# CHAPTER ONE

## 1.0 INTRODUCTION

### 1.1 Background of the Study

Antibiotic resistance, a global issue, threatens the effective prevention and treatment of an ever increasing range of infections. It is an increasingly serious threat to global public health that requires action across all government sectors and society. There are high proportions of antibiotic resistance in bacteria that cause common infections (e.g. urinary tract infections, pneumonia blood stream infections) in all regions of the world. A high percentage of hospital acquired infections (nosocomial infections) are caused by high resistant bacteria such as extended spectrum beta-lactamase producing *Escherichia coli* and *klebsiella pneumoniae(*Paterson and Bonomo, 2005).

Patients with infections caused by drug resistant bacteria are generally at increased risk of worse clinical out comes and death and consume more healthcare resources than patients infected with the same bacteria that are not resistant (Tumbarello, 2006).Antimicrobial resistance is resistance of a microorganism to antimicrobial drugs that was originally effective for treatment of infections caused by it. Resistant microorganisms which include bacteria are able to withstand attack by antimicrobial drugs, so standard treatments become ineffective and infections persist, increasing the rate of spread to others. Antimicrobial resistance makes it difficult to eliminate infections from the body as existing drugs become less effective.

The use and misuse of drugs accelerates the emergence of drug resistant strains (cheesebrogh, 2010). Poor infection control and inappropriate food-handling encourage the further spread of antimicrobial resistance.New resistance mechanisms emerge and spread globally threatening our ability to treat common infectious diseases, resulting in death and disability of individual. As an example, the treatment failures for patients with blood infections caused by bacteria that produce ezymes capable of hydrolyzing third generation cephatosorin (called extended spectrum lactamase (ESBL) like BSBL

producing *K.pneumoniae* infected group was almost as twice as high as that of the non-ESBL producing *K.pneumoniae* infected group (Tumbarello, 2006).

The achievements of modern medicine are put at risk by antimicrobial resistance. Without effective antimicrobial for prevention and treatment of infections, the success of organ transplantation, cancer chemotherapy and major surgery would be compromised.World health organization's (WHO, 2014) report on global surveillance of antimicrobial resistance revealed that antibiotic resistance is no longer a prediction for the future. It is putting at risk the ability to treat common infections in the community and hospitals. Without urgent, coordinated action, the world is heading towards a post-antibiotic era, in which common infections and minor surgeries, which have been treatable for decades, can once again kill. Key tools to tackle antibiotic resistance such as basic systems to track and monitor the problem reveal considerable gaps. The main significant predictor of mortality caused by ESBL-producing *E.coli* is inadequate initial antimicrobial therapy (Oteo *et al*, 2010)

There are three fundamental mechanisms of antimicrobial resistance.
They are:

i. Enzymatic degradation of antibacterial drugs
ii. Alteration of bacterial proteins that are antimicrobial targets
iii. Changes in membrane permeability to antibiotics (Dever and Dermody, 1991).

Bacteria may be intrinsically resistant to one or more than one class of antimicrobial agents or may acquire resistance by mutation or via the acquisition of resistant genes from other organisms. Acquired resistance genes may enable a bacterium to produce enzymes that destroy the antibacterial drug, to express the efflux systems that prevent the drug from reaching its intracellular target, to modify the drugs target site or to produce an alternative metabolic pathway that bypasses the action of the drug. Acquisition of new genetic material by antimicrobial-susceptible bacteria from resistant strains of bacteria may occur through conjugation, transformation or transduction, with transponsons, often

facilitating the incorporation of the multiple resistance genes into the host's genome or plasmid (Tenover, 2005).

Antibiotic resistance can be either plasmid mediated or maintained on the bacterial chromosome. The most important mechanism of resistance to the penicillins and cephalosporin is antibiotic hydrolysis mediated by the bacterial enzyme ,beta lactamase. The expression of chromosomal beta-lactamase can either be induced or stably depressed by exposure to beta lactam drugs. Reduced antibiotics penetration is also a resistance mechanism for several classes of antibiotics, including the beta-lactamase (Dever and Dermody, 1991). Methods to overcome resistance to beta lactam antibiotics include the development of new antibiotics that are stable to beta-lactamse attack and the coadministration of beta-lactamase inhibitors with beta-lactam drugs (Dever and Dermody, 1991).

The plasmid mediated extended spectrum beta lactamases (ESBLs) are of increasing concern. Most are mutants of TEM-and SHV-beta lactamases types. Unlike these parent enzymes, ESBLs hydrolyze oxyimino-cephalosporins such as cefuroxime, cefotaxime, ceftriaxomine, ceftizoxime, ceftazidime, celpirome and cefepime, aztreonam as well as penicillins and other cephalosporin except for cephamycin (cefoxitin and ceftetean) (Susić, 2004). Plasmids responsible for ESBL production tend to be large and carry resistance to several agents, an important limitation in the design of treatment alternatives. The most frequent resistances found in ESBL organisms are aominoglycosides, fluoroquinolones, tetracycline, chloramphenicol and sulfamethoxazole-trimethoprim (Nathisuwan *et al*, 2001).

ESBL producing gram negative organisms in which *E. coli* and *Klebsiella*species are the chief culprit limit therapeutic options as a result of their multidrug resistance (Anago *et al,* 2013). Blood infections caused by ESBL producing *K.pneumoniae* are a major concern for clinicians, since they markedly increase the rates of treatment failure and death (Tumbarello *et al.*, 2006; Cosgrave, 2006).

Some of the ESBL genes are located on plasmids (Iroha *et al.*, 2009) and can be easily transferred between and within bacterial species. ESBL genes are borne on chromosome while others are borne on plasmids. Some of the ESBL genes borne on plasmids can be cured be subjecting the resistant bacteria like *E.coli* to certain chemicals like a low concentration of acridine orange (Adeyankinnu*et al*., 2014). Elimination of the resistant plasmid makes the bacteria to become susceptible to antibiotics.

There is also abundance of ESBL genes in the food chain and this may have a profound effect on future treatment by gram negative bacteria (Grave *et al*., 2010). Antimicrobial resistance is usually brought about by the use and misuse of drugs (Cheesbrough, 2010). Drug resistance in animals is caused mainly by the large amount of antimicrobial drugs used in food production.Grave *et al.*, (2010), reported that Netherlands is one of the highest users of antimicrobial agents in food production for animals which results in high rate of drug resistance among these animals.

Dierikx *et al.*,(2012) observed a high prevalence of birds carrying ESBL producing *E. coli* at Dutch broiler farms and a high prevalence of ESBL producing *E.coli* in farmers. Also there is the dissemination of drug resistant bacteria from animal farms to aquatic environment.

ESBL producing *E.coli* can also be isolated from chicken feeds. ESBL producing multidrug resistant *E.coli* and *K.pneumoniae* were isolated from commercial feeds in Nigeria (Oyinloye and Ezekiel, 2011).

ESBL producing *E.coli* and *Klebsiella pneumoniae* is a major concern for everybody all over the world because of their multidrug resistance,

## 1.2 Statement of the Problem

Antibiotic resistance, a global issue, threatens the effective prevention and treatment of ever increasing range of infections caused by bacteria. It leads to increased rate of patients'/diseased animals' morbidity and mortality as a result of antibiotic treatment failure. This prompted the investigation of antibiotic resistance observed in infections caused by *E.coli* and *K.pneumoniae* in poultry farm environment.

## 1.3 Justification of the study

Antibiotic resistance is now a major global concern. This resistance has been indicated in poultry farm environment infections caused by *E.coli* and *Klebsiella* species. Therefore, the need to determine the cause of this resistance and ways by which it can be remedied .

## 1.4 Aim of the study

The aim of the study is to determine the occurrence of extended spectrum beta lactamase producing *E.coli* and *K.pneumonia*e in poultry farm environment.

## 1.5 Objectives of the Study

**The objectives of the study are to:**

i. Isolate *E.coli* and *klebsiella* species from cloaca of chicks, stools and urine of chicken farmers and the chicken environment.
ii. Determine the beta-lactamase producing strains among the isolates.
iii. Determine the ESBL positive isolates among the betalactamase producing isolates.
iv. Investigate the antimicrobial resistance patterns of the ESBL positive isolates.
v. Determine the moleculate weight of the ESBL positive isolates
vi. Determine the curing rate of the ESBL positive isolates using acridine orange.

## 1.6 Delimitation of the Study

The researcher used the poultry farm environmentsin Jalingo. The cloacae samples of broilers, layers and cockerels confined to their poultry farms were used, environment of the farms (which include the walls, floors, drinkers, feeders, water and feed) were also used for the study. Likewise, the stool and urine of the chicken rearers were assessed. The poultry farms were all located in Jalingo, Taraba State, and North East Nigeria.

# CHAPTER TWO

## 2.0 LITERATURE REVIEW

Poultry farmers face a lot of challenges in treatment of themselves and their farm animals because of treatment failure (Pitout, 2007). Such challenges include increased morbidity, long stay in hospital/ spending more money on drugs and high rate of mortality (Pitout, 2007; Tumbarello *et al.,* 2006). The treatment failure observed in the poultry farmers and their chickens is because the microorganisms causing the disease conditions of the farmers and their animals are resistant to antimicrobials.

### 2.1 Predisposing factors for the development of bacteria resistance to antimicrobial agents.

They include:

**2.1.1 Inappropriate use of antimicrobials:** this is usually as a result of these drugs being freely procured without medical authorization or supervision(Ochei and Kolhatkar, 2000)..

**2.1.2 Wrong selection of drug**: This is usually due to lack guidelines regarding the correct selection of drugs for those prescribing the drug. it may also be due to lack of knowledge regarding a drug resistance by the prescribing a drug resistance by the prescribing official. In addition lack of facility for antibiotic sensibility testing may result in the wrong choice of drugs(Ochei and Kolhatkar, 2000)..

**2.1.3: Inadequate control measures**: Inadequate control and management measures in the use of chemotherapeutic agents in hospitals give rise to the development of resistance to various drugs by bacteria (Ochei and Kolhatkar, 2000)..

**2.1.4: Prophylactic use of antimicrobials**: The practice of frequent prophylactic use of antibiotic may also enhance the development of bacteria drug resistance (Ochei and Kolhatkar, 2000).

## 2.2 Origin of Drug Resistance

The Origin of drug resistance may be non genetic or genetic (Brooks *et.al*, 2008)

### 2.2.1 Non genetic Origin of Drug Resistance

Active replication of bacteria is usually required for most antibacterial drug actions.

Consequently, microorganisms that are metabolically inactive (Non multiplying) may be phenotypically resistance to drugs. However, their offspring are fully susceptible. Example Mycobacteria often survive in tissues for many years after infection yet are restrained by the host's defenses and do not multiply. Such "Persisting" organisms are resistant to treatment and cannot be eradicated by drugs. Yet if they start to multiply (e.g following suppression of cellular immunity in the patient), they are fully susceptible to the same drugs (Brooks *et al.*, 1998).

Microorganisms may lose the specific target structure for a drug for several generations and thus be resistant. Example penicillin-susceptible organisms may change to cell wall-deficient L forms during penicillin administration. Lacking cell walls, they are resistant to cell wall-inhibitor drugs (penicillins cepphalosporins) and may remain so for several generation. When these organisms reverse to their bacterial parent forms by resuming cell wall production, they again become susceptible to penicillin (Brooks*et al*., 1998).

Micro-organisms may infect the host at sites where antimicrobials are excluded or are not active. Example: Amino glycosides such as gentamicine are not effective in treating *Salmonella* enteric fevers because the Salmonellae are intracellular and the amino glycosides do not enter the cells (Brooks *et al.*, 1998)

### 2.2.2 Genetic Origin of Drug Resistance

Most drug-resistant microbes emerge as a result of genetic change and subsequent selection processes by antimicrobial drugs. The presence of the antimicrobial drug serves as a selecting mechanism to suppress susceptible resistant mutants. Spontaneous mutation occurs with a frequency of $10^{-12}$ to $10^{-7}$ and thus is an infrequent cause of the emergence (Brooks *et al.*, 1998)

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#### 2.2.2.1 Chromosomal resistance.

This develops as a result of spontaneous mutation in a locus that controls susceptibility to a given antimicrobial.The presence of the antimicrobial drug serves as a selecting mechanism to suppress susceptible organisms and favour the growth of drugs resistantmutants. Spontaneous mutation occurs with a frequency of $10^{-12}$to $10^{-7}$ and this is an infrequent cause of the emergence of drugs resistancein a given patient Chromosomal mutants are most commonly resistance by virtue of a change in a structural receptor for a drug. Thus the P-12 protein on the 30S subunit of the bacterial ribosome serves as a receptor for streptomycin attachment. Mutation in the gene controlling that structural protein results in streptomycin resistance. Mutation can also result in the loss of (Penicillin binding protein (PBPs) making such mutants resistant to β -lactam drug (Brooks *et al.*, 1998).

