CHAPTER ONE

INTRODUCTION

Antibiotic resistance (especially those mediated by ESBLs, MBLs and *AmpC* β - lactamases) is a global health challenge posing a menace to effectively treat and manage infections caused by organisms – producing these enzymes. The *mcr* gene inclusive of *mcr*-1, *mcr*-2, and the newest being *mcr*-3 gene are plasmid-mediated colistin resistance genes that cause microbial resistance to the polymyxins and colistin. These genes occur in both enteric and non-enteric bacteria. They could compromise the effective treatment of infections caused by extensively drug-resistant bacterial pathogens since they harbour genes for other resistance traits. Extended-spectrum β-lactamases (ESBLs) are beta-lactamase enzymes that hydrolyse extended-spectrum cephalosporins with an oxyimino side chain but are inhibited by clavulanic acid. They occur mostly in Gram-negative bacteria especially the *Enterobacteriaceae*, and they are associated with increased morbidity and mortality.

Metallo-β-lactamases (MBLs) are Carbapenemase that hydrolyse and cause resistance to carbapenems and other β-lactams but not aztreonam, and which are yet inhibited by chelating agents like ethylenediamine tetra-acetic acid (EDTA). *AmpC* β-lactamase is a class C β-lactamase enzyme that is active against β-lactam drugs especially cephamycins. They also confer a great deal of antibiotic resistance on Gram-negative pathogens, and they are located on bacterial chromosomes. Organisms that express ESBLs, MBLs, *AmpC* β-lactamases, and those that harbour genes for colistin and/or polymyxin resistance are indeed a great threat. Clinical importance of these organisms are usually resistant to virtually all β-lactam drugs and some non-β-lactam drugs like aminoglycosides, fluoroquinolones, and co-trimoxazole used in clinical medicine today.

Multidrug-resistant organisms continue to be a major problem globally as their menace are gradually eroding the efficacy of our therapeutic armamentarium. It is therefore very important for microbiology laboratories all over the world to detect these organisms in their routine susceptibility studies using prompt and accurate detection techniques. This work is aimed at characterizing ESBLs, MBLs, *AmpC* β-lactamases, and plasmid-mediated colistin resistance genes (*mcr*-1, *mcr*-2, *mcr*-3) from environmental isolates of *Klebsiella* species, *Escherichia coli,* and *Pseudomonas aeruginosa* from abattoir and chicken samples in Abakaliki metropolis, Ebonyi State, Nigeria.

1.2 Background of the Study

Globally, antibiotic-resistant bacteria still pose a threat to public health. The widespread use of antibiotics in food-animal production including *in-vivo* application in both chicken and livestock as a prophylactic agent in promoting animal growth and tackling infection, allows resistant strains of microbes to evolve (Caprioli *et al.*, 2005). And the contamination of the environment with animal wastes (containing resistant bacteria) is a major route via which human populations become infected by these microbes. The incessant use of antibiotics in animal husbandry and in other agricultural practices allows resistant microbes to evolve. These resistant bacteria can persist in meat, chicken/animal products and animal wastes, and thus serve as the route via which the environment becomes contaminated with them (Van da Bogaard and Stobberingh, 2002).

Environmental microorganisms including *Escherichia coli, Klebsiella* species and *P. aeruginosa* represent the most relevant reservoir of resistance to antibiotics and other antimicrobial agents in the community. This is due in part to their ability to acquire and incorporate into their genome, antibiotic resistance genes from their environment. Gram-negative bacteria from environmental isolates including *Escherichia coli, Klebsiella*

species and *P. aeruginosa* harbour antibiotic resistance genes such as ESBLs, MBLs, *AmpC* and plasmid-mediated colistin resistance genes that give them the exceptional ability to potent antibiotics directed towards them.

P. aeruginosa, *E. coli* and *Klebsiella* species are among the many bacterial pathogens responsible for most nosocomial and community-acquired infections around the world today (Yin *et al.,* 2017; King-Ting *et al.,* 2009; Pattarachai *et al.,* 2008; Iroha *et al.,* 2008), and they also harbour antibiotic resistance genes (including ESBL, MBL, *AmpC*, and plasmid-mediated colistin resistance genes). These organisms encode varieties of β – lactamase enzymes (including ESBLs, MBLs, and *AmpC*) and plasmid-mediated colistin resistance genes that hydrolyse and confer resistance to β-lactam antibiotics (one of the multipurpose class of antibiotics used in clinical medicine).

Extended-spectrum β-lactamases (ESBLs) are plasmid-mediated β-lactamase enzymes that hydrolyse and confer resistance to broad-spectrum cephalosporins including monobactams (e.g. aztreonam) but are yet inhibited by clavulanic acid (Chow *et al.,* 1991). Though some are chromosomally mediated, ESBL-producing bacteria was first isolated in Germany in 1983 (Knothe *et al.,* 1983) and in the United States in 1989 (Quinn *et al.,* 1989), and they have since then spread worldwide – limiting the efficacy of most antibiotics used for treating bacterial infections. The introduction of the third-generation cephalosporins into clinical practice in the early 1980s was foreshadowed as a major breakthrough in the fight against β-lactamase-mediated bacteria resistance (Bradford, 2001). But the emergence of ESBLs has led to the demise of the cephalosporins and has threatened the efficacy of other non-β-lactam agents in treating bacterial infections. Their incidence has been steadily increasing over the years resulting

in the limitation of therapeutic options (Bradford, 2001; Podschun and Ullmann, 1998; Adeleke *et al.,* 2010).

Metallo-β-lactamases (MBLs) are carbapenem-hydrolyzing β-lactamases which belong to Molecular Class B of ambler beta-lactamase classification, and which have the ability to hydrolyse and confer resistance to carbapenems (imipenem, meropenem, ertapenem) and other β-lactam antibiotics (Walsh *et al.,* 2005). The carbapenems are very potent antimicrobial agents used for the treatment of serious Gram-negative infections including those mediated by ESBLs (Walsh *et al.,* 2005). They are mostly used by hospitals worldwide under restricted conditions to treat and manage severe Gram-negative infections because of their broad-spectrum of activity (Franco *et al.,* 2010).

The MBLs are known to confer variable range of high resistance to all β-lactam antibiotics except the monobactams. And their presence in clinically important organisms like *P. aeruginosa* have put the use of the carbapenems as the last treatment option for treatment of serious Gram-negative bacterial infections under threat (Walsh *et al.,* 2005; Franco *et al.,* 2010). MBLs belong to a group of β-lactamases which requires divalent cations of zinc as cofactors for their enzyme activity. And they share four main characteristics namely; activity against carbapenem antibiotics, no clear hydrolysis of monobactams, inhibition by chelating agents (e.g. EDTA), and requirement of zinc ions for enzyme activity (Walsh *et al.,* 2005; Aibinu *et al.,* 2007).

AmpC β-lactamases are clinically important cephalosporinases encoded on the chromosomes of many *Enterobacteriaceae* and a few other bacteria where they mediate resistance to cephalothin, cefazolin, cefoxitin (all cephalosporins) and most penicillins (Jacoby and Munoz-Price, 2005). It is active on cephamycins as well as oxyimino β-lactams (Jacoby and Munoz-Price, 2005) and this differentiates them from other ESBLs.

It is usually encoded by bacterial chromosomal genes in many Gram-negative bacilli, though some are plasmid mediated. The *AmpC* enzyme in *E. coli* is poorly expressed and the *AmpC* gene is missing from the chromosome of *Klebsiella* and *Salmonella* species (Jacoby and Munoz-Price, 2005). Characteristically, *AmpC* β-lactamases are known to mask ESBL production in organisms harbouring both *AmpC* and ESBLs, and they are poorly inhibited by clavulanic acid (Jacoby and Munoz-Price, 2005).

Plasmid-mediated Colistin resistance in Gram-negative bacteria including *Escherichia coli, Klebsiella* species and *P. aeruginosa* pose a serious public health challenge globally due to their ability to render inefficacious the polymyxins used for treating serious infections caused by Gram-negative bacteria. Previously, the *mcr*-1 and *mcr*-2 genes were predominantly responsible for bacterial resistance to the antimicrobial onslaught of Colistin and other polymyxins, but the *mcr*-3 genes recently detected in *E. coli* isolates gives impetus to the devastating effect of these organisms to our therapeutic armamentarium (Yin *et al.,* 2017). According to Yin *et al.* (2017), plasmid-mediated colistin resistance genes inclusive of *mcr*-1, *mcr*-2, and *mcr*-3 are fast spreading across the globe via mobile genetic elements in Gram-negative bacteria such as those in the *Enterobacteriaceae* family and some non-enteric bacteria like Aeromonas species. Colistin and the polymyxins are widely used in veterinary practices and even in human medicine for treatment and other prophylactic measures. It is therefore important to continue to be on the lookout for Gram-negative bacteria from animals and human samples in order to bring their emergence and spread under control and thus protect the clinical efficacy and usage of colistin and the polymyxins.

1.2 Statement of the Problem

Organisms harbouring plasmid-mediated colistin resistance genes and producing ESBLs, MBLs, *AmpC* beta-lactamase are resistant to virtually all β-lactam antibiotics and some non-β-lactam antibiotics as well (Kasap *et al.,* 2010; Yin *et al.,* 2017). The emergence of bacteria producing these enzymes together with their ability to transmit their resistant genes to susceptible strains through plasmids, transposons, integrons and other means of genetic transfer poses a serious problem especially in settings where they still remain undetected. These β-lactamases are now found worldwide, and they are being observed as increasingly important causes of multidrug resistance in Gram-negative bacteria all over the world, and this menace limits the number of drugs available for potential treatment of bacterial infections (Alistair and Julie, 2004).

1.3 Significance of the Study

The prevalence of ESBLs is steadily increasing over the years as shown by some previous works (Amita and Rajesh, 2007; Iroha *et al.,* 2010). Plasmid-mediated colistin-resistance genes inclusive of *mcr-1, mcr-2* and *mcr-3* genes have not been previously reported in Nigerian. The threat posed by Gram-negative bacteria harbouring genes that mediate colistin and polymyxin resistance might continue undetected and cause some infectious disease outbreak if they are left undetected and uncontrolled. The menace posed by multidrug-resistant bacteria necessitates the need to detect by molecular characterization the presence of ESBLs, MBLs and *AmpC* β-lactamases from abattoir and chicken sources – owing to the fact that antibiotic resistance is an increasing problem in health sector worldwide. Also, Gram-negative bacteria including *Escherichia coli, P. aeruginosa* and *Klebsiella* species are fast becoming resistant because they now carry plasmid-mediated resistance genes. These plasmid-mediated resistance genes

mediate bacteria resistance. This project will detect and determine by molecular techniques the presence of these genes in *Escherichia coli, P. aeruginosa* and *Klebsiella* species isolated from abattoirs and chicken sources.

The resistance of microbes to antimicrobial agents is a global phenomenon spanning from one country to another and antibiotic-resistant bacteria knows no border of any nation. Owing to the increased globalization and free movement of people today which conveys these microbes from one place to another. It represents a major and serious problem in the health sector, these makes the antibiotic selection for treatment difficult. Some of these organisms are multidrug resistant in nature – showing resistance to a wide variety of antibiotics used in clinical medicine (Iroha *et al.,* 2008).

The increased prevalence of Extended spectrum beta – lactamases (ESBLs), Metallo Beta – Lactamases (MBLs), *AmpC* β-lactamases, and plasmid-mediated colistin resistant Gram-negative bacteria has created an urgent and very important need. To accurately detect these pathogens in order to keep them at bay. Countries like ours where these organisms are still not detected in hospitals, because phenotypic characterization is not enough and appropriate to establish the reality and presence of all these important enzymes. It is imperative to undertake a molecular characterization (the current Gold Standard) in order to provide a more elaborate and comprehensive epidemiological data. about them in this part of Nigeria. This research work is aimed at characterizing the genetic determinants of ESBLs, MBLs, *AmpC*, and plasmid-mediated colistin resistance in *Escherichia coli, Klebsiella* species and *P. aeruginosa* isolated from abattoir and chicken sources in Abakaliki, Ebonyi State, Nigeria.

1.4 Aim and Objectives of the Study

1.4.1 Aim

This study was designed to characterize resistance enzymes from *Escherichia coli, Klebsiella* species and *Pseudomonas aeruginosa* isolates of abattoir and poultry effluents within Abakaliki metropolis

1.4.2 Objectives of the Study

The specific objectives of this proposed study are to:

1. isolate, characterize and identify *Escherichia coli, Klebsiella* species and *P. aeruginosa* from abattoir and poultry origins.
2. evaluate the antibiogram of the isolates to both beta-lactam and non-beta-lactam antimicrobial agents
3. evaluate the antimicrobial resistance phenotype of the isolates
4. evaluate the isolates for beta-lactamase (ESBL), Metallo beta lactamase (MBL), *AmpC* and mobile colistin resistance (*mcr*) production using disk diffusion technique.
5. determine the multiple antibiotics resistance index of the isolates.
6. Classify the antimicrobial resistance
7. detect the presence of beta-lactamase, Metallo beta-lactamase, *AmpC* and *mcr* resistance genes in the isolates using multiplex PCR.
8. determine the transmissibility of the resistance genes to susceptible isolates by trans-conjugation experiment.

1.5 Study Hypothesis

a. Null hypothesis

1 *Escherichia coli, Klebsiella* species and *Pseudomonas aeruginosa* isolated from abattoir and poultry effluents are not resistant to antibiotics.

2 *Escherichia coli, Klebsiella* species and *Pseudomonas aeruginosa* isolated from abattoir and poultry effluents are not resistant to more than one antibiotics.

3 *Escherichia coli, Klebsiella* species and *Pseudomonas aeruginosa* are not multi-drug resistance.

4 *Escherichia coli, Klebsiella* species and *Pseudomonas aeruginosa* isolated from abattoir and poultry effluents are not ESBL, MBL *AmpC* and *mcr* producers.

5 *Escherichia coli, Klebsiella* species and *Pseudomonas aeruginosa* isolated from abattoir and poultry effluents cannot transfer resistance genes to susceptible bacteria.

6 *Escherichia coli, Klebsiella* species and *Pseudomonas aeruginosa* isolated from abattoir and poultry effluents do not carry resistance genes.

b. Alternative Hypothesis

1 *Escherichia coli, Klebsiella* species and *Pseudomonas aeruginosa* isolated from abattoir and poultry effluents are resistant to antibiotics.

2 *Escherichia coli, Klebsiella* species and *Pseudomonas aeruginosa* isolated from abattoir and poultry effluents are resistant to more than one antibiotic.

3 *Escherichia coli, Klebsiella* species and *Pseudomonas aeruginosa* are multi-drug resistance.

4 *Escherichia coli, Klebsiella* species and *Pseudomonas aeruginosa* isolated from abattoir and poultry effluents are ESBL, MBL *AmpC* and *mcr* producers.

5 *Escherichia coli, Klebsiella* species and *Pseudomonas aeruginosa* isolated from abattoir and poultry effluents can transfer resistance genes to susceptible bacteria.

6 *Escherichia coli, Klebsiella* species and *Pseudomonas aeruginosa* isolated from abattoir and poultry effluents carry's resistance genes.

CHAPTER TWO

REVIEW OF RELATED LITERATURE

The parts of an animal that are not used for the production of food are called abattoir waste and can consist of internal organs, blood, bone, tendons and ligaments (Franke-Whittle and Insam, 2013). It can also include urine, faeces and carcasses (Adeyemi and Adeyemo, 2007). Abattoir waste also includes wastewater originating from an abattoir (Adeyemi and Adeyemo, 2007). The abattoir is a specialized facility approved and registered by the regulatory authority for inspection of animals, hygienic slaughtering, processing and effective preservation and storage of meat products for human consumption (Alonge, 2002). Adequate facilities to ensure safe disposal of abattoir wastes in a manner that will not constitute a potential hazard to public, animal and environmental health is considered very essential. Most abattoirs in Nigeria have no facilities for waste treatment. Wastes are either disposed on open dumps or are discharged into nearby streams, hence constituting an environmental menace (Adeyemo *et al.,* 2002).

The use of contaminated surface water for meat processing by butchers may portend serious challenges to public health and food safety. Several studies have revealed that abattoirs in developing countries have an unhygienic environment (Adeyemo, 2002; Nwanta *et al.,* 2010) and detected the presence of pathogens that are known causes of diarrheal diseases and a possible hazard to human health in the abattoir waste and water contaminated by abattoir waste (Benka-Coker and Ojior, 1995; Abiade-Paul *et al.*2005; Nwanta *et al.,* 2010). It has also been suggested that scavengers feeding on abattoir waste can spread pathogens from the waste to new locations (Adeyemi and Adeyemo, 2007).

Abattoir operation could be very beneficial to man; in that, it provides meat for human consumption and other useful by-products. Still, it can be very hazardous to public health in respect to the waste it generates (Meadows, 1995; Adeyemi and Adeyemo, 2007). Recent studies have shown that zoonoses from abattoir wastes are yet to be fully controlled in more than 80% public abattoirs in Nigeria (Cadmus *et al.*, 1999). Diseases like pneumonia, diarrhoea, typhoid fever, asthma, wool sorter diseases, respiratory and chest diseases were reported to be associated with abattoir activities (Bello and Oyedemi, 2009).

Nwachukwu *et al.* (2011) studied on microbial assessment of surface water and sediment samples from different points (A, B, C) in Otamiri river receiving abattoir wastes. Preliminary identification following the criteria of Holt *et al.* (1994) indicated that the proteolytic bacteria isolates included *Pseudomonas* spp., Bacillus spp., Enterobacter spp., *Escherichia* spp., *Klebsiella* spp., *Streptococcus* spp., *Staphylococcus* spp. and *Proteus* spp. while lipolytic bacteria were *Pseudomonas* spp., *Moraxella* spp., *Acinetobacter* spp., Arthrobacter sp. and *Micrococcus* spp (Holt *et al.,* 1994 and Nwachukwu *et al.,* 2011).

Some are causative agents of gas gangrene, food poisoning, infantile diarrhoea, chronic infections and faecal indicators dehydration, gastrointestinal irritation and infantile of water pollution. These types of diseases can spread from the abattoir to the neighbourhood via vectors or animals. In addition, the isolation of other species may be as a result of poor environmental conditions due to dust and contamination of the water used during slaughtering, because these bacteria organisms are also inhabitants of dairy products, as reported by Talaro (2006). The presence of these organisms is suggestive of impending health hazards. Fresh meats sold to the public in open markets are contaminated with coliform bacteria as well as other bacteria forms (Nandita *et al.*,

2016). Improper handling and improper hygiene might lead to the contamination of fresh meats and this might eventually affect the health of consumers (Okonko *et al.,* 2008).

Effluents generated from abattoirs are characterized by the presence of a high concentration of whole blood of slaughtered food animals and suspended particles of semi-digested and undigested feeds within the stomach and intestine of slaughtered and dressed food animals. Abattoir effluent contains several million colony forming units (CFU) of total aerobic bacteria count and faecal coliforms (Coker *et al.,* 2001). In addition, there may also be the presence of pathogenic microorganisms, such as *Klebsiella* species, *Pseudomonas* species and *E. coli* (including serotype O157: H7), parasite eggs and amoebic cysts (Bull and Roger, 2001).

Several past studies have reported the isolation of pathogenic bacteria and fungi species such as *Staphylococcus aureus*, *Streptococcus* spp, *Salmonella* spp, *Escherichia coli*, *Aspergillus* spp, Mucor, *Saccharomyces* spp, *Klebsiella* species, *Pseudomonas* species and *Penicillium* spp from abattoir wastewater (Coker *et al.,* 2001, Adesomoye *et al.,* 2006 and Adebowale *et al.,* 2016). Pathogens isolated might threaten public health by migrating into ground or surface water, or vectors like animals, birds and arthropods which can help in the dissemination (Gauri, 2004).

Bacterial contamination from abattoir wastes are yet to be fully controlled in more than 80% public abattoirs in Nigeria (Cadmus *et al.,* 1999). This may be linked to the absence or inadequate abattoir waste management facilities. This has consequently led to large solid wastes and untreated effluents being common sites (Adeyemo, 2002). The risk of epidemics, water contamination and pollution, the annihilation of biotic life, global warming and soil degradation by waste materials are real problems confronting

developing countries where issues concerning waste management have been grossly neglected (Adedipe, 2002; Adeyemi and Adeyemo, 2007).

2.1 Animal Agriculture and Potential Environmental Impact

It is projected that the number of people in the world was 7.7 billion by 2020 and 9.4 billion by 2050, with the largest increase coming from the developing world (Castro *et al.,* 2011). Over the last 25 years, per capita meat consumption in developing countries grew at 3 times the rate in developed countries (Sparling *et al.,* 2006). In traditional livestock rearing, manure is vital fertilizer, and animals return nutrients to the soil in forms that plants can readily use. In addition, in some areas, manure is a vital resource to be used as fuel or building materials. In most traditional systems, manure is effectively utilized and is not a disposal issue (Sherman, 2008).

In comparison, modern or industrialized systems are high-input, open-loop systems. These industrialized systems provide significant efficiency in terms of economy of scale, consistency, and value to consumers. Industrialized systems began in the USA over 60 years ago with the chicken industry and have now become the norm for the swine industry also. It is more difficult to industrialize the cattle industries, largely because they are ruminants and benefit from grazing, but beef feedlots and large dairies are examples of industrialization for this species as well (Sherman, 2008). Livestock densities in both the developed and developing worlds range between 5 and >6000 kg/km^2, with the greatest concentrations in India, China, and Europe (Masse and Masse, 2000).

As traditional systems are replaced by industrial agriculture, livestock density increases. As mentioned above, manure disposal from industrialized facilities is problematic. Numerous systems have been developed to dispose of or recycle the manure, but many

have environmental and health considerations. Application to fields is the time-honoured manner of manure disposal, but it can cause considerable problems with nitrate leaching if the ground is frozen, there is excess rain, or the soil is very sandy. Runoff of manure into watersheds causes increased microbial proliferation, high biochemical oxygen demand, and altered aquatic microenvironments (Hooda *et al.,* 2000). Catastrophic events can ensue after natural disasters such as floods or earthquakes, or even because of faulty design. For broilers, virtually all waste goes into stacking pits. After three or four broods are raised in a house, the litter, which contains fibrous material, spilt feed, feathers, and bird excreta is collected and held in a heat-generating pile for some weeks until pathogenic organisms are destroyed. This material is fed to cattle, who consume it free-choice in self-feeders as a source of fibre and protein (Rankins *et al.,* 2002). Beef and dairy faeces are usually collected as solids and later applied to land. There is an anaerobic slurry storage period, which is designed to decrease microbial content, but it has been shown that certain pathogenic organisms can survive beyond the designated period (Hooda *et al.,* 2000).

2.2 Potential Bacterial Pathogens from Abattoir and its Receiving Surface Water

Adebowale *et al.* (2016) reported in their study conducted in Abeokuta, Nigeria observed that open dumps within and around the abattoir are a practice in Lafenwa abattoir in Ogun State Nigeria. There were no structured and adequate drainages. Consequently, effluents were discharged into the nearby surface Ogun River. Most, if not all abattoirs in Nigeria uses these methods of disposal (Adeyemo, 2002). Previous studies conducted had shown that the receiving surface water does not meet international standards and unfit for meat processing (Agbogu *et al.,* 2005; Luga, 2006 and Adebowale *et al.,* 2010).

The risk is further amplified because the receiving surface water is also used for recreational activities including swimming and fishing as well as a source of water for domestic use by people living around the abattoir (Adebowale *et al.*, 2016).

2.3 Slaughter and Waste Handling in Developing Countries

Abattoirs in developing countries are generally less developed compared with the situation for instance in Europe and US (Chukwu, 2008). They can be modern or very simple but many of them disregarding type may constitute a threat to human health because of unsanitary conditions (Verheijen *et al.*,1996). According to Odong *et al.* (2013) two of the abattoirs in the city of Kampala discharge untreated waste into Nakivubo channel. Nakivubo channel falls into Lake Victoria at Inner Murchison Bay (Odong *et al.*, 2013). The inner Murchison Bay is the source for drinking water for Kampala, its suburbs and nearby towns (Water and Environment Sector Performance Report, 2012).

2.4 Potential Pathogens in Abattoir Waste

Approximately 61 % of the known human pathogens in the world are zoonotic (Taylor, 2001). Pathogens in abattoir waste may originate from the digestive tracts or hides of the animals. Most of the pathogens are of enteric sources (Mittal, 2004). *Klebsiella* species, *Pseudomonas* species and *E. coli* are examples of zoonotic bacteria that can cause diseases in humans and can be present in high levels in abattoir waste (Adeyemi and Adeyemo, 2007). Nwanta *et al.* (2010) examined abattoir waste for bacteria with potential risk for human health at an abattoir in Nigeria, and it was found that several bacteria such as *E. coli* O157: H7, *Salmonella* spp. and *Campylobacter* spp. were present in the waste. In addition, *E. coli* O157: H7 was one of the most frequently isolated

bacteria. Several studies in Africa have found *Salmonella* spp. in wastewater at abattoirs (Benka-Coker and Ojior, 1995; Abiade-Paul *et al.,* 2005; Nyamboya *et al.,* 2013).

Salmonella has also been isolated in effluent water from treating facilities at abattoirs (Barros *et al.,* 2007). Also, the presence of *Salmonella* spp. in water has been associated with the dumping of abattoir waste into water bodies (Benka-Coker and Ojior, 1995). Abattoir waste can also contain antibiotics (Adeyemi and Adeyemo, 2007).

2.5 Transmission of Pathogens from Abattoir Waste/Abattoir Waste water

Pathogens can spread from animal to man by several different ways, for example via direct contact, consumption of food or water that is contaminated, indirect contact via objects that are contaminated, and transmission by vectors and by aerosols (Center for Food Security and Public Health, 2008). A study by Adeyemi and Adeyemo (2007) suggested that wild animals can transfer pathogens to humans and other animals from abattoir waste by feeding on the same. Water contaminated with pathogens can also cause infection in animals and humans drinking the water or eating crops or foods contaminated by the water (Mittal, 2004). An example of the latter was shown by Breuer *et al.* (2001), where they connected a multistate outbreak of disease caused by EHEC O157: H7 to seeds of alfalfa sprouts contaminated with the pathogen.

a). Transmission by Chicken Birds

Birds feeding on sewage outfalls, rubbish tips or shellfish that's been contaminated can pick up bacteria and then the bacteria can be distributed to other places by the birds (Wallace *et al.*,1997). In a survey of faecal samples from birds (mostly gulls) in 1997, the results showed that a small percentage of the birds included were carriers of *E. coli* O157 (Wallace *et al.*,1997). In 2006 Ejidokun *et al.* found, when investigating the source

of infection for an outbreak of disease caused by *E. coli* O157 in three humans, that isolates from the humans were identical to isolate found in a sample from wild rooks' faeces. Their results indicated that indirect contact with faeces from wild birds can result in infection with *E. coli* O157 and that the infection thereafter can carry on by person to person transmission. It was suggested that the birds had picked up the pathogen from faeces from livestock (Ejidokun *et al.,* 2006). Several studies have shown that *Salmonella* spp. can be found in several different species of wild birds and that they, therefore, can act as carriers of the bacteria (Vlahović *et al.,* 2004; Nyakundi and Mwangi, 2011). Al-Sallami, (1991) found that the *Salmonella* spp. that was most frequently isolated from humans with the diarrheal disease was found in wild crows in the same area. According to Cízek *et al.* (1994) the main source of infection in wild birds with *Salmonella* spp. is the environment and that birds with links to such an environment can pick up an infection when drinking or eating.

2.6 Water and Waste Water-Related Disease

According to the WHO (2013a), approximately 1,1 billion people lack access to high-quality water supply sources resulting in diarrhoea, which is the cause of 4% of the human deaths in the world. According to Black *et al.* (2010) 16 % of the deaths among children under five years in Uganda during 2008 were caused by gastrointestinal diseases associated with diarrhoea and in the Uganda Demographic and Health Survey for 2011 it was found that 23 % of the children that were included in the study with an age under five years had diarrhoea two weeks prior to the start of the survey. Water contaminated with faeces from animals can cause diarrhoea because animal faeces can contain diarrhoea-causing microorganisms (WHO, 2013a). As an example, animal faeces can contain pathogens such as *E. coli* 0157 and *Salmonella* spp., which can infect humans

2.7 Antibiotic Resistance

Antibiotic resistance means that bacteria can resist the effect of one or more antibiotics (ECDC 2013). Some bacteria are resistant to antibiotics naturally, but bacteria can also acquire resistance (ECDC, 2013). Infections caused by bacteria that are resistant to antibiotics can lead to failure of conventional treatment, longer treatments and death. Antibiotic resistance also leads to higher medical costs and endangers the success of certain treatments (WHO, 2013b). It is well known that animals can harbour antibiotic resistant and zoonotic pathogens (Bywater *et al.,* 2004; Swedres-Svarm, 2012). Multiple drug resistance has been suggested to be defined as when a bacterium has acquired resistance to one or more antibiotics in at least three antimicrobial categories (Magiorakos *et al.,* 2012). Pathogens that are resistant to antibiotics can be transmitted from animals to humans and vice versa (ECDC, 2013).

Antibiotic-resistant bacteria that are non-pathogenic and part of the normal intestinal flora have been shown to be able to transfer resistance genes to pathogenic bacteria (Blake *et al.,* 2003). Resistant bacteria present in animals can also transfer resistance genes to bacteria that are part of the human normal intestinal flora if they are transferred to humans (van den Bogaard and Stobberingh, 2000). There are several studies that have established the presence of antibiotic-resistant bacteria in abattoir waste (Abiade-Paul *et al.,* 2005; Nwanta *et al.,* 2010). Research reports have demonstrated that this bacterial organism harbours multidrug-resistant genes (Nwanta *et al.,* 2010; Byarugaba *et al.,* 2011and Olatoye *et al.,* 2012).

In a study conducted in Sweden by Peter (2014) indicates that abattoir wastewater samples were contaminated with high levels of *Escherichia coli, Klebsiella, Pseudomonas* and Enterococcus spp. which indicates faecal contamination. The source

of the contamination is most likely the abattoir waste that is being washed out into the drainage channel during and after slaughter Peter (2014). Such contamination conducts a hazard to public health since the water can spread to water sources and make such water unfit for human consumption and even transmit disease. This is a potential hazard in the case of the city since the drainage channel may eventually end up where drinking water or edible fruits and vegetables are extracted (Strockbine and Maurelli, 2005). More likely faecal contamination from humans (workers or other people resident at the abattoir area) defecates into the drainage channel (VetBact, 2013).

Trond *et al.* (2013) worked on bacteria surviving practical disinfection and compare their survival abilities with representative isolates of the pathogen *Listeria monocytogenes*. The most dominating bacterial genera based on counts on non-selective agar were *Aerococcus*, *Acinetobacter*, *Pseudomonas*, *Serratia* and *Staphylococcus*. Isolates of Citrobacter, *Enterobacter* and *Serratia* dominated on agar plates selective for *Enterobacteriaceae*. Moschonas *et al.* (2011) identified 431 psychrophilic or psychrotrophic isolates from commercial Irish beef abattoir environments and "blown packs" of vacuum-packed beef, using PCR and 16S rRNA sequencing, and estimated their intraspecies genetic diversity using restriction fragment length polymorphism (RFLP) analysis and spacer region PCR (SR-PCR). Microbial content of wastewater in two abattoirs and the impact on the microbial population of receiving soil was studied in Agege and Ojo Local Government Areas in Lagos State, Nigeria by Adesemoye *et al.* (2006).

Until about 15 years ago the emergence of resistance to a single antimicrobial, or even to all members of a class of antimicrobials, was merely an inconvenience. During the 1970s and 1980s, large numbers of new antimicrobials had been developed, and together these new drugs ensured that infections due to almost any bacteria could be readily cured.

Antibiotic resistance acquisition due to selective pressure is of public health concerns as resistance genes can be disseminated in nature and transferred to pathogenic counterparts of bacterial species by genetic mobile elements (Wellington *et al.*, 2013).

Bacterial resistance is closely associated with the use of antimicrobial agents in clinical practice. Prolonged therapy with antibiotics can lead to the development of resistance in a microorganism that initially is sensitive to antibiotics, but later it can adapt gradually and develop resistance to antibiotics. When an antibiotic attacks bacterium, bacterial cells susceptible to it will die, but those that have some insensitivity will survive. The emergence of a phenotype resistant to antimicrobial agents depends on various factors of a host: degree of resistance expression, the capability of a microorganism to tolerate resistance mechanism, initial colonization site, and other factors. When resistance determinants are on plasmids, they will spread quickly within the genus and even unrelated bacterial genera. When resistance is associated with genes on chromosomes, resistant microorganisms will spread more slowly (Vaičiuvėnas, 2005; Džidic *et al.*, 2008).

An important cause of the spread of antimicrobial resistance is a failure to apply infection control measures in a hospital and outside it. It has been established that methicillin-resistant *Staphylococcus aureus* (*S. aureus*, MRSA) in a hospital and MRSA in the community are often genetically related. Resistant bacteria are transmitted by aerosol transmission, especially during periods of viral upper respiratory infections, frequent hand-nose contacts, and poor hand washing among health care workers (Rice *et al.*, 2003). Antibiotic use in nonhuman niches is another important reason for the spread of resistant bacteria (Martínez and Baquero, 2002). It is known that the use of antimicrobial agents in animal food is related to bacterial resistance; for example, *Salmonella* and Campylobacter acquire resistance to antibiotics and transfer genes of

antibiotic resistance to natural human flora, for example, Enterococcus. High *E. coli* (*E. coli*) resistance to ciprofloxacin is associated with the use of fluoroquinolones in aviculture (Martínez and Baquero, 2002; Džidic *et al.*, 2008).

2.7.1 Untreatable bacterial infections as a rapidly emerging threat

At present, for most infections due to antibiotic-resistant bacteria, a relatively effective treatment is available, even if this treatment has disadvantages with regard to cost or convenience or adverse effects. However, strains of *E. coli* and *Klebsiella* species that produce a carbapenemase, and so cannot be effectively treated with meropenem or ertapenem, have been isolated from relatively small numbers of patients in New Zealand (Duff and Teacher, 2013) and there has been an alarming increase in their prevalence in hospitals overseas. These infections are often untreatable and cause high death rates (Palmore and Henderson, 2013). A similar problem has recently emerged in Russia where excessive antimicrobial consumption has led to resistance to all available antimicrobial agents in over 5% of strains of *P. aeruginosa* (*P. aeruginosa*) isolated from hospital inpatients (Edelstein *et al.*, 2013). If untreatable infections, due to *Escherichia coli, Klebsiella* species, *P. aeruginosa* or other completely drug-resistant organisms become common, this will have major consequences for the healthcare system (Levy, 1998). However, It seems very unlikely that new drugs were developed in time to effectively treat infections caused by the increasing numbers of highly resistant bacteria. Consequently, we must change our strategy from accepting the emergence of bacterial resistance as a minor inconvenience to doing all that is feasible to slow the emergence of resistance.

2.7.2 Antibiotic Resistance as a Growing Problem

The emergence of high rates of resistance, in a wide range of bacterial species, during recent decades, has had significant impacts on patients and on the healthcare system. Resistance to commonly used antimicrobial medicines, in a wide range of bacteria responsible for common diseases, is rapidly emerging as a major threat to health (Fauci and Marston, 2014). In New Zealand for example, during 2012, approximately 4,000 people suffered from infections due to strains of *E. coli* (*E. coli*) or *Klebsiella* species (*K. pneumoniae*) or other closely related organisms that produced an extended-spectrum beta-lactamase (ESBL) (Heffernan and Woodhouse, 2012).

The production of an ESBL by these bacteria confers resistance to almost all penicillin and all cephalosporins. Associated resistance genes in these ESBL producing bacteria frequently confer resistance to many other antimicrobials. The epidemic of ESBL-producing bacteria in New Zealand has grown rapidly in recent years (Fauci and Marston, 2014). Experience in other countries suggests that ESBL-producing bacteria may become much more common in the next few years. For example, between 2000 and 2011, the proportion of isolates of *E. coli* that were resistant to third-generation cephalosporins (an approximate surrogate for the production of an ESBL) increased from 0.1% to 5.7% in the Netherlands, and from 3.6% to 14.9% in Greece (EARS-Net, 2016). The dramatic increase in ESBL-producing *E. coli* and *Klebsiella* species is but one example of the growth in antimicrobial resistance in recent years. Resistance to mupirocin, the active component in Bactroban© ointment, was present in <5% *Staphylococcus aureus* strains isolated from patients in Auckland in 1992, but by 2000 had risen to >20% (Upton *et al.*, 2003).

For most of the approximately 4000 patients who suffered from an infection due to an ESBL-producing *E. coli* or *Klebsiella* species during 2012, the only reliable and safe treatment would have been a carbapenem antibiotic, such as meropenem or ertapenem. Because these antibiotics are not orally absorbed, patients with infections due to an ESBL-producing strain of *E. coli* or *Klebsiella* species commonly require intravenous therapy, usually administered within the hospital (Fauci and Marston, 2014).

2.7.3 Genetics of Antibiotic Resistance

Bacterial resistance to antibiotics can be intrinsic or innate, which is characteristic of a bacterium and depends on the biology of the microorganism (*E. coli* has an innate resistance to vancomycin) and acquired resistance (Vaičiuvėnas, 2005). Acquired resistance occurs from (i) acquisition of exogenous genes by plasmids (conjugation or transformation), transposons (conjugation), integrons and bacteriophages (transduction), (ii) mutation of cellular genes, and (iii) a combination of these mechanisms (Hawkey, 1998; Hawkey, 2008).

