test (CDT) as recommended by CLSI guidelines (CLSI, 2018).

4.8.5. Phenotypic confirmatory test for ESBL

Those isolates that fulfilled the screening test were confirmed for ESBL production by means of CDT using Cefotaxime-clavulanate (30/10 µg) and Ceftazidime-clavulanate (30/10 µg) (BD, USA) along with third generation cephalosporins (Cefotaxime and Ceftazidime) on Mueller-Hinton agar plate. After 16-18 hours of incubation at 35± 2°C, a ≥ 5-mm increase in a zone diameter for either antimicrobial agent tested in combination with clavulanate versus the zone diameter of the agent when tested alone is considered as positive for the ESBL production (CLSI, 2018). Compared to polymerase chain reaction, CDT has sensitivity of 98.0% and specificity of 96.3% (Kohner *et al.*, 2009).

4.8.6. Phenotypic confirmatory test for carbapenemese

Those isolates that showed non-susceptibility to one or more carbapenems were checked for carbapenemase production using modified Carbapenem Inactivation Method (mCIM). The method has > 99% sensitivity and specificity for detection of carbapenemase among *Enterobacteriaceae* isolates (CLSI, 2018). To perform the mCIM, a suspension was made by taking 1µl loopful of bacteria from an overnight blood agar plate and then adding in to 2 ml trypticase soy broth. Subsequently, Meropenem (10 µg) disc was immersed in the suspension and incubated for 4 hours ± 15 minutes at 35°C ± 2°C. After incubation, the disc was removed from the suspension using 10 µl inoculation loops and placed on a Mueller-Hinton agar plate inoculated with a susceptible *E. coli* indicator strain (ATCC 29522). Then, the results were read after 18-24 hours of incubation at 35°C ± 2°C. If the bacterial isolate produced carbapenemase, the meropenem disc was inactivated allowing uninhibited growth of the susceptible indicator strain. Discs incubated in suspensions that do not contain carbapenemases yielded a clear inhibition zone (CLSI, 2018).

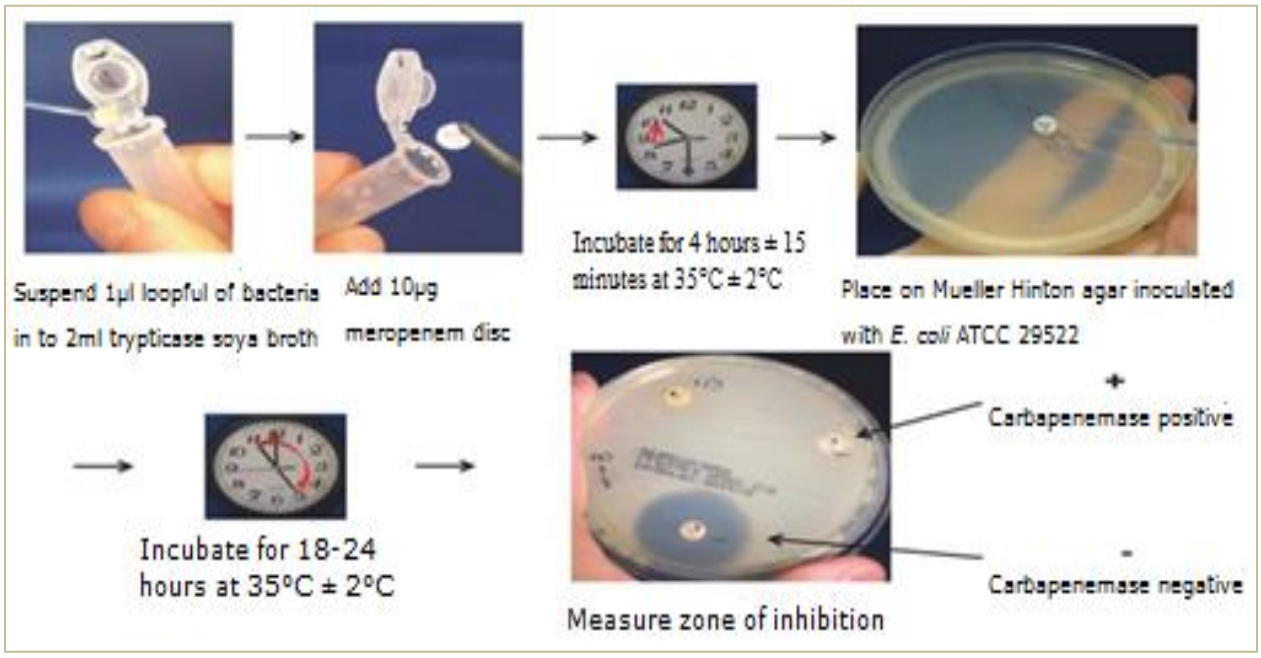


Figure 4.1: Schematic representation of the modified Carbapenemase Inactivation Method (mCIM)

Adopted and modified from (van der Zwaluw *et al.*, 2015)

Laboratory Investigation Work Flow

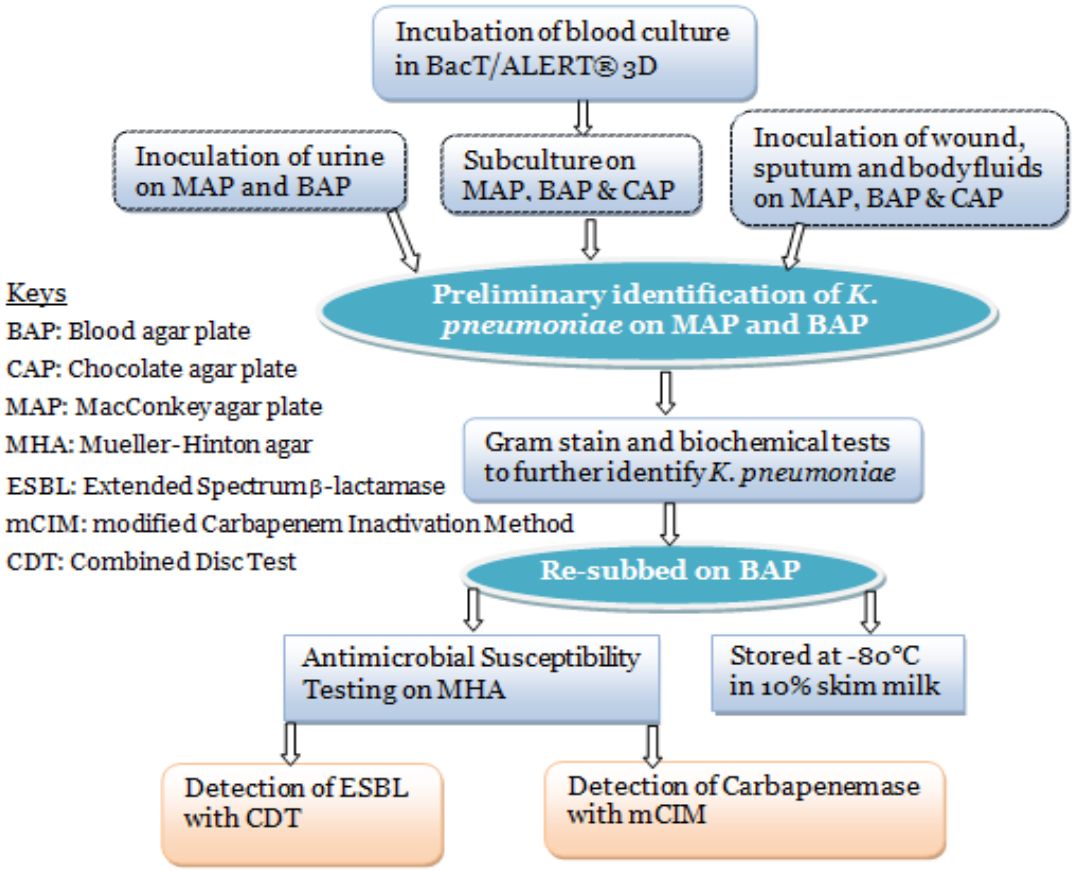


Figure 4.2: Laboratory work flow chart

4.8.7. Data quality assurance

The reliability of the study findings were guaranteed by implementing quality control measures throughout the whole processes of the laboratory work. All materials, equipment and procedures were adequately controlled. Standard operating procedures were strictly followed to evaluate and monitor the quality of the test result and to maintain high standard of accuracy of the result. International control bacterial strains obtained from TASH microbiology and AHRI: *Escherichia coli* ATCC® 25922, *Pseudomonas aeruginosa* ATCC® 27853 (for carbapenems), *Escherichia coli* ATCC® 35218 (for β-lactam/ β-lactamase inhibitor combinations) were used for controlling the potency of the drugs. *K. pneumoniae* ATCC® 700603 and *Escherichia coli* ATCC® 25922 were used as positive and negative control during CDT, respectively. *K. pneumoniae* ATCC® BAA-1705™ and *K. pneumoniae* ATCC® BAA-1706 were used as positive and negative controls for carbapenemase test respectively.

4.8.8. Data analysis

Data was checked, cleaned and double entered in to Epidata software version 3.1, and then it was exported to Statistical Package for Social Sciences (SPSS version 25.0) software for analysis. Binary logistic regression and Chi-Square test or Fisher's exact test were used to compare categorical variables and P-value < 0.05 at 95% confidence interval was considered as statistically significant. Results were summarized and presented in texts, tables and graphs.

4.9. Ethical considerations

Ethical approval was obtained from the Ethics Review Committee of Department of Microbiology, Immunology and Parasitology (DMIP), School of Medicine, College of Health Sciences, Addis Ababa University (AAU) (Reference number: DERC/17/18/02-N) and AHRI/ ALERT ethical review committee (Protocol number: PO12/18). Supportive letter was obtained from TASH. Samples were coded with unique number in order to keep the confidentiality of the study participants. Moreover, prior to commencing the study, a written informed consent/assent was obtained from each *K. pneumoniae* culture-positive participant after he/she has understood the participant information sheet. We have communicated physicians for the better management of patients, when ESBL and carbapenemase positive isolates were detected.

4.10. Result dissemination

While the work is in progress, there was a brief communication (meeting) about the problem with the pediatric ward team. Furthermore, the finding of this study will be presented and submitted to DMIP of College of Health Sciences of AAU and AHRI. It will also be disseminated to TASH as well as made recognized by the scientific community through conference presentation and publication in reputable journals.

5. RESULTS

5.1. Socio-demographic and clinical characteristics

A total of 132 *K. pneumoniae* isolates collected from patients who were admitted or attended different departments of TASH were included. As shown in **Table 5.1** among the total isolates, 83 (62.9%) were from males with male to female ratio of 1.7:1. The majority of the isolates were obtained from blood specimens 63 (47.7%) followed by urine 34 (25.8%), wound 21 (15.9 %), body fluids 10 (7.6 %), and sputum specimens 4 (3.0%).

The average age of the study participants was 13.4 years, varying from at least 1 day to a maximum of 86 years, of which 74 (56.1%) were below 5 years. Majority of *K. pneumoniae* isolates were recovered from hospitalized patients 120 (90.9%), of which the largest number were from patients admitted in pediatrics ward 53 (44.2%) and ICUs 46 (38.3%). Whereas, only 2 isolates were from patients in orthopedics and 2 from emergency wards.

Table 5.1: Distribution of *K. pneumoniae* isolates against demographic and clinical characteristics of study participants

Variables	Category	Frequency	Percentage
Gender	Male	83	62.9
	Female	49	37.1
Age	birth to < 5 years	74	56.1
	5 years to <18years	20	15.2
	18 years to <45 years	25	18.9
	≥ 45 years	13	9.8
Specimen type	Blood	63	47.7
	Urine	34	25.8
	Wound	21	15.9
	Body fluids	10	7.6
	Sputum	4	3.0
Patient setting	Inpatient	120	90.9
	Outpatient	12	9.1
Ward type	Pediatric ward	53	44.2
	ICU	46	38.3
	Medical ward	9	7.5
	Surgical ward	8	6.7
	Other wards	4	3.3

Note: - Other wards: Emergency (2) and orthopedics (2)

5.2. Antimicrobial susceptibility patterns of *K. pneumoniae* isolates

In this study, susceptibility of 132 *K. pneumoniae* isolates to 20 antimicrobial agents belonging to 12 antimicrobial categories was determined. As presented in **Table 5.2** high frequency of resistance was observed to commonly used antimicrobials such as cefotaxime 128/132 (97%), ceftriaxone 128/132 (97%), trimethoprim-sulfamethoxazole 124/132 (93.9%) and cefepime 111/132 (84.1%). Besides, a significant intermediate level of resistance was noted to tobramycin 26.5%, amoxicillin /clavulunate 24.2%, ceftazidime 21.2%, piperacillin-tazobactam 20.5%, aztreonam 22.7%, and ciprofloxacin 18.9%. On the other hand, *K. pneumoniae* isolates showed highest susceptibility to amikacin 123 (93.2%) followed by carbapenem antimicrobials which were imipenem 107 (81.1%), meropenem 96 (72.7%) and ertapenem 93 (70.5%).

Table 5.2: Antimicrobial susceptibility patterns of *K. pneumoniae* isolates

Antimicrobial categories	**Antimicrobial agents**	**Antimicrobial susceptibility pattern**		
		Sensitive n (%)	**Intermediate n (%)**	**Resistant n (%)**
Tetracyclines	Tetracycline	21(15.9)	15(11.4)	96(72.7)
Aminoglycosides	Gentamicin	29(22.0)	8(6.1)	95(72.0)
	Tobramycin	49(37.1)	35(26.5)	48(36.4)
	Amikacin	123(93.2)	5(3.8)	4(3.0)
Fluoroquinolones	Ciprofloxacin	58(43.9)	25(18.9)	49(37.1)
Monobactams	Aztreonam	13(9.8)	30(22.7)	89(67.4)
Antipseudomonal Penicillins+ β-lactamase inhibitors	Piperacillin-tazobactam	55(41.7)	27(20.5)	50(37.9)
Penicillins + β-lactamase inhibitors	Amoxicillin-clavulanate	18(13.6)	32(24.2)	82(62.1)
Folate pathway inhibitors	Trimethoprim-Sulfamethoxazole	6(4.5)	2(1.5)	124(93.9)
Phenicols	Chloramphenicol	59(44.7)	13(9.8)	60(45.5)
1st and 2nd generation cephalosporins	Cefuroxime	2(1.5)	2(1.5)	128(97.0)
	Cefazolin	1(0.8)	1(0.8)	130(98.5)
Cephamycins	Cefoxitin	62(47.0)	12(9.1)	58(43.9)
3rd and 4thgeneration cephalosporins	Cefepime	5(3.8)	16(12.1)	111(84.1)
	Ceftriaxone	4(3.0)	0(0.0)	128(97.0)
	Cefotaxime	4(3.0)	0(0.0)	128(97.0)
	Ceftazidime	17(12.9)	28(21.2)	87(65.9)
Carbapenems	Meropenem	96(72.7)	4(3.0)	32(24.2)
	Imipenem	107(81.1)	9(6.8)	16(12.1)
	Ertapenem	93(70.5)	5(3.8)	34(25.8)

Resistance patterns of *K. pneumoniae* isolates among inpatients and outpatients

To see the difference in resistance patterns, *K. pneumoniae* isolates from patients on different wards and outpatient department were compared. *K. pneumoniae* isolates from inpatients showed highest resistance to ceftriaxone and cefotaxime (98.3%) followed by trimethophrim-sulfamethoxazole (94.2%), cefepime (86.7%) and gentamicin (74.2%) data presented in **Table 5.3**. Among the isolates obtained from outpatients highest resistance was recorded to trimethophrim-sulfamethoxazole (91.7%). Regarding the rate of resistance in each inpatient ward, all isolates from the ICUs were completely resistant to trimethophrim-sulfamethoxazole, 97.8% to ceftriaxone and cefotaxime. 98.1% of the isolates from pediatric wards were resistant to ceftriaxone and cefepime followed by trimethophrim-sulfamethoxazole (92.5%). Overall, isolates from inpatients showed higher resistance to most of the antimicrobials than outpatients.