#### 2.2.2.2 Extrachromosomal Resistance

Bacteria often contain extrachromosomal genetic element called plasmids.R factors are a class of plasmids that carry genes for resistance to one- and often several- antimicrobial drugs and heavy metals. Plasmid genes for antimicrobial resistance often control the formation of enzymes capable of destroying the antimicrobial drugs. Thus, plamids determine resistance to penicillins and cephalosporins by carrying genes for the formation of β-lactamase. Plasmids code for enzymes that destroy chloramphenicol (acetylferase); for enzymes that acetylate, adenylate, or phosphorylate various aminogly

cosides; for enzymes that determine the active transport of tetracyclineacross the cell membrane; and for others (Brooks *et al*., 1998).

## 2.3 Types of Drug resistance

The types of drug resistance are

### 2.3.1 Natural drug resistance

Natural drug resistance is an innate property of the batcterium and is unrelated to previous exposure to the drug. An entire bacterial species may be resistance to an antibiotic even before the introduction of the drug. Example,*streptoccus pyogenes* is resistant to gentamicin. The reasons for this natural resistance include lack of penetration of the drug through the cell wall, lack of suitable cell wall or other target receptor, naturally produced enzymes that may have existed before the introduction of the drug which may be lethal to the drug (Ochei and Kolhatkar, 2000).

### 2.3.2 Acquired Drug resistance

Resistance to some drugs can be acquired by bacteria is two ways. They are: Mutation and Gene transfer (Ochei and Kolhatkar, 2000)

#### 2.3.2.1 Mutation

Mutation is when a gene undergoes chemical alteration in DNA in which one or more bases are changed. Mutation is heritable. Spontaneous mutation in a locus that control susceptibility to a given antimicrobial drug, leads to antimicrobial resistance. The presence of the antimicrobial drug serves as a selecting mechanism to suppress susceptible organisms and favour the growth of drug resistant mutants. Spontaneous mutation occurs with a frequency of $10\text{-}^{12}$ to $10\text{-}^{7}$ and thus is an infrequent cause of the emergence of clinical drug resistance in a given patient. However, chromosomal mutants resistant to rifampin occur with high frequency (about $10^{-7}$ to $10^{-5}$). Consequently, treatment of bacterial infection with rifampin as the sole drug often fails. Mutation can also result in the loss of PBPs, making such mutants resistant to β lactam drugs (Ochei and Kolhatkar, 2000).

#### 2.3.2.2 Gene Transfer

Gene transfer is achieved by transformation, conjugation and transduction (Arora and Arora, 2008). Genetic transfer of antimicrobial resistance can also take place through transposons. These are small pieces of DNA which unlike plasmids cannot replicate themselves, but can "jump" between different between plasmids and chromosomes. An example of an important gene carried by an antibiotic resistant transposon is known as TEM I. It controls the production of beta-lactamase and is incorporated into plasmids which then mediate resistance to β-lactam antibiotics in some strains of *E.coli, Klebsiella, Hemophilus influenza,* N*esseria gonorrhoeae.* The resistance transposon can be transferred from one strain to another (Ochei and Kolhatkar, 2000)

## 2.4 Mechanisms of Antimicrobial Resistance

There are many different mechanisms by which microorganism might exhibit resistance to drugs. They are:

### 2.4.1 Change in Permeability to the Drug

Microorganisms change their permeability to the drug. Examples: Tetracyclines accumulate in susceptible bacteria but not in resistant bacteria. Resistance to polymyxins is also associated with a changein permeability to the drugs. (Brooks *et al.*, 1998)

### 2.4.2 Development of an Altered Structural target for the Drug

Microorganisms develop an altered structural target for the drug. Examples: Chromosomal resistance to aminoglycosides is associated with the loss or alteration of a specific protein in the 30S subunit of the bacterial ribosome that serves as a binding site in susceptible organisms. Resistance to some penicillins and cephalosporins may be a function of the loss or alteration of PBPs. Penicillin resistance in *Streptoccus pneumoniae* and *Enterococci* is due to altered PBPs (Brooks *et al.,* 1998)

### 2.4.3 Development of an Altered Metabolic Pathway

Microorganisms develop on altered metabolic pathway that bypasses the reaction inhibited by the drug. Example: some sulfonamide resistance bacteria do not require extracellular Ƥ-Aminobenzoic acid (PABA) but, like mammalian cells utilize preformed folic acid (Brooks *et al.,* 1998)

### 2.4.4 Development of an Altered Enzyme

Microorganisms develop an altered enzyme that can still perform its metabolic function but is much less affected by the drug. Example: in trimethoprim resistant bacteria, the dihydrotolic acid reductase is inhibited far less efficiently than in trimethoprim susceptible bacteria (Brooks *et al.,* 1998)

### 2.4.5 Production of Enzymes that Destroy the Active Site

Gram negative bacteria resistant to aminoglycosides (by virtue of a plasmid) produce adenylating, phosphorylating or acetylating enzymes that destroy the drug. *Staphylococcus*resistance to penicillin G produce a β – lactamase that destroy the drug.Other β-lactamases like ESBL are produced by Gram negative rods.

## 2.5 Definition of Extended Spectrum $\beta-$ Lactamases

Extended Spectrum beta lactamases (ESBLS) are a rapidly evolving group of $\beta-lactamases$ which share the ability to hydrolyze third generation cephalosporins and aztreonam but are inhibited by clavulanic acid (Phlippon *et al.,* 1989). ESBL is also defined as enzymes produced by certain bacteria that are able to hydrolyse extended spectrum cephalosporin. They are therefore effective against beta-lactam antibiotics like ceftazidime, ceftriaxone, cefotaxime oxyimino and monobactam, carbapenems and cephamycin are effective against ESBL producer strains. ESBLs are inhibited by clavulanic acid and tazobactams (Bradford, 2001). ESBL are encoded by genes that can be exchanged between bacteria. The most currently common genetic variant of ESBL is CTX-Ms (Paterson and Bonomo, 2005). The (CTX-M) enzymes are produced by *E. coli* while SHV and TEM types are produced by *klebsiella* species. CTX-M, SHV and TEM are types of ESBL found on ingreen negative bacteria like *E. coli* and *Klebsiella*species.

SHV and TEM are the major cause of hospital acquired infections, while the CTX-M enzymes have emerged as an important cause of community-onset urinary tract infections in some areas.

## 2.6 Cephalosporins

Cephalosporins are a family of antibiotics originally isolated in 1948 from the fungus *Cephalosporium*. They contain a β – lactam structure that is very similar to that of the penicillins. As might be expected from their structural similarities to penicillins, Cephalosporins also inhibit the transpeptidation reaction during peptidoglycan synthesis. They are broad spectrum drugs frequently given to patients with penicillin allergies (although about 10% of patients allergic to penicillin are also allergic to Cephalosporins) (Willey *et al.*, 2008)

Cephalosporins are β – Lactams with a nucleus of 7-amino cephalosporin acid instead of the penicillins' 6-amino penicillnic acid. Natural cephalosporins have low antibacterial activity, but the attachment of various R-side group resulted in the politferation of enormous array of drugs with varyinging pharmacologic properties and antimicrobial spectra and activity. (Brooks *et al.*, 1998)

### 2.6.1 Mechanism of action of Cephalosporin

The mechanisms of action of cephalosporin include:

1. Binding to specific PBPs that serve as drug receptors on bacteria
2. Inhibiting cell wall synthesis by blocking the transpeptidation of peptidoglycan
3. Activating autolytic enzymes in the cell wall that can produce lesions resulting in bacterial death. (Brooks *et al.*, 1998)

### 2.6.2 Groups/Generation of Cephalosporins

Cephalosporins are arranged into three major groups, or "generations" for easy reference. They are:

#### 2.6.2.1 First Generation Cephalosporin

They are more effective against gram positive pathogens than gram negatives. Anaerobic cocci are often sensitive, but *Bactericides fragilis* is not. *Enterococcus as well as methicillin resistant Staphylococci is* not sensitive.

First generation cephalosporin include cephalothin, cephalexin and others (Brooks *et al*., 1998)

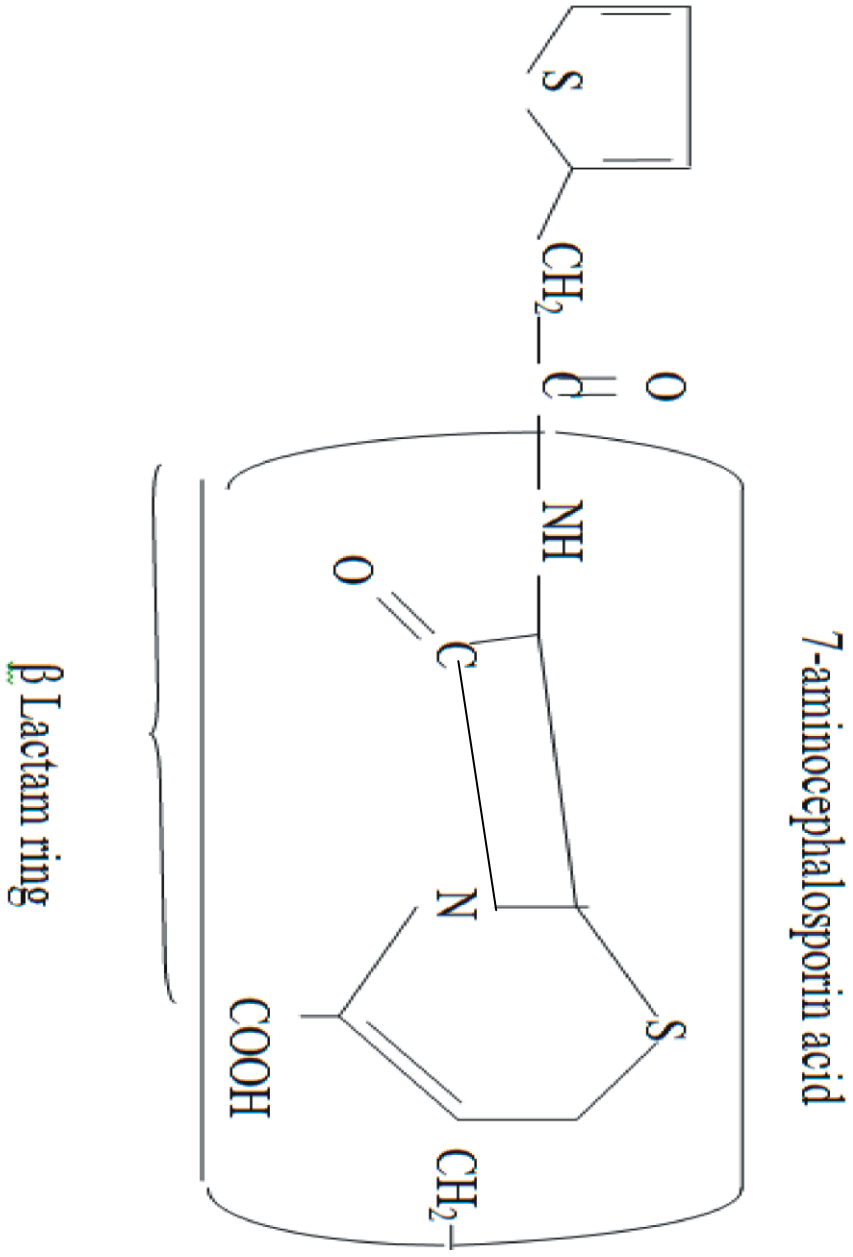


**Figure 2.1:** First generationCephalosporin(Cephalothin)(Willey *et al*., 2008)

#### 2.6.2.2 Second Generation Cephalosporin

The Second generation cephalosporins are a heterogenous group. All are active against organism covered by first generation drugs but have improved effect on gram negative rods – including *Klebsiella* and *proteus* but not *P. aeruginosa*. Examples are cefoxitin and cefotetan (Brooks *et al*., 1998).