Mutations-Spontaneous Mutations: Chromosomal mutations are quite rare (one in a population of 106–108 microorganisms) and commonly determine resistance to structurally related compounds (Rice *et al.*, 2003). These mutations occur as errors of replication or an incorrect repair of damaged DNA. They are called spontaneous mutations or growth-dependent mutations. Resistance to quinolones in *E. coli* is caused by changes in at least seven amino acids in the *gyrA* gene or three amino acids in the *parC* gene (Hawkey, 1998; Levy, 1998; Džidic, 2008), whereas only a single point mutation in the *rpoB* gene is associated with a complete resistance to rifampin (Hawkey, 1998).

A chromosomal mutation in dihydropteroate synthetase results in a reduced affinity for sulphonamides (Hawkey, 1998). Some biochemical resistance mechanisms are the result of mutations. Antibiotic uptake or efflux system can be modified by mutations (Hooper, 2001). Hypermutators; according to the "hypermutable state" model, a small bacterial population during a prolonged nonlethal selection of microorganisms may achieve a short-term state when the population mutates at a very high rate (hypermutable strains or mutators) (Džidic, 2008).

Most hypermutators are found in populations of *Escherichia coli, S. enterica*, *Neisseria meningitidis* (*N. meningitidis*), *H. influenzae, S. aureus, Helicobacter pylori* (*H. pylori*), *Streptococcus* species (*S.* species), and *P. aeruginosa* (Džidic, 2008). Adaptive Mutagenesis: most mutations occur in dividing cells. However, they can also arise in nondividing or slowly dividing cells. Mutations occur only during a nonlethal selection of microorganisms and are called "adaptive mutations." This adaptive process is the only and main source of the antibiotic-resistant mutants to originate under normal conditions. Streptomycin causes a hypermutable phenotype in *Escherichia coli,* and some antibiotics (quinolones) can induce the SOS mutagenic response and increase the rate of emergence of resistance to antibiotics (Erill *et al.,* 2006; Džidic, 2008 and Guerin *et al.,* 2009).

Horizontal Gene Transfer: A transfer of resistance genes from one bacterium to another is called a horizontal gene transfer (Bennett, 2008). The main mechanisms of resistance gene transfer in a bacterium are plasmid transfer, transfer by viral delivery and transfer of free DNA. Genes can be transferred by three main ways: transduction (via bacteriophages and integrons), conjugation (via plasmids and conjugative transposons) and transformation (via incorporation of chromosomal DNA, plasmids into a chromosome. Then genes are incorporated into the recipient chromosome by

recombination or transposition and may have one or several changes in gene sequence (Džidic, 2008; Alekshun and Levy, 1998; Hawkey 2008).

Most plasmids are double-stranded circular DNA whose size may vary from 2–3 kb to plasmids, which encode up to 10% of the host cell chromosome. The transfer of resistance genes is more effective than chromosomal mutation (Alekshun and Levy, 1998). Plasmids encode genes that confer resistance to main classes of antimicrobial agents (cephalosporins, fluoroquinolones, and aminoglycosides) (Bennett, 2008), toxic heavy metals (mercury, cadmium, silver), and virulence determinants that help a cell to survive in the environment of lethal antibiotic doses (Mayer *et al.*, 1995; Hawkey, 2008). MDR genes are in a DNA sequence that is transferred from one plasmid to another or to the genomes, which are called transposons or "jumping gene systems" (Hawkey, 1998). Transposons can be integrated into plasmids or the host's chromosome, encompass small elements called insertion sequences (IS elements), transposons, and transposing bacteriophages.

They have terminal repeat sequences that play a role in recombination and recognize a protein (for example, transposase or recombinase) that is necessary to insert or remove a transposon from specific genome regions (Mayer *et al.*, 1995; Alekshun and Levy, 1998; Bennett, 2004). Transposons are transferred by conjugation, transformation or transduction (e.g., *mecA* gene is found in MRSA) and spread quicker than genes in chromosomes. Conjugative transposons have characteristic features of plasmids and can help to transfer endogenic plasmids from one microorganism to another (Raghunath, 1998; Hawkey, 2008; Tolmasky, 2000).

Bacterial integrons are gene capture systems that instead of transposition use a specific recombination mechanism (Bennett, 2004; Hawkey, 2008). Integron encodes three main

components in the 5´ conserved segments: an enzyme integrase (gene int) that serves as a specific recombination system to insert or to remove a new gene cassette, specific recombination site (*attI* site), and a promoter that starts gene transcription. Most integrons of class I in the 3´ conserved segments have an additional gene puII responsible for resistance to sulphonamides (Hooper, 2001; Roe and Pillai 2003; Daikos *et al.,* 2007).

2.7.4 Biochemical mechanisms of resistance

The main types of biochemical mechanisms that bacteria use for defence are as follows: decreased uptake, enzymatic modification and degradation, altered penicillin-binding proteins (PBPs), efflux, altered target, and its overproduction (Rice *et al.,* 2003; Wright, 2005, Chen *et al.,* 2011). Below will describe the main types of different biochemical mechanisms that are found in clinically important bacteria.

2.8 Antibiotic inactivation or modification

There are three main enzymes that inactivate antibiotics: β-lactamases, aminoglycoside-modifying enzymes, and chloramphenicol acetyltransferases (Mims *et al.,* 2004). Antibiotic Modification by Hydrolysis. β-Lactamases are broadly prevalent enzymes that are classified using two main classification systems: Ambler and Bush-Jacoby-Medeiros (Alekshun and Levy, 1998). It is known that there are about 300 different beta-lactamases. The most clinically important are produced by gram-negative bacteria (Wickens and Wade, 2005) and are coded on chromosomes and plasmids. Genes that encode β-lactamases are transferred by transposons but also, they may be found in the composition of integrons (Jacoby and Munoz-Price, 2005).

β-Lactamases hydrolyse nearly all β-lactams that have ester and amide bond, e.g. penicillins, cephalosporins, monobactams, and carbapenems. Serine β-lactamases – cephalosporinases, e.g. *AmpC* enzyme – are found in Enterobacter spp. and *P. aeruginosa* and penicillinases in S. aureus (Bush *et al.,* 1995; Alekshun and Levy, 1998; Lambert, 2002; Garau *et al.*, 2004; Thomson and Bonomo, 2005). Metallo-β-lactamases (MBLs) found in *P. aeruginosa,* K. pneumoniae, *Escherichia coli, Proteus mirabilis* (*P. mirabilis*), Enterobacter species have the same role as serine β-lactamases and are responsible for resistance to imipenem, new-generation cephalosporins and penicillins. MBLs are resistant to inhibitors of β-lactamases but sensitive to aztreonam (Bush *et al.,* 1995; Alekshun and Levy, 1998; Lambert, 2002; Garau *et al.*, 2004, Thomson and Bonomo, 2005; Vatopoulos *et al.,* 2008).

Specific *A. baumannii* carbapenem-hydrolyzing oxacillinase (OXA) enzymes that have low catalytic efficiency together with porin deletion and other antibiotic resistance mechanisms can cause high resistance to carbapenems (Jacoby and Munoz-Price, 2005). The resistance of *Klebsiella* species carbapenemases (KPC-1) to imipenem, meropenem, amoxicillin/clavulanate, piperacillin/tazobactam, ceftazidime, aztreonam, and ceftriaxone is associated with the nonconjugative plasmid coded bla gene (Babic *et al.,* 2006).

Extended-spectrum β-lactamases (ESBL) – T*EM, SHV, OXA, PER, VEB-1, BES-1, GES, IBC, SFO* and *CTX* – mainly are encoded in large plasmids. They can be transferred in connection of two plasmids or by transposon insertion. ESBL are resistant to penicillins (except temocillin), third-generation oxyimino-cephalosporins (e.g., ceftazidime, cefotaxime, ceftriaxone), aztreonam, cefamandole, cefoperazone, but they are sensitive to methoxy-cephalosporins, e.g., cephamycins and carbapenems, and can be inhibited by

inhibitors of β-lactamases, e.g., clavulanic acid, sulbactam, or tazobactam (Livermore, 1995; Jacoby and Munoz-Price, 2005; Bonnet, 2004; Paterson and Bonomo, 2005).

Strains producing ESBL are commonly resistant to quinolones, but their resistance depends not on multiple resistance plasmids but on mutations in *gyrA* and parC genes (Vitkauskienė *et al.,* 2006). Such strains are found among *Escherichia coli, K. pneumoniae,* and *P. mirabilis* (Džidic *et al.,* 2008). The number of known ESBLs reaches 200 (Paterson and Bonomo, 2005; Vitkauskienė *et al.,* 2006; Govinden *et al.,* 2007).

a) Target Modification

An interaction between an antibiotic and a target molecule is very specific so even small changees in a target molecule can influence antibiotic binding to a target. Sometimes, in the presence of a modification in a target, other changes in the cell are needed to compensate an altered target (Hartman and Tomasz, 1986; Džidic *et al.,* 2008).

i) Peptidoglycan Structure Alteration

Inhibition of cell wall synthesis is performed by β-lactams, e.g., penicillins, cephalosporins, carbapenems, monobactams, and glycopeptides, e.g., vancomycin and teicoplanin. The presence of a mutation in PBPs leads to a reduced affinity to β-lactam antibiotics. It results in resistance of *E. faecium* to ampicillin and S. pneumoniae to penicillin. *S. aureus* resistance to methicillin and oxacillin is associated with the integration of a mobile genetic element – "staphylococcal cassette chromosome mec" (*SCCmec*) – into the chromosome of *S. aureus* that contains resistance gene mecA. *mecA* gene encodes PBP2a protein, a new penicillin-binding protein, that is required to change a native staphylococcal PBP (Alekshun and Levy, 1998; Džidic *et al.,* 2008). PBP2a

shows high resistance to β-lactam antibiotics (they do not bind to β-lactams) and ensures cell wall synthesis at lethal β-lactam concentrations (Hawkey, 1998; Lencastre *et al.*, 2007). S. aureus strains resistant to methicillin can be cross-resistant to all β-lactam antibiotics, streptomycin, and tetracycline and in some cases to erythromycin (Grudmann *et al.*, 2006).

b) Protein Synthesis Interference

Antibiotics (aminoglycosides, tetracyclines, macrolides, chloramphenicol, fusidic acid, mupirocin, streptogramin, and oxazolidinones) can interfere with protein synthesis at its different stages; for example, during transcription via RNA polymerase, rifamycins modify a specific target (Walsh, 2000). Aminoglycosides (gentamicin, tobramycin, amikacin) bind to the 30S ribosomal subunit (Lambert, 2002) while chloramphenicol binds to the 50S ribosomal subunit and suppresses protein synthesis (Tenover, 2006). Macrolides, lincosamides, and streptogramin B (MLS antibiotics) block protein synthesis in Gram-negative bacteria by binding to the 50S ribosomal subunit. Then the 50S subunit undergoes a posttranscriptional modification (methylation). RNA methyltransferase involves RNA that is close to or in the binding place of antibiotics. Mutations in 23S rRNA, the same as nonmethylated rRNA, are associated with resistance to MLS (Džidic *et al.*, 2008). Nonmethylated 23S rRNA and 16S rRNA at U2584 position in *Haloarcula marismortui* cause resistance to kasugamycin and sparsomycin. A nonreactive rluC gene is responsible for resistance to clindamycin, linezolid, and tiamulin. Oxazolidinones interfere with proteins synthesis at several stages:

(i) inhibit protein synthesis by binding to 23S rRNA of the 50_S subunit and

(ii) suppress 70S inhibition and interaction with peptidyl-tRNA (Alekshun and Levy, 1998; Mims *et al.*, 2004).

c) DNA Synthesis Interference

This mechanism of resistance is a modification of two enzymes: DNA gyrase (also known as topoisomerase II) (genes *gyrA* and *gyrB*) (Kim *et al.*, 2002) and topoisomerase IV (*parC* and *parE*). Mutations in genes *gyrA* and *parC* are followed by replication failure, and then quinolones/ fluoroquinolones cannot bind. The most common mutation in *E. coli* gyrA causes a reduced drug affinity for modified-DNA complex, and MIC is higher (Alekshun and Levy, 1998; Rice *et al.*, 2003, Martinez-Martinez *et al.*, 2003; Vester and Long, 2009). Quinolones (ciprofloxacin) bind to DNA gyrase A subunit (Garau *et al.*, 2004). Usually, resistance to quinolones is associated with mutations in chromosomes, but plasmid-mediated (Bush, 1996; Martinez-Martinez *et al.*, 2003; Wang *et al.*, 2004) and point mutation-related (in genes *gyrA* and *parC*) resistance to quinolones (Anderson, 2005) were reported as well.

d) Efflux Pumps and Outer Membrane Permeability

Membrane proteins that export antibiotics from the cell and maintain their low intracellular concentrations are called efflux pumps. Reduced outer membrane (OM) permeability results in reduced uptake of antibiotics (Džidic *et al.*, 2008).

i) Efflux Pumps: In analysing resistance to antibiotics, identification and characterization of efflux pumps are one of the most difficult problems. Single component efflux systems transfer their substrates across the cytoplasmic membrane. Multicomponent pumps found in gram-negative bacteria and together with a periplasmic membrane synthesis protein (MFP) component and an OM protein (OMP) component transfer substrates across the cell envelope (Hawkey, 1998; Walsh, 2000; Džidic *et al.*, 2008). Antibiotics of all classes except polymyxins are susceptible to the activation of efflux systems (Lambert, 2002).

Efflux pumps can be specific to antibiotics, most of them are multidrug transporters that are capable of pumping a wide range of unrelated antibiotics – macrolides, tetracyclines, fluoroquinolones – and thus significantly contribute to MDR (Džidic *et al.,* 2008). Bacteria resistant to tetracyclines often produce increased amounts of membrane proteins that are used as export or efflux pumps of antimicrobial drugs (Speer *et al.,* 1992). To eliminate toxic compounds from the cytoplasm and periplasm, *P. aeruginosa* uses more than four powerful MDR efflux pumps (MEX) (Schweizer, 2003; Siegel, 2008; Strateva and Yordanov, 2009). *MexV-MexW-OprM* MDR efflux pumps are responsible for resistance to fluoroquinolones, tetracyclines, chloramphenicol, erythromycin, ethidium bromide, and acriflavine (Strateva and Yordanov, 2009). Increased expression of *MexAB-OprM* efflux pumps results in higher inhibitory concentration against penicillins, broad-spectrum cephalosporins, chloramphenicol, fluoroquinolones, macrolides, novobiocin, sulfonamides, tetracycline, trimethoprim, dyes and detergents (Livermore, 2002; Thomson and Bonomo, 2005).

ii) Changes in Outer Membrane Permeability (OMP)

The outer membrane (OM) in Gram-negative bacteria contains an inner layer that has phospholipids and an outer layer that has the lipid A. Such OM composition reduces drug uptake to a cell and transfer through the OM (through porin proteins, e.g., *OmpF* in *E. coli* and *OprD* in *P. aeruginosa*). Drug molecules to a cell can be transferred by the following mechanisms:

(i) diffusion through porins,

(ii) diffusion through the bilayer, and

(iii) by self-promoted uptake.

This type of entry depends on the chemical composition of a drug molecule (Džidic *et al.,* 2008). Acquired resistance to all antibiotic classes in *P. aeruginosa* is due to low OM permeability. Small hydrophilic molecules (*β*-lactams and quinolones) can cross the OM only through porins. Aminoglycosides and colistin cannot be transferred to the cell through porins; therefore, self-promoted uptake to the cell is initiated by binding to lipopolysaccharides of the outer side of the OM (Lambert, 2002). Acquired resistance is characteristic of high resistance to almost all aminoglycosides (especially to tobramycin, netilmicin, and gentamicin) (Ferguson and Cahill, 2007).

iii) Bypass of Antibiotic Inhibition

The fourth mechanism of bacterial resistance to antibiotics is specific. Bacteria produce an alternative target (usually an enzyme) that is resistant to inhibition by the antibiotic (for example, MRSA produces an alternative PBP). At the same time, bacteria produce a native target too, which is sensitive to antibiotics. An alternative targ*et al*lows bacteria to survive by adopting the role of a native protein. Resistance to trimethoprim and sulphonamides is caused by reduced sensitivity and affinity of altered enzymes dihydropteroate synthetase (DHPS) and dihydropteroate reductase (DHFR) to trimethoprim and sulphonamides (Jacoby and Munoz-Price, 2005).

2.9 High-levels of Antibiotics Consumption, Prime cause of High Rates of Antibiotic-Resistant Bacteria

The emergence and proliferation of bacteria resistant to an antibacterial are directly related to the amount of that antibacterial in the organism's environment. As a result, the prevalence of strains of bacteria resistant to an antimicrobial class increases more rapidly in those countries where large amounts of antimicrobials within that class are consumed (Fauci and Marston, 2014). For example, strains of S. pneumoniae with reduced

susceptibility to penicillins are common in Spain and France, where large amounts of penicillins are consumed (EARS-Net, 2016, ESAC, 2015). In contrast, strains of S. pneumoniae with reduced susceptibility to penicillins are unusual in Germany and the Netherlands, where lower amounts of penicillins are consumed. Similarly strains of *E. coli* resistant to ciprofloxacin are common in Spain and Italy, where large amounts of fluoroquinolones are consumed (EARS-Net, 2016, ESAC, 2015) In contrast, strains of *E. coli* resistant to fluoroquinolones are less common in Norway, Sweden and Finland, where lower amounts of fluoroquinolones are consumed (EARS-Net, 2016, ESAC, 2015).

2.10 Risk Involved in Detecting Pathogenic Organisms from Abattoirs

Food-borne pathogens have been extensively incriminated worldwide as common causes of bacterial infections in humans with food animals serving as important reservoirs (Akoachere *et al.,* 2009;). Food contamination with antibiotic-resistant bacteria can, therefore, be a major threat to public health, as the antibiotic resistance determinants can be transferred to other bacteria of human clinical significance (Van *et al.*, 2007; Adesiji *et al.,* 2011). In industrialized countries, microbiological food-borne illnesses were reported in up to 30% of the population (WHO, 2011).

Most of the morbidity and mortality related to foodborne infections are caused by bacterial agents (Buzby and Roberts, 2009; CDC, 2011). Food poisoning is commonly manifested as diarrheal diseases which are often triggered either by toxin production by the microbe or by the host's reaction to the infection (CDC, 2011). A few pathogenic bacteria have been associated with food of animal origin; these include amongst others *Salmonella* spp., *Campylobacter* spp., *Staphylococcus aureus, Listeria monocytogenes, Clostridium perfringens, Clostridium botulinum*, *E. coli* 0157: H7, and

enterohemorrhagic *E. coli* (EHEC) (Akoachere *et al.,* 2009; Olaoye, 2011; Nyenje *et al.,* 2012).

E. coli occur as normal flora in the gastrointestinal tract of humans and animals. However, pathogenic *E. coli* strains have been reported to cause life-threatening infections in humans worldwide (Bonardi *et al.,* 2003; Ateba and Mbewe, 2011). Antibiotic resistance remains a major challenge in human and animal health. Resistance is increasingly being recognized in pathogens isolated from food (Arlet *et al.,* 2006).

Furthermore, the transfer of these resistant bacteria to humans has significant public health implications by increasing the number of food-borne illnesses and the potential for treatment failure (Adesiji *et al.,* 2011). Food of animal origin could be contaminated from the farm, a situation which may be further compounded if the food is not properly handled during slaughtering and processing giving way for pathogens to multiply (Ghosh *et al.,* 2007). The conditions under which these foods are handled raise questions regarding their microbiological quality. Studies conducted in different countries to investigate the microbiological quality of food of animal origin reported the presence of potential human pathogens (Davies *et al.,* 2004; Akoachere *et al.,* 2009).

In most countries in the world, a large proportion of the population relies on beef and pork as their source of protein which could expose them to infection if contaminated (Movassagh *et al.,* 2010; Ateba and Mbewe, 2011). Foods contaminated with enteropathogenic bacteria are an important factor contributing to the high incidence of diarrhoea in developing countries (EI-Rami *et al.,* 2012). Pathogenic *Escherichia coli,* nontyphoid *Salmonella* serovars, and S. aureus remain a potential threat to human health with beef, broiler chickens, and pork serving as possible sources of these organisms in the environment (Bonardi *et al.,* 2003; Davies *et al.,* 2004; Ateba and Mbewe, 2011).

The clinical significance of these pathogens cannot be overemphasized. Pathogenic *E. coli* is recognized as an important pathogen in outbreaks of acute diarrhoea especially in developing countries (CDC, 2011). In 2015, a study by Nicoline *et al.* was conducted to investigate the prevalence and antibiogram of these pathogens in a bid to provide baseline data for epidemiological surveillance. In all, *E. coli* 104/154 (67.5%) they isolated was the most detected pathogen followed by S. aureus 50/154 (32.5%). Their findings corroborate those of other studies that equally reported a high prevalence of either *Escherichia coli,* S. aureus, or both (Adesiji *et al.,* 2011; Ghosh *et al.,* 2007; Manguiat and Fang, 2013). Several studies have reported the presence of *E. coli* 0157: H7 in beef and pork carcasses (Bonardi *et al.,* 2003; Ateba and Mbewe, 2011; Zarei *et al.,* 2013).

2.11 Programs that Encourage and Reduced Antimicrobial Consumption

Various measures to reduce antimicrobial consumption, and thus slow the increase in the prevalence of resistance, have been used overseas. In 2002, France conducted a nationwide campaign *Antibiotics are not automatic* that over the subsequent 5 years resulted in a greater than 25% per capita reduction in antibiotic prescriptions (Sabuncu *et al.,* 2009). In 2010, the Swedish Government set a target to reduce total antimicrobial consumption by 36% over 4 years, from 39 antibiotic prescriptions per 100 population per year to less than 25 antibiotic prescriptions per 100 population per year (SICDC, 2009). Systematic reviews suggest that a variety of educational strategies targeted at the prescribing doctor and/or their patients can result in an average of 25% reduction in the proportion of patients who antibiotics are prescribed (Ranji *et al.,* 2008).

Patients commonly appreciate the dramatic benefits of antibiotic treatment of serious bacterial infections, but commonly are unaware of the potential harms of antimicrobial consumption (Fauci and Marston, 2014). As a result, patients may unrealistically

exaggerate the benefits and neglect the disadvantages of antibiotic treatment for a wide range of relatively minor illnesses (Fauci and Marston, 2014). One disadvantage of an unnecessary antimicrobial treatment that patients may neglect is the increased risk that future infections were due to an antimicrobial resistant bacterium. Antibiotic treatment significantly increases the risk that subsequent infection is due to an antibiotic-resistant bacterium. A meta-analysis of four studies has shown that antibiotic treatment for a urinary tract infection results in a 2.5 times greater risk due to an antibiotic resistant *E. coli* (Fauci and Marston, 2014).

2.12 Antimicrobial Resistance from Farms Animals

The ongoing increase in antimicrobial resistance (AMR) in enteric bacteria in production animals and their potential transmission to humans represent a major threat to public health (Huttner *et al.,* 2013). Commensal enteric bacteria such as *E. coli* (*E. coli*), which reside for prolonged periods in the intestinal tract, potentially represent an important reservoir of AMR in the food chain, and moreover, they make good representative markers for investigating dynamic changes in AMR genes (Kaspar, 2006). Even though the source of AMR has not always been identified using molecular epidemiological analysis or DNA-based data, bacteria from livestock are believed to be a major source of AMR in the environment, and resistant bacteria and resistance genes may be acquired by the human gut microbiome (Lhermie *et al.,* 2016).

2.13 Public Health risk of ESBL Producing *Enterobacteriaceae* of Animal Origin

The widespread use of extended-spectrum cephalosporins creates a reservoir of resistant bacteria. Moreover, multi-resistance frequently associated with strains carrying ESBLs, which could dramatically reduce the treatment options. Resistance against β- lactams is increasingly being reported and is on the rise in *Enterobacteriaceae* from both humans

and animals. Resistance may be transferred directly from animal to human and may possibly be acquired indirectly, through the transfer of resistance genes from bacteria of animal origin to bacteria infecting humans (Urumova, 2015).

Bacterial resistance to β-lactams can be due to a few mechanisms. One of the mechanisms consists of mutations in genes encoding PBPS, the acquisition of alternative PBPS. These altered PBPS have a reduced affinity for β-lactams. Another mechanism consists of a change in the permeability of the cell wall. This may be due to alterations in the expression of porins or active efflux. The hydrolysis of beta-lactam antibiotics by beta-lactamase enzymes is the most encountered essential mechanism of resistance exhibited by clinically relevant Gram-negative bacteria (Urumova, 2015). Serine active-site enzymes possess high conformation flexibility with respect to substrates. Zn^{++} metalloproteins use histidine- or cysteine-bound divalent ions for acting on the carboxyl group (Gniadkowski *et al.*, 2001; Chaudary *et al.*, 2004).

Beta-lactamases are produced by various Gram-positive and Gram-negative microbial species. Some of them are exocellular, i.e. are secreted out of the microbial cell (staphylococcal penicillinases) whereas others, especially in Gram-negative bacteria, are located in the periplasmic space and are available only after cell wall breakdown. In a number of bacterial species, they are inducible, while in others they are constitutively synthesized. Enzyme inactivation is of special interest due to its clinical significance, ecological aspects and evolutionary development (Ambler, 1980).

Genes determining beta-lactamase production are most commonly extra-chromosomal – on plasmids but could be also found within the bacterial chromosome. Chromosomal-mediated inducible beta-lactamases in some representatives of family *Enterobacteriaceae* and genus *Pseudomonas* are of special concern and major clinical

significance, as are the plasmid-encoded broad-spectrum beta-lactamases of *Klebsiella* genus and other Gram-negative bacteria, conferring resistance to novel cephalosporins (Urumova, 2015). It is acknowledged that plasmids play an essential role in the transmission of bacterial resistance and epidemic outbreaks caused by resistant bacterial species in both men and animals (Samaha-Kfour and Araj, 2003). Since 2009, the number of unique protein sequences associated with beta-lactamases has exceeded 890 (Bush and Jacoby, 2010).

The intricacy of therapeutic approaches using third- and fourth-generation cephalosporins in infections caused by *Enterobacteriaceae* could also be attributed to the multi-resistance of strains. In some bacteria producing extended-spectrum beta-lactamases (ESBL), such as *AmpC* beta-lactamase producers, the presence of plasmids could determine the co-transfer of resistance to aminoglycosides. Unfortunate, the alternative therapy including the application of carbapenems in ESBL producers during the last years is also becoming inefficient due to the increasing spread of carbapenem-resistant Stenotrophomonas spp., *Pseudomonas* spp (Urumova, 2015). strains. Another important epidemiological issue of ESBL producing bacteria spread is the existence of similar ESBL phenotypes in human and animal isolates, with the significance of representatives of resident intestinal microflora in animals. Epidemiology of ESBL-producing bacteria is complex with resources between hospitals and the community. *E. coli* producing *CTX-M* – beta-lactamases are mainly the community ESBL producers which differ from the *Klebsiella* spp. producing *TEM*- and *SHV* types of ESBL (Pitout 2010 and Urumova, 2015).

2.19 Beta-Lactam Antibiotics Structural Classification, Mechanism of Action and use in Veterinary Practice

Beta-lactams include several groups of compounds with a β-lactam ring. According to their structure, they could be classified as follows, penams, carbapenems, oxapenams or clavams, carbapenems, penems, cephems (cephalosporins), carbacephems, oxacephems (moxalactam), monobactams (aztreonam) (Fernades *et al.,* 2015).

The similarity in the mode of action of beta-lactams is their ability to inhibit peptidoglycan complex of bacterial cell wall via action on penicillin-binding proteins (PBPs). PBPs are transpeptidases and carboxypeptidases involved in the last stage of peptidoglycan synthesis. The effect of beta-lactams on them consists of cell integrity loss. Interpeptide bonds are formed under the influence of specialised acetyltransferases, which are immobilised by penicillins (Fernades *et al.,* 2015). Penicillins impede the regulation of cell autolysis control, resulting in autolysis of bacterial cell. Data about the use of beta-lactam antibiotics in animals from numerous monitoring programmes carried in different parts of the world (NARMS, 2012) as well as facts reported by research teams (Li *et al.,* 2005; Mcewan and Fedorka-Cray, 2006; Lioyd, 2007; Guardabassi *et al.,* 2008; Hammerum and Heuer, 2009) provide a detailed information on their leading role amongst antimicrobial drugs used in veterinary medical practice.

In veterinary practice, penicillins, biosynthetic and semi-synthetic derivatives of the 6-amino penicillanic acid, first- to four-generation cephalosporins and some members of beta-lactamase inhibitors group are of clinical significance (Schwarz *et al.,* 2001; Timmerman *et al.,* 2009).

2.14.1 Classification of Beta-Lactamases

The first beta-lactamases have been discovered in soil bacteria, natural producers of penicillins several years before their introduction in clinical practice (Livermore and Woodford, 2000). The first plasmid-determined beta-lactamase detected in Gram-negative bacteria is TEM-1. The nomenclature is associated with the name of a patient - Temoniera, from whose blood culture was isolated the *E. coli* producing strain (Sturenburg *et al.,* 2003). SHV-1 is another type of plasmid-determined beta-lactamase in *E. coli* and *Klebsiella pneumoniae.* Later, several different enzyme classes were established – penicillinases, cephalosporinases, carbapenemases etc. They are distinguished by their physicochemical properties, hydrolytic activity, genetic determination etc. but all are capable to hydrolyse the beta-lactam ring of the antibiotic and hence, to inactivate it (Sturenburg *et al.,* 2003).

Novel beta-lactamases, these are the extended-spectrum beta-lactamases (ESBL), are active against third and fourth-generation cephalosporins as well as against carbapenems and monobactams. First facts about them date back to the early 1980s (Philippon *et al.,* 2002). For instance, in Germany, a clinical isolate with an ESBL profile was first described in 1983 (Kliebe *et al.,* 1985).

ESBL possess a broad range of point mutations altering the active sites configuration of original beta-lactamases such as *TEM-1, TEM-2, SHV-1*. ESBL-producing bacteria are often multi-drug resistant as the genes determining the resistant to other classes of chemotherapeutics are located in the same plasmids where ESBL genes are situated. As mentioned above with respect to this statement, some of the ESBL-producing bacteria are resistant to aminoglycosides, 4-quinolones etc. Extended-spectrum beta-lactamases are active against most beta-lactam antibiotics, including oxyimino-beta-lactams,

ceftazidime, ceftiofur, aztreonam, could be inactivated by clavulanic acid, but some of them are not active against cephamycins, cefoxitin and carbapenems. Their extended spectrum could be regarded as a consequence of the increased sensitivity of ESBL to beta-lactamase inhibitors (Bradford, 2001).

According to Jacoby *et al.* (2004), ESBL is detected with especially high frequency in *Klebsiella* spp. and *E. coli* resistant to cefotaxime, ceftazidime, ceftriaxone, aztreonam and other oxyimino beta-lactams (Jacoby *et al.*, 2004). One of the most commonly encountered ESBL profiles are associated with point mutations, in which lysine substitutes glutamine, serine substitutes arginine and lysine – glycine (Bradford, 2001). Amino acid substitutions with respect to ESBL result in altered active site configuration which permits the access to oxyimino beta-lactams and expands the spectrum of beta-lactam antibiotics, which could be hydrolyzed (Jacoby and Minoz-Price, 2005). As a result of enzymatic active site modification and the variety of beta-lactam substrates prone to hydrolysis, increased the sensitivity of isolates to beta-lactam inhibitors, clavulanic acid, sulbactam and tazobactam is observed (Jacoby and Minoz-Price, 2005).

a) *SHV* and *TEM* ESBLs

Before 2000 *SHV* and *TEM* types of ESBLs were the predominant variants found in *Klebsiella* and *E. coli* that caused nosocomial infections. These ESBLs developed by mutations of the broad spectrum *TEM*-1 and *SHV*-1 and 2 genes that were transferred between bacteria by plasmids, which were in turn spread by clonal distribution between hospitals and countries through patient mobility (Baraniak *et al.*, 2005; Damjanova *et al.*, 2007; Jacoby and Munoz-Price, 2005; Paterson and Bonomo, 2005).

b) Beta-Lactamases from the *TEM* Class

They are among the beta-lactamases most frequently produced by Gram-negative bacteria. According to Livermore (1995), almost 90% of ampicillin-resistant *E. coli* strains produce *TEM*- 1 (Livermore, 1995). This enzyme is capable to hydrolyse penicillins as well as first-generation cephalosporins. Its first derivative is *TEM*-2, and the difference between both enzymes is one substituted amino acid resulting in an altered isoelectric point of the enzyme, but not in a change in its substrate profile (Bradford, 2001). *TEM*-3, however, is the first representative of the *TEM*-type belonging to ESBLs, reported in 1989 (Sougakoff *et al.*, 1988). *TEM*-52 (Spain, Portugal, Belgium, Netherlands, Ireland, Denmark and Germany); *TEM*-20 (The Netherlands, Ireland and Germany), *TEM*-106 (Belgium) and *TEM*-126 (France).

According to these results, *CTX-M*-1 appears to be well disseminated in food-producing animals and food in most of the European countries for which reports exist. Amino acid substitutions of *TEM* enzymes are observed in a limited number of positions. The combinations of substitutions result in a number of complex changes in the ESBLs phenotype – substrate profile, isoelectric point etc. (Bradford, 2001). According to Bush and Jacoby (2010), the described diversity of about 150 *TEM* enzymes allows for investigation of the prevalence if individual genetic determinants conferring resistance to beta-lactams (Bush, 2008). It is therefore clear that *TEM*-enzymatic profile is transformed into ESBLs profile – for instance after replacement of arginine at position 163 with histidine and serine, or glutamine at position 238 with serine and alanine, glycine at position 240 with lysine.

c) *SHV* – Beta-Lactamases Class

The first reported member of this group is *SHV*-1 (Bush, 2008). It is chromosomally determined and detected in a number of *Klebsiella* species. In *E. coli* bacteria, it is plasmid-determined (Hammerum and Heuer, 2009). Derivatives of *SHV*-1 are *SHV* extended-spectrum beta-lactamases, in which glycine at position 238 is replaced by serine. According to Al-Jasser (2006), *SHV*-type ESBL is the most encountered among clinical isolates (Al-Jasser, 2006). At present, more than 114 *SHV* enzymes are described (Jacoby and Bush, 2008); they are also the most frequently seen in *Klebsiella* species (Hopkins *et al.,* 2006).

These class were frequently detected throughout the EU, especially *SHV*-12 and *SHV*-2 (Blanc *et al.,* 2006; Bortolaia *et al.,* 2010b; Boyle *et al.,* 2010; Brinas *et al.,* 2003a; Brinas *et al.,* 2003b; Chiaretto *et al.,* 2008; Cortes *et al.,* 2010; Costa *et al.,* 2009; Dierikx *et al.,* 2010a; Escudero *et al.,* 2010; Goncalves *et al.,* 2010; Hasman *et al.,* 2005; Hopkins *et al.,* 2006; Kolar *et al.,* 2010; Machado *et al.,* 2008; Madec *et al.,* 2008; Morris *et al.,* 2009; Riano *et al.,* 2006). In relation to the ESBLs of the *TEM* class, the most frequently detected throughout the EU was *TEM*-52 (Blanc *et al.,* 2006; Brinas *et al.,* 2005; Carneiro *et al.,* 2010; Cloeckaert *et al.,* 2007; Costa *et al.,* 2009; Dierikx *et al.,* 2010a; Jensen *et al.,* 2006; Machado *et al.,* 2008; Morris *et al.,* 2009; Randall *et al.,* 2011; Rodriguez *et al.,* 2009; Smet *et al.,* 2008). To a lesser extend *TEM*-20 was detected in countries like Netherlands, Ireland and Germany (Boyle *et al.,* 2010; Dierikx *et al.,* 2010a; Hasman *et al.,* 2005; Hopkins *et al.,* 2006; Rodriguez *et al.,* 2009).

