MOLECULAR DETECTION OF ANTIBIOTIC RESISTANCE GENES FROM EXTENSIVELY ANTIBIOTIC-RESISTANT *ESCHERICHIA* AND *KLEBSIELLA* SPECIES ISOLATED FROM PATIENTS ATTENDING GENERAL HOSPITAL MINNA

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DEPARTMENT OF MICROBIOLOGY
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A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL FEDERAL UNIVERSITY OF TECHNOLOGY, MINNA, NIGERIA IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF TECHNOLOGY IN MEDICAL MICROBIOLOGY

MARCH, 2023

DECLARATION

I hereby declare that this thesis titled**: "**MOLECULAR DETECTION OF ANTIBIOTIC RESISTANCE GENES FROM EXTENSIVELY ANTIBIOTIC-RESISTANT *ESCHERICHIA* AND *KLEBSIELLA* SPECIES ISOLATED FROM PATIENTS VISITING GENERAL HOSPITAL MINNA**"** is a collection of my original research work and it has not been presented for any other qualification anywhere. Information from other sources (published or unpublished) has been duly acknowledged.

ABUBAKAR, Obomile Aliyu _____
MTech/SLS/2018/9289 **Signature & Date**

CERTIFICATION

The thesis titled: **"Molecular detection of antibiotic resistance genes from extensively antibiotic-resistant *Escherichia* and *Klebsiella* species isolated from patients visiting General Hospital Minna"** by ABUBAKAR, Obomile Aliyu (MTech/SLS/2018/9289) meets the regulations governing the award of the degree of Masters (MTech) of Technology in Federal University of Technology, Minna and it is approved for its contribution to scientific knowledge and literary presentation.

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DEDICATION

This research is dedicated to Almighty God, the pillar and rock of my life for making this journey a success.

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ABSTRACT

Reduced antibiotics susceptibility of extensively drug resistance enterobacterial strains limit treatment options and emerge as an important public health problem. Therefore, this research focused on molecular detection of antibiotic resistant genes from extensively antibiotic resistant *Escherichia* and *Klebsiella* species from urine and stool samples of patients attending General Hospital Minna. A total of 192 clinical samples (urine and stool) was analysed using standard microbiological techniques. The isolated *Escherichia* and *Klebsiella* species were investigated using guidelines prescribed by the Clinical and Laboratory Standard Institute (CLSI) to identify extensively drug resistant isolates and their molecular basis for resistance was determined using the 16S rRNA molecular sequencing technique. Out of the 76 gram negative bacteria isolated,45 (23.4%) and 31 (16.1%) were *Escherichia* and *Klebsiella* species respectively. Majority of the isolated *Escherichia* species resisted Ceporex (97.8%), Tetracycline (82.2%), Colistin (77.8) and Nalidixic acid (71.1%). Similarly, *Klebsiella* species resisted Ceporex (87.1%), Tetracycline (83.9%), Colistin (80.6%) and Nalidixic acid (61.3%). Both isolates were susceptible to Imipenem and Fosfomycin. Result revealed extensive antibiotic resistant *Escherichia* spp. (3.9%) and *Klebsiella* spp. (2.6%). This study has shown that there are multiple antibiotic resistant *Escherichia* and *Klebsiella* species in urine and stool samples obtained in General Hospital Minna. Molecular analysis revealed the presence of Tet A, Tet B, TEM genes in all the isolates that were extensively drug resistant. Findings of the present study showed that *Escherichia coli* and *Klebsiella pneumoniae* isolated from the urine and stool samples harboured Tet A, Tet B, TEM and CTX genes and may have used other mechanisms like the use of drug efflux, modification of the target site or selective membrane permeability in resisting the antibiotic colistin from the polymyxin class. These findings accentuate the need to implement strict measures to limit the imprudent use of antibiotics particularly, tetracycline, colistin and ceporex in the study area.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

The emergence and spread of antibiotic-resistant bacteria (ARB), antimicrobial resistant genes (ARGs), and antimicrobial-resistant gene determinants (ARGDs) have been portrayed as one of the leading challenges of the 21st century and this issue is rapidly expanding worldwide especially in less developed countries (Ekwanzala *et al.,* 2018).

Antibiotic-resistance is the ability of bacteria or other microbes to resist the effects of an antibiotic. The resistance to an antibiotic occurs when bacteria change in some ways that reduces or eliminates the effectiveness of drugs, chemicals, or other agents designed to cure or prevent infections. Ever since, from the start of antibiotic development Bilal *et al.,* 2021, there was a continuous worry about the resistance of bacteria to antibiotics (). It is one of the significant hazards developed by bacteria because, it not only causes deadly infections but also causes antibiotic treatment failure, high budget outlay and increased morbidity (Bilal *et al.,* 2021).

Each year in Europe, about 400,000 patients experience ill effects due to infection by antibiotic-resistant microorganisms, with an associated mortality of 25,000 patients. More and more enteric bacteria are being reported as being drug resistant in the USA, where antibiotic resistance (AR) is accountable for greater than 2 million hospitalizations and at least 23,000 deaths annually (Ekwanzala *et al.,*2018).

The poor management, unhygienic environment, untrained professionals, overuse, and misuse of antibiotics are the factors that lead to the development of theses panic situations in the form of adopting or acquiring resistant genes by bacteria (Partridge *et al.,* 2018). In bacterial genomes, capture, accumulation and dissemination of antibiotic resistance

determinants are often associated with mobile genetic elements (MGEs) like plasmids, transposons and insertion sequences (ISs) (Xanthopoulou *et al.,* 2020).

Infections caused by drug-resistant Gram-negative bacteria (GNB), particularly the hospital-acquired antibiotic-resistant infections pose a significant threat to global public health (Exner *et al.,* 2017). Organisms expressing in vitro resistance to three or more antimicrobial classes are referred to as multidrug-resistant organisms. According to the Centers for Disease Control and Prevention (CDC), more than 70% of the bacteria causing hospital-acquired infections are resistant to at least one of the antimicrobial agents that are commonly used to treat them (Alkofide *et al.,*2020). There are three types of antimicrobial resistance exhibiting microorganisms: multidrug-resistant (MDR), extensively drug-resistant (XDR), and pandrug-resistant (PDR). Multidrug resistance (MDR) microorganisms acquired non-susceptibility to at least one agent in three or more antimicrobial classes. Extensively drug resistance (XDR) microorganisms are non-susceptible to at least one agent in all but susceptible to only one or two categories. Microorganisms with non-susceptibility to all agents in all antimicrobial classes are referred to as pan drug resistance (PDR) (Alkofide *et al.,*2020).

Gram-negative enteric bacteria such as *Klebsiella pneumoniae* and *Escherichia coli* are natural inhabitant of the gastrointestinal tract of humans and animals and are widely distributed in the environment and increasingly reported as a cause of invasive infections in healthcare settings, particularly in immunocompromised patients (Bi *et al.,* 2017). Antimicrobial resistance in *Klebsiella pneumoniae* and *Escherichia coli* is increasing, particularly beta-lactamases and carbapenemases having been well-characterized as increasing the infection threat (Mathers *et al.,* 2015; Campos *et al.,* 2016; Lee *et al.,* 2016). This seriously antibiotic management problem is now frequently seeing both nosocomial and community associated infections. Infections caused by extensively drug-

resistant (XDR) *K. pneumoniae* and *Escherichia coli* such as pneumonia, urinary tract infections, septicemia, meningitis, peritonitis and bloodstream infections (BSIs) (Girometti *et al*., 2014; Paczosa and Mecsas, 2016), have been closely related to increased morbidity, mortality, long hospital stay, and high healthcare costs (Bi *et al.*, 2017).

Klebsiella pneumoniae and *Escherichia coli* is recognized as a major pathogen of hospital acquired infections. In the past several years, clinicians have witnessed a remarkable increase in the drug resistance rate of *Klebsiella pneumoniae* and *Escherichia coli* strains from clinical isolates. The dissemination of XDR *Klebsiella pneumoniae* and *Escherichia coli* is now causing difficult to-treat infections worldwide, bringing with its tremendous challenges to the clinical therapeutic options (Lim *et al.*, 2015). Although carbapenems possess good antibacterial activity to Gram-negative bacteria, the rates of carbapenem resistance among *Klebsiella pneumoniae* escalated from 0.7% in 2006 to 10% in 2013 (Hu *et al.*, 2016). The availability of alternative, effective antimicrobial agents is limited (Tang *et al.*, 2016). Several mechanisms are known to mediate antibiotic resistance to commonly used antimicrobial agents, including extended-spectrum β-lactamases (ESBLs) and carbapenemases, as well as plasmid-mediated quinolone resistance (PMQR) genes, aminoglycoside-modifying enzymes (AMEs), and 16S rRNA methyltransferase (16S-RMTase) (Hu *et al.*, 2014; Findlay *et al*., 2015; Buruk *et al.*, 2016). This current study focused on pinpointing the antibiotic resistance determinants of XDR *Klebsiella pneumoniae* and *Escherichia coli* isolated from General Hospital Minna attendees.

1.2 Statement of the Research Problem

Escherichia and *Klebsiella* species are the major culprit responsible for difficult to treat hospital and community acquired bacterial infection globally (Kaper *et al*., 2004; Allocati *et al*., 2013). This situation is even more worrisome in developing countries including Nigeria due to indiscriminate use of antibiotics and scarcity of data.

Extensively antibiotic resistant bacteria are the leading cause of prolong hospital stay, increased health care cost and antibiotic treatment failure (Moini *et al*., 2015).

1.3 Aim and Objectives of the Study

The study was aimed at molecular detection of antibiotic resistant genes from extensively antibiotic resistant *Escherichia* and *Klebiella* species isolated from patients attending General hospital Minna.

Objectives of the study were to:

i. Isolate and identify *Escherichia* spp. and *Klebsiella* spp. from clinical samples
ii. Determine the antibiotic susceptibility patterns of *Escherichia* spp. and *Klebsiella* spp.
iii. Identify the isolated extensively antibiotic-resistant *Escherichia* spp. and *Klebsiella* spp. using the 16S RNA.
iv. Detect antibiotic resistance genes in extensively drug resistance *Escherichia* spp. and *Klebsiella* spp.

1.4 Justification for the Study

Surveillance studies of bacterial resistance and trends are the best approaches for controlling the emergence and dissemination of multidrug resistant bacteria as well as the possible epidemic (Vranic and Uzunovic, 2016).

However, to the best of our knowledge, there is little or no information on the prevalence of extensively antibiotic-resistant *Escherichia* and *Klebsiella* from the study Area.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Biology of *Klebsiella* spp. and *Escherichia* spp.

Klebsiella spp. is a Gram-negative, non-motile, encapsulated, lactose-fermenting, facultative anaerobic, rod-shaped bacterium. It appears as a mucoid lactose fermenter on MacConkey agar. Although found in the normal flora of the mouth, skin, and intestines, it can cause destructive changes to human and animal lungs if aspirated, specifically to the alveoli resulting in bloody, brownish or yellow colored jelly like sputum. In the clinical setting, it is the most significant member of the genus *Klebsiella* of the Enterobacteriaceae. *Klebsiella oxytoca* and *Klebsiella rhinoscleromatis* have also been demonstrated in human clinical specimens (Lee *et al*., 2016).

It naturally occurs in the soil, and about 30% of strains can fix nitrogen in anaerobic conditions, as a free-living diazotroph its nitrogen-fixation system has been much studied and is of agricultural interest. *Klebsiella pneumoniae* has been demonstrated to increase crop yields in agricultural conditions. It is closely related to *Klebsiella oxytoca* from which it is distinguished by being indole-negative and by its ability to grow on melezitose but not 3-hydroxybutyrate. Illness affects middle-aged and older men with debilitating diseases. This patient population is believed to have impaired respiratory host defenses, including persons with diabetes, alcoholism, malignancy, liver disease, chronic obstructive pulmonary diseases, glucocorticoid therapy, kidney failure, and certain occupational exposures (such as paper mill workers). Many of these infections are obtained when a person is in the hospital for some other reason (Davies, 2001).

In addition to pneumonia, *Klebsiella* can also cause infections in the urinary tract, lower biliary tract, and surgical wound sites. The range of clinical diseases includes pneumonia, thrombophlebitis, urinary tract infections, cholecystitis, diarrhea,

upper respiratory tract infection, wound infection, osteomyelitis, meningitis, and bacteremia, and sepsis. Patients with an invasive device in their bodies, contamination of the device becomes a risk; neonatal ward devices, respiratory support equipment, and urinary catheters put patients at increased risk. In addition, the use of antibiotics can be a factor that increases the risk of nosocomial infection with *Klebsiella* bacteria. Sepsis and septic shock can follow entry of the bacteria into the blood (Centre for Disease Control (CDC), 2008).

Klebsiella ranks second to *Escherichia coli* for urinary tract infections in older people. It is also an opportunistic pathogen for patients with chronic pulmonary disease, enteric pathogenicity, nasal mucosa atrophy, and rhinoscleroma. New antibiotic resistant strains of *Klebsiella pneumoniae* are appearing (World Health Organization (WHO), 2011)

To get a *Klebsiella pneumoniae* infection, a person must be exposed to the bacteria. In other words, *Klebsiella pneumoniae* must enter the respiratory tract to cause *pneumoniae,* or the blood to cause a bloodstream infection. In healthcare settings, *Klebsiella pneumoniae* bacteria can be spread through person-to-person contact (for example, contaminated hands of healthcare personnel, or other people via patient to patient) or, less commonly, by contamination of the environment; the role of transmission directly from the environment to patients is controversial and requires further investigation (Spellberg, 2011). However, the bacteria are not spread through the air. Patients in healthcare settings also may be exposed to *Klebsiella pneumoniae* when they are on ventilators, or have intravenous catheters or wounds. These medical tools and conditions may allow *Klebsiella pneumoniae* to enter the body and cause infection (Denyer, 2011)

Growth of *Klebsiella pneumoniae* carbapenem resistant enterobacteriaceae (CRE) from positive blood culture on MacConkey agar in Tuscany, where an outbreak was reported starting in November 2018 of strains producing NDM carbapenemase. (Levin, 2007).

Infection with carbapenem-resistant Enterobacteriaceae (CRE) or carbapenemase-producing Enterobacteriaceae is emerging as an important challenge in health-care settings. One of many CREs is carbapenem-resistant *Klebsiella pneumoniae* (CRKP). Over the past 10 years, a progressive increase in carbapenem-resistant *Klebsiella pneumoniae* has been seen worldwide; however, this new emerging nosocomial pathogen is probably best known for an outbreak in Israel that began around 2006 within the healthcare system there (Mcnulty, 2011). In the US, it was first described in North Carolina in 1996; since then carbapenem-resistant *Klebsiella pneumoniae* has been identified in 41 states and is routinely detected in certain hospitals in New York and New Jersey. It is now the most common carbapenem resistant enterobacteriaceae species encountered within the United States (Spellberg, 2011).

Carbapenem-resistant *Klebsiella pneumoniae* is resistant to almost all available antimicrobial agents, and infections with carbapenem-resistant *Klebsiella pneumoniae* have caused high rates of morbidity and mortality, in particular among persons with prolonged hospitalization and those critically ill and exposed to invasive devices (e.g., ventilators or central venous catheters). The concern is that carbapenem is often used as a drug of last resort when battling resistant bacterial strains. New slight mutations could result in infections for which healthcare professionals can do very little, if anything, to treat patients with resistant organisms (Marshall and Levy, 2011).

2.2 Biology of *Escherichia* spp.

Escherichia spp. is a Gram-negative, facultative anaerobic, rod-shaped, coliform bacterium of the genus *Escherichia* that is commonly found in the lower intestine of warm-blooded organisms (endotherms). Most *Escherichia coli* strains are harmless, but some serotypes (EPEC, ETEC etc.) can cause serious food poisoning in their hosts, and are occasionally responsible for food contamination incidents that prompt product

recalls. The harmless strains are part of the normal microbiota of the gut, and can benefit their hosts by producing vitamin K_2, (which helps blood to clot) and preventing colonisation of the intestine with pathogenic bacteria, having a symbiotic relationship (Levin, 2007). *Escherichia coli* is expelled into the environment within faecal matter. The bacterium grows massively in fresh faecal matter under aerobic conditions for 3 days, but its numbers decline slowly afterwards.

Escherichia coli and other facultative anaerobes constitute about 0.1% of gut microbiota, and faecal oral transmission is the major route through which pathogenic strains of the bacterium cause disease. Cells are able to survive outside the body for a limited amount of time, which makes them potential indicator organisms to test environmental samples for faecal contamination. A growing body of research, though, has examined environmentally persistent *Escherichia coli* which can survive for many days and grow outside a host (Krause *et al*., 2016).

The bacterium can be grown and cultured easily and inexpensively in a laboratory setting, and has been intensively investigated for over 60 years. *Escherichia coli* is a chemoheterotroph whose chemically defined medium must include a source of carbon and energy. *Escherichia coli* is the most widely studied prokaryotic model organism, and an important species in the fields of biotechnology and microbiology, where it has served as the host organism for the majority of work with recombinant DNA. Under favorable conditions, it takes as little as 20 minutes to reproduce (Haruta *et al*., 2000).

2.3 Antibiotics

Antibiotics are mainly used both in human and veterinary medicine to insure human and animal health worldwide. Beside medical therapy, antibiotics have also been used to improve aquaculture and agricultural production. However, the emergence of resistant bacteria to commonly used effective antibiotics, resulted in the need for stronger drugs

and more costly therapy. New forms of antibiotic resistance and transmission of genes can easily spread across boundaries and between continents. World health leaders have described antibiotic resistant microorganisms as "nightmare bacteria" that "pose a catastrophic threat" to people in every country in the world (Wang *et al*., 2012).

Table 2.1 A list of antimicrobial agents and their modes of action

Antimicrobial Agents	Groups	Mode of Action
Ampicillin, Augmentin, Amoxylin	Penicillins	Inhibitor of cell wall synthesis
Ceftriaxone	Cephalosporins	Inhibitor of cell wall synthesis
Chloramphenicol	Chloramphenicol	Inhibitor of cell wall synthesis
Erythromycin	Macrolides	Inhibitor of cell wall synthesis
Azithromycin		
Gentamycin, streptomycin	Aminoglycosides	Inhibitor of cell wall synthesis
Oxytetracycin	Tetracycline	Inhibitor of DNA synthesis
Nalidixicacid	Quinolones	Inhibitor of DNA synthesis
Ciprofloxacin	Quinolones	Inhibitor of DNA synthesis
Sulphamethazine	Sulfonamide	Competitive Inhibitors of folic acid synthesis
Trimethopim	Sulfonamide	Competitive Inhibitors of folic acid synthesis

Source: Wang *et al*. (2012)

Some studies on bacterial resistance have shown that there is a huge diversity of resistance mechanisms, in which the distribution and interaction is mostly complex and unknown. However, there are varieties of biochemical and physiological mechanisms that are responsible for the development of antibiotic resistance. The mechanism of resistance may be evolution of either genetically inherent or the result of the microorganism being exposed to antibiotics. Most of the antibiotic resistance has emerged as a result of

mutation or through transfer of genetic material between microorganisms. Several of various recent studies revealed that almost 400 different bacteria have demonstrated about 20,000 possible resistant genes (Davies, 2010). The resistances that evolve within bacteria that affect animals have the potential to affect humans. Zoonosis of the resistant strains is able to occur, posing a risk to human health. People who are employed at farms or food animal production facilities are at a higher risk of infection with a resistant strain of bacteria (Sarmah *et al*., 2016).

Antibiotic resistant infections occur too often and with increasing frequency, interfering with the effective treatment of people and animals. Antibiotic resistance has increased due to the introduction of antibiotics into an environment. In general practice, there are concerns about some common infections which are becoming difficult to treat an illness with antibiotic resistant bacteria which may take longer to resolve. To preserve the effectiveness of antibiotics, it is critical to examine the uses of these drugs, in both humans and animals. Several new initiatives are being put in place to halt the alarming trend of resistance to antibiotics and to deal with the ever-increasing number of infections caused by resistant bacteria (CDC, 2008).