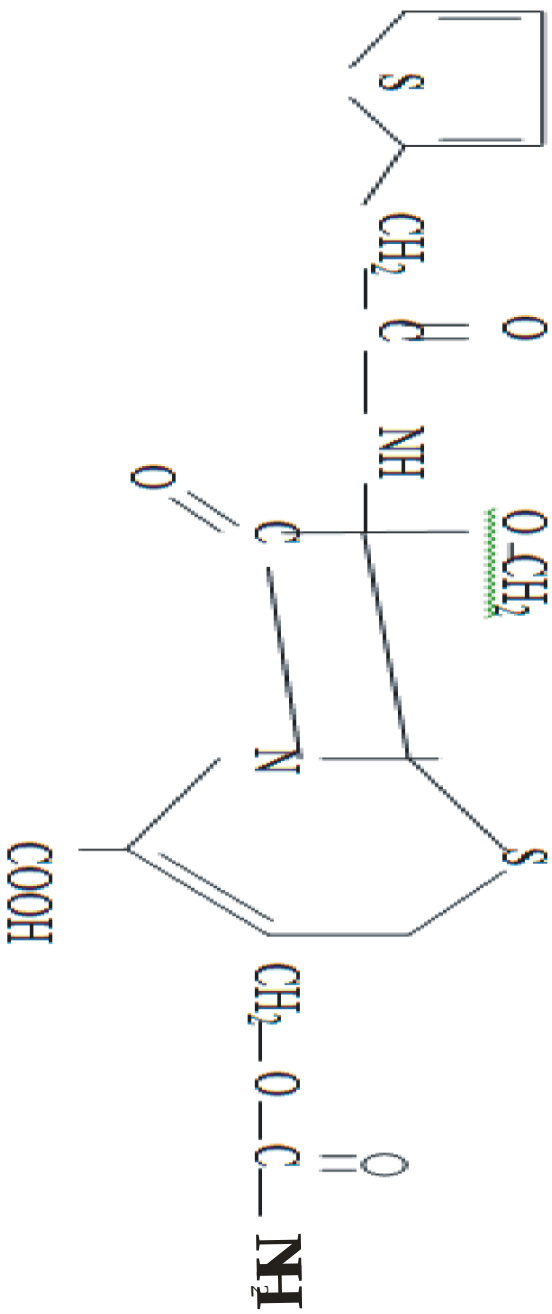


**Fig 2.2:** Second generation cephalosporin (cefoxitin)

### 2.6.2.3 Third Generation Cephalosporin

They are particularly effective against gram-negative pathogens. Whereas second generation drugs tend to fail against *P.aeruginosa* ceftazidime or cefoperazone may succed.

Another important distinguishing feature of several third generation drugs-except cefoperazone-is the ability to reach the central nervous system and to appear in the spinal fluid in sufficient concentration to treat meningitis caused by gram negative rods. Examples of third generation cephalosporin include cefotaxime and ceftriaxone (Brooks *et al.*, 1998)

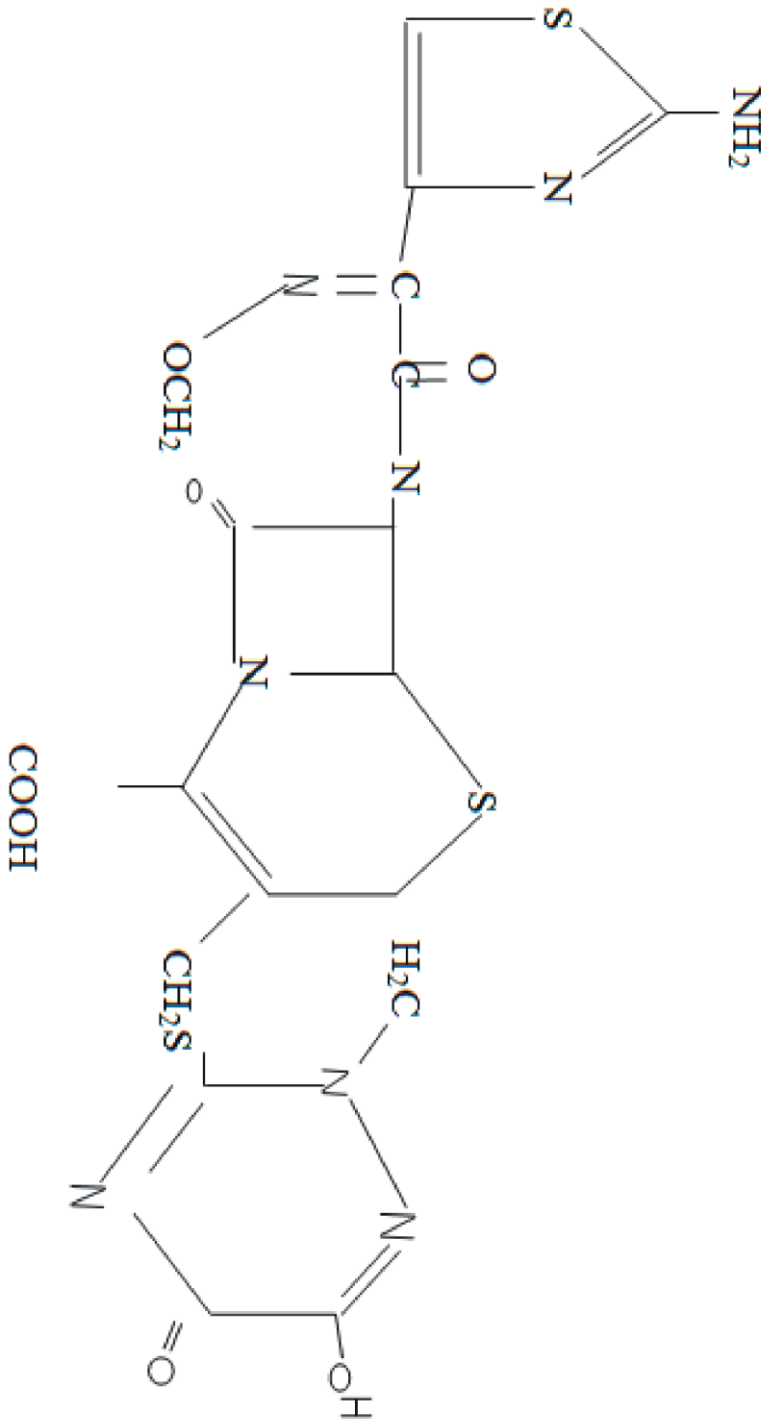


**Fig 2.3:** Third generation cephalosporin (ceftriaxone) Willey et al., 2008

#### 2.6.2.4 Fourth Generation Cephalosporin

They are broad spectrum with excellent activity against gram positive and gram negative bacteria. Like their third generation predecessors, they inhibit the growth of the difficult opportunistic pathogen *Pseudomonas aeruginosa*. Cefepime is the only fourth generation cephalosporin in clinical use (Brooks et *al.*, 1998)

### 2.6.3 Resistance to Cephalosporins

Resistance to cephalosporin can be attributed to:

1. Poor permeation of bacteria by the drug
2. Lack of PBP for a specific drug
3. Degradation of drug by β – Lactamasess(Brooks *et al.,* 1998)

## 2.7 Classification of Beta Lactamases

Beta- lactamases are enzymes produce by bacteria including staphyloc colcusaces, *Escherichia coli, Klebsiella pneumonae* that are responsible for resistance to penicullus and cephalosporus they are commonly classifiesd according to two general schemes.

**TheAmbler molecular classification**

The Ambler molecular classification divides $\beta-$ lactamases into four major classes (A to D). The basis of this classification scheme rests upon protein homology (amino acid similarity) and not phenotypic characteristics. In the Ambler classification scheme, $\beta-$ lactamases of classes A, C and D are serine $\beta-$ lactamases In contrast, the classB enzymes are metallo$\beta-$ lactamases. With the exception of OXA – type enzymes (which are class D enzymes) the ESBLs are of molecular class A (Rawat and Nair, 2011).

**The Bush-Jacoby- Medeiros Functional Classification**

This takes into account substrate and inhibitor profiles in an attempt to group the enzymes in ways that can be correlated with their phenotype in clinical isolates (Bush and Jacoby, 2010).

According to the Bush, Jacoby and Medeiros scheme, beta-lactamases are divided into four groups as shown in Table 2.1. The first scheme, introduced by Ambler, which is also widely used (Ambler *et al.*, 1991), is shown in table 2.1 as well.

### 2.7. 1 Group I (Ambler Class C) Beta-Lactamases (Also known as amp C enzymes as AMPC Enzyme)

This group is resistant to beta-lactamase inhibitors like clavulunate and mostly is found on chromosomes (Minami *et al.*, 1980). In this class, the enzyme is inducible. Thus any exposure of bacteria to beta-lactamase antibiotics leads to an increase in enzyme production. As beta- lactam antibiotics are different, they are able to stimulate different levels of beta-lactamase production. The enzymes in group 1 are found in the Enterobactericeae family. Studies have also shown the shift of enzymes from chromosomes to plasmid in some strains such as *E. coli* and *klebsiella* spp (Sanders and Sanders, 1992). The group I producer beta-lactamases are resistant to beta-lactam/beta-lactamase inhibitor combinations, pencillins, cephamycins as well as 1st, 2nd and 3rd generations cephalosporins. They are sensitive to cefepime and carbapenems (Sanders *et al.*, 1996).

### 2.7.2 Group 2 (Ambler Class A) Enzymes

As the enzymes classified into group 2 are harbored by plasmid, they could easily be transmitted into different bacterial cells, causing rapid resistance to such enzymes. The beta-lactamase inhibitors such as clavulanic acid, sulbactam and tazobactam inhibit the original group 2 enzymes.

The main group 2 enzymes are TEM and SHV. TEM – 1 was first identified in 1965 in the Enterobacteriaceae family. SHV – 1 was discovered in 1979 and is commonly found in *klebsiella* Spp and *E. coli (*De Champs *et al., 1991).* Group 2 enzymes could hydrolyse ampicillin and 1st, 2nd and 3rd generation cephalosporins as well as monobactams (the extended spectrum beta-lactamases) (Livermore, 1995).

### 2.7.3 Group 3 (Ambler Class B) Enzymes

These are metallo-enzymes of destroying carbapenems. These enzymes are frequently found in *Pseudomonas aeruginosa, Bacteriodes fragilis and Sstenotrophomonas maltophilia* (Ghafourian *et al.,* 2015).

### 2.7.4 Group 4 Beta-Lactamases

Group 4 beta-lactamases contains those unusual penicillinases not inhibited by clavulanic acid. Four of these enzymes exhibit high rates of hydrolysis with carbenicillin and/or cloxacillin. Several of them exhibit unusual behaviour with respect to metal ion involvement. Whether these enzymes represent another molecular class of beta-lactamase is not known (Ghafourian *et al.,* 2015).

## 2.8 Types of ESBLs

The types of ESBLs that are important are as follows:

### 2.8.1 TEM-Beta-Lactamases

The TEM-types ESBL are derivatives from TEM-1 and TEM-2.TEM – 3 was first discovered in *K. pneumoniae* in France in 1984. Initially, it was known as CTX – 1, Gbecause of its activity against cefotaxime (Burn-Buisson *et al.,* 1987). Now it is called TEM – 3, which is different from TEM – 2, which is a replacement of two amino acids. The number of TEM type beta-lactamases currently exceeds 100. All of them, with the exception of TEM – 1 and TEM – 2 are ESBLs. The most common TEM type ESBL is found in *E. coli* and *K. pneumoniae.* However, they could appear in the other gram negative bacteria (*Livermore,* 1995) and also in different genera of Enterobacteriaceae *(Enterobacteraerogenes*, *Enterobactercloacae*, *Morganellamorganii*, *Proteusmirabilisand Salmonella Spp*) (Marchandin *et al.,* 1999). In Non-Enterobacteriaceae, they are in *P. aeruginosa (*Nordmannand Guibert, 1998*).*

### 2.8.2 SHV Beta – Lactamases

The SHV is more prevalent than the other types of ESBLs in clinical isolates of bacteria (Jacoby, *1997*). Unlike TEM – type beta-lactamases, fewer SHV mtype beta-lactamases

are derived from SHV – 1. Most strains have SHV on their plasmid, through the replacement of serine to glycine at position 238, is essential for hydrolyzing ceftazidime and the lysine residue is critical for hydrolyzing cefotaxime. More than 100 SHV varieties are known worldwide. Currently SHV – type ESBLs are found in a wide range Enterobacteriaceae (Huang *et al.,* 2004; Porel *et al.,* 2004).

### 2.8.3 CTX – Beta-Lactamases

A new family of β – lactamases that preferentially hydrolyze cefotaxime has arisen. It has been found in isolates of *Salmonella enterica serovar Typhimurinum, E. coli* mainly and some other species of Enterobacteriaceae. These are not very closely related to TEM or SHV $\beta - lactamases$(Tzouvelekis *et al.,* 2000). In addition to the rapid hydrolysis of cefotaxime, another unique feature of these enzymes is that they are better inhibited by the $\beta - lactamase$ inhibitor tazobactam than by sulbactam and clavulanate (Bradford *et al.,* 1998; Ma *et al.,* 1998).

CTX – M β – lactamases are found exclusively in the functional group 2 (Bush and Jacoby, 2010) and thought to originate from chromosomal ESBL genes found in *Kluyvera* Spp. (Bush and Jacoby, 2012), an opportunistic pathogen of the Enterobacteriaceae found in the environment. The first CTX – M protein were discovered in the late 1980s and today more than 100 variants have been sequenced. Based on their amino acid sequences, they can be divided into five groups (CTX – M 1, 2, 8, 9 and 25) (Bonnet, 2004).

The origin of the CTX – M enzymes is different from that of TEM and SHV ESBLs. While SHV ESBLs and TEM – ESBLs were generated by amino acid substitutions of their parent enzymes, CTX – M ESBLs were acquired by the horizontal gene transfer from other bacteria using genetic apparatuses such as conjugative plasmid or transposon.