Table 5.3: Resistance patterns of *K. pneumoniae* isolates among inpatients and outpatient

Antimicrobials	Inpatients						Outpatient
	ICUs n=46(%)	**Surgical wards n=8(%)**	**Pediatric wards n=53(%)**	**Medical wards n=9(%)**	**Other wards n=4(%)**	**Total n=120(%)**	**OPD n=12(%)**
Tetracycline	31(67.4)	5(62.5)	39(73.6)	8(88.9)	3(75.0)	86(71.7)	10(83.3)
Gentamicin	35(76.1)	8(100.0)	37(69.8)	6(66.7)	3(75.0)	89(74.2)	6(50.0)
Tobramycin	16(34.8)	6(75.0)	17(32.1)	5(55.6)	0(0.0)	44(36.7)	4(33.3)
Amikacin	1(2.2)	0(0.0)	2(3.8)	1(11.1)	0(0.0)	4(0.03)	0(0.0)
Ciprofloxacin	14(30.4)	5(62.5)	19(35.8)	7(77.8)	1(25.0)	46(38.3)	3(25.0)
Aztreonam	29(63.0)	7(87.5)	39(73.6)	7(77.8)	2(50.0)	84(70.0)	6(50.0)
Piperacillin-Tazobactam	18(39.1)	4(50.0)	19(35.8)	6(66.7)	0(0.0)	47(39.2)	3(25.0)
Amoxicillin-clavulanate	30(65.2)	6(75.0)	34(64.2)	6(66.7)	2(50.0)	78(65.0)	4(33.3)
Cotrimoxazole	46(100.0)	6(75.0)	49(92.5)	8(88.9)	4(100.0)	113(94.2)	11(91.7)
Chloramphenicol	17(37.0)	7(87.5)	25(47.2)	3(33.3)	3(75.0)	55(45.8)	5(41.7)
Cefoxitin	19(41.3)	4(50.0)	23(43.4)	7(77.8)	1(25.0)	54(45.0)	4(33.3)
Cefepime	38(82.6)	8(100.0)	47(88.7)	9(100.0)	2(50.0)	104(86.7)	7(58.3)
Ceftriaxone	45(97.8)	8(100.0)	52(98.1)	9(100.0)	4(100.0)	118(98.3)	10(83.3)
Cefotaxime	45(97.8)	8(100.0)	52(98.1)	9(100.0)	4(100.0)	118(98.3)	10(83.3)
Ceftazidime	30(65.2)	8(100.0)	37(69.8)	6(66.7)	0(0.0)	81(67.5)	6(50.0)
Meropenem	10(21.7)	3(37.5)	12(22.6)	6(66.7)	0(0.0)	31(25.8)	1(8.3)
Imipenem	8(17.4)	1(12.5)	6(11.3)	1(11.1)	0(0.0)	16(13.3)	0(0.0)
Ertapenem	12(26.1)	2(25.0)	13(24.5)	6(66.7)	0(0.0)	33(27.5)	1(8.3)

Note: - Other wards: Emergency (2) and Orthopedics (2), ICU: Intensive Care Units, OPD: Outpatient Department, Cotrimoxazole: Trimethophrim-sulfamethoxazole

5.3. ESBL producing *K. pneumoniae* isolates

Magnitude of ESBL production among *K. pneumoniae* isolates

To determine the magnitude of ESBL among isolates, screening of the isolates following CLSI guideline was done primarily and those with potential ESBL production capacity were confirmed with Combined Disc Test (CDT). Primarily 128/132 (97%) of the isolates were identified as potential ESBL producers with a zone of inhibitions of cefotaxime ≤ 27 mm, ceftriaxone ≤ 25, ceftazidime ≤ 22 mm and/or aztreonam ≤ 27mm. Among the potential ESBL producers the large majority 102/128 (79.7%) were ESBL positive with CDT. As shown in **Figure 5.1** generally, ESBL positivity rate from the total isolates was 102/132 (77.3%).

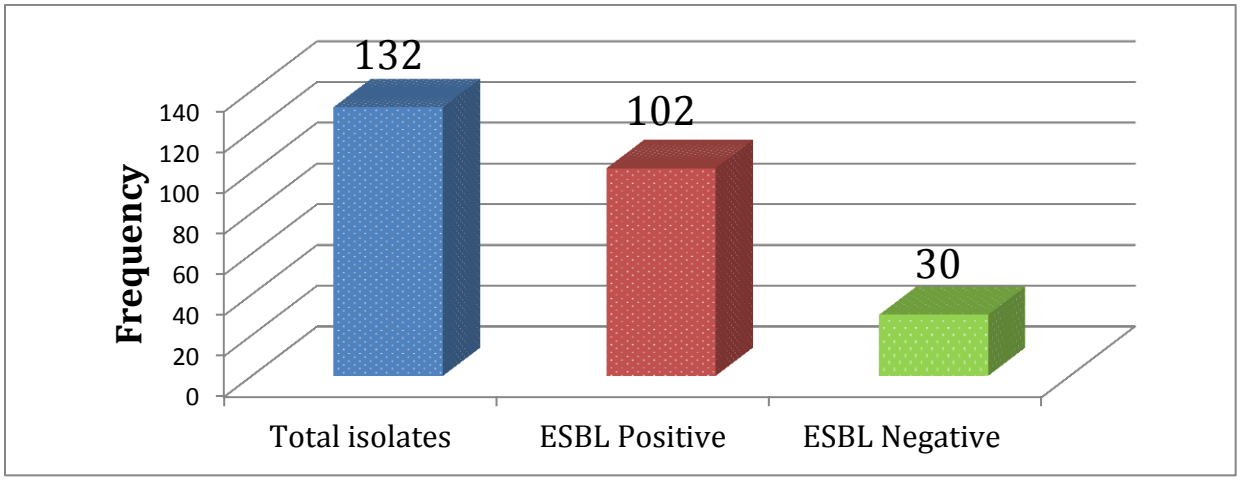
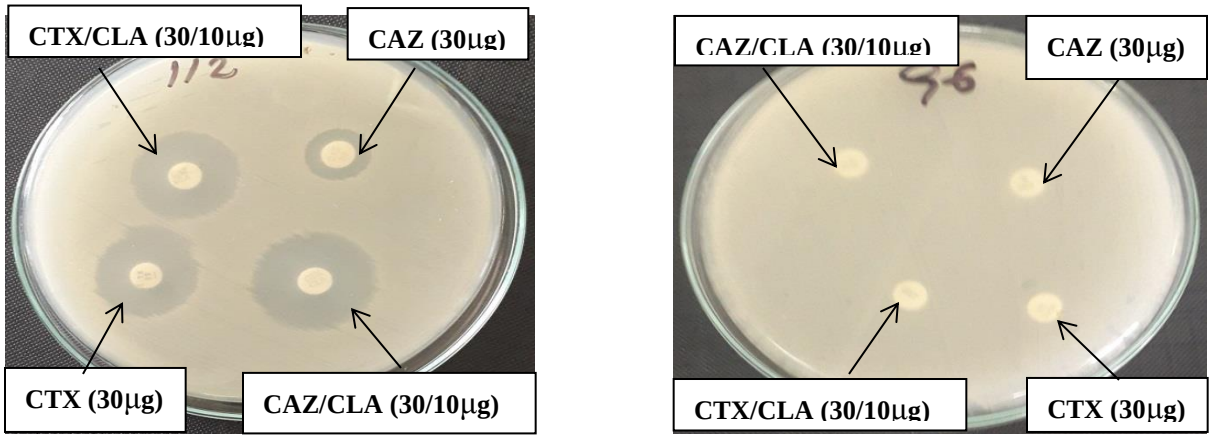


Figure 5.1: Frequency of ESBL producing and non-producing *K. pneumoniae* isolates



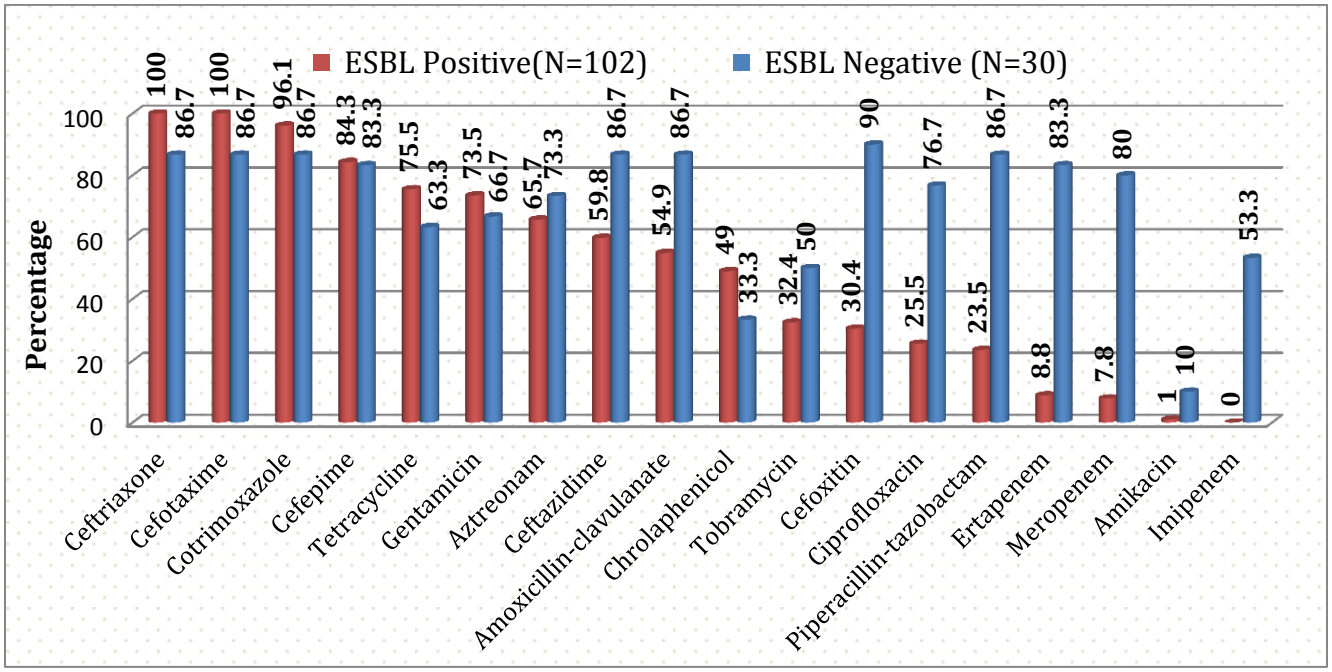
Note: - CTX: Cefotaxime, CAZ: Ceftazidime, CTX/CLA: Cefotaxime/ clavulanate, CAZ/CLA: Ceftazidime/ clavulanate

Figure 5.2: ESBL positive (left) and negative (right) *K. pneumoniae* isolates with Combined Disc Test (CDT)

Antimicrobial resistance patterns of ESBL producing and non-producing *K. pneumoniae* isolates

The antimicrobial resistance patterns of ESBL producers and non-producers were checked. As shown in **Figure 5.3** ESBL positive isolates were 100% resistant to two of third-generation cephalosporins namely ceftriaxone and cefotaxime. Co-resistance of ESBL positive isolates to other non β-lactam antimicrobials was higher to trimethophrim-sulfamethoxazole 98 (96.1%) followed by tetracycline 77 (75.5%), gentamicin 75 (73.5%), aztreonam 67 (65.7%) and amoxicillin-clavulanate 56 (54.9%). However, these isolates showed high susceptibility to imipenem 100 (98.0%), amikacin 98 (96.1%), meropenem 91 (89.2%), and ertapenem 88 (86.3%).

Of particular concern, as presented in **Figure 5.3** ESBL negative isolates had higher resistant profiles to more than half of the antimicrobials tested than that of ESBL positive isolates. ESBL negative isolates were highly resistant to carbapenems (53.3% to 83.3%) and cefoxitin (90.0%). Moreover, 26/30 (86.7%) of non-ESBL producers were resistant to piperacillin-tazobactam, amoxicillin-clavulanate, ceftriaxone, cefotaxime, ceftazidime and trimethophrim-sulfamethoxazole. Both ESBL producers and non-producers were highly susceptible to amikacin with a susceptibility rates of 98/102 (96.1%) and 25/30 (83.3%), respectively.



Note: - Cotrimoxazole: Trimethophrim-sulfamethoxazole

Figure 5.3: Antimicrobial resistance patterns of ESBL producing and non-producing *K. pneumoniae* isolates

Association of socio-demographic and clinical characteristics with ESBL production

As the large majority of the isolates were ESBL producers, association of sex, patient setting ward type and age with ESBL production was assessed. As shown in **Table 5.4** the proportion of ESBL producing *K. pneumoniae* isolates in females 39/49 (79.6%) was comparable with their male counterparts 63/83 (75.9%). Age and ward type has been associated with ESBL production in bivariate analysis. Higher proportion of *K. pneumoniae* isolates from patients in ICUs 37/46 (80.4%) and pediatric wards 42/53 (79.2%) were ESBL producers compared to those from medical wards 4/9 (44.4%). Similarly, ESBL producers were more frequently encountered among patients less than 5 years 64/74 (86.5%) than those 18 to <45 years of age (64.0%). Age and ward type were subjected for multivariate analysis. However, none of them were associated with ESBL production in multivariate analysis

Table 5.4: Association of age, sex, patient setting, and ward type with ESBL production

Variables	Extended spectrum β-lactamase		Bivariate analysis		Multivariate analysis	
	Positive n (%)	Negative n (%)	P- value	COR (95%CI)	P- value	AOR (95%CI)
Sex						
Male (n=83)	63(75.9)	20(24.1)	0.626	0.81 (0.34- 1.9)		
Female (n=49)	39(79.6)	10(20.4)	R			
Age group						
<5 years (n=74)	64(86.5)	10(13.5)	**0.017***	3.6 (1.26-10.33)	0.116	3.86 (0.72-20.77)
5 to <18years (n=20)	12(60.0)	8(40.0)	0.783	0.84 (0.25- 2.83)	0.959	0.95 (0.15- 6.11)
18 to <45 years (n=25)	16(64.0)	9(36.0)	R			
≥45 years (n=13)	10(76.9)	3(23.1)	0.420	1.88 (0.41-8.63)	0.474	1.93 (0.32- 11.6)
Patient setting						
Inpatients (n=120)	94(78.3)	26(21.7)	0.363	1.81 (0.5- 6.48)		
Outpatient (n=12)	8(66.7)	4(33.3)	R			
Ward type						
ICUs (n=46)	37(80.4)	9(19.6)	**0.033***	5.14 (1.14-23.1)	0.421	2.15 (0.33-13.8)
Pediatric ward (n=53)	42(79.2)	11(20.8)	**0.038***	4.77 (1.09-20.82)	0.445	2.21 (0.29-16.77)
Medical ward (n=9)	4(44.4)	5(56.6)	R			
Surgical ward (n=8)	7(87.5)	1(12.5)	0.086	8.75 (0.74- 103.8)	0.106	7.83 (0.65- 94.9)
Emergency (n=2)	2(100.0)	0(0.0)	0.999			
Orthopedics (n=2)	2(100.0)	0(0.0)	0.999			

Note: - R: Reference, *: Significant P<0.05, COR: Crude Odds Ratio, AOR: Adjusted Odds Ratio, 95%CI: 95% Confidence Interval, ICUs: Intensive Care Units

5.4. Carbapenemase producing *K. pneumoniae* isolates

Magnitude of Carbapenemase producing *K. pneumoniae* isolates

Similar to ESBL production, the magnitude of Carbapenemase production among the isolates was also determined. As shown in **Figure 5.4** from the total isolates, 39/132 (29.6%) showed non susceptibility to one or more carbapenems. To this end, we determined the production of carbapenemase by the *K. pneumoniae*. Out of these isolates, 28/39 (71.8%) were carbapenemase positive using modified Carbapenem Inactivation Method (mCIM). The overall magnitude of carbapenemase production from the total isolates was 28/132 (21.2%).

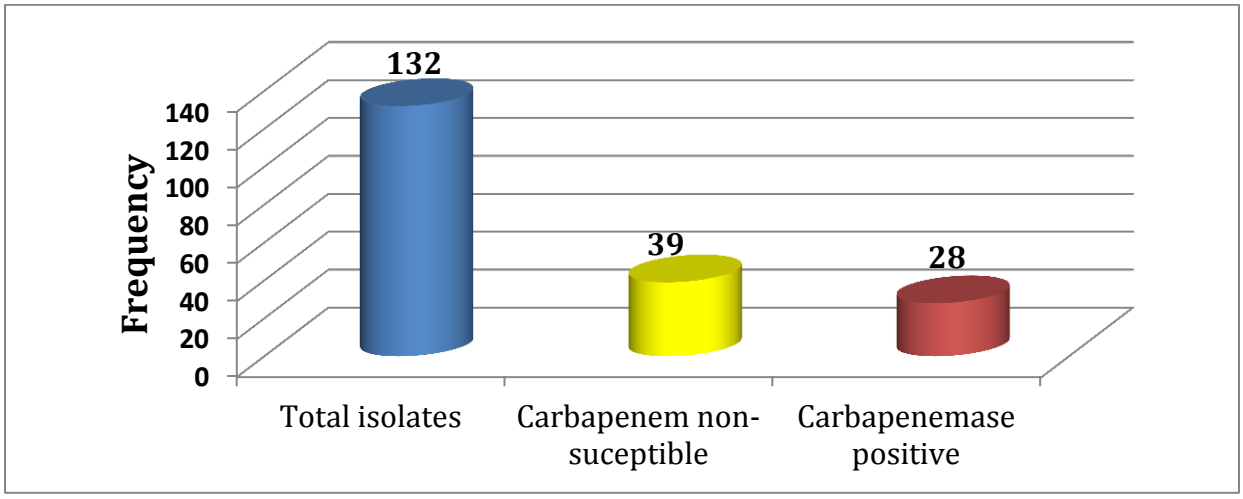
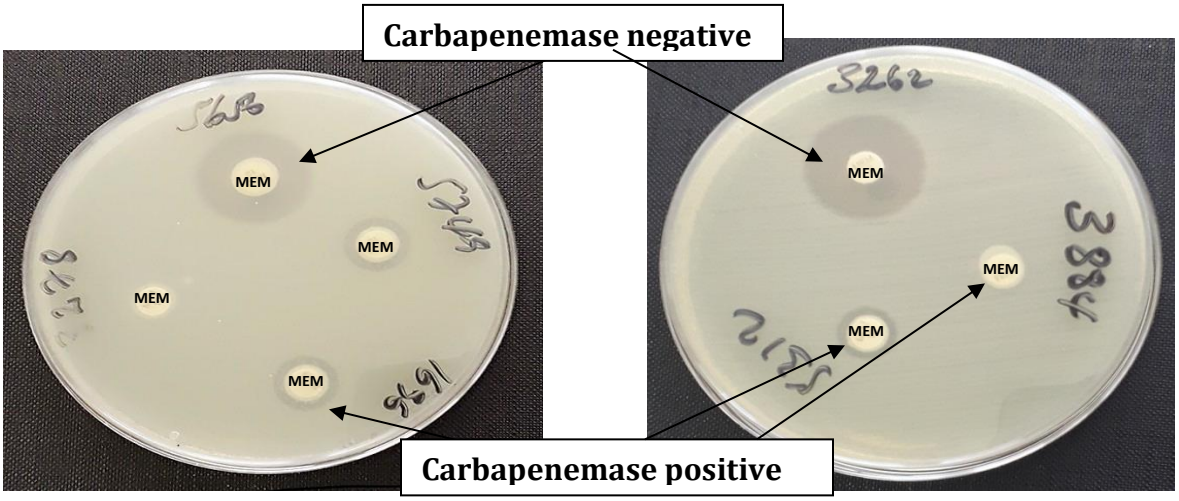


Figure 5.4: Frequency of carbapenemase producing *K. pneumoniae* isolates



Note: -MEM: Meropenem

Figure 5.5: Carbapenemase positive and negative *K. pneumoniae* isolates with modified Carbapenem Inactivation Method (mCIM)

Antimicrobial Susceptibility patterns of carbapenemase producing and non-producing *K. pneumoniae* isolates

Carbapenemase producing isolates were highly resistant to β-lactams as well as to other classes of antimicrobials except amikacin. As displayed in **Table 5.5** carbapenemase producers were 100% non-susceptible to aztreonam, pipracillin-tazobactam, amoxicillin-clavulanate, cefoxitin, ceftriaxone, cefotaxime, ceftazidime, cefepime, meropenem and ertapenem. From carbapenems least resistance was noted to imipenem with resistance rates of 13/28 (46.4%). The susceptibility of carbapenemase producers to amikacin was 20/28 (7l.4%). However, non-carbapenemase producing isolates showed high susceptibility not only to amikacin 103/104 (99.0%) but also to carbapenems (89.4% to 97.1%).