Bradford, (2001) affirms that they determine about 20% of plasmid-mediated resistance to ampicillin in this microbial species (Bradford, 2001). The presence of serine at position 238 is critical for the efficient hydrolysis of ceftazidime while lysine at position

240 – for the efficient hydrolysis of cefotaxime. One *SHV*-10 variant possesses inhibitor-resistant phenotype profile. This enzyme is a derivative of *SHV*-5 as it has an additional serine substitution for glycine at position 130. An interesting fact is that inhibitor-resistant phenotype profile conferred to the presence of serine-140/glycine mutation makes ineffective the strict ESBL profile if enzymes with glycine-238/serine and glutamate-240-lysine substitutions. Apart from *Klebsiella* species, *SHV* enzymes are also observed in other microbial species –*Citrobacter diversus, Escherichia coli, P. aeruginosa* (Bradford, 2001) *CTX-M* family beta-lactamases: These are the most commonly encountered enzymes with ESBL profile according to Jacoby and Minoz-Price, (2005) (Heritage *et al.*, 1999). The investigation of Canton and Coque, (2006) demonstrates that at the beginning of the new millennium, *CTX-M* cefotaximases are the most prevalent ESBL in Europe and South America (Canton and Coque, 2006). They exhibit a 40% identity with *TEM* and *SHV* enzymes (Bradford, 2001).

d) *CTX-M* ESBLs Class

Since the early 2000s the *CTX-M* group of genes, named after their ability to produce enzymes capable of hydrolysing cefotaxime, emerged in human isolates. These genes were also located on highly transmissible plasmids, thus facilitating fast and efficient spread of resistance (Bonnet, 2004; Canton and Coque, 2006; Canton *et al.*, 2008; Hunter *et al.*, 2010; Pitout *et al.*, 2005a). Bacteria that express *CTX-M* enzymes are also commonly co-resistant or multi-drug resistant, exhibiting resistance to multiple antimicrobials including fluoroquinolones (Jacoby *et al.*, 2006). In the last decade, the epidemiology of ESBLs in humans has changed. Successful international bacterial clones harbouring members of the *CTX-M* family have emerged and spread globally. As a result, the *CTX-M*-ß-lactamases have become the most prevalent ESBLs in human

Enterobacteriaceae worldwide (Livermore *et al.,* 2007; Pitout *et al.,* 2005; Pitout *et al.,* 2005). The epidemiology of bacteria that produce *CTX-M* enzymes has also changed. Since the early 2000s, *E. coli* producing *CTX-M* enzymes (specifically *CTX-M*-15) have increasingly been found in the community in uncomplicated and complicated (including bacteraemias) community-acquired urinary tract infections, as well as in serious intra-abdominal and skin and soft-tissue infections (Canton *et al.,* 2008; Livermore *et al.,* 2007; Peirano *et al.,* 2010; Pitout and Laupland, 2008; Rodriguez-Bano *et al.,* 2008; Rodriguez-Bano *et al.,* 2004; Rodriguez-Bano *et al.,* 2006; Woodford *et al.,* 2004).

CTX-M-1 (Spain, Portugal, France, Belgium, Denmark, Italy, Netherlands, UK, Germany and Czech Republic), *CTX-M*-2 (Belgium, Denmark, Netherlands, UK, Ireland), *CTX-M*-9 (Spain, France, Denmark, UK), *CTX-M*-14 (Spain, Portugal, France, Belgium, Denmark and UK), *CTX-M*-15 (France, Belgium, UK, Germany, Denmark), *CTX-M*-32 (Spain, Portugal, Italy and Greece), and other *CTX-M*- variants (*CTX-M*-53 in France, *CTX-M*-3, -8, -17/18, -20 in UK); ii) *SHV* class: *SHV*-2 (Spain, Portugal, and Netherlands), *SHV*-5 (Spain), and *SHV*-12 (Spain, France, Italy, Netherlands, Denmark, Ireland and Czech Republic).

On the other hand, *CTX-M*-14 and *CTX-M*-32 appear more associated with food-producing animals or food in the Mediterranean and Southern European countries (Blanc *et al.,* 2006; Bortolaia *et al.,* 2010a; Bortolaia *et al.,* 2010b; Brinas *et al.,* 2005; Brinas *et al.,* 2003b; Carneiro *et al.,* 2010; Costa *et al.,* 2009; Madec *et al.,* 2008; Politi *et al.,* 2005), although *CTX-M*-14 was also found in food-producing animals in UK and Belgium (Hopkins *et al.,* 2006; Hunter *et al.,* 2010; Smet *et al.,* 2008; Warren *et al.,* 2008). *CTX-M*-2 was observed in Central and Northern European countries, including UK and Ireland (Bertrand *et al.,* 2006; Bortolaia *et al.,* 2010a; Boyle *et al.,* 2010;

Cloeckaert *et al.,* 2007; Dhanji *et al.,* 2010; Dierikx *et al.,* 2010a; Hasman *et al.,* 2005; Hopkins *et al.,* 2006; Liebana *et al.,* 2006; Morris *et al.,* 2009; Randall *et al.,* 2011; Smet *et al.,* 2008; Warren *et al.,* 2008). *CTX-M*-15, the ESBL considered to have spread in a pandemic fashion in humans, was only detected incidentally in food-producing animals or food (Liebana *et al.,* 2004; Madec *et al.,* 2008; Rodriguez *et al.,* 2009; Smet *et al.,* 2008 and Randall *et al.,* 2011).

The ESBLs detected in food-producing animals or food in other non-European countries were: China (*CTX-M*-3, -13, -14, -15, -22, -24, -55, -64, -65; SHV-2, -12; TEM-52), Japan (*CTX-M*-2 and -14), Korea (*CTX-M*-15), USA (*CTX-M*-1, -29, *SHV*-12), Tunisia (*CTX-M*-1, *CTX-M*-8, *CTX-M*-14; *SHV*-5; *TEM*-20) and Senegal (*SHV*-12) (Ben Slama *et al.,* 2010; Cardinale *et al.,* 2001; Doi *et al.,* 2009; Duan *et al.,* 2006; Jouini *et al.,* 2007; Kojima *et al.,* 2005; Li *et al.,* 2010; Li *et al.,* 2010b; Lim *et al.,* 2009; Matsumoto *et al.,* 2007; Rayamajhi *et al.,* 2008; Shiraki *et al.,* 2004; Tian *et al.,* 2008; Wittum *et al.,* 2010). These data show that mainly in Asian countries a higher diversity of *CTX-M* variants was observed. The presence of *CTX-M*-15 in *E. coli* of healthy and sick chicken and pigs in China and Korea may reflect different epidemiology of ESBLs in these countries (Li *et al.,* 2010a; Li *et al.,* 2010b; Lim *et al.,* 2009; Tian *et al.,* 2008).

A high degree of homology with chromosomal *AmpC* enzymes of Kluyvera ascorbate is determined. The group is most closely related to chromosomally determined cephalosporinases produced by *K. oxytoca*, *C. diversus*, *Proteus vulgaris* and *Serratia fonticola*. Their prevalence is not limited to nosocomial infections caused by *Klebsiella* spp. only, but they are also found in *Escherichia coli*, and what's more, in an out-hospital environment (Mushtaq *et al.,* 2004).

Unlike *TEM* and *SHV* type emerging following point mutations, it is believed that *CTX-M* enzymes with ESBL profile appear consequently to combinations of chromosomal ESBL of Kluyvera spp. into a mobile plasmid (Bonnet, 2004). *CTX-M* genes could be classified on the basis of amino acid sequences. Bonnet, (2004), Pitout *et al.*(2007), and GenBank data outline five large groups of *CTX-M* enzymes (Pitout *et al.*, 2007); *CTX-M*-1 group, consisting of six plasmid-determined enzymes (*CTX-M*-1,-3,-10,-12-15, FEC-1) and enzymes *CTX-M*-22, -23, -28; *CTX-M*-2 group, including plasmid-determined enzymes (*CTX-M*-2, -4, -5, -6, -7,- 20, Toho-1); *CTX-M*-8 group including one plasmid-determined enzyme; CTX-M-9 group comprising nine plasmid-determined enzymes (*CTX-M*-9,-13, -14, -16, -17, -19, -21, -27, Toho- 2) and *CTX-M*-24; *CTX-M*-25 group consisting of CTX-M-25 and *CTX-M*-26. *CTX-M* beta-lactamases are more sensitive to tazobactam compared to clavulanic acid, but also show a higher activity to cefotaxime than to ceftazidime (Walther-Rasmussen and Hoiby, 2004; Jacoby and Minoz-Price, 2005; Babic *et al.*, 2006). They were originally discovered in *Salmonella* enterica and *Escherichia coli*, but are also described in other *Enterobacteriaceae* species (Bradford, 2001).

The presence of serine at position 237 in all *CTX-M* enzymes is essential for their broad phenotype profile. The arginine at position 276 in *CTX-M*, similar to arginine-244 in *TEM* and SHV types of ESBL is also significant for hydrolytic activity against oxyimino-cephalosporins. The members of class B (or group 3 from the Bush classification) are plasmid-determined and detected in a number of *Enterobacteriaceae* representatives, but not in *E. coli* (Mushtaq *et al.*, 2004). They are metalloenzymes resistant to clavulanic acid (Samaha-Kfour and Araj, 2003). Ambler's class C is the second largest one comprising group 1 of Bush classification (Bulychev and Morbashery, 1999).

According to Tenover *et al.* (2003), these enzymes were initially determined by chromosomal genes in a number of intestinal and other Gram-negative bacteria (Tenover *et al.*, 2003). The expression of chromosomal *AmpC* genes is constitutively weak but could be induced in the presence of beta-lactams (Hanson and Sanders, 1999). All, as per Perez-Perez and Hanson, (2002) are resistant to all beta-lactams except carbapenems and fourth-generation cephalosporins (Perez-Perez and Hanson, 2002).

e) *AmpC* β-Lactamases Class

The 'acquired' or 'plasmidic' *AmpC* enzymes fall into six phylogenetic groups (www.bellinghamresearchinstitute.com), *CMY-2* being the most commonly-found enzyme. Resistance due to the production of these enzymes is also a significant public health concern. Several of these plasmidic *AmpC* enzymes have also been found with increasing frequency in *Enterobacteriaceae* isolated from food-producing animals and food (Jacoby, 2009; Smet *et al.*, 2009).

Wild *E. coli* strains possess a low constitutive level of resistance to beta-lactams determined by chromosomal beta-lactamases (Jaurin and Normark, 1983). *AmpC* genes of *E. coli* are regulated by a weak promoter and reduced transcription results in low expression of *AmpC*–beta-lactamase (Fernandez-Cuenca *et al.*, 2005). Low Amp C beta-lactamase expression is however not related to clinically significant resistance to beta-lactams (Siu *et al.*, 2003; Fernandez-Cuenca *et al.*, 2005).

Overexpression of Amp C- beta-lactamases occur following mutations, base substitutions in regions -35 and -10 (included in Amp C promoter), which optimize the transcription of 17 bp region or created new -10 and -35 regions with enhanced expression (Jaurin and Normark, 1983; Siu *et al.*, 2003; Mulvey *et al.*, 2005). The phenotype profile of strains exhibiting overexpression of Amp C- beta-lactamases is

similar to that of ESBL. Unlike ESBL, such strains are however slightly inhibited by a combination of beta-lactam and beta-lactam inhibitor and could hydrolyse cephamycins (Nelson and Elisha, 1999). *SHV*, *TEM* and *CTX-M* ESBL-producing *Enterobacteriaceae* (mainly *E. coli* and Salmonella) have, in the last decade, also been increasingly reported in food-producing animals and food.

f) Plasmid-Mediated Beta-Lactamases from Ambler Class C

Plasmids carrying genes encoding *AmpC* beta-lactamase production often possess other resistant genes conferring resistant to aminoglycosides, chloramphenicol, sulfonamides, tetracyclines, trimethoprim and mercury ions (Philippon *et al.,* 2002). Clinical strains producing *AmpC* beta-lactamases and other beta-lactamases, whose genes are in the same or another plasmid. Hanson (2003) affirms that the phenotype features of plasmid-determined *AmpC* beta-lactamases are difficult for determination (Hanson, 2003).

Plasmid-determined Amp C beta-lactamases are classified into 5 families (C1-C5) according to amino acid sequences (Bauernfined *et al.,* 1999). The most commonly encountered member of this group is CMY-2 beta-lactamase. Ambler's class D also includes ESBL from group 2 of Bush classification. The difference is that class D enzymes could hydrolyse cloxacillin (Gniadkowski, 2001). The primary place in this class is occupied by OXA- beta-lactamase family which, at present, comprises 11 enzymes with ESBL profile known to belong to this family. They originate from *OXA*-10, *OXA*- 1 *и OXA*-2 after amino acid substitutions (Bush and Jacoby, 2010).

According to Bradford (2001) they confer resistance to ampicillin and cephalothin, as well as possess a high hydrolytic ability against oxacillin and cloxacillin. These enzymes are poorly inhibited by clavulanic acid (Bradford, 2001). *OXA*-enzymes with ESBL phenotype is encountered mainly in *P. aeruginosa* (Babic *et al.,* 2006). They differ from

TEM and SHV-types in that they belong to Ambler's class D and functional group 2d (Bush *et al.,* 1995). Many *P. aeruginosa* and *A. baumannii* strains producing *OXA* beta-lactamases are isolated in Turkey and France (Babic *et al.,* 2006). ESBLs associated with *OXA*-10 have undergone one or two substitutions of asparagine for serine at position 73 or glycine at position 157 (Babic *et al.,* 2006).

2.15 Propagation of ESBL-Producing *Enterobacteriaceae* spp

An essential question in modern epidemiological research on ESBL producing *Enterobacteriaceae* spp. is the existence of similar ESBL phenotypes in human and animal isolates (from farm animals and pets) (Shiraki *et al.,* 2004; Weill *et al.,* 2004 and Carattoli *et al.,* 2005). There are different literature sources which presented information about the bacterial ESBL producers isolated from diseased and healthy animals as well as farmers (Brinas *et al.,* 2003; Li *et al.,* 2005; Machado *et al.,* 2008). So far, *CTX-M*-1 and *CTX-M*-15 *E. coli* beta-lactamase producers are mostly isolated from chicken, swine and cattle (Meunier *et al.,* 2006).

Numerous reports have witnessed various ESBL types among *Enterobacteriaceae* spp. isolated from chicken reared in intensive production systems. Brinas *et al.* (2003) were the first to publish data about the occurrence of ESBL-producing *E. coli* in the faeces of healthy birds in Spain (Brinas *et al.,* 2003). Thus, the authors reported 1.6% ESBL coli strains, producing *CTX-M*-14 and SHV-12 in particular. Two years later, Brinas *et al.* (2005) higher percentage (5%) of *E. coli* ESBL producers of subtypes *CTX-M*-9, *CTX-M*-14, *SHV*-12 (Brinas *et al.,* 2005).

Also, in 2005, Aarestrup *et al.* reported about a *Salmonella* enterica serovar Virchow strain producing *CTX-M*-9, isolated from quail meat imported from France (Aarestrup *et al.,* 2005). In Italy Bortolaia *et al.* (2010) detected ^{bla}*CTX-M*-1, ^{bla}*CTX-M*-32 and ^{bla}*SHV*-

12 genes in chicken *E. coli* isolates. Chicken *TEM*-52 and *SHV*-12 producing *E. coli* and other *Enterobacteriaceae* spp. strains are reported only in European countries: Spain ((Brinas *et al.,* 2003) Italy (Chiarettog *et al.,* 2008), Belgium (Cloeckaert *et al.,* 2007), Portugal (Coasta *et al.,* 2008); The Netherlands (Hasman *et al.,* 2005) and The Czech Republic (Kolar *et al.,* 2010).

Evidence for the presence of *CTX-M* producers from farm animals in France was provided by Meunier *et al.* (2006) and later by Madec *et al.* (2008). With regard to their prevalence among coli bacteria and *Salmonella* spp. isolated from chicken, cattle and swine in Portugal, Belgium, the Netherlands, France, Spain and Italy, researchers discuss an especially frequent spread of types as TEM-52, TEM-106, *CTX-M*-1, *CTX-M*-2, *CTX-M*-9, *CTX-M*-14, *CTX-M*-15, *SHV*-2, *SHV*-12, *CMY*-2 (Warren *et al.,* 2008; Smet *et al.,* 2009; Hunter *et al.,* 2010). Authors from the Netherlands, Ireland and Germany emphasize on the presence of animal isolates producing *TEM*-20, although less frequently (Hasman *et al.,* 2005; Liebana *et al.,* 2006; Hopkins *et al.,* 2006 and Morris *et al.,* 2009)

Apart chicken strains, Wu *et al.* (2008) analyzed data from the implementation of the Danmap project dedicated to antimicrobial resistance surveillance, including the spread of ESBL, and reported increased prevalence of *CTX-M*-1 enzymes in swine *E. coli* isolates (Wu *et al.,* 2008). They discussed the possibility for a selective pressure of ceftiofur use in pigs on the spread of ESBL-producing *E. coli* strains. Again, in a study on the prevalence of ESBL in Portugal with regard to the widespread utilisation of beta-lactams in intensive pig production systems, Gonçalvas *et al.*(2010) have established that *CTX-M*-1 was the most frequently encountered ESBL type in *E. coli* pig isolates and that pigs are an essential reservoir of ESBL-producing coli bacteria (Gonçalvas *et al.,* 2010). At the same time in Spain, Escudero *et al.* (2010) published comparable results from a

survey of 80 pig farms in 13 Spanish provinces and reported ESBL prevalence rates of 41% *SHV*-12, 10% *CTX-M*-1, 10% *CTX-M*-9, 10% *CTX-M*-14 among coli bacterial isolates (Escudero *et al.*, 2010).

As to the widely spread among *Enterobacteriaceae* ESBL type *CTX-M*-15 in human strains, there are data for its occurrence in strains obtained from healthy birds in Belgium and the United Kingdom, as well as in *E. coli* isolated from diseased French cattle (Madec *et al.*, 2009; Smet *et al.*, 2009; Randall *et al.*, 2011). Animal *Salmonella* isolates producing *CTX-M*-15 are reported by Rodriguez *et al.* (2009), in particular, *Salmonella* enterica serovar Typhimurium, isolated from a horse in Germany (Rodriguez *et al.*, 2009).

As pets are concerned, most data about ESBL-producing microbial pathogens, primarily *E. coli* are reported in dogs, between 2001 and 2006. For some EC countries as Portugal and Italy, the prevalence ranges from 7% to 20% and the types *TEM*-52, *CTX-M*-1, *CMY*-2 were most commonly reported. Almost at the same time, Feria *et al.* (2002), as well as Pomba *et al.* (2009) in Portugal, communicated that there were ESBL-producing members of the family *Enterobacteriaceae* spp. isolated from dogs with chronic urological infections (Feria *et al.*, 2002; Pomba *et al.*, 2009). *CTX-M*-1 produced by commensal coli bacterial isolates from dogs and cats are reported in Latin America (Moreno *et al.*, 2009).

From the epidemiological point of view, reports for ESBL-producing *Enterobacteriaceae* strains isolated from wild animals are also of interest. For example, in Portugal, Costa *et al.* (2006) and Poeta *et al.* (2008) described the prevalence of faecal E. coli isolates from wild birds, producing *CTX-M*-1, *CTX-M*-14, *TEM*-52 (Costa *et al.*, 2006; Poeta *et al.*, 2008).

So far, according to available information about ESBL production by *Salmonella* spp., strains from farm animals and respective foodstuffs, it is far less frequent than that observed in *E. coli* strains. Data of the Spanish Veterinary Antimicrobial-Resistance Surveillance Network demonstrate prevalence rates of 0.2% and 2.5% in *Salmonella* spp. isolates from pigs and chicken, respectively (Riano *et al.*, 2006). According to a study by Chiaretto *et al.* (2008), their prevalence ranges between 0.5% and 0.6% (Chiaretto *et al.*, 2008).

2.16 ESBL from Human *Enterobacteriaceae* spp

Most commonly isolated intestinal bacterial ESBL producers are *E. coli* and *Klebsiella* species. ESBL-producing bacteria from the *Enterobacteriaceae* family were isolated for the first time from in-hospital patients during the 1980s. After 2000, their spread in the out-hospital environment is increasingly discussed (Babic *et al.*, 2006; Livermore *et al.*, 2007; Bush, 2008). The report of Talbot *et al.* presented before the Infectious Diseases Society of America in 2006 sets ESBL-producing bacterial species as priority bacterial species which necessitate immediate novel therapeutic approaches (Talbot *et al.*, 2006).

Numerous researchers have concluded that the presence of ESBL-producing strains depends on various factors including the nature of bacterial infections: nosocomial or not, apart from the species and geographic location specific traits (Bradford, 2001; Livermore *et al.*, 2007; Carattoli, 2008; Livermore, 2008). In humans, the spectrum of affected cohorts is various, with the first place being held by patients from intensive care units, paediatric wards and clinics, including neonatology sectors. Often, such strains are isolated also from cancer and burn patients, in rehabilitation and geriatric care units (Rupp and Fey, 2003).

During the last two decades, apart from *Escherichia coli,* a higher prevalence of *Salmonella* spp. strains possessing plasmid-mediated ESBL from the *CTX-M* family is observed, in particular, S. enterica serovar Typhimurium. Reports indicate that some *Enterobacteriaceae* spp. strains were most commonly responsible for faecal outbreaks of infections in East Europe (Latvia, Poland, Russia), South America and Japan (Ewers *et al.,* 2012). These strains have been preferentially determined to be involved in the aetiology of human out-hospital urological tract infections and bloodstream infections unlike *TEM*- and *SHV*-producing bacteria which are traditionally associated with nosocomial infections.

ESBL-producing bacteria have an important clinical significance also from the point of view of bacterial MDR. Some authors presume the spread of MDR *Enterobacteriaceae* spp. and MDR-encoding genes from farm animals to humans (Chiarettog *et al.,* 2008; Dhanjii *et al.,* 2010). Plasmid-mediated quinolone resistance (PMQR) has been reported by the acquisition of the qnr, *qepA*, and *aac*(6)- *Ib-cr* genes (Poirel *et al.,* 2008). PMQR is also associated with ESBLs and aminoglycoside resistance genes on the same plasmid. The circulation of these multidrug resistance plasmids among *Enterobacteriaceae* strains has an important influence on the empirical treatment of urological infections in human patients (Paterson, 2006).

2.17 Public Health Risks of ESBLs Producing Bacterai in Food and Food-Producing Animals

Exposure to ESBL producing microorganisms can transpire through other means but the hospital has always been thought to be the greatest risk, especially in intensive care units (ICUs) (Babini and Livermore, 2000; Lautenbach *et al.,* 2001; Ikegbunam *et al.,* 2014). In the last decade a variety of plasmid-mediated beta (ß)-lactamases, have emerged in

Gram-negative bacteria, resulting in reduced susceptibility to broad-spectrum ß-lactams. These ß-lactamases included both extended spectrum ß-lactamases (ESBLs) and *AmpC* ß-lactamases (*AmpC*). More specifically, ESBLs are plasmid-encoded enzymes in Enterobacteriaceae, frequently found in *E. coli* and *Klebsiella* pneumoniae, but also present in other members of this bacterial family. ESBLs confer resistance to a variety of ß-lactam antibiotics, including penicillins, 2nd-, 3rd- and 4th-generation cephalosporins and monobactams (eg aztreonam), but usually not the carbapenems or the cephamycins (e.g. cefoxitin). ESBL-producing organisms are frequently co-, or multi-resistant, exhibiting resistance to other antimicrobial classes such as fluoroquinolones, aminoglycosides and, trimethoprim-sulphamethoxazole due to associated resistance mechanisms, which may be either chromosomally- or plasmid-encoded (Jacoby and Munoz-Price, 2005; Paterson and Bonomo, 2005).

The most frequently encountered ESBLs in *Enterobacteriaceae* belong to the TEM, SHV and *CTX-M* families (Paterson and Bonomo, 2005). *AmpC* β-lactamases are intrinsic cephalosporinases found on the chromosomal DNA of many Gram-negative bacteria, including many members of the *Enterobacteriaceae* (but, notably, not in *Klebsiella* or Salmonella), and opportunistic pathogens such as *Pseudomonas* and Acinetobacter. These enzymes confer resistance to penicillins, 2nd- and 3rd-generation cephalosporins including β-lactam/inhibitor combinations, cephamycins (cefoxitin), but usually not to 4th-generation cephalosporins (cefepime, cefquinome) and carbapenems. It is a serious concern that a growing number of *AmpC* enzymes have "escaped" on to plasmids. These are the so-called 'acquired' or 'plasmidic' *AmpC*s. Over the past decade another type of clinically important ß-lactamases, the carbapenemases, have emerged and spread in Enterobacteriaceae. *Klebsiella*-producing carbapenemase (KPC) was the first carbapenemase reported in a *Klebsiella* species isolate (Nordmann *et al.*, 2009).

The emergence and spread of carbapenemase-producing *Enterobacteriaceae* is a matter of great concern for the treatment of human infections, because carbapenemases hydrolyse all ß-lactams, including carbapenems and frequently aztreonam, making these organisms extremely drug–resistant. Moreover, all types of carbapenemases that so far have been found in *Enterobacteriaceae* are spreading globally (Nordmann *et al.,* 2009; Struelens *et al.,* 2010; Walsh, 2008). Such carbapenemase-producing strains are usually only susceptible to the polymyxins (e.g. colistin), fosfomycin and variably susceptible to tigecycline, although there are recent reports of colistin-resistant *Enterobacteriaceae* (Endimiani *et al.,* 2008; Nordmann *et al.,* 2009; Zarkotou *et al.,* 2010).

The broad resistance profile in bacteria that follows the production of ESBL/*AmpC* β-lactamases (and also those that produce carbapenemases), is significant in human infections and poses an ongoing and worrisome public health threat (Pitout and Laupland, 2008; Rodriguez-Bano *et al.,* 2010). This is primarily because of many community infections, and also infections treated or transmitted within hospitals, are caused by bacteria that are no longer sensitive to 2nd, 3rd and 4th generation cephalosporins (Ben-Ami *et al.,* 2006). These antimicrobials are administered many times as first-line therapy for a wide variety of infections found in the community and in the hospital, which include mild to extremely severe infections, ranging from an uncomplicated cellulitis or urinary tract infection, to pyelonephritis, bacteraemia or septic shock (Ben-Ami *et al.,* 2006; Marchaim *et al.,* 2010; Rodriguez-Bano *et al.,* 2008; Rodriguez-Bano and Navarro, 2008; Rodriguez-Bano *et al.,* 2004; Rodriguez-Bano *et al.,* 2006; Rodriguez-Bano *et al.,* 2010).

The multi-resistant nature of bacteria that produce ESBLs and *AmpC*s can affect the selection and timely administration of appropriate antimicrobials for community-

acquired and healthcare-associated infections since many first-line antimicrobials are no longer active against them. Examples are, fluoroquinolones, frequently used in urinary tract infections (UTI) and cephalosporins, as part of an antimicrobial regimen for intra-abdominal infections. Furthermore, infections with such resistant organisms are associated with poorer patient outcomes, increased morbidity, mortality, increased length of stay and increased costs (Anderson *et al.,* 2006; Ben-Ami *et al.,* 2009; Cosgrove *et al.,* 2003; Ibrahim *et al.,* 2000; Lautenbach *et al.,* 2001; Roberts, 2008; Schwaber and Carmeli, 2007).

a) The incidence of Human Infection

The total burden of human infection of ESBL-producing bacteria is not entirely known, nor is the prevalence of human faecal carriage. The data on frequency of occurrence in invasive infections in humans in Europe come from the European Antibiotic Resistance Surveillance System (EARS-Net (formerly EARSS). EARS-Net reports annual rates of antimicrobial resistance in bloodstream infections (BSI) and infections of cerebrospinal fluid (CSF) from hospitals in Europe. Such reports have demonstrated a steady increase in the rates of invasive *E. coli* and *Klebsiella* species isolates that are resistant to 3rd generation cephalosporins since 2000. In 2009, 28 countries reported *E. coli* isolates to EARS-Net, of which 7.4% were resistant to 3rd generation cephalosporins (a surrogate marker for the presence of an ESBL/*AmpC*-producing bacteria).

Trends over the period 2006-2009 showed a rapid and substantial increase in the proportions of *E. coli* isolates resistant to 3rd-generation cephalosporins in 16 of the 28 countries included in this surveillance system. Third-generation cephalosporin resistance in human clinical *Klebsiella* species isolates has also increased substantially. Trends in the faecal carriage of ESBL/*AmpC*-producing *Enterobacteriaceae* (predominantly *E.*

coli), in the community, are also of relevance. Since these resistant organisms have emerged globally as a significant pathogen in the community and in hospitals. Various studies report faecal carriage of ESBL/*AmpC*-producing *Enterobacteriaceae* to be 3.7-5.5% in non-hospitalized patients (Valverde *et al.,* 2004), or 10.8% in patients admitted to the hospital (Ben-Ami *et al.,* 2006). In a study 2006, Ben-Ami *et al.* reported 14% of blood-stream infections in non-hospitalised patients to be caused by *Enterobacteriaceae* resistant to 3rd- generation cephalosporins (Ben-Ami *et al.,* 2006).

Another report by Rodriguez-Bano *et al.* (2008) states that the estimated population-based incidence of community-acquired infection due to ESBL-producing organisms was 2.2 cases per 100,000 populations per year. The risk of human colonisation or infection with ESBL and/or *AmpC* ß-lactamase-producing organisms is a complex matter. From observational studies, risk factors for the acquisition of these bacteria were related to human health care and involved prior use of antimicrobial agents. Breaches in infection control practices, and mobility of patients, invasive medical devices, lengthy hospital stay and compromised host defences. Overall, prior use of antimicrobials is a known risk factor for patient colonisation or infection with ESBL-producing organisms. Many antimicrobials have been associated with either or both of these, but those most frequently found in various studies are oxyimino ß-lactams (cefuroxime, cefotaxime, ceftriaxone, ceftazidime, or aztreonam), fluoroquinolones, ß-lactam-ß- lactamase inhibitor combinations (Paterson *et al.,* 2004; Rodriguez-Bano and Navarro, 2008; Wener *et al.,* 2010).

2.17.1 Possible Reservoirs of ESBL/*AmpC* β- Lactamase-Producing-Bacteria

Although person-to-person spread is recognised as the main method of spread of ESBL/*AmpC*-β- lactamase-containing *E. coli* both in hospitals and the community, the

primary reservoirs of such organisms are contentious. ESBL-producing *E. coli* have been isolated from food animals in many European countries, particularly chicken and cattle, and farm animals are now recognised as important carriers of ESBL/*AmpC*-producing *E. coli* and *Salmonella* (Carattoli, 2008). Similarly, there has been an increasing number of reports of ESBL-producing *E. coli* being isolated from foods of animal origin (Bergenholtz *et al.,* 2009). This raises questions about the possible role of animal- and food-related reservoirs on this phenomenon.

2.17.2 Mobile Genetic Elements involved in Transmission of ESBL and/or *AmpC* Resistance Integrons

Integrons are genetic tools that play a role in the acquisition of resistance genes, and also in their expression. Noteworthy, they are not self-mobile genetic platforms and are usually identified onto transposon and/or plasmid elements that may vehiculate these integrons. Even if integrons are common vehicles for some ESBL determinants (encoding VEB, GES, or BEL enzymes), they do not correspond to those involved in the acquisition of ESBL determinants identified among ESBL producing animal isolates (namely *TEM*, *SHV*, and *CTX-M*). ESBL and *AmpC* genes originally came into the chromosomes of different species of Enterobacteriaceae. Mobilization of these *bla* homologues housekeeping genes from original chromosomal backgrounds are mediated by widespread integrases (class1 integrases), transposases (IS*Ecp*1, IS*26,* IS*CR1*), and to a lesser extent phage-related and repeated Re*Av* elements.

Further successful spread of ESBL and *AmpC* genes occurs by different lateral genetic transfer processes involving insertion sequences, transposons, Class 1 integrons and overall, the transferable plasmids in which they are located (Eckert *et al.,* 2006; Girlich *et al.,* 2011; Poirel *et al.,* 2008; Toleman *et al.,* 2006). Among animal isolates, studies

focused on these genetic features have shown that, overall, these mobile elements were the same as those identified in human isolates (Carattoli, 2009; Cloeckaert *et al.,* 2007; Leverstein-van Hall *et al.,* 2011).

a) ESBL/*AmpC* Encoding Resistance Genes

A large number of genes encode ESBL and *AmpC* enzymes conferring reduced susceptibility to broad-spectrum ß-lactams, a site that contains additional information and GenBank accession number references for β-lactamases from various functional groups). Despite this, not all of such genes are equally prevalent among human and animal bacteria. In the last few years, some ESBLs relevant to human medicine have been described in isolates from animals. By far the most common genes associated with this resistance have been those encoding *CTX-M* enzymes (the most commonly identified ESBL), followed by ^{bla}*TEM*-52 and ^{bla}*SHV*-12 (Bortolaia *et al.,* 2010; Chiaretto *et al.,* 2008; Cloeckaert *et al.,* 2007; Escudero *et al.,* 2010; Hasman *et al.,* 2005; Jensen *et al.,* 2006; Machado *et al.,* 2008; Randall *et al.,* 2011; Smet *et al.,* 2008). The ESBL enzymes associated with animals correspond to *CTX-M* (-1, -2, -3, -8, -9, -14, -15, -17/18, -20, -32, -55), *TEM* (*TEM*-20, -52, -106, -126) and *SHV* (*SHV*-2, -5 and -12). PER variants have been identified among fish and environmental samples (Carattoli, 2008).

Among the *AmpC*-type β-lactamases, ^{bla}*CMY*-2 is the most common, while ^{bla}*ACC*-1 and ^{bla}*DHA*-1 have only been scarcely reported (Arlet *et al.,* 2006; Carattoli, 2008; Coque *et al.,* 2008a; Cortes *et al.,* 2010; Dierikx *et al.,* 2010a; Hasman *et al.,* 2005; Rayamajhi *et al.,* 2010; Rayamajhi *et al.,* 2008; Rodriguez *et al.,* 2009).

Noteworthy, whereas ESBL producers have been mostly found in Europe, *AmpC* producers have been mostly encountered in the US, mirroring the trends observed among human isolates. Broad-spectrum *SHV*-1-, *TEM*-1- and *OXA*-type (e.g. *OXA*-1) β-

lactamases have been frequently recovered from animals and food of animal origin in EU countries. It is important to note that *OXA*-1- like enzymes may confer reduced susceptibility to 4th-generation cephalosporins, and in addition, they confer a high level of resistance to ß-lactam/ß-lactamase inhibitor combinations. Since the ^{bla}*OXA*-1 gene is commonly associated with plasmids carrying the ^{bla}*CTX-M*-15 ESBL gene, carriage of this gene significantly adds to the overall ß-lactam resistance pattern of the corresponding producers. In addition, ^{bla}*OXA*-1 has also commonly been identified in association with many other ß-lactamase genes among all enterobacterial species. As mentioned above, *OXA*-1-like enzymes may confer reduced susceptibility to 4th-generation cephalosporins, and in addition, they confer a high level of resistance to ß-lactam/ß-lactamase inhibitor combinations. Isolates belonging to *OXA*-1-producing *S. Typhimurium* have been described over several years in Portugal, Spain and the UK as a cause of food-borne infections (Antunes *et al.,* 2010; Herrero *et al.,* 2009; Herrero *et al.,* 2008; Hopkins *et al.,* 2006).

In addition, *OXA*-1 was shown to be responsible for high-level resistance to ampicillin in *S. Typhimurium* isolates recovered from fish in India (Ruiz *et al.,* 1999). Recently, a study conducted in Japan showed that among a series of ampicillin-resistant enterobacterial isolates recovered from traditional Egyptian Domiati cheese, some were producing *OXA*-1 (Hammad *et al.,* 2009).

2.18 Epidemiology of Acquired Resistance to Cephalosporins in Food-Producing Animals and Food.

In order to harmonise monitoring of AMR, the establishment of optimum phenotypic testing systems for sensitive, specific and rapid detection of ESBL- and *AmpC*-producing organisms has been recognised as a very important component of antimicrobial

resistance monitoring programmes (Livermore *et al.*, 2001). The European Food Safety Authority (EFSA, http://www.efsa.europa.eu) has prepared detailed specifications on minimum requirements for harmonised monitoring of antibiotic resistance in food-producing animals to obtain comparable data across the EU.

Guidelines for monitoring of AMR in *Salmonella* and Campylobacter and also in indicator *E. coli* and enterococci are available on the EFSA website. To enable the comparison of the occurrence of resistance between different countries the EFSA guidelines request harmonization of

(i) protocols on sampling strategies,

(ii) the method of susceptibility testing,

(iii) the antibiotics to be tested and

(iv) the criteria for categorising isolates as susceptible or resistant, as well as quality control and reporting.

The EFSA guidelines state that cefotaxime is a good substrate for what are currently the most common and important ESBLs in humans in Europe, the *CTX-M* enzymes. This was confirmed in a recent study, in which it was concluded that for detection of ESBL- and *AmpC*-producing *E. coli* or *Salmonella* harbouring *TEM*, *SHV*, CTX-M, and various *AmpC* gene families, usage of cefotaxime, cefpodoxime or ceftriaxone with epidemiological cut-off values (ECOFFs) > 0.5, > 2 and > 0.125 mg/L, respectively, were the most efficient cephalosporins for detection of these ß-lactamase gene families. Ceftazidime, ceftiofur and cefquinome were less efficient (Aarestrup *et al.*, 2010).

In the 2007-2009 Member State (MS) reports the occurrence of resistance is given, where available, for cefotaxime, ceftazidime and ceftiofur. The recent implementation of EFSA's recommendations by MSs has resulted in more frequent monitoring of resistance to third-generation cephalosporins, as based on cefotaxime susceptibility patterns, over the period 2007-2009, this is an important improvement to the EU-wide surveillance programme. Since reports cover only phenotypic monitoring, it is not possible to determine the class or exact type of ß-lactamase enzyme which is likely to confer the resistance detected to 3rd-generation cephalosporins. Since in the tables per country and animal species, data are included if as a minimum 10 isolates are tested, the results need to be interpreted with care.

Other cefotaxime-resistant serovars found in Dutch chicken or chicken meat products include Virchow, Hadar, Infantis, Kottbus, Mbandaka, Agona, Cubana, Rissen, Senftenberg and Heidelberg (MARAN, 2008). In chicken raw meat products, Belgium reported a high occurrence of resistance (26%) in 2008, while the Netherlands reported a moderate occurrence of resistance (18% for *Salmonella* and 15% for *E. coli*) (MARAN, 2008). From 2003 to 2008, a dramatic increase in the occurrence of cefotaxime-resistant *E. coli* and *Salmonella* isolates from Dutch broiler chickens has been observed (3% to 18% for *E. coli* randomly isolated from faecal samples using non-selective plates) (MARAN, 2008).