Kinetic studies have shown that CTX – M type$\beta$ – lactamases hydrolyze cephalothin or cephalorine better than benzyl penicillin and they preferentially hydrolyze cefotaxime over ceftazidime (Tzouvelekis *et al.,* 2000; Bradford *et al.*, 1998). Although there is some hydrolysis of ceftazidime by these enzymes, it is usually not enough to provide clinical resistance to organisms in which they reside. It has been suggested that the serine residue at position 237, which is present in all of the CTX-M enzymes, play an important role in the extended spectrum activity of the CTX-M-type $\beta$ – lactamases(*T*zouvelekis *etal*., 2000). Although it has been shown not to be essential, the Arg – 276 residue lies in a position equivalent to Arg – 244 in TEM – or SHV – type *ESBLs,* as suggested by molecular modelling, and may also play a role in the hydrolysis of oxyimino cephalosporins (Gazouli *et al.,* 1998).

### 2.8.4 OXA Beta-Lactamses

The OXA – type$\beta$ – lactamases are so named because of their oxacillin – hydrolyzing abilities. These $\beta$ – lactamasesare characterized by hydrolysis rate for cloxacillin and oxacillin greater than 50% as that for benzyl penicillin (Bush*et al.,* 1995). They predominantly occur in *P. aeruginosa* (Weldhagen *et al.,* 2003) but have been detected in many other Gram – negative bacteria. In fact the most OXA – type$\beta$ – lactamases, OXA – 1 has been found in 1 – 10% of *E. coli* isolates (Livermore, 1995). The OXA – type ESBLswere originally discovered in *P. aeruginosa* isolates from a single hospital in Ankara, Turkey. In France, a novel derivative of OXA – 10 (Numbered OXA – 28) was found in a *P. aeruginosa* isolates (Poirel *et al.,* 2001). A novel *ESBL* (OXA - 18) and an extended – spectrum derivative of the narrow spectrum OXA – 13 $\beta - lactmase$ (Numbered OXA - 19) have also been discovered in France in *P. aeruginosa* isolates (Philippon *et al.,* 1997). The evolution of ESBLOXA – type$\beta$ – lactamasesfrom parent enzymes with narrow spectra has many parallels with the evolution of SHV – and TEM – type ESBLs. Unfortunately there are very few epidemiologic data on the geographical spread of OXA – type *ESBLs.*(Philippon *et al.,* 1997).

### 2.8.5 PER Beta-Lactamases

The PER – type ESBLsshare only around 25 – 27% homology with known TEM and SHV – typeESBLs.PER – 1 β – lactamase efficiently hydrolyze penicillin and cephalosporins and is susceptible to clavulanic acid inhibition. PER – 1 was first detected in *P. aeruginosa* (Neuhauser *et al*., 2003) and later in *S. enterica* serovar *typhimurium* and *Acinetobacter* isolates as well (Vahaboglu *et al.*, 2001). PER – 2 which shares 86% homology to PER – 1 has been detected in *S. enterica* serovar *typhimurium*, *E. coli, K. pneumoniae,Proteus mirabilis* and *Vibrio cholerae* 01 E1 Tor (Petroni *et al.*, 2002).

### 2.8.6 GES Beta-Lactamases

GES – I was initially described in a *K. pneumoniae* isolate from a neonatal patient just transferred to France from French Guiana (Poirel *et al.,* 2000). GES – 1 has hydrolytic activity against penicillins and extended – spectrum cephalosporins but not against cephamycins or carbapenems, and is inhibited by β – lactamase inhibitors. These enzymatic properties resemble those of other class A ESBLs*:* thus, GES – 1 was recognized as a member of ESBLs.

### 2.8.7 VEB – 1, BES – 1 and other ESBL Type Beta-Lactamases

Other unsual enzymes having *ESBL* have also been described (e.g. BES – 1, CME – 1, VE – B – 1, PER, SFO and GES - 1) (Bradford, 2001). These novel enzymes are found infrequently (Naas *et al.*, 2008)

## 2.9 ESBL Evolution and Dissemination

β – lactamases may be chromosomally encoded and universally present in a species or plasmid mediated. The chromosomal enzymes are believed to have evolved from penicillin binding proteins (PBPS) with which they show same-sequence homology. This was probably a result of the selective pressure exerted byβ – lactam- producing soil organisms found in the environment (Bradford, 2001).

The first plasmid mediated-$\beta - lactamases$ in gram- negative bacteria TEM 1 was described in the early 1960s (Bradford, 2001). It was so designated as it was isolated from the blood culture of a named Temoniera in Greece. Being plasmid and transposon mediated, TEM-1 enzymes spread worldwide and are now found in many different species of the family Enterobacteriaceae, *Pseudomonas aeruginosa, Hemophilus influenza* and *Neisseria gonorrhea*. SHV-I (for sulphydral variable type 1) is another $\beta -$ lactamasecommonly found in *Klebsiella* and *Escherichia coli*. Over the years, the use of newer $\beta -$ lactam antibiotics has enabled selection of new variants of $\beta -$ lactamases.

In the early 1980s,the third generation, or oxy-imino, cephalosporins were introduced into clinical practice in response to the increasing prevalence and spread of the $\beta -$ lactamases. Resistance to these extended- spectrum cephalosporins emerged quickly and the first report of an SHV-2 enzyme which was capable of hydrolyzing these antibiotics was published as early as 1983 from Germany.

These enzymes, called extended-spectrum $\beta -$ lactamases because of their increased activity, especially against the oxyimino cephalosporins are several groups of ESBL with similar behaviour but different evolutionary histories. The largest groups are the mutants of TEM and SHV $\beta -$ lactamaseswith over 150 members. The mutations which affect a small number of critical amino acids enlarge the enzymes active site and enable it to deflect the oxymino substitute, which normally shield the$\beta -$ lactam ring. As a result, whereas the classical TEM and SHV enzymes are unable to significantly hydrolyze the oxyimino cephalosporins, the mutants can do so conferring resistant to their host strains (Livermore and Paterson, 2006).

The second largest group of ESBLs is the CTX – M enzymes. Based on sequence homology these are divided into five subgroups with around 40 members. Most of these

subgroups have evolved as a result of the chromosomal β – lactamases genes escaping from *kluvera* Spp, an Enterobacterial genus of little clinical importance. Having migrated to mobile DNA, the CTX – Mβ – lactamases may evolve further. Enterobacteriaceae (mostly *Escherichacoli*) producing the CTX – M enzymes have been identified, predominantly from the community as a cause of urinary tract infections (Paterson and Bonomo, 2005). Various reports suggest that the CTX – M- type ESBL may now actually be the most frequent ESBL type worldwide.

The OXA – typeβ – lactamases (group 2d) are so named because of their oxacillin – hydrolyzing abilities. They predominantly occur in *pseudomonas aeruginosa* but have been detected in many other gram negative bacteria (Livermore, 1995; Weldhagen, 2014). The OXA – type ESBLs were originally discovered in *Pseudomonasaeruginosa* isolates from Turkey. The evolution of ESBL OXA – Typeβ – lactamases from parent enzymes with narrower spectra has many parallels with the evolution of SHV – and TEM – type ESBLs. OXA – 10 hydrolyzes (Weakly) cefotaxime, ceftriaxone and aztreonam, giving most organisms reduced susceptibility to these antibiotics; but OXA – 11, - 14, - 16, - 17, - 19, - 15, - 18, - 28, - 31, - 32, - 35, and – 45 confer frank resistance to cefotaxime and sometimes ceftazidime and aztreonam (Toleman *et al.,* 2003; Danel *et al.,* 1998). The simultaneous production of a carbapenem – hydrolyzing metalloenzyme and an aztreonam hydrolyzing OXA enzymes can readily lead to resistance to allβ – lactam antibiotics (Toleman *et al.,* 1996).

A variety of other β – lactamases (PER, VEB, GES. BES. TLA, SFO, IBC groups) which are plasmid mediated or integron associated class A enzymes have been discovered. They are not simple point mutant derivatives of any knownβ – lactamases and have been found in a wide range of geographic locations. Novel chromosomally encoded ESBLs have also been described (Bellais *et al.,* 2001; Beuernfiend *et al.,* 1996).

## 2.10 Structure OFBeta − Lactamases and Mechanism of Action

All ESBLs have serine at their active sites for a small (but rapidly growing) group of metalloβ − lactamases belonging to class B. They share several highly conserved amino acid sequences with penicillin binding proteins (PBPS) (Medeiros, 1997).

β − lactamases Attack the amide bond in the $\beta - lactamring$ of penicillins and cephalosporins, with subsequent production of penicillinoic acid and cephalosporic acid, respectively, ultimately rendering the compounds antibacterial inactive (Ayyagari and Bhargava, 2001).

Plasmids responsible for ESBL production tend to be large and carry resistance to several agents, an important limitation in the design of treatment alternatives. The most frequent co-resistances found in ESBL organisms are aminoglycosides, fluroquinolones, tetracyclines, chloramphenicol and sulfamethoxazole-trimethoprim (Nathisuwan *et al.*, 2001).

Except for one brief report, none of these enzymes have been shown to be transposable, the usual transmissibility of the responsible plasmids, however allows resistance to spread readily to other pathogens so that extended spectrum enzymes have been found in nearly all species of Enterobacteriaceae (Jacoby and Medeiros, 1991).

Since, ESBL production is usually plasmid mediated, it is possible for one specimen to contain both ESBL producing and non ESBL producing cells of the same species. This suggests that for optimal detection, several colonies must be tested from a primary culture plate (Coudron *et al.*, 1997).

## 2.11 Methods for ESBL Detection

ESBL testing involves two important steps. The first is a screening test with an indicator cephalosporin which looks for resistance or diminished susceptibility, thus identifying isolates likely to be harboring ESBLs. The second one tests for synergy between an oxyimino cephalosporin and clavulanate, distinguishing isolates with ESBLs from those that are resistant for other reasons.

### 2.11.1 Screening for ESBL Producers

**Disk – Diffusion Methods**

The clinical and laboratory standards institute (CLSI) has proposed disk – diffusion methods for screening ESBL production by *klebsiellapneumoniae*, *K. oxytoca, Escherichia coli and Proteusmirabilis*. Laboratories using disk – diffusion methods for antibiotic susceptibility testing can screen for ESBL production by noting specific zone diameters which indicate a high level of suspicion for ESBL production. Cefpodoxime, ceftazidime, aztreonam, cefotaxime or cefotriaxone disks are used. Since the affinity of ESBLs for different substratesis variable, the use of more than one of these agents for screening improves the sensitivity of detection (Wayne, 2009).However, it is adequate to use cefotaxime, which is consistently susceptible to CTX – M; and ceftazidime which is a consistently good substrate for TEM and SHV variants. If only one drug can be used, then the best indication has been found to be cefpodoxime (Livermore, 2006).

If isolates show resistance or diminished susceptibility to any of these five agents, it indicates suspicion for ESBL production, and phenotypic confirmatory test should be used to ascertain the diagnosis.

**Screening by Dilution Antimicrobial Susceptibility Tests**

The CLSI has proposed dilution methods for screening for ESBL production by *Klebsiellapneumoniae* and *K. oxytoca, E. coli and P. mirabilis.*ceftazidime, aztreonam, cefotaxime or ceftriaxone can be used at a screening concentration of 1m*g*/ml or

cefpodoxime at a concentration of 1*ug*/*ml* for *P. mirabilis*; 4mg/ml, for the others. Growth at or above this screening antibiotic concentration is suspicious of ESBL production and is an indication for the organism to be tested by a phenotypic confirmatory test (Wayne, 2009).

### 2.11.2 Phenotypic Confirmatory Tests for ESBL Production

**The combination Disk Test (CDT)**

Use of cefotaxime (30*ug*) or ceftazidime (30*ug*) disks with or without clavulanate (10*ug*) are used for phenotypic confirmation of the presence of ESBLs in *Klebsiellae* and *E. coli, P. mirabilis* and *salmonella* species. The disk tests are performed with confluent growth on Mueller- Hinton agar. A difference of ≥ 5mm between the zone diameters of either of the cephalosporin disks and their respective cephalosporin/clavulanate disks is taken to be phenotic confirmation of ESBL production (Wayne, 2009).

*For Enterobacter spp,C. freundii, Morganella, Providentia* and *Serratia* spp, it is better to use cefepime or cefpirome in the confirmatory tests as they are less prone to attck by the chromosomal AMP C beta lactamases, which may be induced by clavulanate in these species (Livermore, 2006).