2.4 Antibiotic Resistance and its Mechanism of Development

Antibiotics are chemical agents that prevent bacterial growth by stopping the bacterial cell from dividing (bacteriostatic) or by killing them (bactericidal). The terms antibiotic and antimicrobial are often used interchangeably but are not synonymous. Antibiotics are substances of microbial origin (such as penicillin) while “antimicrobial” refers to any substance including synthetic compounds which destroys microbes (Guardabasse and Courvalin, 2006). Antibiotics are used to treat and or prevent disease in human and animals. The reductions in death afforded by effective antibiotics for bacterial infections of all types, ranging from simple skin infections to infections of the bloodstream, lung,

abdomen, as well as brain, so enormous that the lives of both human and animals are saved due to treatment by using antibiotics (Spellberg, 2011).

2.5 Mechanism of Action of Antibiotics

In order to appreciate the mechanisms of resistance, it is important to understand how antimicrobial agents act. One of the most common mechanisms of action is targeting the cell wall, which is present in bacteria (prokaryotic cells) but absent in humans (eukaryotic cells). Thus, antimicrobial agents act selectively on vital microbial functions with minimal effects or without affecting host functions. Different classes of antibiotics possess specific modes of action by which they inhibit the growth or kill bacteria.

2.6 Antibiotic Resistance

Antibiotic resistance is the ability of a bacterium or other microorganisms to survive and reproduce in the presence of antibiotic doses that were previously thought effective against them (WHO, 2011). The origin of antibiotic resistance genes are unclear; however, studies using clinical isolates collected before the introduction of antibiotics demonstrated susceptibility, although, conjugative plasmids were present (Denyer *et al*., 2011).

Normally, most cells in a naive, susceptible bacterial population which can cause an infection are susceptible to particular antibiotic upon exposure. However, there is always a minute sub-population of resistant bacterial cells that will be able to multiply at higher concentrations in insufficient antibiotic concentration which kill the subpopulation so that micro-organisms survives in the environment (Smith, 2005). Resistance is often associated with reduced bacterial fitness, and it has been proposed that a reduction in antibiotic use will pose selective pressure to acquire resistance would benefit the fitter susceptible bacteria, enabling them to outcompete resistant strains over time.

Antibiotic resistant bacteria are a growing public health emergency since infections from resistant bacteria are more hard and costly to treat. For instance, since the 1990s, some strains of Salmonella became resistant to a range of antibiotics. Resistance is supposed to be occurred from the use of antibiotics in human and animal husbandry. The major problem in the clinical practice today is the emergence of multiple-drug resistance, which is resistance to several types of antimicrobial agent (Amenu, 2014).

2.7 Factors that Engender Antibiotic Resistance

The use of antibiotics at recommended dosage levels to treat confirmed bacterial infections is a type of exposure for which the benefit far outweighs the risk of selecting resistant strains (Amenu, 2014). Unfortunately, much of the antibiotic therapy is not laboratory oriented or even laboratory extrapolated. This coupled with the high proportion of life-threatening infections that require immediate treatment. So the prescribed antibiotic should be with first-line drugs such as ampicillin, ampiclox, cotrimoxazole, chloramphenicol, erythromycin, gentamicin, penicillin, tetracycline and metronidazole The treatment of infected people in many parts of Africa is further challenged by the fact that prohibitive cost of newer second-line antimicrobials like amoxicillin-clavulanate, cefuroxime, ceftriaxone, ofloxacin, ciprofloxacin, azithromycin, amikacin and others, when available, places them out of the reach for the majority of patients (Amenu, 2014). Since there is no broad enough selection profile of the second-line drugs, there is usually no cost effective customization of empiric antibiotic therapy.

Other challenges include the use of sub-therapeutic doses (mainly by improper prescription or patient non-compliance) which creates a situation whereby highly resistant strains are selected sequentially; and the supply of poor quality (substandard) drugs of which neither the prescriber nor the patient is aware that provides sub-inhibitory selective pressure to kill bacteria. Other problems include man-made conditions (warm, moist and

unhygienic environments) which are not only conducive to the spread of pathogens but also good for the resistant organisms that carry resistant genes e.g. resistance in clinical *Escherichia coli*, *Salmonella* or *Shigella species* (Moran *et al*., 2005).

The other factor is the poor storage which leads to drug degradation by heat and/or humidity during the course of distribution. In addition, overcrowding and lack of resources for effective infection control in many healthcare facilities fuelling hospital epidemics of resistant organisms such as methicillin resistant staphylococci, multiple resistant rods, vancomycin resistant strains and others.

2.7.1 Antibiotic Inactivation

On some occasions cell may gain resistance to antibiotics by making an enzyme that renders the drug inactive, or that decreases the functionality of the antibiotics. The best example is beta lactamases which is capable of breaking the beta-lactam rings of beta lactam antibiotics such as penicillin. In such manner, the breakage of the beta-lactam ring stops the antibiotic from being able to attach to the peptidoglycan precursors. But it will be less likely that penicillin or other similar drugs will be able to disrupt the integrity of the cell wall, as long as the organism produces beta lactamases (Wright *et al*., 2016). This method of resistance can be transferred from one bacterium to another through the production of the R-plasmids, and is common in strains of methicillin resistant *Staphylococcus aureus* (MRSA).

2.7.2 Reduced Membrane Permeability

Another common way of interfering with antibiotics is through the prevention of entrance of the drug into the cell. Gram negative bacteria have an outer cell membrane, and drugs must pass through the cell pores, which are channels that span the outer membrane and allow the entry and exit of materials into or out of the cell. In order to enter the cell or

interact with the cell wall, the drugs must be able to pass through the pores (Wright *et al*., 2016).

A gene mutation can result in altered pores, usually by changing the electrical charge or the physical structure which can make it more difficult for antibiotics to enter the cell. The antibiotic is still functionally active, but it will fail to reach its target site. A microorganism can develop resistance to multiple drug classes at once in this manner. But some Gram-negative bacteria are innately resistant to large drugs like vancomycin, which is too large to pass through the pores even before mutation occurs (WHO, 2000).

2.7.3 Modification of Target site

Many antibiotics act by binding to a target molecular component of the microorganism. A microorganism can decrease the effectiveness of a drug if the target molecule changes slightly in its structure so that antibiotic may no longer be able to bind to the target molecule. For example, tetracyclines block the transfer RNA access site by binding to it. In turn slight changes in the access site may result in microbial resistance to tetracyclines (Acar and Rostel, 2001).

2.7.4 Efflux or Transport of Antibiotic

Another mechanism by which microorganisms can become resistant to antibiotics is by utilizing an efflux pump. An efflux pump is a biological pump that can force the antibiotic out of the cell, so that it cannot reach or stay in contact with its target. This method of antimicrobial resistance may often create resistance to more than one class of antibiotics, especially the macrolides, tetracyclines, and fluoroquinolones because these antibiotics inhibit different aspects of protein and DNA biosynthesis and therefore must be intracellular to exert their effect (Wang *et al*., 2012).

2.8 Economic Significance of Antibiotic Resistance

The detrimental health effects produced by antibiotic resistance go hand in hand with a negative impact on the budget of health systems and, more broadly, on the economy. From the micro-level to the macro-level, antimicrobial resistant micro-organisms (ARMs) have a direct negative impact on many actors and economic dimensions. First, by requiring more intensive therapies, antibiotic resistance increases health expenditures. Second, patients and their families may undergo additional non-healthcare related expenditures (e.g. travel time) or suffer from income loss due to ill-health. At the societal level, antibiotic resistance negatively impact labor market outcomes due to absence from work which, down the line, negatively affect the broader economic performances of countries (AMR-Review, 2015).

The main drivers underlying the additional expenditure are: More intensive medical procedures as, for example, an increased likelihood of undergoing surgery among patients infected with resistant organisms. Surgery may range from debridement of infected tissue to amputation. Excess length of stay or treatment until the infection is eradicated. This entails additional medical and nurse care (and, consequently, time) as well as use of other additional hospital resources. Changes in physicians' prescribing habits that may start prescribing second-line antibiotics even to patients with first-line antibiotic susceptible infections, if the prevalence of ARMs is perceived as increased (McNulty, 2011).

2.9 Transmission of Antibiotic Resistant Bacteria to Man

Many antibiotics that are used in animal feed are also used to treat diseases in man. Such use of antibiotics in feed raised the concern among public health authorities and consumers because such level use of the drug may cause occurrence of bacterial resistance in the gastrointestinal tract (GIT) of these animals. Such resistance can also transfer to bacterial inhabitants of the GIT through food chain (Marshal and Levy, 2011).

The feeding of low levels of antibiotics such as tetracycline and penicillin in poultry, swine and calves to promote growth has resulted in a great increase in the reservoir of resistant bacteria. These resistant bacteria from animals may reach the human population. This is well established with Salmonella infections. Antibiotic resistant bacteria spread from animals to human indirectly via food (e.g. by contamination of carcasses during slaughter), or less commonly by direct contact (farmers, abattoir workers)

2.10 Control of Antibiotic Resistances

2.10.1 Responsible use

Guidelines exist for responsible (proper, appropriate, prudent, or judicious) use of antibiotics in veterinary and human medicine, and are similar in the medical and agricultural sectors (Jacoby, 2009). Veterinary and animal producer organizations in many countries have developed and implemented responsible use of guidelines. These address use in various species, including poultry, swine, dairy and beef from cattle, and sheep. International organizations such as WHO have developed principles or codes of practice to contain antibiotic resistance. The WHO published global principles for the containment of antimicrobial resistance in animals intended for food (WHO, 2000).

The OIE issued five documents concerning antibiotic resistance, including guidelines for the responsible and prudent use of antimicrobial agents in Veterinary Medicine. The other four documents deal with risk analysis methodology, monitoring of use quantities, surveillance programs, and laboratory methodologies (Acar and Rostel, 2001).

2.10.2 Alternative Practice

Herd, flock, and other health management programs overseen by veterinary or other professionals attempt to minimize infectious disease outbreaks by using non-antibiotic interventions early in the life of the animals. The rationale is to promote healthy animals that do not become ill and are, thus, unlikely to be treated with an antibiotic agent. Several

current approaches are available. These non-antibiotic approaches have led to a need to establish performance standards for regulatory and commercial purposes.

2.10.3 Preventing Infectious Diseases in Animals

Focus should be given to the continuous implementation of appropriate measures for disease prevention, to decrease the need for antibiotics. To minimize infection in food, animal production and decrease the amount of antibiotics used, efforts should aim to improve animal health, thereby eliminating or reducing the need for antibiotics for treatment or prophylaxis. This can be achieved by improving hygiene, biosecurity and health management on farms and preventing disease through the use of vaccines and other measures such as probiotics (beneficial bacteria found in various foods), prebiotics (non-digestible foods that help probiotic bacteria grow and flourish) or competitive exclusion products (intestinal bacterial flora that limit the colonization of some bacterial pathogens). Vaccines have been a key component of disease prevention for many years because they have many favorable attributes such as low cost, ease of administration, efficacy, multiple agent efficacy (viruses, bacteria, mycoplasma, and parasites), and safety (worker, animal, environmental, lack of food residue). Adjuvants are sometimes included with vaccines to enhance the immune response. Various delivery systems or routes of administration (for example muscle injection or, aerosol, topical, or oral (mucosal) are used to administer the vaccine into the animal (Lee *et al*., 2016).

Future research in veterinary vaccine adjuvants will focus on particle delivery to antigen presenting cells and immune stimulatory adjuvants to affect a higher and longer lasting state of immune response (Krause *et al*., 2016). New oral delivery systems, such as plant-based vaccines, are being developed that offer ease of administration, production, and other benefits, although the regulatory acceptance of these products remains to be clarified (Krause *et al*., 2016). Bacteriophages have been used successfully to prevent

and treat bacterial diseases in humans and animals in Russia, but have failed to gain acceptance in Western countries owing to the focus on antibiotic use. Anti-infective, bacteriophages have several attractive attributes including specificity because, bacteriophage is directed toward a single kind of bacterium (limited host range), but its lethality, projected low cost, and no residues in the food product is rewarding. However, questions surrounding the safety of using recombinant therapies, environmental containment, and phage resistance remain unresolved (Krause *et al*., 2016).

The other way is through use of Competitive exclusion in which direct-fed microbial products containing live microorganisms (known as probiotics) or products containing enzymes as the active ingredient are currently marketed in many countries. Probiotics, which contain one or more types of microorganisms and are administered orally, are currently approved for use in food, animals in Europe and other countries, but as for the use of antibiotics for growth promotion, their mode of action is not fully understood. Probiotic bacteria could affect normal gut microflora by competitive exclusion of pathogenic bacteria, production of antibacterial products or enzymes that act on gut bacteria, or production of other metabolites that affect gut commensals (Guillard *et al*., 2015).

Bacteriocins have also been investigated for their potential use in the control of certain zoonotic pathogens in the avian intestinal tract because they are pore-forming antibacterial proteins produced by microorganisms. One bacteriocin, nisin, has been approved for use in several food products (Haruta *et al*., 2000).

2.10.4 Molecular Studies of Antibiotics Resistant Bacteria

Antibiotics have been used in clinical practice for about 80 years and, throughout that period the problems posed by resistant bacteria have escalated at a pace that has forced near continuous development of new antibacterial drugs. A large variety of antibiotics are

currently being used in human and veterinary medicine, but their efficacy has been threatened by microbial resistance. Currently, there is concern over the possible spread of resistance determinants to antimicrobials. Antibiotic resistant bacteria are an increasing threat to public health, as highlighted by a recent estimate that in the US methicillin-resistant *Staphylococcus aureus* (MRSA) may contribute to more deaths than HIV2. Methicillin-resistant strains of *S. aureus* were initially documented in the 1960s and have been associated with higher mortality rates than their drug-sensitive counterparts (Lee *et al*., 2016).

The prevalence of multi-antibiotic resistant bacteria in recent decades makes an interest to search for new alternative and effective antibiotics. The antibiotic resistant bacteria are identified that are not killed by commonly used antibiotics. When bacteria are exposed to the same antibiotics over and over, the bacteria can change and are no longer affected by the drug due to changing of bacterial membrane, secretion of enzymes by target organisms, modification of site receptors and/or due to genetic reasons. Antibiotic resistance can result also from large genomic changes, such as the acquisition of entire plasmids or mobile elements encoding resistance factors. To study the antibiotic resistance ability, the key steps are the isolation and purification of the antibiotic resistant strains (Leavitt *et al*., 2009).

Identification of the isolated antibiotic resistant organism using molecular techniques such as 16S rRNA, Random Amplification of Polymorphic DNA (RAPD) and plasmid profile causing infectious processes is usually essential for effective antimicrobial and supportive therapy. Initial treatment may be empiric, based on the microbiological epidemiology of the infection and the patient symptoms (Lee *et al*., 2016).

However, the identification of the infectious organism guides the physician in treatment of the disease, because the necessary data on the causal pathogen including its phenotypic and biochemical characters facilitate the choice of suitable and effective antibiotic.

2.10.5 Molecular Characteristics of *Klebsiella pneumoniae*

Klebsiella pneumoniae has been medically recognized as one of the most important opportunistic pathogens, causing hospital-acquired and healthcare-associated pulmonary system, urinary tract, circulating system and soft tissue infections worldwide. However, *Klebsiella pneumoniae* has become a clinically important microorganism, particularly in last two decades due to its tendency to develop antibiotic resistance and cause fatal outcomes (Yigit *et al.*, 2001).

In recent years, *Klebsiella pneumoniae* has been identified as a major cause of community-acquired pneumonia (CAP) and is responsible for approximately 10% of all hospital acquired infections, ranking second among Gram-negative pathogens. The fatality rate of *Klebsiella pneumoniae*-induced pneumonia in elderly people was 15% to 40%, equal to or even greater than that of *Haemophilus influenzae*. The production of extended-spectrum lactamases (ESBL) in this organism contributes to the emergence and dissemination of *Klebsiella pneumoniae* infections. Effective anti-infective drugs, such as aminoglycosides, fluoroquinolones, and carbapenems, have been used to treat ESBL-producing *Klebsiella pneumoniae* infections. In particular, the use of carbapenems against ESBL-producing Gram negative micro-organisms poses a serious problem in the management of healthcare-associated infections because the abuse of antibiotics might lead to the emergence of carbapenem-resistant organisms (Yigit *et al.*, 2001). Notably, the selective pressure posed by the extensive use of antibiotics has facilitated the emergence of multidrug-resistant (MDR) *Klebsiella pneumoniae*. Furthermore,

conjugational transmission of antibiotic resistance genes across bacterial species and genera has aggravated the problem of *Klebsiella pneumoniae* antibiotic resistance.

Multidrug resistant *Klebsiella pneumoniae* was first reported in the United States, followed by Europe, South America, and Asia (Yigit *et al.*, 2001). At present, infections caused by multidrug resistant *Klebsiella pneumoniae* have become a major problem, as few antibiotics are available, resulting in higher morbidity, longer hospitalization, increased mortality rates, and excessive health care costs compared with infections associated with antibiotic- susceptible micro-organisms. However, the prevalence of antibiotic resistant bacteria significantly varies according to region, country, and susceptible population, as the seriousness of this problem is significantly associated with the measures applied to control the spread of drug-resistant bacteria.

2.10.6 *Klebsiella pneumoniae* Resistome

The resistome is defined as the collection of all the genes that confer antibiotic resistance. The antibiotic resistant genes and the mechanisms that confer resistance to five important classes of antibiotics used to treat *Klebsiella pneumonia* infections including β-lactams, aminoglycosides, quinolones, tigecycline and polymyxins. *Klebsiella* resistome encompassing all antibiotic resistance genes reported in this species over the years since the first use of β-lactam antibiotics in the 1940s.

2.10.7 β-lactam resistance genes

β-lactam antibiotics, in clinical use since the 1940s, are a major class of antibacterial agents prescribed in human medicine. Since their introduction, hundreds of different *β*-lactamases have evolved among enteric pathogens including *Klebsiella pneumoniae*, reaching an astonishing number (>2000) and diversity (Bush and Jacoby, 2010).

2.10.8 Broad-spectrum and extended-spectrum β-lactamases

Resistance to penicillin in *Klebsiella pneumoniae* was reported early in the 1960s, leading to the identification of the first *β*-lactamase genes, *bla*SHV-1 and *bla*TEM-1 (Yigit *et al.*, 2001). Two decades later, the first extended spectrum *β*-lactamase (ESBL) gene, *bla*SHV-2, was identified in *Klebsiella pneumoniae* recovered from an ICU patient in Germany (Kliebe *et al.* 1985). The gene exhibited an extended-spectrum activity against *β*-lactams, including third-generation cephalosporins and monobactams, and was defined as the first ESBL gene. Shortly after, another plasmid-mediated ESBL variant *bla*TEM-3 was reported from this pathogen in France (Sirot *et al.* 1987). Since the emergence of ESBLs in *Klebsiella pneumoniae*, during the 1990–2000s, this pathogen has become the major ESBL-carrying pathogen associated in nosocomial outbreaks. In Israel and Spain, a prevalence of 40% of ESBL production among the total *Klebsiella pneumoniae* hospital isolates was reported (Cant´on *et al.* 2008). During that period, *Klebsiella pneumonia* strains harboured mainly TEM and SHV *β*-lactamases (Chong, 2011), with high occurrence and spread of various alleles in numerous countries (Livermore, 2012).

In the 2000s, there was a shift in the type of ESBLs present in *K. pneumoniae*, causing hospital outbreaks, due to acquisition of plasmids and transposons encoding *bla*CTX-M-type ESBLs, that led to the dominance of CTX-M-producing strains (Calbo and Garau, 2015).

Additional groups of ESBL genes were transferred to *K. pneumoniae* by horizontal gene transfer (HGT) including *bla*OXA-type ESBLs, and the rare genes *bla*GES and *bla*SFO, or *bla*PER, *bla*TLA and *bla*VEB (Philippon *et al.*, 2016). Moreover, inhibitor-resistant *β*-lactamase genes have emerged, encoding enzymes that are partially inhibited by clavulanic acid and tazobactam (Bush and Jacoby, 2010). After 30 years since their emergence, the occurrence of ESBL producing *K. pneumonia* is increasing worldwide,

reaching an epidemic proportion in many countries (Calbo and Garau, 2015), although prevalence and ESBL gene content varies between different geographical regions. According to World Health Organization (WHO), the occurrence of ESBL-producing *K. pneumonia* has reached now endemic rates of up to 50% in many parts of the world, and up to 30% resistance rates in the community demonstrating the widespread nature of this resistance.