In Sweden, since 2008 healthy food animals are screened for ESBL- or *AmpC*-producing *E. coli* by the culture of intestinal content on media supplemented with cefotaxime. In 2010, ESBL and /or *AmpC* producing *E. coli* were found in 34% of the samples from broilers (SVARM, 2010). In 2009 eight MS and two non-MS tested more than 10 isolates of *E. coli* for cefotaxime resistance in chicken (*Gallus gallus*) isolates.

Resistance to cefotaxime for the eight reporting MSs was 8.5%, although the occurrence of resistance reported by individual MSs ranged from 0% to 26%. The resistance ranged from the low level of around 3% (Austria, Germany and France) to high level of 26% (Spain) with the Netherlands and Poland reporting moderate proportions of 18% and 11%, respectively.

A large number of research studies have been performed in different European countries as well as in countries in other continents, focused on the analysis of the prevalence of ESBL- and *AmpC*-carrying *Enterobacteriaceae* (especially *E. coli* and non-typhoidal Salmonella) in faecal samples from food-producing animals or in food derived from them. Reviews about this topic have been published by different authors (Torres and Zarazaga, 2007; Li *et al.*, 2007; Carattoli, 2008; Smet *et al.*, 2009).

In a study by Bortolaia *et al.* (2008) ESBL-producing *E. coli* isolates were also detected in two of four flocks of chicken in farms with no previous antibiotic use in Denmark (Bortolaia *et al.*, 2010). In a study in Belgium Smet *et al.* (2008), 45% of 295 ceftiofur-resistant *E. coli* isolates obtained from 489 cloacal samples collected at five different Belgian broiler farms were ESBL producers. The prevalence of ESBLs among *Salmonella* isolates of food-producing animals or derived food is much lower than that among *E. coli* (Smet *et al.*, 2008). In a study in Germany of 22,679 *Salmonella* isolates of the National *Salmonella* Laboratory Collection, sixteen isolates produced *CTX-M* enzymes (1 *CTX-M*-15; 15 *CTX-M*-1) and four isolates produced the *TEM*-52 or *TEM*-20 enzymes located on IncI1 plasmids. The ESBL-producers represented only 0.09% of the total number of isolates tested (Rodriguez *et al.*, 2009).

In another study in Spain among isolates obtained through the Spanish Veterinary-Antimicrobial-Resistance-Surveillance Network (VAV), 4 of 556 (0.7%) *Salmonella*

organisms obtained from faecal samples of healthy food animals (pigs and chicken) at the slaughterhouse level were ESBL producers, representing 0.2% in the case of pigs and 2.5% of all isolates in the case of chicken (Riano *et al.,* 2006). ESBL-producing *Salmonella* prevalence of 0.5-0.6% was detected in Italy, Korea and Japan (Chiaretto *et al.,* 2008; Hur *et al.,* 2010; Matsumoto *et al.,* 2007). In a pilot study in 2010 on 26 broilers production farms in the Netherlands, the prevalence of ESBL and/or *AmpC*-producing *E. coli* was determined. Faecal samples were collected from the cloaca from 25 to 41 animals per farm. All farms were found positive. In 85% of the farms, 80.0% or more of the chickens examined were positive for ESBL- and/or *AmpC*-producing *E. coli* (Dierikx *et al.,* 2010b).

2.19 Transmission of ESBL/*AmpC*-Resistant Bacterial Strains/Genes to Humans Handling of Contaminated Food.

The emergence and spread of ESBL/*AmpC*-producing microorganisms in the environment is a complex process and has mainly been due to point mutations that arise and are selected (Knox, 1995), and to the spread of resistant mobile genetic elements through the environment, especially plasmids between bacteria (Bonnet, 2004; Carattoli, 2001, 2008).

The role of antibiotic selection pressure is also important, from agents used in both animal husbandry and human medicine (Blazquez *et al.,* 2000; Jorgensen *et al.,* 2007; Medeiros, 1997; Rice *et al.,* 1996) in the selection of resistance in commensal bacteria in the gut of food animals (van den Bogaard *et al.,* 2001). Resistance can also emerge in bacteria in soil as a result of pressure from antibiotic residues resulting from the use of antimicrobials in animal husbandry. Such residues have been found in all components of the ecosystem, including the farm environment (Sayah *et al.,* 2005), with recent reports

of ESBL producing *E. coli* found along with antibiotic residues in animal manure (Furtula *et al.,* 2010).

Evidence for the transmission of MDR *Enterobacteriaceae* resulting from food consumption to humans has been documented and is well established for resistant *Salmonella* and *E. coli* (Gerner- Smidt and Whichard, 2009). Multiple reports exist, showing the direct association of food with outbreaks of multi-drug-resistant *Salmonella* in humans. Outbreaks associated with raw milk (Bezanson *et al.,* 1983; Cody *et al.,* 1999; Villar *et al.,* 1999), and raw, ground beef (Dechet *et al.,* 2006), pork (Molbak *et al.,* 1999), and breakdowns in pasteurization (Walker *et al.,* 2000) have been reported.

With regard to the possibility of bacteria that produce ESBLs and/or *AmpC* being transmitted to humans, there are reports that provide circumstantial evidence that ESBL-producing *E. coli* can be associated with its transmission from food to humans (Lavilla *et al.,* 2008), studies whose findings suggest transmission of *E. coli* that produce ESBL, from chicken to humans (Leverstein-van Hall *et al.,* 2011), but also evidence of direct association of transmission of *Salmonella* resistant to 3rd generation cephalosporins during an outbreak in humans (Zansky *et al.,* 2002).

a) *AmpC*-Carrying Organisms of Chicken Origin

In 2005, a study by Brinas *et al.*from chicken samples indicates that the percentage of *AmpC*-carrying *E. coli* isolates ranged from 0.8 to 3.3% in different European countries (Spain, Belgium, Netherlands, Czech Republic) (Brinas *et al.,* 2005; Brinas *et al.,* 2003b; Dierikx *et al.,* 2010a; Smet *et al.,* 2008) and non-European countries (Japan, Taiwan and China) (Kojima *et al.,* 2005; Li *et al.,* 2010b; Yan *et al.,* 2004). For *Salmonella,* percentages of detection in the range of 0.1-10% were identified for chicken or derived

food in Ireland, USA, Canada or Japan (Allen and Poppe, 2002; Boyle *et al.,* 2010; Taguchi *et al.,* 2006; Zhao *et al.,* 2008).

Imported meat could be an important source of ESBL/*AmpC*- *E. coli* producers. A recent survey of frozen chicken meat imported into Sweden showed that 92% of meat from South America had ESBL/*AmpC E. coli* producers, compared with 19% for meat from elsewhere in Europe. Moreover, *E. coli* from South American meat were resistant to a wider range of antibiotics and had a higher diversity of ESBL/*AmpC* genes (Borjesson *et al.,* 2011). Other studies conducted in chicken meat imported into Denmark and the United Kingdom from the same geographical region indicated an occurrence of ESBL/*AmpC E. coli* producers in 30-36% of samples analyzed (Bergenholtz *et al.,* 2009; Dhanji *et al.,* 2010).

b) *AmpC*-Carrying Organisms of Cattle Origin

Allen and Poppe in their study reported that cattle samples analysed, *AmpC* β-lactamases were detected in 0.01% of 8,426 *Salmonella* isolates in a Canadian study (Allen and Poppe, 2002). In samples of pig origin, the percentages of *AmpC* carrying *Salmonella* or *E. coli* isolates ranged from 2.4 to 23% in Canada, Taiwan and México (Yan *et al.,* 2004; Zaidi *et al.,* 2007; Kozak *et al.,* 2009). The type of *AmpC* β-lactamase detected in food-producing animals or derived food has been almost always the *CMY*-2 variant, and the DHA-1 variant was also identified in *Klebsiella* species of pig origin in Korea (Rayamajhi *et al.,* 2008). ACC-1 has also been reported to occur on unidentified plasmids in *S.* Braenderup (Dierikx *et al.,* 2010a; Hasman *et al.,* 2005).

2.20 Transmission of Resistant Bacterial Strains/Genes to Humans via Food Animal Production Environment

There is limited evidence for the spread of ESBL/*AmpC*-carrying organisms via direct contact with animals or indirectly via the environment. In cases where a similar gene pattern in humans and animals has been documented it is often not clear whether this has been spread through contact or via food (Fey *et al.,* 2000). It seems as if the "one medicine concept" applies which means that an increase in the number of ESBL/*AmpC* carrying organisms in either animals or humans (or in different animal populations) could make resistance emerge in other human or animal populations but the links might be indirect and thus difficult to map.

In general, the ecosystem acts as a reservoir, a "resistome" (Wright, 2007). Resistant bacteria which can be found in faeces and soil in the farm environment (Cobbold *et al.,* 2006; Goncalves *et al.,* 2010), aquatic systems (Machado *et al.,* 2009), plants (Ruimy *et al.,* 2009) can be transferred from these sources to animals and humans through the food chain (Silbergeld *et al.,* 2008). These bacteria can infect animals or colonise their gut (Hinton *et al.,* 1982; Alexander *et al.,* 2009). A natural consequence of animal gut colonisation is shedding of resistant bacteria into the farm environment. Although the duration of persistence and shedding is not known, in an outbreak in cows caused by ESBL-producing Salmonella, shedding continued for 68 days after the start of the outbreak (Lanzas *et al.,* 2009).

Strong evidence supporting the potential for transmission and colonisation of *Enterobacteriaceae* between animals and farm staff has been provided. Reports where farmers were colonised with indistinguishable strains of antimicrobial-resistant *E. coli* from food animals on the farm. Such colonisation was initially reported for *E. coli* from

chicken and humans followed by many subsequent reports on *E. coli* (Linton *et al.,* 1977; Ojeniyi, 1989; van den Bogaard *et al.,* 2001; Price *et al.,* 2007). Transmission of *CTX-M*-1 between pigs and pig farmers has also been reported (Moodley and Guardabassi, 2009). (Dierikx *et al.,* 2010a) have demonstrated that people working with chicken have a higher risk for intestinal carriage of ESBL-producing bacteria. The ESBL prevalence in chicken farmers was higher than in the general population (around 30% versus 5% respectively), which suggests that direct transmission from chicken to humans may also be a possible route of transmission.

a) Clonal Transmission of Resistance Genes

Expansion of *E. coli* clones (producing ESBL) and *Salmonella* (producing ESBL or *AmpC*) with zoonotic potential has been reported. Recent studies have shown that *E. coli* isolates from chicken and pig farms differ with respect to ESBL and CMY-2 enzymes, phylogenetic group, virulence genes, and serotype (Cortes *et al.,* 2010). It has been suggested that *E. coli* isolates from chicken are genetically-related to human pathogenic *E. coli*. In a study comparing genetic similarities of *E. coli* derived from humans and chicken, antibiotic-resistant *E. coli* isolates from both reservoirs were more frequently genetically related than antibiotic-susceptible isolates (Johnson *et al.,* 2007; Vincent *et al.,* 2010).

2.21 Abattoirs as Non-Hospital Source of Extended-Spectrum Beta-Lactamase Producing Pseudomonas

Extended-spectrum beta-lactamase-producing *P. aeruginosa* has been reported to be a potential human hazard in the world (Mahmoud *et al.,* 2017). The increasing resistance of potentially pathogenic bacteria to multiple conventional antibiotics is an urgent problem in global public health (Strauß *et al.,* 2015). *P. aeruginosa* is one of the major

causes of diseases such as otitis, mastitis, endometritis, hemorrhagic pneumonia and urinary tract infections in both livestock and companion animals (Kidd *et al.,* 2012; Salomonsen *et al.,* 2013). The multiple-drug-resistant (MDR) *Pseudomonas* can be transmitted from different sources to humans and also to the environment through horizontal gene, the emergence and occurrence of MDR *P. aeruginosa* strains are growing in the world, leading to limited therapeutic options (Breidenstein *et al.,* 2011; Tavajjohi *et al.,* 2011). The prevalence of MDR *Enterobacteriaceae* in slaughterhouses, including swine and chicken environments, has been reported in several studies building a growing alarm about their effect on animal and human health (Miko *et al.,* 2005; Schwaiger *et al.,* 2012). Recently encountered the emergence of livestock-associated ESBL-producing *P. aeruginosa* in cow, chicken, pigs and Camel (Odumosu *et al.,* 2016; Zulfluh *et al.,* 2016; Mahmoud *et al.,* 2017).

Accordingly, livestock-associated ESBL-producing gram-negative bacteria become new alarm for emerging infectious pathogens to human and animals ((Mahmoud *et al.,* 2017). Livestock may be an important vehicle for the community-wide dissemination of antimicrobial-resistant Enterobacteriaceae, also *P. aeruginosa* especially ESBL-producing type isolates have been found in increasing numbers in food-producing animals (Odumosu *et al.,* 2016; Zulfuh *et al.,* 2016; Mir *et al.,* 2016). Accordingly, the hypothesis that animals might become infection sources or even natural persistent sources acting as risky reservoirs of infection leading to the spread of these bacteria specifically multidrug-resistant types in the community (Watkins *et al.,* 2016). There are essential needs for monitoring or surveillance studies incorporating veterinary medicine to identify transmissible pathogens to human and its risk factors. Mahmoud *et al.* reported the prevalence of *P. aeruginosa* in camel to be at about 22.5% (45/200). Another scientific report has it that fish and cow in Switzerland and Nigeria also harbour

these resistance genes (Odumosu *et al.,* 2016; Zulfuh *et al.,* 2016). Class A (ESBLs) are typically identified in *P. aeruginosa* isolates and showing resistance to the extended-spectrum cephalosporin (ESCs) (Fadlelmula *et al.,* 2016). This resistance is often due to the production of β-lactamases. Clinically, ESBLs are generally encoded by plasmid-mediated bla genes; three major clinically relevant β-lactamase genes are *bla*SHV, *blaTEM* and bla*CTX-M* 2 and 4 (Bush, 2013).

a) Metallo-Beta-Lactamase (MBL) Producing *E. coli* from Abattoir

Metallo-β-lactamases (MBLs) are a group of β-lactamase enzymes that have zinc ion (Zn+) in their active site and are active hydrolyzers of carbapenems including meropenem, imipenem and ertapenem. However, MBLs are inactivated by chelating agents such as Ethylenediamine tetra-acetic acid (EDTA) (Ejikeugwu *et al.,* 2016). MBLs, which are a type of carbapenemases, is an emerging public health problem among clinically important Gram-negative organisms and community isolates including *P. aeruginosa*, *A. baumannii* and the *Enterobacteriaceae* (Thompson, 2010; Franco *et al.,* 2010). The carbapenems are potent antimicrobial agents used for the treatment of serious Gram-negative bacterial infections including those that are caused by bacteria harbouring extended-spectrum beta-lactamases (ESBLs). The MBLs are known to confer a variable range of high resistance to all beta-lactam antibiotics except the monobactams and their presence in both community and clinically important Gram-negative bacteria have put the use of the carbapenems under threat (Thompson, 2010; Tortola *et al.,* 2005).

Genetically, the MBLs are either plasmid-mediated or chromosomally-mediated, and those that are plasmid-mediated (or encoded by transferable genes or elements such as integrons and transposons) are found in more resistant bacteria such as *P. aeruginosa*, A. baumannii, and the *Enterobacteriaceae* while those that are chromosomally-mediated are

found in bacterial strains such as *Bacillus cereus* and *Stenotrophomonas maltophilia* and in obscure nonclinical bacteria such as Aeromonas species (Toleman *et al.,* 2005; Walsh *et al.,* 2005; Thompson, 2010). However, MBL genes are important resistance determinants considering the fact that most of these genes are carried as mobile gene cassettes (which can easily be integrated into the chromosomes of other susceptible organisms). One integrons with the potential to spread to other non-MBL-producing bacteria in either the community or hospital environment. And because the MBL genes are mainly plasmid-borne, there spread to the population of pathogenic organisms is of great concern and a menace to our ability to fight and treat a wide variety of Gram-negative infections (Toleman *et al.,* 2005; Walsh *et al.,* 2005).

Opportunistic organisms from the environment are also known to ubiquitously express MBLs chromosomally, and the reason for this is still esoteric (Walsh *et al.,* 2005; Tortola *et al.,* 2005). The MBLs belong to a group of beta-lactamases which requires divalent cations (e.g. zinc ions) as cofactors for their enzyme activity, and enzymes in this category are similar in that they require zinc ions for enzyme activity and they are inhibited by EDTA and other chelating agents (Toleman *et al.,* 2005; Varaiya *et al.,* 2008). The advent of MBL-producing bacteria calls for concerted effort to detect and contain their spread in either the community or hospital environment in order to sustain the efficacy of some available potent antimicrobial agents (Ejikeugwu *et al.,* 2016).

Metallo beta-lactamase (MBL) production is one of the mechanisms of resistance among bacterial isolates especially the members of *Enterobacteriaceae* and other Gram-negative bacteria such as *P. aeruginosa* and *Klebsiella* species (Ejikeugwu *et al.,* 2016). This mechanism of resistance has tremendous public health implications as they limit treatment options for bacterial infections caused by multidrug-resistant bacteria (Bashir

et al., 2011). There were similar rates of susceptibility and resistance of *E. coli* isolates from environmental samples that were previously reported (Moore *et al.,* 2014; Ejikeugwu *et al.,* 2014; Ejikeugwu *et al.,* 2016). The occurrence of MBL positive *E. coli* in the community amongst Gram-negative bacilli have previously been reported; and they also account for the spread of drug resistance genes in these settings (Johnson *et al.,* 2013; Ejikeugwu *et al.,* 2014; Walsh *et al.,* 2005).

Poor handling of meat in abattoirs could predispose consumers to the acquisition of pathogenic *E. coli* that may harbour drug resistance genes. Recently, research report conducted in Nigeria indicates that there is a high prevalence of *E. coli* (80 %) proliferation form anal swab samples of cow analysed, and *E. coli* is a common bacterial organism that is associated with a variety of human infections including those that are nosocomial in origin, and those that are acquired from the community (Ejikeugwu *et al.,* 2016). The rapid spread of resistance among bacteria may be attributed to the widespread and inappropriate use of antibiotics in the environment. Also, non-medical uses of antibiotics in some area such as animal husbandry, fish farming as feed additives and in the treatment of certain plant diseases may also contribute to the spread of antimicrobial resistance in the community (Claude, 2013).

b) Metallo- Beta-Lactamase (MBL)-Producing *Klebsiella* Species from Abattoir

The current surge in the emergence and spread of microbial resistance in the community has impacted negatively on the most important discoveries of modern medicine. Antibiotic resistance within the non-hospital environment is an emerging public health threat that has put the efficacy of some available antimicrobial agents at risk and this phenomenon is due in part to the misuse of antibiotics for both human and non-human purposes (Liu *et al.,* 1992). Antibiotics have over the years saved an untold number of

people from morbidity and mortality due to infectious diseases, but this important discovery of modern medicine is under threat due to microbial resistance which now occurs in the hospital and non-hospital environment. The emergence of carbapenem-resistant Gram-negative bacteria in both the community and hospital environments constitutes an alarming development in the field of infectious disease management and control (Walsh, 2005, Walsh, 2010; Bush and Jacoby, 2010; Ejikeugwu *et al.*, 2014).

This peril foretells with major public health implications since they jeopardize the clinical significance of potent antibiotics used to treat serious infections (Ejikeugwu *et al.*, 2016). Antimicrobial resistance in *Klebsiella* species has become increasingly prevalent, and this has been partly attributed to the efflux pump, decreased outer membrane permeability and production of beta-lactamase enzymes by this organism. The increase in antibiotic resistance among bacteria, most notably *Klebsiella* species by the production of β-lactamases has led to the increased use of carbapenem antibiotics. However, most strains of this organism are resistant to the carbapenems since they produce MBLs (Ejikeugwu *et al.*, 2016).

Klebsiella species typically cause opportunistic infections in soft tissues, wounds, urinary tract and in the bloodstream (Podschun and Ullamann, 1998). They cause community and hospital-acquired infections in human and therefore poses challenges to public health. Microbes develop resistance to antimicrobial agents following mutation and selective pressure imposed on them by the incessant use of antibiotics (Chakraborty *et al.*, 2010). Resistance to carbapenems among these bacteria remains remarkably rare in most countries. However, the situation still remains unreported in many parts of the developing world where access to quality healthcare is still poor (Zurfluh *et al.*, 2013). More intensive efforts are urgently required to elucidate the epidemiological and

infection control issues related to multidrug-resistant organisms and to improve measures aimed at stopping their emergence and spread in any environment. The use of antibiotics in the rearing and production of livestock and chicken birds as well as in other veterinary purposes has contributed significantly to the emergence and spread of drug-resistant bacteria in the community (Aibinu *et al.,* 2003). *Klebsiella* species is responsible for a handful of both community-acquired and nosocomial infections and this Gram-negative bacterium is notoriously resistant to some commonly available antibiotics (Peterson *et al.,* 2004).

The production of MBL has previously been reported in which MBL was detected in *Klebsiella* species from both clinical and community samples (Yusuf *et al.,* 2011) Also in a recent study conducted in Japan has reported a higher prevalence of MBL-producing *Klebsiella* species. Okazaki *et al.* (2016) reported the occurrence of *Klebsiella* species positive for MBL production in a non-hospital environment. There has been also a report on the occurrence of MBL from the bird in which 5 (41.7%) isolates out of 24 isolates of *Klebsiella* species produced MBL phenotypically (Ejikeugwu *et al.,* 2017). This demonstrates the impact of antibiotic misuse in the community.

2.22 Emergence of Colistin Resistance Producing Organisms of Abattoir Origin

Colistin, also known as polymyxins E is an old cyclic and cationic polypeptide antibiotic with considerable in vitro effects against *P. aeruginosa* that are highly active against many Gram-negative bacteria, including *P. aeruginosa* (Michalopoulos and Karatza, 2010). It is bactericidal to Gram-negative bacteria. They exert their bactericidal activity through disruption of the cell membrane leading to leakage of cell contents and cell death and these organisms can expand resistance to this agent via adaptation mechanisms or mutation (Falagas *et al.,* 2005). This is achieved at least in part by binding to

lipopolysaccharide (LPS), a major component of the Gram-negative cell surface, through interactions with phosphates and fatty acids of LPS core and lipid A moiety (Moskowitz *et al.,* 2004). The operon pmrH-M is directly controlled by pmrAB, and the gene products are responsible for the synthesis of N-4-aminoarabinose that binds to lipid A and reduces binding of polymyxins to LPS, whereby resistance arises (Trent *et al.,* 2001).

Following on from the publication of the nucleotide sequence of *mcr*-1, numerous research groups were able to rapidly screen archived bacterial DNA sequences for the presence of the gene. This resulted in a rash of reports of the detection of the *mcr*-1 gene in different countries on five continents: Asia (China) (Liu *et al.,* 2016 and Hu *et al.,* 2016), Cambodia (Stoesser *et al.,* 2016) Japan (Suzuki *et al.,* 2016), Laos (Olaitan *et al.,* 2016) Malaysia (Petrillo *et al.,* 2016, Yu *et al.,* 2016 and Hu *et al.,* 2016), Taiwan (Kuo *et al.,* 2016) Thailand (Olaitan *et al.,* 2016) and Vietnam (Malhotara-Kumar *et al.,* 2016, Nguyen *et al.,* 2016 and Pham *et al.,* 2016), Europe (Belgium (Malhotra-Kumar *et al.,* 2016 and Xavier *et al.,* 2016) Denmark (Hasman *et al.,* 2016), France (Olaitan *et al.,* 2016; Webb *et al.,* 2016, Haenni *et al.,* 2016 and Perrin_Guyomard *et al.,* 2016), Germany (Falgenhauer *et al.,* 2016), Great Britain (Doumith *et al.,* 2016 and Anjum *et al.,* 2016), Italy (Cannatelli *et al.,* 2016 and Giufre *et al.,* 2016), Poland (Izdebski *et al.,* 2016) Portugal (Figueiredo *et al.,* 2016 and Hu *et al.,* 2016) Spain (Quesada *et al.,* 2016 and Prim *et al.,* 2016), Switzerland (Zurfuh *et al.,* 2016) and The Netherlands (Arcilla *et al.,* 2016; Kluytmans *et al.,* 2016 and Veldmann *et al.,* 2016), Africa (Algeria (Olaitan *et al.,* 2016) Egypt (Elnahriry *et al.,* 2016 and Elnahriry *et al.,* 2016), Nigeria Olaitan *et al.,* 2016), South Africa (Coetzee *et al.,* 2016; Poriel *et al.,* 2016 and Perreten *et al.,* 2010 and Tunisia (Grami *et al.,* 2016), South America (Argentina)

Rapoport *et al.*, 2016 and Liakopoulos *et al.*, 2016 and Brazil (Fernands *et al.*, 2016) and North America (Canada (Mulvey *et al.*, 2016) and the USA (McGann *et al.*, 2016).

In recent years, several reports have called attention to the emergence and spreading of clones of *P. aeruginosa* showing MDR characteristics with high epidemic risk in various hospitals worldwide (Mulet *et al.*, 2011). For example, clones with sequence types ST111, ST175, ST235, ST253 and ST274 and the Liverpool epidemic strain (LES-1). Initially it appeared at one location and were later found to have undergone significant global spreading, causing high mortality rates (Mulet *et al.*, 2011; McCarthy *et al.*, 2015 and Oliver *et al.*, 2015). *P. aeruginosa* is reported to be the main cause of nosocomial infections in the USA (7.1%) and European countries (8.9%) (McCarthy *et al.*, 2015). Population genetics studies of microorganisms of clinical importance have been essential for understanding and predicting their evolution. Several authors have reported that *P. aeruginosa* exhibits a non-clonal epidemic population structure and that much of its genetic variation is the result of recombination events (Maatallah *et al.*, 2011; Kidd *et al.*, 2012; McCarthy, 2015).

Thus, surveillance protocols need to be developed in all health institutions to prevent and control nosocomial infections worldwide. Nevertheless, the molecular characterization of these microorganisms was crucial for identifying their evolutionary processes (Deplano *et al.*, 2005; Cholley *et al.*, 2011) and how important characteristics such as resistance to antibiotics change over time within the hospital environment. At a pediatric tertiary care hospital in Mexico City in 2007, *P. aeruginosa* clones emerged, spread and produced high infant mortality rates. This analysis revealed the presence of sensitive, multi-resistant, extremely resistant and pan-resistant strains among these isolates (Aguilar-Rodea, 2013). However, no study was conducted during this period to understand the genetic relationships and evolutionary dynamics of these strains.

a) Colistin Resistance Organisms from Abattoir

Antimicrobial drug resistance in the food chain is an emerging public health problem that needs to be curtailed. The occurrence of drug-resistant bacteria in food-producing animals presents a serious concern for infection control management both in the food chain and in the healthcare system (Ejikeugwu *et al.*, 2017; Ugwu *et al.,* 2018).

The antibiotic resistance genes can be transferred among bacteria of varying taxonomic groups; and the transmission of resistant microbes from animals to humans is well established (Malini and Aditi, 2016). The most important mechanisms for resistance among the foodborne pathogens include the production of colistin resistance mechanism (*mcr*-1) gene, Metallo β-lactamases (MBLs), *AmpC* enzymes and extended spectrum β-lactamases (ESBLs) (Geser *et al.,* 2012; Malini and Aditi, 2016). Since the spread to humans cannot be disregarded, the increasing incidence of multidrug-resistant (MDR) microbes among food-producing animals have powered interest in the inheritances and mechanisms of resistance progress by bacteria to frustrate the effects of antibiotics. ESBLs are a group of enzymes that break down β-lactam antibiotics including the penicillins and oxyimino-cephalosporins, and render them ineffective (EFSA, 2011).

P. aeruginosa is one of the most common hospital-acquired pathogens and causes pneumonia, urinary tract infections, surgical site infections and bloodstream infections (Lister *et al.,* 2009). It has the ability to develop resistance to multiple classes of antibiotics, and multidrug-resistant (MDR). *P. aeruginosa* infections are difficult to treat (Hancock and Speert, 2000). Polymyxins, polymyxin B and colistin (Cst), are regarded as one of the last resorts to treat MDR Gram-negative pathogens, including *P. aeruginosa* (Nation and Li, 2009). These drugs comprise a family of antimicrobial cyclic oligopeptides synthesized by *Bacillus polymyxa* that bind to the lipopolysaccharides

(LPS) of the Gram-negative outer membrane, invoking membrane permeabilization and diffusion of peptides across the periplasm (Evans *et al.,* 1999). Disruption of cellular respiration through the insertion of polymyxins results in cell lysis and death (Andersson *et al.,* 2016).

Although polymyxin resistance rates are relatively low, the emergence of polymyxin-resistant *P. aeruginosa* isolates has been reported in many parts of the world with the increased use of polymyxins (Landman *et al.,* 2005; Lee *et al.,* 2011). It is known that Colistin resistance (CstR) occurs by modification of lipid A, a component of the LPS, resulting in a reduction in the net negative charge of the outer membrane (Moskowitz *et al.,* 2004; Miller *et al.,* 2011; Lee *et al.,* 2016). In *P. aeruginosa,* the arnBCADTEF-ugd (or pmrHFIJKLME) operon plays a critical role in CstR through the synthesis and addition of 4-amino-4-deoxy-L-arabinose (L-Ara4N) to lipid A (Lee *et al.,* 2014).

The gene formerly known as pmrH (meaning 'polymyxin resistance') was renamed arnB (meaning 'LAra4N biosynthesis') to provide a description of the gene product through updated annotation, based on genome analysis of *E. coli* K-12 (Riley *et al.,* 2006). It is regulated by a two-component regulatory system, including PhoPQ, PmrAB, ParRS and CprRS in *P. aeruginosa* (Mcphee *et al.,* 2006; Schurek *et al.,* 2009; Fernandez *et al.,* 2010, Muelle *et al.,* 2011; Fernandez *et al.,* 2012). A report has proven that CstR is developed through alternative or compensatory pathways, even if one of these two-component regulatory systems is inactivated (Lee *et al.,* 2014).

All the previously isolated CstR mutants in the absence of those four two-component systems expressed increased amounts of the arnB encoding uridine 5¢-(beta-1- threo-pentapyranosyl-4-ulose diphosphate) aminotransferase (Lee *et al.,* 2014). Although previous studies have exhibited the importance of arnB for CstR in *P. aeruginosa*

(Fernandez *et al.,* 2010; Lee *et al.,* 2014). In most studies, CstR can occur by a change of charge in the outer membrane due to the addition of L-Ara4N or phosphoethanolamine to the lipid A of the LPS, including the recently found *mcr*-1 in plasmid (Moskowitz *et al.,* 2004; Miller *et al.,* 2011; Lee *et al.,* 2016; Liu *et al., 2*016). However, the mechanisms of resistance have not yet been revealed in some CstR isolates (Park *et al.,* 2011). Modification of the LPS and cell membrane due to arnB deletion may affect the physiological responses to various environmental stresses. The physiological changes after arnB deletion and in-vitro selection of CstR might be very complex. Tolerance against osmotic stress (5% NaCl) increased in both CstR mutants irrespective of arnB deletion, which might be due to changes in the cell membrane that occurred during the acquisition of CstR (Chung *et al.,* 2017). It is probable that the arnB operon might be functionally associated with many other genes that influence diverse physiological activities, including virulence, in *P. aeruginosa*. In addition, the development of CstR might be accompanied by diverse physiological changes in the bacteria, many of which are probably associated with cell membrane changes (Chung *et al.,* 2017).

i) Prevalence of *mcr*-1 Genes (Plasmid-Mediated Colistin Resistance)

Polymyxins (polymyxin B and colistin) are a last-resort treatment for infections caused by multidrug-resistant (MDR) Gram-negative bacteria (Olaitan *et al.,* 20141). In veterinary use, colistin is administered with food in pig and chicken farming to prevent infections caused by pathogens (Kempf *et al.,* 2013). The *mcr*-1 gene, which confers plasmid-mediated colistin resistance to Enterobacteriaceae, was identified in an Inc I2 plasmid from *E. coli* and *Klebsiella* species in China in 2016 (Liu *et al.,* 2016).

The *mcr*-1 gene found in *E. coli* (Malhotra-Kumar *et al.,* 2016), *Klebsiella* species (Du *et al.,* 2016), and *Salmonella* spp. (Yang *et al.,* 2016) has been proven to disseminate

ubiquitously. The transmission of *mcr*-1-mediated colistin resistance between animals and human has been a threat to human health. It has also been demonstrated that the *mcr*-1 gene can co-exist with ^{bla}*CTX-M*, ^{bla}*NDM* (Yao *et al.,* 2016), and other resistance genes (Malhotra-Kumar *et al.,* 2016), which threatens a return of untreatable infections worldwide. Previous reports described the unique *mcr*-1 gene sequence compared with that of the originally published sequence (Liu *et al.,* 2013), which indicates that *mcr*-1 is relatively conserved. Recently, a point mutation of A to T at position 8 in *mcr*-1 was identified in *Klebsiella* species (Di *et al.,* 2016).

In 2015, Liu *et al.* reported the first description of plasmid-mediated colistin resistance (*mcr*-1 gene) in food animals, food and humans in China (Liu *et al.,* 2016). In this issue, Kluytmans-van den Bergh *et al.* reported on their finding of the *mcr*-1 gene in *E. coli* (Kluytmans-van den Bergh *et al.,* 2016). This was done by whole genome sequencing of all *E. coli* isolates and then screening for the presence of the *mcr*-1 gene by comparing the assembled sequences with sequence data from two databases. Their study added to the already long list of articles on plasmid-mediated colistin resistance (Battisti, 2014; Falgenhauer *et al.,* 2016; Haenni *et al.,* 2016; Arcilla *et al.,* 2016). Within a short while, it was observed that the *mcr*-1 gene (i) had spread to most continents, (ii) had been found in bacteria isolated from various food animals, from the environment including river, water, from various types of meat and vegetables, and from infected patients and asymptomatic human carriers including international travellers, (iii) had been found in various bacterial species, mostly *Escherichia coli,* and on several different plasmids, and (iv) was highly transferrable with in-vitro transfer.

Plasmid-mediated colistin resistance lies at the interface between animal health and human health. Polymyxins and colistin (in particular) have been used, both in human and veterinary medicine, for more than 50 years, although their parenteral usage in humans

has been limited because of concerns about nephrotoxicity and neurotoxicity. In veterinary medicine, colistin is widely used, especially for controlling diarrhoeal diseases in pig and chicken production (Kempf *et al.,* 2013). However, its use varies widely between countries; in Europe, from 0 mg (Finland, Iceland, Norway) to more than 20 mg (Italy, Spain) per kg animal biomass were used in 2013 (CVMP, 2015). Data from other parts of the world are more-scarce, however Liu *et al.* reported that the market value for colistin for veterinary usage increased from USD 8.7 billion (EUR 8.0 billion) in 1992 to a projected USD 43 billion (EUR 39.6 billion) in 2018, with China being the largest user of a projected 12,000 tonnes in 2015 (Liu *et al.,* 2016).

ii) High Rate of *mcr-1*–Producing *E. coli* and *Klebsiella* species

The progressive global increase of antimicrobial drug resistance in *Enterobacteriaceae* is worrisome and adding to the concern is the recent discovery of the plas¬mid-mediated mobile colistin resistance (*MCR*) genes *mcr*-1 and *mcr*-2 (Liu *et al.,* 2016; Xavier *et al.,* 2016). These genes encode phosphoethanolamine transferases, which add a phosphoethanolamine group to the lipid A of the lipopolysaccharide, leading to gram-negative bacteria resistance to polymyxins (Poirel *et al.,* 2017). Since its discovery, the *mcr*-1 gene has been identified almost worldwide, mostly in animal and environmental samples (Poirel *et al.,* 2017) and to a lesser extent in human clinical samples (Liassine *et al.,* 2016). The *mcr*-1 gene has often been identified from *E. coli* strains recovered from pigs (Perrin-Guyomard *et al.,* 2007; Irrgang *et al.,* 2016; Poirel *et al.,* 2017; Guenther *et al.,* 2017; Huang *et al.,* 2017). More recently, the *mcr*-2 gene, which shares 76.8% identity with *mcr*-1, has been identified from a single *E. coli* isolate recovered from a pig in Belgium (Xavier *et al.,* 2016). The genetic element related to the *mcr*-2 gene are possibly involved in its acquisition is insertion sequence (IS) ISEc69 (Kieffer *et al.,* 2017).

The *mcr*-1 gene has been identified on a large variety of plasmids, such as IncI2, IncX4, IncHI2, IncP, IncFI, IncFII, IncFIB, and IncY (Poirel *et al.,* 2016; Xavier *et al.,* 2016 and Zhang *et al.* (2017). The genetic context of the *mcr*-1 gene always includes the *mcr*-1 cassette, as previously described (Poirel *et al.,* 2016; Zurfluh *et al.,* 2016). In addition, ISApl1 is often found upstream of the *mcr*-1 gene. It has been recently shown that the second copy of ISApl1 may be found downstream of the *mcr*-1 gene, therefore bracketing the 2.6-kb *mcr*-1 cassette and forming the composite transposon Tn6330, demonstrated to be functional and responsible for the transposition of *mcr*-1 (Zurfluh *et al.,* 2016; Poirel *et al.,* 2017).