**Double Disk Synergy Test (DDST)**

Discs containing cephalosporin (cefotaxime or ceftriaxone, ceftazidime, cefepime) are applied next to a disc with clavulanic acid, amoxicillin + clavulanic acid or ticarcillin + clavulanic acid. Positive result is indicated with when the inhibition zones around any of the cephalosporin discs are augmented in the direction of the disc containing discs containing clavulanic acid. The distance between the discs is critical and 20mm centre to centre has been found to be optimal for cephalosporin 30*ug* discs; however it may be reduced (15mm) or expanded (30mm) for strains with very high or low resistance level respectively (Liofichem, 2014)

**Broth Microdilution**

Phenotypic confirmatory testing can also be performed by broth microdilution assays using ceftazidime (0.25-128*ug*/ml), ceftazidime plus clavulanic acid (025/4-128/4*ug*/ml), cefotaxime (0.25 – 64*ug*/ml), or cefotaxime plus clavulanic acid (0.25/4-64/4*ug*/ml) (Queenan *et al.*, 2004). Broth microdilution is performed using standard methods. Phenotypic confirmation is considered as ≥3 two-fold serial-dilution decreases in minimum inhibitory consideration (MIC) of either cephalosporin in the presence of clavulanic acid compared to its MIC when tested alone.

Steward *et al.*, 2001 suggested using cefoxitin susceptibility in isolates with positive screening tests but negative confirmatory tests as a means of deducing the mechanism of resistance. ESBL- producing isolates appear susceptible, while those with plasmid AMP C enzymes are resistant. However, resistance to cefoxitin seems to be increasing in ESBL-producing isolate due to efflux or permeability changes or coexistence ESBLs with AMP C enzymes. The usefulness of this screen test may thus be diminishing (Rawat, and Nair, 2010).

### 2.11.3 Implications OfPositive Phenotypic Confirmatory Tests

According to CLSI guidelines, isolate which have a positive phenotypic confirmatory test should be reported as resistance to all cephalosporins (except the cephamycins, cefoxitin and cefotetan) and aztreonam, regardless of the MIC of that particular cephalosporin. Penicillins (for example, piperacillin or ticarcillin) are reported as resistant regardless of MIC, but $\beta-$ lactam/ $\beta-$ lactamase inhibitor combinations (for example, tircacillin – clavulanate or piperacillin – tazobactam are reported, as susceptible if MICs or zone diameters are within the appropriate range). (Rawat and Nair, 2010)

### 2.11.4 Other Methods of ESBL Prodution

**Three Dimensional Test.**

The three dimensional test gives phenotypic evidence of ESBL- induced inactivation of extended spectrum cephalosporins or aztreonam without relying on demonstration of inactivation on the $\beta-$ lactamases by a $\beta-$ lactamase inhibitor (Thomson and Sanders, 1992). In this test, the surface of the susceptibility plate is inoculated by standard methods for disk-diffusion testing, but additionally a circular slit is cut in the agar concentric with the margin of the plate. A heavy inoculum of the test organism ($10^9$ to $10^{10}$ CFU of cells) is pipetted into the slit. $\beta-$ lactam $-$ impregnated disks are then placed on the surface of the agar 3mm outside of the inoculated circular slit. $\beta-$ lactamase $-$ induced inactivation of each test antibiotic is detected by inspection of the margin of the zone of inhibition in the vicinity of its intersection with the circular three dimensional inoculations. The presence of $\beta-$ lactamase $-$ induced drug inactivation is visualized as a distortion or discontinuity in the usually circular inhibition zone or as the production of discrete colonies in the vicinity of the inoculated slit (Rawat and Nair, 2010).

**Inhibitor-Potentiated Disk-Diffusion Test**

Antibiotic disks containing ceftazidime (30*ug*), cefotaxime (30*ug*), ceftriaxone (30*ug*) and aztreonam (30*ug*) are placed on the clavulanate – containing agar plates and regular clavulanate-free Mueller-Hinton agar plates (Ho et al., 1998). A difference in $\beta-$ *lactam* zone width of $\geq$10mm in the two media was considered positive for ESBL production. A major drawback of the method is the need to freshly prepare clavulanate – containing plates. The potency of clavulanic acid begins to decrease after 72 hours (Rawat and Nair, 2010).

**Cephalosporin/Clavulanate Combination Disks on Iso - Sensitest Agar**

The British society for antimicrobial chemotherapy has recommended the disk-diffusion method for phenotypic confirmation of ESBL presence using ceftazidime – clavulanate and cefotaxime – clavulanate combination disks, with semi confluent growth on Iso-

sensitest agar (rather than confluent growth on Mueller-Hinton agar). A ratio of cephalosporin/clavulanate zone size to cephalosporin zone size of 1.5 or greater was taken to signify the presence of ESBL activity. Using this method, the sensitivity of the test for detecting ESBLs was 93% using both ceftazidime and cefotaxime. The test did not detect ESBL production by strains producing SHV – 6 (M'zali *et al.*, 2000).

**Disk Approximation Test**

Cefoxitin (inducer) disk is placed at a distance of 2.5cm from cephalosporin disk (Revathi and Singh, 1997). Production of inducible $\beta-$lactamase is indicated by flattening of the zone of inhibition of the cephalosporin disk towards inducer disk by >1mm (Rawat and Nair, 2010).

### 2.11.5. Commercially available Methods for ESBL Detection

**Vitek ESBL Test**

A specific card which induces tests for ESBL production has now been (food and drugadministration) FDA approved. The vitek ESBL test (Biomerieux Vitek, Hazelton, Missouri) utilizes cefotaxime and ceftazidime, alone (at 0.5*ug*/ml) and in combination with clavulanic acid (4*ug*/ml). Inoculation of the cards is identical to that performed for regular Vitek cards. Analysis of all wells is performed automatically once the growth control well has reached a set threshold (4-15 hours of incubation). A predetermined reduction in the growth of cefotaxime or ceftazidime wells containing clavulanic acid, compared with the level of growth in the well with the cephalosporin alone, indicates presence of ESBL. Sensitivity and specificity of the method exceed 90% (Sanders *et al.*, 1996).

**E Test**

The E test ESBL strip (AB Biodisk, Solna, Sweden) carries two gradients; on the one end ceftazidime, and on the opposite end ceftazidime plus clavulanic acid (Vercauteren *et al.*, 1997). MIC is interpreted at the point of intersection of the inhibition ellipse with the E test strip edge. A ratio of ceftazidime MIC to ceftazidime – clavulanic acid MIC equal to or greater than 8 indicates the presence of ESBL. The reported sensitivity of the method

as a phenotypic confirmatory test for ESBL was 87% to 100% (Ho *et al*, 1998; Cormican *et al.,* 1996). And the specificity is 95% to 100%. The availability of cefotaxime strip, as well as ceftazidime strip, improves the ability to detect ESBL types which preferentially hydrolyze cefotaxime, such as CTX-M-type enzymes (Paterson and Bonomo, 2005)**Microscan Panels**

Microscan panels (Dade Behring Microscan, sacramento, CA) comprise dehydrated panels for microdilution antibiotic susceptibility. Those used for ESBL detection which contain combination of ceftazidime or cefotaxime plus $\beta-$ lactamase inhibitors have received food and Drug Administration approval and in studies of large numbers of ESBL producing isolates, they have appeared to be highly reliable (Komatsu *et al.,* 2003; Pagani *et al.,* 2002)

**Becton Dickinson (BD) Phoenix Automated Microbiology System**.

Becton Dickinson biosciences (sparks, md) have introduced a short-incubation system for bacterial identification and susceptibility testing, known as BD-Phoenix. (Sanguinetti *et al.,* 2003; Sturrenbury *et al.,* 2003). The phoenix ESBL test uses growth response to cefpodoxime, ceftazidime, Ceftriaxone and cefotaxime with or without clavulanic acid, to detect the production of ESBLs. The test algorithm has been delineated by Sanguinetti *et al,* 2003. Results are usually available within 6 hours. The BD phoenix ESBL detection method detected ESBL production in greater than 90% of strains genotypically confirmed to produce ESBLs. The method correctly detected ESBL production by *Enterobacter, Proteus Citrobacter* spp. in addition to *Klebsiella* and *E. coli*. (Sanguinetti *et al.,* 2003)

## 2.12 Genotypic Detection

The determination of whether a specific ESBL present in a clinical isolate is related to TEM and SHV enzymes is a complicated process because point mutations around the active sites of the TEM and SHV sequences have led to amino acid changes that increase the spectrum of activity of the parent enzymes, such as in TEM1, TEM 2 and SHV 1 (Bradford, 2001). The molecular method commonly used is the PCR amplification of the $bla_{TEM}$ and $bla_{SHV}$ genes with oligonucleotide primers, followed by sequencing is essential to discriminate between the non- ESBL parent enzymes (e.g. TEM 1, TEM 2 or

SHV 1) and different variants of TEM or SHV ESBLs (e.g. TEM 3, SHV 2, etc) (Bradford, 2001).

The PCR amplification of CTX-M- specific products without sequencing, in an isolate that produces an ESBL, usually provides sufficient evidence that a $bla_{CTX-M}$ gene is responsible for this phenotype. This is unlike TEM and SHV types of ESBLs. Several recent studies have described various molecular approaches for the rapid screening of ESBL- positive organisms for the presence of different $bla_{CTX-M}$ genes (pitout *et al.,* 2004), amplification of a universal DNA fragment specific for most of the different groups of CTX-M $\beta - lactamases$ , duplex PCR (pitout, 2007), multiplex PCR (Woodford *et al.,* 2006), real time PCR, pyrosequencing and reverse line hybridization. Molecular techniques undoubtedly have the potential to play an essential part in the laboratory setting for the screening tracking and monitoring of the spread of large number of organisms producing CTX – M enzymes from the community and hospital setting in real time.

## 2.13 ESBL Epidemiology

The epidemiology of ESBL is quite complicated. Initially, there are certain levels to consider: the wider geographical area, the country, the hospital, the community and the host (in most cases a single patient or a healthy carrier). Additionally, there are various reservoirs, including the environment (e.g. soil and water), wild animals, and farm animals and pets. The final component entails transmission from food and water and via direct or indirect contact (person to person) (Carotolli, 2008). The epidemiology of ESBL in different parts of the world is different. Some of the epidemiology studies are as follows:-

### Europe

The first report of ESBL producing strain was from Germany in 1983 (knothe *et al.,* 1983) but it was found in France in 1985 (Knothe *et al.,* 1983; Rice *et al.,* 1990). According to a national surveillance report, there was an increase in ESBL producer strains in northern European countries such as Denmark, Norway and Sweden. The studies also showed prevalence of ESBL positive strains in Spain and Portugal. In the last

ten years, Italy also showed an increase in ESBL positive strains (Luzzaro *et al.,* 2006). The frequency of the occurrence of ESBL positive strains was more than 10% in Eastern Europe countries such as Hungary, Poland, Romania, Russia and Turkey. In all the mentioned countries, *K. pneumoniae* was found to be dominantly ESBL positive (Damjanova *et al.,* 2007; Korten *et al.,* 2007; Markovska *et al.,* 2008). The enzymes mostly responsible for ESBL production in Eastern Europe countries are; CTX-M-3, SHV-2 and SHV-5. It is evident that there is an increasing prevalence of CTX-M- 15 and it constitutes the epidemiology of ESBL in all the European countries (Oteo *et al.,* 2006).

**South and Central America**

SHV-2 and SHV-5 were first reported to harbor *K. pneumoniae* during the period, 1988 to 1989 in chile and Argentina (EARSS, 2011). The studies revealed that ESBLs positive were identified is 30 to 60 % of Spp in Brazil, Colombia and Venezuela (Mendes *et al.,* 2000). The prevalence of ESBL producing*E.coli* and *Klebsiella* in Latin America showed an increase in 2008 compared to the previous years. Generally, 26% of *E. coli* and 35% of *K. pneumoniae* in Latin American were ESBL producers in 2008. In 2003, 10% of *E. coli* and 14% of *K. pneumoniae* were positive for ESBL production, while in 2004, it was 10% of *E. coli* and 18% *K. pneumoniae (Rossi et al., 2006).*

**North America**

The first ESBL positive reported in 1988 in the United State, was *K. pneumoniae* with TEM 10(Jacoby et al., 1988). This was followed by TEM- 12 and TEM- 26 (Bush, 2008).

In 2001, it was reported that 5.6% of strains were ESBL positive in the United States (Winokur *et al.,* 2001). In 2009, a survey on *E. coli* reported that 9% of *E. coli*were ESBL producers (Bhusal *et al.,* 2011). Further, there were also reports of outbreaks of SHV type ESBLs. Data obtained from the Surveillance Network concerning in vitro antimicrobial resistance is US outpatients between 2000 and 2010 and their results showed that resistance to ceftriaxone rose from 0.2% to 2.3% and resistance to cefuroxime increased from 1.5% to 5%, but the bacterial isolates in focus were not tested for ESBLs (Sanchez *et al.,* 2010).

**Africa**

There have been some studies which showed a high prevalence of ESBL producing *K. pneumoniae* in South Africa. A survey conducted during a one year period, between 1998 and 1999, in a South African hospital indicated that 36.1% of *K. pneumoniae* were ESBL producers (Bell *et al.*, 2002; Cotton et al., 2000)).