2.10.9 Plasmid-mediated AmpC genes

The remarkable versatility of *K. pneumoniae* to incorporate *β*-lactamase genes onto transferrable plasmids that enable their spread, gave rise to the emergence and spread of plasmid-mediated AmpC-like cephalosporins in this species (Jacoby, 2009; Bush, 2010). These genes emerged during the late 1980s and early 1990s, in parallel with the explosion of ESBL genes, and they are entirely plasmid-borne in *K. pneumonia* (Jacoby, 2009). The most abundant *bla*AmpC gene families in this species belong to the CMY, DHA, FOX and MOX types, and their first years of occurrence in *Klebsiella pneumoniae*. Plasmid-encoded *bla*ACT, *bla*MIR, *bla*ACC and *bla*LAT seem to be highly rare AmpC genes in *Klebsiella pneumoniae*, and were not added to the time line. However, evolutionary tendency to incorporate resistant genes onto the chromosome occurred, and the first chromosomal AmpC *bla*CMY-2 was identified in *Klebsiella pneumoniae* in 2009 (Zamorano *et al.* 2015). *Klebsiella pneumoniae* strains showing enhanced resistance to *β*-lactams due to the existence of *bla*AmpC combined with porin loss, or increased efflux, were also reported as with the case of *bla*ACT-1. These genes can also be easily overexpressed on plasmids due to multiple copies, or increased promoter strength of plasmid genes, and even lead to carbapenem resistance (Jacoby, 2009).

2.11 Carbapenem Resistome

The endemic occurrence of ESBL-producing *Klebsiella pneumoniae* in various parts of the globe was reflected by ongoing exponential evolvement of new ESBL-types and alleles within this species, as well as in other *Enterobacteriaceae,* acquired by horizontal transfer of ESBL-encoding plasmids and transposons. The multidrug resistant phenotype characteristics of these ESBL-producing *Klebsiella pneumonia* strains have led to a significant increase in carbapenem use, which became the last resort antibiotics to treat ESBL producing *Klebsiella pneumoniae* (Livermore and Woodford, 2006). The extensive use of carbapenems has resulted in the evolution of plasmid-mediated carbapenemases, i.e. enzymes that hydrolyze all *β*-lactams including the last-line carbapenems. Their appearance in *Enterobacteriaceae* led to carbapenem-resistant *Enterobacteriaceae* (CREs). Probably due to its hospital association, *Klebsiella* turned out to be the major carbapenem resistant enterobacteriaceae to have spread worldwide (Cant´on *et al.*, 2012), posing a significant public health threat. Imipenem was the first carbapenem antibiotic used to treat *Klebsiella pneumoniae* infections back in 1983 (Baumgartner and Glauser, 1983), and imipenem-resistant strains were recognized already after 2 years. The earliest detected carbapenemase was IMP-1 metallo-enzyme, detected in *Klebsiella pneumoniae* from 1991 in Japan (Haruta *et al.,* 2000). Several years later, in 1996, the first *Klebsiella pneumoniae* carbapenemase, named KPC, has emerged in the United States (Yigit *et al.,* 2001). Although other carbapenemase genes occured in *K. pneumonia* and were even reported in this species for the first time, such as *bla*OXA-48 and *bla*NDM-1 (Yong *et al.,* 2009), it is undoubtable that *bla*KPC became the most prevalent and highly impacting carbapenemase in *Klebsiella pneumoniae.* Strains carrying *bla*KPC have been described in all continents, with *bla*KPC-2 and *bla*KPC-3 being the most common genes, highly prevalent in strains involved in hospital outbreaks in many countries (Mathers *et al.*,

2015). The main driving force for spread of these genes was and is still clonal expansion of *K. pneumoniae* ST258, which, since its first report in the United states, has become endemic in many parts of the world (Munoz-Price *et al*., 2013), as will be discussed further. Along with clonal dissemination, *bla*KPC genes reside in a unique Tn*4401* transposon variants (Naas *et al.,* 2012), and are inserted into plasmids of various replicon types, which facilitates the dissemination of the gene to other bacterial species (Chmelnitsky *et al.,* 2014). An interesting example of interspecies transfer of transposon-encoded *bla*KPC-3 gene from *Klebsiella pneumonia* to *Escherichia coli* was reported in a patient from the United States. A *Serratia marcescens* isolate carrying *E. coli* plasmid harboring this transposon was sequentially recovered from the same patient, demonstrating *Klebsiella pneumoniae* as a source for KPC genes in multiple pathogens (Yigit *et al.*, 2001).

Along with their activity against all *β*-lactam antibiotics and high transferability, KPCs often possess resistance to common *β*-lactamase inhibitors, posing a clinical challenge. Increased mortality was shown for infections caused by KPC-producing *Klebsiella pneumoniae* (Munoz-Price *et al*., 2013). With the increasing use of carbapenems to treat ESBL producing *Klebsiella pneumoniae* infections, numerous processes occurred:

(i) carbapenem resistance emerged without the actual carriage of a carbapenemase gene as a result of permeability alterations due to porin loss and over expression of efflux pumps.

The first description of this phenomenon was in 1988, and was further demonstrated by a combination of high-level plasmid-mediated AmpC *bla*ACT-1 together with porin loss, decreased susceptibility to ertapenem by a combination of ESBL and loss of OmpK36 (Leavitt *et al.,* 2009), and the involvement of AcrAB efflux pump (Padilla *et al*., 2010).

(ii) The emergence of unique ESBL genes with wider spectrum of activity, such as

*bla*GES. The first GES-type carbapenemase acquired in *Klebsiella pneumoniae* was *bla*GES-4, identified in 2002. These enzymes are relatively rare in *Klebsiella pneumoniae*, and were mostly reported from Greece, Finland, South Korea and Brazil (Lee *et al*., 2016). (iii) New plasmid encoded carbapenemase genes like *bla*OXA-48 and *bla*VIM arrived in *K. pneumoniae* now reported as endemic in different countries. (iv) Translocation of carbapenemase-encoding genes, such as *bla*VIM-1 and *bla*NDM-1, from plasmids onto *Klebsiella pneumoniae* chromosome, by MGEs that were unique for these ARGs, making these resistances merely impossible to control (Lee *et al*., 2016).

2.11.1 Multiple β-lactamase-encoding *Klebsiella pneumoniae*

Carriage of multiple *β*-lactamase genes in the same strain is a known ability of *Klebsiella pneumoniae* and may contribute to the selective success of this pathogen. Combinations of all types of *bla* genes were reported in this species (Lee *et al.,* 2016). This may be due to carriage of an antibiotic-resistant plasmid encoding an array of ARGs due to acquisition of transposons containing different *bla* genes on the same plasmid.

2.11.2 Aminoglycoside resistance genes

Aminoglycosides were actively used from mid-1940s to 1980s until they were replaced with third-generation cephalosporins, carbapenems and fluoroquinolones (Krause *et al.*, 2016). During that period of use, *Klebsiella pneumoniae* gained the main resistance mechanisms against this group, involving drug modification enzymes with different activities, such as acetylation, adenylation or phosphorylation. Within 10 years, plasmid-mediated resistance genes of all classes, *aac*, *ant* (grouped with *aad*), and *aph* gene families, were identified in *Klebsiella pneumonia.* Decrease in aminoglycoside usage slowed down evolution of new resistance mechanisms until the discovery of 16S rRNA methylase, belonging to the *armA* gene family,that encodes enzymes which prevent aminoglycosides to bind to their 16S rRNA target (Doi *et al*., 2016). These genes are

plasmid-encoded in *Klebsiella pneumoniae* (Galimand *et al*., 2003), and while drug-modifying enzymes have a narrow spectrum of activity, 16S rRNA methylases confer resistance to practically all aminoglycosides, including plazomicin, the most recent aminoglycoside compound developed (Poulikakos and Falagas, 2013). Chromosomal location of *armA* was described in *Klebsiella pneumoniae* only once. Other known plasmid mediated 16S rRNA methylases including Rmt family and NpmA were also found in *Klebsiella pneumoniae* (Krause *et al.*, 2016), with no evidence of chromosomal location.

Chromosomal resistance mechanisms against aminoglycosides in *Klebsiella pneumoniae* include modifications in cell permeability due to alterations in AcrAB-TolC and KpnEF efflux pump systems, and due to loss of putative porin, KpnO. Disruptions in AcrAB-TolC increased susceptibility to tobramycin and gentamicin (Padilla *et al*., 2010), whereas *kpnEF* mutant showed strong change in resistance to tobramycin and spectinomycin, but affected only slightly resistances to gentamicin and streptomycin. This may suggest different affinities of the permeability apparatus to different aminoglycosides. Direct involvement in aminoglycoside resistance was reported *in vitro* for KpnO porin, which upon loss caused resistances to tobramycin, streptomycin and spectinomycin (Yigit *et al.*, 2001).

Mutations which confer resistance via target modification, such as *rrs* or *rpsL*, have not been found yet in clinical strains of *Klebsiella pneumoniae*. It is possible that *rpsL* mutations are connected with high fitness cost and reduced virulence, so they are less preferable. Multiple copies of *rrs* in *Klebsiella pneumoniae* chromosome may also complicate the development of resistance due to mutations in this gene (Yigit *et al.*, 2001).

2.11.3 Quinolone resistance genes

Quinolones target bacterial topoisomerases blocking bacterial DNA replication. These drugs have been used in clinical practice since the 1960s, but their use increased extensively after the introduction of the first fluoroquinolones in the 1980s, which has led to development of bacterial quinolone resistance mechanisms (Naeem *et al.,* 2016). *Klebsiella pneumoniae* resistome combines all the resistance mechanisms known for quinolone resistance in Gram-negative bacteria (Redgrave *et al.,* 2014), including target-site gene mutations, increased production of MDR efflux pumps, modifying enzymes and/or target protection proteins.

Cases of *Klebsiella* treated with nalidixic acid, the first clinically approved quinolone and norfloxacin, the first fluoroquinolone used against *Klebsiella pneumoniae* (Guerra *et al.,* 1983), were accompanied by the first evidences of chromosomal resistances to nalidixic acid. The first and major resistance mechanism is chromosomal mutations in the quinolone binding targets, DNA gyrase (*gyr*A-*gyr*B subunits) and topoisomerase IV (*par*C-*par*E subunits). Mutations in *gyrA* and *parC* in *K. pneumonia* were recognized earlier than mutations in *gyrB* (Nam *et al.,* 2013) and *parE* (Guillard *et al.,* 2015), and are probably more common (Nam *et al.,* 2013).

Changes in *Klebsiella pneumoniae* cell permeability have been reported to be involved in quinolone resistance, similar to their role in *β*-lactam resistance. These included OmpK36 deficiency (Mart´ınez-Mart´ınez *et al.,* 1996), overexpression of *acrAB*, the multidrug efflux pump gene (Mazzariol *et al.,* 2002) and unaltered production of *kdeA.* In *vitro* transferring of these genes into sensitive *Escherichia coli* increased. Another important pump in *Klebsiella pneumoniae*, OqxAB, assumed to originate from the *Klebsiella pneumoniae* chromosome, has now been recognized to be involved in plasmid-mediated quinolone resistance (PMQR), and to mobilize and spread among other bacteria. Efflux

pump regulators were also reported to be involved in quinolone resistance in *K. pneumoniae*; the years in which the first resistance was identified are indicated, as well as the alteration in expression found in quinolone-resistant *Klebsiella pneumoniae* strains. Another group of quinolone resistance genes include PMQR determinants, which occur in *Klebsiella pneumoniae*, as in other *Enterobacteriaceae* (Jacoby *et al.,* 2014). These genes encompass members of the *qnr* genes, which encode a family of proteins that physically protect DNA gyrase and topoisomerase IV from the inhibitory activity of quinolones. The first *qnr* gene was discovered on a plasmid of *Klebsiella pneumoniae* isolated in 1994 in the United States. There is no evidence of *qnr* genes on the *K. pneumoniae* chromosome, although these proteins are chromosomally encoded in other Gram negative bacteria including disease-causing species like *Shewanella algae, Citrobacter* spp., *Stenotrophomonas maltophilia, Vibrionaceae* and *Serratia marcescens*. Another PMQR gene, aac(6_)-Ib-cr, is the single gene responsible for quinolone modification in *K. pneumoniae*. It deactivates narrow spectrum quinolones such as ciprofloxacin and norfloxacin, which carry the unsubstituted piperazinyl group, a substrate of this enzyme. Initially, it was plasmid-encoded in *Klebsiella pneumoniae*, but was recently found on chromosome of this species. Aminoglycoside resistance is often accompanied with co-resistance to *β*-lactams and fluoroquinolones. These conferred resistance to both aminoglycosides (tobramycin, amikacin and kanamycin) and fluoroquinolones (ciprofloxacin and norfloxacin). PMQR gene *qepA* encodes an efflux pump protein and it has yet to be recognized on *K. pneumoniae* chromosome. Mechanisms provided by expression of PMQR genes produce low or moderate levels of resistance to quinolones, but they create favorable conditions for appearance of chromosomal gene mutants (Jacoby *et al.*, 2014).

2.11.4 Polymyxin resistance genes

Polymyxin disrupts membrane integrity through displacement of cations (Ca+2/Mg+2) in the outer membrane, by binding to the negatively charged lipopolysaccharides (LPS) and leading to cell lysis (Falagas and Kasiakou, 2005). The history of polymyxin resistance in *Klebsiella pneumoniae* is shorter compared to other classes of antibiotics, owing to restricted use in human medicine between 1980s and 2000s, due to recognized toxicity (Jacoby *et al.,* 2014). The first clinical isolate of colistin-resistant *Klebsiella aerogenes* (now classified as *pneumoniae*) was isolated during the first period of usage. In the early 2000s, with the increasing occurrence of XDR carbapenemase-producing *Klebsiella pneumonia* (CPKP) strains, therapy often relied on polymyxins, which became one of the last line of drugs (Falagas and Kasiakou, 2005).

The first hospital outbreak of colistin non-susceptible MDR *K. pneumoniae* was reported in 2004 from Greece (Antoniadou *et al.,* 2007), and since then an increasing number of reports on the recovery of colistin-resistant strains emerged from the clinical setting. The major mechanism of polymyxin resistance in *K. pneumonia* is target modification, achieved by chromosomal mechanisms, and is referred to as the 'LPS modification system'. Strains equipped with this multifaceted system alter the LPS structure, resulting in decreased anionic charge interfering with polymyxins binding. These changes in LPS are provided by mutations in several core genes, responsible for the maturation of lipid A (*lpxM* and its regulator *ramA*) (Clements *et al.,* 2007) and by neutralization of lipid A, by additional binding of amino arabinose (*pbgP*, *pmrE*), phosphoethanolamine (*pmrC*) or palmitate (*pagP*) (Llobet *et al.,* 2011). Resistance also involves increased activity of numerous LPS-modifying gene regulators, such as *phoPQ*, *pmrA* and *pmrD*). Mutation in one of two other regulation genes, leading to *pmrB* overexpression or *mrgB* deactivation, is already sufficient to cause resistance to polymyxins. Another potential

pathway for LPS modification involves TupA-like/glycosyltransferase and CrrAB regulatory system described by (Wright *et al*., 2016) These numerous pathways that lead to colistin resistance in *K. pneumoniae* in the clinical setting are complexed, may derive independently in a specific strain genetic background and are less common via patient-to-patient spread of resistant strains (Wright *et al.,* 2016).

Additional mechanisms found to be involved in colistin resistance in *K. pneumoniae* are capsule polysaccharides (CPS) that may mask charged molecules on the outer membrane, and an increased expression of efflux pumps AcrAB-TolC and KpnEF due to positive regulation of their transcription by RarA (Jacoby *et al.,* 2014).

Plasmid-mediated polymyxin resistance was reported only recently, with the identification of the *mcr-1* gene in China (Liu *et al.,* 2016). This gene encodes a phosphoethanolamine transferase enzyme family that modifies lipid A by connecting it with phosphoethanolamine, similarly to the activity of PmrC (Liu *et al.,* 2016). The *mcr-1*-encoding plasmid, although originally found in *E. coli*, was conjugated and expressed *in vitro* in *K. pneumoniae*. Shortly after, widespread occurrence of this gene was reported in various countries across the globe. It was suggested to originate from *E. coli* with chicken origin, presumably due to extensive colistin use in the poultry (Jacoby *et al.,* 2014).

For CRKP, only tigecycline, colistin and some aminoglycosides still show favourable *in vitro* activities. Their XDR profile results in increased usage of colistin, which in turn leads to emergence of strains with induced resistance. This was demonstrated by widespread of colistin-resistance among KPC producing *Klebsiella pneumoniae* (Lee *et al*., 2016). Colistin-resistant *Klebsiella pneumoniae* was found to be significantly higher in prevalence as compared to other *Enterobacteriaceae* species.

Co-selection of colistin resistance was observed also as a result of a broad-spectrum cephalosporin treatment in ESBL producing *Klebsiella pneumoniae,* when resistance to colistin was achieved due to disruption of *mgrB* by IS*Ecp1-bla*CTX-M-15 or IS*Ecp1-bla*OXA-181 (Zowawi *et al.,* 2015). High rates of colistin resistance (up to 36%) were found in carbapenem resistant *K. pneumoniae* in endemic areas like Italy linked to higher mortality (Capone *et al.,* 2013). Description of CRKP isolates carrying *mcr-1* has just been started, but spread of this gene is already considered to be a serious threat resulting in strains that are pan-resistant.

2.12 The Animal and Human face of Antimicrobial use

Over the past half century, the use of antimicrobials to treat infections in human and animals has generated an enormous antimicrobial pressure not only on targeted pathogens but also on commensal bacteria. Response to therapeutic antimicrobial pressure, the intestinal flora may undergo dramatic changes, including reductions in the orders of *Bifido bacteriales, Clostridiales, Campylobacterales*, but an exponential and sudden increase of *Enterobacteriales* (*Escherichia*) and *Lactobacillales* (*Enterococcus*) as described very recently in case of streptomycin and/or tetracycline therapy of laying hens (Capone *et al.,* 2013). The accumulating effect of traditional antimicrobials was completed by the continuous discovery and introduction of new therapeutical drugs, which drives bacteria to be trained to constant changes by selecting appropriate antimicrobial resistance pheno- and genotypes. Once armed with the required set of antimicrobial resistance genes, bacterial strains may have the advantage to survive and spread both in animal and human populations, since with few exceptions the same antimicrobial classes are used to treat infections in animals and humans (Guardabassi and Courvalin, 2006). Although antimicrobial classes are common in veterinary and human medicine, their importance may vary according to the species and application. Majority

of the antibacterial compounds are generally used to treat a wide range of animals and infections, but there are drugs with applications restricted to certain groups of species e.g difloxacintoavian infections. On the other hand, some antimicrobial classes such as cephalosporins (first to fourth generation) are represented by a large number of compounds for treating serious infections in humans, while only few of them has veterinary application (Jacoby *et al.,* 2014). In addition, based on the importance in medication, availability of alternatives, selection of cross resistance, and frequency of use, antimicrobials are ranked into critically, or highly important drugs. Obviously, these categories do not necessarily overlap. There are further remarkable differences in the use of antibacterial agents in humans and animals, especially in food animals. In humans drugs are generally administered directly to sick (Jacoby *et al.*, 2014).