Table 5.5: Antimicrobial susceptibility patterns of carbapenemase producing and non-producing *K. pneumoniae* isolates

Antimicrobial agents	**Carbapenemase positive (n=28)**			**Carbapenemase negative (n=104)**		
	S	**I**	**R**	**S**	**I**	**R**
Tetracycline	5(17.9)	4(14.3)	19(67.9)	16(15.4)	11(10.6)	77(74.0)
Gentamicin	4(14.3)	0(0.0)	24(85.7)	25(24.0)	8(7.7)	71(68.3)
Tobramycin	4(14.3)	5(17.9)	19(67.9)	45(43.3)	30(28.8)	29(27.9)
Amikacin	20(71.4)	4(14.3)	4(14.3)	103(99.0)	1(1.0)	0(0.0)
Ciprofloxacin	1(3.6)	2(7.1)	25(89.3)	57(54.8)	23(22.1)	24(23.1)
Aztreonam	0(0.0)	3(10.7)	25(89.3)	13(12.5)	27(26.0)	64(61.5)
Piperacillin-tazobactam	0(0.0)	0(0.0)	28(100.0)	55(52.9)	27(26.0)	22(21.2)
Amoxicillin-clavulanate	0(0.0)	1(3.6)	27(96.4)	18(17.3)	31(29.8)	55(52.9)
Trimethophrim-sulfamethoxazole	3(10.7)	1(3.6)	24(85.7)	3(2.9)	1(1.0)	100(96.2)
Chloramphenicol	7(25.0)	10(35.7)	11(39.3)	52(50.0)	3(2.9)	49(47.1)
Cefoxitin	0(0.0)	0(0.0)	28(100.0)	62(59.6)	12(11.5)	30(28.8)
Cefepime	0(0.0)	0(0.0)	28(100.0)	5(4.8)	16(15.4)	83(79.8)
Ceftriaxone	0(0.0)	0(0.0)	28(100.0)	4(3.8)	0(0.0)	100(96.2)
Cefotaxime	0(0.0)	0(0.0)	28(100.0)	4(3.8)	0(0.0)	100(96.2)
Ceftazidime	0(0.0)	0(0.0)	28(100.0)	17(16.3)	28(26.9)	59(56.7)
Meropenem	0(0.0)	3(10.7)	25(89.3)	96(92.3)	1(1.0)	7(6.7)
Imipenem	6(21.43)	9(32.1)	13(46.4)	101(97.1)	0(0.0)	3(2.9)
Ertapenem	0(0.0)	2(7.1)	26(92.9)	93(89.4)	3(2.9)	8(7.7)

Note:- S: Sensitive, I: Intermediate, R: Resistance

Association of antimicrobial agent use and carbapenemase production

The number of study participants who have took antimicrobials within the past 3 months and the possible association with carbapenemase production was assessed. As revealed in **Table 5.6** from the total study participants, 81/132 (61.9%) have took 3rd or 4th generation cephalosporins. 20/132 (15.2%) of the total study participants have took carbapenems, of them 11/20 (55%) were positive for carbapenemase. There was statistically significant association between carbapenem use and carbapenemase production (P<0.001).

Table 5.6: Association of antimicrobial use and carbapenemase production

Variables		Carbapenemase positive n=28(%)	Carbapenemase negative n=104(%)	P-value
Antimicrobial therapy	Yes(n=116)	27(23.3)	89(76.7)	0.191[b]
	No (n=16)	1(6.3)	15(93.8)	
Carbapenems	Yes (n=20)	11(55.0)	9(45.0)	**<0.001*[b]**
	No (n=112)	17(15.2)	95(84.8)	
3rd or 4th GCs	Yes (n=83)	21(25.3)	62(74.7)	0.135[a]
	No (n=49)	7(14.3)	42(85.7)	
Quinolones	Yes (n=14)	4(28.6)	10(71.4)	0.495[b]
	No (n=118)	24(20.3)	94(79.7)	
Aminoglycosides	Yes (n=43)	12(27.9)	31(72.1)	0.191[a]
	No (n=89)	16(18.0)	73(82.0)	

Note: - 3rd or 4th GCs: Third or fourth generation cephalosporins, *: Significant P<0.05, a: Chi-Square test, b: Fisher's exact test

5.5. Co-production of ESBL and Carbapenemase

From the total isolates, 6/132 (4.6%) were positive for both carbapenemases and ESBL, simultaneously. Unfortunately, only 8/132 (6.1%) of *K. pneumoniae* isolates were positive for neither ESBLs nor carbapenemases. Overall, 124/132 (93.9%) isolates produced either ESBLs, carbapenemases or both. Among the total ESBL negative isolates, 22/30 (73.3%) were carbapenemase positive **(Table 5.7)**.

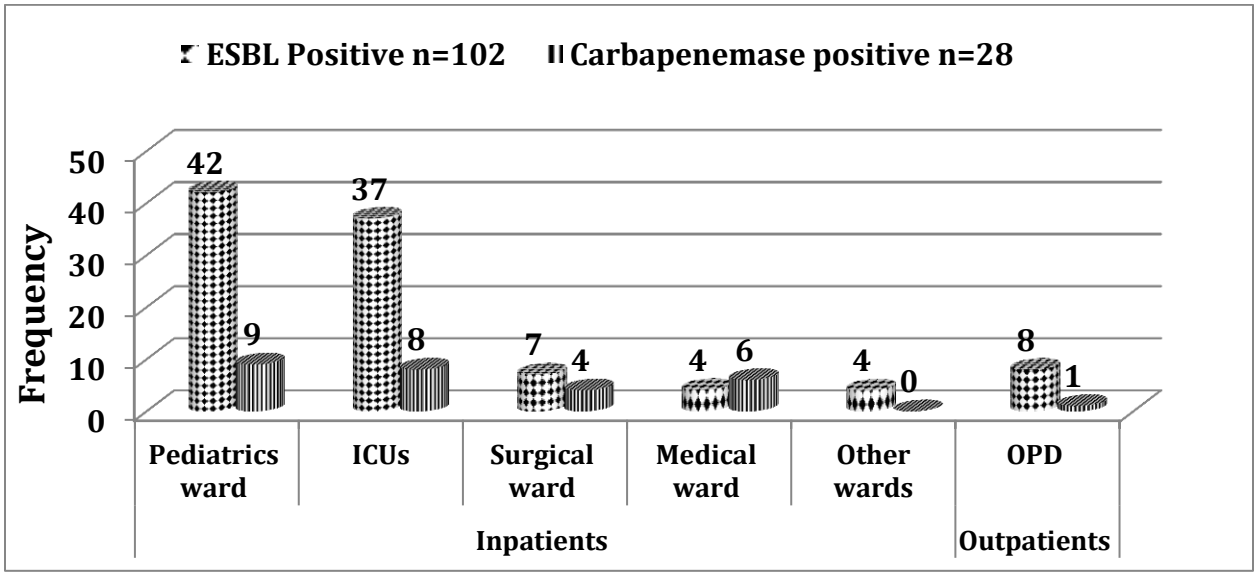
Table 5.7: Frequency of ESBL and carbapenemase co-producing *K. pneumoniae* isolates

ESBL production	Carbapenemase production		Total
	Carbapenemase positive	Carbapenemase negative	
ESBL positive	6	96	**102**
ESBL negative	22	8	**30**
Total	**28**	**104**	**132**

5.6. Distribution of ESBL and carbapenemase producing *K. pneumoniae* isolates

Distribution of ESBL and carbapenemase producing isolates on the basis of patient location/wards

As shown in **Figure 5.6** from the total 102 ESBL producers, 42 (41.2%) and 37 (36.3%) were from patients admitted in the ICUs and pediatrics wards, respectively. Similarly, 9/28 (32.1%) and 8/28 (28.6%) of carbapenemase producing isolates were recovered from patients admitted in the ICUs and pediatrics wards, respectively. Outpatients comprise 8/102 (7.8%) and 1/28 (3.6%) of the total ESBL and carbapenemase producing *K. pneumoniae* isolates, respectively.



Note: - Other wards: Emergency and orthopedics, ICUs: Intensive Care Units, OPD: Outpatient Department

Figure 5.6: Distribution of ESBL and carbapenemase producing *K. pneumoniae* isolates on the basis of patient location/wards

Age-wise distribution of ESBL and carbapenemase producing *K. pneumoniae* isolates

As presented in **Figure 5.7** more than half 64/102 (62.7%) of ESBL positive isolates were recovered from patients less than 5 years of age and 10/102 (9.8%) of them were from patient's ≥45 years. Among carbapenemase positives, maximum number 11/28 (39.2%) was isolated from patients in the age group of 18 to <45 years. While, patients in the age group of 5 to <18 years and under 5 years comprised 7/28 (25%) of carbapenemase positive isolates each.

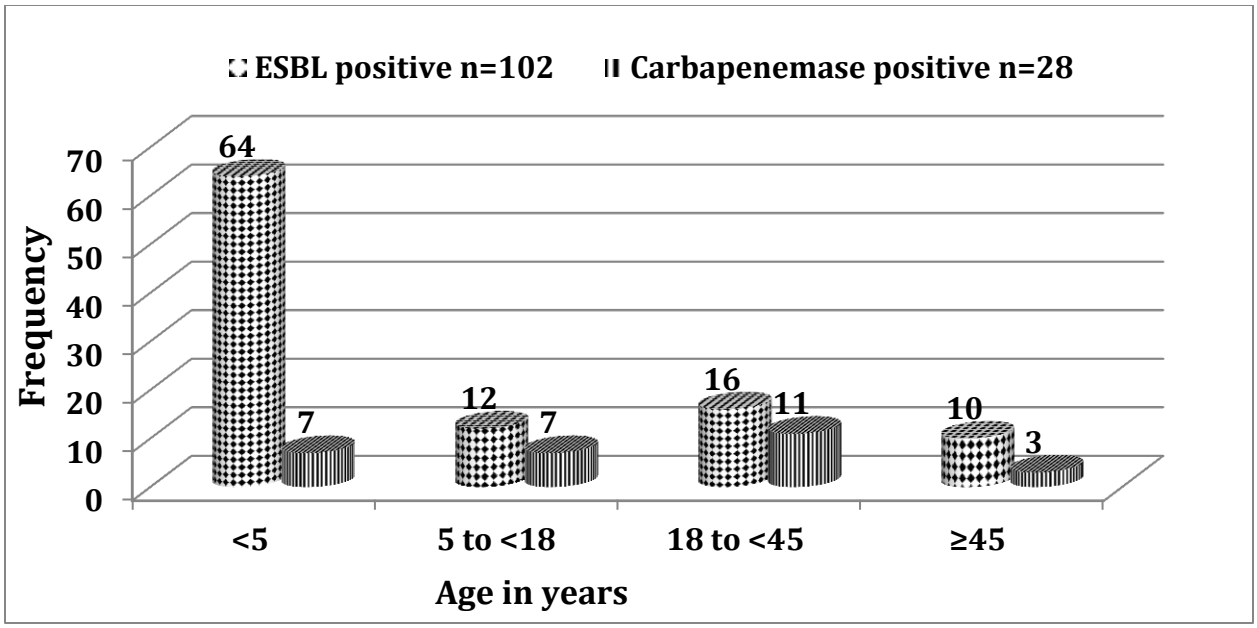


Figure 5.7: Age-wise distribution of ESBL and carbapenemase producing *K. pneumoniae* isolates

Specimen-wise distribution of ESBL and carbapenemase producing *K. pneumoniae* isolates

As displayed in **figure 5.8** blood was the major specimen from which ESBL-producing *K. pneumoniae* were isolated 54/102 (52.9%) followed by urine 24/102 (23.5%). Whereas, urine was the major source of carbapenemase producing *K. pneumoniae* with 10/28 (35.7%), while only 1/28 (3.6%) of carbapenemase producing *K. pneumoniae* was isolated from sputum.

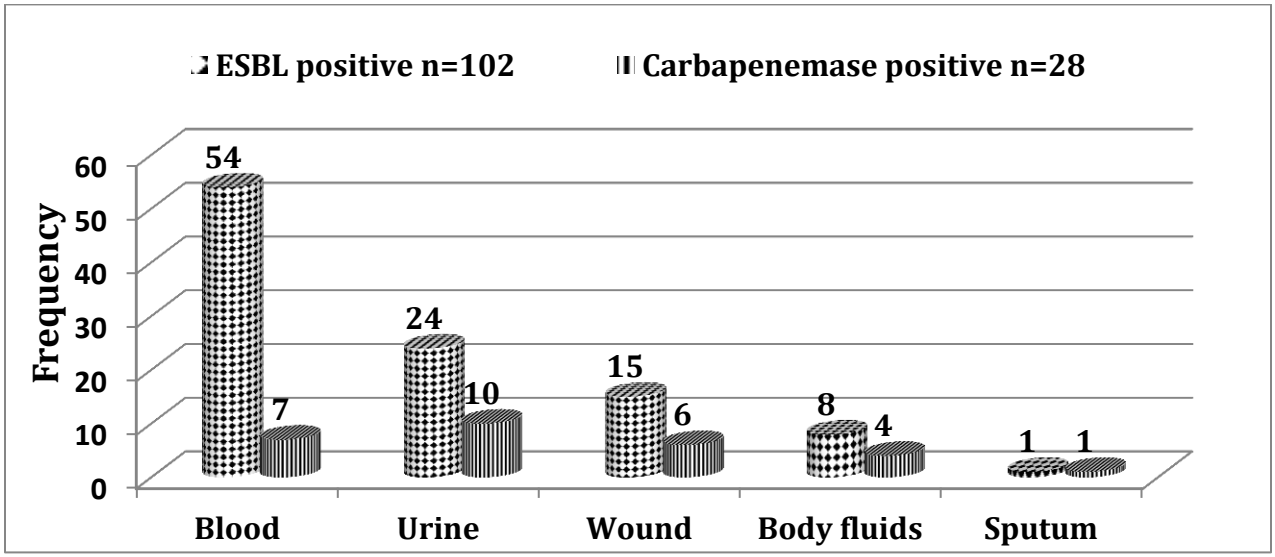


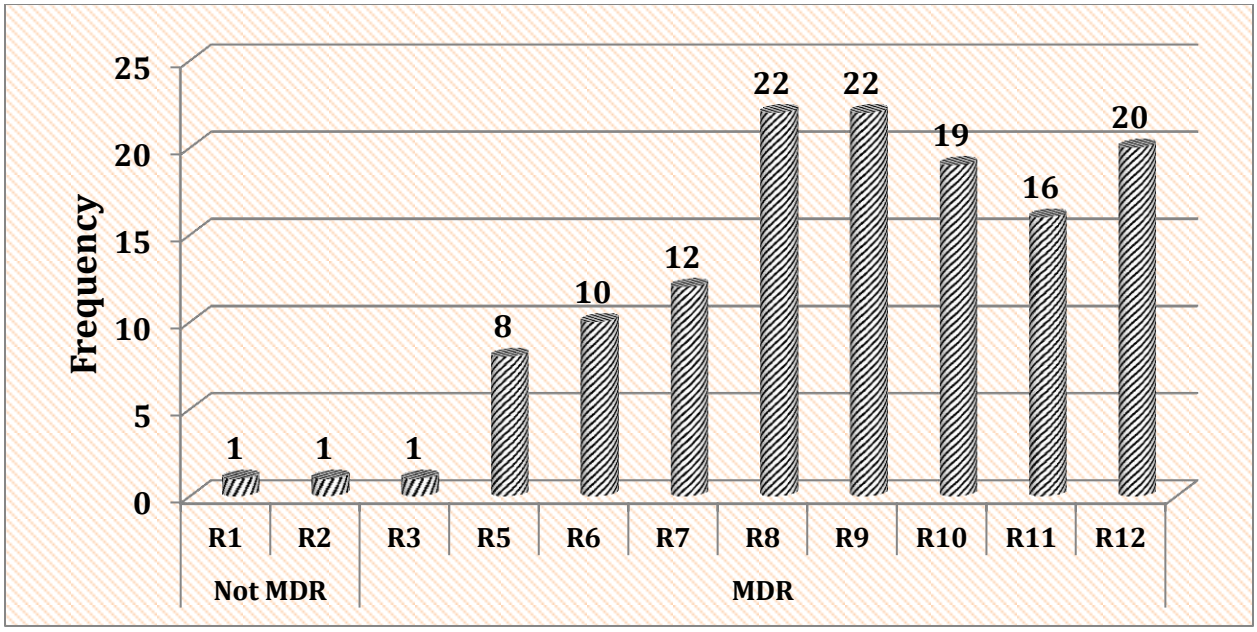
Figure 5.8: Specimen-wise distribution of ESBL and carbapenemase producing *K. pneumoniae* isolates

5.7. Multidrug resistance in *K. pneumoniae* isolates

Magnitude of Multidrug resistance among *K. pneumoniae* isolates

Almost all 130/132 (98.5%) of the isolates were non-susceptible to ≥3 antimicrobials in different categories and hence defined as MDR, where only two isolates were not MDR. All ESBL positive and carbapenemase positive *K. pneumoniae* isolates were MDR.