Worldwide, colistin is widely used in veterinary medicine for different purposes, including treatment of enteric infections, prophylaxis or metaphylaxis (Rhouma *et al.,* 2016), and as a growth promoter in several countries (Katsunuma *et al.,* 2007). Despite this selective pressure, studies reporting the identification of colistin-resistant *Enterobacteriaceae* in veterinary medicine remain scarce, although an overall low prevalence of those resis¬tant strains was noticed in Europe (Perrin-Guyomard *et al.,* 2016; Xavier *et al.,* 2016; Quesada *et al.,* 2016; Falgenhauer *et al.,* 2016).

iii) Genetic Features of *mcr-1*-Producing Colistin-Resistant organisms

The recent identification of a plasmid-encoded polymyxin resistance mechanism (*mcr*-1) among human and animal enterobacterial isolates is a source of concern (Liu *et al.,* 2016). Actually, polymyxins (colistin and polymyxin B) are the last-resort antibiotics for treating infections caused by carbapenemase producers (Poirel *et al.,* 2016). *MCR*-1 is a phosphoethanolamine transferase that modifies the lipopolysaccharide by adding phosphoethanolamine to lipid A, leading to resistance to polymyxins (Liu *et al.,* 2016). This resistance trait is transferable and has been reported so far mostly in

Enterobacteriaceae from animal isolates but also in those from human isolates and from food products (Figueiredo *et al.,* 2015; Webb *et al.,* 2016; Arcilla *et al.,* 2016; Haenni *et al.,* 2016). First identified in China as published in November 2015 (Liu *et al.,* 2016), *MCR*-1-producing isolates are mostly *E. coli* strains that have been reported in many different countries scattered throughout Europe, Asia, and North America. In Africa, PCR and in silico analysis identified a few *MCR*-1-positive *E. coli* isolates from chicken from Algeria and from a single human *E. coli* isolate from Nigeria (Olaitan *et al.,* 2016).

In a study in South Africa which initiated the isolation of seven colistin-resistant enterobacterial *E. coli* isolates from patients hospitalized in different hospitals in Johannesburg and Pretoria, South Africa, and also from community patients in Johannesburg from March 2014 to June 2015. The report of the study of clonal relationships of the isolates conducted in Johannesburg and Pretoria, from isolates obtained from patients hospitalized in different hospitals were first evaluated by pulsed-field gel electrophoresis analysis as described previously by Peirano *et al.* (2014). Multilocus sequence typing they performed as described previously Peirano *et al.* (2014), two out of the isolates obtained (Af31 and Af45) remained susceptible to all ß-lactams while isolates Af23 and Af24 exhibited a penicillinase phenotype related to *TEM*-1 production, and isolates Af40 and Af49 exhibited an extended-spectrum-lactamase (ESBL) phenotype related to *CTX-M*-55 (Poirel *et al.,* 2014).

According to molecular analyses conducted by Poirel *et al.* (2017) in their study, isolate Af48 exhibited an *AmpC*-type cephalosporinase phenotype related to CMY-2. Interestingly, isolate Af31 was resistant to florfenicol and possessed the floR gene that we previously identified in another *MCR*-1-positive *E. coli* isolates from Switzerland (Poirel *et al.,* 2016). Another scientific report indicates that all isolates that possess these

resistant genes are resistant to sulphonamides, tetracyclines, and fluoroquinolones, which are antibiotics that are extensively prescribed in veterinary medicine (Catry *et al.,* 2015).

Therefore, expression of *mcr*-1 may be driven by a promoter which is part of the mobile cassette, thus making this element autonomous in term of transcription. In addition, by analysis of the sequences located upstream of the *mcr*-1 cassette in plasmids, thus that common region for expression of antibiotics resistance in mrc-1 is located at the ISApl1 insertion sequence element could be identified at 9 bp upstream of the *mcr*-1 cassette (Rodriguez-Martinez *et al.,* 2006).

Until recently, resistance to colistin was thought to only rely on a chromosomally mediated modification of lipid A (Landman *et al.,* 2008). However, plasmid-mediated colistin resistance mechanism *mcr*-1 was described in November 2015 in *E. coli* strains isolated from pigs in China, as well as in raw meat and inpatients (Liu *et al.,* 2016). Since then, the *mcr*-1 gene has been described in *Escherichia coli, Klebsiella* species and *Salmonella* spp. from animals and humans in Asia, Africa, South America and Europe (Skov *et al.,* 2016).

Recently, *Salmonella* spp. that harbour *mcr*-1 positive gene was isolated from pigs and one likely produced a plasmidic cephalosporinase (El Garch *et al.,* 2016). Nevertheless, the *mcr*-1 gene was present but unseen for years in *E. coli* from European food-producing animals. El Garch *et al.* (2016) suggest that the *mcr*-1 gene is borne by animal-associated clones. The high diversity of pulsotypes indicates that no epidemic strains are spreading and supports the dissemination of *mcr*-1 via plasmids (El Garch *et al.,* 2016).

2.23 Transferable Resistance to Colistin

Although *mcr*-1 was initially identified in *Escherichia coli,* several reports confirmed its presence in other Enterobacteriaceae, including Enterobacter aerogenes and Enterobacter cloacae (Zeng *et al.,* 2016) *Klebsiella* species (Liu *et al.,* 2016; Du *et al.,* 2016), *Shigella somnei* (Pham *et al.,* 2016). The location of *mcr*-1 on mainly conjugative plasmids, which transfer in part at rather high frequencies (Liu *et al.,* 2016) may explain its occurrence in different enterobacterial species. Plasmid analysis revealed that occasionally plasmids similar in structure to the original plasmid pHNSHP45 (Liu *et al.,* 2016) have been detected in *Salmonella typhimurium* (Anjum *et al.,* 2016), *Klebsiella* species and *E. coli* (Stoesser *et al.,* 2016). Also, resistance to colistin is infrequently observed despite a daily selective pressure in patients receiving colistin by inhalation (Zavascki *et al.,* 2007). However, increasing administration of colistin for antibiotic therapy of infections by MDR organisms may lead to the emergence of colistin-resistant strains in some countries (Bialvaei and Samadi Kafil, 2015). The worldwide prevalence of *P. aeruginosa* resistance to colistin is low and may be different between regions and over time.

Other reports, however, detected *mcr*-1 on structurally diverse plasmids in *E. coli* (Sun *et al.,* 2016,

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

3.1.1 Equipment

The following equipment was used: Microscope Autoclave Hot air oven, Refrigerator, Incubator, Water bath, Weighing balance, Colony counter, System Thermocycler\ Genetic Analyzer.

3.1.2 Instruments

The following instruments were used: Sterile bottles, Test tubes, Durham tubes, Aluminium Foil paper, Forceps, Cotton wool, Meter rule, Conical flasks, Beakers, Coverslip, Hand gloves, Stirring Rods, Glass slides, Injection syringes, Wire gauze, Tripod stand, Inoculating loop, Test tube rack, Swab sticks, Masking tape, Slide, Glass rod, Whatman number (1) Filter paper, McCartney bottles, Bijou bottles, Micropipette, Disposable Petri dishes, Sterile test tubes, Sterile universal bottles, Measuring cylinder, Meter rule, Wire loop, spatula, Sterile cotton swabs, Filter paper, Bunsen burner, Nose mask, Syringes, Staining rack, Test tube rack, Wire gauze, indelible Marker.

3.1.3 Chemicals and Reagents

The following chemicals were used: Crystal Violet, Safranine, Lugol's iodine, Oxidase reagents, Kovac’s reagent, Hydrogen peroxide, Normal saline, Buffer solution, tetraoxosulphate (vi) acid, Barium chloride, Acetone reagent, Creatinine, Phenol peptone water, Sodium hydroxide, Ethyl-dimethyl tetramine (EDTA), Immersion oil, Peptone pellets, Ethidium bromide, Sodium acetate, ethanol, Methanol, Acetone, TE Buffer, 10 mM Tris- HCl, model 2000, Agarose gel, CTAB solution, Chloroform, Isoamyl alcohol,

Isopropanol, GoTaq green reaction buffer, Magnesium chloride, EDTA solution, BigDye Terminator v3.1 cycle sequencing kit.

3.1.4 Media

The following media were used: Nutrient agar, MacConkey agar, Mueller-Hinton agar, Cysteine Lactose Electrolyte Deficient (CLED) agar, Eosin-Methylene Blue agar, Luria Bertani broth, Nutrient broth, Peptone water, Mueller-Hinton broth, cetrimide agar.

Sugars: The following sugars were used: Fructose, Lactose, Galactose, Sucrose, Maltose and Glucose.

3.2 Methods

3.2.1 Ethical Consideration

Ethical approval (EBS/MENV/AD/40/222) was collected from the Research and Ethics Committee of Ebonyi State Ministry of Environment, Abakaliki. This study was carried out in line with the World Medical Association (WMA) Declaration of Helsinki on the principles for medical research involving human subjects, animal subjects and identifiable human/animal material/data. The research work was carried out in collaboration with the Ministry of Agriculture/animal breeding Abakaliki Ebonyi State, Nigeria.

3.2.2 Determination of Sample Size

The Sample size for this study was determined using Cochran's formula. This was based on previously reported prevalence rates of Gram-negative bacteria producing multidrug-resistant enzymes including Extended-spectrum beta-lactamase (ESBL) in Abakaliki (Ejikeugwu *et al.,* 2017; Nwankwo *et al.,* 2015). These studies reported prevalence's of between 28 – 41 % of ESBL and MBL production phenotypically amongst Gram-

negative bacteria from environmental samples. In order to get the sample size for this study, we pegged the prevalence rate of the production of ESBLs at 34 %. The sample size was done at 95 % confidence interval (CI) at a 0.05 precision, and the following formula (Cochran's formula) was used to evaluate this:

$$\mathbf{n = \frac{Z^2pq}{e^2}}$$

Where:

n = sample size

Z = standard normal deviation at a 95 % confidence interval (which was 1.96)

p = proportion of target population (prevalence estimated at 34 %; this implies 34/100 = 0.34)

q = alternate proportion (1-p), which was calculated as: 1 – 0.3 = 0.7

e = desired level of precision (degree of precision/significance). This was taken as 0.05

Thus, using the sample size determination formula: $\mathbf{n=Z^2pq/e^2}$

10 % miscellaneous which is 32 was added, therefore **n= 354**

3.2.3 Sample Collection and Processing

The specific environmental samples that were collected include cloacal swab samples from the cloacae region of chicken birds and anal swab samples from the anal region of cows respectively. The dignity of the animals was taken into consideration during sample collection in order to ensure that minimal or no pain was incurred by these animals as samples are collected from them. Each of the samples was collected using sterile swab sticks and was inoculated into 5 ml double strength nutrient broth and incubated for 24 h at 37^oC prior to sub-culturing onto solid culture media including eosin methylene blue (EMB), MacConkey agar (MCA) and cetrimide selective agar (Ejikeugwu *et al.*, 2017).

3.2.4 Isolation and Identification of Bacterial Isolates

Selective cultured plates with grown organisms were further characterized bacteriologically using conventional microbiological techniques such as coagulase test, Indole test, oxidase test, methyl red test, Voges Proskauer's test, Simon citrate test, catalase test, sugar fermentation test (Cheesbrough, 2006).

3.2.5 Preparation of 0.5 McFarland Turbidity Standards

Turbidity standard equivalent to 0.5 McFarland Standard was prepared by adding 1 ml of concentrated H_2SO_4 to 99 ml of distilled water and dissolving 0.5 g of dehydrated barium chloride ($BaCl_2.2H_2O$) in 50 ml of distilled water in separate reaction flasks respectively. Barium chloride solutions (0.6 ml) was added to 99.4 ml of the H_2SO_4 solution in a separate test tube and was mixed well to obtain 0.5 McFarland turbidity standards. A small portion of the turbid solution was transferred to a capped test tube stored at room temperature (28^0C). This was used to adjust and to compare the turbidity of the test bacteria in order to get a confluent growth on culture plate (Cheesbrough, 2006) when performing antimicrobial susceptibility testing (AST).

3.2.6 Standardization of Test Bacteria

All test bacteria were standardized individually before use by inoculating a 5 ml normal saline in sterile test tubes with loopful of a 24 h young culture of the test organism from a nutrient agar slant. Afterwards, dilutions using loopful of the test bacterium and sterile water was carried out in order to get the microbial population of 10^5 colonies forming unit per millilitre (cfu/ml) by comparing it with 0.5 McFarland turbidity standards (Esimone *et al.*, 2008).

3.2.7 Antimicrobial Susceptibility Testing

The antimicrobial resistance and susceptibility patterns of the isolates were determined by the Kirby – Bauer susceptibility test techniques as recommended by the NCCLS, now CLSI (2015). An overnight culture of the test bacteria grown in nutrient broth (Oxoid, UK) was adjusted to 0.5 McFarland turbidity standards. The inoculum was aseptically inoculated on the surface of Mueller-Hinton (MH) agar plate(s) using sterile swab sticks. Single antibiotic disks from different classes were aseptically impregnated on the surface of the inoculated Mueller- Hinton agar. The antibiotics discs include aztreonam (30 µg), colistin (10 µg), Cefepime (30 µg) cefoxitin (30 µg), ceftazidime (30 µg), cefotaxime (30 µg), Ceftriaxone (30 µg) imipenem (10 µg), meropenem (10 µg), ertapenem (10 µg), amoxicillin/clavulanic acid (30 µg), ofloxacin (5 µg), cloxacillin (5 µg) Gentamicin (30 µg), Tobramycin (30 µg) and ciprofloxacin (5 µg) (Oxoid, UK). The plates were incubated at 37^{o}C for 24 h, and the inhibition zone diameters (IZDs) produced by the antibiotic disks was measured with a meter rule and recorded and the inhibition zone diameter was compared to the standard breakpoints of the Clinical and Laboratory Standard Institute (CLSI, 2015).

3.2.8 Phenotypic determination of Extended Spectrum Bete Lactamases Production

The evaluation of Extended Spectrum Bete Lactamases (ESBL) production by the test isolates in this study was done using the double disc synergy test (DDST) technique as was previously described (Iroha *et al.,* 2008; Iroha *et al.,* 2010; Bradford, 2001; CLSI, 2005). Briefly, isolates suspected of producing ESBL after being screened with any of the cephalosporins: ceftazidime (30µg), ceftriaxone (30µg), cefotaxime (30µg) aztreonam and cefuroxime (30µg) was swabbed on a Mueller-Hinton (MH) agar

plate(s). A disk containing amoxicillin-clavulanic acid (10µg) was placed on the center of the MH agar plate (s), ceftazidime (30µg), ceftriaxone (30µg), cefotaxime (30µg) aztreonam and cefuroxime (30µg) were placed adjacent to the central disk at a distance of 15mm. After an overnight incubation at 37^{o} C, a ≥5mm increase in the inhibition zone diameter for either of the cephalosporins tested in combination with the central disk versus its zone when tested alone confirms ESBL production phenotypically by the DDST method (Iroha *et al.,* 2008; Iroha *et al.,* 2010; Bradford, 2001; CLSI, 2005).

3.2.9 Evaluation of *AmpC* β-lactamase Production

AmpC β-lactamase production was phenotypically detected on only isolates screened for ESBL production and found to be positive for ESBL production according to CLSI criteria (CLSI, 2005). All ESBL positive isolates were screened and tested for *AmpC* β-lactamase production using the inhibitor-based test methods of Gunjan *et al.* (2014). Ceftazidime disk (30 µg) was placed at the center on the plate and imipenem (10 µg), cefoxitin (30 µg) and amoxicillin-clavulanate (20/10 µg) disks were each placed at a distance of 20 mm from ceftazidime disk. Any blunting or flattening of the zones of inhibition between the ceftazidime disk and the inducing substrate (imipenem, cefoxitin and amoxicillin/clavulanate disk) is considered as a positive result for *AmpC* production (Gunjan *et al.,* 2014).

3.2.10 Phenotypic Detection of Metallo – Beta lactamase Production

The presence of MBL was evaluated phenotypically on the test bacterial isolates by the inhibition-based assay using EDTA. These organisms were first testes for sensitivity using carbapenem antibiotics (meropenem, imipenem and ertapenem) in order to determine their susceptibility patterns. All the organisms that were previously resistant to imipenem, meropenem and ertapenem were subjected to this test (MBL assay) using

disk diffusion technique. Antibiotic disks containing 10 µg each of meropenem and imipenem (with and without) ethylenediamine tetra-acetic acid (EDTA) was employed in the EDTA-based experiment for MBL detection. Each of the antibiotics (Meropenem, imipenem) were placed 25 mm apart. One of the antibiotics contained EDTA while the other did not. Suspected MBL positive isolates shows clear zones of inhibition (≤ 23 mm) apart with the one that contain the ethylenediamine tetra-acetatic acid (EDTA) (Ejikeugwu *et al.,* 2014, Javeed *et al.,* 2011, Walsh *et al.,* 2005; Pitout *et al.,* 2007; Varaiya *et al.,* 2008; Franco *et al.,* 2010).

3.2.11 Phenotypic Detection of Colistin Resistance in the Bacterial Isolates

All the isolated bacteria (*E. coli, Klebsiella* species and *P. aeruginosa*) from abattoir and chicken origin was phenotypically screened for resistance to colistin by determining their antimicrobial susceptibility to colistin (10 µg) (Oxoid, UK).

3.2.12 Determination of Multiple antibiotic Resistance Index (MARI)

Multiple antibiotics resistance (MAR) Indics is defined as resistance to 2 or more of the antibiotics determined as was described by Akinjogunla *et al.* (2010) and Ejikeugwu *et al.* (2017) as follows

$$\text{MARI} = \frac{\text{No of antibiotics isolates is resistant to}}{\text{No of antibiotics the organisms were teste}}$$

3.2.13 Extraction of Plasmid DNA (Genes) and Genomic DNA (gDNA) from the Bacterial Isolates

The isolation of plasmid DNA (genes) from the *Escherichia coli, Klebsiella* species and *P. aeruginosa* isolates were carried out using Zymo Plasmid miniprep kit (Epigenetics Company, USA) according to manufacturer's instruction. Genomic DNA was isolated

and prepared from the *E. coli, Klebsiella* species and *P. aeruginosa* cultures on a tryptone soya agar. Two colonies of each of the isolates were resuspended in 100 µl of distilled water and heated to 99°C for 15 min. After removing cellular debris by centrifugation at 13000×g for 2 min, the supernatant of the resultant solution was used as the template DNA for amplification of genes. The quality of the extracted chromosomal and plasmid DNA was confirmed by running the extracts on 1 % agarose gel at 100 Volts, 400 amps for 2 h.

3.2.14 Trans conjugation Study

Liquid mating methods (Van *et l.,* 2007) was used in the conjugation experiments with different donor-recipient combinations. Selected strains were used, including *E. coli*, *Pseudomonas aeruginosa* and *Klebsiella* species from abattoir and chicken isolate. Recipient laboratory strains and strains isolated for this study were grown separately overnight and then 0.5 ml and 1.0 ml of overnight-incubated donor and recipient broth cultures, respectively, were mixed in 10 ml of LB broth. The mixtures were then incubated overnight without shaking. Then, 0.2-ml volumes of each mixture at different concentration were spread onto LB-agar plates containing both of the selected antibiotics. Colonies from the selector plates were picked off and identified again after plates were incubated at 37°C for 24 h and their antibiotic resistance phenotypes were determined.

3.2.15 Molecular Detection of Extended Spectrum Beta-Lactamase Genes

This analysis aims at detecting different ESBL genes that encode ESBL production in the test bacterial isolates. The ESBL genes (*TEM*, *SHV* and *CTX-M*) were detected by polymerase chain reaction (PCR) using specific reverse and forward primers as described by previous authors (Kiratisin *et al.,* 2008; King-Ting Lim *et al.,* 2009; Bali *et*

al., 2010). A conventional PCR technique was used to identify the *TEM*, *SHV* and *CTX-M* beta-lactamase genes in the bacterial isolates using specific primers shown in Table 1. All amplified genes were sequenced in order to validate their identities, and a 1.5% agarose gel electrophoresis was run to determine the bands of the PCR products using Fermentase SM0241 molecular marker (with the size range of 80-1000kbp) as was previously described (Bali *et al.,* 2010).

Table 1: Primers for Detection of Extended – Spectrum Beta – Lactamase Genes

Primer name	Target gene	Primer sequence	Product size (bp)	PCR conditions	Reference
TEM	*bla**TEM*	F-5´-ATGAGTATTCAACATTTCCG-3´ R-5´-CCAATGCTTAATCAGTGAGC-3´	966	1 cycle of 5min at 96^{o}C, 35 cycles of 1min at 96^{o}C (**Denaturation**), 1min at 58^{o}C, 1min at 72^{o}C (**annealing**), and 1 cycle of 10min at 72^{o}C (**extension**).	Colon *et al.,* 2013
SHV	*bla**SHV*	F-5´-CTTTACTCGCTTTATCG-3´ R-5´-TCCCGCAGATAAATCACCA-3´	1007	1 cycle of 5min at 96^{o}C, 35 cycles of 1min at 96^{o}C (**Denaturation**), 1min at 58^{o}C, 1min at 72^{o}C (**annealing**), and 1 cycle of 10min at 72^{o}C (**extension**)	Gangoué-Piéboji *et al.*, 2016
CTX-M	*bla**CTX*-M	F-5´-CCCATGGTTAAAAAATCACTG-3´ R-5´-CCGTTTCCGCTATTACAAAC-3´	891	1 cycle of 5min at 96^{o}C, 35 cycles of 1min at 96^{o}C (**Denaturation**), 1min at 58^{o}C, 1min at 72^{o}C (**annealing**), and 1 cycle of 10min at 72^{o}C (**extension**).	Kaftandzieva *et al.,* 2017

Key: F = Forward, R = Reverse

3.2.15 Molecular detection of Metallo- Beta Lactamases (MBL) Genes

MBL genes including *bla*IMP-1, *blaIMP*-2, *blaVIM*-1, and *blaVIM*-2 were determined according to previously described methodology (Shibata *et al*, 2003; Franco *et al.*, 2010; Woodford *et al.*, 2004). Specific primers for MBL genes was used to detect the presence of MBL genes in the *Escherichia coli*, *Klebsiella* species and *P. aeruginosa* isolates (Table 2). Overall, the initial denaturation temperature was 95^{o}C for 2 mins, followed by 25 cycles of DNA denaturation at 95^{o}C for 30 secs. The primer annealing was carried out at 48^{o}C for 30 secs, and primer extension at 72^{o}C for 30 secs. After the last cycle, a final extension step was carried out at 72^{o}C for 2 mins. A 100 bp DNA molecular marker was used as the positive control DNA (marker) during the gel electrophoresis process while the negative control was a PCR master mix containing distilled water in place of the template DNA molecule. Gel electrophoresis of the multiplex PCR products was carried out in 1.5 % agarose gel (Inqaba Biotechnical Industries Ltd, South Africa) for 2 h at 80 V.

3.2.16 Multiplex PCR Detection of *AmpC* β-Lactamase Genes

Multiplex PCR was used to confirm the presence of *AmpC* β-lactamase genes in all the *Escherichia coli, Klebsiella* species and *P. aeruginosa* isolates as was previously described (Perez – Perez and Hanson, 2002). The primers that were used to run the multiplex PCR is shown in Table 3. All the bacterial isolates that were phenotypically positive for *AmpC* enzyme production was tested by multiplex PCR technique for the identification of specific *AmpC* genes carried on the bacterial DNA including *FOX* gene, *ACC* gene, *DHA* gene and *CMY* gene. Overall, the initial denaturation temperature was at 94 ^{o}C for 3 mins, followed by 25 cycles of DNA denaturation at 94 ^{o}C for 30 secs. The primer annealing was carried out at 64 ^{o}C for 30 secs, and primer extension at 72 ^{o}C for 1 min. After the last cycle, a final extension step was at 72^{o}C for 7 mins. A known resistant

DNA was used as a molecular marker as a positive control (marker) during the gel electrophoresis while the negative control was a PCR master mix containing nuclease-free water in the place of the template DNA molecule. Gel electrophoresis of the multiplex PCR products was carried out using 1.5 % agarose gel.

3.2.17 PCR detection of Mobile Colistin Resistance (*mcr-1, mcr-2* and *mcr-3*) Genes

Plasmid-mediated colistin-resistance genes inclusive of *mcr*-1, *mcr*-2 and *mcr*-3 genes have not been previously studied in Nigerian bacteria isolates from either the community or hospital environment. The detection of *mcr*-1, *mcr*-2 and *mcr*-3 genes in all the *Escherichia coli, Klebsiella* species and *P. aeruginosa* isolates was carried out using PCR technique as was previously described (Yin *et al.,* 2017; Liu *et al.,* 2016; Xavier *et al.,* 2016). The specific primers that were used to run the PCR and the PCR conditions are shown in Table 4.

3.2.18 Statistical Analysis

Statistically, all variables in this study were compared and analyzed using the latest version of the statistical package for social sciences (SPSS version 23.0).

Table 2. Primers for the Detection of MBL Genes

Primer name	Target gene	Primer sequence	Product size	Reference
IMP-1	*bla*$_{IMP-1}$	F1 (5'-ACC GCA GCA GAG TCT TTG CC-3')	587	Ellington *et al.*, 2016
		R1 (5'-ACA ACC AGT TTT GCC TTA CC-3')		
IMP-2	*bla*$_{IMP-2}$	F2 (5'-GTT TTA TGT GTA TGC TTC C-3')	678	Ellington *et al.*, 2016
		R2 (5'-AGC CTG TTC CCA TGT AC-3')		
VIM-1	*bla*$_{VIM-1}$	F3 (5'-AGT GGT GAG TAT CCG ACA G-3')	261	Ellington *et al.*, 2016
		R3 (5'-ATG AAA GTG CGT GGA GAC-3')		
VIM-2	*bla*$_{VIM-2}$	F4 (5'-ATG TTC AAA CTT TTG AGT AAG-3')	801	Ellington *et al.*, 2016
		R4 (5'-CTA CTC AAC GAC TGA GCG-3')		

Key: F = Forward, R = Reverse

Table 3. Primers for Multiplex PCR of *AmpC* genes

Primer name	Target gene	Primer sequence	Product size	PCR Condition	Reference
MOXM	CMY-1	F-5'-GCT GCT CAA GGA GCA CAG GAT-3' R-5'-CAC ATT GAC ATA GGT GTG GTG C-3'	520	PCR was performed using thermal cycler with cycling condition of initial denaturation step at 95^{o}C for 5 min followed by 30 cycles of Denaturation at 94^{o}C for 45sec. Annealing at 62^{o}C for 45 sec. Extension at 72^{o}C for 1 min and final extension at 72^{o}C for 5 min. Cyclic repetition resulted in exponential amplification of the DNA that lied between the oligopeptides used.	EL-Hady and Adel, 2015Ghonaim and Moaety, 2018.
DHAM	DHA-1	F-5'- AAC TTT CAC AGG TGT GCT GGG T-3' R-5'- CCG TAC GCA TAC TGG CTT TGC-3'	405	PCR products were separated on a 2% agarose gel. 3 µl of loading dye was added to 6 µl (ladder) 100bp DNA ladder (Invitrogen, life technologies), He PCR marker was also loaded into one of the wells.	Weifeng *et al.*, 2013
ACCM	ACC	F-5'- AAC AGC CTC AGC AGC CGG TTA-3' R-5'- TTC GCC GCA ATC ATCC CT AGC-3'	346	PCR was performed using thermal cycler with cycling condition of initial denaturation step at 95^{o}C for 5 min followed by 30 cycles of Denaturation at 94^{o}C for 45sec. Annealing at 62^{o}C for 45 sec. Extension at 72^{o}C for 1 min and final extension at 72^{o}C for 5 min. Cyclic repetition resulted in exponential amplification of the DNA that lied between the oligopeptides used.	Japoni-Nejad *et al.*, 2014
FOXM	FOX-1	F-5'- AAC ATG GGG TAT CAG GGA GAT G-3' R-5'- CAA AGC GCG TAA CCG GAT TGG-3'	190	Each PCR is of 20 µL volume, comprising 1× PCR buffer (pH 8.3), 1.5 mM of $MgCl_2$, 200 nM each of the deoxynucleotide triphosphates, and 40 pmol each of the forward primer. The reaction was carried out in a Techne™ thermal cycler (TC-312; Thermo Fisher Scientific,	Akinyemi *et al.*, 2017

Key: F = Forward, R = Reverse

Table 4. Primers for PCR Detection of Colistin Resistance *mcr-1, mcr-2* and *mcr-3* Genes

Primer name	Target gene	Primer sequence	Product size (bp)	PCR conditions	Ref
*MCR*1	*mcr-1*	F-5´-ATGAGTATTCAACATTTCCG-3´ R-5´-CCAATGCTTAATCAGTGAGC-3´	966	30 cycles of 95^{o}C for 30 s, 50^{o}C for 30 s, and 72^{o}C for 45 s; followed by 1 cycle of 72^{o}C for 7 min.	Yen *et al.*, (2017)
*MCR*2	*mcr-2*	F-5´-CTTTACTCGCTTTATCG-3´ R-5´-TCCCGCAGATAAATCACCA-3´	1007	30 cycles of 95^{o}C for 30 s, 50^{o}C for 30 s, and 72^{o}C for 45 s; followed by 1 cycle of 72^{o}C for 7 min.	Liu *et al.*, (2016)
*MCR*3	*mcr-3*	F-5´-TTGGCACTGTATTTTGCATTT-3´ R-5´-TTAACGAAATTGGCTGGAACA-3´	542	30 cycles of 95^{o}C for 30 s, 50^{o}C for 30 s, and 72^{o}C for 45 s; followed by 1 cycle of 72^{o}C for 7 min.	Xavier *et al.*, (2016)

Key: F = Forward, R = Reverse

CHAPTER FOUR

RESULTS

4.1 Morphology, Microscopical and Biochemical Characterization of Bacteria Pathogens isolated from Effluents from Abattoir (cow) and Chicken market in Abakaliki

The isolated bacterial pathogens were identified and characterized based on Gram staining, colony morphology and biochemical test as shown in table 5.

4.2 Recovery of Bacteria and Occurrence of Bacteria Species

Of all the 354 samples collected, 265 bacterial (74.9 %) isolates were recovered as shown in figure 1. *Escherichia coli* had the highest recovery rate at 46 % while *Klebsiella* species was the lowest at 25 %. The occurance of bacterial species in the samples is as shown in table 6.

Table 5: Characterization of Bacteria Pathogens from Effluents Samples in Abakaliki

Morphology of organisms	Indole test	Coagulase test	Catalase test	Citrate test	Voges-Proskauer test (VP)	Methyl Red test	Motility test	Oxidase test	Gram staining test	Organism suspected
Rods	+	_	+	_	_	+	+	_	_	***E. coli***
Rods	_	_	+	+	+	_	_	_	_	***Kleb***
Rods	_	_	+	+	_	_	+	+	_	***Pseudo***

Key: - = Negative, + = Positive, *E. coli = Escherichia coli, Pseudo = P. aeruginosa, Kleb = Klebsiella* species.

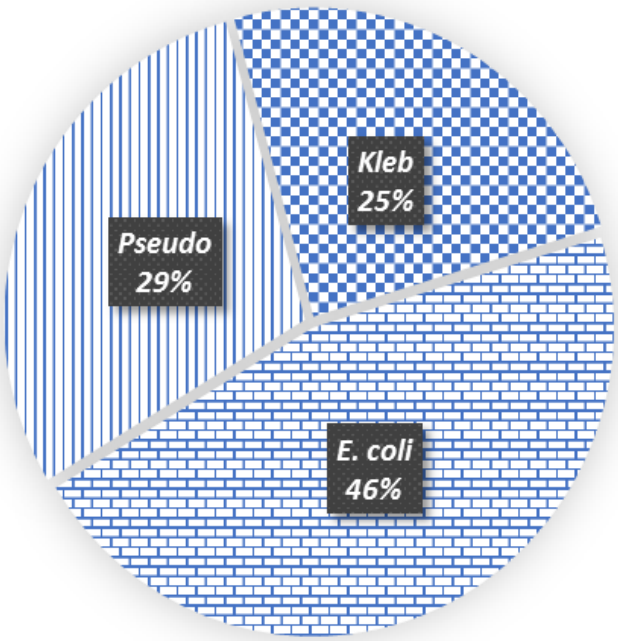

Figure 1: Percentage Prevalence of Bacterial isolated form Abattoir and Chicken Effluents.

Key: *E. coli = Escherichia coli*, *Pseudo = P. aeruginosa*, *Kleb = Klebsiella* species.

Table 6: Frequency Distribution of Bacterial Isolation from Effluents According to Collection Points

ORGANISMS	**No (%) form Abattoir**					**No (%) form Chicken Market**				
	BS	**RS**	**DS**	**DCS**	**Total No.**	**BS**	**RS**	**DS**	**DCS**	**Total No.**
E. coli (n = 122)	8 (72.7)	15(53.6)	16 (43.2)	21 (42.0)	60 (49.1)	7 (46.7)	13 (44.8)	22 (45.8)	20 (42.5)	62 (50.8)
P. aeruginosa (n = 78)	3(27.3)	11 (39.3)	10 (27.1)	14 (28.0)	38 (48.7)	5 (33.3)	8 (27.6)	14 (29.2)	13 (27.7)	40 (51.2)
Klebsiella SPP (n = 65)	3 (4.6)	4 (6.2)	9 (13.8)	12 (18.5)	28 (43.0)	3 (20.0)	8 (27.6)	12 (25.0)	14 (29.8)	37 (57.0)
TOTAL (265)	**14** (100)	**30** (100)	**35** (100)	**47** (100)	**126 (48.0)**	**15** (100)	**29** (100)	**48** (100)	**47** (100)	**139 (52.0)**

Key: BS = Butchering spot, RS = Rinsing spot, DS = Dung spot, DCS =Discharging spot, n = number, % = Per cent

4.3 Antibiotics Resistance Profile of Bacteria isolated from Effluents from Abattoir (Cow) and Chicken Markets in Abakaliki

The distribution of antibiotic resistance of bacteria in relation to collection sites are shown in tables 7 (*E. coil*), 8 (*Pseudomonas aeruginosa*) and 9 (*Klebsiella* species) below. Table 7 shows the percentage resistance of *E. coli* isolated from all the sampling points (butchering, rinsing, dung and discharging points) collected. The results indicate that the organisms express high resistance profiles among the tested antibiotics.

4.4 Multiple Antibiotic Resistance (MAR) Index of bacterial organisms

The misuse and over the counter availability of broad-spectrum antibiotics has led to the emergence of multidrug-resistant pathogens. In our study, the bacteria strains isolated showed resistance to more than two (2) classes of antibiotics and this qualifies them to be classified as multi-drug resistant organisms (MDRO). MARI value of *E, coli, Klebsiella* species and *Pseudomonas aeruginosa* isolated from abattoir and poultry markets were between 0.4 to 1 which means that the test organisms were resistance to at least more than 4 classes of antibiotics. This is a clear suggestion of misuse of antibiotics which has exposed the bacterial strains to several antibiotics, instigating the development of resistance and possibly the transfer of these resistance genes.

Table 10: Multiple Antibiotics Resistance Index of Bacterial isolated from Abattoir Effluents

S/N	*E. coli*				*Klebsiella* species				*P. aeruginosa*			
	BS	**RS**	**DS**	**DCS**	**BS**	**RS**	**DS**	**DCS**	**BS**	**RS**	**DS**	**DCS**
1	0.9	0.9	0.8	1.0	0.5	0.4	0.9	0.8	0.9	0.8	0.7	0.8
2	0.8	0.9	1.0	0.9	0.7	0.8	0.7	0.7	0.9	0.8	0.6	0.6
3	0.8	0.9	1.0	0.9	0.6	0.8	0.5	0.4	1.0	0.9	0.8	0.9
4	0.7	0.9	1.0	0.9		0.8	0.8	0.6		0.7	0.8	0.8
5	0.7	0.9	0.8	0.8			0.8	0.8		0.7	0.8	0.7
6	0.8	1.0	0.9	0.9			0.7	0.8		0.9	0.9	0.8
7	1.0	0.9	0.9	0.9			0.8	0.6		0.8	0.9	0.7
8	0.9	0.9	0.7	0.9			0.8	0.6		0.8	0.9	0.8
9		0.9	0.9	1.0			0.9	0.8		0.8	0.8	0.8
10		1.0	0.8	1.0				0.7		0.8	0.9	0.7
11		1.0	0.8	0.9				0.7		0.8		0.7
12		0.9	1.0	0.9				0.6				0.7
13		1.0	1.0	0.9								0.9
14		1.0	0.6	1.0								0.8
15		1.0	0.9	0.9								
16			0.9	0.8								
17				0.8								
18				0.9								
19				1.0								
20				0.9								
21				1.0								

Key: *E. coli = Escherichia coli, P. aeruginosa = P. aeruginosa, Klebsiella* species = *Klebsiella* species, BS = Butchering spot, RS = Rinsing spot, DS = Dung spot, DCS =Discharging spot, n = number, % = Per cent.