2.12.1 Resistant Gene in *Escherichia coli*

Due to the introduction of antimicrobials as growth promoters and/or as therapeutic agents combating bacterial infections, targeted pathogenic *Escherichia coli* strains and their commensal counterparts habituating the intestine are similarly exposed to the effect of various antimicrobial compounds, thereby being forced to develop different strategies to survive and grow in the newly established toxic environment. The most efficient and sophisticated defense mechanism is the acquisition of MDR, characterized by the complex interaction of different mechanisms (e.g drug efflux, enzymatic inactivation) conferring simultaneous resistance to a wide range of older or new antimicrobial compounds or drug classes. Recently, MDR became widely established especially in Gram-negative bacteria such as *E. coli*, being a "versatile" species encompassing different pathotypes, but also as a member of the normal intestinal flora (Krause *et al.*, 2016). Therefore, *Escherichia coli* may play a special role in the accumulation and inter play between resistance traits. In contrast to pathogenic strains, which are in the focus of the

therapy, commensal strains are generally marginalized in many respects, due to their reduced clinical significance. Tackled as potential reservoirs of resistance determinants, the prevalence of antimicrobial resistance in commensal *Escherichia coli* from food animals is monitored regularly (European Food Safety Authority and European Centre for Disease Prevention and Control [EFSA and ECDC]. However, their genetic attributes, such as the co-existence and spread of resistance genes, and their ability to colonize the human intestine are not adequately considered (Krause *et al.*, 2016).

CHAPTER THREE

3.0 Materials and Methods

3.1 Study Area

The study was carried out in Minna, Niger State (Figure 1). Minna is a city in middle belt Nigeria and lies on latitude 9.5836^0 N and longitude 6.5463^{0}E at an altitude of 256 m above sea level. It has a landmass of 76469.903 square kilometres (about 10% of the total land area of Nigeria) out of which about 85% is arable. It is one of the largest cities in the country with three major ethnic groups (Nupe, Gbagyi and Hausa). It has numerous settlers from other parts of the country living peacefully and contributing their quota to the development of the state. Majority of the populace are farmers while others are involved in other vocations such as white-collar jobs, business, craft and arts.

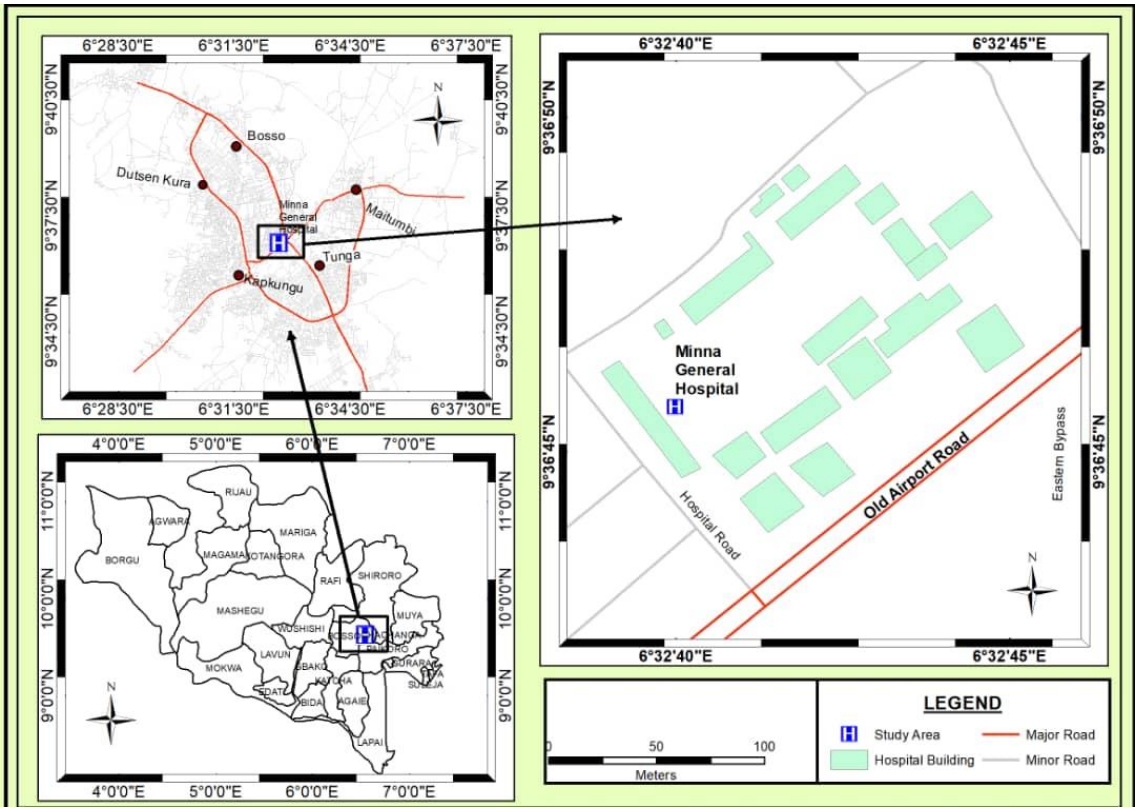


Figure 1: Map of the study area (Source: Department of Geography, Federal University of Technology, Minna)

3.2 Study Population

The population sampled comprises of in and out patients of both genders within the age range of 1-70 years attending General Hospital Minna, Niger State.

3.3 Ethical Consideration

Ethical approval was obtained from the Ethics and Research Committee of General Hospital, Minna, Niger state.

3.4 Sample Size Determination

Single population proportion using the prevalence reported by Olowo-Okere *et al.* (2019) was used to determine the sample size. The sample size was calculated using equation 3.1

$$n = \frac{Z^2 p(1-p)}{d^2} \quad (3.1)$$

where n is sample size,

p = prevalence rate = 14.6% by Olowo-Okere *et al.* (2019)

z = 1.96 for confidence level at 95%

d = marginal tolerable error at 0.05%

$$n = \frac{(1.96)^2 \; x \; 0.146 \; x \; (1 - 0.146)}{(0.05)^2}$$

$$n = \frac{0.479}{0.0025} = 191.6$$

Total number of samples = 192 samples

3.5 Collection and Transportation of Samples

Urine and stool samples were collected from patients that agreed to participate in the research and were instructed on the standard procedures for sample collection. Thereafter, they were issued sterile sample bottles. The samples collected were stored in ice pack and then transported to Centre for Genetic Engineering and Biotechnology (CGEB)

laboratory, Federal University of Technology Minna, where they were processed based on standard microbiological procedures.

3.6 Bacterial Isolation

Urine and stool samples were inoculated onto MacConkey agar and incubated at 37°C for 24 hours. Characteristically distinct colonies obtained after incubation were sub cultured onto Eosin Methylene Blue agar (EMB) repeatedly to obtain pure cultures. Gram negative bacterial isolates obtained were then stored on nutrient agar slant for further identification and analysis.

3.7 Identification of Isolates

Gram negative bacterial isolates stored on agar slants were identified with the help of colony morphology and conventional biochemical tests including Catalase test, Urease test, Citrate test, Indole test, Methyl red test, Oxidase test, Motility test, Voges Proskauer test.

3.8 Susceptibility Testing

Antimicrobial susceptibility was determined using the Kirby Bauer disk diffusion method. Suspension of the test bacteria was adjusted in normal saline to the turbidity of 0.5 McFarland standard. A loopful of the adjusted bacteria solution was swab onto Muller-Hinton agar. Single disc antimicrobial disc of Imipenem (10µg), Colistin (10µg), Trimethoprim-sulfamethoxazole (25µg), Amoxicillin-clavulanic acid (30µg), Fosfomycin (50µg), Gentamicin (10µg), Ciprofloxacin (10µg), Nalidixic acid (30µg), Tetracycline (10µg**)**, Erythromycin **(**30µg**)**, Ceporex (10µg**)**, Ampicillin (30µg**)**, Chloramphenicol (30µg), Streptomycin (30µg**)**, and Ofloxacin (10µg), was aseptically place onto the surface of the inoculated plates. The plates were allowed to sit for a while at room temperature and then incubated at 37 degree for 18-24 hours. Results were recorded by measuring the diameter of the zones of inhibition around the antibiotics and

interpreted in accordance with the break-points and criteria recommended by the Clinical Laboratory Standard Institute (CLSI, 2018).

Multiple antibiotic resistance index was calculated as the ratio of the number of antibiotics to which the isolate displaced resistance, to the number of antibiotics to which the isolate had been evaluated for susceptibility (Krumperman, 1983).

3.9 Molecular Identification of Bacterial Isolates

3.9.1 DNA Extraction

Genomic DNA extraction was carried out with column-based JENA Bioscience Bacteria DNA Preparation Kit following manufacturer's instructions. Bacteria cells were harvested from 500µl aliquot of bacteria broth culture using a microcentrifuge at 10,000 rpm for 1minute. The residual pellet was resuspended in 300µl of Resuspension Buffer and 2µl of Lysozyme Solution. The mixture was homogenized by inverting several times thereafter incubated at 37°C for 1 hour. Resuspended cells were recovered by centrifugation and lysed by adding 300µl of Lysis Buffer, 2µl RNase A and 8µl proteinase K solution were added, followed by 10 minutes incubation at 60 °C. The tube was cooled on ice for 5 minutes and 300µl binding buffer was added to the mixture and vortexed briefly. The mixture was cooled on ice for 5 minutes and thereafter centrifuged at 10,000 rpm for 5 minutes. The supernatant was transferred directly into the spin column and centrifuged at 10,000 rpm for 1minute to trap the DNA. The trapped DNA was washed twice with washing buffer after which it was eluted with 50µl elution buffer into a clean Eppendorf tube.

3.9.2 Polymerase Chain Reaction (PCR)

3.9.2.1 16S rRNA Amplification

Each PCR reaction mixture consist of 12.5µl mastermix (2x JENA Ruby hot start mastermix),1µl (10pmol) each of forward primer 27F 5' AGA GTT TGA TCM TGG

CTC AG3’ and reverse primer 1492R-5’ TAC GGY TAC CTT GTT ACG ACT T 3’ 1µl DNA template and 9.5 µl sterile nuclease free water to make up a total reaction volume of 25 µl. PCR amplification was carried out in an Applied Biosystem 2720 Thermocycler. The mixture was subjected to an initial denaturation at 94°C for 3minutes, followed by 35 cycles of denaturation at 94°C for 3 minutes followed by 35 cycles of denaturation at 94°C for 45 seconds , annealing at 55°C for 60 seconds and extension at 72°C for 60 seconds; and a final extension at 72°C for 10minutes.

3.9.3 Gel Electrophoresis

PCR products were visualized on a 2 % agarose gel containing ethidium bromide in 0.5x Tris-borate buffer (pH 8.0).

3.9.4 Sequencing

PCR products were purified and sequenced by Sanger sequencing method using AB1 3730XL sequencer and done by Inqaba biotec, Pretoria, South Africa. The primer sequences and protocols for amplification is shown in table 3.1. The extracted DNA was used to detect the presence of some antibiotic resistance genes from the bacterial isolates by PCR

Table 3.1 Primer Sequence and PCR Protocol for Amplification of Antibiotic Resistant Genes

Genes	Primer sequences	PCR amplification
TEM	F: 5'GCGGAACCCCTATTTG 3' R: 5'ACCAATGCTTAATCAGTGAG3'	• Initial denaturation at 94°C for 3minutes • Finial denaturation 94°C for 60seconds • Annealing at 50°C for 60seconds • Extension at 72°C for 60seconds • Finial extension at 72°C for 10minutes
CTX	F:5'ATGTGCAGYACCAGTAARGTKATGGC3' R:5'TGGGTRAARTARGTSACCAGAAYSAGCGG3'	• Initial denaturation at 94°C for 3minutes • Finial denaturation 94°C for 60seconds • Annealing at 60°C for 60 seconds • Extension at 72°C for 60seconds • Finial extension at 72°C for 10minutes
Tet A	F: 5'GTAATTCTGAGCACTGTCGC 3' R:5'CTGCCTGGACAACATTGCTT 3'	• Initial denaturation at 94°C for 3minutes • Finial denaturation 94°C for 60seconds • Annealing at 57°C for 60seconds • Extension at 72°C for 60seconds • Finial extension at 72°C for 10minutes
Tet B	F: 5'CTCAGTATTCCAAGCCTTTG 3' R:5'ACTCCCCTGAGCTTGAGGGG 3'	• Initial denaturation at 94°C for 3minutes • Finial denaturation 94°C for 60seconds • Annealing at 52°C for 60seconds • Extension at 72°C for 60seconds • Final extension at 72°C for 10minutes
Mcr 1	F: 5'AGTCCTTTGTTCTTGTGGC 3' R: 5'AGATCCTTGGTCTCGGCTTG 3'	• Initial denaturation at 94°C for 15 minutes • Finial denaturation 94°C for 30seconds • Annealing at 58°C for 90seconds • Extension at 72°C for 60seconds • Finial extension at 72°C for 10minutes
Mcr 2	F: 5'CAAGTGTGTTGGTCGCAGTT 3' R: 5'TCTAGCCCAACAAGCATACC 3'	• Initial denaturation at 94°C for 15 minutes • Finial denaturation at 94°C for 60seconds • Annealing at 57°C for 60seconds • Extension at 72°C for 60seconds • Finial extension at 72°C for 10minutes

3.9.5 Gel Electrophoresis

The PCR products were visualized on a 2 % agarose gel containing ethidium bromide in 0.5x Tris-borate buffer (pH 8.0).

3.10 Data Analysis

Data generated from this study was analyzed using the Statistical packages for social sciences (SPSS) version 20.0.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Results

4.1.1 Prevalence of *Escherichia* and *Klebsiella* species in the Study Area

Out of the 96 Urine and Stool samples each screened, *Escherichia* spp. (23.4%) and *Klebsiella* spp. (16.1%) were obtained (Table 4.1). From the 96 urine sample screened 23 (12.0%) *Escherichia* spp. and 13 (6.8%) *Klebsiella* spp. were isolated. In the stool samples 22 (11.5%) *Escherichia* spp. and 18 (9.4%) *Klebsiella* spp. were isolated. The Chi-square analysis revealed no significant difference between the urine and stool sample of *E. coli* and *Klebsiella spp*. ($p>0.05$). The prevalence of *Escherichia* and *Klebsiella* species in the study area is shown in table 4.1.

Table 4.1: Prevalence of *Escherichia* and *Klebsiella* species in the Study Area

		Escherichia species		*Klebsiella* species	
Sample	NSS	NPS	Prevalence (%)	NPS	Prevalence (%)
Urine	96	23	12.0	13	6.8
Stool	96	22	11.5	18	9.4
Total	192	45	23.4	31	16.1

χ^2cal=0.036 χ^2tab=3.841 df=1.0

Keys: NSS: Number of samples screened, NPS: Number of positive samples

4.1.2 Prevalence of *Escherichia* and *Klebsiella* Species According to Gender

The prevalence of *Escherichia* and *Klebsiella* species according to gender is shown in table 4.2. Result revealed that out of the 76 isolated bacteria, 39 (51.3%) were obtained from females while 37 (48.7%) were isolated from males. *Klebsiella* spp. 18 (23.7%) had higher prevalence in females compared to 13 (17.1%) in males, while *Escherichia* spp. 24 (31.6%) was more common in male than in females 21 (27.6%). The Chi-square analysis revealed no significant difference between the prevalence of *E. coli* and *Klebsiella spp*. ($p>0.05$) in males and females.

Table 4.2: **Prevalence of *Escherichia* and *Klebsiella* species according to gender**

Bacterial isolate	No of isolate	Male	Female	Total
Escherichia spp.	45	24(31.6)	21(27.6)	59.2
Klebsiella spp.	31	13(17.1)	18(23.7)	40.8
Total	76	37(48.7)	39(51.3)	100

χ^2cal=0.26 χ^2tab=5.991 df=2.0

4.1.3 Prevalence of *Escherichia* and *Klebsiella* Species by Age

Highest prevalence of *Escherichia* spp. was observed in the age group of 31-40 years with a prevalence of 12 (15.8%) followed by age groups 21-30 and 41-50 years both having a prevalence of 11 (14.5%). On the other hand, *Klebsiella* spp. was found to be more prevalent among the age group 21-30 years with a prevalence of 8 (10.5%) followed by the age groups 11-20 and 31-40 years both having a prevalence of 7 (9.2%). The Chi-square analysis showed significant difference between the prevalence of *E. coli* and *Klebsiella spp*. ($p>0.05$) in different age groups. The prevalence of *Escherichia* and *Klebsiella* species by age is shown in table 4.3.

Table 4.3: Prevalence of *Escherichia* and *Klebsiella* species by Age

Age (years)	No of isolate	*Escherichia* spp.	*Klebsiella* spp.
1-10	1	1 (1.3)	0 (0.0)
11-20	15	8 (10.5)	7 (9.2)
21-30	19	11 (14.5)	8 (10.5)
31-40	19	12 (15.8)	7 (9.2)
41-50	14	11 (14.5)	3 (3.9)
51-60	6	1 (1.3)	5 (6.6)
61-70	2	1 (1.3)	1 (1.3)
Total	**76**	**45**	**31**

χ^2cal=36.84 χ^2tab=21.026 df=12.0

4.1.4 Antibiotic Susceptibility Profile of *Escherichia* and *Klebsiella* Species

The susceptibility profile of *Escherichia* species to antibiotics is shown in table 4.4. *Escherichia* species isolates showed high level of resistance to Ceporex 44 (97.8%), Tetracycline 37 (82.2%), Nalidixic acid 32 (71.1%), Colistin 35 (77.8%), Ampicillin 28 (62.2%), Trimetoprim-sulfamethoxazole 26 (57.8%) and Chloramphenicol 23 (51.1%), although susceptible to Fosfomycin 45 (100.0%), Amoxicillin Clavulanic acid 37 (82.2%), Tarivid 37 (82.2%), Gentamicin 31 (68.9%) and Ciprofloxacin 31 (68.9%). The Chi-square analysis showed significant difference between different antibiotics used against *E. coli* ($p>0.05$).

Similarly, in *Klebsiella* isolates the Chi-square analysis showed significant difference between different antibiotics used against *Klebsiella spp.* ($p>0.05$). High level of resistance was observed in Ceporex 27 (87.1%), Tetracycline 26 (83.9%), Colistin 25 (80.6%), ampicillin 21 (67.7%) and Trimethoprim-sulfamethoxazole 17 (54.8%) while the isolates were highly susceptible to Fosfomycin 28 (90.3%), Tarivid 28 (90.3%), Imipenem 25 (80.6%), Ciprofloxacin 25 (80.6%), Amoxicillin-clavulanic acid 23 (74.2%), Streptomycin 19 (61.3%) and Gentamicin 18 (58.1%). The antibiotic susceptibility profile of *Klebsiella* species is shown in table 4.5

Table 4.4: Antibiotic Susceptibility Profile of *Escherichia* Species

Antibiotics	Susceptible (%)	Intermediate (%)	Resistant (%)
PN	11 (24.4)	6 (13.3)	28 (62.2)
CEP	1 (2.2)	0 (0.0)	44 (97.8)
CH	16 (35.6)	6 (13.3)	23 (51.1)
CN	31 (68.9)	0(0.0)	14 (31.1)
NA	7 (15.6)	6(13.3)	32 (71.1)
E	21 (46.7)	6(13.3)	18 (40.0)
CPX	31 (68.9)	4 (8.9)	10 (22.2)
OFX	37 (82.2)	1 (2.2)	7 (15.6)
S	31 (68.9)	4 (8.9)	9 (20.0)
AMC	37 (82.2)	4 (8.9)	4 (8.9)
SXT	15 (33.3)	4 (8.9)	26 (57.8)
FOS	45 (100)	0 (0.0)	0 (0.0)
IPM	37 (82.2)	3 (6.7)	5 (11.1)
CT	6 (13.3)	4 (8.9)	35 (77.8)
TET	3 (6.7)	5 (11.1)	37 (82.2)

χ^2cal=78.996 χ^2tab=41.337 df=28.0

PN: Ampicillin; CEP: Ceporex; CH: Chloramphenicol; CN: Gentamycin; NA: Nalidixic Acid; E: Erythromycin CPX: Ciprofloxacin; OFX: Tarivid; S: Streptomycin; AMC: Clavulanate; SXT: Sulphamethoxazole; FOS: Fosfomycin; IPM: Imipenem; CT: Colistin; TET: Tetracycline

Table 4.5: Antibiotic Susceptibility Profile of *Klebsiella* Species

Antibiotics	Susceptible (%)	Intermediate (%)	Resistant (%)
PN	7 (22.6)	3(9.7)	21 (67.7)
CEP	1 (3.2)	3 (9.7)	27 (87.1)
CH	12 (38.7)	6 (19.4)	13 (41.9)
CN	18(58.1)	3 (9.7)	10 (32.3)
NA	7 (22.6)	15 (48.4)	19 (61.3)
E	14 (45.2)	5 (16.1)	12 (38.7)
CPX	25 (80.6)	3 (9.7)	3 (9.7)
OFX	28 (90.3)	0(0.0)	3 (9.7)
S	19 (61.3)	3 (9.7)	9 (29.0)
AMC	23 (74.2)	3 (9.7)	5 (16.1)
SXT	11 (35.5)	3 (9.7)	17 (54.8)
FOS	28 (90.3)	0 (0.0)	3 (9.7)
IPM	25 (80.6)	2 (6.5)	4 (12.9)
CT	4 (12.9)	2 (6.5)	25 (80.6)
TET	3 (9.7)	2 (6.5)	26 (83.9)

χ^2cal=121.023 χ^2tab=41.337 df=28.0

PN: Ampicillin; CEP: Ceporex; CH: Chloramphenicol; CN: Gentamicin; NA: Nalidixic Acid; E: Erythromycin CPX: Ciprofloxacin; OFX: Tarivid; S: Streptomycin; AMC: Amoxicillin-clavulanic acid; SXT: Trimetoprim-sulfamethoxazole; FOS: Fosfomycin; IPM: Imipenem; CT: Colistin; TET: Tetracycline

4.1.5 Resistance Category and MARI of *Escherichia* and *Klebsiella* Species

Out of 45 *Escherichia* spp. isolates, 42 (93.3%) were multi-drug resistant while only 3 (6.7%) were extensively drug resistant. About 91.1% of the *Escherichia* spp. isolates had MAR index ranging from 0.2 to 0.5 while 8.9% had MAR index of greater than or equal to 0.6. The MAR index of all *Escherichia* isolates is shown in Table 4.6 and 4.7. In *Klebsiella* isolates only 2 (6.5%) were extensively drug resistant from the 31 *Klebsiella* spp. isolates, 28 (90.3%) were multi-drug resistant, 87.1% of the *Klebsiella* spp. isolates had MAR index ranging from 0.2 to 0.5 while 9.6% had MAR index of greater than or equal to 0.6. The MAR index of all *Klebsiella* spp. isolates is shown in Table 4.8 and 4.9.