In our case the MDR range is wide, which encompasses resistance to three (R3) to twelve (R12) antimicrobial categories. Consequently, it was necessary to clearly indicate the number of isolates in each level of MDR. Based on this, from the total isolates, 20/132 (15.2%) showed non-susceptibility to at least one antimicrobial agent in all categories. Of them, two isolates showed complete non-susceptibility to all antimicrobial agents. In addition, 16/132 (12.1%) isolates were non-susceptible to at least one antimicrobial agent in 11 antimicrobial categories. only one isolate was non-susceptible to at least one antimicrobial agent in merely three antimicrobial categories, which is the least MDR (**Figure 5.9**). The details of multidrug resistance patterns of *K. pneumoniae* isolates were presented in **Figure 5.10**. Please note that majority of non-susceptibility observed in the aminoglycoside category in **Figure 5.10** was resulted from gentamicin and tobramycin not due to amikacin as it is presented in **Table 5.2**.



Note:- MDR: Multidrug Resistance, $\mathbf{R_{n:}}$ non-susceptibility to at least one antimicrobial agent in **"n"** antimicrobial categories, where **"n"** is the number of antimicrobial categories.

Figure 5.9: Multidrug resistance levels of *K. pneumoniae* isolates

Level of resistance	Resistance pattern												Frequency
	A	B	C	D	E	F	G	H	I	J	K	L	
R12													20
R11													2
R11													2
R11													3
R11													5
R11													4
R10													1
R10													3
R10													1
R10													1
R10													2
R10													1
R10													3
R10													3
R10													4
R9													1
R9													1
R9													1
R9													6
R9													5
R9													1
R9													3
R9													2
R9													2
R8													1
R8													1
R8													1
R8													1

Level of resistance	Resistance pattern												Frequency
	A	B	C	D	E	F	G	H	I	J	K	L	
R8													1
R8													2
R8													5
R8													2
R8													8
R7													1
R7													1
R7													1
R7													1
R7													1
R7													1
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R6													2
R6													1
R6													3
R6													1
R5													5
R5													1
R5													1
R5													1
R3													1
R2													1
R1													1

The isolates were susceptible to all antimicrobial agents in each antimicrobial category

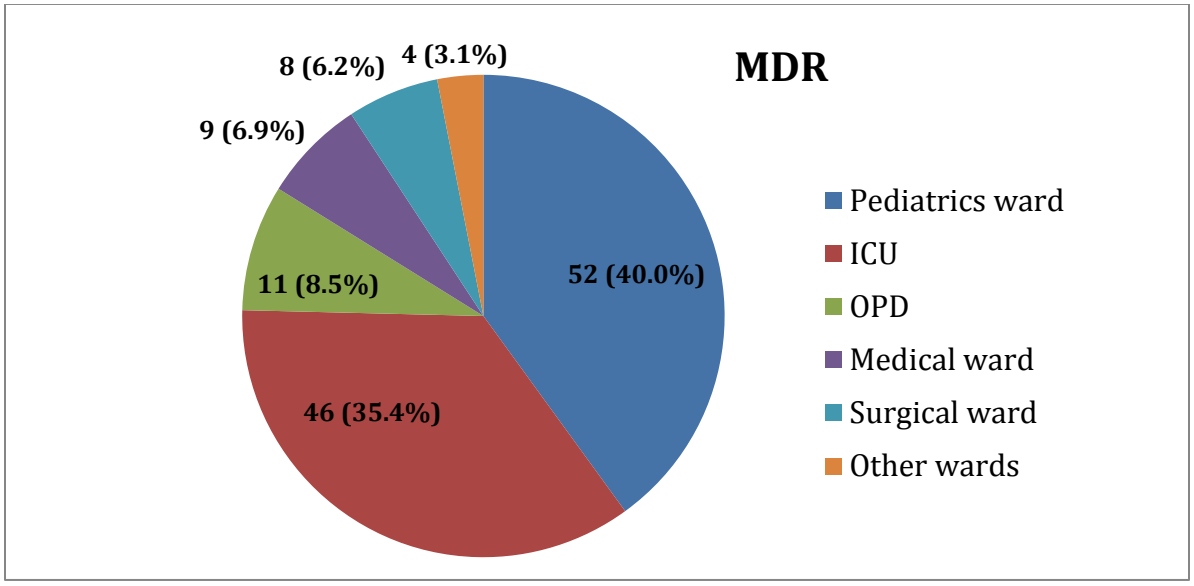
The isolates were non-susceptible to at least one antimicrobial agent in each antimicrobial category

Antimicrobial categories based on Magiorakos *et al.* (2012)**: - A:** Tetracyclines, **B:** Aminoglycosides, **C:** Fluoroquinolones, **D:** 1st and 2nd generation cephalosporins, **E:** Monobactams, **F:** Antipseudomonal penicillins+ß-lactamase inhibitors, **G:** Penicillins+ß-lactamase inhibitors, **H:** Folate pathway inhibitors, **I:** Phenicols, **J:** Cephamycins, **K:** 3rd and 4th generation cephalosporins, **L:** Carbapenems

Figure 5.10: Multidrug resistance patterns of *K. pneumoniae* isolates

Distribution of MDR *K. pneumoniae* isolates among inpatient wards and outpatient

To see the location of MDR *K. pneumoniae* isolates, the distribution of the isolates on different wards and outpatient department were examined. From the total MDR *K. pneumoniae* isolates, the large majority were isolated from patients in pediatric wards 52/130 (40%) and ICUs 46/130 (35.4%) followed by outpatient department 11/130 (8.5%). The least proportion 4/130 (3.1%) of MDR isolates was obtained from those in orthopedics and emergency wards.



Note: - ICUs: Other wards: Emergency (2) and Orthopedics (2), Intensive Care Units, OPD: Outpatient Department

Figure 5.11: Distribution of MDR *K. pneumoniae* isolates among inpatients and outpatient

6. DISCUSSION

Klebsiella pneumoniae is one of the multidrug resistant microorganisms identified as an urgent threat to human health by the World Health Organization (Tacconelli *et al.*, 2017) and Centers for Disease Control and Prevention (CDC, 2013). It has acquired an extensive and versatile range of β-lactamase enzymes capable of hydrolyzing penicillins, cephalosporins and even carbapenems over the past decade (Pendleton *et al.*, 2013). Therefore, it is rapidly becoming untreatable using even last-line antimicrobials especially in hospital settings (Holt *et al.*, 2015) suggesting the need for continuous surveillance of antimicrobial resistance patterns. This work focuses on the magnitude of antimicrobial resistance, ESBL and carbapenemase production rates among clinical *K. pneumoniae* isolates.

6.1. Antimicrobial resistance patterns of *K. pneumoniae* isolates

Third generation cephalosporins have been the treatment of choice for Gram-negative bacteria including *K. pneumoniae* but nowadays they are largely ineffective due to the emergence of ESBL-producing bacteria (Pistella and Santini, 2016). This is also true in our study at which one of the most frequent antimicrobial resistance was against 3rd and 4th generation cephalosporins; ceftriaxone (97%), cefotaxime (97%), cefepime (84.1%) and ceftazidime (65.9%). Likewise, high resistance rates to these antimicrobials was also observed in other recent studies in Ethiopia; 86.4% to cefotaxime, 85.4% to ceftazidime and cefepime in Addis Ababa (Teklu *et al.*, 2019) and 97.6% to ceftazidime, 94.1% to cefepime and 88.2% to ceftriaxone in Bahir Dar (Moges *et al.*, 2019). However, Badamchi *et al*. (2018) from Iran reported resistance rate of 60.2% to cefriaxone, 32.6% cefotaxime and 54.8% cefepime which is lower than the results of our study.

In this study, more than half of the study participants had taken 3rd and 4th generation cephalosporins prior to recruitment to the study. Additionally, a previous study by Gutema *et al.* (2018) found that, three out of four patients in the hospital where we did our study were prescribed antimicrobials empirically, of whom almost 90% were prescribed broad spectrum β-lactams. Therefore, the high resistance to broad spectrum β-lactams might be attributed to the indiscriminate use of these antimicrobials which creates a selective pressure.

Resistance to trimethoprim-sulfamethoxazole was 93.9%, which is comparable with different studies in Ethiopia; 86.4% in Addis Ababa (Teklu *et al.*, 2019), 96.5% in Bahir Dar (Moges *et al.*, 2019), also there was a consistent report from Tunisia 94.06% (Alibi *et al.*, 2015).

Nevertheless, it is higher than a study conducted in Bangladesh 44% (Chakraborty *et al.*, 2016) and Nepal 51.3% (Nepal *et al.*, 2017). High resistance to trimethoprim-sulfamethoxazole may be due to variations in prescription practices and self-medication because of affordability and availability (Geresu *et al.*, 2014).

In the current study, from all tested antimicrobials the best susceptibility (93.2%) was observed to amikacin. Low resistance of *K. pneumoniae* to amikacin was also reported from previous studies in Ethiopia (Siraj *et al.*, 2015;Teklu *et al.*, 2019). The lower resistance to amikacin may be due to its absence of use as empirical therapy and non-existence of considerable cross resistance with β-lactam antimicrobials (Magalhães and Blanchard, 2009).

K. pneumoniae isolates were more susceptible to carbapenems next to amikacin with an overall non-susceptibility rate of 29.6%. Imipenem was the most active carbapenem and revealed a susceptibility rate of 81.1% followed by meropenem 72.7% and ertapenem 70.5%. It is in accordance with a previous study done in Egypt, which reported susceptibility rates of 75% to meropenem and imipenem (El-Badawy *et al.*, 2017). In a study in Addis Ababa, Ethiopia (Teklu *et al.*, 2019), 10.7% of *K. pneumoniae* isolates were resistance to meropenem which is lower than our study. Moreover, there are also studies (Alibi *et al.*, 2015;Henson *et al.*, 2017) at which no resistance was detected to carbapenems, but our study showed a considerable carbapenem resistance considering that carbapenems are the last resort antibiotics. In this study, 15.2% of the study participants had taken carbapenems prior to recruitment to the study. Empirical prescription of carbapenems particularly meropenem was very common in the hospital (unpublished study), which may create resistance and further the emergence of carbapenemase producing bacteria.

Generally, in our study *K. pneumoniae* showed high resistance to most of the antimicrobials tested which is very alarming. A study in *K. pneumoniae* by Breurec *et al*. (2013) in five African and two Vietnamese major towns stated uncontrolled consumption of antimicrobial agents through self-medication, inappropriate antibiotic prescription, the substandard quality of some drugs, and a lack of effective measures to prevent nosocomial infections as key factors for facilitating the spread of antimicrobial resistance among studied countries. Since these conditions could be present in Ethiopia, the high drug resistance observed in the current study could also be due to these factors.

6.2. ESBL producing *K. pneumoniae* isolates

In this study, the magnitude of ESBL producing *K. pneumoniae* from the total isolates was 77.3%. This finding is in a close harmony with other studies in Ethiopia at which ESBL producing *K. pneumoniae* was 78.6% in Addis Ababa (Teklu *et al.*, 2019), 70.4 % in Jimma (Siraj *et al.*, 2015) and 76% in Bahir Dar (Moges *et al.*, 2019). Similarly, a comparable finding was reported from other countries such as 68.3% in South Africa (Perovic *et al.*, 2014), 72.7 % in Uganda (Kateregga *et al.*, 2015) and 81.39% in Iraq (Aljanaby and Alhasnawi, 2017). Conversely, it is higher than studies from Ghana (61.5%) (Obeng-Nkrumah *et al.*, 2013) and India (48.27%) (Shashwati *et al.*, 2014). The high ESBL could be due to the frequent use of broad-spectrum β-lactams which creates a selective pressure and results in emergence of stable, rapidly proliferating ESBL-producing clones with continued horizontal gene transfer across genera (Lukac *et al.*, 2015).

Moreover, a previous study done in the same hospital where we did the current study noted that gastrointestinal colonization of ESBL producing *K. pneumoniae* was as high as 100% in hospitalized neonates, 88% in children and 50% in adults (Desta *et al.*, 2016). Gut colonization with ESBL producing bacteria can lead to extra intestinal infections (Karanika *et al.*, 2016). Given the majority of *K. pneumoniae* isolates in our study were recovered from inpatients and the over crowdedness of the hospital the high magnitude of ESBL producing *K. pneumoniae* may also be due to lack of strong infection control measures and effective antimicrobial resistance surveillance.

In this study, the susceptibility of ESBL producing *K. pneumoniae* to carbapenems was high; 98.0% to imipenem, 89.2% to meropenem and 86.3% to ertapenem. Carbapenems are the drugs of choice to treat ESBL producing *K. pneumoniae*, but a considerable carbapenem resistance was observed in our study. Therefore, they should be used as a therapy of choice for only severe infections with ESBL-producing organisms as recommended by Tamma and Rodriguez-Baño (2017). Utilizing non-carbapenem antimicrobial for the treatment of ESBL producing organisms intends to preserve the therapeutic value of these precious drugs.

In the present study, when the resistance rates of ESBL producing *K. pneumoniae* isolates to β-lactam/β-lactamase inhibitor combinations was compared, resistance to piperacillin/tazobactam (23.5%) was much lower than amoxicillin/clavulanate (54.9%). It was consistent with other studies (Shashwati *et al.*, 2014;Desta *et al.*, 2016). ESBLs are better inhibited by the β-lactamase inhibitor tazobactam than by sulbactam and clavulanate showing clinical resistance to amoxicillin/clavulanate than piperacillin/tazobactam (Bradford, 2001). However, as reported elsewhere β-lactam/β-lactamase inhibitor consumption had a significant temporal correlation with increased resistance to piperacillin/tazobactam in *K. pneumoniae*, suggesting that reducing the misuse of β-lactam/β-lactamase inhibitors may help to use piperacillin/tazobactam as alternative to carbapenems (Ryu *et al.*, 2018) for low to moderate severity infections (Tamma and Rodriguez-Baño, 2017).On the other hand, a recent review highlights piperacillin/tazobactam as suitable alternative to carbapenems for the treatment of many invasive infections caused by ESBL producers if it is active *in vitro* and the dose is adjusted (Rodriguez-Bano *et al.*, 2018).

Of particular concern, non-ESBL producing isolates had higher resistance rates to more than half of the antimicrobials used in our study than that of ESBL producers, which magnifies the burden of drug resistance *K. pneumoniae* in the study setting. The opposite was true in several other studies, in which ESBL producers had higher resistance rates than non-ESBL producers (Obeng-Nkrumah *et al.*, 2013;Sheemar *et al.*, 2016;Teklu *et al.*, 2019). In this study, ESBL negative isolates were highly resistant to carbapenems 53.3% to 83.3% and cefoxitin 90.0%. Moreover, 86.7% of ESBL negative isolates were resistant to piperacillin-tazobactam, amoxicillin-clavulanate, ceftriaxone, cefotaxime, ceftazidime and trimethophrim-sulfamethoxazole. This worrisome resistance profile of *K. pneumoniae* could be due to the circumstance that majority (73.3%) of the ESBL negative isolates in our study were carbapenemase producers. In fact, carbapenemase producing Gram-negatives in particular are resistant to all or almost all beta-lactams, fluoroquinolones and/or aminoglycosides concomitantly (Meletis, 2016).

In our study, the proportion of ESBL positive *K. pneumoniae* was higher in those aged below 5 years than in those aged 18 to < 45 years. This could be because, as it was reported by Desta *et al.* (2016) the proportion of ESBL producing *K. pneumoniae* colonization was higher in these age groups which could result in infection with these bacteria, if infection control measures were not adequately implemented.

Higher proportion of ESBL production was observed among those admitted to the intensive care units than those in medical wards. A study by Marra *et al*. (2006) also reported a higher proportion of patients with ESBL-producing nosocomial blood stream infections in the ICUs (Marra *et al.*, 2006). This could be due to the reason that hospitalized patients in the ICU are more likely to be exposed to heavy load of antimicrobials, long-term hospitalization and extensive usage of invasive devices.