The first study of ESBLs in Tanzania was performed in 2001 – 2002 and in analyzed blood isolates from neonates, was found 25% of the *E. coli* and 17% of the *K. pneumoniae* produced ESBLs, mainly the CTX – M – 15 and TEM – 63 types (Blomberg *et al.*, 2005). In a more recent investigation conducted at a tertiary hospital in Mwanza, Tanzania, the overall prevalence of ESBLs in all gram negative bacteria (377 clinical isolates) was 29%. The ESBL prevalence was 64% in *K. pneumoniae* but 24% in *E. coli* (Kariuki *et al.*, 2007). Also, in a small study at an orphanage in Mali, 63% of the adults and 100% of the children were found to carry ESBL producing Enterobacteriaceae that showed extensive co-resistant to other antibiotics (Tande *et al.*, 2009).

In investigations conducted in Nigeria, one of which was conducted by Afunwa *et al.*, 2011 at a tertiary hospital in Nigeria, among the overall ESBL producing isolates, 35% being community origin and 65% from hospitals. The ESBL isolates showed high resistance to tetracycline, gentamicin, pefloxacin, ceftriaxone, cefuroxime, ciprofloxacin, and augmentin.

Also, in another study, by Adeyankinnu *et al.*, (2014) in Abeokuta township South West Nigeria, 1997 isolates of *Escherichia coli* and *klebsiellapneumoniae* were selected and tested for ESBL production and antimicrobial susceptibility. ESBL prevalence was 26.4% for all isolates tested, with *E. coli* having a greater proportion. There was absolute resistance to ampicillin, tetracycline and co-trimoxazole among tested isolates. There was above average susceptibility to the $2^{nd}$ and $3^{rd}$ generation cephalosporins. The study

shows an upsurge in ESBL acquisition by gram negative bacteria and evidence of co circulation of varying subtypes of ESBL with both plasmid transmissible and chromosome ecoded subtypes.

Also in another investigation on poultry feeds in Nigeria, 20.7% of the multi drug resistance (MDR) strains were positive for ESBL enzyme expression with *salmonella enterica* ser. typhi having the highest incidence of ESBL expression (50%) although it recorded the least MDR incidence 6.9% (Oyinloye and Ezekiel, 2011).Chah and Oboegbulem, 2007 reported one hundred and seventy-two ampicillin resistant *E. coli* strains isolates from commercial chickens in Enugu, Nigeria, 170 (98.9%) of which produced beta-lactamase enzyme. Sixteen (9.4%) were phenotypically confirmed to produce ESBLs.

**Middle East**

The overall data on ESBL-producing Enterobacteriaceae in the countries of the Middle East are extremely worrisome and this region might indeed be one of the major epicenters of the global ESBL pandemic. Investigations conducted in that country showed that 61% of *E. coli* produced ESBLs of the CTX – M – 14, CTX – M 15 and CTX – M – 27 types, and all of the strains harbored the TEM enzyme (Al-Agamy *et al.,* 2006). In a study of inpatients in Saudi Arabia in 2008, it was found that 26% of *K. pneumoniae* isolates produced ESBLs, the majority of which were SHV – 12 and TEM – 1 enzymes, and 36% were CTX – M – 15 (Tawfik *et al.,* 2011).

Another investigations conducted in the same country in 2004 – 2005 showed that 10% of clinical urinary *E. coli* isolates from patients and 4% of such isolates from outpatients were ESBL producers (Khanfar *et al.,* 2009). It was also observed in analyzed fecal samples in Lebanon in 2003 and noted that ESBL carriage differed somewhat between patients (16%), health care workers (3%) and healthy subjects (2%) and also that there was a predominance of the CTX – M – 15 enzyme (83%) (Moubareck *et al.,* 2005). Data

collected over three years in kuwait showed that the levels of ESBLs were lower in community isolates of *K. pneumonia*e (17%) and *E. coli* (12%) than in the corresponding hospital isolates (28% and 26%, respectively) (Al. Benwan *et al.,* 2010).

**Australia**

The first report of an ESBL positive in Australia was found in *klebsiella* Spp (gentamicin resistance) in a study done between 1986 and 1988 (Mulgrave, 1990). They later found SHV was responsible for ESBL production in *Klebseilla* Spp (Mulgrave and Attwood, 1993). In the last decade, ESBL positive strains were also identified in all the regions in Australia. It is estimated that 5% of isolates in Australia are positive for ESBL production. (Bell *et al.,* 2002)

**Asia**

The first isolates of *K. pneumoniae* harboring SHV – 2 were reported from China in 1988 (Rossi *et al.,* 2006). Only lately has the understanding of the extent of the ecological disaster related to ESBL producing Enterobacteriaceae in parts of Asia and the Indian subcontinent, and the number of reports of very high frequency of such bacteria in those regions continues to rise. It is likely that some of the successful ESBL – producing clones originate from Asia. Deficient sewage routines (the "Delhi belly") and poor quality of drinking water, in combination with a lack of control over prescription and sales of antibiotics, are probably major factors that have promoted the development of resistance. A few articles published as early as the end of the 1980s, and the beginning of the 1990s have reported occurrence of the SHV–2 and Toho –1 (CTX – M – 44) enzymes in China and Japan (Hawkey, 2008). According to SENTRY surveillance program there have been rapid increase in ESBL – producing *K. pneumoniae* (up to 60%) and *E. coli* (13 – 35%) in different parts of China, with a predominance of the CTX – M – 14 and CTX – M – 3 enzymes (Hawkey, 2008; Hirakata *et al.,* 2005). It has been found that 66% of third generation cephalosporin resistant *E. coli* and *K. pneumoniae* from three medical centres in India harbored the CTX – M – 15 type of ESBL, which was also the only CTX – M enzyme found and an investigation of 10 other centres in that country showed that rates of ESBL – producing Enterobacteriaceae reached 70% (Mathai *et al.,* 2002). Recently

ESBL production was observed in 48% of *E. coli*,44% of *K. pneumoniae* and 50% of *P. aeruginosa* isolates in a tertiary hospital in Patiala, Punjab (Rupinder *et al*., 2013). Also, Sankar *et al.,* 2012 reported observing ESBL rate of 46% and 50% in out and in patients respectively. Investigations from India and Pakistan show an alarming and rapid increase in the prevalence of Enterobacteriaceae with NDM-I with prevalence rate from 6.9% in a hospital in Varanasi, India, to 18.5% in Rawalpindi, Pakistan (Perry *et al.,* 2011) and perhaps the spread of these enzymes could be even more rapid than the spread of the CTX – M enzymes.

## 2.14. Risk Factors for Colonization and Infection with ESBLProducers

Patients at high risk for developing colonization or infection with ESBL-producing organisms are often seriously ill patients with prolonged hospital stays and in whom invasive medical devices are present (urinary catheters, endotracheal tube, central venous lines) for a prolonged duration (Paterson and Bonomo, 2005). Other risk factors include the presence of nasogastric tubes, gastrostomy or jejunostomy tubes or arterial lines, administration of total parenteral nutrition, recent surgery, hemodialysis, decubitus ulcers and poor nutritional status (Lucet *et al.,* 1996; Weldhagen and Prinsloo, 2004; Pena *et al.,* 1997).

Heavy antibiotic use is also a risk factor for acquisition of an ESBL- producing organism (Lautenbach *et al.,* 2001). Several studies have found a relationship between third-generation cephalosporin use and acquisition of an ESBL producing strain (Pessoa- silva *et al.,* 2003). However, perhaps the greatest risk factor for nosocomial acquisition of an ESBL- producing organism is accommodation in a ward or room with other patients with ESBL-producing organism (Livermore and Paterson, 2006).

Risk factors for colonization or infection with ESBL- producing organisms, especially the CTX - M producers, include history of recent hospitalization, treatment with cephalosporins, penicillins and quinolones; age 65 years or higher; dementia and diabetes (Paterson and Bonomo, 2005). Although there is no conclusive evidence, one potential

source of colonization with the ESBL producers in the community may be the uses of veterinary oxyimino cephalosporins like ceftiofur in livestock (Livermore and Paterson, 2006). Over the past few years the researchers (Tham *et al.,* 2010) have found evidence that international travel to highly endemic areas (i.e. Asia, the Middle East and Africa) represents one of the most important risk factors for ESBL carriage especially in the community.

## 2.15. Treatment

The carbapenems (imipenems, meropenem, ertrapenem, doripenem) are still the first choice of treatment for serious infection with ESBL- producing *E. coli and K. pneumoniae.* It has been reported that > 98% of the ESBL producing *E. coli, K. pneumoniae and P. mirabilis* are still susceptible to these drugs (Perez *et al.,* 2007). But with the emergence of the carbapenem- resistant Enterobacteriaceae, the "magic bullet" is actually difficult to find. There are some older drugs which can be used to treat the ESBL- producing *E. coli or K. pneumoniae* infections. Fosfomycin was reported of having admirable in vitro activity against the ESBL- producing *E. coli or K. pneumoniae*. In Hong Kong, most of the ESBL- producing E. *coli* isolates were reported to be sensitive to fofomycin (Ho *et al*., 2010). Colistin is another choice which can be considered for the treatments of infections with these organisms. Although once considered as a toxic antibiotic, it is a last resort that can be considered at the present moment as there is no new anti gram negative antibiotics available for the treatment of these multidrug resistant organisms. Other than ESBL producing organisms, actually colistin is used in the treatment of multidrug resistant *P. aeruginosa,* carbapenem resistant *Acinetobacter baumannii.* Close monitoring for the development of side effects can improve the safety margin when prescribing the drug. Tigecycline is also one of the drugs in the pipeline which can be considered for treatment (Perez *et al*., 2007).

Although ESBL activity is inhibited by clavulanic acid, the only infection that can be treated with $\beta-$ lactam / $\beta-$ lactamase inhibitor combination are those involving the urinary tract. In this instance, $\beta-$ lactamase inhibitor concentration is high enough to

counteract the hydrolytic activity (Nordmann, 1998). By inhibiting ESBL, β – lactamaseinhibitors appear to impair the emergence and spread of *Klebsiella* carrying resistant plasmids. Furthermore, administration of inhibitors may exert in vitro pressure on ESBL, thereby facilitating their reverse mutation to less harmful enzymes (Piroth *et al.*, 1998)

Non- β – lactam antimicrobial agents (aminoglycosides, fluoroquinolones) may be beneficial; however, coresistance rates against these agents are frequent (Nathisuwan *et al.*, 2001)

## 2.16. Prevention

Proper infection-control practices and barriers are essential to prevent spreading and outbreak of ESBL producing bacteria. The reservoir for these bacteria seems to be the gastrointestinal tract of patients. Alternative reservoirs could be the oropharynx,colonized wounds and urine. The contaminated hands and stethoscopes of health care providers are important factors in spreading infection between patients (Samaha- K Foury and Araj, 2003).

Essential infection-control practices should include avoiding unnecessary use of invasive devices such as indwelling urinary catheters or intra venous lines, hand washing by hospital personnel, increased barrier precautions and isolation of patients colonized or infected with ESBL-producers (Rawat and Nair, 2015)

Institutions with endemic ESBL-producing organisms need to determine whether there is a high rate of cephalosporin use, especially third generation cephalosporins. Several studies have shown that by limiting the use of these agents alone or in combination with infection control measures, the frequency of ESBL isolates can be reduced substantially (Chaudharg and Aggarwal, 2004).

Some authors have suggested that use of β – lactam / β – lactamase inhibitor combinations, rather than cephalosporins as workhorse empirical therapy for infections suspected as being due to gram negative bacilli, may facilitate control of ESBL producers. However, many organisms now produce multiple β – lactamase, which may reduce the effectiveness of β – lactam / β – lactamase inhibitor combinations (Chanawong *et al.*, 2002; Baraniak *et al.*, 2002)

# CHAPTER THREE

## 3.0 MATERIALS AND METHODS

### 3.1Study Area

Jalingo was the study area chosen. It is the head quarter of Jalingo Local Government Area and the capital of Taraba State which is located in the North-East geopolitical zone of Nigeria.Jalingo is in the northern Guinea Savanna zone of the vegetative cover of Nigeria. It located between latitude $8^0$ 47′ North and $90^0$1′ North; longitude $11^0$9′, East and $11^0$30′ East. It has a population of approximately 118,000 people (2006 census) and a land mass of 3,871 sq km. The annual precipitation fall is 1053 while the temperature averages $27.3^0$ C (KÖpper Geiger, 1936)

### 3.2 Collection of Sample

Six hundred and nineteens sample were collected from 7 poultry farm environments. The poultry farms studied were:

Tartius's farm, Tutu's farm, Ijaja's farm, Bello's farm, Baltic Farm Jakadafari,Baltic farm, Sabon line and Madam Fibi's farm.