Table 4.6 Resistance pattern of *Escherichia* species

Isolate code	Antibiotic resistant pattern	MARI	Number of antibiotics classes resistant to	Number of antibiotics resistant to	Resistance category
GHU3	PN, CEP, NA, SXT, CT, TET	0.4	5	6	MDR
GHU6	PN, CEP, CH, CN, NA, E, CPX, OFX, AMC, SXT, CT, TET, S	0.8	8	13	XDR
GHU7	PN, CEP, CH, NA, E, CPX, OFX, SXT	0.5	5	8	MDR
GH U8	CEP, CH, NA, E, CPX, OFX, SXT, TET	0.5	6	8	MDR
GH U13	PN, CEP, CH, CN, NA, E, CPX, OFX, S, AMC, SXT, CT, TET	0.8	8	13	XDR
GH U14	PN, CEP, CH, CN, NA, E, CPX, OFX, S, AMC, SXT, CT, TET	0.8	8	13	XDR
GH U22	PN, CEP, E, CPX	0.2	3	4	MDR
GH U24	PN, CEP, CH, NA, E, CPX, OFX, SXT, CT	0.6	6	9	MDR
GH U36	CEP, NA, SXT, CT, TET	0.3	5	5	MDR
GH U43	CN, NA, S, SXT, CT, TET	0.3	5	6	MDR
GH U46	PN, CEP, NA, OFX, SXT, TET	0.4	4	6	MDR
GH U51	PN, CEP, CH, NA, SXT, CT, TET	0.4	6	7	MDR
GH U65	PN, CEP, CN, SXT, CT, TET	0.4	5	6	MDR
GH U66	CEP, CH, CN, CT	0.2	4	4	MDR

GH U69	CEP, CH, NA, SXT, JPM, CT	0.4	6	6	MDR
GH U75	PN, CEP, CN, E, CPX, S, TET	0.4	5	7	MDR
GH U76	PN, CEP, NA, E, SXT, CT	0.4	5	6	MDR
GH U77	CEP, CN, NA, E, IPM, TET	0.4	6	6	MDR
GH U78	PN, CEP, CH, CN, SXT, CT, TET	0.4	6	7	MDR
GH U79	CEP, NA, E, S, TET	0.3	5	5	MDR
GH U80	CEP, CH, NA, S, CT, TET	0.4	6	6	MDR
GH U81	PN, CEP, CN, E, IPM, CT, TET	0.4	6	7	MDR
GH U91	PN, CEP, CN, CPX, CT, TET	0.4	5	6	MDR
GH ST1	PN, CEP, NA,CT	0.2	3	4	MDR
GH ST3	PN, CEP, NA, SXT, CT, TET	0.4	5	6	MDR
GH ST7	CEP, CH, NA, SXT, CT, TET, S	0.4	7	7	MDR
GH ST8	PN, CH, CN, NA, SXT, CT, TET	0.4	7	7	MDR
GH ST12	CEP, CH, NA, S, SXT, CT, TET	0.4	7	7	MDR
GH ST17	CEP, CH, NA, S, SXT, CT, TET	0.4	7	8	MDR
GH ST 18	PN, CEP, E, SXT, CT	0.3	4	5	MDR
GH ST20	CEP, SXT, TET	0.2	3	3	MDR

GH ST26	CEP, SXT, TET	0.2	4	4	MDR
GH ST28	CEP, NA, IPM, CT, TET	0.3	5	5	MDR
GH ST30	PN, CEP, CT, TET	0.2	3	4	MDR
GH ST31	CEP, CN, E, SXT, CT, TET	0.4	6	6	MDR
GH ST33	PN, CEP, CH, NA, S, CT, TET	0.4	6	7	MDR
GH ST34	CEP, CH, CT, TET	0.2	4	4	MDR
GH ST35	PN, CEP, NA, CT, TET	0.3	4	5	MDR
GH ST40	PN, CEP, NA, E, SXT, CT, TET	0.4	6	7	MDR
GH ST46	CEP, CH, CN, E, CT	0.3	5	5	MDR
GH ST51	PN, CEP, E, AMC, TET	0.3	3	5	MDR
GH ST71	PN, CEP, CH, E, SXT, CT, TET	0.4	6	7	MDR
GH ST76	PN, CEP, CN, NA, CT, TET	0.4	5	6	MDR
GH ST80	CEP, CH, NA, TET	0.2	4	4	MDR
GH ST81	PN, CEP, CH, NA, CT, TET	0.4	5	6	MDR

PN: Ampicillin; CEP: Ceporex; CH: Chloramphenicol; CN: Gentamicin; NA: Nalidixic Acid; E: Erythromycin CPX: Ciprofloxacin; OFX: Tarivid; S: Streptomycin; AMC: Clavulanate; SXT: Sulphamethoxazole; FOS: Fosfomycin; IPM: Imipenem; CT: Colistin; TET: Tetracycline; MDR: Multidrug resistance; XDR: Extensively drug resistance

Table 4.7: Multiple Antibiotic Resistance index (MARI) of *Escherichia* Species.

MARI	Number of isolates	Percentage
0	0	0.0
0.1	0	0.0
0.2	8	17.8
0.3	8	17.8
0.4	23	51.1
0.5	2	4.4
0.6	1	2.2
0.7	0	0.0
0.8	3	6.7
0.9	0	0.0
1.0	0	0.0
Total	**45**	**100**

Table 4.8 Resistance pattern of *Klebsiella* species

Isolate Code	Antibiotic Resistance Pattern	MARI	Number of Antibiotic classes resistant to	Number of Antibiotic resistant to	Resistance Category
GH U15	PN, CEP, CH, CN, NA, E, CPX, OFX, S, SXT, CT, TET,	0.8	8	12	XDR
GH U17	CEP	0.1	1	1	-
GHU19	PN, CEP, OFX, CH, CN, NA, E, CPX, S, SXT, CT, TET	0.8	8	12	XDR
GH U52	CH, NA, S, FOS, CT, TET	0.4	6	6	MDR
GH U55	CEP, CH, NA, CT, TET	0.3	5	5	MDR
GH U57	PN, CEP, NA, SXT, IPM, CT, TET	0.4	6	7	MDR
GH U58	CEP, CH, NA, SXT, CT, TET	0.4	6	6	MDR
GH U59	PN, CEP, NA, SXT, IPM, TET	0.4	5	6	MDR
GH U60	CEP, CH, NA, SXT, CT, TET	0.4	6	6	MDR
GH U67	PN, CEP, CH, E, S, TET	0.4	5	6	MDR
GH U 82	PN, CEP, NA, E, SXT, TET	0.4	5	6	MDR
GH U85	PN, CEP, CN, S, CT, TET	0.4	4	6	MDR
GH U89	CEP, E, S, CT, TET	0.3	5	5	MDR
GH U95	PN, CEP, CH, E, CT	0.3	4	5	MDR
GH ST2	PN, CEP, NA, CT, SXT	0.3	4	5	MDR

GH ST4	PN, CH, CN, NA, SXT, CT, TET	0.5	7	8	MDR
GH ST5	CEP, CN, NA, S, AMC, SXT, FOS, CT, TET	0.6	7	9	MDR
GH ST6	CEP, CH, CN, E, SXT, CT, TET	0.4	7	7	MDR
GH ST15	PN, CEP, NA, S, SXT, CT, TET	0.4	6	7	MDR
GH ST16	PN, NA, SXT, CT, TET	0.3	5	5	MDR
GH ST19	PN, CEP, E, CT, TET	0.4	4	5	MDR
GH ST24	CEP, CN, NA, SXT, CT, TET	0.4	6	6	MDR
GH ST27	PN, CEP, NA, OFX, SXT, TET	0.4	4	6	MDR
GH ST 29	PN, CEP, NA, S, CT,	0.3	4	6	MDR
GH ST32	PN, CEP, CN, E, SXT, IPM, CT	0.4	6	7	MDR
GH ST38	PN, CEP, NA, CT, TET	0.3	4	5	MDR
GH ST57	PN, CEP, CH, OFX, CT, TET	0.4	5	6	MDR
GH ST58	CEP, CH, E, AMC, CT, TET	0.4	5	6	MDR
GH ST66	PN, CEP, CN, AMC, TET	0.3	3	5	MDR
GH ST67	PN, CN, E, CPX, SXT, CT	0.4	6	6	MDR
GH ST78	PN, CEP, CH, NA, E, CT, TET	0.4	6	7	MDR

PN: Ampicillin; CEP: Ceporex; CH: Chloramphenicol; CN: Gentamicin; NA: Nalidixic Acid; E: Erythromycin CPX: Ciprofloxacin; OFX: Tarivid; S: Streptomycin; AMC: Amoxicillin-clavulanic acid; SXT: Trimetoprim-sulfamethoxazole; FOS: Fosfomycin; IPM: Imipenem; CT: Colistin; TET: Tetracycline; MDR: Multidrug resistance; XDR: Extensively drug resistance

Table 4.9: Multiple Antibiotic Resistance index (MARI) of *Klebsiella* species.

MARI	Number of isolates	Percentage
0	0	0.0
0.1	1	3.2
0.2	0	0
0.3	8	25.8
0.4	18	58.1
0.5	1	3.2
0.6	1	3.2
0.7	0	0.0
0.8	2	6.5
0.9	0	0.0
1.0	0	0.0
Total	**31**	**100**

4.1.6 Prevalence of MDR and XDR

Among the 76 *Escherichia* and *Klebsiella* spp. isolates obtained, 75 (98.7%) showed high level of antibiotic resistance. 70 (92.1%) were multidrug resistant (MDR) while 5 (6.5%) were extensively drug resistance (XDR). The prevalence of MDR and XDR is shown in table 4.10

Table 4.10 Prevalence of MDR and XDR

Isolate	No of isolate	MDR (%)	XDR (%)
Escherichia spp.	45	42 (60.0)	3 (60.0)
Klebsiella spp.	31	28 (40.0)	2 (40.0)
Total	**76**	**70 (100)**	**5 (100)**

MDR: Multidrug resistance; XDR: Extensively drug resistance

4.1.7 Molecular Identification of Isolates and Detection of Antibiotic Resistance genes

Identification of the five selected isolates with MARI value of 0.8 was carried out using the 16S rRNA sequence analysis. The gel electrograph of the selected isolates is shown in plate 1 and plate 2. The obtained sequences were queried in the GenBank of NCBI and the result revealed the test organisms were *Escherichia coli* strain NBRC 102203, *Escherichia coli* strain U 5/41 and *Klebsiella pneumoniae* strain DSM 30104 (Table 4.11). The gel electrophoresis plates of PCR performed using extracted DNA from isolates U6, U13, U14, U15, U19 indicated the presence of TEM, Tet A, Tet B and CTX genes with 964bp, 956bp, 414bp and 593bp amplicon size (Plate 4, 5, 6, 7). There was no amplification in Mcr 1 and Mcr 2 genes as indicated by no visible band in the gel electrophoresis image (Plate 3)

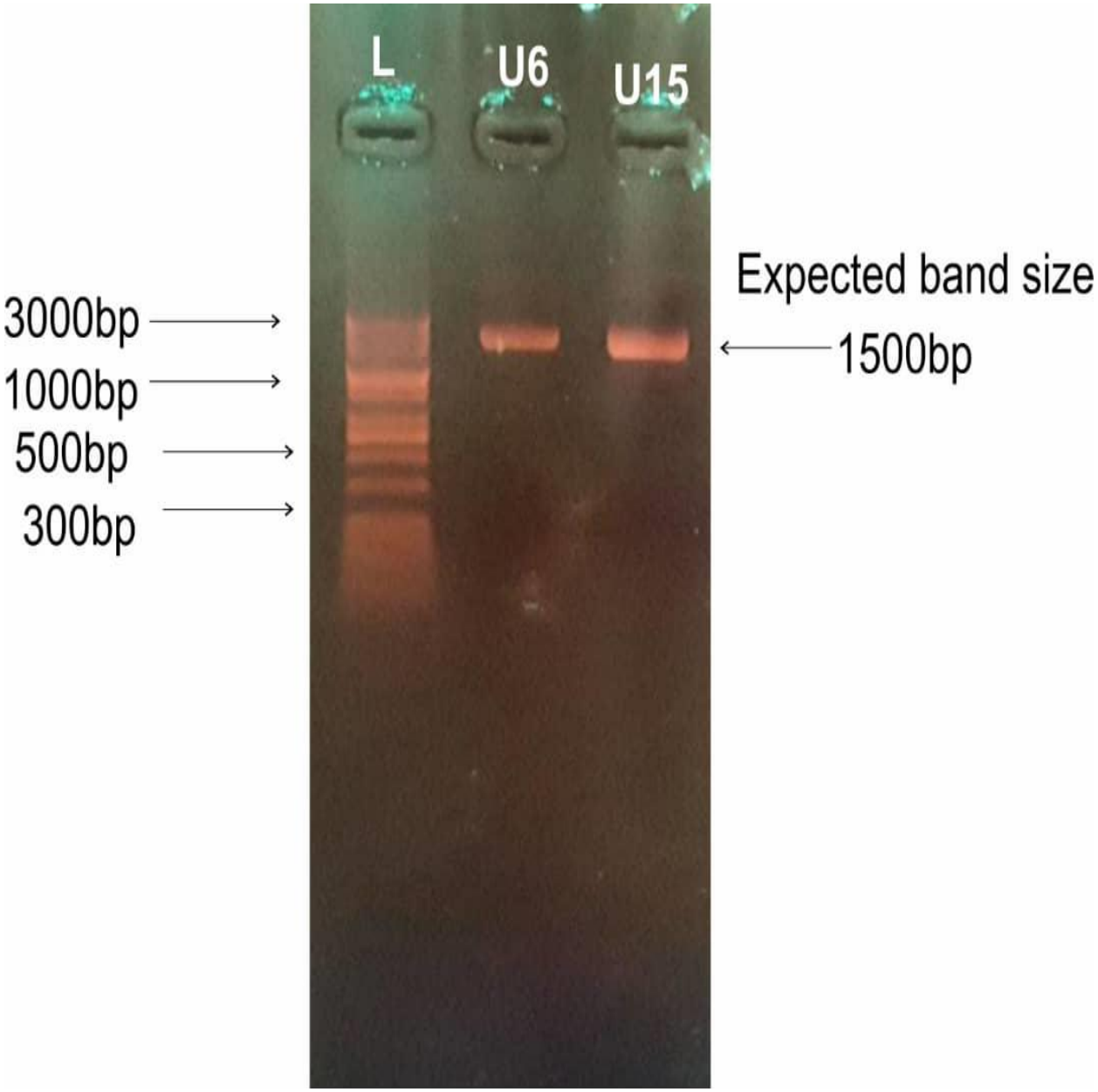
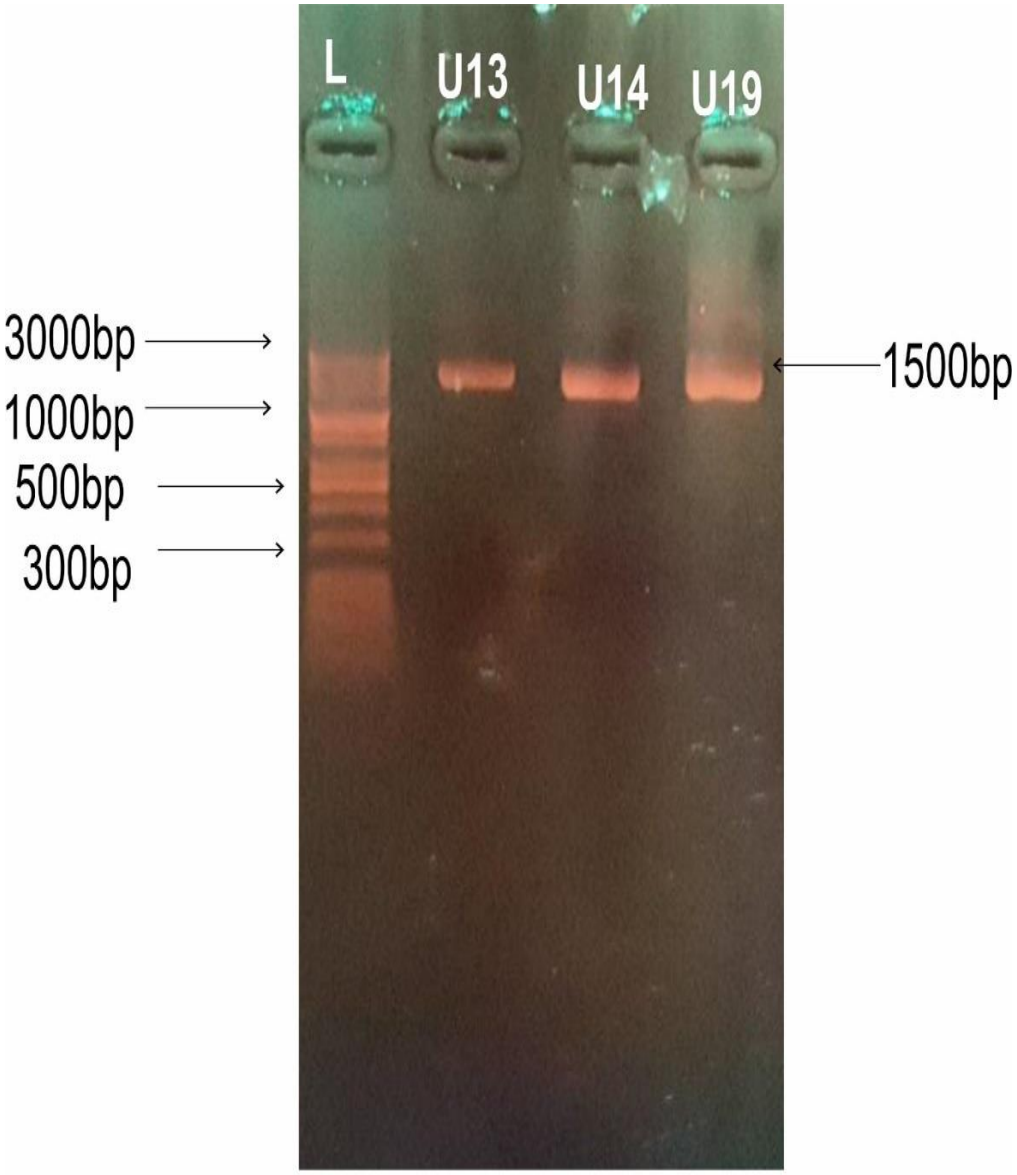