6.3. Carbapenemase-producing *K. pneumoniae* isolates

There were few studies on carbapenemases in Ethiopia and almost all of them noted *K. pneumoniae* as the most common carbapenemase producer compared to other bacterial isolates (Eshetie *et al.*, 2015;Legese *et al.*, 2017;Moges *et al.*, 2019). In our study, the magnitude of carbapenemase producing *K. pneumoniae* was 21.2%, which is in close agreement with a surveillance study in Italy, where 17.5% of *K. pneumoniae* were carbapenemase producers (Corcione *et al.*, 2015). It is lower than a report by Kazemian *et al.* (2019) from Iran at which 43.3% of *K. pneumoniae* from hospitalized patients were carbapenemase producers, and 48 % in Greece surveillance study (Spyropoulou *et al.*, 2016). Nevertheless, our finding is higher than 10.8% in Bahir Dar, Ethiopia (Moges *et al.*, 2019) and 11.4% in Tunisian and Libyan hospitals (Mathlouthi *et al.*, 2016). Given the association of carbapenem use with carbapenemase production in this study; the higher carbapenemase production could be due to selective pressure created by the indiscriminate use of carbapenems. Moreover, it might be due to improper infection control practices as the emergence of carbapenemase-producing *K. pneumoniae* was previously noted in the hospital (Desta *et al.*, 2016;Legese *et al.*, 2017).

From the total isolates, 6 (4.6%) were positive for both carbapenemases and ESBL, simultaneously. Combined production of ESBL and carbapenemase in *K. pneumoniae* isolates was also observed by Ibrahim *et al.* (2017). In fact, genes encoding carbapenemase and ESBL can be located on the same plasmids (Pitout *et al.*, 2015;Lee *et al.*, 2016).

In our study, relatively higher susceptibility (71.4%) of carbapenemase producers was noted to amikacin, which is comparable (78.8%) with a study in Taiwan (Chiu *et al.*, 2018) suggesting the possible use of this drug against carbapenemase producers. Nonetheless, complete non-susceptibility of carbapenemase producing *K. pneumoniae* isolates to aztreonam, amoxicillin-

clavulanate, pipracillin-tazobactam, cefoxitin, ceftriaxone, cefotaxime, ceftazidime, cefepime, meropenem and ertapenem was observed, which is in line with a report from Taiwan (Chiu *et al.*, 2018). This might be due to the simultaneous presence of several resistance genes in these isolates.

Karaiskos and Giamarellou (2014) reported that the world-wide emergence of carbapenemases mediated carbapenem resistance in *Klebsiella pneumoniae*, *Acinetobacter baumannii* and *Pseudomonas aeruginosa* with a mortality exceeding 50%, is attributed mainly to the lack of effective antimicrobial regimens. Although they have their own limitations in accessibility, resistance, clinical efficacy and adverse effects; colistin, tigecycline, fosfomycin, temocillin and newer underdevelopment antimicrobials including carbapenemase inhibitors alone or in combination exhibit promising and/or effective antibacterial activity in vitro and some in vivo against infections caused by carbapenemase producing bacteria (Karaiskos and Giamarellou, 2014;Pitout *et al.*, 2015).

6.4. Multidrug resistance among *K. pneumoniae* isolates

In our study almost all (98.5%) *K. pneumoniae* isolates were MDR, which is comparable with a study conducted at Gondar, Ethiopia (95.6%) from patients with urinary tract infections (Eshetie *et al.*, 2015) and Iraq 100% (Aljanaby and Alhasnawi, 2017). On the contrary, our result is higher than studies conducted in Addis Ababa, 83.5% (Teklu *et al.*, 2019) and Bahir Dar, 87.6% (Moges *et al.*, 2019). This high prevalence might be because the majority (90.91%) of *K. pneumoniae* isolates were collected from hospitalized patients, where many antimicrobials being prescribed in the hospital, which serves as a selective pressure for drug resistance. Moreover, in this study, more antimicrobial agents were covered in the assessment based on recommendations of Magiorakos *et al*. (2012), which increases the detection of MDR.

All ESBL-producing *K. pneumoniae* isolates were MDR, which is in agreement with a study in China (Li *et al.*, 2012). Similarly, all carabapenemase producers were MDR. This could be explained by the fact that ESBL and carbapenemse genes are mostly located on plasmids with other resistance genes, leaving only a few effective drugs available for treatment (Pitout *et al.*, 2015;Navon-Venezia *et al.*, 2017). To this end, the implementation of strong antimicrobial stewardship programs together with effective infection control and antimicrobial surveillance practices is highly needed.

Limitation of the study

- ✓ Last line antimicrobials such as polymyxins, tigecycline and fosfomycin were not tested due to unavailability.
- ✓ Detection of AmpC β-lactamase was not done due to unavailability of necessary/required antimicrobial discs for performing the test.

Strength of the study

- ✓ The study included a large number of isolates
- ✓ Antimicrobial susceptibility test was done using a wide variety of antimicrobial agents to increase identification of multidrug resistance isolates based on recommendations of Magiorakos *et al*. (2012).
- ✓ Confirmation of ESBL and carbapenemase production was done using combined disc test and modified carbapenemase inactivation method, respectively, based on CLSI (2018) recommendation.

7. CONCLUSIONS AND RECOMMENDATIONS

7.1. Conclusions

K. pneumoniae isolates showed high resistance to most of the antimicrobials used. Better susceptibility was observed to amikacin and carbapenems. The magnitude of ESBL and carbapenemase production as well as multidrug resistance among *K. pneumoniae* isolates was very alarming. Almost all (98.5%) of the isolates were multidrug resistant. Similarily, most (93.9%) of the isolates were positive to either ESBL, carbapenemase or both. Of particular concern, majority of ESBL negatives were carbapenemase positive. Carbapenems and amikacin, followed by pipracillin-tazobactam, were the most active drugs against ESBL producing isolates. However, only amikacin was relatively active against carbapenemase producing isolates. Higher proportion of ESBL production was observed in those aged below 5 years than those 18 to 45 years and among those admitted to the intensive care units (ICUs) and pediatric wards than those in medical wards. Previous use of carbapenems was associated with carbapenemase production.

7.2. Recommendations

Based on the above findings, the following recommendations are made:-

- ✓ Better prevention and control strategies should be designed in the hospital to curtail the problem
- ✓ Detection of ESBL and carbapenemase production should be done in the hospital regularly for the better management of patients and to monitor further progress in control of antimicrobial resistance
- ✓ The hospital managers/clinical directors should work hand-in-hand with the appropriate stakeholders in strengthening the antimicrobial stewardship program to reduce the magnitude of multidrug resistant *K. pneumoniae*
- ✓ Strengthening the antimicrobial surveillance program is highly needed
- ✓ Large scale studies on multiple study sites and molecular characterization of resistance genes should be conducted

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Unpublished study by Theodros Fenta, Addis Ababa University Repository (2018)

ANNEXES

Annex Ia: Participant Information Sheet for Adults (English version)

Title of the study: Phenotypic Antimicrobial Resistance Profiles of *Klebsiella pneumoniae* Isolated from Patients at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia**.**

Principal Investigator: Tewachew Awoke

Name of the Sponsor: Addis Ababa University and Armauer Hansen Research Institute

Introduction: Hello**.** We are doing a research on Phenotypic Antimicrobial Resistance Profiles of *Klebsiella pneumoniae* Isolated from Patients at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia**.** This is MSc research work. Please read or has study staff read this text to you and take your time to decide to participate in this study.

Purpose of the study: The purpose of this study is to determine the antimicrobial resistance patterns and associated resistance genes of multidrug resistant *K. pneumoniae* isolated from patients in the Hospital. The result of this study will help policy makers and hospital managers for planning, implementing and evaluating various interventions for management of drug resistance that further prevent possible occurrence of nosocomial outbreaks due to this and other bacteria. Since its drug resistance pattern in the study area is still remain poorly understood; this study will indicate antimicrobials that should be used for the empirical treatment of infections caused by these bacteria in case of urgency and limited resources.

Procedure: For this study to be successful we need your participation provided that you are willing that the isolated bacteria to be used for this research, to respond questionnaires and to take clinical information from your medical records which are relevant to the study.

Confidentiality: All personal information you give and data obtained from laboratory analysis will be kept confidential. All the data will be coded with numbers without names.

Expected benefits: There is no payment that you will be given for participating in this research. However, the result will be reported to your physician for appropriate treatment and management. The study will have importance in the management of future patients. Moreover, your participation in this study will have a great value on preventive measures in hospitals and in the community.

Risks: There is no more than minimal risk for participating in this study except that you will spend a maximum of 15 minutes for interview.

Right to Refusal or Withdraw: Your participation in the study is absolutely voluntary; you have full right to refuse from participating in this research. You can ask any question which is not clear for understanding and skip any or all the questionnaires, if you don't want to respond and this will not affect you on using any kind of services from the hospital.

Person to Contact: Thank you for taking time to read the information sheet/ listen as it is being read to you. If you have question or problem related with the present study, you can contact the principal investigator at any time using the following address:

Mr. Tewachew Awoke Mobile: +251918149901 **E-mail:** tewaslike@gmail.com
Department Medical Microbiology, Immunology and Parasitology, College of Health Sciences, Addis Ababa University

Or you can contact ALERT /AHRI Ethical Review Committee 0113481289

Annex Ib፡<u>በአማርኛ የተዘጋጀ ዕድሜያቸዉ 18 እና ከዚህ በላይ ለሆኑ የጥናቱ ተሳታፊዎች የመረጃ ቅጽ</u>

የጥናቱ ርዕስ፡ በአዲስ አበባ ከተማ በጥቁር አንበሳ እስፔሻላይዝድ ሆስፒታል የመጡ ታካሚዎች ላይ የተገኘ ከለብሴላ ኒሞኔ ባክቴሪያን መዳኒትን የመቋቋም ዉጫዊ እና ዉስጣዊ ባህሪይዉን ማጥናት

የዋና አጥኝው ስም፡ ተዋቸዉ አወቀ

የጥናቱ ደጋፊ ፡- አዲስ አበባ ዩንቨርሲቲ እና አርማወር ሀንሰን የጥናት እና የምርምር ማከል

አጠቃላይ መረጃ፡ ጤና ይስጥልኝ ፡ በጥቁር አንበሳ እስፔሻላይዝድ ሆስፒታል የመጡ ታካሚዎች ላይ የተገኘ ከለብሴላ ኒሞኔ ባክቴሪያን መዳኒትን የመቋቋም ዉጫዊ እና ዉስጣዊ ባህሪይዉን ለማዎቅ የሚካሄድ የማስተርስ ጥናት ነው፡፡ እባከዎ በጥናቱ ለመሳተፍ ከመወሰንዎ በፊት ይህንን ቅጽ በትክክል ያንብቡ ወይም ሲነበብልዎ በትክክል ያድምጡ፤ እንዱሁም ግልፅ ያልሆነለዎትን ነገር በሙሉ ነፃነት ይጠይቁ።

የጥናቱ አላማ፡ በጥቁር አንበሳ እስፔሻላይዝድ ሆስፒታል የመጡ ታካሚዎች ላይ የተገኘ ከለብሴላ ኒሞኔ ባክቴሪያን መዳኒትን የመቋቋም አቅሙን፤ የትኞችን መዳኒቶች እነደሚቋቋም እና እንዲቋቋም ያስቻላዉን ዉስጣዊ ባህርይ ምን እንደሆነ አዉቆ ለህዋሱ ተመራጭ የሆኑትን መድሃኒቶችን ለማመልከት እና የሁስፒታሉ ሃላፊዎች አንዲሁም የህብረተሰቡን ጤና ለመጠበቅ ለተቋቋሙ አካላት ስርጭቱን እና በዚህ ባክቴሪያ ምክኒያት ሊመጣ የሚችል ወረርሽኝን ለመግታት አስፈላጊዉን እረምጃ እንዲወስዱ ለማሳወቅ ነዉ፡፡ በጥቁር አንበሳ እስፔሻላይዝድ ሆስፒታል የዚህ ባክቴሪያ መዳኒትን የመቋቋም አቅም በዉሉ አይታወቅም፡፡ ስለሆነም ይህ ጥናት በዚህ ባክቴሪያ ምክኒያት የሚመጣ በሽታን ለማከም የትኛዉ መዳኒት ተመራጭ እንደሆነ ያሳያል፡፡

የአሰራር ሂደት፡- በጥናቱ በሙሉ-ፈቃደኝነት እንዲሳተፉ እየጠየቆምን በጥናቱ በመሳተፍ ፈቃደኛ ከሆኑ ከእርስዎ የተገኘዉን ባክቴሪያ ለምረምሩ እንድንጠቀምበት፤ ከህክምና ካርድዎ ለጥናቱ አስፈላጊ ከሆነ መረጃ እንድንወስድ እንዲሁም ከህመምዎት ጋር በተያያዘ ለሚቀርብለዎት መጠይቅ ምላሽ እንዲሰጡ በትህትና እንጠይቃለን።

ሚስጥራዊነት፡- የሚሰጡት መረጃ በጥናቱ ወቅትም ሆነ ከዛ በኃላ ባሉት ጊዜያት ሙሉ በሙሉ ሚስጥራዊነቱ የሚጠበቅና መረጃውም የሚያዘዉ በስም ሳይሆን በመለያ ቁጥር ይሆናል፡፡

በጥናቱ በመሳተፍዎ ያለው ጉዳት ፡- በዚህ ጥናት ላይ በመሳተፍዎ በእርስዎ ላይ ሊደርስብዎ የሚችል ምንም ጉዳት አይኖርም፡፡ ለአስራ አምስት ደቂቃ ብቻ ከኛ ጋር ይቆያሉ፡፡

የሚያገኙት ጥቅም፡- በጥናቱ ለሚሳተፉ ፍቃደኛ ተሳታፊዎች ምንም አይነት የገንዘብ ክፍያ የለም። ነገር ግን ዉጤቱ ለሚከታተሉዋቸው ሀኪም እንዲደርሰዉ ይደረጋል፡፡ በተጨማሪም ለወደፊት የሚመጡ ታካሚዎችን አያያዝ ለማስተካከል ይረዳል፡፡የእርሰዎ በዚህ ጥናት ተሳታፊ መሆን በሆስፒታሉ ላሉ ታካሚዎች እንዲሁም ለማህበረሰቡ በዚህ ባክቴሪያ እንዳይያዙ ቅድመ መከላከል ለማድረግ ከፍተኛ ጠቀሜታ አለው፡፡

የመዉጣት (የማቋረጥ) መብት ፡- በጥናቱ በሙሉ ፈቃደኝነት እንዲሳተፉ እያስታወስን በጥናቱ ላይ እያሉ ለሚጠየቁት ጥያቄ ግለጽ ያልሆነልት ካለ የመጠየቅ፤ መልስ ያለመስጠት፤ አንዲሁም በፈለጉት ጊዜ የማቆም ወይም የማቋረጥ መብት አለዎት፡፡ ይህን በማድረግዎ ከሆስፒታሉ የሚያገኙትን ማነኛውንም አገልግሎት አይገድብም፡፡

አድራሻ፡ ይህን የመረጃ ቀጽ ስላነበቡ ወይም ሲነበብልዎ ስላዳመጡ እያመሰገን ማንኛውም ጥያቄ ካለዎት ይህንን አድራሻ ይጠቀሙ፡

የዋናው አጥኝዉ አድራሻ፡

ተዋቸዉ አወቀ ስልክ፡<u>+251918149901</u> **ኢ-ሜይል:** tewaslike@gmail.com

አዲስ አበባ ዩንቨርስቲ ጤና ሳይንስ ኮሌጅ ሜዲካል ማይክሮባዮሎጅ፣ኢሚኖሎጅ እና ፓራስቶሎጅ ት/ት ክፍል

አህሪ/አለርት ኢቲክስ ሪቪው ኮሚቴ ስልክ፡0113 48128

Annex IIa: Participant Information Sheet for Adolescents (12-17 years old) (English version)

Title of the study: Phenotypic Antimicrobial Resistance Profiles of *Klebsiella pneumoniae* Isolated from Patients at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia**.**

Principal Investigator: Tewachew Awoke

Name of the Sponsor: Addis Ababa University and Armauer Hansen Research Institute

Introduction: Hello**.** We are doing a research on Phenotypic Antimicrobial Resistance Profiles of *Klebsiella pneumoniae* Isolated from Patients at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia**.** This is MSc research work. We will ask your parents/guardian to approve your participation in this study. Please read or has study staff read this text to you and take your time to decide to participate in this study.

Purpose of the study: The purpose of this study is to determine the antimicrobial resistance patterns and associated resistance genes of multidrug resistant *K. pneumoniae* isolated from patients in the Hospital. The result of this study will help policy makers and hospital managers for planning, implementing and evaluating various interventions for management of drug resistance that further prevent possible occurrence of nosocomial outbreaks due to this and other bacteria. Since its drug resistance pattern in the study area is still remain poorly understood; this study will indicate antimicrobials that should be used for the empirical treatment of infections caused by these bacteria in case of urgency and limited resources.

Procedure: For this study to be successful we need your participation provided that you are willing that the isolated bacteria to be used for this research, to respond questionnaires and to take clinical information from your medical records which are relevant to the study.

Confidentiality: All personal information you give and data obtained from laboratory analysis will be kept confidential. All the data will be coded with numbers without names.