Table 11: Multiple Antibiotics Resistance Index of Bacterial Isolated from Effluents Samples from Chicken Market in Abakaliki

S/N	*E. coli*				*Klebsiella* species				*P. aeruginosa*			
	BS	**RS**	**DS**	**DCS**	**BS**	**RS**	**DS**	**DCS**	**BS**	**RS**	**DS**	**DCS**
1	0.8	0.9	0.9	0.8	0.6	0.8	0.5	0.7	0.9	0.8	0.8	0.9
2	0.8	0.9	0.9	0.7	0.6	0.8	0,6	0.8	0.7	0.8	0.9	0.8
3	1.0	0.8	0.9	0.6	0.5	0.9	0.3	0.9	0.7	0.7	0.9	0.8
4	0.7	0.9	0.8	0.6		0.9	0.7	0.8	0.9	0.7	0.8	0.8
5	0.7	0.9	0.9	0.7		0.9	0.7	0.7	0.9	0.8	0.9	0.6
6	1.0	0.9	0.8	0.5		0.4	0.8	0.6		0.8	0.8	0.6
7	1.0	0.9	0.8	0.8		0.6	0.5	0.9		0.8	0.7	0.8
8		0.8	0.8	0.8		0.6	0.7	0.9		0.8	0.8	0.7
9		0.8	0.8	0.8			0.8	0.8			0.6	0.9
10		0.8	0.8	0.7			0.8	0.4			0.9	0.8
11		0.7	0.8	0.8			0.6	0.6			0.5	0.8
12		0.9	0.9	0.8			0.6	0.8			0.8	0.6
13		0.9	0.8	0.6				0.8			1.0	0.8
14			0.6	0.7				0.4			0.8	
15			0.8	0.6								
16			0.9	0.6								
17			0.9	0.7								
18			0.8	0.9								
19			0.7	0.7								
20			0.7	0.8								
21			0.8									
22			1.0									

Key: *E. coli = Escherichia coli, P. aeruginosa = P. aeruginosa, Klebsiella* species = *Klebsiella* species, BS = Butchering spot, RS = Rinsing spot, DS = Dung spot, DCS =Discharging spot, n = number, % = Per cent.

4.5 Prevalence of Beta Lactam and Colistin Resistance Bacteria Isolated

From the susceptibility pattern of the bacterial isolates, we selected the strains of these bacteria (*Escherichia coli*, *P. aeruginosa* and *Klebsiella* specie) that showed high resistance profile and screen for the presence of the notable resistance genes that may perhaps prompt the recorded high resistance among the test bacteria.

Results from the *AmpC* resistance determination showed that these organisms harbour resistance *AmpC* genes with chicken market having the highest number (56) while abattoir recorded 49. *E. coli* (29) recorded highest among the test bacteria followed by *Klebsiella* species (15) and Pseudomonas aeruginosa (11).

Table 13 shows the results of the ESBL screening test conducted. Isolates from all the sampling points showed the presence of ESBL production. Similarly, all the isolates from the sampling points recorded high resistance profile against colistin.

In Table 15 below, results obtained from the screening for Metallo beta-lactam production indicate that all the test bacteria strains harbour MBL genes except *Klebsiella* species isolated from butchering point. From the results obtained, we observed a significant increase in the frequency of resistance genes to commercially available antibiotics, this could be attributed to the incessant use of antibiotics in veterinary settings within the study locations thus the incidence of recurrent of more resistant strains.

In table 16 below test bacteria organisms were found to be resistance to Metallo- beta – lactamase (MBL). From the result, abattoir effluents harbour more MBL resistance strains than chicken effluents.

From the results obtained among these organisms, *E. coli* harbour more resistant genes followed by *P. aeruginosa* and the *Klebsiella* pneumoniae. This may not be surprising since these resistant genes (*AmpC*, ESBL, Colistin and MBL) can be transferred possibly through conjugation and horizontal gene transfer. This implies that this study area harbours resistant organisms.

Table 12: Prevalence of *AmpC* producing Organisms from abattoir and chicken market in Abakaliki

ORGANISMS	ABATTOIR					CHICHKEN MARKET				
	BS	**RS**	**DS**	**DCS**	**TOTAL**	**BS**	**RS**	**DS**	**DCS**	**TOTAL**
E. coli	3	5	7	9	24	5	6	7	9	29
Kleb.	2	2	3	4	11	2	2	6	5	15
Pseudo	1	5	3	5	14	3	3	4	4	11
TOTAL	6	12	13	18	49	10	11	17	18	56

Key: BS = Butchering spot, RS = Rinsing spot, DS = Dung spot, DCS =Discharging spot,

Table 13: Prevalence of Extended Spectrum Beta Lactamases (*ESBL*) Producing Organisms from Abattoir and Chicken Market in Abakaliki

ORGANISMS	ABATTOIR					CHICHKEN MARKET				
	BS	**RS**	**DS**	**DCS**	**TOTAL**	**BS**	**RS**	**DS**	**DCS**	**TOTAL**
E. coli	3	5	4	7	19	1	4	7	7	19
Kleb.	1	1	4	3	9	2	3	6	8	18
Pseudo	0	4	4	3	11	1	1	2	3	7
TOTAL	4	10	12	13	39	4	8	15	18	44

Key: BS = Butchering spot, RS = Rinsing spot, DS = Dung spot, DCS =Discharging spot,

Table 14: Prevalence of Mobile Colistin Resistant *(mcr)* Producing Organisms from Abattoir and Chicken Market in Abakaliki

ORGANISMS	ABATTOIR					CHICHKEN MARKET				
	BS	**RS**	**DS**	**DCS**	**TOTAL**	**BS**	**RS**	**DS**	**DCS**	**TOTAL**
E. coli	0	7	9	8	24	4	5	7	4	19
Kleb.	3	3	3	5	15	0	2	4	5	11
Pseudo	3	3	7	9	21	0	2	3	4	9
TOTAL	6	13	19	22	60	4	9	13	13	39

Key: BS = Butchering spot, RS = Rinsing spot, DS = Dung spot, DCS =Discharging spot,

Table 15: Prevalence of Metallo-Beta Lactamases (MBL) Producing Organisms from Abattoir and Chicken Market in Abakaliki

ORGANISMS	ABATTOIR					CHICHKEN MARKET				
	BS	**RS**	**DS**	**DCS**	**TOTAL**	**BS**	**RS**	**DS**	**DCS**	**TOTAL**
E. coli	2	5	8	12	27	1	4	3	3	11
Kleb.	0	2	2	4	8	0	1	1	3	4
Pseudo	0	4	3	4	11	1	1	3	3	8
TOTAL	2	11	13	20	46	2	6	7	9	23

Key: BS = Butchering spot, RS = Rinsing spot, DS = Dung spot, DCS =Discharging spot

4.6 The Transferability of Resistance Genes amongst the Test Bacteria Strains

The transferability of resistance genes amongst the test bacteria strains (*Escherichia coli, P. aeruginosa* and *Klebsiella* species) isolates was done by conjugation studies. There were successful transconjugants of both in *Escherichia coli, P. aeruginosa* and *Klebsiella* species isolated from all the sampling points' samples as seen in table 17

Table 17: Successful Resistant Gene transferred by Conjugation Confirmed by DDST among Bacterial isolated from Abattoir (Cow)

Isolates	Resistance Markers Transferred	
	Abattoir	**Chicken Market**
Escherichia coli	CAZ, IPM, CTX, FEP, CN, CIP, OB, FOX, CRO	MEM, OB, ATM, CAZ, IPM, ETP, CTX, FOX, CRO, AMC
Klebsiella species	CAZ, CTX, ETP, OB, CRO, CIP, OFX. CT	CAZ, OB, FOX, CTX, ATM, ETP, OB, CRO, CIP, OFX. MEM, CT, AMC,
Pseudomonas aeruginosa	CAZ, AMC, CTX, OFX, ATM, CRO, OB, ETP, MEM, CT, FOX	CAZ, FOX, MEM, AMC, CTX, OFX, ATM, CRO, OB, IPM, CT

Key: FEP = Cefepime, CT = Colistin sulphate, CAZ = Ceftazidime, FOX = Cefoxitin, IPM = Imipenem, CTX = Cefotaxime, OB = Cloxacillin, MEM = Meropenem, CRO = Ceftriaxone, ETP = Ertapenem, ATM = Aztreonam, CIP = Ciprofloxacin, AMC = Amoxicillin/clavulanic acid, OFX = Ofloxacin, CN = Gentamicin, TOB = Tobramycin, *Escherichia coli, Pseudomonas aeruginosa, Klebsiella* species

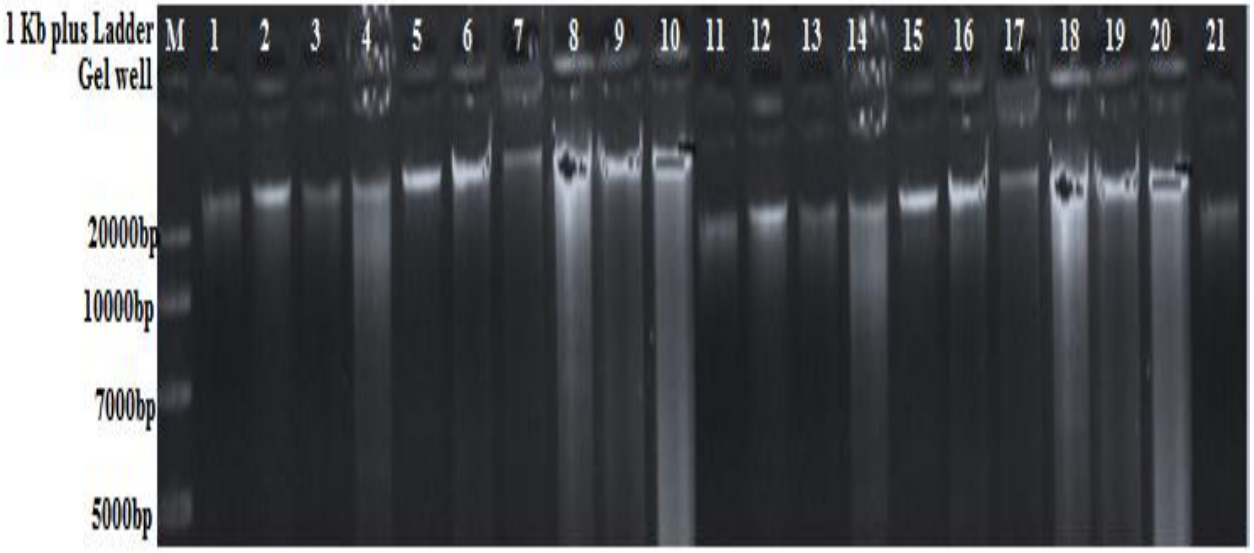

Plate 1**:** DNA Bands Extracted from *Escherichia coli, Klebsiella* species and *P. aeruginosa*

Keys: M = 1kb plus ladder, Lanes 1 – 7 = *E. coli* isolates (1 – 3 = *E. coli* from abattoir, 4 – 7 = *E. coli* from chicken), Lanes 8 – 14 = *Klebsiella* isolates (8 – 10 = *Klebsiella* from abattoir, 11 – 14 = *Klebsiella* from Chicken) and Lanes 15 – 21 = *Pseudomonas* isolates (15 – 17 = *Pseudomonas* from abattoir, 18 – 21 = *Pseudomonas* from Chicken)

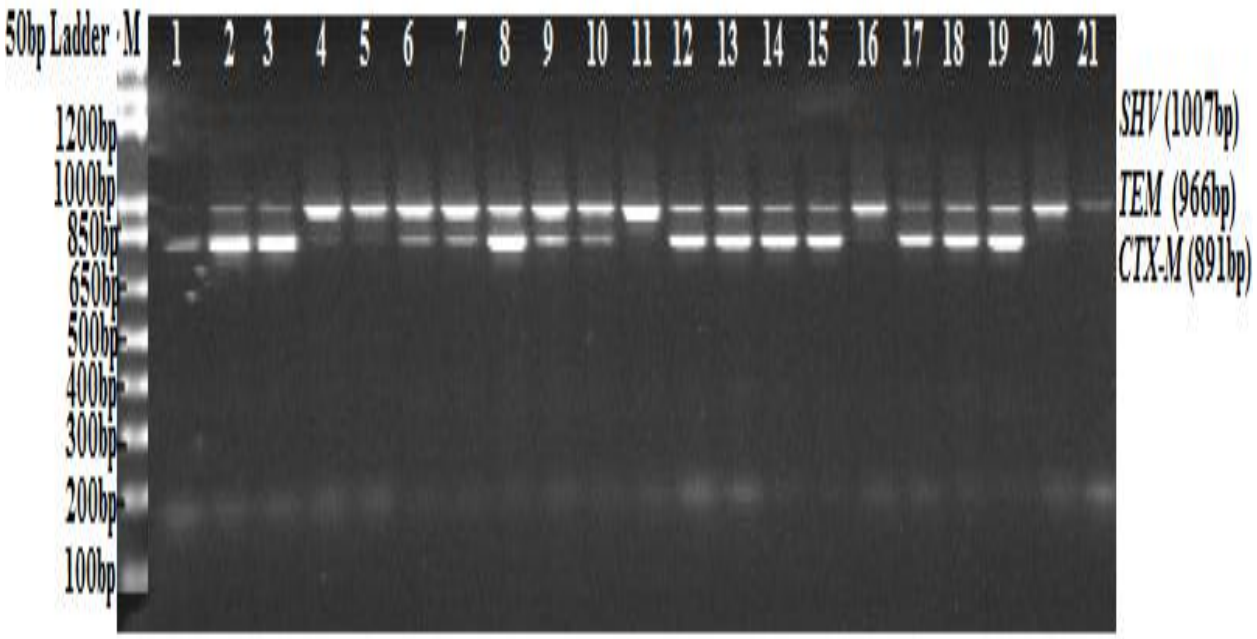

Plate 2: Molecular Characterization of Beta-lactamases Genes among *Escherichia coli, Klebsiella* species and *P. aeruginosa* isolated from abattoir and chicken effluents within Abakaliki metropolis

Keys: M = 1kb plus ladder, Lanes 1 – 7 = *E. coli* isolates (1 – 3 = *E. coli* from abattoir, 4 – 7 = *E. coli* from chicken), Lanes 8 – 14 = *Klebsiella* isolates (8 – 10 = *Klebsiella* from abattoir, 11 – 14 = *Klebsiella* from Chicken) and Lanes 15 – 21 = *Pseudomonas* isolates (15 – 17 = *Pseudomonas* from abattoir, 18 – 21 = *Pseudomonas* from Chicken)

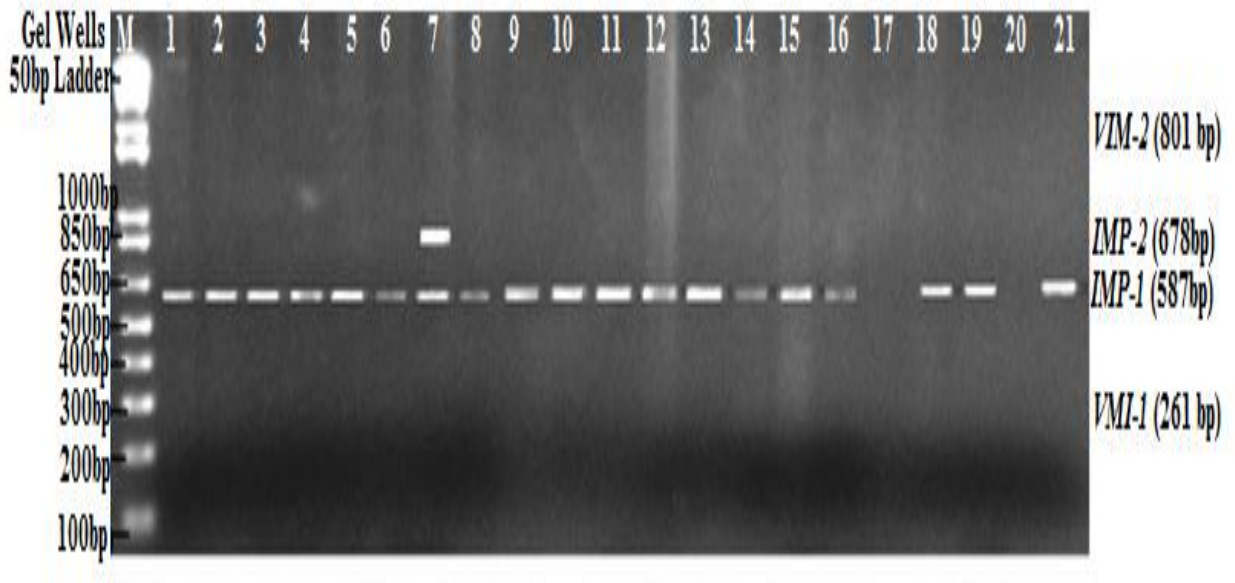

Plate 3: Molecular Characterization of MBL Genes from *Escherichia coli, Klebsiella* species and *P. aeruginosa* isolated from abattoir and chicken effluents within Abakaliki metropolis

Keys: M = 1kb plus ladder, Lanes 1 – 7 = *E. coli* isolates (1 – 3 = *E. coli* from abattoir, 4 – 7 = *E. coli* from chicken), Lanes 8 – 14 = *Klebsiella* isolates (8 – 10 = *Klebsiella* from abattoir, 11 – 14 = *Klebsiella* from Chicken) and Lanes 15 – 21 = *Pseudomonas* isolates (15 – 17 = *Pseudomonas* from abattoir, 18 – 21 = *Pseudomonas* from Chicken)

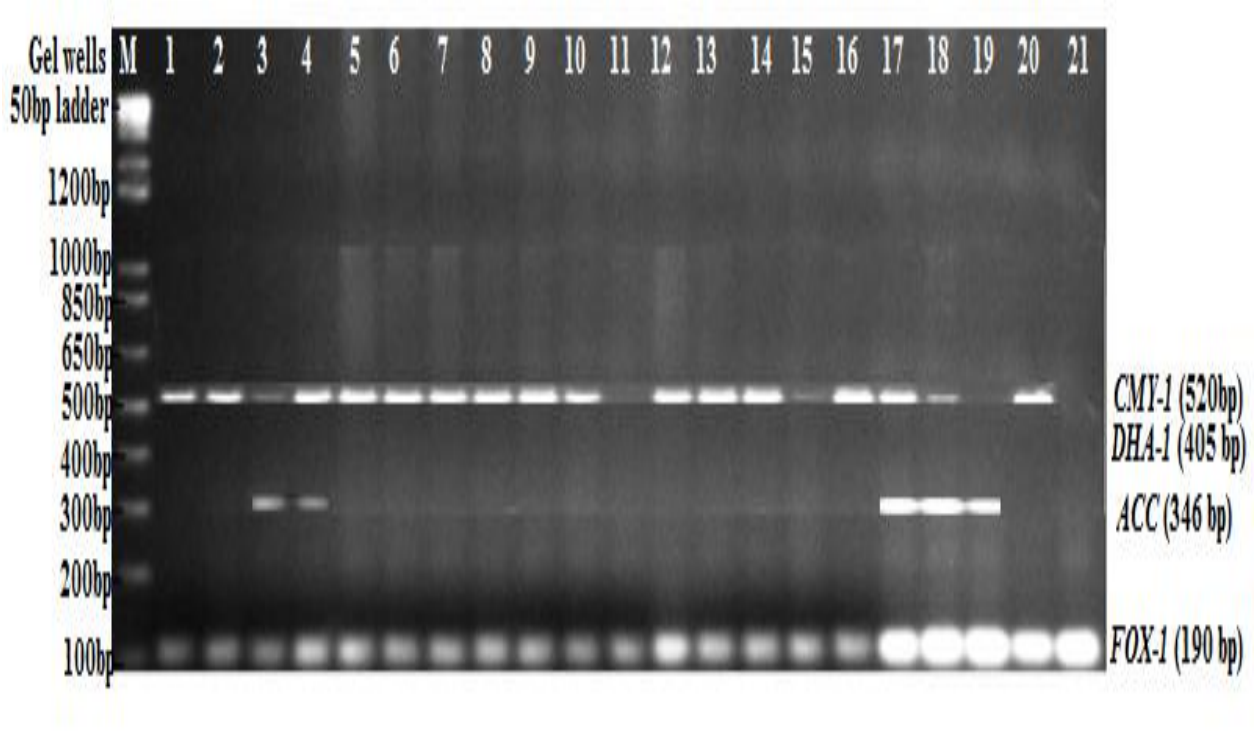

Plate 4: Molecular Characterization of *AmpC* Genes among *Escherichia coli, Klebsiella* species and *P. aeruginosa* isolated from abattoir and chicken effluents within Abakaliki metropolis

Keys: M = 1kb plus ladder, Lanes 1 – 7 = *E. coli* isolates (1 – 3 = *E. coli* from abattoir, 4 – 7 = *E. coli* from chicken), Lanes 8 – 14 = *Klebsiella* isolates (8 – 10 = *Klebsiella* from abattoir, 11 – 14 = *Klebsiella* from Chicken) and Lanes 15 – 21 = *Pseudomonas* isolates (15 – 17 = *Pseudomonas* from abattoir, 18 – 21 = *Pseudomonas* from Chicken)

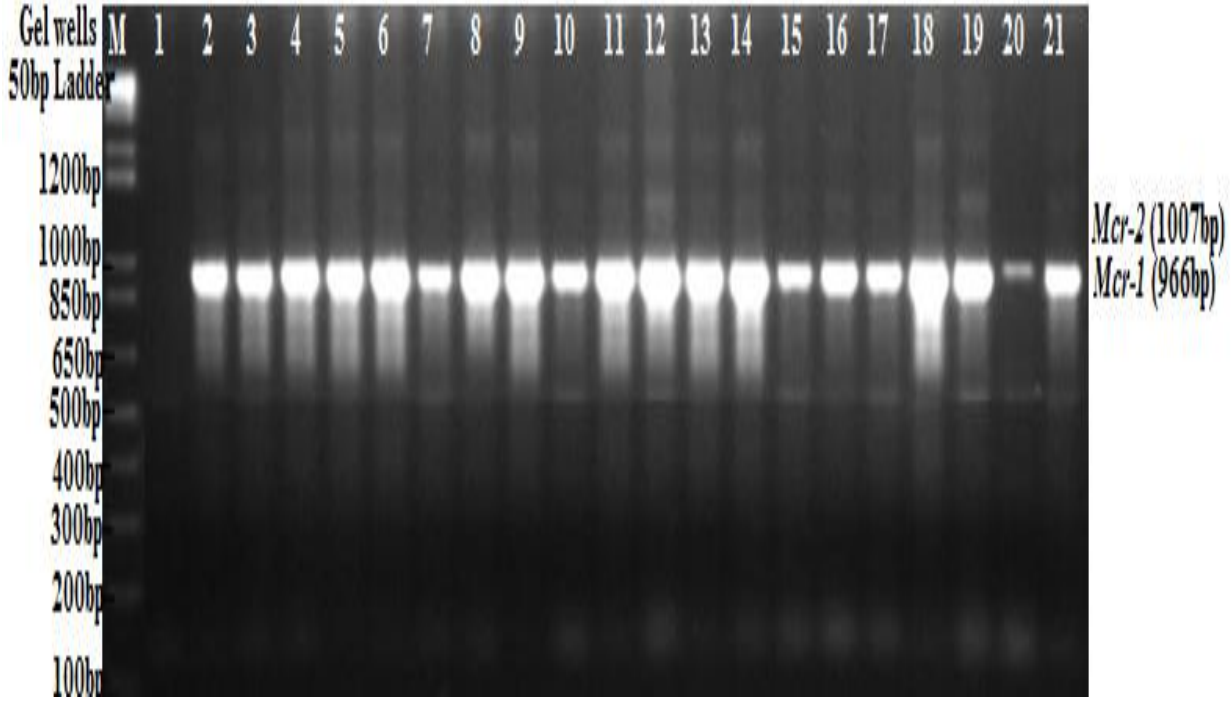

Plate 5: Molecular Characterization of Colistin Resistances Genes among *Escherichia coli, Klebsiella* species and *P. aeruginosa* isolated from abattoir and chicken effluents within Abakaliki metropolis

Keys: M = 1kb plus ladder, Lanes 1 – 7 = *E. coli* isolates (1 – 3 = *E. coli* from abattoir, 4 – 7 = *E. coli* from chicken), Lanes 8 – 14 = *Klebsiella* isolates (8 – 10 = *Klebsiella* from abattoir, 11 – 14 = *Klebsiella* from Chicken) and Lanes 15 – 21 = *Pseudomonas* isolates (15 – 17 = *Pseudomonas* from abattoir, 18 – 21 = *Pseudomonas* from Chicken)

CHAPTER FIVE

DISCUSSION, CONCLUSION, RECOMMENDATIONS AND CONTRIBUTION TO KNOWLEDGE

5.1 Discussion

Abattoir effluents are wastewater generated from the slaughtering activities of animals in the abattoirs and usually consist of intestinal contents, blood and water (Akano *et al.,* 2013; Olutayo *et al.,* 2016). Although operations in the abattoirs are of benefits to man in the provision of meat for human consumption. It can be hazardous to public health with respect to the untreated generated wastes which are discharged into the environment (Adeyemo and Adeyemo, 2007). In Nigeria, many abattoirs discharge their effluents directly into streams and rivers without any form of treatment and the slaughtered animals are washed with the same water (Adesoji *et al.,* 2016). The case was not different as we observed the same thing during sampling.

Analysis of the occurrence of *Escherichia coli, P. aeruginosa* and *Klebsiella* species from an abattoir and chicken market in Abakaliki metropolis, was carried out. The result obtained showed that *E. coli* was more prominent (46%) followed by *P. aeruginosa* (29 %) and then *Klebsiella* species (25 %) from abattoir and chicken samples in Abakaliki (Figure 1). Among the effluent samples collected *E. coli* isolates were more predominant in chicken samples (51 %) than abattoir samples (49 %). *P. aeruginosa* was also predominate with (51 %) from chicken effluent samples than abattoir effluent samples (49 %). *Klebsiella* species was also higher in chicken (57 %) in effluent samples than that of abattoir (43 %) in effluent samples. The results obtained in this study showed that chicken effluent samples harbour these organisms more than the abattoir effluents samples analysed.

Many studies have indicated that cattle are the major reservoir of *E. coli* (Armstrong *et al.,* 1996; Hancock *et al.,* 1998). Thus, this pathogen has been isolated from apparently healthy animals during an investigation of sources of human infection (Ostroff *et al.,* 1990; Chapman *et al.,* 1997; Wells *et al.,* 1999). Cattle harbour this pathogen on their hides and in their intestinal contents, and carcass contamination may occur from either hides or the leakage of faecal material during intestine removal (Grau, 1987; Ayres, 1996; Elder *et al.,* 2000). The observed incidence rate of *E. coli* in abattoir samples evaluated in this study is in line with other studies in Nigeria Iroha *et al.* (2016) and Itelima and Agina, (2011) who reported the prevalence rate of 33.3. % and 6.29 % respectively. Similar report exits in Ethiopia (Taye *et al.,* 2013), South Africa (McCluskey *et al.,* 1999 and Tanih *et al.,* 2015).

This study supports the study by Hiko *et al.* (2008) in Ethiopia, Rahimi *et al.,* 2008 and Hashemi *et al.,* 2010 in Iran. Similarly, our study supports the study by Elder *et al.* (2000) who reported a higher *E. coli* isolation rate of 27.8% from slaughtered cattle in the USA. Similarly, Dahiru *et al.* (2008) reported a very high isolation rate of 53% from Kano state, Nigeria same as in this study. The finding from our study agrees with the findings of Olayinka *et al.* (2013). In his study, he confirmed that abattoir effluents have environmental pollution propensity and abattoir effluents on surface water alters the microbial characteristic of the receiving water. On the other hand, our study disagrees with a study conducted in Egypt by El-Gamal and EL-Bahi, (2016) who reported 0.0% *E. coli* isolation from abattoir samples investigated. Nkanga and Uraih, (1981) in a study reported high prevalence rate of *E. coli* in raw meat samples from an abattoir and traditional open markets, with a prevalence rate of 85.65 %.

In Nigeria, the abattoir industry is an important component of the livestock industry providing domestic meat supply to over 150 million people and employment opportunities for teaming population (Nafaranda *et al.,* 2011). They are usually situated near aquatic environment were different untreated waste are discharged (Sangodoyin *et al.,* 1992; Benka - coker and Ojior, 1995; Adelegan, 2002) and constitute public health concern to authorities.

The emergence of strains of *Pseudomonas* species with variable and growing levels of antimicrobial resistance has generated considerable concern and various studies have sought to characterize this resistance and establish risk parameters (Nfongeh *et al.,* 2018). Isolation of *Pseudomonas* species from the effluents samples of the present study supports previous studies from abattoir samples in Nigeria (Nwankwo *et al.,* 2015 and Iroha *et al.,* 2016). In our study, *P. aeruginosa* and *Klebsiella* species isolated were observed to be 29 % and 26 % respectively. Our study agrees with the study conducted in Ibadan, Nigeria by Falodun and Adekanmbi (2016), Igbinosa *et al.,* 2011 who also isolated *Pseudomonas* species from the Bodija, Ibadan abattoir.

Abattoirs are generally known all over the world to pollute the environment either directly or indirectly from their various processes (Adelegan, 2002). Wastewaters are usually released from abattoirs directly into the ecosystems without adequate treatment process (Mittal, 2006; Arvanitoyannis and Ladas, 2008). Thus, posing serious threats to surface water quality, general environmental safety and health. Microorganisms isolated from abattoir facilities in this study have been earlier found in foods, environments and other places (Enabuelele and Uriah, 2009).

The presence of these organisms in abattoir facilities depicts a deplorable state of poor hygienic and sanitary practices employed in the slaughtering, processing, and packaging

of fresh meats. This agrees with previous reports by Okonko *et al.* (2008). Most of the organisms found in this study are those commonly found in soil and water. Presence of *E. coli* is an indication of feacal contamination of the product (Atuanya *et al.,* 2012). Sobukola *et al.* (2009), Okonko *et al.* (2008) reported the presence of *E. coli* as possible contaminant of fresh meats and meat products. Their report suggested that the contamination is possible due to unhygienic handling of the meat right from the slaughtering and beef processing.

Bacteria of the *Klebsiella* genus are responsible for a variety of diseases in animals and humans (Brisses *et al.,* 2006). Of the four disease-causing *Klebsiella* species, *Klebsiella* species is the medically most important species. *Klebsiella* species has both clinical and non-clinical habitats (Abbott, 2015). Surface water, drinking water, soil, plants, sewage, and industrial effluent are the environmental reservoirs of *Klebsiella* species (Struve *et al.,* 2004). In fact, due to its widespread nature, even in the environments apparently free from obvious faecal contamination, *Klebsiella* species is usually considered as a member of total coliforms with insignificant public health risk (WHO, 2004). However, Padschun *et al.* (2001) isolated potentially pathogenic *Klebsiella* species from the aquatic environment in Germany that possess virulence factors such as pili, serum resistance and siderophore. These virulence factors are commonly present in the clinical isolates which therefore suggest that the *Klebsiella* species of environmental origin could have public health implications (Lightfoot, 2003). In our study, twenty-five (25 %) of the organisms isolated were *Klebsiella pneumoniae*. This study supports the study by Anis *et al.* (2016) who isolated 55 % *Klebsiella* species from Malaysia. Similarly, Kim *et al.* isolated resistance *Klebsiella* species from farm animal in Oklahoma USA (Kim *et al.,* 2005).

Chicken is one of the most widespread food industries worldwide. Chicken is the most commonly farmed species, with over 90 billion tons of chicken meat produced per year (FOA, 2017). A large diversity of antimicrobials is used to raise chicken in most countries (Sahoo *et al.,* 2010; Landers *et al.,* 2012; Boamah *et al.,* 2016). Our study is in line with a study carried out in Ghana which also proved that *P. aeruginosa* is present in chicken litter (Odoi, 2016). Similarly, our study supports the study conducted in Nigeria by Aniokete *et al.,* (2016) and elsewhere by Zhang *et al.,* (2017) who also demonstrated isolation of *Pseudomonas* from chicken environment.

E. coli is a Gram-negative bacterium that has been known for ages to easily and frequently exchange genetic information through horizontal gene transfer with other related bacteria. Hence, it may exhibit characteristics based on the source of isolation. *E. coli* is a commensal organism living in the intestines of both humans and animals. However, some strains have been reported to cause gastrointestinal illnesses (Tenaillon *et al.,* 2010). In Netherland, van den Bogaard *et al.* (2001) reported high prevalence of *E. coli* from broiler and turkey. Thus, in our study 51 % of the organisms isolated from chicken sample were *E. coli*. Consequently, our study also supports a study carried out in Ghana by Yao, (2015) with a prevalence of 46.98% *E. coli* among the other bacteria isolated from chicken samples.

The spread of antimicrobial resistance (AMR) in pathogenic bacteria is one of the key public-health concerns and national security risks of the modern era (Smith and Caost, 2002). A lot of reports have associated the infection of *E. coli* around the world with having contact with infected cattle, and/or eating undercooked ground beef, cider, vegetables, fruits or other products (Crump *et al.,* 2002; Robinson *et al.,* 2004). Enterohaemorrhagic *E. coli* (EHEC) has been reported to persist and remain infectious

for several weeks in sewage sludge, pasture land, farmyard manure and slurries (Duffy, 2003).

Treatment of humans and animals using antibiotics affects not only the targeted pathogenic bacteria but also the complex commensal microbial communities (Nahid *et al.,* 2006). This contributes to the emergence of resistance among commensal bacteria and create serious side effect of antibiotic usage in human and veterinary medicine (Kucheria *et al.,* 2005). Possibly transfer genetic resistance elements to other members of the microbiota (Levy, 2000).

The result obtained in this study showed high resistant profile to polymyxin, cephalosporins and carbapenem used. There is tendency of these organisms to acquire or transfer resistance plasmid to susceptible ones. The susceptibility of *E. coli* to ofloxacin and ciprofloxacin observed in this study also contradicts with the study of Kemebradikumo *et al.* (2012) in Bayelsa. On the same note, *E. coli* strains isolated from rinsing point, dump point and discharging points showed high resistant profile against the test antibiotics. Thus, our study agrees with the report in Benin by Okunola *et al.* (2012), who reported that *E. coli* associated infections are becoming highly untreatable due to antibiotics resistant, especially to the first line empirical antimicrobials such as beta-lactams.

Pathogenic *E. coli* remain a potential threat to human health with beef, broiler chickens, and pork serving as possible sources of these organisms in the environment (Ateba and Mbewe, 2011). The clinical significance of these pathogens cannot be overemphasized. Pathogenic *E. coli* is recognized as an important pathogen in outbreaks of acute diarrhoea especially in developing countries (Boisen *et al.,* 2013). In this study, *E. coli* strains isolated from chicken effluent from all the sampling points harbour resistant

genes against all the classes of antibiotics used. Carbapenem (imipenem and meropenem) were 65 % active against test organisms isolated from the discharge point. Our study contradicts the study in China which reported that all the *E. coli* isolates in their study were susceptible to all the test antibiotics (Yassin *et al.,* 2017).

Cross-contamination is another possible source of antibiotic resistance (Smith *et al.,*2007). Shared facilities such as product and slaughter from either poultry or abattoir could promote cross-contamination and possibly antibiotic resistance strains could be spread among organism and environments (Graham *et al.,* 2008; Moodley *et al.,* 2009). For example, companies with both conventional and organic products may slaughter in the same facilities, promoting cross-contamination. Production facilities that convert from one practice to another could also experience residual contamination, though there is evidence that converting from conventional to organic can reduce the frequency of resistance (Sapkota *et al.,* 2011). In all, it is noteworthy that the antibacterial resistance rates of isolates of *E. coli* from chickens were significantly higher than in abattoir (cow). This could be due to regular use of antibiotics as growth promoter in poultry than in livestock rearing.

Pseudomonas aeruginosa is being recognized as an increasing emerging organism causing opportunistic infection of clinical importance. One of the significant characteristics of public health *Pseudomonas* possessed is its reduced antibiotic susceptibility (Igbinosa and Odjadjare, 2015). This abridged susceptibility is usually associated to a strenuous action of multiple antibiotic efflux pumps coupled with chromosomally mediated antibiotic resistance determinants (such as mexXY, mexAB-oprM) and the reduced permeability of the bacterial cellular envelopes by antimicrobial agents (Igbinosa *et al.,* 2012). In our study, it indicates that environmental *P. aeruginosa*

isolated from an abattoir effluent has significant high levels of antibiotic resistance in both the abattoir and chicken effluents.

Our study correlates with the previous reports which have found comparable prevalence levels of resistant *P. aeruginosa* in both the hospital and larger community (Bodey *et al.,* 2008; Lister *et al.,* 2009). Elsewhere, our study disagrees with the study of Igbinosa *et al.* (2012) who reported 11.8% resistance of *P. aeruginosa* isolated from abattoir environment while *P. aeruginosa* isolated showed resistance profile of 28 and 38 % in abattoir and chicken respectively. This differences in percentage might be because of the sample size, our sample size is bigger than their sample size. Our study agrees with the study of Ejikeugwu *et al.* who reported in their study that *P. aeruginosa* isolates were highly resistant to ceftriaxone, ceftazidime, ampicillin, gentamicin, and cefoxitin (Ejikeugwu *et al.,* 2015).