Plate 1: **Gel electrophoresis of the PCR product for sample U6 (***Escherichia coli***) and U15 (***Klebsiella pneumoniae***)**

Key: L=Midrange ladder



Keys: L= Midrange ladder

Plate 2: **Gel electrophoresis of the PCR product for sample U13 (***Escherichia coli***), U14 (***Escherichia coli***) and U19 (***Klebsiella pneumoniae***)**

Table 4.11: **Sequencing Result Alignment for the Selected Isolates**

Sample Code	Scientific Name	Max Score	Total Score	Query Cover	E Value	Percentage Identity	Acc Length	Accession
U6	*Escherichia coli*	1656	1656	100%	0.0	98.34%	1467	NR_114042.1
U13	*Escherichia coli*	1315	1315	100%	0.0	96.63%	1450	NR_024570.1
U14	*Escherichia coli*	1322	1322	99%	0.0	96.03%	1467	NR_114042
U15	*Klebsiella pneumoniae*	1622	1622	100%	0.0	99.56%	1530	NR_117683.1
U19	*Klebsiella pneumoniae*	1423	1423	100%	0.0	99.25%	1462	NR_113240.1

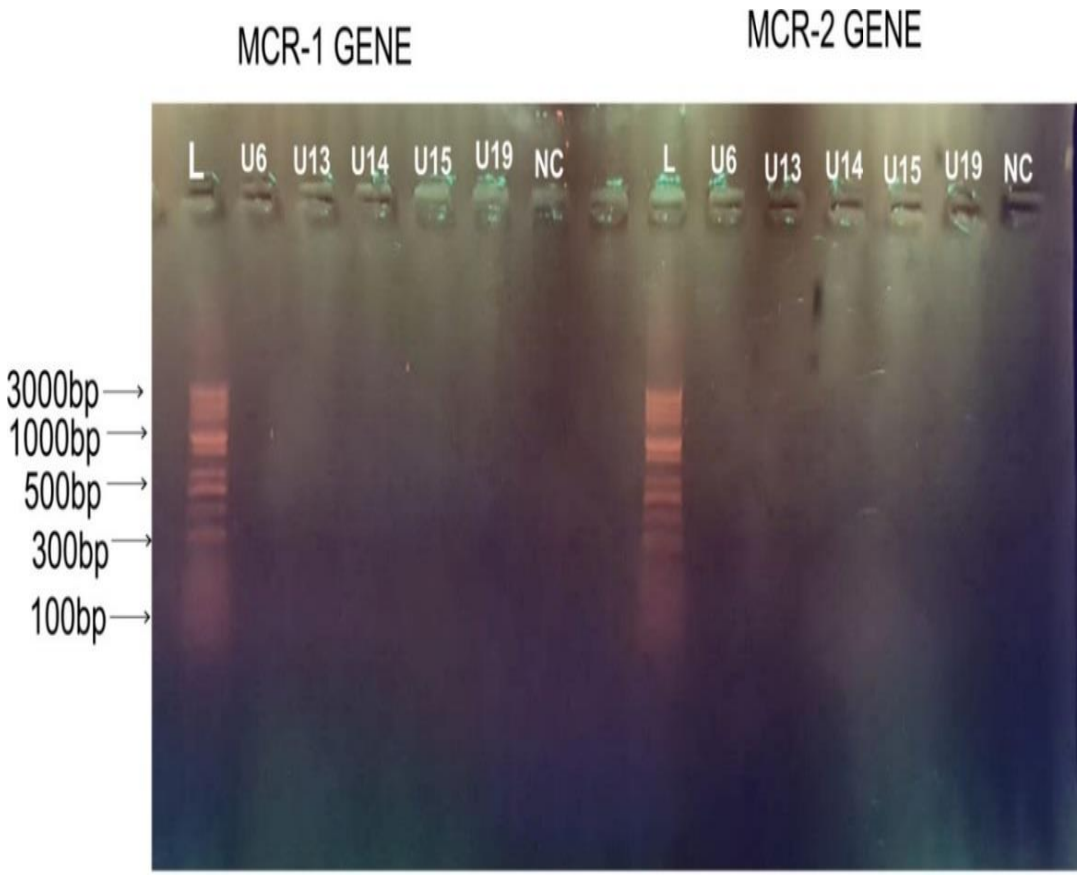
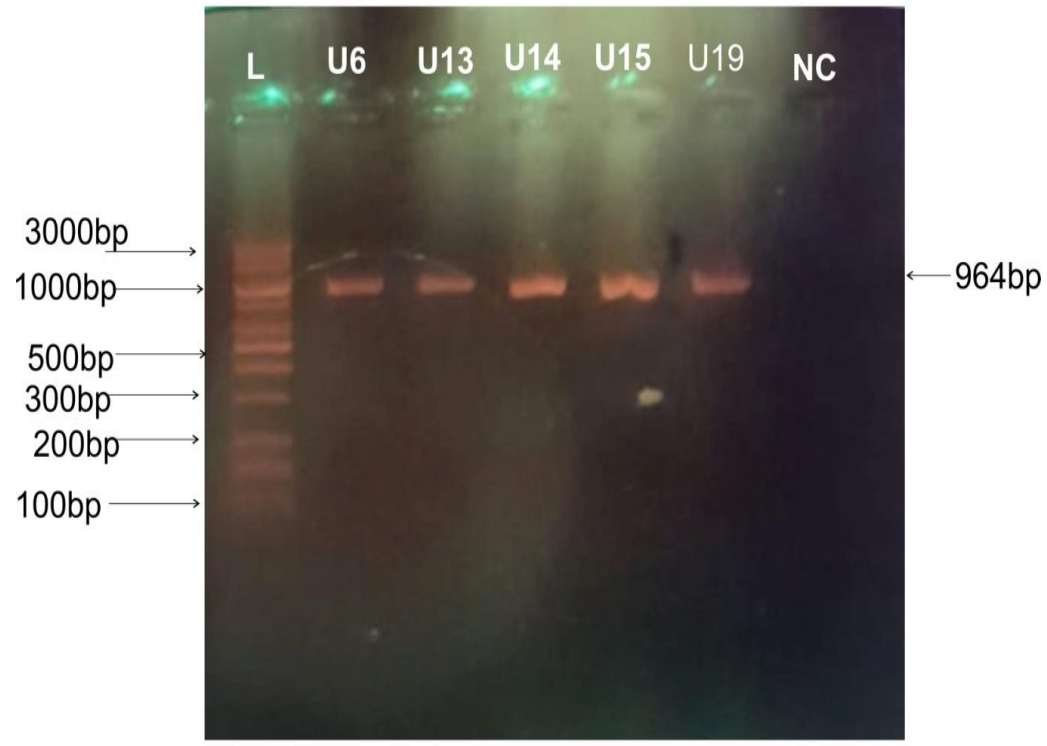


Plate 3: Gel plate of MCR 1 and MCR 2 showing no amplification.

Key: Midrange Ladder, NC: Negative Control

U6, U13, U14 = *Escherichia coli*

U15, U19 = *Klebsiella pneumoniae*



Key: Midrange Ladder; NC: Negative Control
Plate 4: Gel plate of amplified TEM gene

U6, U13, U14 = *Escherichia coli*

U15, U19 = *Klebsiella pneumoniae*

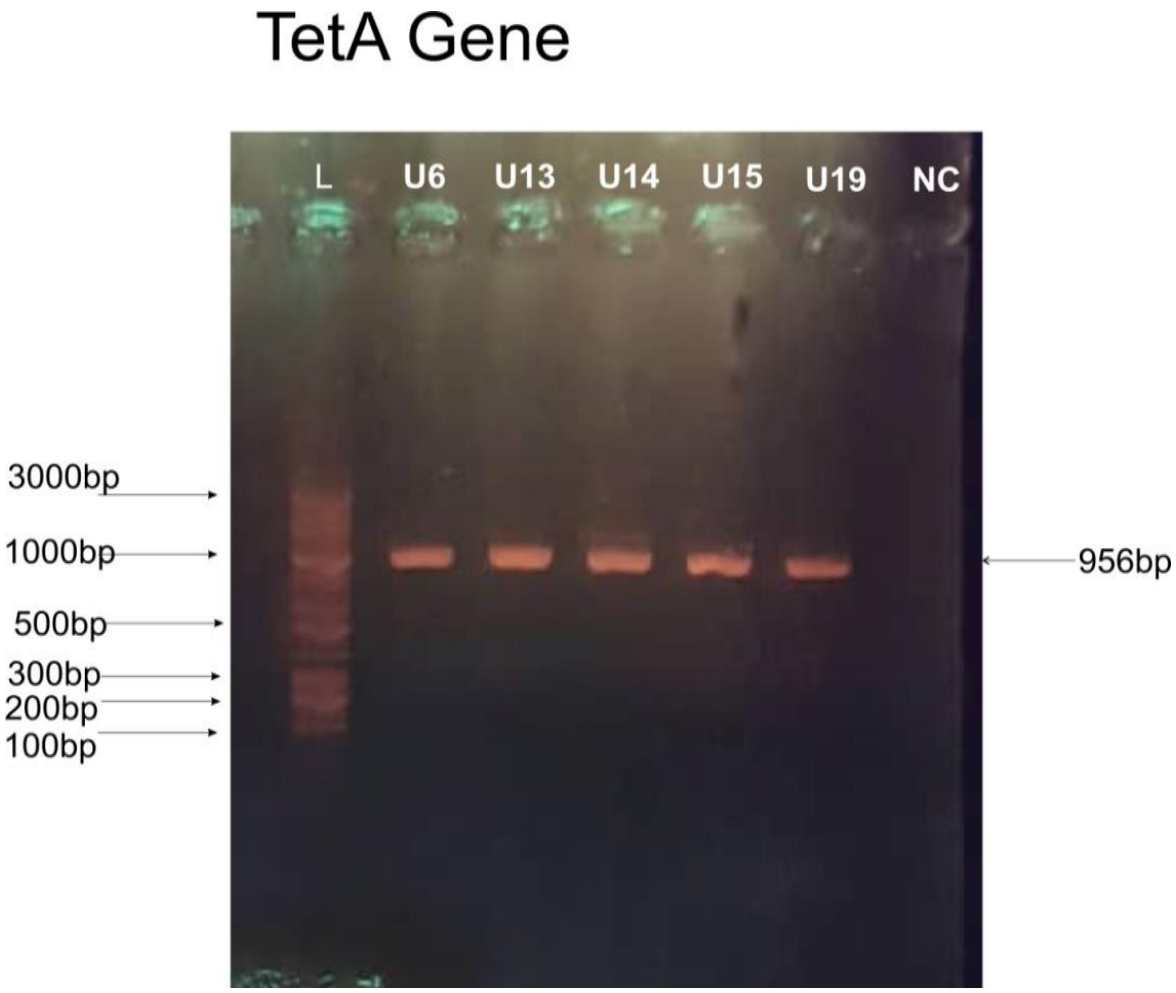


Plate 5: Gel plate of amplified Tet A gene

Key: L =Midrange ladder, NC: Negative Control

U6, U13, U14 = *Escherichia coli*

U15, U19 = *Klebsiella pneumoniae*

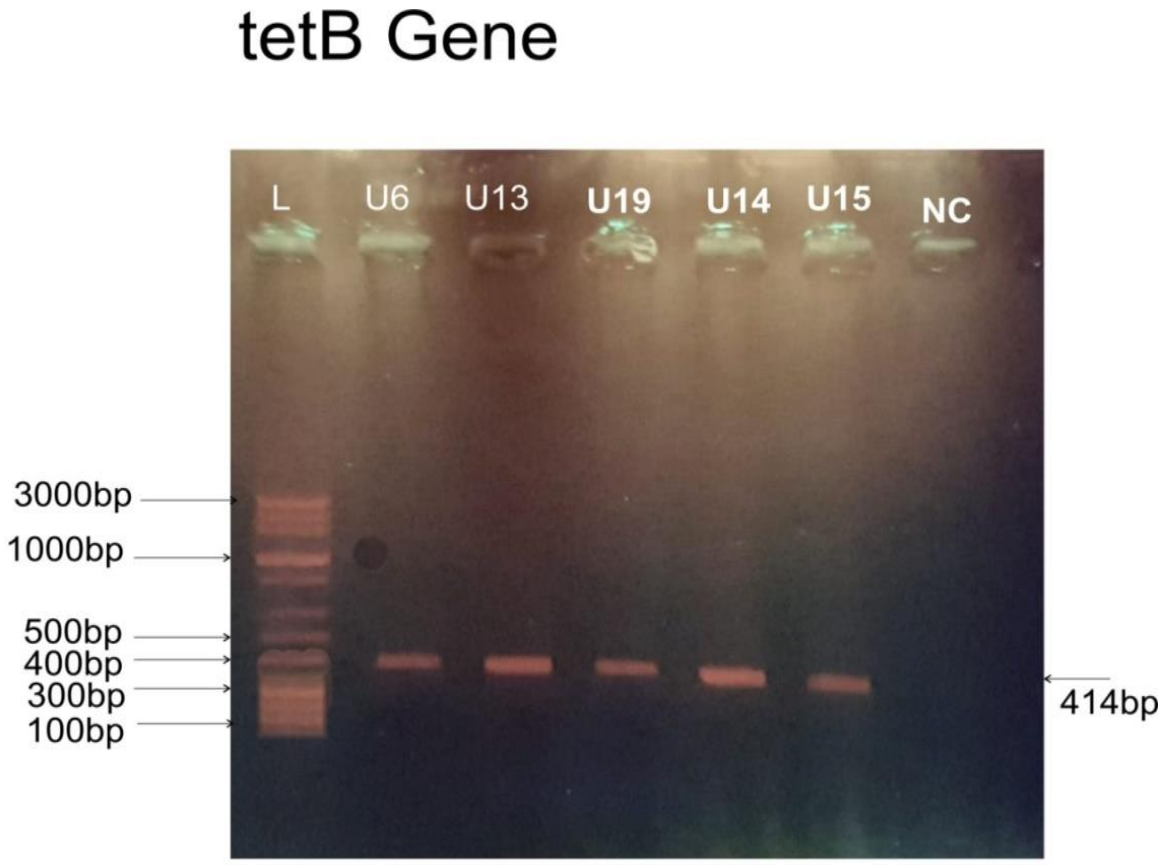


Plate 6: Gel plate of the amplified Tet B gene

Keys: L=Midrange ladder; NC: Negative Control

U6, U13, U14 = *Escherichia coli*

U15, U19 = *Klebsiella pneumoniae*

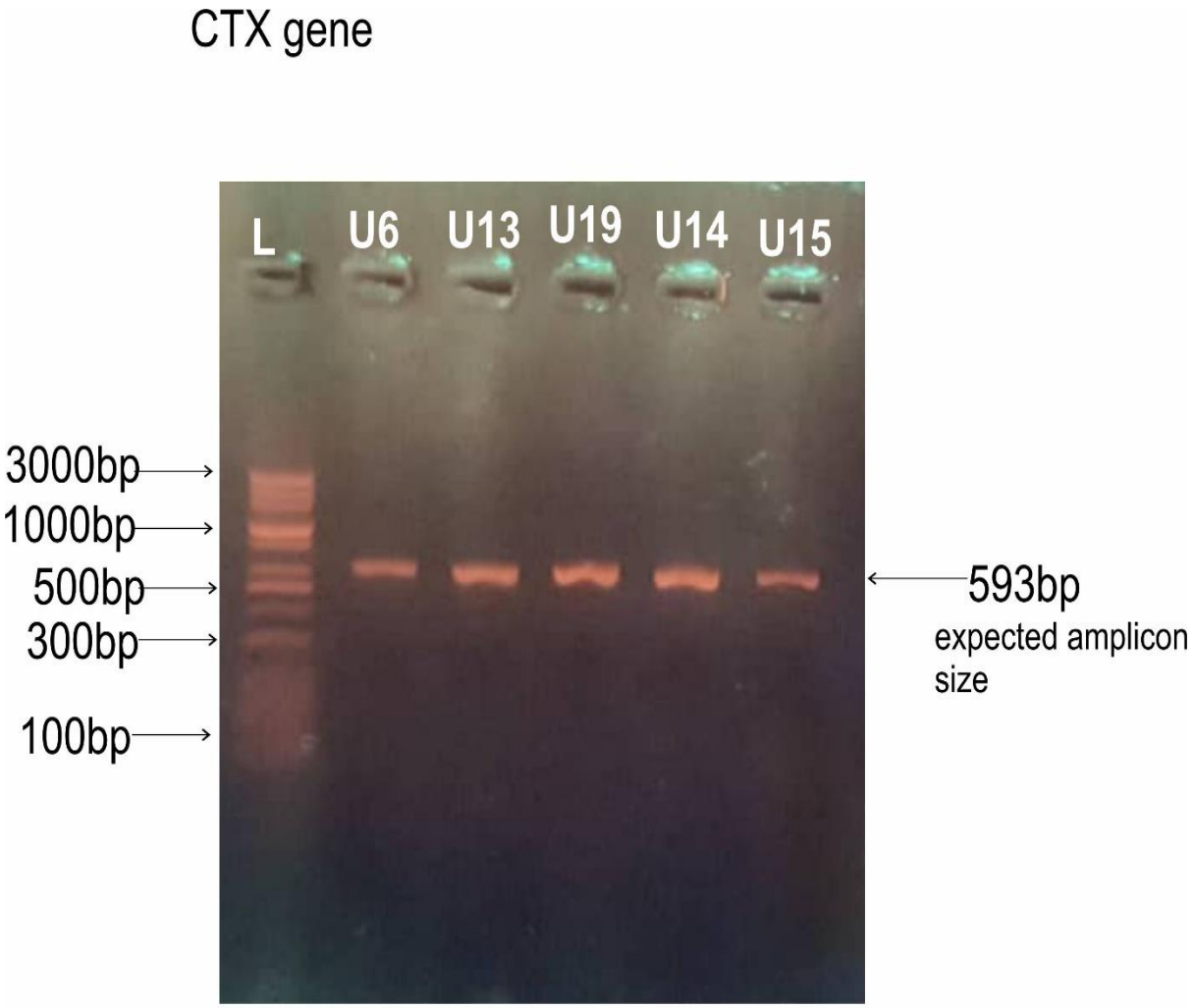


Plate 7: Gel plate of the amplified CTX gene

Key: L= Midrange ladder

U6, U13, U14 = *Escherichia coli*

U15, U19 = *Klebsiella pneumoniae*

4.2 Discussion

4.2.1 Prevalence of *Escherichia* and *Klebsiella* species in the Study Area

Antimicrobial resistance poses a serious threat to human health. The normal microbial flora of the urinary and gastrointestinal tract is becoming a reservoir of antibiotic resistant organisms and genes (Zakia *et al*., 2021). Out of the 192 samples screened, 45 (23.4%) and 31 (16.1%) of the Urine and Stool samples respectively yielded *Escherichia* and *Klebsiella* growth. *Escherichia* had a prevalence of 23 (12.0%) and 22 (11.5%) while *Klebsiella* spp had a prevalence of 13 (6.8%) and 18 (9.4%) from the Urine and Stool samples respectively. Majority of the positive culture came from *Escherichia* and *Klebsiella* species. This is consistent with the findings of Mingyu *et al.* (2020) who reported that *Escherichia* and *Klebsiella* species were the major clinical bacteria that were pathogenic causing significant morbidity and mortality. The high number of *Escherichia* and *Klebsiella* species indicated high level of contamination by bacteria from the family *Enterobacteriaceae*. This family is well known as the representative bacteria causing nosocomial infections (Zakia *et al.*, 2021).