Expected benefits: There is no payment that you will be given for participating in this research. However, the result will be reported to your physician for appropriate treatment and management. The study will have importance in the management of future patients. Moreover, your participation in this study will have a great value on preventive measures in hospitals and in the community.

Risks: There is no more than minimal risk for participating in this study except that you will spend a maximum of 15 minutes for interview.

Right to Refusal or Withdraw: Your participation in the study is absolutely voluntary. You have full right to refuse from participating, even if your parents/guardian permits to be enrolled in this study. You can ask any question which is not clear for understanding and skip any or all the questionnaires, if you don't want to respond and this will not affect you on using any kind of services from the hospital.

Person to Contact: Thank you for taking time to read the information sheet/ listen as it is being read to you. If you have question or problem related with the present study, you can contact the principal investigator at any time using the following address:

Mr. Tewachew Awoke Mobile: +251918149901 E-mail: tewaslike@gmail.com
Department Medical Microbiology, Immunology and Parasitology, College of Health Sciences, Addis Ababa University

Or you can contact ALERT /AHRI Ethical Review Committee 0113481289

Annex IIb: <u>በአማርኛ የተዘጋጀ ዕድሜያቸዉ ከ12 እስከ 17 ዓመት ለሆኑ ታዳጊዎች የጥናቱ ተሳታፊዎች የመረጃ ቅጽ</u>

የጥናቱ ርዕስ: በአዲስ አበባ ከተማ በጥቁር አንበሳ እስፔሻላይዝድ ሆስፒታል የመጡ ታካሚዉች ላይ የተገኘ ክለብሴላ ኒሞኔ ባክቴሪያን መዳኒትን የመቋቋም ዉጫዊ እና ዉስጣዊ ባህሪይዉን ማጥናት

የዋና አጥኝው ስም: ተዋቸዉ አወቀ

የጥናቱ ደጋፊ :- አዲስ አበባ ዩንቨርሲቲ እና አርማወር ሀንሰን የጥናት እና የምርምር ማከል

አጠቃላይ መረጃ: ጤና ይስጥልኝ ፡ በጥቁር አንበሳ እስፔሻላይዝድ ሆስፒታል የመጡ ታካሚዉች ላይ የተገኘ ክለብሴላ ኒሞኔ ባክቴሪያን መዳኒትን የመቋቋም ዉጫዊ እና ዉስጣዊ ባህሪይዉን ለማዎቅ የሚካሄድ የማስተርስ ጥናት ነው፡፡ ወላጆችህን/ወላጆችሽን ወይም ሞግዚትህን/ሞግዚትሽን በጥናቱ ውስጥ መሳተፍ/አለመሳተፍ እንደምትችል/እንደምትችይ እንጠይቃለን፡፡ በጥናቱ ለመሳተፍ ከመወሰንዎ በፊት ይህንን ቅጽ በትክክል ስላነበብህ/ሽ ወይም ሲነበብህ/ሽ በትክክል አዳምጥ/ጭ፤ እንዲሁም ግልፅ ያልሆነልህን/ሽን ነገር በሙሉ ነፃነት ጠይቅ/ቂ።

የጥናቱ አላማ: በጥቁር አንበሳ እስፔሻላይዝድ ሆስፒታል የመጡ ታካሚዉች ላይ የተገኘ ክለብሴላ ኒሞኔ ባክቴሪያን መዳኒትን የመቋቋም አቅሙን፤ የትኞችን መዳኒቶች እነደሚቋቋም እና እንዲቋቋም ያስቻላዉን ዉስጣዊ ባህርይ ምን እንደሆነ አዉቆ ለህዋሱ ተመራጭ የሆኑትን መድሃኒቶችን ለማመልከት እና የሁስፒታሉ ሃላፊዎች አንዲሁም የህብረተሰቡን ጤና ለመጠበቅ ለተቋቋሙ አካላት ስርጭቱን እና በዚህ ባክቴሪያ ምክኒያት ሊመጣ የሚችል ወረርሽኝን ለመግታት አስፈላጊዉን እረምጃ እንዲወስዱ ለማሳወቅ ነዉ፡፡በጥቁር አንበሳ እስፔሻላይዝድ ሆስፒታል የዚህ ባክቴሪያ መዳኒትን የመቋቋም አቅም በዉሉ አይታወቅም፡፡ስለሆነም ይህ ጥናት በዚህ ባክቴሪያ ምክኒያት የሚመጣ በሽታን ለማከም የትኛዉ መዳኒት ተመራጭ እንደሆነ ያሳያል፡፡

የአሰራር ሂደት:- በጥናቱ በሙሉ-ፈቃደኝነት እንድትሳተፍ/ፊ እየጠየቆምን በጥናቱ ለመሳተፍ ፈቃደኛ ከሆንህ/ሽ ከአንተ/አንች የተገኘዉን ባክቴሪያ ለምረምሩ እንድንጠቀምበት፤ ከህክምና ካርድዎ ለጥናቱ አስፈላጊ ከሆነ መረጃ እንድንወስድ እንዲሁም ከህመምህ/ሽ ጋር በተያያዘ ለሚቀርብልህ/ሽ መጠይቅ ምላሽ እንድትስጥ/ጭ በትህትና እንጠይቃለን።

ሚስጥራዊነት:- የምትሰጠዉ/ጭዉ መረጃ በጥናቱ ወቅትም ሆነ ከዛ በኃላ ባሉት ጊዜያት ሙሉ በሙሉ ሚስጥራዊነቱ የሚጠበቅና መረጃውም የሚያዘዉ በስም ሳይሆን በመለያ ቁጥር ይሆናል፡፡

በጥናቱ በመሳተፍዎ ያለው ጉዳት :- በዚህ ጥናት ላይ በመሳተፍህ/ሽ ሊደርስብህ/ሽ የሚችል ምንም ጉዳት አይኖርም፡፡ ለአስራ አምስት ደቂቃ ብቻ ከኛ ጋር ትቆያለህ፡፡

የሚያገኙት ጥቅም:- በጥናቱ ለሚሳተፉ ፈቃደኛ ተሳታፊዎች ምንም አይነት የገንዘብ ክፍያ የለም።ነገር ግን ዉጤቱ ለሚከታተሊቸዉ ሀኪም እንዲደርሰዉ ይደረጋል፡፡ በተጨማሪም ለወደፊት የሚመጡ ታካሚዎችን አያያዝ ለማስተካከል ይረዳል፡፡የእርሰዎ በዚህ ጥናት ተሳታፊ መሆን በሆስፒታሉ ላሉ ታካሚዎች እንዲሁም ለማህበረሰቡ በዚህ ባክቴሪያ እንዳይያዙ ቅድመ መከላከል ለማድረግ ከፍተኛ ጠቀሜታ አለው፡፡

የመዉጣት (የማቋረጥ) መብት :- በጥናቱ በሙሉ ፈቃደኝነት እንድትሳተፍ/ፊ እያስታወስን ወላጆችህ/ወላጆችሽ ወይም ሞግዚትህ/ሞግዚትሽ ቢፈቅድልህም/ሽም በጥናቱ ያለመሳተፍ መብት አለህ/አለሽ፡፡ በጥናቱ ላይ እያሉ ለሚጠየቁት ጥያቄ ግለጽ ያልሆነልት ካለ የመጠየቅ፤ መልስ ያለመስጠት፤ አንዲሁም በፈለጉት ጊዜ የማቆም ወይም የማቋረጥ መብት አለዎት፡፡ ይህን በማድረግዎ ከሆስፒታሉ የሚያገኙትን ማነኛውንም አገልግሎት አይገድብም፡፡

አድራሻ: ይህን የመረጃ ቅጽ ስላነበብህ/ሽ ወይም ሲነበብህ/ሽ ስላዳመጥህ/ሽ እያመሰገን ማንኛውም ጥያቄ ካለህ/ሽ ይህንን አድራሻ ተጠቀም/ሚ:

የዋናው አጥኝዉ አድራሻ:
ተዋቸዉ አወቀ ስልክ:+251918149901 ኢ-ሜይል: tewaslike@gmail.com
አዲስ አበባ ዩንቨርስቲ ጤና ሳይንስ ኮሌጅ ሜዲካል ማይክሮባዮሎጅ፣ኢሚኖሎጅ እና ፓራስቶሎጅ ት/ት ክፍል
አህሪ/አለርት ኢቲክስ ሪቪው ኮሚቴ ስልክ:0113 481285

Annex IIIa: Participant Information Sheet for Parent/Guardian (English version)

Title of the Study: Phenotypic Antimicrobial Resistance Profiles of *Klebsiella pneumoniae* Isolated from Patients at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia**.**

Principal Investigator: Tewachew Awoke

Name of the Sponsor: Addis Ababa University and Armauer Hansen Research Institute

Introduction: Hello**.** We are doing a research on Phenotypic Antimicrobial Resistance Profiles of *Klebsiella pneumoniae* Isolated from Patients at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia**.** This is MSc research work. Please read or has study staff read this text to your daughter's/son's and take your time to decide on your daughter's/son's participation in this study.

Purpose of the study: The purpose of this study is to determine the antimicrobial resistance patterns and associated resistance genes of multidrug resistant *K. pneumoniae* isolated from patients in the Hospital. The result of this study will help policy makers and hospital managers for planning, implementing and evaluating various interventions for management of drug resistance that further prevent possible occurrence of nosocomial outbreaks due to this and other bacteria. Since its drug resistance pattern in the study area is still remain poorly understood; this study will indicate antimicrobials that should be used for the empirical treatment of infections caused by these bacteria in case of urgency and limited resources.

Procedure: For this study to be successful we need your daughter's/son's participation provided that you are willing that the isolated bacteria to be used for this research, to respond questionnaires and to take clinical information from your daughter's/son's medical records which are relevant to the study.

Confidentiality: All personal information about your daughter's/son's and data obtained from laboratory analysis will be kept confidential. All the data will be coded with numbers without names.

Expected benefits: There is no payment that your daughter's/son's will be given for participating in this research. However, the result will be reported to your daughter's/son's physician for appropriate treatment and management. The study will have importance in the management of future patients. Moreover, your daughter's/son's participation in this study will have a great value on preventive measures in hospitals and in the community.

Risks: There is no more than minimal risk for participating in this study except that you will spend a maximum of 15 minutes for interview.

Right to Refusal or Withdraw: Your daughter's/son's participation in the study is absolutely voluntary; you have full right to refuse from participating in this research. you can ask any question which is not clear for understanding and skip any or all the questionnaires, if you don't want to respond and this will not affect your daughter's/son's on using any kind of services from the hospital.

Person to Contact: Thank you for taking time to read the information sheet/ listen as it is being read to you. If you have question or problem related with the present study, you can contact the principal investigator at any time using the following address:

Mr. Tewachew Awoke Mobile: +251918149901 E-mail: tewaslike@gmail.com
Department Medical Microbiology, Immunology and Parasitology, College of Health Sciences, Addis Ababa University

Or you can contact ALERT /AHRI Ethical Review Committee 0113481289

Annex IIIb፡<u>በአማርኛ የተዘጋጀ በወላጅ/አሳዳጊ የሚሰጥ የመረጃ ቅጽ</u>

የጥናቱ ርዕስ፡ በአዲስ አበባ ከተማ በጥቁር አንበሳ እስፔሻላይዝድ ሆስፒታል የመጡ ታካሚዎች ላይ የተገኘ ክለብሴላ ኒሞኔ ባክቴሪያን መዳኒትን የመ**ቋቋ**ም ዉጫዊ እና ዉስጣዊ ባህሪይዉን ማጥናት

የዋና አጥኝው ስም፡ ተዋቸዉ አወቀ

የጥናቱ ደጋፊ :- አዲስ አበባ ዩንቨርሲቲ እና አርማወር ሀንሰን የጥናት እና የምርምር ማከል

አጠቃላይ መረጃ፡ ጤና ይስጥልኝ ፡ በጥቁር አንበሳ እስፔሻላይዝድ ሆስፒታል የመጡ ታካሚዎች ላይ የተገኘ ክለብሴላ ኒሞኔ ባክቴሪያን መዳኒትን የመ**ቋቋ**ም ዉጫዊ እና ዉስጣዊ ባህሪይዉን ለማዎቅ የሚካሄድ የማስተርስ ጥናት ነው፡፡ እባክዎ ልጅዎ በጥናቱ እንዲሳተፍ ከመወሰንዎ በፊት ይህንን ቅጽ በትክክል ያንብቡ ወይም ሲነበብልዎ በትክክል ያድምጡ፤ እንዱሁም ግልፅ ያልሆነለዎትን ነገር በሙሉ ነፃነት ይጠይቁ።

የጥናቱ አላማ፡ በጥቁር አንበሳ እስፔሻላይዝድ ሆስፒታል የመጡ ታካሚዎች ላይ የተገኘ ክለብሴላ ኒሞኔ ባክቴሪያን መዳኒትን የመ**ቋቋ**ም አቅሙን፤ የትኞችን መዳኒቶች እነደሚ**ቋቋ**ም እና እንዲ**ቋቋ**ም ያስቻላዉን ዉስጣዊ ባህርይ ምን እንደሆነ አዉቆ ለህዋሱ ተመራጭ የሆኑትን መድሃኒቶችን ለማመልከት እና የሁስፒታሉ ሃላፊዎች አንዲሁም የህብረተሰቡን ጤና ለመጠበቅ ለተ**ቋቋ**ሙ አካላት ስርጭቱን እና በዚህ ባክቴሪያ ምክኒያት ሊመጣ የሚችል ወረርሽኝን ለመግታት አስፈላጊዉን እረምጃ እንዲወስዱ ለማሳወቅ ነዉ፡፡በጥቁር አንበሳ እስፔሻላይዝድ ሆስፒታል የዚህ ባክቴሪያ መዳኒትን የመ**ቋቋ**ም አቅም በዉሉ አይታወቅም፡፡ስለሆነም ይህ ጥናት በዚህ ባክቴሪያ ምክኒያት የሚመጣ በሽታን ለማከም የትኛዉ መዳኒት ተመራጭ እንደሆነ ያሳያል፡፡

የአሰራር ሂደት፡- በጥናቱ በሙሉ-ፈቃደኝነት እንዲሳተፉ እየጠየቆምን በጥናቱ በመሳተፍ ፈቃደኛ ከሆኑ ከልጅዎ የተገኘዉን ባክቴሪያ ለምረምሩ እንድንጠቀምበት፤ ከህክምና ካርድ ለጥናቱ አስፈላጊ ከሆነ መረጃ እንድንወስድ እንዲሁም ከህመሙ ጋር በተያያዘ ለሚቀርብለዎት መጠይቅ ምላሽ እንዲሰጡ በትህትና እንጠይቃለን።

ሚስጥራዊነት፡- የሚሰጡት መረጃ በጥናቱ ወቅትም ሆነ ከዛ በኃላ ባሉት ጊዜያት ሙሉ በሙሉ ሚስጥራዊነቱ የሚጠበቅና መረጃውም የሚያዘዉ በስም ሳይሆን በመለያ ቁጥር ይሆናል፡፡

በጥናቱ በመሳተፍዎ ያለው ጉዳት ፡- በዚህ ጥናት ላይ በመሳተፍዎ በእርስዎም ሆነ በልጅዎ ላይ ሊደርስ የሚችል ምንም ጉዳት አይኖርም፡፡ ለአስራ አምስት ደቂቃ ብቻ ከኛ ጋር ይቆያሉ፡፡

የሚያገኙት ጥቅም፡- በጥናቱ ለሚሳተፉ ፍቃደኛ ተሳታፊዎች ምንም አይነት የገንዘብ ክፍያ የለም። ነገር ግን ዉጤቱ ለሚከታተሊቸዉ ሀኪም እንዲደርሰዉ ይደረጋል፡፡ በተጨማሪም ለወደፊት የሚመጡ ታካሚዎችን አያያዝ ለማስተካከል ይረዳል፡፡የእርሰዎ በዚህ ጥናት ተሳታፊ መሆን በሆስፒታሉ ላሉ ታካሚዎች እንዲሁም ለማህበረሰቡ በዚህ ባክቴሪያ እንዳይያዙ ቅድመ መከላከል ለማድረግ ከፍተኛ ጠቀሜታ አለው፡፡

የመዉጣት (የማቋረጥ) መብት ፡- በጥናቱ በሙሉ ፈቃደኝነት እንዲሳተፉ እያስታወስን በጥናቱ ላይ እያሉ ለሚጠየቁት ጥያቄ ግለጽ ያልሆነልት ካለ የመጠየቅ፤ መልስ ያለመስጠት፤ አንዲሁም በፈለጉት ጊዜ የማቆም ወይም የማቋረጥ መብት አለዎት፡፡ ይህን በማድረግዎ ከሆስፒታሉ የሚያገኙትን ማነኛውንም አገልግሎት አይገድብም፡፡

አድራሻ፡ ይህን የመረጃ ቅጽ ስላነበቡ ወይም ሲነበብልዎ ስላዳመጡ እያመሰገን ማንኛውም ጥያቄ ካለዎት ይህንን አድራሻ ይጠቀሙ፡

የዋናው አጥኝዉ አድራሻ፡

ተዋቸዉ አወቀ **ስልክ፡**+251918149901 **ኢ-ሜይል:** tewaslike@gmail.com

አዲስ አበባ ዩንቨርስቲ ጤና ሳይንስ ኮሌጅ ሜዲካል ማይክሮባዮሎጅ፣ኢሚኖሎጅ እና ፓራስቶሎጅ ት/ት ክፍል

አህሪ/አለርት ኢቲክስ ሪቪው ኮሚቴ **ስልክ፡0113 481285**

Annex IVa: Informed Consent Form for Adults (English version)

The consent to participate in the "Phenotypic Antimicrobial Resistance Profiles of *Klebsiella pneumoniae* Isolated from Patients at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia" has been read to me/ I have read. I have been fully informed and understand about the research project objectives. I have been informed that all the information that I provide to the interviewer will be kept confidential. I also knew that I have the right to withhold information; skip questions to answer or to withdraw from the study at any time and none of my actions will have any bearing at all on my overall health care and hospital access. 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 the principal investigator.