Tartius's farm is located in Jolly Nyame garden city (Technobat Quarters) Mile Six. The chickens reared were Golden comet layers.

Tutu's farm is located in Abuja phaseI.Both Australorp and Rhode Isand red layers were reared but only the Australorp farm was studied.

Bello's farm is located in Teacher Collge (TC) Quarters, Nyabukaka. Cornish cross broilers were reared in their farm. The broilers studied were those that were ≤ 7 weeks old.

Ijaja's farm is located in Teacher Collge (TC) Quarters, Nyabukaka. Cornish cross broilerswerereared in their farm. The broilers studied were those that were ≤ 7 weeks old.

Baltic farm studied were located at Sabon Line and Jaikada-Fari. The farm in Jaikada-Fari is where Cornish cross Broilers were reared. The broilers studied were 7 weeks old.

The other Baltic farm located at Sabon Lineis were white leghorn cockerels were reared. The cockerels investigated were 3 weeks old.

Madam Fibi's farm was located at Nyamusala. The cockerels studied were 2 days old white leghorn.

The sa.mples were chicken cloacae, floor, wall, drinker and feeder swabs. Others were, stool and urine of the poultry farmers as well as the drinking water and feed of the chickens. The samples were grouped into three. They are chicken (comprising of the cloacal swab), chicken environment (comprising of swabs from floors, wall, drinkers, feeder and also the drinking water and the feed) and chicken rearers (comprising of stool and urine samples).

### 3.2.1 Collection of Sample from chicken Cloacae

A swab stick was soaked in sterile distilled water and was inserted into the cloaca of each of the randomly selected chickens in each of the poultry farm environment. While still in the cloaca, the swab stick was rotated three times before it was finally put back into its container.

### 3.2.2 Collection of Sample from the Floor of the Poultry

Four swab sticks were soaked in sterile distilled water. Two were separately used to swab the floor horizontally at opposite ends of the poultry while another two were separately used to swab vertically opposite ends of the floor. Each swab stick was then put back into its container.

### 3.2.3 Collection of Sample from the Wall of the Poultry

Four swab sticks were soaked in sterile distilled water. Two were individually used to swab horizontally the two opposite walls of the poultry while another two were separately used to swab the other opposite walls. Then each swab stick was put back into its container.

### 3.2.4 Collection of Sample from Drinkers

Two swab sticks were soaked in sterile distilled water and each was used to swab a drinker and then put back into its container.

### 3.2.5Collection of Sample from Feeders

Two swab sticks were soaked in sterile distilled water and each was used to swab a feeder and then put back into its container.

### 3.2.6Collection of Sample of chickens' Drinking water

Two 50ml of drinking water of the chicken were collected with sterile containers. The water sample was collected from containers.

### 3.2.7Collection of Sample of Feed

Ten grams of feed was collected from two different feed bags in the poultry farm. Each feed sample was dissolved in 90ml of sterile distilled water.

### 3.2.8Collection of Urine and Stool Sample from Chicken Rearers

A sterile container was given to each chicken rearer whom he/she used to collect his urine and stool sample

## 3.3 Bacterial Isolation and Identification

All samples collected were cultured within 2 hours of collection on MacConkey agar and incubated at $37^0$C for 18 hours. Then, the MacConkey agar plates were examined and colonies were subcultured to obtain distinct colonies and incubated for 18h at $37^0$C for 18 hours. The next day, distinct colonies were examined for their morphology and ability to ferment lactose. Lacstose fermenting colonies were further subcultured on Eosin methylene blue (EMB) agar and incubated for 18h at $37^0$C. All the isolates (both lactose

and non lactose fermenters) were gram stained and subjected to biochemical tests.Also,(deoxyribonucleic acid)DNA sequencing of the isolates was performed.

### 3.3.1 Gram Reaction

A smear of the test bacterial species was done on a glass slide and heat fixed. The slide was placed on a level surface and then crystal violet stain was poured on the smear and allowed to stand for one minute. Then, the stain was washed off under running water. After which lugol's iodine solution was poured on the smear (while the slide was on a level surface) and allowed to stand for 30 seconds. The slide againwas washed under running water. The slide was then put in a slanting position and lugol's alcohol was applied in drops for 30 seconds for decolourization. After 30 seconds, the alcohol was washed off under running water. Finally, safranin was poured on the slide and washed off after 30 seconds and then the slide was then placed back on the rack for the smear to air dry and then observed under the microscope using 40x objective and the oil immersion objective. The colour and shape of the bacteria were noted(Ochei and Kolhatkar, 2000.)

### 3.3.2 Biochemical Tests

The biochemical testsdone include indole test, methyl red test, urease test, motility test, voges-proskauer test, and citrate test (Ochei and Kolhatkar, 2000).

#### 3.4.2.1Indole Test

The test bacterial species was obtained from 24 hours culture on nutrient agar.
The test organism was grown in peptone water for 24 hours.
Three drops of kovac's reagent was added to the 24 hours peptone water culture and observed for the appearance of a red ring above the peptone water.

#### 3.4.2.2 Triple Sugar Iron Agar (TSI) Test

Each test organism was obtained from 24 hours culture on nutrient agar. The isolates were individually inoculated on TSI agar. Both butt and slant inoculations were done. After inoculation, it was incubated at $37^0$C for 24 hoursand then examined.

### 3.4.2.3. Urease Test

Each test organism was obtained from24 hours culture on nutrient agar, the isolates were each heavily inoculated into urease agar contained in a test tube and incubated at $37^0$C for 24 hours, and then examined for the ability to hydrolyse urea which is indicated by the appearance of a pink colouration.

### 3.4.2.4 Motility Test

The test organism was also obtained from 24 hours culture on nutrient agar. A semi solid medium containing 0.3% nutrient agar dissolved in distilled water containing peptone water was used. Using a straight wire, the semi solid medium was stab inoculated with the test organism and incubated at $35^o$C for 48 hours and then observed for motility.

### 3.4.2.5 Voges Proskauer Test

The test organism was obtained from 24 hours culture on nutrient agar. The test organism was grown in peptone water for 48 hours at $37^o$C. Then 1ml of 10% of KOH was added and left for one hour and then observed for the development of a pink colour which indicated that the organisms is Voges Prokauer positive.

### 3.4.2.6 Citrate Test

The test organism was obtained from 24 hours culture on nutrient agar.A light suspension of the organism was made in saline. Citrate agar was stab inoculated and incubated for 24 hours and thereafter observed for the development of a deep blue colour which indicates citrate positive.

### 3.4.2.7 Methyl Red Test

The test organism was obtained from 24 hours culture on nutrient agar. The peptone water was lightly inoculated and incubated at $35^o$C for 48 hours. Then 5 drops of the indictor (methyl red solution) were added to the culture and then observed for a change in colour to red to indicate methyl red positive.

### 3.4.2.8 Oxidase Test

The test organism was obtained from 24 hoursculture on nutrient agar. A piece of filter paper ws put in a clean and sterile petri dish. Then 3 drops of freshly prepared oxidase reagent were added. Using a piece of sterile glass rod, a colony of the test organism was smeared on the filter paper. Appearance of blue-purple colour within 10 seconds indicates a positive oxidase test.

### 3.4.3.0 DNA Sequencing

Molecular analysis of the isolates was based on (PCR) polymerase Chain Reaction and metigenomics analysis. DNA extraction was performed at a commercial laboratory; Teddy and Thaddeus Nig. Co. Lagos Anaerobe laboratory. Sequencing and bioinformatics analysis at Ingaba biotechnology pty South Africa

## 3.5 Detection of Beta-lactamase producers

Isolates were tested for beta-lactamase production using acidimetric method as described by Cheesbrough, (2010). A strip of what man No.1 filter paper was placed in the bottom of a petri dish and a few drops of buffered crystalline penicillin bromocresol purple solution was added to it until the paper was saturated. Using a sterile wire loop, seven colonies of same species of the test organism were spread on the filter paper, covering an area approximately 5mm in diameter. The petri dish was covered and then incubated at room temperature for 30 minutes and then observed for the development of a yellow colour which indicated the ability to produce betalactamase.

## 3.6 Phenotypic Confirmation of Extended Spectrum Beta Lactamse (ESBL) Production

All beta-lactamase producing isolates were screened for ESBL production by double disk synergy test (DDST) according to Liofichem, (2014). Seven colonies of same 24 hour species of organism on nutrient agar was touched with sterilized inoculating wire loop and then suspended in 4ml sterile normal saline and the inoculums density adjusted to 0.5 Mc Farland turbidity standards. Surfaces of Mueller-Hinton agar were flooded with the standardized bacterial suspension and allowed to dry for 4 minutes. With a sterile forcep, ceftazidime disk (30mg) and ceftriaxone (30mg) were applied next to a disc with

clavulanic acid, amoxicillin + clavulanic acid (20/10mg). Positive result was indicated when the inhibition zones around any of the cephalosporin disc were augumented in the direction of the disc containing clavulanic acid. The distance between the discs was 20mm centre to centre

### 3.7 Antimicrobial Susceptibility Testing

All the *ESBL* positive isolates were subjected to antimicrobial susceptibility test. *Antimicrobial* susceptibility was determined by Kirby-Bauer disk diffusion method as described by Cheesbroigh, (2010). The antibiotics used include ampicillin (*10ug*), ceftazidime (*30ug*), ceftriaxone (*30ug*), ciprofloxacin (*5ug*), gentamicin (*10ug*), chloramphenicol (*30ug*) and tetracycline (*30ug*).

Seven colonies of same 24 hours species of organism on nutrient agar were touched with sterilized inoculating loop and then suspended in 4ml sterile normal saline and the inoculum density adjusted to 0.5 McFarland turbidity standards. Surfaces of Mueller-Hinton agars were flooded with the standardized bacterial suspension and allowed to dry for 4 minutes. With a sterile forcep, the aforementioned antibiotics discs were placed on the inoculated plates. The plates were incubated at $37^0$C for 24 hours. After incubation, the diameters of zones of inhibition around each antibacterial disc were measured with a foot translucent rule and recorded to the nearest whole millimeter. The species were interpreted as susceptible, intermediateor resistant by using a table that relates zone diameter to the degree of microbial resistance.

### 3.8 Plasmid Profile of Extended Spectrum Betalactamse (ESBL) Positive Isolates

Out of the 93 *ESBL* positive isolates obtained, plasmid profiling was carried out on 15 randomly selected *E. coli* isolates and the 2 *klebsiella pneumoniae* isolates. Plasmid extraction was done using TENS-mini prep (for gram negative bacteria). Briefly 1.5ml of 24 hours culture was spinned for 1 minute in a micro-centrifuge to pellet cells. Supernatant was gently decanted, leaving 10-100*ul* together with cell pellets and vortexed at high speed to re-suspend cells completely, 300*ul* of TENS was added and

mixed by inverting tubes 3 – 5 times until the mixture became sticky. To prevent the degradation of chromosomal DNA which may co-precipitate with plasmid DNA, if more than 10 minutes would be needed before moving to the next step, it is better to set samples on ice. 150*ul* of 3.0m sodium acetate pH 5.2 was added and vortexed to mix completely. To pellet cell debris and chromosomal DNA, the mixture was spinned for 5 minutes in micro-centrifuge. The supernatant was transferred in a fresh tube and mixed well with 900*ul* of ice-cold absolute ethanol. To pellet plasmid DNA, it was spinned for 10 minutes and white pellet was observed. The supernatant was discarded and the pellet rinsed twice with 1*ml* of 70% ethanol and then the pellet dried. Pellet was re-suspended in 20 – 40*ul* of TE buffer or distilled for further use (TEN compositions: Tris 5mm, EDTA 10mm, NaOH O.1N and SDS 0.5%).

Extracted DNA plasmids were electrophorosed on 0.8% agarose gel and stained with 14*ul/g* Ethidium bromide, gel pictures were photographed with a Polaroid camera under the view of (ultraviolet) UV transilluminator. Molecular weights and distances were determined according to Kim *et al*., (2002).

### 3.9 Pasmid Curing

ESBL positive isolates were selected and subjected to acridine orange as described by Iroha *et al.*, (2009). Each tested organism was grown in a solution of 5ml double strength nutrient brothsupplemented with *0.1µg/ml* of acridine orange and incubated at $37^0$C for 24 hours. After incubation test organisms were retested for ESBL production using double disk synergy test.

### 3.10 Statistical Analysis

The data obtained during the investigations were subjected to analysis of variance and chi-square and inferences made at $p \leq 0.05$ using statistical package for social sciences (SPSS) version 21.0.

# CHAPTER FOUR

## 3.0 RESULTS

A total of 619 different samples were collected from 7 poultry farm environment. Three hundred and ninty seven isolates which were 365 *Escherichia coli*, 20 *Klebsiella pneumoniae* and 12 *Klebsiella oxytoca* were isolated and used for this study. Other isolates are 107 *Enterobacter*, 22 *Salmonella*, 24 *Shigella*,18 *Proteus*, 92 *Pseudomonas aeroginosa* and 46 *Achromobacter*.The results obtained from this study are shown in the following tables (4.1 to 4.31) figures (4.1 to 4.12) and plates (4. 1a and 4.16).