Also noteworthy is the remarkable resistance of *P. aeruginosa* isolated in our study against cloxacillin which is not often used to treat infections but can be administered in cases of drug resistance. Ejikeugwu *et al.* (2015), reported that cloxacillin were active against *P. aeruginosa* but in our study cloxacillin had no activity against the test bacteria strains. The astonishing results from our study are the nudging cases of carbapenem-resistant expressed by these organisms from both chicken and abattoir effluents. The results obtained in our study is similar to the report of Ejikweugwu *et al.* (2017) who also in their study reported that *P. aeruginosa* was 50 % resistant to carbapenem-resistant which include imipenem (66.7%), ertapenem (61.2%) and meropenem (60.5%).

In similar studies conducted by Oduyebo *et al.* (1997) and Kesah *et al.* (1999) reported that *P. aeruginosa* strains were found to be highly susceptible to the quinolones (96%). But in our study, data obtained shows that there were high records of resistance to

quinolones such as ciprofloxacin and ofloxacin by the test organisms. Our study contradicts also the study by Aibinu *et al.* (2007) who reported that ofloxacin and ciprofloxacin were highly active against *P. aeruginosa*. The insignificant discrepancy in the sensitivity percentages observed in this study might be due to the abuse of ciprofloxacin and ofloxacin because of its availability over the counter. The zoonotic transfer of *Klebsiella* species between humans and animals foretell risk to public health because of the implication in several bacterial infections in humans (Ejikeugwu *et al.*, 2018).

The high resistant to beta-lactam antibiotic (Penicillins and cephalosporins) by *Klebsiella* species might be due to the production of beta-lactamase as was shown in the resistance pattern of the beta-lactamase producers. Beta-lactamases are recognized to hydrolyse the amide bond of the β-lactam ring of which the resultant effect is an inactive compound. Many of these β-lactamases are encoded by transposons, some of which may also carry resistance determinants to several other antibiotics: quaternary ammonium compounds, dyes (acriflavine and ethidium bromide) or heavy metals (lead, mercury and cadmium) (Pantosti *et al*, 2007). The drift of which antibiotics are becoming inactive against test bacteria as observed in our study also concurs with the significant rising trend in resistance to antibiotics as carried out by Daniel *et al.* (2012). *Klebsiella* species having recorded a high percentage of resistance in our study supports the findings of Christopher *et al.* (2013) who also reported in their study high resistant strains of *Klebsiella* species they proposed that the isolates ought to have originated from a high-risk source of contamination somewhere that antibiotics are often used indiscriminately.

The high incidence of resistant organisms as recorded in our study from both the abattoir and chicken effluents may be due to a combination of microbial characteristics such as

selective pressure on antimicrobial usage, societal and technological changes that enhance the transmission of drug-resistant organisms (Orozova *et al.,* 2008). Other reasons could be due to an increase in irrational administration and abuse of antibiotics in both hospital and non-hospital settings which might support the transmission of resistant genes among organisms or vis-a-vis between humans.

Today, it is steadily familiar that colonizing opportunistic pathogens such as extraintestinal pathogenic *Escherichia coli, Enterococcus* species, and *Clostridium difficile* may also be transmitted from food animals to humans via retail meat (Johnson *et al.,* 2005; Kim *et al.,* 2005; Pesavento *et al.,* 2014). Furthermore, antibiotic resistance has been increasing among *Enterobacteriaceae* that contaminate retail meats, particularly chicken products (NARM, 2012). Thus, there is a continued need to characterize more fully the breadth and public health relevance of bacterial pathogens in our food supply. *Klebsiella* species is a colonizing opportunistic pathogen of humans and animals, and a common contaminant of retail meat (Kim *et al.,* 2005). In animals, *Klebsiella* species causes disease in cows, horses, and companion animals (Brisse *et al.,* 2009).

In humans, *K.* species frequently colonize the gut and sporadically causes extraintestinal infections (Podschun *et al.,*1998). Additionally, increasing multidrug resistance among *Klebsiella* species strains makes the clinical management of these infections more challenging (Shon *et al.,* 2013; Sanchez *et al.,* 2013). Our study supports the study by Kitchel *et al.* (2009) who reported that the notoriety of *Klebsiella* species as a multidrug-resistant pathogen can be attributed, in part, to successful lineages such as the carbapenem-resistant *Klebsiella* species. In our study, we observed that all the *Klebsiella* species isolated showed high resistance profile to carbapenem antibiotics. This could be due to diverse genetical makeup as reported by Diancourt *et al.* (2005) and Baraniak *et*

al. (2013). Thus, it is essential to characterize the origins and epidemiology of both epidemic and sporadic *Klebsiella* species strains, of which abattoir or chicken effluents may be an important reservoir.

Extended-spectrum beta-lactamases (ESBL) are enzymes that confer resistance to most beta-lactam antibiotics, including penicillins, cephalosporins, and the monobactam aztreonam. Infections with ESBL-producing organisms have been associated with poor outcomes (Ben-Ami *et al.*,2009). In this study, we evaluated for the presence of ESBL using double disc diffusion method. In our study, the multidrug resistance *E. coli*¸ *Klebsiella* species and *P. aeruginosa* respectively that were resistant to at least four (4) and above groups of antibiotics tested were screened for ESBL production. Results obtained showed that 37 % (19), 53 % (9) and 48 % (11) of *Escherichia coli, Klebsiella* species and *P. aeruginosa* (respectively) were ESBL producers.

The ESBL producing *E. coli* as observed in this study concurs with the reports of Irinth *et al.* (2007) who reported 56.7%, Husam *et al.* (2009) reported 83% and Wani *et al.* (2009) who reported 95.5% ESBL producing *E. coli* isolates from nosocomial and community-acquired infections. Also, Xiong *et al.* (2002) had explained that ESBL producing species are also resistant to other commonly used antibiotics which supports our findings. Some producers achieve outbreak status spreading among patients and locals, perhaps owing to particular pathogenicity traits (Wani *et al.*, 2009). This character severely limits the therapeutic options of patients infected with this organism. CLSI had suggested that better designing of susceptibility reports to correlate better antibiotic dosages and drug users could be the way towards accomplishing this goal (CSLI, 2015).

Phenotypic evaluation for the presence of *AmpC* genes was also carried out in this study. The result obtained from abattoir effluents showed that *E. coli* 57 % (24), *Klebsiella*

species 58 % (11) and *P. aeruginosa* 52% (14) MDR producing ESBL were both cefoxitin resistant and *AmpC* gene producers. Also, in this study, organisms from chicken effluents express *AmpC* production. The high percentages of *AmpC* beta-lactamase gene producers among ESBL producing organisms as observed in this study concur with the report of Polsfuss *et al.* (2011), who reported 69.2% *AmpC* producers among *Enterobacteriaceae*. Our study is contradicting with the study elsewhere by Bradford, (2001) who claimed that cefoxitin would be the drug of choice besides carbapenems. This might be because of the high cost of carbapenem which is not as common as cefoxitin for the treatment of MDR isolates

Although, Coudron *et al.* (1997) proposed combining antibiotics that will serve as an augmentin for the treatment of infections by bacterial (*Enterobacteriaceae*) resistant strains. His idea might be as a result of the lower occurrence of colonization observed in ESBL-producing strain reported by Piroth *et al.* (1998), where β-lactam antibiotics and β-lactam inhibitors (oxyimino-cephalosporins and amoxicillin-clavulanic acid) were used concurrently against MDR isolates that produced high susceptibility of the resistant isolates. *AmpC* beta-lactamases have been linked with high degradation of penicillins, expanded-spectrum cephalosporins (except cefepime and cefpirome), cephamycins, monobactams, and beta-lactam inhibitors (Polsfuss *et al.,* 2011). In strains with loss of outer membrane porins, high resistance to carbapenems has been recorded (Jacoby, 2009). Failure to detect these enzymes during routine laboratory practice has contributed to their hysterical spread and occasionally to therapeutic fiascoes (Thomson, 2001). *AmpC* beta-lactamases are inhibited by boronic acid and cloxacillin (Tan *et al.,* 2009). In *Escherichia coli,* regulation of chromosomal *AmpC* expression is by a weak promoter and strong attenuator genes encoded in a plasmid which results in a constitutive low-level *AmpC* expression. Mutations at the promoter site which could influence

overexpression that is different from the mode of expression in other *Enterobacteriaceae* has been reported (Mulvey *et al.,* 2005; Jacoby, 2009).

In our study, *AmpC* producing strains were found to be resistance to ceftriaxone, ceftazidime and cefotaxime. Our findings concur with the study by Jacoby (2009) who reported that overexpression of *AmpC* beta-lactamases confers resistance to broad-spectrum cephalosporins including cefotaxime, ceftazidime, and ceftriaxone. This is a problem especially in infections due to *Enterobacter aerogenes* and *Enterobacter cloacae*, where an isolate initially susceptible to these agents may become resistant upon therapy (Jacoby, 2009).

Multiple antibiotics index of the test organisms was conducted for both isolates from abattoir and chicken markets effluents. Recall that bacteria organisms became resistant to multiple antibiotics only when they harbour genes that can confer resistance to different antibiotics classes. The results of MARI obtained from our study showed that test organisms MARI value ranges between 0.4 to 1. This implies that the test organisms were resistant to more than 4 classes of antibiotics. The results obtained in our study correlates with that of van den Bogaard *et al.* (2001) Ogunleye *et al.* (2008) and Majalija *et al.* (2010).

Hetero-resistance refers to phenotypic heterogeneity of microbial clonal populations under antibiotic stress, accruing due to mutation and adaptation whose mechanism is unknown. In broad terms, hetero-resistance is defined as resistance to certain antibiotics expressed by a subset of a microbial population that is generally considered to be susceptible to these antibiotics according to traditional *in-vitro* susceptibility testing (Falagas *et al.,* 2008). Resistance to antibiotics associated with hetero-resistance is

believed to have evolved as a result of differences in susceptibilities displayed by such subsets of bacterial cells to antibiotics (Omar *et al.,* 2013).

The high percentage (58 % and 52 %) of the MDR *Klebsiella* species and *P. aeruginosa* respective isolates were observed to produced hetero-resistance to cefoxitin, imipenem, meropenem and colistin in this study. Sun *et al.* (2014) in China reported a similar prevalence (25 %) of the heteroresistant formation to imipenem, ertapenem, and meropenem. Factors such as antibiotic use and bacterial extended-spectrum β-lactamase (ESBL) production have been noted to contribute to invasive infections by hetero-resistance. Thus, Hetero-resistance could give rise to the development of intrinsic and high-level resistance to virtually all antimicrobials agents available for clinical use especially in immunocompromised patients (Omar *et al.*,2013).

Carbapenems, such as imipenem and meropenem are a class of β-lactam antibiotics with the broadest spectrum of activity compared to other β-lactam classes. They are effective against MDR nosocomial *Acinetobacter baumannii* and *P. aeruginosa* producing β-lactamase enzymes (Despande *et al.,* 2006). Although carbapenem resistance is mediated by a variety of mechanisms, it has been rarely reported (Tenovar *et al.,* 2003). Carbapenem producing strains have been reported to exhibit difficulty in treatment using β-lactamase inhibitors and resistance can spread widely into various Gram-negative bacilli (Hanan *et al.,* 2003). In this study high phenotypical resistance profiles of the test bacteria to carbapenems used is alarming.

The results obtained from the MBL test carried out showed that *Escherichia coli, Klebsiella* species and *P. aeruginosa* screened were resistance to carbapenems phenotypically. Overall carbapenem results obtained showed that the test bacterial isolates (*Escherichia coli, Klebsiella* species and *P. aeruginosa*) isolated from abattoir

effluents were above 70 % resistant to carbapenem drugs used. While isolates *Escherichia coli, Klebsiella* species and *P. aeruginosa*) from chicken effluents were above 80 % resistance to carbapenem tested. In order words, our findings correlate with the antibiotic susceptibility study result by Ugwu *et al.* (2018) who reported resistance MBL producing *E. coli* and *Klebsiella* species respectively. This implies that abuse of antibiotics in poultry. Also, our study quite disagrees with their study with respect to imipenem susceptibility. Similar increases in Gram-negative isolates from chicken had been reported in southern Europe, with Greece reporting an incidence of carbapenem resistance upwards of 60% in *Klebsiella* species isolates (ECDC, 2014).

Our report supports another study by Castanheira *et al.* (2011) who reported the presence of carbapenem producing organisms among the test bacterial isolates from effluents. Interestingly, these results reported in this study were very significant as CDC (2009) had early published that guidelines should be established in conjunction with CLSI guidelines; in order to detect resistance and carbapenemase production particularly in *Enterobacteriaceae* and immediately report to the epidemiology and infection control staff members if identified (CDC, 2009).

Evaluation for the presence of colistin resistance was conducted and overall results from abattoir effluents showed that *E. coli* organisms were 68.3 % resistant to colistin, *Klebsiella* species 53.6 % and *P. aeruginosa* 71 %. While isolates from chicken effluents showed *E. coli* 66.8 %, *Klebsiella* species 62.2 % and 60 % for *P. aeruginosa*. Colistin has been re-assessed as a critically important antimicrobial in humans due to its efficacy against multi-drug resistant Gram-negative bacteria, in particular, *P. aeruginosa*, *A. baumannii*, and *Klebsiella* species. The recent discovery of mobile colistin resistance (*mcr*) determinants in humans and animals has brought concerns regarding the future of

this antimicrobial (Apostolakos and Piccirillo, 2018). Our study is in line with the study conducted in Argentina by Dominguez *et al.* (2017) who isolated colistin resistant *E. coli* from fresh chicken faecal samples collected randomly from clinically healthy chickens (aged 4 – 6 weeks). The analysis showed that almost half of them (49 %) were found to be resistant to colistin.

In Europe, sporadic colistin resistance rates among *E. coli* and *Salmonella* of abattoir and chicken origins are in general low (Chakraborty *et al.,* 2010). Absence of resistance or very low rates has been recorded in countries where colistin is either not employed (e.g. Norway) or used in minimal amounts (e.g. Denmark) in food-producing animals (Aarestrup *et al.,* 2000; Naseer *et al.,* 2010). In large chicken meat producing countries, such as China and Brazil, the widespread use of colistin has resulted in the dissemination of resistance determinants in diverse bacterial species (Liu *et al.,* 2015). Worryingly, these bacteria are often co-resistant to other clinically important antimicrobials, such as extended-spectrum cephalosporins.

Colistin is considered a last-line antimicrobial agent retaining activity against multi-resistant bacteria recovered from humans. Even when resistance was sporadically reported, it was assumed to be obtained by mutation of regulatory genes (Olaitan *et al.,* 2014). However, a gene conferring resistance to colistin was recently reported in conjugative plasmids (Liu *et al.,* 2016). This gene (*mcr*-1) was most frequently found in *E. coli* but also in other species such as *Klebsiella* species and *Salmonella* (Doumith *et al.,* 2016). Up to date, a large number of publications demonstrated its presence in isolates collected mainly from animal samples, and to a lesser extent, in samples of human origin (Livermore *et al.,* 2007; Machado *et al.,* 2008; Nation and Elisha, 2009; Monaco *et al.,* 2014).

In our study, colistin-resistant strains of *Escherichia coli, Klebsiella* species and *P. aeruginosa* screened were confirmed as *mcr*-1 producers by PCR. In Argentina, the *mcr*-1 determinant was previously reported in *E. coli* clinical strains isolated from inpatients (Rapoport *et al.,* 2016). Our study correlate with the study in Nigeria by Ugwu *et al.* (2018) who reported presence of *mcr*-1 gene from the abattoir effluents. It should be noted that in our country, many producers of both livestock and poultry products have no idea of the havoc these resistant strains are causing to our environment and clinic. In this regard, the awareness should be carried out to educate the chicken farmer and cattle rarer of the implication of treating their livestock with colistin. Since World Health Organization recommends that the use of colistin be limited for the treatment of clinically affected animals.

In China, Liu *et al.* (2016) reported that plasmid-mediated (*mcr*-1 gene) colistin resistance *E. coli* isolates were recovered from animal, food and human in China. Also, the isolation of colistin resistance strains of organisms in our study is of concern because this drug is one of the last effective drugs for the treatment of multidrug-resistant Gram-negative infections. In Canada, Yao *et al.* (2016) isolated colistin resistant *E. coli* that is multidrug resistant and OXA - 48 produce from a patient that had lived in Egypt (Yao *et al.,* 2016). This indicates that soon, *mcr* resistance genes will disseminate and as such transfer the resistant genes to other organisms.

Evaluation for the presence of antibiotic resistance genes from the genome of 21 MDR organisms (*Escherichia coli, Klebsiella* species and *P. aeruginosa*) isolated from chicken and abattoir effluents were determined as shown in (Plate 1 – 5). Molecular characterization of ESBL genes of *E. coli* isolates from the abattoir and chicken effluents from Abakaliki showed remarkable results. The predominant genes amplified among *E.*

coli strains isolated from abattoir were *TEM* and *CTX-M*. Interestingly, test bacterial isolates from chicken effluents harbour *CTX-M* and *TEM* genes unlike the isolates from abattoir. Our study contradicts an investigation which reported that *SHV* and not *TEM* was mainly found during sampling in 2011 and 2012 in Austria (Zarfel *et al.*, 2013). In our study, *TEM* gene was present while SHV gene was absent. Recently, a higher prevalence of ESBL producing *E. coli* was determined in 5-week-old broilers compared with 3-week-old broilers, suggesting an increase during the production period (Schwaiger *et al.*, 2013). Our study correlated with the study conducted in Canada by Agunos *et al.* (2013) who reported isolating *E. coli* that were ESBL and *AmpC* producers from both chicken and turkey products in Canada (Agunos *et al.*, 2013).

The overall results of *AmpC* conducted showed that most isolates from chicken origin harbour *CMY-1* genes. Similarly, *FOX*-1 genes were expressed in all the isolates from both abattoir and chicken effluents. Interestingly, one *E. coli* isolate from both abattoir and chicken harbours *ACC* genes while three *P. aeruginosa* isolated from chicken harbours *ACC* the rest of the isolates do not harbour *ACC* genes. This indicates that these organisms can acquire or transfer this resistance gene through plasmid.

Furthermore, our study agrees with a survey of clinical isolates of *E. coli* from dogs and horses in the Netherlands. The study revealed that *E. coli* isolated were *AmpC* producers (Denisuik *et al.,* 2007). Besides, our study varies with a recent study conducted by Börjesson *et al.* (2013) in Sweden. Their study reported presence of *CMY-2* genes producing *E. coli* isolated from the broiler. Our study correlates with the study in the Czech Republic by Kolar *et al.* (2010). Their study reported one strain of ESBL-producing *E. coli* and two strains of *AmpC* producing *E. coli*. Based on PCR assays on *E. coli* isolates which phenotypically confirmed ESBL production, our results disagree with

the report of Kolar *et al.* (2010) who reported positive amplicon with *SHV* and *CTX-M* genes. In our study, only the *CTX-M* gene was amplified. The most frequent extended-spectrum beta-lactamases amplified were *CTX-M* and *TEM* associated ESBL genes.

Likewise, in both cases (chicken and abattoir isolates), we also find out that *AmpC* genes were carried on plasmids of different incompatibility groups by the presence of *CMY-1* as indicated in our study. Our study is in line with the findings of Lei *et al.* (2013) who reported that ^{bla}*CTX-M* is the most widely distributed gene encoding extended-spectrum β-lactamases globally. On the contrary, Canton and Coque, (2006) reported that within the past few years, the nature of ESBL dissemination has changed. *E. coli* is now the most frequently isolated ESBL-carrying bacterium. *CTX-Ms* have become the most frequently isolated ESBLs (Canton and Coque, 2006). Also, Igwe *et al.,* (2013) reported high prevalence of *E. coli* producing ESBL (*CTX-M* and *TEM*) genes among UTIs, diarrhoea, diabetics and wound samples in Nigeria.

Genes for *AmpC* beta-lactamases have also recently been found on plasmids that transfer non-inducible cephalosporin resistance to *Klebsiella* species. Antimicrobial resistance in *Enterobacteriaceae* isolates from the community especially from food-producing animals as shown in this study has been reported worldwide and increasing rates of resistance among *Klebsiella* spp. is a growing concern in both developed and developing countries (Aarestrup *et al.,* 2000; Acar and Rostel, 2001). According to Perez-Perez and Hanson (2002), plasmid-mediated *AmpC* enzyme producers are known to be resistant to multiple antibiotics; and this leaves few therapeutic options for the treatment of bacterial-related infections especially those caused by *AmpC* enzyme producers. Coudron, (2005) also opined that plasmid-mediated *AmpC* β-lactamases pose a big

challenge to infection control because *AmpC* gene can be expressed in larger amounts and has high transmissibility to other bacterial species.

AmpC beta-lactamase production in both enteric and non-enteric bacteria is of clinical significance because of their resistance to some available antibiotics used in clinical medicine. The low susceptibility levels of *P. aeruginosa* isolates to antibiotics as reported in this study is in accordance with the study conducted elsewhere (Abd El-Baky *et al.,* 2013). Also, Akinniyi *et al.* (2012) reported that *P. aeruginosa* isolated were also found to be resistant to commonly used antibiotics. Also, Ejikeugwu *et al.* (2016) reported a prevalence of thirty-six per cent (36 %) of *P. aeruginosa* isolates which were phenotypically confirmed to produce *AmpC* beta-lactamase. Previous studies on plasmid-mediated and/or chromosomal *AmpC* beta-lactamase production in bacteria have mainly been conducted on clinical isolates (Hanson, 2003). Our study corelates with the study of Akinniyi *et al.* (2012) who reported the production of *AmpC* enzymes in enteric bacteria in Abeokuta, Nigeria.

Other studies conducted by Amita and Rajesh (2007) and Adeleke *et al.* (2010) in Asia and Nigeria respectively have also reported that there are higher β-lactam drugs resistance in *Enterobacteriaceae* isolates. Ejikeugwu *et al.* (2016) have previously reported high resistance profile among bacteria isolates particularly, *E. coli* and *Klebsiella* isolated from animal sources. The patterns of resistance in the *Klebsiella* spp. to the tested antimicrobial agents used in this study may be due to the widespread and lengthy use of antibiotics in animals for growth purposes. Previous studies have shown that the use of antimicrobial agents for animal production allow drug-resistant microbes to emerge and spread in the community (Wegener, 2003; Winokur *et al.,* 2000; Waters *et al.,* 2011).

Phenotypic testing for ESBL-positive strains was confirmed by PCR detection of genes encoding the appropriate beta-lactamases. Taylor (2008) had noted that mutation(s) in bacteria might be responsible for some instances of drug resistance when faced with antibiotic therapy. Until recently, most ESBLs in clinical samples are from the hospital environment and they belonged to the family of *TEM* or *SHV* β-lactamase and they are produced by *Klebsiella* spp., *Enterobacter* spp., and *E. coli* (Bradford, 2001).

The emergence of carbapenem-resistant Gram-negative bacteria in both the community and hospital environments constitutes an alarming development in the field of infectious disease management and control (Walsh *et al.,* 2005; Bush and Jacoby, 2010; Walsh, 2010; Ejikeugwu *et al.,* 2014). Metallo-β-lactamases (MBLs) which belong to class B beta-lactamase of Ambler classification are enzymes that hydrolyse and confer on bacteria the exceptional ability to resist the antimicrobial action of the carbapenems such as imipenem and meropenem (Ejikeugwu *et al.*, 2016). In our study, we screened all the MDR isolates (*Escherichia coli, P. aeruginosa* and *Klebsiella pneumoniae*) obtained for the Metallo-beta – lactamase production. Our study correlates with that of Enwuru *et al.* (2017) who also reported a higher prevalence of MBL-producing *Klebsiella* species from both clinical and community samples in their study. Also, in a recent study conducted in Japan, Okazaki *et al.* (2016) reported the occurrence of *Klebsiella* species positive for MBL production in a non-hospital environment.

The occurrence of MBL-producing *E. coli* isolates in this present study is similar to the work of Leung *et al.* (2013), who reported in Australia the occurrence of MBL-producing *E. coli* from environmental samples. Chakraborty *et al.* (2010), also reported a similar prevalence of *E. coli* isolates positive for MBL production in India. Our result is corelates with the work of Bashir *et al.* (2011), who recorded a higher prevalence of

MBL producing *E. coli* isolates in their study in Kashmir. The prevalence of MBL-producing *E. coli* isolates in our study also agreed with the work of Chouchani *et al.* (2011), who reported the occurrence of MBL-producing *E. coli*. The occurrence of MBL-producing *Klebsiella* species in the present study agrees with the study conducted in Nigeria, Asia and Europe (Chakraborty *et al.,* 2010, Deshpande *et al.,* 2010; Ejikeugwu *et al.,* 2014).

In this study, results obtained from *P. aeruginosa* isolates that produce MBL enzymes corelate with a report in Asia by Abd El-Baky *et al.* (2013). In their study, 31 isolates of *P. aeruginosa* was phenotypically detected to produce MBL enzymes in Asia. Akinduti *et al.* (2012), also reported a presence of MBL-positive *P. aeruginosa* isolates in a study carried out in Southwest Nigeria. Shibata *et al.* (2003) also reported a higher epidemiological rate of MBL-producing *P. aeruginosa* isolates in Japan. Nevertheless, the result of MBL enzyme production amongst the *P. aeruginosa* isolates screened in this study also corelates with the report of Saderi *et al.* (2008) who reported a similar prevalence of MBL-producing *P. aeruginosa* isolates in their study carried out in Iran.

This resistance menace portends with major public health implications since they jeopardize the clinical significance of potent antibiotics used to treat serious infections. Microbes develop resistance to antimicrobial agents following mutation and selective pressure imposed on them by the incessant use of antibiotics (Akinduti *et al.,* 2012, Chakraborty *et al.,* 2010). The use of antibiotics in the rearing and production of livestock and chicken birds as well as in other veterinary purposes has contributed significantly to the emergence and spread of drug-resistant bacteria in the community (Ejikeugwu *et al.,* 2017). Our study agrees with the study by Akinduti *et al.* (2012), who reported in their study that *Escherichia coli, Klebsiella* species and *P. aeruginosa* were

the most prevalent organisms isolated from environmental samples including samples from chicken farms.

Evaluation of colistin resistance genes among the test bacterial organisms (*Escherichia coli, Klebsiella* species and *P. aeruginosa*) was conducted in our study. Here, we reported colistin-resistant *Escherichia coli, Klebsiella* species and *P. aeruginosa* strains producing *mcr*-1 that was recovered from a chicken and abattoir effluents samples. Results obtained showed that only *mcr*-1 gene was amplified among *mcr* -1, *mcr*-2 and *mcr*-3 screened. The emergence of plasmid-mediated colistin resistance gene *mcr*-1 raised great concern that the world was on the cusp of the post-antibiotic era (Liu *et al.*, 2016 and Peterson *et al.,* 2016). Polymyxins, including colistin, are the last resort antibiotics to treat serious infections caused by carbapenem-resistant *Enterobacteriaceae* (Li *et al.,* 2006). A similar result was reported by Hong *et al.* (2016) who isolated *mcr*-1 positive strains of *E. coli* from meat sample in China.

The antibiotic resistance genes can be transferred among bacteria of varying taxonomic groups; and the transmission of resistant microbes from animals to humans is well established (Malini and Aditi, 2016; Ugwu *et al.,* 2018).

The most important mechanisms for resistance among the foodborne pathogens include production of colistin resistance mechanism (*mcr*-1) gene, Metallo β-lactamases (MBLs), *AmpC* enzymes and extended spectrum β- lactamases (ESBLs) (Geser *et al.,*2011; Geser *et al.,* 2012; Gelinski *et al.,* 2014; Ugwu *et al.,* 2018). Since the spreading to humans is possible, the increasing incidence of multidrug-resistant (MDR) microbes among food-producing animals have fuelled curiosity in the genetics and mechanisms of resistance evolved by bacteria to thwart the effects of antibiotics.

Surveillance cultures revealed that the organism could be transmitted through the contaminated environment and the hands of personnel (Duggett *et al.,* 2017). The hyperactive resistance to antibiotics expressed by MDR ESBL observed in this study also concur with the report of Duggett *et al.* (2017) from the pig. Although, there are reports of colistin resistance in different bacterial species such as *Salmonella* from both livestock and human (Hasman *et al.,* 2015; Webb *et al.,* 2015; Doumith *et al.,* 2016; Li *et al.,* 2016). This study concurs with the study of Zurfluh *et al.* (2016) and Kluytmans-van den Bergh *et al.* (2016). In their study, they reported *mcr*-1 plasmid amplification in *Escherichia coli*, *Klebsiella* species and *Salmonella* Typhimurium, isolated from humans, meat products and pig slurry between 2012 and 2015 globally, indicating the dissemination of a stable plasmid.

Environmentally induced adaptive resistance without observable changes in genotype or mutational changes has been associated with bacterial outer membrane porin proteins, which encourages a reduction in the cell to envelop permeability induced by modification (formation of capsule or regulation), thereby causing an intrinsic multidrug resistance (Ganal *et al.,* 2007). The presence of these genes in environmental isolates should indeed be of public health concern.

5.2 Conclusion

Antibiotics resistance invasion to abattoir and chicken houses with bacterial harbouring drug-resistant enzymes such as MBL, *mcr*-1, ESBL and *AmpC* genes foretell danger for public health. The present study evaluates by molecular and phenotypic methods prevalence of antibiotics resistance genes of *Escherichia coli, Klebsiella* species and *P. aeruginosa* encoded with *mcr, MBL ESBL* and *AmpC* genes isolated from abattoir and

chicken effluent sample from different points (butchering, rinsing, dumping and discharging points).

In this present study, effluents samples from abattoir analysed showed that the predominant bacterial organisms isolated are *E. coli* (122), followed by *P. aeruginosa* (78) and *Klebsiella* species (65). The overall antibiotics susceptibility patterns of *Escherichia coli, Klebsiella* species and *P. aeruginosa* from abattoir effluents indicates that the test organisms were highly resistant. The overall antibiotics susceptibility patterns of *Escherichia coli, Klebsiella* species and *P. aeruginosa* from abattoir and chicken effluents indicates that the test organisms were highly resistant to commercially available antibiotics. This implies that antibiotics abuse in both abattoir and poultry sector. The result of *AmpC* resistance genes evaluated showed that *E. coli* recorded the highest percentage resistance followed by *P. aeruginosa* and then *Klebsiella* species. However, *P. aeruginosa, Klebsiella* species and *E. coli* express ESBL resistance. While For the mobile colistin resistance genes evaluated, *E. coli* showed 39 %, followed by *Klebsiella* species 27 % and *P. aeruginosa* 34 %. All the isolates that were tested for MBL resistance genes showed high resistance profile of the MBL enzymes. *E. coli* showed 49 %, *Klebsiella* species and *P. aeruginosa* had 22.4 % and 28.6 % respectively.

Molecular characterization of ESBL, *AmpC mcr*-1 and MBL genes in MDR *Escherichia coli, Klebsiella* species and *P. aeruginosa* isolates from abattoir and chicken effluents in Abakaliki metropolis, Nigeria showed that *CTX-M* and *TEM* were the predominant ESBL genes among MDR *Escherichia coli, Klebsiella* species and *P. aeruginosa* tested in Abakaliki, while the *IMP – 1* of MBL resistant genes was also amplified. *VIM* – 2 and only one (1) *E. coli* strain amplified *IMP* – 2 gene others organisms could express *IMP – 2* genes. *FOX*-1 genes were expressed by all the test isolates (*Escherichia coli, Klebsiella*

species and *P. aeruginosa*) while *CMY* – 1 gene dominated among the *AmpC* genes amplified. Three *Pseudomonas* isolates and 2 *E. coli* isolates only express ACC gene. In colistin gene expression, only *mcr*-1 genes were expressed by all the organisms except the 1 isolate of *E. coli*.

5.3 Recommendations

However, from the results reported in this study, we suggest as thus;

1. That stringent measures should be set by the government in order to monitor and curtail the spread of resistance strains in non-clinical sectors such as livestock and poultry sectors.

2. Antibiotics resistance needs to be seen from an ecological and environmental perspective in the sense that a new sustainable global model for the discovery, development, and distribution of antibiotics needs be developed in which the private and public sectors work in partnership.

3. Public enlightenment campaign programmes on indiscriminate drug prescription and usage (abuse of antimicrobials) and should be carried out regularly to curtail alarming increase in mortality and morbidity, increased in cost of medical treatment, diagnostic uncertainties.

4. Antimicrobial surveillance team and monitoring units should be on the global political agenda to develop a global plan to tackle the antibiotic crisis and share responsibilities for its implementation.

5.4 Study Limitations and Avenues for Future Research

This study was limited by several factors;

1) Due to the nature of the environment, we were unable to determine the species of chicken that harbour these resistant genes, there is need to exploit the wide variability of birds' densities over time to look more closely at the correlation of transmissibility between bird species.

2) Our analysis relies on phenotypic resistance data, and we, therefore, did not have the opportunity to account for the multiple genetic determinants and expression patterns that encourage resistance.

3) We used only three (3) organisms viz *Escherichia coli, Klebsiella* species and *P. aeruginosa* as a sentry organism and bank on culture-based methods, capturing only a fraction of the complex, multilevel interactions between environment, host, microorganism, and horizontally transferred of genetic elements. Similar studies in the future could use a metagenomic approach to characterize the diversity of environmental and animal reservoirs as well as the patterns and mechanisms of resistance gene exchange between these bacterial communities.

In other words, the effective monitoring of the development and spread of antimicrobial resistance in zoonotic pathogens especially those that produce high profile antibiotic-degrading enzymes such as MBLs *mcr*-1 and ESBL are critical to the containment of any disease outbreak due to these microbes. Proper sanitization, better hygienic practices and immunization should be employed in the rearing and production of food-producing animals instead of using antibiotics in order to forestall the emergence and spread of drug-resistant bacteria.

5.5 Contributions to Knowledge

1. This study is the first to report presence of *mcr*-1 gene from abattoir and chicken effluents in Abakaliki, Ebonyi State.
2. This study is the first to report *ACC* gene that mediate *AmpC* resistance among *Escherichia coli, Klebsiella* species and *P. aeruginosa* isolated from abattoir and chicken effluents in Abakaliki, Ebonyi State, Nigeria.
3. This study is the first to report *TEM* and *CTX-M* mediated resistant genes of *Escherichia coli, Klebsiella* species and *P. aeruginosa* from abattoir and chicken effluents in Abakaliki
4. This present study also confirms *IMP – 1* gene as the predominant resistant gene among metallo-beta lactamases tested.