For Adult participant:

I am willing that, the isolated bacteria to be used for this research and to respond questionnaires that are relevant to the study. By signing below I agree to participate in this study.

Signature of study participant _____ Date_____

If Participant is illiterate:

Add name of independent literate witness (if possible, this person should be selected by the participant and should have no connection to the study team).

Name of witness: _____Signature of witness: _____ Date: _____

For the study staff:

I have read/explained the study to the above named participant in a language that he/she understands well. I am certain that the participant has understood the information and is going to answer questions on his/her own free will.

Name of study staff: _____Signature: _____Date: _____

Thanks for your participation!!!

Annex IVb: <u>በአማርኛ የተዘጋጀ ዕድሜያቸዉ 18 እና ከዚህ በላይ ለሆኑ የጥናት ተሳታፊዎች የፈቃደኝነትማረጋገጫ ቅፅ</u>

በአዲስ አበባ ከተማ በጥቁር አንበሳ እስፔሻላይዝድ ሆስፒታል የመጡ ታካሚዎች ላይ የተገኙ ከለብሴላ ኒሞኔ ባክቴሪያዎችን መዳኒትን የመቋቋም ዉጫዊ እና ዉስጣዊ ባህሪያቸዉን ማሳየት በሚል ርዕስ ላይ ለማጥናት በተመለከተ በሚደረገዉ ጥናት ላይ ለመሳተፍ መሆኑ፤ የጥናቱ ዓላማና ጥቅም ተገልጾልኛል፡፡ ጥናቱ ዉስጥ አለመሳተፍ መብቴ እደሆነና በማንኛዉም ጊዜ ከጥናቱ በራሴ ዉሳኔ መዉጣት እንደምችልና በዚህም ምክንያት ከሆስፒታሉ ማገኘት ባለብኝ የህክምና አገልግሎት ላይ ምንም አይነት ችግር እንደማያደርስብኝ በሚገባ ተረድቻለሁ፡፡ ማንኛውንም ያልገባኝን ነገር የመጠየቅ ዕድል ተሰጥቶኝ ተረድቻለሁ፡፡ ስለ እኔ የተሰበሰበው መረጃ ሚስጥራዊ እንደሚሆን እና ጥያቄ ካለኝ እና መወያየት ከፈለኩኝ ማንን ማግኘት እንዳለብኝ ተነግሮኛል፡፡

ይህን ሁሉ በማገናዘብ ባክቴሪያዉ ለምረምሩ እንዲዉል አና ለጥናቱ አስፈላጊ የሆነ መረጃም ለመስጠት ተስማምቻለሁ፣ ይህንንም በፊርማየ አረጋግጣለሁ፡፡

ተሳታፊው አዋቂ ከሆነ፡

የተሳታፊ ፊርማ ፡ _____ ቀን፡ _____

ማንበብና መፃፍ ለማይችሉ ተሳተፊዎች ወይም ውሳኔ ሰጪወች፡

ተሳታፊው ወይም ውሳኔ ሰጪው ማንበብና መፃፍ የማይችሉ ከሆኑ የተማረ ነፃ ምስክር ስም ያስገቡ (ከተቻለ ይህ ግለሰብ በተሳታፊ ተመርጦ ከጥናት ቡድኑ ጋር ግንኙነት የሌለው መሆንይኖርበታል፡፡)

የምስክር ስም፡ _____የምስክር ፊርማ፡ _____ ቀን፡ _____

<u>ለጥናቱ ሰራተኛ</u>

ጥናቱን ከላይ ለተገለጹት ተሳታፊ በደንብ በሚረዱት ቋንቋ አንብቤያለሁ/አብራርቻለሁ፡፡ መረጃውን ተሳታፊ ስለመረዳቱ እርግጠኛነኝ፡፡በነፃ ፍቃዳቸውም መጠይቁን እንደሚሞሉ ተረድተዋል፡፡

የጥናቱ ሰራተኛ ስም _____የጥናቱ ሰራተኛ ፊርማ_____ቀን_____

ስለ መልካም ትብብርዎ እናመሰግናለን!!

Annex Va: Assent Form for Adolescents (12-17 years old) (English version)

The consent to participate in the "Phenotypic Antimicrobial Resistance Profiles of *Klebsiella pneumoniae* Isolated from Patients at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia" has been read to me/ I have read. I have been fully informed and understand about the research project objectives. I have been informed that all the information that I provide to the interviewer will be kept confidential. My parents or guardian have to say to choose if I want to be in the study. I also knew that I have full right to refuse from participating, even if my parents/guardian permits to be enrolled in this study. I also told that I have the right to withhold information; skip questions to answer or to withdraw from the study at any time and none of my actions will have any bearing at all on my overall health care and hospital access. 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 the principal investigator.

For Adolescent participant

I know my participation will also be approved by my parents/guardian. Therefore, I am willing that, the isolated bacteria to be used for this research and to respond questionnaires that are relevant to the study. By signing below I agree to participate in this study.

Signature of study participant _____ Date_____

If Participant is illiterate:

Add name of independent literate witness (if possible, this person should be selected by the participant and should have no connection to the study team).

Name of witness: _____Signature of witness: _____ Date: _____

For the study staff:

I have read/explained the study to the above named participant in a language that he/she understands well. I am certain that the participant has understood the information and is going to answer questions on his/her own free will.

Name of study staff: _____Signature: _____Date: _____

Thanks for your participation!!!

Annex Vb: <u>በአማርኛ የተዘጋጀ ዕድሜያቸዉ ከ12 እስከ 17 ዓመት ለሆኑ ታዳጊ ወጣት የጥናት ተሳታፊዎች የፈቃደኝነት ማረጋገጫ ቅፅ</u>

በአዲስ አበባ ከተማ በጥቁር አንበሳ እስፔሻላይዝድ ሆስፒታል የመጡ ታካሚዉች ላይ የተገኙ ከለብሴላ ኒሞኔ ባክቴሪያዎችን መዳኒትን የመቋቋም ዉጫዊ እና ዉስጣዊ ባህሪያቸዉን ማሳየት በሚል ርዕስ ላይ ለማጥናት በተመለከተ በሚደረገዉ ጥናት ላይ ለመሳተፍ መሆኑ፤ የጥናቱ ዓላማና ጥቅም ተገልጾልኛል፡፡ ወላጆቼ /ሞግዚቴ በጥናቱ ውስጥ መሳተፍ/አለመሳተፍ ምርጫው የእኔ መሆኑን አሳውቀውኛል፡፡ጥናቱ ዉስጥ አለመሳተፍ መብቴ እደሆነና በማንኛዉም ጊዜ ከጥናቱ በራሴ ዉሳኔ መዉጣት እንደምችልና በዚህም ምክንያት ከሆስፒታሉ ማገኘት ባለብኝ የህክምና አገልግሎት ላይ ምንም አይነት ችግር እንደማያደርስብኝ በሚገባ ተረድቻለሁ፡፡ ማንኛውንም ያልገባኝን ነገር የመጠየቅ ዕድል ተሰጥቶኝ ተረድቻለሁ፡፡ ስለ እኔ የተሰበሰበው መረጃ ሚስጥራዊ እንደሚሆን እና ጥያቄ ካለኝ እና መወያየት ከፈለኩኝ ማንን ማግኘት እንዳለብኝ ተነግሮኛል፡፡

ይህን ሁሉ በማገናዘብ ባክቴሪያዉ ለምረምሩ እንዲዉል እና ለጥናቱ አስፈላጊ የሆነ መረጃም ለመስጠት ተስማምቻለሁ፣ ይህንንም በፊርማየ አረጋግጣለሁ፡፡

ተሳታፊው ታዳጊ ከሆነ፡

የተሳታፊ ፊርማ ፡ _____ ቀን፡ _____

ማንበብና መፃፍ ለማይችሉ ተሳተፊዎች ወይም ውሳኔ ሰጪወች፡

ተሳታፊው ወይም ውሳኔ ሰጪው ማንበብና መፃፍ የማይችሉ ከሆኑ የተማረ ነፃ ምስክር ስም ያስገቡ (ከተቻለ ይህ ግለሰብ በተሳታፊ ተመርጦ ከጥናት ቡድኑ ጋር ግንኙነት የሌለው መሆን ይኖርበታል፡፡)

የምስክር ስም፡ _____የምስክር ፊርማ፡ _____ ቀን፡ _____

ለጥናቱ ሰራተኛ

ጥናቱን ከላይ ለተገለጹት ተሳታፊ በደንብ በሚረዱት ቋንቋ አንብቤያለሁ/አብራርቻለሁ፡፡ መረጃውን ተሳታፊ ስለመረዳቱ እርግጠኛነኝ፡፡በነፃ ፍቃዳቸውም መጠይቁን እንደሚሞሉ ተረድተዋል፡፡

የጥናቱ ሰራተኛ ስም _____የጥናቱ ሰራተኛ ፊርማ_____ቀን_____

ስለ መልካም ትብብርዎ እናመሰግናለን!!

Annex VIa: Informed Consent Form for Parent/ Guardian (English version)

The consent my daughter's/son's to participate in the "Phenotypic Antimicrobial Resistance Profiles of *Klebsiella pneumoniae* Isolated from Patients at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia" has been read to me/ I have read. I have been fully informed and understand about the research project objectives. I have been informed that all the information that I provide to the interviewer regarding my daughter/son will be kept confidential. I also knew that I have the right to withhold information; skip questions to answer or to withdraw from the study at any time and none of my actions will have any bearing at all on my daughter's/son's overall health care and hospital access. 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 the principal investigator.

For Parent/Guardian:

I am willing that, the isolated bacteria to be used for this research and to respond questionnaires that are relevant to the study. By signing below I agree my daughter's/son's participation in this study.

Signature of parent/guardian _____ Date_____

If Parent/ Guardian is illiterate:

Add name of independent literate witness (if possible, this person should be selected by the participant and should have no connection to the study team).

Name of witness: _____Signature of witness: _____ Date: _____

For the study staff:

I have read/explained the study to the above participant in a language that he/she understands well. I am certain that the participant has understood the information and is going to answer questions on his/her own free will.

Name of study staff: _____Signature: _____Date: _____

Thanks for your participation!!!

Annex VIb: <u>በአማርኛ የተዘጋጀ በወላጅ/አሳዳጊ የሚሰጥ የፈቃደኝነት ማረጋገጫ ቅፅ</u>

በአዲስ አበባ ከተማ በጥቁር አንበሳ እስፔሻላይዝድ ሆስፒታል የመጡ ታካሚዎች ላይ የተገኙ ክለብሴላ ኒሞኔ ባክቴሪያዎችን መዳኒትን የመቋቋም ዉጫዊ እና ዉስጣዊ ባህሪያቸዉን ማሳየት በሚል ርዕስ ላይ ለማጥናት በተመለከተ በሚደረገዉ ጥናት ላይ ልጄ እንዲሳተፍ መሆኑ፤ የጥናቱ ዓላማና ጥቅም ተገልጾልኛል፡፡ ጥናቱ ዉስጥ አለመሳተፍ መብቴ እደሆነና በማንኛዉም ጊዜ ከጥናቱ በራሴ ዉሳኔ መዉጣት እንደምችልና በዚህም ምክንያት ከሆስፒታሉ ልጄ ማገኘት ባለበት/ባለባት የህክምና አገልግሎት ላይ ምንም አይነት ችግር እንደማያደርስ በሚገባ ተረድቻለሁ፡፡ ማንኛውንም ያልገባኝን ነገር የመጠየቅ ዕድል ተሰጥቶኝ ተረድቻለሁ፡፡ ስለ ልጄ የተሰበሰበው መረጃ ሚስጥራዊ እንደሚሆን እና ጥያቄ ካለኝ እና መወያየት ከፈለኩኝ ማንን ማግኘት እንዳለብኝ ተነግሮኛል፡፡

ይህን ሁሉ በማገናዘብ ባክቴሪያዉ ለምረምሩ እንዲዉል እና ለጥናቱ አስፈላጊ የሆነ መረጃም ለመስጠት ተስማምቻለሁ፣ ይህንንም በፊርማየ አረጋግጣለሁ፡፡

ወላጅ/አሳዳጊ፡

የወላጅ/የአሳዳጊ ፊርማ ፡ _____ ቀን፡ _____

ማንበብና መፃፍ ለማይችሉ ተሳተፊዎች ወይም ውሳኔ ሰጪዎች፡

ወላጅ/አሳዳጊ ማንበብና መፃፍ የማይችሉ ከሆኑ የተማረ ነፃ ምስክር ስም ያስገቡ(ከተቻለ ይህ ግለሰብ በተሳታፊ ተመርጦ ከጥናት ቡድኑ ጋር ግንኙነት የሌለው መሆንይኖርበታል፡፡)

የምስክር ስም፡ _____የምስክር ፊርማ፡ _____ ቀን፡ _____

<u>ለጥናቱ ሰራተኛ</u>

ጥናቱን ከላይ ለተገለጹት ወላጅ/አሳዳጊ በደንብ በሚረዱት ቋንቋ አንብቤያለሁ/አብራርቻለሁ፡፡ መረጃውን ወላጅ/አሳዳጊ ስለመረዳቱ እርግጠኛ ነኝ፡፡በነፃ ፍቃዳቸውም መጠይቁን እንደሚሞሉ ተረድተዋል፡፡

የጥናቱ ሰራተኛ ስም _____የጥናቱ ሰራተኛ ፊርማ _____ቀን_____

ስለ መልካም ትብብርዎ እናመሰግናለን!!

Annex VIIa: Questionnaire (English Version)

A study conducted in collaboration with Addis Ababa University and Armauer Hansen Research Institute

Study Number: _____ **Study period: September 2018 – February 2019**

QUESTIONNAIRES: Administered for investigation of **Phenotypic Antimicrobial Resistance Profiles of *Klebsiella pneumoniae* Isolated from Patients at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia.** We would be most grateful if you could answer the following questions.

Questionnaire code_____ Date _____Specimen type_____

S. No	Question	Answers	Remark
1	Gender	1. Male 2. Female	
2	How old are you?	_____ years	
3	What is your Educational status?	1. Can't read and write 2. Read and write 3. Primary education 4. Secondary education 5. College or University	
4	Have you been taking antimicrobials in the past three months?	1. Yes 2. No	
5	If the answer is 'yes' for question number 4 what are these?	1. Carbapenems 2. 3rd or 4th generation cephalosporins 3. Aminoglycosides 4. Quinolones 5. β-lactam/β-lactamase inhibitors 6. Others	
6	Are you admitted yet?	1. Yes 2. No	
7	If 'yes' for question number 6 in which ward you are admitted?	1. Intensive care units 2. Pediatric ward 3. Emergency ward 4. Obstetrics and gynecology 5. Medical ward 6. Surgical ward 7. Orthopedics 8. Oncology	
8	If 'yes' for question number 6 for how long have you stayed?	1. <= 7days 3. 15 to 30 days 2. 7 to14 days 4. >30 days	

Study number_____

9	Have you been admitted to hospital in the last three months?	1. Yes 2. No	
10	If 'yes' for question number 9 for how long?	1. ≤ 7days 3. 15 to 30 days 2. 7 to14 days 4. >30 days	
11	Have you been admitted to intensive care unit ward in the last six months?	1. Yes 2. No	
12	If 'yes' for question number 11 for how long?	1. ≤ 7days 3. 15 to 30 days 2. 7 to14 days 4. >30 days	
13	Do you have underlying medical conditions?	1. Yes 2. No	
14	If 'yes' for question number 13 which one from the listed	1. Diabetic mellitus 2. Hypertension 3. Cancer 4. Renal disease 5. Cardiac disease 6. Chronic viral diseases (Hepatitis, HIV….) 7. Others	
15	Do you have impregnated foreign body medical device?	1. Yes 2. No	
16	Do you have a history of surgery in the past three months?	1. Yes 2. No	

Thank you for completing questionnaires

Annex VIIb: አማርኛ መጠይቅ ገፅ 1

ከአዲስ አበባ ዩኒቨርሲቲ እና ከአርማወር ሐንሰን የምርምር ተቋም ጋር በመተባበር የሚደረግ ጥናት ነዉ

የጥናቱ መለያ ቁጥር _____ጥናቱ የሚካሄድበት ጊዜ፡ ከ መስከረም 2011- የካቲት 2011 ዓ.ም

መጠይቁ በአዲስ አበባ ከተማ በጥቁር አንበሳ ስፔሻላይዝድ ሆስፒታል የመጡ ታካሚዎች ላይ የተገኙ ክለብሴላ ኒሞኔ ባክቴሪያዎችን መዳኒትን የመቋቋም ዉጫዊ እና ዉስጣዊ ባህሪያቸዉን ለማወቅ የሚደረግ ጥናት ነዉ፡፡ የሚከተለዉን መጠይቅ ቢሞሉልን በጣም ደስ ይለናል፡፡