4.2.2 Prevalence of *Escherichia* and *Klebsiella* Species According to Gender

According to gender, the bacterial isolates were more prevalent in females 39 (51.3%) than males 37 (48.7%). *Klebsiella* spp. 18 (23.7%) had higher prevalence in females compared to males 13 (17.1%) while *Escherichia* spp. 24 (31.6%) was more common in males than in females 21 (27.6%). This high prevalence could be attributed to physiological and anatomical differences in both sexes. Urinary tract infections occur more frequently in women than men because the shorter, wider, female urethra appears to be less effective in preventing access of bacteria to the bladder (Vasudevan, 2014). Study have also demonstrated that women who are prone to urinary tract infections,

possess epithelial cells with significantly more receptors for uropathogenic bacteria than healthy controls (Stamm, 2001).

4.2.3 Prevalence of *Escherichia* and *Klebsiella* Species by Age

By age group, *Escherichia* spp. was observed to be more prevalent in the age group of 31-40 years with a prevalence of 12 (15.8%) followed by the age groups 21-30 and 41-50 both having a prevalence of 11 (14.5%). On the other hand, *Klebsiella* spp. was more prevalent among the age group 21-30 with a prevalence of 8 (10.5%) followed by the age groups 11-20 and 31-40 both having a prevalence of 7 (9.2%). This is partly in line with a similar study where urinary tract infection was most prevalent in age group 38-42 (Battikhi *et al*., 2015). The high prevalence recorded in the above age groups, is related to the indiscriminate sexual activity and high pregnancy rate (among females) that characterize these groups.

4.2.4 Antibiotic Susceptibility Profile of *Escherichia* and *Klebsiella* Species

Antibiotic susceptibility results of the *Escherichia* and *Klebsiella* isolates showed high level of resistance to Ceporex, Tetracycline, Nalidixic acid, Colistin, Ampicillin, Trimetoprim-sulfamethoxazole and Chloramphenicol. Most of these antibiotics are traditional first-line drugs used against Gram negative bacterial infections (Delgado-Valverde *et al*., 2013). Various studies have reported resistance to these antibiotics worldwide (Liu *et al*., 2017; Tadesse *et al*., 2017). Results of this study follows a similar trend. *Escherichia* and *Klebsiella* isolates showed resistance rates of 97.8% and 87.1% respectively against Ceporex, 82.2% and 83.9% against Tetracycline, 62.2% and 67.7% against Ampicillin, 57.8% and 54.8% against Trimetoprim-sulfamethoxazole. While only *Escherichia* isolates showed significant resistance to Nalidixic acid (71.1%) and Chloramphenicol (51.1%). Resistance to these common antibiotics poses a major public health threat. Similar finding was observed by Iramiot *et al*. (2018) who reported a

resistance rate of 83.7% to Ceporex and 52.2% to Tetracycline. Manel *et al*. (2014) reported a significantly higher level of resistance to Ampicillin (98.1%). Resistance rate to Trimetoprim-sulfamethoxazole (57.8% and 54.8%) observed in this study is similar to the finding of Sharma *et al*. (2013) and Ya'aba *et al*. (2020) who reported resistance rate of 54.1% and 51.0% respectively. *Escherichia* isolates resistance rate to Nalidixic acid (71.1%) is comparable to the study of Sharma *et al*. (2013) and Iramiot *et al*. (2018) who reported resistance rates of 78.9% and 76.5% respectively. The high resistance rates to these commonly used antibiotics observed in this study clearly indicates frequent prescription and injudicious use of these antibiotics in the study area resulting in development of resistance to these antimicrobial agents (Saleh *et al*., 2009).

The *Escherichia* and *Klebsiella* isolates were highly susceptible to Fosfomycin (100.0% and 90.3%) respectively, 82.2% and 90.3% for Tarivid, 68.9% and 80.6% for Ciprofloxacin, 82.2% and 74.2% for Amoxicillin clavulanic acid, 82.2% and 80.6% for Imipenem, 68.9% and 58.1% for Gentamycin. Similar susceptibility rate to these antibiotics was reported in other studies. Kibret and Abera, (2011) reported a susceptibility rate of 87.0% to Gentamicin. The effectiveness of Gentamicin could be attributed to its nature of administration, Gentamicin are administered intravenously as such they are not easily abused. Engda *et al*. (2018) and Ya'aba *et al*. 2020 reported a susceptibility rate of 100% and 61.2% to amoxicillin-clavulanic acid respectively. The lower resistance rate to Amoxicillin/clavulanic acid observed could be due to the effectiveness of the drug combinations, as the clavulanic acid targets β-lactamases that are responsible for resistance against β-lactams and other antibiotics (Odongo *et al.*, 2020). The susceptibility rate to Imipenem (82.2% and 80.6%) observed in this study is consistent with the findings of Malik *et al*. (2021) that reported susceptibility rate of 75.3% to Imipenem. Higher susceptibility rate to Imipenem (100%) was reported by Al-

Salamy, (2012). High susceptibility rates of isolates to Imipenem observed in this study could be attributed to their limited use and unavailability in the study area. High susceptibility rate to Fosfomycin (100.0% and 90.3%) was observed in this study. Similarly, Seo *et al*. (2014) reported 100% susceptibility rate to Fosfomycin. Fosfomycin is structurally unrelated to any other antimicrobial agent, therefore chances of cross-resistance is low (Sastry and Doi, 2016).

4.2.5 Resistance Category and MARI of *Escherichia* and *Klebsiella* Species

Multiple antibiotic resistance (MAR) index is helpful in analyzing health risk, as well as checking the level of antibiotic resistance (Saba *et al*., 2011). A MARI value greater than 0.2 indicates that the isolate is from a high risk source of contamination where antibiotics are frequently used. About 96.8% and 100% of the *Klebsiella* and *Escherichia* isolates obtained in this study had MARI of 0.2 or above which indicates that the bacterial isolates have been previously exposed to several antibiotics. This could be due to the uncontrolled usage of antibiotics in the study area.

4.2.6 Prevalence of MDR and XDR

Findings from the present study also showed that 36.8% and 55.3% of the *Klebsiella* and *Escherichia* isolates were resistant to at least three classes of antibiotics, and are classified as Multidrug resistant (MDR). While 3.9% of *Escherichia* isolates and 2.6% of *Klebsiella* isolates were resistant to 8 or more classes of antibiotics and were classified as Extensively drug resistant (XDR). The presence of these drug resistant organisms could be attributed to improper sanitation, unhygienic practices and indiscriminate use of antibiotics which are easily procured over the counter (Joseph *et al*., 2017).

In general, extensively drug resistant bacteria screening is common to humans and the hospital environment, although recent reports suggest the emergence of such resistant pathogens in livestock. (Ghatak *et al*., 2013; Pruthvishre *et al*., 2017). There is an urgent

need for routine screening for XDR resistant bacteria in human, since these mechanisms of resistance are transferable and are of public health significance.

4.2.7 Antibiotic Resistance Genes

In this study, extensively resistant *Escherichia* and *Klebsiella* isolates were all found to have Tet A, Tet B, CTX and TEM genes at varying bands. However, Mcr1 and Mcr 2 genes were not detected in any of the isolates. Colistin has become a viable antimicrobial against aggressive infections due to multidrug resistant bacteria. Its resistance is mainly driven by alterations in the Gram-negative outer membrane lipopolysaccharides and is caused in most cases by mutation. However, the recent emergence of plasmid-encoded colistin resistance among *Enterobacteriaceae* strains represents a serious threat to global public health. Although, Colistin resistant genes were not detected in the bacterial isolates by Polymerase chain reaction (PCR). It may be due to the fact that the bacteria isolates utilise other mechanism of resistance like selective membrane permeability, modification of the target site or the use of efflux pump.

The presence of Tet A and Tet B genes in these organisms is a strong indication of their resistance to antibiotics belonging to Tetracycline family. Tetracycline is an antibiotic that fights infection caused by bacteria.

Tetracycline is an antibiotic which is commonly believed to be more effective than penicillin. In some cases, tetracycline is used when penicillin or another antibiotic cannot be used to treat infections such as Anthrax, Listeriosis, and others. It is mostly recommended as stronger alternative whenever penicillin refuse to work. Resistance of Bacteria to Tetracycline in clinical environment is a serious public health issue considering the wide usage of Tetracycline to treat many different bacterial infections of

the skin, intestines, respiratory tract, urinary tract, genitals, lymph nodes, and other body systems.

Furthermore, the presence of TEM and CTX genes in all the XDR isolates in this study revealed their strong resistance to antibiotics belonging to β-Lactam family. A similar finding was reported by Karimian *et al.* (2015) in Iran whose results shows the presence of CTX and TEM genes in most of the isolates. Other studies in clinical isolates showed the absence of TEM and CTX genes in *Escherichia* and *Klebsiella* isolates. The difference in the prevalence rate of TEM and CTX genes between the present study and other studies, might be due to the difference in the sample population and antibiotic susceptibility pattern of the isolates, the type of study design and sample size. The genetic diversity of CTX and TEM genes in the isolates, suggested that resistance genes can easily move from one species to another with the possibility of easy interspecies transfer.

Resistance is generally encoded by plasmid or chromosomal genes which are easily transferred from one bacterium to another, without any consideration of species or genus limits.

High prevalence of (XDR) resistant Gram-negative isolates having multiple antibiotic resistant index of $\geq$0.2 indicated that the isolates were gotten from sources of high antibiotic usage. This is an indication of complex nature of emerging antibiotic resistance by Enterobacteriaceae and its implication on the limited resourced health facilities in the study area. The high antibiotic resistance of these organisms may be an indication of the resistance levels among the *Enterobacteriaceae* and perhaps indiscriminate ingestion of antibiotics provides selective pressure, leading to a higher prevalence of resistant bacteria which is very common in developing countries like Nigeria. These organisms are not only

potential causes of infections but they are also reservoir of resistance genes that could be transferred to other bacterial pathogens.

CHAPTER FIVE

5.0 CONCLUSION, RECOMMENDATIONS AND CONTRIBUTION TO KNOWLEDGE

5.1 Conclusion

The prevalence of *Escherichia* and *Klebsiella* species isolated from urine and stool samples of patients visiting General Hospital was 23.4% and 16.1%.

Most of the *Escherichia* and *Klebsiella* isolates were highly resistant to Ceporex, Tetracycline, Colistin, Nalidixic acid, Ampicillin and Trimethoprim-Sulfamethoxazole. However, they were highly susceptible to Fosfomycin, Imipenem, Clavulanate, Tarivid, Gentamicin and Ciprofloxacin.

This study showed that there are multiple antibiotic resistant *Escherichia* and *Klebsiella* species in the Urine and Stool samples of patients attending General Hospital Minna, Niger State. Extensively drug resistant *Escherichia* spp. 3 (60.0%) and *Klebsiella* 2 (40.0%) were identified

Various antibiotic resistance gene were including the *Bla*CTX, *Bla*TEM, TetA and TetB encoding resistance to beta-lactam and Tetracyclines were identified. However molecular analysis of the resistant isolates revealed that Mcr 1 and Mcr 2 genes were absent.

5.2 Recommendations

Based on the findings of this study, the following recommendations were made:

i. Antibiotics should only be used when needed and only when prescribed by health professionals.
ii. An extensive study on susceptibility profile of extensively drug resistant *Escherichia* and *Klebsiella* species is recommended to confer solution against the rising of highly resistant isolates.

iii. People should avoid self-medications in their homes. They should visit hospitals for proper medications.

iv. Antibiotics such as Fosfomycin, Imipenem should be used on patients with extensively drug resistant *Escherichia* and *Klebsiella* isolates.

v. Attention from health policy makers is demanded for improvement in promoting the use of antibiotics in health care in order to monitor changes in antibiotic resistance pattern.

5.3 Contribution to Knowledge

This study has shown that, there are extensive antibiotic resistant *Escherichia* (3.9%) and *Klebsiella* (2.6%) species in urine and stool samples obtained from patients visiting General Hospital, Minna, Niger state. The presence of antibiotic resistant genes such as Tet A, Tet B, TEM and CTX genes encoding resistance to tetracycline and beta lactam antibiotics were demonstrated in all the isolates that are extensively drug resistant.

This study showed that bacteria use various mechanisms in resisting antibiotics like antibiotic inactivation, reduced membrane permeability, modification of target site and the use of efflux or transport of antibiotics.

The result of this study also contributed that the distribution of antibiotic resistant bacteria throughout the biosphere are as a result of many years of unremitting selection pressure from human applications of antibiotics, via underuse, overuse and misuse of antibiotics.

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APPENDICES

APPENDIX A

CULTURING AND ISOLATION

URINE SAMPLES

S/N	Sample Code	Sample Source	Colony Morphology on MacConkey Agar	Colony Morphology on Eosine Methylene Blue Agar	Suspected Organisms
1.	GH U 1	Urine	No growth	-	-
2.	GH U 2	Urine	No growth	-	-
3.	GH U 3	Urine	Pink	Dark purple with green metallic sheen	*Escherichia* species
4.	GH U 4	Urine	No growth		-
5.	GH U 5	Urine	No growth		-
6.	GH U 6	Urine	Pink	Dark purple with green metallic sheen	*Escherichia* species
7.	GH U 7	Urine	Pink	Dark purple with green metallic sheen	*Escherichia* species
8.	GH U 8	Urine	Pink	Dark purple with green metallic sheen	*Escherichia* species
9.	GH U 9	Urine	No growth	-	-

10.	GH U 10	Urine	No growth	-	-
11.	GH U 11	Urine	No growth	-	-
12.	GH U 12	Urine	No growth	-	-
13.	GH U 13	Urine	Pink	Dark purple with green metallic sheen	*Escherichia* species
14.	GH U 14	Urine	Pink	Dark purple with green metallic sheen	*Escherichia* species
15.	GH U 15	Urine	Pink and mucoid	Pink to purple and mucoid	*Klebsiella* species
16.	GH U 16	Urine	No growth	-	-
17.	GH U 17	Urine	Pink and mucoid	Pink to purple and mucoid	*Klebsiella* species
18.	GH U 18	Urine	No growth	-	-
19.	GH U 19	Urine	Pink and mucoid	Pink to purple and mucoid	*Klebsiella* species
20.	GH U 20	Urine	No growth	-	-
21.	GH U 21	Urine	Colourless	Colourless	Non- lactose fermenter
22.	GH U 22	Urine	Pink	Dark purple with green metallic seen	*Escherichia* species
23.	GH U 23	Urine	Colourless	Colourless	Non lactose fermenter
24.	GH U 24	Urine	Pink	Dark purple with green metallic	*Escherichia* species

25.	GH U 25	Urine	No growth	-	-
26.	GH U 26	Urine	No growth	-	-
27.	GH U 27	Urine	No growth	-	-
28.	GH U 28	Urine	No growth	-	-
29.	GH U 29	Urine	Colourless	Colourless	Non- lactose fermenter
30.	GH U 30	Urine	No growth	No growth	No growth
31.	GH U 31	Urine	Colourless	Colourless	Non lactose fermenter
32.	GH U 32	Urine	No growth	-	-
33.	GH U 33	Urine	No growth	-	-
34.	GH U 34	Urine	Colourless	Colourless	Non lactose fermenter
35.	GH U 35	Urine	Pink	Dark purple with green metallic sheen	*Escherichia* species
36.	GH U 36	Urine	No growth	-	-
37.	GH U 37	Urine	No growth	-	-
38.	GH U 38	Urine	Colourless	Colourless	Non lactose fermenter
39.	GH U 39	Urine	No growth	-	-

40.	GH U 40	Urine	Colourless	Colourless	Non lactose fermenter
41.	GH U 41	Urine	No growth	-	-
42.	GH U 42	Urine	Colourless	Colourless	Non lactose fermenter
43.	GH U 43	Urine	Pink	Dark purple with green metallic sheen	*Escherichia* species
44.	GH U 44	Urine	Colourless	Colourless	Non lactose fermenter
45.	GH U 45	Urine	No growth	-	-
46.	GH U 46	Urine	Pink	Dark purple with green metallic sheen	*Escherichia* species
47.	GH U 47	Urine	No growth	-	-
48.	GH U 48	Urine	No growth	-	-
49.	GH U 49	Urine	No growth	-	-
50.	GH U 50	Urine	Colourless	Colourless	Non lactose fermenter
51.	GH U 51	Urine	Pink	Dark purple with green metallic sheen	*Escherichia* species
52.	GH U 52	Urine	Pink and mucoid	Pink to purple and mucoid	*Klebsiella* species
53.	GH U 53	Urine	No growth	-	-
54.	GH U 54	Urine	No growth	-	-

55.	GH U 55	Urine	Pink and mucoid	Pink to purple and mucoid	*Klebsiella* species
56.	GH U 56	Urine	No growth	-	-
57.	GH U 57	Urine	Pink and mucoid	Pink to purple and mucoid	*Klebsiella* species
58.	GH U 58	Urine	Pink and mucoid	Pink to purple and mucoid	*Klebsiella* species
59.	GH U 59	Urine	Pink and mucoid	Pink to purple and mucoid	*Klebsiella* species
60.	GH U 60	Urine	Pink and mucoid	Pink to purple and mucoid	*Klebsiella* species
61.	GH U 61	Urine	No growth	-	-
62.	GH U 62	Urine	No growth	-	-
63.	GH U 63	Urine	No growth	-	-
64.	GH U 64	Urine	No growth	-	-
65.	GH U 65	Urine	Pink	Dark purple with green metallic sheen	*Escherichia* species
66.	GH U 66	Urine	Pink	Dark purple with green metallic sheen	*Escherichia* species
67.	GH U 67	Urine	Pink and mucoid	Pink to purple and mucoid	*Klebsiella* species
68.	GH U 68	Urine	No growth	-	-
69.	GH U 69	Urine	Pink	Dark purple with green metallic sheen	*Escherichia* species

70.	GH U 70	Urine	No growth	-	-
71.	GH U 71	Urine	No growth	-	-
72.	GH U 72	Urine	No growth	-	-
73.	GH U 73	Urine	No growth	-	-
74.	GH U 74	Urine	No growth	-	-
75.	GH U 75	Urine	Pink	Dark purple with green metallic sheen	*Escherichia* species
76.	GH U 76	Urine	Pink	Dark purple with green metallic sheen	*Escherichia* species
77.	GH U 77	Urine	Pink	Dark purple with green metallic sheen	*Escherichia* species
78.	GH U 78	Urine	Pink	Dark purple with green metallic sheen	*Escherichia* species
79.	GH U 79	Urine	Pink	Dark purple with green metallic sheen	*Escherichia* species
80.	GH U 80	Urine	Pink	Dark purple with green metallic sheen	*Escherichia* species
81.	GH U 81	Urine	Pink	Dark purple with green metallic sheen	*Escherichia* species
82.	GH U 82	Urine	Pink and mucoid	Pink to purple and mucoid	*Klebsiella* species
83.	GH U 83	Urine	No growth	-	-
84.	GH U 84	Urine	No growth	-	-

85.	GH U 85	Urine	Pink and mucoid	Pink to purple and mucoid	*Klebsiella* species
86.	GH U 86	Urine	No growth	-	-
87.	GH U 87	Urine	No growth	-	-
88.	GH U 88	Urine	No growth	-	-
89.	GH U 89	Urine	Pink and mucoid	Pink to purple and mucoid	*Klebsiella* species
90.	GH U 90	Urine	Colourless	Colourless	Non lactose fermenter
91.	GH U 91	Urine	Pink	Dark purple with green metallic sheen	*Escherichia* species
92.	GH U 92	Urine	Colourless	Colourless	Non lactose fermenter
93.	GH U 93	Urine	No growth	-	-
94.	GH U 94	Urine	Colourless	Colourless	Non lactose fermenter
95.	GH U 95	Urine	Pink and mucoid	Pink to purple and mucoid	*Klebsiella* species
96.	GH U 96	Urine	No growth	-	-
97.	GH U 97	Urine	Colourless	Colourless	Non lactose fermenter