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APPENDIX I

Table 3: Antibiotics Susceptibility patterns of *E. coli* isolated from Effulents at Cow Butcharying Spot

	ANTIBIOTICS (Break points)															
Org	FEP 18	CT 11	CAZ 21	FOX 18	IPM 16	CTX 26	OB 16	MEM 16	CRO 23	ETP 19	ATM 18	CIP 21	AMC 18	OFX 16	CN 15	TOB 15
1	22	18	16	10	8	12	10	11	16	10	10	18	14	13	09	19
2	17	10	17	18	10	11	11	18	18	11	16	16	10	16	10	0
3	21	11	18	16	12	17	20	11	19	12	15	19	8	14	09	0
4	18	9	11	14	11	14	17	14	24	13	18	23	9	13	10	00
5	19	19	8	13	14	13	13	10	25	14	19	20	11	18	11	0
6	16	7	13	12	13	14	14	20	20	18	18	24	20	10	10	0
7	13	10	12	14	14	16	10	11	18	17	17	13	12	9	9	0
8	19	7	17	10	13	15	9	10	19	15	14	11	14	14	8	0

Key: FEP = Cefapime, CT = Colistin sulphate, CAZ = Ceftazidime, FOX = Cefoxitin, IPM = Imipenem, CTX = Ceffotaxime, OB = Cefotetan, MEM = Meropenem, CRO = Ceftriaxozone, ETP = Ertapenem, ATM = Azeteronam, CIP = Ciprofloxacin, AMC = Amoxicillin/clavulanic acid, OFX = Ofloxacin, CN = Gentamicin, TOB = Tobramycin

Table 4: Antibiotics Susceptibility patterns of *E. coli* isolated from Effulents at Cow Rinsing Spot

	ANTIBIOTICS															
Org	FEP 18	CT 11	CAZ 21	FOX 18	IPM 16	CTX 26	OB 16	MEM 16	CRO 23	ETP 19	ATM 18	CIP 21	AMC 18	OFX 16	CN 15	TOB 15
1	17	9	16	10	11	11	10	10	18	17	17	21	10	12	10	11
2	16	8	15	11	12	14	16	11	11	18	18	9	12	14	9	7
3	18	9	14	17	13	15	14	12	20	11	14	10	11	14	10	11
4	19	10	10	18	14	17	10	11	21	12	18	11	12	13	11	10
5	22	9	10	10	15	18	11	10	14	14	11	14	13	15	12	9
9	11	9	12	11	10	10	12	10	12	18	12	15	14	16	11	11
7	14	10	13	16	11	11	14	11	13	19	11	17	10	12	10	10
8	16	14	14	12	12	17	15	9	20	21	10	16	9	14	10	12
9	12	15	14	13	14	20	10	11	18	21	12	14	8	13	9	9
10	11	10	15	15	15	21	11	8	17	14	13	12	9	14	9	9
11	11	10	20	9	14	20	14	11	16	18	14	13	10	10	8	9
12	10	11	23	11	19	11	10	10	15	16	14	14	14	10	9	9
13	10	9	14	12	11	12	12	10	10	15	15	16	15	11	9	10
14	11	11	13	14	14	14	13	12	11	11	16	10	10	11	10	10
15	11	12	16	15	12	15	11	14	9	10	10	10	11	11	11	12

Key: FEP = Cefapime, CT = Colistin sulphate, CAZ = Ceftazidime, FOX = Cefoxitin, IPM = Imipenem, CTX = Ceffotaxime, OB = Cefotetan, MEM = Meropenem, CRO = Ceftriaxozone, ETP = Ertapenem, ATM = Azeteronam, CIP = Ciprofloxacin, AMC = Amoxicillin/clavulanic acid, OFX = Ofloxacin, CN = Gentamicin, TOB = Tobramycin

Table 5: Antibiotics Susceptibility patterns of *E. coli* isolated from Effulents at Cow Dung Spot

	ANTIBIOTICS															
Org	**FEP 18**	**CT 11**	**CAZ 21**	**FOX 18**	**IPM 16**	**CTX 26**	**OB 16**	**MEM 16**	**CRO 23**	**ETP 19**	**ATM 18**	**CIP 21**	**AMC 18**	**OFX 16**	**CN 15**	**TOB 15**
1	16	9	21	17	10	21	17	14	20	11	17	22	11	18	9	10
2	15	10	18	14	11	11	10	10	11	10	16	11	17	11	11	10
3	14	12	17	12	10	14	12	9	12	10	10	12	21	14	12	10
4	12	11	14	13	9	11	18	10	14	10	11	18	11	10	14	11
5	11	9	13	18	8	10	21	12	15	9	12	17	22	11	13	12
6	10	08	12	21	11	9	11	13	11	12	9	16	24	10	14	14
7	17	8	14	24	10	12	10	14	24	13	9	14	10	10	11	11
8	10	18	15	20	12	12	12	15	25	11	10	20	21	9	11	15
9	11	9	21	11	11	11	11	16	10	11	10	21	14	9	12	14
10	18	10	22	15	11	12	12	19	11	10	11	11	17	9	13	13
11	21	10	24	14	12	14	14	11	12	9	14	12	18	11	14	14
12	14	9	10	13	14	15	10	12	14	9	10	14	17	12	14	14
13	14	9	10	13	14	15	10	12	14	9	10	14	17	12	14	14
14	14	9	10	14	10	10	10	18	24	9	18	24	16	14	15	16
15	16	8	12	10	9	24	11	9	11	11	21	11	9	10	16	9
16	15	11	13	9	9	11	12	9	19	8	8	9	11	11	11	11

Key: FEP = Cefapime, CT = Colistin sulphate, CAZ = Ceftazidime, FOX = Cefoxitin, IPM = Imipenem, CTX = Ceffotaxime, OB = Cefotetan, MEM = Meropenem, CRO = Ceftriaxozone, ETP = Ertapenem, ATM = Azeteronam, CIP = Ciprofloxacin, AMC = Amoxicillin/clavulanic acid, OFX = Ofloxacin, CN = Gentamicin, TOB = Tobramycin

Table 6: Antibiotics Susceptibility patterns of *E. coli* isolated from Effulents at Cow Effluents Discharging Spot

	ANTIBIOTICS															
Org	**FEP 18**	**CT 11**	**CAZ 21**	**FOX 18**	**IPM 16**	**CTX 26**	**OB 16**	**MEM 16**	**CRO 23**	**ETP 19**	**ATM 18**	**CIP 21**	**AMC 18**	**OFX 16**	**CN 15**	**TOB 15**
1	14	14	18	17	10	14	14	14	14	11	11	11	11	11	14	10
2	13	12	20	16	11	15	16	13	20	10	12	18	12	12	10	12
3	14	11	16	10	9	16	17	12	21	9	14	14	10	14	11	12
4	11	10	17	15	11	17	10	14	22	9	15	15	21	11	14	10
5	21	9	18	10	12	14	11	14	24	9	16	16	20	12	14	12
6	11	11	11	10	14	10	11	13	25	11	11	14	11	14	13	10
7	10	10	10	11	10	10	12	17	11	12	12	11	14	10	15	10
8	14	10	10	18	11	9	11	10	18	14	14	16	19	11	11	11
9	12	9	12	12	10	9	10	11	10	14	13	14	17	12	9	11
10	11	9	14	14	12	10	14	12	11	13	12	12	14	14	9	9
11	14	8	15	13	14	11	13	11	10	10	10	11	15	16	11	13
12	15	9	11	14	13	12	12	10	9	11	11	13	13	17	12	12
13	16	9	10	9	10	21	14	15	11	14	12	14	10	18	11	11
14	17	10	11	10	9	24	14	13	10	15	9	11	11	11	10	12
15	10	10	18	11	9	23	13	16	14	12	9	9	14	12	10	13
16	10	10	20	19	11	26	11	19	15	11	11	9	15	14	14	10
17	11	11	22	12	10	27	10	11	16	18	12	10	10	24	11	9
18	12	9	24	13	12	11	10	12	11	1	14	11	11	9	11	9
19	14	10	10	10	12	14	9	11	10	14	15	12	10	10	10	9
20	11	12	11	10	10	18	9	10	12	15	10	14	15	11	9	9
21	12	14	12	11	11	21	11	14	14	11	11	13	16	11	9	9

Key: FEP = Cefapime, CT = Colistin sulphate, CAZ = Ceftazidime, FOX = Cefoxitin, IPM = Imipenem, CTX = Ceffotaxime, OB = Cefotetan, MEM = Meropenem, CRO = Ceftriaxozone, ETP = Ertapenem, ATM = Azeteronam, CIP = Ciprofloxacin, AMC = Amoxicillin/clavulanic acid, OFX = Ofloxacin, CN = Gentamicin, TOB = Tobramycin

Table 7: Antibiotics Susceptibility patterns of *E. coli* isolated from Effulents at Chicken Butcharying Spot

	ANTIBIOTICS															
Org	FEP 18	CT 11	CAZ 21	FOX 18	IPM 16	CTX 26	OB 16	MEM 16	CRO 23	ETP 19	ATM 18	CIP 21	AMC 18	OFX 16	CN 15	TOB 15
1	14	8	20	14	19	20	14	26	11	16	19	14	15	14	11	10
2	13	9	19	12	18	18	8	24	10	17	18	13	14	13	10	9
3	12	8	14	17	14	13	11	10	15	14	10	14	13	10	12	9
4	18	11	13	16	16	14	17	19	14	13	11	9	10	14	11	9
5	19	9	11	18	18	17	14	18	13	10	12	10	11	12	10	9
6	16	10	10	20	11	11	13	14	10	11	14	11	10	14	11	9
7	15	9	9	11	10	9	12	10	11	12	13	10	9	15	10	9

Key: FEP = Cefapime, CT = Colistin sulphate, CAZ = Ceftazidime, FOX = Cefoxitin, IPM = Imipenem, CTX = Ceffotaxime, OB = Cefotetan, MEM = Meropenem, CRO = Ceftriaxozone, ETP = Ertapenem, ATM = Azeteronam, CIP = Ciprofloxacin, AMC = Amoxicillin/clavulanic acid, OFX = Ofloxacin, CN = Gentamicin, TOB = Tobramycin

Table 8: Antibiotics Susceptibility patterns of *E. coli* isolated from Effulents at Chicken Rinsining Spot

	ANTIBIOTICS															
Org	FEP 18	CT 11	CAZ 21	FOX 18	IPM 16	CTX 26	OB 16	MEM 16	CRO 23	ETP 19	ATM 18	CIP 21	AMC 18	OFX 16	CN 15	TOB 15
1	21	10	20	10	14	24	14	19	10	10	10	18	10	15	9	14
2	17	11	19	11	17	22	13	12	20	20	12	20	9	14	10	13
3	18	9	17	12	16	18	18	21	17	11	14	11	9	13	10	10
4	16	9	12	14	18	17	10	10	14	18	13	14	11	12	11	8
5	14	9	13	10	20	14	11	28	15	14	12	10	9	11	11	9
6	13	9	12	11	11	13	12	11	20	10	10	21	8	10	11	9
7	14	10	14	14	12	14	13	24	21	11	19	20	11	10	11	9
8	15	13	12	12	14	10	16	20	22	12	21	11	10	12	12	9
9	16	14	11	13	13	11	14	11	21	11	23	12	10	10	13	9
10	12	13	10	18	10	12	10	12	11	21	20	14	9	10	10	10
11	21	15	10	20	20	16	9	14	10	22	10	15	11	11	9	10
12	10	8	10	11	11	18	9	15	9	11	9	10	12	21	11	11
13	11	9	10	10	10	10	20	9	16	10	9	9	10	11	10	11

Key: FEP = Cefapime, CT = Colistin sulphate, CAZ = Ceftazidime, FOX = Cefoxitin, IPM = Imipenem, CTX = Ceffotaxime, OB = Cefotetan, MEM = Meropenem, CRO = Ceftriaxozone, ETP = Ertapenem, ATM = Azeteronam, CIP = Ciprofloxacin, AMC = Amoxicillin/clavulanic acid, OFX = Ofloxacin, CN = Gentamicin, TOB = Tobramycin

Table 9: Antibiotics Susceptibility patterns of *E. coli* isolated from Effulents at Chicken Dung Spot

	ANTIBIOTICS															
Org	FEP 18	CT 11	CAZ 21	FOX 18	IPM 16	CTX 26	OB 16	MEM 16	CRO 23	ETP 19	ATM 18	CIP 21	AMC 18	OFX 16	CN 15	TOB 15
1	11	14	18	16	18	22	14	18	10	10	18	14	18	14	10	11
2	10	10	14	12	17	12	13	11	11	11	10	13	17	13	10	11
3	21	11	15	10	10	18	12	14	21	14	11	12	14	14	10	12
4	12	12	20	11	20	14	11	15	24	15	14	14	13	15	10	11
5	14	9	21	12	11	10	11	10	23	10	10	13	14	17	9	10
6	13	9	11	10	24	14	10	16	12	14	11	10	15	18	9	11
7	14	13	13	10	26	15	11	18	14	10	12	17	16	20	11	11
8	14	9	14	11	28	16	9	21	13	11	14	18	11	21	12	12
9	12	11	15	12	30	21	9	24	15	12	13	21	12	11	10	13
10	13	12	16	14	11	26	9	26	10	14	12	10	18	10	10	15
11	12	14	10	14	11	26	9	26	10	14	12	10	18	10	10	15
12	11	10	11	16	28	10	12	11	12	14	21	14	10	11	10	18
13	16	10	11	17	26	29	14	16	14	15	10	17	12	12	9	10
14	17	15	12	18	25	28	16	17	15	14	21	18	13	14	12	13
15	20	9	14	21	12	11	18	11	16	15	18	20	14	13	12	14
16	11	14	13	20	14	11	14	14	18	10	17	18	15	14	12	12
17	11	14	16	21	14	10	17	12	14	10	16	11	16	15	11	12
18	10	11	10	11	18	9	18	12	9	10	11	12	17	10	11	15
19	10	11	11	10	10	9	10	14	9	21	10	12	18	17	16	13
20	10	18	11	18	21	9	11	14	10	24	12	14	20	18	10	14
21	10	19	12	17	22	11	11	13	11	25	12	14	11	11	11	10
22	10	10	14	16	11	12	10	14	11	11	11	11	10	10	11	11

Key: FEP = Cefapime, CT = Colistin sulphate, CAZ = Ceftazidime, FOX = Cefoxitin, IPM = Imipenem, CTX = Ceffotaxime, OB = Cefotetan, MEM = Meropenem, CRO = Ceftriaxozone, ETP = Ertapenem, ATM = Azeteronam, CIP = Ciprofloxacin, AMC = Amoxicillin/clavulanic acid, OFX = Ofloxacin, CN = Gentamicin, TOB = Tobramycin

Table 10: Antibiotics Susceptibility patterns of *E. coli* isolated from Effulents at Chicken Discharging Spot

	ANTIBIOTICS															
Org	FEP 18	CT 11	CAZ 21	FOX 18	IPM 16	CTX 26	OB 16	MEM 16	CRO 23	ETP 19	ATM 18	CIP 21	AMC 18	OFX 16	CN 15	TOB 15
1	11	9	20	16	20	18	10	19	20	18	10	18	10	14	17	11
2	18	15	20	16	20	18	10	19	20	18	10	18	10	14	17	11
3	17	10	21	14	24	20	18	19	21	22	17	14	12	11	15	17
4	10	11	14	13	25	21	14	21	22	24	10	13	14	20	14	18
5	16	11	15	14	11	24	15	26	24	26	16	14	15	18	15	10
6	15	9	10	12	20	25	17	18	21	27	14	15	10	17	18	11
7	14	11	11	13	18	10	18	29	22	18	15	16	12	14	10	12
8	10	12	2	14	17	10	10	25	11	19	13	18	18	15	11	14
9	11	14	14	13	14	9	11	27	18	20	12	14	21	16	14	15
10	10	15	15	15	19	21	12	26	16	24	11	14	17	17	15	14
11	11	10	21	14	21	28	14	9	20	22	12	13	15	14	11	10
12	11	11	22	18	11	11	15	10	18	11	18	10	14	12	10	11
13	18	12	11	19	10	29	18	10	13	10	14	11	10	12	18	18
14	19	10	12	10	9	31	18	18	14	21	15	12	11	14	11	11
15	20	11	14	11	21	11	17	21	15	12	16	14	21	11	11	16
16	11	14	22	12	14	10	16	24	16	14	18	13	22	11	10	14
17	11	13	24	14	28	12	10	22	21	17	14	14	18	10	9	13
18	11	10	12	15	26	10	11	21	22	18	13	15	11	11	9	10
19	10	11	18	16	11	11	11	10	24	11	14	16	24	11	18	10
20	10	11	14	17	24	11	11	16	21	10	15	11	11	10	10	10

Key: FEP = Cefapime, CT = Colistin sulphate, CAZ = Ceftazidime, FOX = Cefoxitin, IPM = Imipenem, CTX = Ceffotaxime, OB = Cefotetan, MEM = Meropenem, CRO = Ceftriaxozone, ETP = Ertapenem, ATM = Azeteronam, CIP = Ciprofloxacin, AMC = Amoxicillin/clavulanic acid, OFX = Ofloxacin, CN = Gentamicin, TOB = Tobramycin

Table 11: Antibiotics Susceptibility Patterns of *Pseudomonas aeruginosa* isolated from Effulents at Cow Butcharying Spot

	ANTIBIOTICS															
Org	FEP 18	CT 11	CAZ 21	FOX 18	IPM 16	CTX 26	OB 16	MEM 16	CRO 23	ETP 19	ATM 18	CIP 21	AMC 18	OFX 16	CN 15	TOB 15
1	14	9	14	15	19	10	13	14	21	10	13	18	12	17	10	9
2	15	8	13	13	15	14	12	21	15	12	15	17	14	12	11	9
3	12	10	16	10	12	17	15	15	16	11	12	13	11	14	10	9

Table 12: Antibiotics Susceptibility Patterns of *Pseudomonas aeruginosa* isolated from Effulents at Cow Rinsing Spot

	ANTIBIOTICS															
Org	FEP 18	CT 11	CAZ 21	FOX 18	IPM 16	CTX 26	OB 16	MEM 16	CRO 23	ETP 19	ATM 18	CIP 21	AMC 18	OFX 16	CN 15	TOB 15
1	15	10	17	14	18	13	10	16	13	13	13	13	13	13	13	15
2	13	8	16	17	17	17	15	15	17	15	15	15	14	15	15	16
3	16	10	19	16	29	21	14	19	20	18	16	16	17	12	12	13
4	17	9	20	19	25	17	12	13	21	12	13	12	21	18	10	14
5	19	8	21	16	27	16	10	16	24	14	17	14	13	10	11	12
6	21	9	23	14	11	19	11	14	21	11	13	16	15	12	12	11
7	20	13	14	11	13	10	14	16	151	12	15	14	13	14	10	10
8	12	8	17	10	14	24	16	13	18	16	18	18	16	17	13	7
9	13	10	19	11	16	26	13	18	16	10	13	13	13	13	15	10
10	23	8	13	13	18	25	14	13	15	11	19	21	11	11	12	11
11	15	9	11	14	19	11	16	15	14	15	14	22	12	12	11	11

Key: FEP = Cefapime, CT = Colistin sulphate, CAZ = Ceftazidime, FOX = Cefoxitin, IPM = Imipenem, CTX = Ceffotaxime, OB = Cefotetan, MEM = Meropenem, CRO = Ceftriaxozone,

ETP = Ertapenem, ATM = Azeteronam, CIP = Ciprofloxacin, AMC = Amoxicillin/clavulanic acid, OFX = Ofloxacin, CN = Gentamicin, TOB = Tobramycin

Table 13: Antibiotics Susceptibility Patterns of *Pseudomonas aeruginosa* isolated from Effulents at Cow Dung Spot

	ANTIBIOTICS															
Org	FEP 18	CT 11	CAZ 21	FOX 18	IPM 16	CTX 26	OB 16	MEM 16	CRO 23	ETP 19	ATM 18	CIP 21	AMC 18	OFX 16	CN 15	TOB 15
1	14	10	21	19	14	21	13	19	21	21	10	19	10	18	13	12
2	15	11	12	17	18	13	14	18	22	14	13	18	19	17	15	13
3	16	10	14	15	1	15	15	10	12	15	15	16	21	15	16	15
4	11	9	15	12	11	21	12	20	13	10	12	13	23	12	14	12
5	11	8	16	17	12	23	11	21	14	16	13	21	12	14	12	17
6	12	9	12	13	13	18	13	113	15	21	12	22	15	14	11	10
7	13	8	12	14	14	19	21	15	18	23	11	13	16	12	10	12
8	21	9	11	15	12	20	10	17	21	17	11	14	18	14	11	11
9	14	10	21	21	11	26	17	12	11	13	11	11	11	15	9	10
10	12	11	22	11	10	11	13	11	10	22	10	10	12	16	10	9

Key: FEP = Cefapime, CT = Colistin sulphate, CAZ = Ceftazidime, FOX = Cefoxitin, IPM = Imipenem, CTX = Ceffotaxime, OB = Cefotetan, MEM = Meropenem, CRO = Ceftriaxozone, ETP = Ertapenem, ATM = Azeteronam, CIP = Ciprofloxacin, AMC = Amoxicillin/clavulanic acid, OFX = Ofloxacin, CN = Gentamicin, TOB = Tobramycin

Table 14: Antibiotics Susceptibility Patterns of *Pseudomonas aeruginosa* isolated from Effulents at Cow Discharging Spot

	ANTIBIOTICS															
Org	FEP 18	CT 11	CAZ 21	FOX 18	IPM 16	CTX 26	OB 16	MEM 16	CRO 23	ETP 19	ATM 18	CIP 21	AMC 18	OFX 16	CN 15	TOB 15
1	13	10	22	12	10	11	19	14	12	19	12	21	15	13	12	13
2	14	23	21	18	11	12	17	16	14	13	19	12	18	15	14	15
3	15	11	19	13	12	14	15	15	15	12	15	14	19	17	11	14
4	21	14	18	14	14	15	18	12	12	14	13	15	20	13	12	16
5	20	11	18	15	16	16	13	21	16	15	21	16	21	15	10	10
6	12	10	12	16	17	18	13	24	13	17	17	14	21	12	11	12
7	11	8	11	21	18	21	17	22	21	19	19	11	13	19	12	12
8	10	9	15	19	13	26	13	10	22	12	10	12	15	17	10	9
9	12	12	13	10	14	27	12	19	11	14	11	13	16	14	12	10
10	15	10	12	18	19	22	11	18	26	16	13	12	12	18	14	11
11	17	11	14	16	17	21	10	14	21	22	12	10	14	19	15	13
12	18	13	15	17	18	15	13	14	14	18	12	19	17	21	17	14
13	10	12	12	11	13	12	15	16	11	12	11	1	11	13	12	12
14	19	11	19	13	15	11	12	12	10	11	14	23	12	14	11	16

Key: FEP = Cefapime, CT = Colistin sulphate, CAZ = Ceftazidime, FOX = Cefoxitin, IPM = Imipenem, CTX = Ceffotaxime, OB = Cefotetan, MEM = Meropenem, CRO = Ceftriaxozone, ETP = Ertapenem, ATM = Azeteronam, CIP = Ciprofloxacin, AMC = Amoxicillin/clavulanic acid, OFX = Ofloxacin, CN = Gentamicin, TOB = Tobramycin

Table 15: Antibiotics Susceptibility Patterns of *Pseudomonas aeruginosa* isolated from Effulents at Chicken Butcharying Spot

	ANTIBIOTICS															
Org	FEP 18	CT 11	CAZ 21	FOX 18	IPM 16	CTX 26	OB 16	MEM 16	CRO 23	ETP 19	ATM 18	CIP 21	AMC 18	OFX 16	CN 15	TOB 15
1	12	9	19	15	18	13	12	14	15	18	13	16	13	13	14	11
2	11	8	11	23	19	14	15	19	21	21	16	19	14	15	16	14
3	12	10	21	15	14	15	13	14	23	22	12	21	12	17	12	12
4	14	9	12	9	13	17	17	12	21	15	19	11	18	12	12	12
5	17	8	15	10	11	12	11	11	17	16	18	10	14	18	17	19

Table 16: Antibiotics Susceptibility Patterns of *Pseudomonas aeruginosa* isolated from Effulents at Chicken Rinsing Spot

	ANTIBIOTICS															
Org	FEP 18	CT 11	CAZ 21	FOX 18	IPM 16	CTX 26	OB 16	MEM 16	CRO 23	ETP 19	ATM 18	CIP 21	AMC 18	OFX 16	CN 15	TOB 15
1	13	11	23	15	15	21	10	12	21	21	12	17	13	13	13	13
2	12	14	17	12	16	14	13	15	12	14	14	15	19	15	16	14
3	19	16	23	15	17	16	16	13	14	16	15	13	10	16	12	12
4	12	17	21	12	12	17	13	18	12	12	21	12	13	12	13	16
5	17	13	12	16	19	21	12	14	23	13	11	11	14	13	14	12
6	15	15	10	17	12	23	10	12	21	19	12	23	17	11	16	14
7	19	12	14	12	17	11	11	10	10	10	17	22	19	6	13	12
8	21	11	13	11	18	10	12	11	20	11	12	16	12	12	12	11

Key: FEP = Cefapime, CT = Colistin sulphate, CAZ = Ceftazidime, FOX = Cefoxitin, IPM = Imipenem, CTX = Ceffotaxime, OB = Cefotetan, MEM = Meropenem, CRO = Ceftriaxozone, ETP = Ertapenem, ATM = Azeteronam, CIP = Ciprofloxacin, AMC = Amoxicillin/clavulanic acid, OFX = Ofloxacin, CN = Gentamicin, TOB = Tobramycin

Table 17: Antibiotics Susceptibility Patterns of *Pseudomonas aeruginosa* isolated from Effulents at Chicken Dung Spot

	ANTIBIOTICS															
Org	FEP 18	CT 11	CAZ 21	FOX 18	IPM 16	CTX 26	OB 16	MEM 16	CRO 23	ETP 19	ATM 18	CIP 21	AMC 18	OFX 16	CN 15	TOB 15
1	12	10	23	12	13	23	14	13	23	13	13	13	21	13	13	12
2	14	9	24	15	14	21	12	13	21	14	12	15	11	12	14	13
3	15	11	22	16	11	22	16	12	22	17	21	12	14	14	12	15
4	16	9	17	10	10	21	19	21	25	13	16	14	15	13	15	12
5	12	10	19	11	19	16	10	18	10	12	12	12	16	10	12	11
6	11	12	21	12	14	17	11	10	12	11	17	16	10	21	10	10
7	18	16	17	2	9	18	12	11	11	10	19	10	13	22	11	9
8	19	12	16	14	18	0	21	12	10	9	10	12	12	11	13	12
9	21	15	14	12	13	12	13	21	13	19	21	17	21	14	12	18
10	12	9	16	11	14	21	15	13	14	21	11	16	22	15	14	12
11	21	8	23	16	10	13	16	16	15	22	13	10	19	19	15	10
12	17	9	25	18	11	10	12	10	17	19	14	13	10	12	12	11
13	16	10	12	13	13	12	11	10	11	11	15	15	11	14	10	9
14	17	11	21	12	21	13	10	9	19	10	10	12	19	17	9	9

Key: FEP = Cefapime, CT = Colistin sulphate, CAZ = Ceftazidime, FOX = Cefoxitin, IPM = Imipenem, CTX = Ceffotaxime, OB = Cefotetan, MEM = Meropenem, CRO = Ceftriaxozone, ETP = Ertapenem, ATM = Azeteronam, CIP = Ciprofloxacin, AMC = Amoxicillin/clavulanic acid, OFX = Ofloxacin, CN = Gentamicin, TOB = Tobramycin

Table 18: Antibiotics Susceptibility Patterns of *Pseudomonas aeruginosa* isolated from Effulents at Chicken Discharging Spot

	ANTIBIOTICS															
Org	FEP 18	CT 11	CAZ 21	FOX 18	IPM 16	CTX 26	OB 16	MEM 16	CRO 23	ETP 19	ATM 18	CIP 21	AMC 18	OFX 16	CN 15	TOB 15
1	12	9	19	12	14	21	10	12	21	12	13	9	18	14	12	19
2	17	9	17	14	15	13	18	14	13	22	15	9	21	15	13	12
3	13	9	16	18	16	15	16	15	14	12	12	9	14	12	15	13
4	14	10	10	19	12	12	13	12	14	14	21	9	16	17	12	16
5	15	10	22	21	14	11	19	13	21	10	19	10	12	13	19	19
6	16	13	21	20	18	18	13	17	17	13	10	11	17	18	10	12
7	21	12	12	14	13	10	15	12	20	21	11	21	14	13	11	11
8	22	11	11	16	12	19	16	13	21	15	12	12	19	19	11	8
9	25	10	13	17	18	12	13	14	22	12	14	13	10	13	13	10
10	21	9	14	10	10	14	12	12	26	16	16	23	11	15	10	9
11	19	10	17	9	9	12	15	15	17	19	13	24	21	12	12	8
12	10	9	19	18	17	16	12	12	19	18	18	27	23	15	16	14
13	12	9	14	12	11	17	18	19	11	12	12	12	24	17	10	12

Table 19: Antibiotics Susceptibility Patterns of *Klebsiella pneumoniae* isolated from Effulents at Cow Butcharying Spot

	ANTIBIOTICS															
Org	FEP 18	CT 11	CAZ 21	FOX 18	IPM 16	CTX 26	OB 16	MEM 16	CRO 23	ETP 19	ATM 18	CIP 21	AMC 18	OFX 16	CN 15	TOB 15
1	16	13	21	20	18	18	13	17	17	13	10	11	17	18	10	12
2	21	12	12	14	13	10	15	12	20	21	11	21	14	13	11	11
3	22	11	11	16	12	19	16	13	21	15	12	12	19	19	11	10

Key: FEP = Cefapime, CT = Colistin sulphate, CAZ = Ceftazidime, FOX = Cefoxitin, IPM = Imipenem, CTX = Ceffotaxime, OB = Cefotetan, MEM = Meropenem, CRO = Ceftriaxozone, ETP = Ertapenem, ATM = Azeteronam, CIP = Ciprofloxacin, AMC = Amoxicillin/clavulanic acid, OFX = Ofloxacin, CN = Gentamicin, TOB = Tobramycin

Table 20: Antibiotics Susceptibility Patterns of *Klebsiella pneumoniae* isolated from Effulents at Cow Rinsing Spot

	ANTIBIOTICS															
Org	FEP 18	CT 11	CAZ 21	FOX 18	IPM 16	CTX 26	OB 16	MEM 16	CRO 23	ETP 19	ATM 18	CIP 21	AMC 18	OFX 16	CN 15	TOB 15
1	21	8	23	16	10	13	16	16	15	22	13	10	19	19	15	10
2	14	9	12	9	13	17	17	12	21	15	19	11	18	12	12	12
3	14	10	10	19	12	12	13	12	14	14	21	9	16	17	12	16
4	17	9	25	18	11	10	12	10	17	19	14	13	10	12	12	11

Table 21: Antibiotics Susceptibility Patterns of *Klebsiella pneumoniae* isolated from Effulents at Cow Dung Spot

	ANTIBIOTICS															
Org	FEP 18	CT 11	CAZ 21	FOX 18	IPM 16	CTX 26	OB 16	MEM 16	CRO 23	ETP 19	ATM 18	CIP 21	AMC 18	OFX 16	CN 15	TOB 15
1	13	11	23	15	15	21	10	12	21	21	12	17	13	13	13	13
2	22	11	11	16	12	19	16	13	21	15	19	12	19	19	11	9
3	19	16	23	15	17	16	16	13	14	16	15	13	10	16	12	12
4	14	10	10	19	12	12	13	12	14	14	21	9	16	17	12	16
5	14	9	12	9	13	17	17	12	21	15	19	11	18	12	12	12
6	11	8	11	23	19	14	15	19	21	21	16	19	14	15	16	14
7	13	11	10	16	14	21	16	13	23	15	12	12	19	10	11	0
8	12	14	17	12	16	14	13	15	12	14	14	15	19	15	16	14
9	12	9	19	15	18	13	12	14	15	18	13	16	13	13	14	11

Key: FEP = Cefapime, CT = Colistin sulphate, CAZ = Ceftazidime, FOX = Cefoxitin, IPM = Imipenem, CTX = Ceffotaxime, OB = Cefotetan, MEM = Meropenem, CRO = Ceftriaxozone, ETP = Ertapenem, ATM = Azeteronam, CIP = Ciprofloxacin, AMC = Amoxicillin/clavulanic acid, OFX = Ofloxacin, CN = Gentamicin, TOB = Tobramycin

Table 22: Antibiotics Susceptibility Patterns of *Klebsiella pneumoniae* isolated from Effulents at Cow Discharging Spot

	ANTIBIOTICS															
Org	FEP 18	CT 11	CAZ 21	FOX 18	IPM 16	CTX 26	OB 16	MEM 16	CRO 23	ETP 19	ATM 18	CIP 21	AMC 18	OFX 16	CN 15	TOB 15
1	13	10	21	10	13	13	15	21	21	23	13	23	15	13	14	12
2	16	10	23	13	15	14	13	22	13	21	21	22	12	12	13	13
3	13	9	22	15	12	16	21	24	14	25	21	24	18	21	19	11
4	12	9	12	12	16	18	16	15	12	24	23	21	14	14	18	11
5	14	12	13	13	17	21	14	12	17	21	10	10	15	17	10	12
6	15	13	16	16	13	25	13	23	12	22	11	13	17	12	10	10
7	17	11	18	17	18	27	11	25	24	19	14	13	12	19	9	18
8	19	10	19	19	19	10	10	21	21	10	14	14	19	20	9	10
9	21	13	10	17	10	13	17	23	19	11	16	17	13	12	9	17
10	22	11	11	10	11	21	16	11	25	13	12	15	21	13	12	10
11	24	10	12	11	21	22	19	10	21	23	16	19	15	15	11	13
12	19	10	21	21	10	20	21	9	23	21	12	13	17	13	10	16

Key: FEP = Cefapime, CT = Colistin sulphate, CAZ = Ceftazidime, FOX = Cefoxitin, IPM = Imipenem, CTX = Ceffotaxime, OB = Cefotetan, MEM = Meropenem, CRO = Ceftriaxozone, ETP = Ertapenem, ATM = Azeteronam, CIP = Ciprofloxacin, AMC = Amoxicillin/clavulanic acid, OFX = Ofloxacin, CN = Gentamicin, TOB = Tobramycin

Table 23: Antibiotics Susceptibility Patterns of *Klebsiella pneumoniae* isolated from Effulents at Chicken Butcharying Spot

	ANTIBIOTICS															
Org	FEP 18	CT 11	CAZ 21	FOX 18	IPM 16	CTX 26	OB 16	MEM 16	CRO 23	ETP 19	ATM 18	CIP 21	AMC 18	OFX 16	CN 15	TOB 15
1	11	8	11	23	19	14	15	19	21	21	16	19	14	15	16	14
2	21	13	10	17	10	13	17	23	19	11	16	17	13	12	9	17
3	13	9	22	15	12	16	21	24	14	25	21	24	18	21	19	11

Table 24: Antibiotics Susceptibility Patterns of *Klebsiella pneumoniae* isolated from Effulents at Chicken Rinsing Spot

	ANTIBIOTICS															
Org	FEP 18	CT 11	CAZ 21	FOX 18	IPM 16	CTX 26	OB 16	MEM 16	CRO 23	ETP 19	ATM 18	CIP 21	AMC 18	OFX 16	CN 15	TOB 15
1	14	10	10	19	12	12	13	12	14	14	21	9	16	17	12	16
2	21	12	12	14	13	10	15	12	20	21	11	21	14	13	11	11
3	12	10	19	11	19	16	10	18	10	12	12	12	16	10	12	11
4	25	10	13	17	18	12	13	14	22	12	14	13	10	13	13	10
5	13	9	22	15	12	16	21	24	14	25	21	24	18	21	19	11
6	16	13	21	20	18	18	13	17	17	13	10	11	17	18	10	12
7	15	10	22	21	14	11	19	13	21	10	19	10	12	13	19	19
8	22	11	11	16	12	19	16	13	21	15	12	12	19	19	11	8

Key: FEP = Cefapime, CT = Colistin sulphate, CAZ = Ceftazidime, FOX = Cefoxitin, IPM = Imipenem, CTX = Ceffotaxime, OB = Cefotetan, MEM = Meropenem, CRO = Ceftriaxozone, ETP = Ertapenem, ATM = Azeteronam, CIP = Ciprofloxacin, AMC = Amoxicillin/clavulanic acid, OFX = Ofloxacin, CN = Gentamicin, TOB = Tobramycin

Table 25: Antibiotics Susceptibility Patterns of *Klebsiella pneumoniae* isolated from Effulents at Chicken Dung Spot

	ANTIBIOTICS															
Org	FEP 18	CT 11	CAZ 21	FOX 18	IPM 16	CTX 26	OB 16	MEM 16	CRO 23	ETP 19	ATM 18	CIP 21	AMC 18	OFX 16	CN 15	TOB 15
1	13	13	23	12	13	21	14	21	21	14	13	21	23	23	12	12
2	14	9	21	16	17	12	17	23	24	13	14	24	14	22	13	10
3	12	15	19	21	19	14	19	25	12	17	16	21	15	24	15	21
4	11	10	13	22	21	15	12	23	16	19	12	25	12	14	12	9
5	15	15	15	24	24	16	13	17	17	10	15	12	16	17	11	10
6	17	9	10	13	12	19	15	12	12	23	18	11	17	16	10	13
7	21	10	11	18	11	11	21	19	18	22	12	16	18	19	18	14
8	23	9	13	19	19	12	20	10	16	14	19	14	13	15	12	12
9	13	12	14	10	13	25	18	11	25	15	12	19	12	12	11	11
10	14	10	10	11	12	28	10	14	15	21	15	15	16	23	10	10
11	21	9	21	13	17	31	11	24	10	24	17	13	13	21	13	14
12	14	9	25	12	15	19	15	26	24	16	12	15	19	23	14	19

Key: FEP = Cefapime, CT = Colistin sulphate, CAZ = Ceftazidime, FOX = Cefoxitin, IPM = Imipenem, CTX = Ceffotaxime, OB = Cefotetan, MEM = Meropenem, CRO = Ceftriaxozone, ETP = Ertapenem, ATM = Azeteronam, CIP = Ciprofloxacin, AMC = Amoxicillin/clavulanic acid, OFX = Ofloxacin, CN = Gentamicin, TOB = Tobramycin

Table 26: Antibiotics Susceptibility Patterns of *Klebsiella pneumoniae* isolated from Effulents at Chicken Discharging Spot

	ANTIBIOTICS															
Org	FEP 18	CT 11	CAZ 21	FOX 18	IPM 16	CTX 26	OB 16	MEM 16	CRO 23	ETP 19	ATM 18	CIP 21	AMC 18	OFX 16	CN 15	TOB 15
1	22	11	11	16	12	19	16	13	21	15	12	12	19	19	11	8
2	17	9	17	14	15	13	18	14	13	22	15	9	21	15	13	12
3	25	10	13	17	18	12	13	14	22	12	14	13	10	13	13	10
4	13	10	21	10	13	13	15	21	21	23	13	23	15	13	14	12
5	18	13	15	17	18	15	13	14	14	18	12	19	17	21	17	14
6	16	13	21	20	18	18	13	17	17	13	10	11	17	18	10	12
7	12	9	19	12	14	21	10	12	21	12	13	9	18	14	12	19
8	10	12	12	11	13	12	15	16	11	12	11	1	11	13	12	12
9	13	9	16	18	16	15	16	15	14	12	12	9	14	12	15	13
10	13	9	22	15	12	16	21	24	14	25	21	24	18	21	19	11
11	21	14	24	11	14	25	18	19	21	14	16	17	19	21	13	10
12	21	12	12	14	13	10	15	12	20	21	11	21	14	13	11	11
13	14	10	10	19	12	12	13	12	14	14	21	9	16	17	12	16
14	13	9	22	15	12	16	21	24	14	25	21	24	18	21	19	11

Key: FEP = Cefapime, CT = Colistin sulphate, CAZ = Ceftazidime, FOX = Cefoxitin, IPM = Imipenem, CTX = Ceffotaxime, OB = Cefotetan, MEM = Meropenem, CRO = Ceftriaxozone, ETP = Ertapenem, ATM = Azeteronam, CIP = Ciprofloxacin, AMC = Amoxicillin/clavulanic acid, OFX = Ofloxacin, CN = Gentamicin, TOB = Tobramycin