የመረጃ ማሰባሰቢያ ቅጽ ኮድ_____ ቀን_____የናሙና አይነት_____

ተ.ቁ	ጥያቄ	መልስ	ምርመራ
1	ጾታ	1. ወንድ 2. ሴት	
2	እድሜዎ ስንት ነዉ?	_____አመት	
3	የትምህርት ደረጃዎ ምን ያክል ነው?	1. ማንበብና መፃፍ አልችልም 2. ማንበብና መፃፍ አችላለሁ 3. አንደኛ ደረጃ 4. ሁለተኛ ደረጃ 5. ኮሌጅ ወይም ዩኒቨርሲቲ	
4	በዚህ ሶስት ወር ዉስጥ ጸረ-ባክቴሪያ መድሃኒት ወስደው ያውቃሉ?	1. አዎ 2. አላዉቅም	
5	ለጥያቄ ቁጥር 4 መልስዎ ‘አዎ’ ከሆነ ምን አይነት?	1. ካርባፔኔም 2. ሴፉሎስፖሪን 3. አሚኖግላይኮሳይድ 4. ኪኖሎንስ 5. ቤታላክታም/ቤታላክታሜዝ ኢንሂቢተር 6. ሌሎች	
6	ተኝቶ ታካሚ ነዎት?	1. አዎ 2. አይደለሁም	
7	ለጥያቄ ቁጥር 6 መልስዎ ‘አዎ’ ከሆነ ምን ክፍል ዉስጥ?	1. የጽኑ ህሙማን ማቆያ ክፍል 2. ህጻናት ክፍል 3. ድንገተኛ ክፍል 4. ማዋለጃ ክፍል 5. ሜዲካል ክፍል 6. ሰርጅካል ክፍል 7. የአጥንት ህክምና ክፍል 8. የካንሰር ህክምና ክፍል	
8	ለጥያቄ ቁጥር 6 መልስዎ ‘አዎ’ ከሆነ ምን ያክል ጊዜ ሆነዎት?	1. 7 ቀን ወይም ከዚያ በታች 2. ከ 7 እስከ 14 ቀናት 3. ከ 15 እስከ 30 ቀናት 4. ከ 30 ቀን በላይ	

ገፅ 2

የጥናቱ መለያ ቁጥር _____

9	በዚህ ሦስት ወር ዉስጥ ሆስፒታል ተኝተው ያውቃሉ?	1. አዎ 2. አላዉቅም	
10	ለጥያቄ ቁጥር 9 መልስዎ 'አዎ' ከሆነ ለምን ያክል ጊዜ?	1. 7 ቀን ወይም ከዚያ በታች 2. ከ 7 እስከ 14 ቀናት 3. ከ 15 እስከ 30 ቀናት 4. ከ 30 ቀን በላይ	
11	በዚህ ስድስት ወር ዉስጥ የጽኑ ህመማን ማቆያ ክፍል ተኝተው ያውቃሉ?	1. አዎ 2. አላዉቅም	
12	ለጥያቄ ቁጥር 11 መልስዎ 'አዎ' ከሆነ ለምን ያክል ጊዜ?	1. 7 ቀን ወይም ከዚያ በታች 2. ከ 7 እስከ 14 ቀናት 3. ከ 15 እስከ 30 ቀናት 4. ከ 30 ቀን በላይ	
13	ሌላ አይነት ህመም አለብዎት?	1. አዎ 2. የለብኝም	
14	ለጥያቄ ቁጥር 13 መልስዎ አዎ ከሆነ ምን አይነት?	1. ስኳር 2. ደም ብዛት 3. ካንሰር 4. የኩላሊት በሽታ 5. የልብ ህመም 6. ስርየሰደደ የቫይረስ ብሽታ (ሄፓታይቲስ ኤች. አይ. ቪ.…) 7. ሌሎች	
15	ሰውነትዎ ላይ የገባ ባዕድ የሆነ የህክምና መሳሪያ አለ?	1. አዎ 2. የለም	
16	በዚህ ሦስት ወር ዉስጥ የቀዶ ጥገና ህክምና አድርገው ያውቃሉ?	1. አዎ 2. አላዉቅም	

መጠይቁን በመሙላት ስለተባበሩን ከልብ እናመሰግናለን!

Annex VIII: Laboratory procedures

We identified *K.pneumoniae* from blood, urine, sputum, wound and body fluids collected from patients attending Tikur Anbessa Specialized Hospital for routine bacteriological culture through inoculation of the specimens on MacConkey agar, 5% sheep blood agar and chocolate agar, Gram stain and biochemical tests.

Culture media preparation

Materials and reagents

Culture media	Autoclave	Heater
Petri dishes	Flasks	Refrigerater
Test tubes	Measuring cylinders	PH meter
Bunsen burner	Distelled water	Quality control strain
Incubator	Balance	Dispenser
Water bath	Aluminum foil	Spatula

Procedure

1. Weighing and dissolving of culture media: - Weigh and add the appropriate amount of culture media according to the manufacturer instruction. Mix and stir while heating if heating is required to dissolve the medium
2. Sterilization and sterility testing: - Sterilize with autoclave at a pressure of 15 lb/in^2 and temperature of 121^oC for 15 minutes.
3. Addition of heat sensitive ingredients such as blood: - Refrigerated heat sensitive ingredients should be first warmed at room temperature before added to a molten agar medium
4. PH testing of culture media: - Test the pH of a culture medium using narrow range papers or a pH meter
5. Dispensing of the culture media: - Most fluid media, unlike solid media, are first dispensed into screw capped bottles or tubes, and then sterilized by autoclaving. After it is cooled to 50°C in water bath, media should be dispensed in a clean draught-free room using aseptic technique and sterile container. Approximately 25 ml of media was dispensed to have a uniform depth of 4 mm.

6. Quality assurance of culture media: - Incubate at least one uninoculated plate/tube from each batch overnight or longer to verify sterility of the medium. Use at least one control strain to test for ability to support growth and identification of the target pathogen
7. Storage of culture media: - Plates of culture media should be stored at 2 – 8^{o}C, preferably in sealed plastic bags to prevent loss of moisture.

Culture

Purpose: For preliminary identification of *K. pneumoniae*

Materials and reagents

- Required culture media plate
- Sterile inoculating loops
- Incubator with correct atmosphere at appropriate temperature
- Bio-safety cabinet

Procedure

1. Inoculate the specimen on the plate using a sterile innoculating loop.
2. Incubate plates aerobically at 35-37ºC for 18-24 hours.
3. Examine plates for colony morphology and pigment production.

❖ Pink or yellow to colorless mucoid colonies (due to fermentation of lactose) from MacConkey agar and further confirmed by the pattern of biochemical profiles

Gram stain

Purpose: To look for gram stain characterstics, shape and arrangement of the bacteria

Principle: Bacteria stain either Gram-positive or Gram-negative on the basis of differences in their cell wall compositions and architectures. Gram-positive cell wall is unaffected by alcohol decolorization and retain the initial stain, appearing deep violet. Gram-negative cell wall is damaged by the alcohol decolorizer, allowing the crystal violet-iodine complex to leak out and be replaced by the counterstain

Materials and reagents

- Gram stain reagents: Crystal violet, Gram's iodine, Decolorizer, Safranin
- Slides, sterile applicator sticks, sterile normal saline
- Bunsen burner

Procedure

1. After making a smear, leave the slide in a safe place for the smear to air-dry then fixed by heat.

2. Cover the fixed smear with crystal violet stain for 30–60 seconds.

3. Rapidly wash off the stain with clean tape water.

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 tape water.

6. Decolorize rapidly (few seconds) with acetone–alcohol. Wash immediately with clean tape water.

Caution: Acetone–alcohol is highly flammable; therefore use it well away from an open flame.

7. Cover the smear with safranin stain for 1 minute.

8. Wash off the stain with clean water.

9. Wipe the back of the slide clean with a guaze, 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

❖ *K. pneumoniae* is Gram-negative rod shaped bacteria

Biochemical tests

Purpose: To identify *K. pneumoniae*

Materials and reagents

- Inoculation loops
- Incubator with correct atmosphere at appropriate temperature
- Nutrient broth
- Required biochemical media
- Bio-safety cabinet

Procedure

1 Prepare a suspension of 3-4 colony of test organism in 5 ml nutrient broth.

2 A loop full of the bacterial suspension is inoculated in to indole, citrate agar, triple sugar iron agar, lysine decarboxylase agar, mannitol, manolate, urea agar and motility medium.

3 Incubate at 35-37°C for 18-24 hours.

4 Look for color change (turbidity for motility) of the medium

5 Identify the test organism by considering the result of the eight biochemical tests

❖ An isolate is identified as *K. pneumoniae* when it is indole negative, gas and acid producer, hydrogen sulfide negative, citrate (Simmons) positive, mannitol fermenter, malonate positive, lysine decarboxylase positive, urea slow producing and non-motile

Antimicrobial Susceptibility Testing: Disc diffusion

Purpose

To measure the susceptibilities of *K. pneumoniae* to appropriate antimicrobials by a standardized disc diffusion method

Principle

Standard amounts of antimicrobials impregnated onto absorbent paper are placed onto a Muller-Hinton agar plate seeded with a known concentration of bacteria. These plates are then incubated for 16-18 hours at 35±2^{o}C. The antimicrobial diffuses through the medium from the disc and if the bacteria are sensitive to the antimicrobial then growth will be inhibited leaving a zone of clearing. The diameter of this zone is measured and compared with CLSI guidelines for zone sizes to determine whether the bacteria should be classified as sensitive, intermediately sensitive or resistant to the antimicrobial.

Reagents and materials

- Mulleur Hinton agar (3-4 mm deep)
- Antimicrobial impregnated discs
- Sterile saline (2-5ml)
- Sterile cotton tipped swabs
- Inoculation loops
- Forceps
- Bio-safety cabinet
- Incubator with correct atmosphere at appropriate temperature
- Densitometer
- Ruler or caliper

Procedure

1. Make 0.5 McFarland standard suspension of test strain in normal saline
2. Within 15 minutes sample was taken from the suspension using sterile swab (the swab squeezed against the side of the test tube to remove the excess fluid)
3. Swab entire surface of the agar plate three times, rotating plate approximately 60^{o} between streaking to ensure even distribution. As a final step, swab the rim of the agar.

4. Allow inoculated plate to stand 3 -15 minutes (no longer than 15 minutes) before applying discs.
5. Using a sterile forceps, the antimicrobial discs was placed on the inoculated plate and incubated at 35±2^{o}C for 16-18 hours.
6. The test was read after checking that the bacterial growth is neither heavy nor light. The radius of the inhibition zone will be measured with caliper
7. The reaction of the test organism to each antimicrobial was interpreted as sensitive, intermediate, or resistance based on CLSI guidelines

Combined Disc Test (CDT)

Purpose

To confirm the bacteria produces Extended Spectrum β–lactamase (ESBL)

Principle

A disc of cefotaxime-clavulanate (30/10 µg) and ceftazidime-clavulanate (30/10 µg) along with third generation cephalosporins (cefotaxime and ceftazidime) placed at a distance of 25 mm, center to center, on a MHA plate inoculated with a bacterial suspension of 0.5 McFarland turbidity standards and incubated at 35±2°C for 16-18 hours. An increase in the inhibition zone diameter of ≥5 mm for a combination disc versus third generation cephalosporin disc alone confirms ESBL production

Reagents and Materials

- Agar plates (3-4 mm deep)
- Antimicrobial discs
    a. Ceftazidime 30 µg
    b. Ceftazidime-clavulanic acid 30/10 µg
    c. Cefotaxime 30 µg
    d. Cefotaxime-clavulanic acid 30/10 µg
- Sterile saline (2-5ml)
- Sterile cotton tipped swabs
- Inoculation loops
- Forceps
- Bio-safety cabinet
- Incubator with correct atmosphere at appropriate temperature
- Ruler

Procedure

1. Using a fresh pure culture; prepare a suspension of the test organism equal to 0.5 McFarland Standard.
2. Using a sterile cotton swab; spread the adjusted suspension over the entire area of a Mueller Hinton agar plate.
3. Apply the discs onto the inoculated plate, ensuring sufficient space between individual discs to allow for proper measurement of inhibition zones.
4. Incubate at 35±2°C for 16-18 hours.
5. Measure zone inhibition diameters

Interpretation

A ≥ 5 mm increase in diameter of zone of inhibition for either of the cephalosporin/ clavulanate disc versus the zone diameter of the respective cephalosporin discs was considered positive and the isolate was interpreted as ESBL producer.

Modified Carbapenem Inactivation Method (mCIM)

Purpose

It is used for confirmation of carbapenemase producing bacteria

Principle

When a meropenem disc is placed in a suspension of carbapenemase-producing bacteria and incubated for a few hours, the carbapenem in the disc will be hydrolyzed by the carbapenemase. When the disc is then transferred to a plate that has just been inoculated with a meropenem-susceptible *E. coli* strain and the plate is incubated for 18-24 hours, there will be no zone or a very small zone of inhibition around the meropenem disc. In contrast, if the test bacterium is not a carbapenemase producer, the meropenem disc will retain its activity and be available to inhibit growth of *E. coli* as evidenced by a zone of inhibition around the disc.

Reagents and materials

- Trypticase Soy Broth (TSB) (2 mL aliquots)
- Meropenem disc (10 μg)
- 1-μL and 10-μL inoculation loops
- Nutrient broth (eg, Mueller-Hinton, TSB) or normal saline (3.0–5.0 mL aliquots)
- Mueller-Hinton Agar plates (100 mm or 150 mm)
- Meropenem-susceptible indicator strain – *E. coli* ATCC®[a] 29522

- Forceps
- Bio-safety cabinet
- Incubator with correct atmosphere at appropriate temperature
- Ruler

Procedure

1. Transfer 1-µL loopful of test bacteria from an overnight agar plate to a tube containing 2 mL of trypticase soy broth (TSB) and vortex the suspension 10–15 seconds.
2. Add a standard 10-µg meropenem disc in to the suspension. Ensure the entire disc is immersed in the suspension.
3. Incubate for 4 hours ± 15 minutes at 35°C ± 2° C in ambient air.
4. Just prior to completion of the 4 hour incubation cycle, prepare a 0.5 McFarland suspension of *E. coli* ATCC® 25922 and inoculate onto a MHA plate following the routine disc diffusion procedure.
5. Remove the meropenem disc from the TSB suspension using a 10-µL loop, taking care to remove excess liquid from the disc.
6. Place the meropenem disc immediately on the Mueller-Hinton Agar (MHA) plate that has been inoculated with *E. coli* ATCC® 25922.
7. Incubate the plate at 35°C ± 2° C in ambient air for 18-24 hours.
8. Measure the zone of inhibition around the meropenem disc.

Result Interpretation

Carbapenemase positive: - inhibition zone 6-15 mm or presence of colonies within a 16-18 mm zone.

Carbapenemase negative:- inhibition zone ≥ 19 mm.

Indeterminate:- inhibition zone 16-18 mm (unable to confirm if isolate is or is not a carbapenemase producer)

For indeterminate results:

- Check test isolate and *E. coli* ATCC® 25922 indicator strain for purity.
- Check meropenem disc integrity by confirming acceptable results were obtained when disc were subjected to routine disc diffusion test QC.
- Repeat the mCIM for test isolate and QC strains.
- If the repeat test is indeterminate, consider performing a test for carbapenemase genes.

Annex IX: Laboratory data

Code: _____

Antimicrobial susceptibility testing result

Antimicrobials	**S (mm)**	**I (mm)**	**R (mm)**
• Tetracycline (30µg)	_____	_____	_____
• Gentamicin (10 µg)	_____	_____	_____
• Tobramycin (10µg)	_____	_____	_____
• Amikacin (30µg)	_____	_____	_____
• Ciprofloxacin (5µg)	_____	_____	_____
• Naldixic Acid (5µg)	_____	_____	_____
• Aztreonam (30µg)	_____	_____	_____
• Piperacillin-tazobactam (100/10µg)	_____	_____	_____
•Amoxicillin-clavulanate (20/10µg)	_____	_____	_____
• Trimethoprim-sulfamethoxazole (25µg)	_____	_____	_____
• Chloramphenicol (30µg)	_____	_____	_____
• Cefuroxime (30µg)	_____	_____	_____
• Cefazolin (5µg)	_____	_____	_____
• Cefoxitin (30µg)	_____	_____	_____
• Cefepime (30µg)	_____	_____	_____
• Ceftriaxone (30µg)	_____	_____	_____
• Cefotaxime (30µg)	_____	_____	_____
• Ceftazidime (30µg)	_____	_____	_____
• Meropenem (10µg)	_____	_____	_____
• Imipenem (10µg)	_____	_____	_____
• Ertapenem (10µg)	_____	_____	_____

ESBL production: Positive ☐ Negative ☐

Carbapenemase production: Positive ☐ Negative ☐

Declaration

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

Name of the principal investigator: Tewachew Awoke (BSc, MSc candidate)

Signature: _____Date of submission: _____

This thesis has been submitted with our approval as advisors.

Name of Advisors	Signature	Date
Tamrat Abebe (PhD, Assistant Professor), DMIP, CHS, AAU	_____	_____
Brhanu Teka (MSc, PhD Fellow), DMIP, CHS, AAU	_____	_____
Adane Mihret (DVM, PhD, Associate Professor), AHRI	_____	_____
Abraham Aseffa (MD, PhD, Senior Scientist), AHRI	_____	_____
Biruk Yeshitela (MSc, PhD Fellow), AHRI	_____	_____