Stool Samples

S/N	Sample Code	Sample Source	Colony Morphology on MacConkey	Colony Morphology on Eosin Methylene Blue	Suspected Organisms
1.	GH St 1	Stool	Pink	Dark purple with green metallic sheen	*Escherichia* species
2.	GH St 2	Stool	Pink and mucoid	Pink to purple and mucoid	*Klebsiella* species
3.	GH St 3	Stool	Pink	Dark purple with green metallic sheen	*Escherichia* species
4.	GH St 4	Stool	Pink and mucoid	Pink to purple and mucoid	*Klebsiella* species
5.	GH St 5	Stool	Pink and mucoid	Pink to purple and mucoid	*Klebsiella* species
6.	GH St 6	Stool	Pink and mucoid	Pink to purple and mucoid	*Klebsiella* species
7.	GH St 7	Stool	Pink	Dark purple with green metallic sheen	*Escherichia* species
8.	GH St 8	Stool	Pink	Dark purple with green metallic sheen	*Escherichia* species
9.	GH St 9	Stool	No growth	-	-
10.	GH St 10	Stool	No growth	-	-
11.	GH St 11	Stool	No growth	-	-
12.	GH St 12	Stool	Pink	Dark purple with green metallic sheen	*Escherichia* species

13.	GH St 13	Stool	No growth	-	-
14.	GH St 14	Stool	No growth	-	-
15.	GH St 15	Stool	Pink mucoid	Pink to purple and mucoid	*Klebsiella* species
16.	GH St 16	Stool	Pink mucoid	Pink to purple and mucoid	*Klebsiella* species
17.	GH St 17	Stool	Pink	Dark purple with green metallic sheen	*Escherichia* species
18.	GH St 18	Stool	Pink	Dark purple with green metallic sheen	*Escherichia* species
19.	GH St 19	Stool	Pink mucoid	Pink to purple and mucoid	*Klebsiella* species
20.	GH St 20	Stool	Pink	Dark purple with green metallic sheen	*Escherichia* species
21.	GH St 21	Stool	Colourless	Colourless	Non lactose fermenter
22.	GH St 22	Stool	Colourless	Colourless	Non lactose fermenter
23.	GH St 23	Stool	Colourless	Colourless	Non lactose fermenter
24.	GH St 24	Stool	Pink and mucoid	Pink to purple and mucoid	*Klebsiella* species
25.	GH St 25	Stool	Colourless	Colourless	Non lactose fermenter
26.	GH St 26	Stool	Pink	Dark purple with green metallic sheen	*Escherichia* species
27.	GH St 27	Stool	Pink and mucoid	Pink to purple and mucoid	*Klebsiella* species

28.	GH St 28	Stool	Pink	Dark purple with green metallic sheen	*Escherichia* species
29.	GH St 29	Stool	Pink and mucoid	Pink to purple and mucoid	*Klebsiella* species
30.	GH St 30	Stool	Pink	Dark purple with green metallic sheen	*Escherichia* species
31.	GH St 31	Stool	Pink	Dark purple with green metallic sheen	*Escherichia* species
32.	GH St 32	Stool	Pink and mucoid	Pink to purple and mucoid	*Klebsiella* species
33.	GH St 33	Stool	Pink	Dark purple with green metallic sheen	*Escherichia* species
34.	GH St 34	Stool	Pink	Dark purple with green metallic sheen	*Escherichia* species
35.	GH St 35	Stool	Pink	Dark purple with green metallic sheen	*Escherichia* species
36.	GH St 36	Stool	No growth	-	-
37.	GH St 37	Stool	No growth	-	-
38.	GH St 38	Stool	Pink and mucoid	Pink to purple and mucoid	*Klebsiella* species
39.	GH St 39	Stool	Colourless	Colourless	Non lactose fermenter
40.	GH St 40	Stool	Pink	Dark purple with green metallic sheen	*Escherichia* species
41.	GH St 41	Stool	No growth	-	-
42.	GH St 42	Stool	No growth	-	-

43.	GH St 43	Stool	No growth	-	-
44.	GH St 44	Stool	Colourless	Colourless	Non lactose fermenter
45.	GH St 45	Stool	No growth	-	-
46.	GH St 46	Stool	Pink	Dark purple with green metallic sheen	*Escherichia* species
47.	GH St 47	Stool	No growth	-	-
48.	GH St 48	Stool	No growth	-	-
49.	GH St 49	Stool	No growth	-	-
50.	GH St 50	Stool	Colourless	Colourless	Non lactose fermenter
51.	GH St 51	Stool	Pink	Dark purple with green metallic sheen	*Escherichia* species
52.	GH St 52	Stool	No growth	-	-
53.	GH St 53	Stool	No growth	-	-
54.	GH St 54	Stool	Colourless	Colourless	Non lactose fermenter
55.	GH St 55	Stool	No growth	-	-
56.	GH St 56	Stool	No growth	-	-
57.	GH St 57	Stool	Pink and mucoid	Pink to purple and mucoid	*Klebsiella* species

58.	GH St 58	Stool	Pink and mucoid	Pink to purple and mucoid	*Klebsiella* species
59.	GH St 59	Stool	No growth	-	-
60.	GH St 60	Stool	No growth	-	-
61.	GH St 61	Stool	No growth	-	-
62.	GH St 62	Stool	No growth	-	-
63.	GH St 63	Stool	No growth	-	-
64.	GH St 64	Stool	No growth	-	-
65.	GH St 65	Stool	No growth	-	-
66.	GH St 66	Stool	Pink and mucoid	Pink to purple and mucoid	*Klebsiella* species
67.	GH St 67	Stool	Pink and mucoid	Pink to purple and mucoid	*Klebsiella* species
68.	GH St 68	Stool	No growth	-	-
69.	GH St 69	Stool	No growth	-	-
70.	GH St 70	Stool	Colourless	Colourless	Non lactose fermenter
71.	GH St 71	Stool	Pink	Dark purple with green metallic sheen	*Escherichia* species
72.	GH St 72	Stool	No growth	-	-

73.	GH St 73	Stool	No growth	-	-
74.	GH St 74	Stool	No growth	-	-
75.	GH St 75	Stool	No growth	-	-
76.	GH St 76	Stool	Pink	Dark purple with green metallic sheen	*Escherichia* species
77.	GH St 77	Stool	No growth	-	-
78.	GH St 78	Stool	Pink and mucoid	Pink to purple and mucoid	*Klebsiella* species
79.	GH St 79	Stool	No growth	-	-
80.	GH St 80	Stool	Pink	Dark purple with green metallic sheen	*Escherichia* species
81.	GH St 81	Stool	Pink	Dark purple with green metallic sheen	*Escherichia* species

APPENDIX B

BIOCHEMICAL TESTS

S/N	Isolate Code	Mr	Vp	Indo	Cit	Cata	Urase	Moti	Sucrose	Glucose	Fructose	Lactose	Maltose	Gram Staining	Oxidase
1.	GH U 3	+ve	-ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve	G – ve rod	-ve
2.	GH U 6	+ve	-ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve	G – ve rod	-ve
3.	GH U 7	+ve	-ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve	G – ve rod	-ve
4.	GH U 8	+ve	-ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve	G – ve rod	-ve
5.	GH U 13	+ve	-ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve	G – ve rod	-ve
6.	GH U 14	+ve	-ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve	G – ve rod	-ve
7.	GH U 15	-ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	G – ve rod	-ve
8.	GH U 17	-ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	G – ve rod	-ve
9.	GH U 19	-ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	G – ve rod	- ve
10.	GH U 22	+ve	-ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve	G – ve rod	- ve
11.	GH U 24	+ve	-ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve	G – ve rod	- ve

12.	GH U 35	+ve	-ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve	G – ve rod	-ve
13.	GH U 43	+ve	-ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve	G – ve rod	-ve
14.	GH U 46	+ve	-ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve	G – ve rod	-ve
15.	GH U 51	+ve	-ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve	G – ve rod	-ve
16.	GH U 52	-ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	G – ve rod	-ve
17.	GH U 55	-ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	G – ve rod	-ve
18.	GH U 57	-ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	G – ve rod	-ve
19.	GH U 58	-ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	G – ve rod	-ve
20.	GH U 59	-ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	G – ve rod	-ve
21.	GH U 60	-ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	G – ve rod	-ve
22.	GH U 65	+ve	-ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve	G – ve rod	-ve
23.	GH U 66	+ve	-ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve	G –ve rod	-ve
24.	GH U 67	-ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	G – ve rod	-ve
25.	GH U 69	+ve	-ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve	G – ve rod	-ve
26.	GH U 75	+ve	-ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve	G – ve rod	-ve

27.	GH U 76	+ve	-ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve	G – ve rod	-ve
28.	GH U 77	+ve	-ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve	G – ve rod	-ve
29.	GH U 78	+ve	-ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve	G – ve rod	-ve
30.	GH U 79	+ve	-ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve	G – ve rod	-ve
31.	GH U 80	+ve	-ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve	G – ve rod	-ve
32.	GH U 81	+ve	-ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve	G – ve rod	-ve
33.	GH U 82	-ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	G – ve rod	-ve
34.	GH U 85	-ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	G – ve rod	-ve
35.	GH U 89	-ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	G – ve rod	-ve
36.	GH U 91	+ve	-ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve	G – ve rod	-ve
37.	GH U 95	-ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	G – ve rod	-ve
38.	GH St 1	+ve	-ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve	G – ve rod	-ve
39.	GH St 2	-ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	G – ve rod	-ve
40.	GH St 3	+ve	-ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	G – ve rod	-ve
41.	GH St 4	-ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	G – ve rod	-ve

42.	GH St 5	-ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	G – ve rod	-ve
43.	GH St 6	-ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	G – ve rod	-ve
44.	GH St 7	+ve	-ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	G – ve rod	-ve
45.	GH St 8	+ve	-ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	G – ve rod	-ve
46.	GH St 12	+ve	-ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	G – ve rod	-ve
47.	GH St 15	-ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	G – ve rod	-ve
48.	GH St 16	-ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	G – ve rod	-ve
49.	GH St 17	+ve	-ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	G – ve rod	-ve
50.	GH St 18	+ve	-ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	G – ve rod	-ve
51.	GH St 19	-ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	G – ve rod	-ve
52.	GH St 20	+ve	-ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	G – ve rod	-ve
53.	GH St 24	-ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	G – ve rod	-ve
54.	GH St 26	+ve	-ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	G – ve rod	-ve
55.	GH St 27	-ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	G – ve rod	-ve
56.	GH St 28	+ve	-ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	G – ve rod	-ve

57.	GH St 29	-ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	G – ve rod	-ve
58.	GH St 30	+ve	-ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	G – ve rod	-ve
59.	GH St 31	+ve	-ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	G – ve rod	-ve
60.	GH St 32	-ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	G – ve rod	-ve
61.	GH St 33	+ve	-ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	G – ve rod	-ve
62.	GH St 34	+ve	-ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	G – ve rod	-ve
63.	GH St 35	+ve	-ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	G – ve rod	-ve

S/N	**Isolate Code**	**Gram staining**	**MR**	**VP**	**Indo**	**Cit**	**Cata**	**Urease**	**Oxidase**	**Moti**	**Sucrose**	**Glucose**	**Fructose**	**Lactose**	**Maltose**
64.	GH St 38	G – ve rod	-ve	+ve	-ve	+ve	+ve	+ve	- ve	-ve	+ve	+ve	+ve	+ve	+ve
65.	GH St40	G – ve rod	+ve	-ve	+ve	-ve	+ve	-ve	- ve	+ve	+ve	+ve	-ve	+ve	+ve
66.	GH St 46	G – ve rod	+ve	-ve	+ve	-ve	+ve	-ve	- ve	+ve	+ve	+ve	-ve	+ve	+ve
67.	GH St 51	G – ve rod	+ve	-ve	+ve	-ve	+ve	-ve	- ve	+ve	+ve	+ve	-ve	+ve	+ve
68.	GH St 57	G – ve rod	-ve	+ve	-ve	+ve	+ve	+ve	-ve	-ve	+ve	+ve	+ve	+ve	+ve

69.	GH St 58	G – ve rod	-ve	+ve	-ve	+ve	+ve	+ve	- ve	-ve	+ve	+ve	+ve	+ve	+ve
70.	GH St 66	G – ve rod	-ve	+ve	-ve	+ve	+ve	+ve	- ve	-ve	+ve	+ve	+ve	+ve	+ve
71.	GH St 67	G – ve rod	-ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve
72.	GH St 71	G – ve rod	+ve	-ve	+ve	-ve	+ve	-ve	- ve	+ve	+ve	+ve	- ve	+ve	+ve
73.	GH St 76	G – ve rod	+ve	-ve	+ve	-ve	+ve	-ve	- ve	+ve	+ve	+ve	-ve	+ve	+ve
74.	GH St 78	G – ve rod	-ve	+ve	-ve	+ve	+ve	+ve	-ve	-ve	+ve	+ve	+ve	+ve	+ve
75.	GH St 80	G – re rod	+ve	-ve	+ve	-ve	+ve	-ve	- ve	+ve	+ve	+ve	-ve	+ve	+ve
76.	GH St 81	G – re rod	+ve	-ve	+ve	-ve	+ve	-ve	- ve	+ve	+ve	+ve	-ve	+ve	+ve

APPENDIX C

Antibiotic Susceptibility Test Result

S/N	Sample Code	PN	CEP	CH	CN	NA	E	CPX	OFX	S	AMC	SXT	FOS	IPM	CT	TET
1.	GH U3	R	R	I	S	R	S	S	S	S	S	R	S	S	R	R
2.	GH U6	R	R	R	R	R	R	R	R	R	R	R	S	S	R	R
3.	GH U7	R	R	R	S	R	R	R	R	S	S	R	S	S	I	S
4.	GH U8	S	R	R	R	R	R	R	R	I	I	R	S	S	I	R
5.	GH U13	R	R	R	R	R	R	R	R	R	R	R	S	S	R	R
6.	GH U14	R	R	R	R	R	R	R	R	R	R	R	S	S	R	R
7.	GH U15	R	R	R	R	R	R	R	R	R	I	R	S	S	R	R
8.	GH U17	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S
9.	GH U19	R	R	R	R	R	R	R	R	R	R	R	S	S	R	R
10.	GH U22	R	R	R	S	I	R	R	S	S	I	S	S	S	I	I
11.	GH U24	R	R	R	S	R	R	R	R	S	I	R	S	S	R	I
12.	GH U35	I	R	S	S	R	S	S	S	S	S	R	S	S	R	R

13.	GH U43	S	S	S	R	R	S	S	S	R	S	R	S	I	R	R
14.	GH U46	R	R	I	S	R	S	S	R	S	S	R	S	S	I	R
15.	GH U51	R	R	R	S	R	S	I	S	S	S	R	S	S	R	R
16.	GH U52	I	I	R	S	R	S	S	S	R	S	S	R	S	R	R
17.	GH U55	S	R	R	S	R	I	S	S	S	S	I	S	S	R	R
18.	GH U57	R	R	I	S	R	S	S	S	I	S	R	S	R	R	R
19.	GH U58	I	R	R	S	R	S	S	S	S	S	R	S	S	R	R
20.	GH U59	R	R	I	S	R	I	S	S	S	I	R	S	R	I	R
21.	GH U60	S	R	R	S	R	S	S	S	S	S	R	S	S	R	R
22.	GH U65	R	R	S	S	R	I	S	S	S	S	R	S	S	R	R
23.	GH U66	S	R	R	S	R	S	I	S	I	S	S	S	I	R	I
24.	GH U67	R	R	R	I	I	R	S	S	R	S	I	S	S	S	R
25.	GH U69	I	R	R	S	R	S	S	S	S	S	R	S	R	R	S
26.	GH U75	R	R	S	R	S	R	R	S	R	S	S	S	S	S	R
27.	GH U76	R	R	S	S	R	R	I	S	S	S	R	S	S	R	S

28.	GH U77	I	R	I	R	R	R	S	I	I	S	I	S	R	S	R
29.	GH U78	R	R	R	R	S	S	S	S	S	I	R	S	S	R	R
30.	GH U79	I	R	S	S	R	R	S	S	R	S	S	S	S	S	R
31.	GH U80	S	R	R	S	R	S	S	S	R	S	S	S	S	R	R
32.	GH U81	R	R	S	R	S	R	S	S	S	S	S	S	R	R	R
33.	GH U82	R	R	S	S	R	R	S	S	S	S	R	S	S	S	R
34.	GH U85	R	R	S	R	I	S	S	S	R	R	S	S	S	R	R
35.	GH U89	I	R	I	S	S	R	S	S	R	S	S	S	I	R	R
36.	GH U91	R	R	S	R	S	I	R	I	S	S	I	S	S	R	R
37.	GH U95	R	R	R	S	I	R	S	S	S	S	S	S	S	R	S
38.	GH ST 1	R	R	S	S	R	S	S	S	S	S	S	S	S	R	I
39.	GH ST 2	R	R	S	S	S	S	S	S	S	S	S	S	S	R	R
40.	GH ST 3	R	R	S	S	S	S	S	S	S	S	R	S	S	R	R
41.	GH ST 4	R	I	R	R	R	S	S	S	S	S	R	S	S	R	R
42.	GH ST 5	S	R	S	R	R	S	S	S	R	R	R	R	S	R	R

43.	GH ST 6	S	R	R	R	R	S	S	S	S	S	R	S	S	R	R
44.	GH ST 7	S	S	R	S	R	S	S	S	R	S	R	S	S	R	R
45.	GH ST 8	R	R	R	R	R	I	S	S	I	S	R	S	S	R	R
46.	GH ST 12	S	S	R	S	R	I	S	S	R	S	R	S	S	R	R
47.	GH ST 15	R	R	I	S	R	S	I	S	R	S	R	S	S	R	R
48.	GH ST 16	R	R	S	I	R	S	S		I	S	R	S	S	R	R
49.	GH ST 17	S	S	R	S	R	R	S	S	R	S	R	S	S	R	R
50.	GH ST 18	R	R	I	S	I	R	S	S	S	S	R	S	S	R	I
51.	GH ST 19	R	R	S	S	I	R	S	S	S	S	S	S	S	R	R
52.	GH ST 20	I	I	S	S	S	I	S	S	S	S	R	S	S	R	R
53.	GH ST 24	S	R	S	R	R	I	S	S	I	S	R	S	S	R	R
54.	GH ST 26	I	R	R	S	S	S	R	S	S	S	S	S	S	S	R
55.	GH ST 27	R	R	S	S	R	S	S	R	S	S	R	S	S	I	R
56.	GH ST 28	I	R	I	S	R	S	S	S	S	S	S	S	R	R	R
57.	GH ST 29	R	R	S	S	R	S	S	S	R	S	I	S	I	R	S

58.	GH ST 30	R	R	S	S	I	S	I	S	S	S	S	S	I	R	R
59.	GH ST 31	S	R	I	R	S	R	S	S	S	S	R	S	S	R	R
60.	GH ST 32	R	R	S	R	S	R	S	S	S	S	S	S	S	R	I
61.	GH ST 33	R	R	R	S	R	I	S	S	R	S	S	S	R	R	R
62.	GH ST 34	S	S	R	S	I	S	S	S	S	S	I	S	S	R	R
63.	GH ST 35	R	R	S	S	R	S	S	S	S	S	S	S	S	R	R
64.	GH ST 38	R	R	I	S	R	I	S	S	S	S	S	S	S	R	R
65.	GH ST 40	R	R	S	S	R	S	S	S	S	S	R	S	I	R	R
66.	GH ST 46	S	R	R	R	I	R	S	S	S	I	I	S	I	R	I
67.	GH ST 51	R	R	I	S	I	R	S	S	S	R	S	S	S	S	R
68.	GH ST 57	R	R	R	S	I	I	S	R	S	S	S	S	S	R	R
69.	GH ST 58	S	R	R	S	S	R	I	S	S	R	S	S	S	S	R
70.	GH ST 66	R	R	S	R	S	S	I	S	S	R	S	S	S	S	R
71.	GH ST 67	R	S	I	R	S	R	R	S	S	I	R	S	S	R	R
72.	GH ST 71	R	R	R	S	I	R	S	S	S	S	R	S	S	R	R

73.	GH ST 76	R	R	S	R	R	S	S	S	S	S	S	S	S	R	R
74.	GH ST 78	R	R	R	I	R	R	S	S	S	S	S	S	S	R	R
75.	GH ST 80	S	R	R	S	R	I	S	S	S	S	S	S	S	I	R
76.	GH ST 81	R	R	R	I	R	S	I	S	S	S	I	S	S	R	R

APPENDIX D

Antibiotic susceptibility test plate