The isolates obtained were*E.coli, K.pneumoniae ,K.pneumoniae, Enterobacter, Salmonella, Proteus, Shigella,Achromobacter and Pseudomonas aeruginosa*.Their biochemical characteristics are as shown in Table 4.1.

**Table 4.1: Biochemical Characteristics of the Isolates**

| on Nutrient Pigmentaion | Methy/Red | Voges Proskauer | Indole Production | Motility | Oxidase | Citrate | Urease | TSI Slant | TSI BUTT | $H_2S$ | Test / Probable Isolate |
|---|---|---|---|---|---|---|---|---|---|---|---|
| - | + | - | + | ± | - | - | - | A (Alk) | AG | - | *E.coli* |
| | - | + | - | - | | + | + | A | AG | - | *K.pneumoniae* |
| - | - | + | + | - | - | + | + | A | AG | - | *K.oxytoca* |
| | + | - | ± | + | | ± | + | ALK | AG | + | *Proteus* |
| - | - | + | - | + | - | + | - | A | AG | - | *Enterobacter* |
| - | + | - | - | + | - | ± | - | ALK; G | A | + | *Salmonella* |
| | + | - | ± | - | | - | - | ALK | A | - | *Shigella* |
| - | + | + | - | + | - | + | + | ALK | ALK | - | *Pseudomonas aeruginosa* |
| ++ | | + | - | + | | + | + | A | ALK | - | *Achromobacter* |

A – Acid, G – Gas, Alk – Alkaline

The highest frequency of isolation (77.5%) of *E. coli* was obtained from the chickens while the least (3.9%) was obtained from the chickens rearers. The frequency of isolation of*K. pneumoniae* obtainedfrom the chickens was 5.9%. Also, 3.9% of *K. oxytoca*was obtained from chickens. The frequency of occurrence of *E.coli*obtained from chickens, chickens environment and chicken rearers were 77.5%, 8.8% and3.9% respectively while that of *K.pneumoniae*was 5.9%, 0%, 0%. The frequency of isolation of *K. oxytoca* was 3.9%, 0%, 0% from chickens' chickens' environment and chickens' rearers respectively. However there is no stastiscal difference in the frequency of isolation of the isolates in farm I ($P>0.05$). This is as presented in Table 4.2.

***Table 4.2: Frequency of Isolation of the Isolates fromTutu's Farm***

| | ***E. coli*** | | | ***K. pneumoniae*** | | ***K. oxytoca*** | |
|---|---|---|---|---|---|---|---|
| Sample | Number of Isolates | Number Isolated | Frequency of Isolation (%) | Number Isolated | Frequency of Isolation (%) | Number Isolated | Percentage of Isolation (%) |
| Chickens | 89 | 79 | 77.5 | 6 | 5.9 | 4 | 3.9 |
| Chickens' Environment | 9 | 9 | 8.8 | 0 | 0 | 0 | 0 |
| Rearers | 4 | 4 | 3.9 | 0 | 0 | 0 | 0 |
| **Total** | **102** | **92** | **90.2** | **6** | **5.9** | **4** | **3.9** |

The highest isolation rate (53.7%) was obtained from chickens while the least (7.4%)was obtained from the rearers. The highest frequency of isolation (7.4%) of *K. pneumoniae* was also obtained from chickens while the least (1.9%) was from the rearers. Only 5.5% isolatesof *K. oxytoca*was obtained from the chickens. The isolation rates of *E. coli* obtained from chickens, chickens' environment and chickens rearers were respectively 53.7%, 18.5% and 7.4% while those of *K. pneumoinae* were respectively 7.4%, 5.5% and 1.9%,. However, there is no stastistical difference in the frequency of isolation of the isolates from farm II ($P>0.05$). This is presented in Table 4.3.

***Table 4.3: Frequency of Isolation of the Isolates from Tartiu's Farm***

| | | ***E. coli*** | | ***K. pneumoniae*** | | ***K. oxytoca*** | |
|---|---|---|---|---|---|---|---|
| Sample | Number of Isolates | Number Isolated | Frequency of Isolation (%) | Number Isolated | Frequency of Isolation (%) | Number Isolated | Frequency of Isolation (%) |
| Chickens | 36 | 29 | 53.7 | 4 | 7.4 | 3 | 5.5 |
| Chickens' Environment | 13 | 10 | 18.5 | 3 | 5.5 | 0 | 0 |
| Rearers | 5 | 4 | 7.4 | 1 | 1.9 | 0 | 0 |
| **Total** | **54** | **43** | **79.5** | **8** | **14.8** | **3** | **5.5** |

The highest frequency of isolation (66.7%) of *E. coli* was obtained from the chickens while 27.1% was the lowest frequency of isolation obtained from the chicken environment and no *E. coli* was obtained from the rearers. Only 6.2% isolates of *K. pneumoniae* was obtained from the chickens. No *K. oxytoca* was obtained from farm III. The frequencies of isolation of *E. coli, K. pneumoinae and K. oxytoca* in chickens were 66.7%, 4.1% and 0% respectively while that obtained in chickens' environment were respectively 27.1%, 2.1% and 0%. No isolates of *E. coli, K. pneumoniae and K. oxytoca* was obtained from the rearer.There is no significance difference ($P>0.05$) in the frequency of isolation of the isolates in farm III as presented inTable 4.4.

***Table 4.4: Frequency of Isolation of the isolates from Bello's Farm***

| | | ***E. coli*** | | ***K. pneumoniae*** | | ***K. oxytoca*** | |
|---|---|---|---|---|---|---|---|
| Sample | Number of Isola0tes | Numbe r of Isolated | Frequency of Isolation (%) | Number of Isolates | Frequenc y of Isolation (%) | Numbe r of Isolates | Frequency of Isolation (%) |
| Chickens | 35 | 32 | 66.7 | 2 | 4.1 | 0 | 0 |
| Chickens' Environme nt | 13 | 13 | 27.1 | 1 | 2.1 | 0 | 0 |
| Rearers | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Total** | **48** | **45** | **93.8** | **3** | **6.2** | **0** | **0** |

The highest frequency of isolation (67.3%) of *E. coli* isolates was obtained from chickens while the least frequency of isolation( 3.6%)was obtained from the rearers. The isolation rate (27.3%) was obtained from the chicken environment.Only1.8% isolate of *K. pneumoniae was* obtained from chicken. No *K. oxytoca* was obtained from the farmand chickens' rearers respectively. Meanwhile, there is stastical difference in the frequency of isolation of the isolates from farm IV ($P<0.05$) as presented in Table 4.5.

***Table 4.5: Frequency of Isolation of the Isolates from Ijaja's Farm***

| | | ***E. coli*** | | ***K. pneumoniae*** | | ***K. oxytoca*** | |
|---|---|---|---|---|---|---|---|
| Sample | Number of isolates | Number of Isolates | Frequency of Isolation (%) | Number of Isolates | Frequency of Isolation (%) | Number of Isolates | Frequency of Isolation (%) |
| Chickens | 38 | 37 | 67.3 | 1 | 1.8 | 0 | 0 |
| Chickens' Environment | 15 | 15 | 27.3 | 0 | 0 | 0 | 0 |
| Rearers | 2 | 2 | 3.6 | 0 | 0 | 0 | 0 |
| **Total** | **55** | **54** | **98.2** | **1** | **1.8** | **0** | **0** |

The highest frequency of isolation (54.3%) of *E. coli* was obtained from the chickens, 28.6% was obtained from the farm environment while the least frequency of isolation 8.6% was obtained from the rearers. No isolate of *K. pneumoniae* was obtained from the farm. The frequency of isolation (5.7%) of *K. oxytoca* was obtained from the chicken environment and 2.9 % was obtained from the chickens. No *K. oxytoca* was obtained from the rearers. The frequency of isolation of isolates obtained from the chickens', chickens environment and chickens rearers were 57.2%, 34.3% and 8.6% respectively. However there is no statistical difference in the frequency of isolation of the isolates from farm V ($P>0.05$) as presented in Table 4.6.

***Table 4.6: Frequency of Isolation of the Isolates from Battic Farm ,Jaikada-Fari***

| | | ***E. coli*** | | ***K. pneumoniae*** | | ***K. oxytoca*** | |
|---|---|---|---|---|---|---|---|
| Sample | Number of Isolates | Number of Isolates | Frequency of Isolation (%) | Number of Isolates | Frequency of Isolation (%) | Number of Isolates | Frequency of Isolation (%) |
| Chickens | 20 | 19 | 54.3 | 0 | 0 | 1 | 2.9 |
| Chickens' Environment | 12 | 10 | 28.6 | 0 | 0 | 2 | 5.7 |
| Rearers | 3 | 3 | 8.6 | 0 | 0 | 0 | 0 |
| **Total** | **35** | **32** | **91.5** | **0** | **0** | **3** | **8.6** |

The highest frequency of isolation (59.4%)of*E. coli* was obtained from the chickens while the least frequency (12.5%) was obtained from the rearers. The isolation rate obtained from the chicken environment was 18.8%, while 3.1% of *K. pneumoniae* was obtained from the chicken. The isolation rate of *K. oxytoca* obtained from this farm was 6.2%. The total isolation rates of the isolates from chickens, chickens' environment and chicken rearers were 62.5%, 21.9% and 18.7% respectively. However, there is no satastical significance difference in the frequency of isolation of the isolates from farm VI ($P>0.05$). This is presented in Table 4.7.

***T able 4.7: Frequency of Isolation of the Isolates from Madam Fibi's Farm***

| Sample | Number of isolates | ***E. coli*** Number of Isolates | Frequency of Isolation (%) | ***K. pneumoniae*** Numbe r of Isolates | Frequency of Isolation (%) | ***K. oxytoca*** Numbe r of Isolates | Frequency of Isolation (%) |
|---|---|---|---|---|---|---|---|
| Chicken s | 19 | 19 | 59.4 | 0 | 0 | 0 | 0 |
| Chickens' Environment | 7 | 6 | 18.8 | 0 | 0 | 1 | 3.1 |
| Rearers | 6 | 4 | 12.5 | 1 | 3.1 | 1 | 3.1 |
| **Total** | **32** | **29** | **90.7** | **1** | **3.1** | **2** | **6.2** |

The highest frequency of isolation (70.4%) of *E. coli* was obtained from the chickens, 22.5 % was obtained from the chicken environment while the least (5.6%)was obtained from the rearers. Only 1.4% isolate of *K. pneumoniae* was obtained from the farm .No *K. oxytoca* was obtained. The total isolation rate of 71.8% was obtained from the chickens' while 22.5% and 5.6% were obtained from chicken environment and chicken rearers respectively.Meanwhile there is no statistical difference ($P>0.05$) the frequency of isolation of the isolates from farm VII as presented in Table 4.8.

***Table4.8: Frequency of Isolation of the Isolates from Battic, Sabon Line Farm***

| Sample | ***E. coli*** | | ***K. pneumoniae*** | | ***K. oxytoca*** | | |
|---|---|---|---|---|---|---|---|
| | Number of Isolates | Number of Isolates | Frequency of Isolation (%) | Number of Isolates | Frequency of Isolation (%) | Number of Isolates | Frequency of Isolation (%) |
| Chickens | 51 | 50 | 70.4 | 1 | 1.4 | 0 | 0 |
| Chickens, Environment | 16 | 16 | 22.5 | 0 | 0 | 0 | 0 |
| Rearers | 4 | 4 | 5.6 | 0 | 0.0 | 0 | 0 |
| **Total** | **71** | **70** | **98.5** | **1** | **1.4** | **0** | **0** |

The highest frequency of isolation (*23.2%*) of *E. coli* isolates was obtained from farm I while the least (*7.3%*) was obtained from farm VI. The highest frequency of isolation*2%* of the *K. pneumoniae* isolates was obtained from farm II and the least *0.3% K. pneumoniae* was obtained separately from farmsIV, VI and VII. The highest frequency of isolation (*1%*) of *K. oxytoca*was obtained from farm I while *0.5%* was the lowest obtained. This least frequency of isolation was from farm VI. No *K. oxytoca* was obtained from farms III, IV and VII. Meanwhile, there is statistical difference ($P<0.05$) in the frequency of isolation of the isolates in relation to sites as presented inTable 4.9.

***Table 4.9: Frequency of Isolation of the Isolates In Relation To Sites***

| Study Site | ***E. coli*** | | ***K. pneumoniae*** | | ***K. oxytoca*** | | |
|---|---|---|---|---|---|---|---|
| | Number of Isolates | Number of Isolates | Frequency of Isolation (%) | Number of Isolates | Frequency of Isolation (%) | Number of Isolates | Frequency of Isolation (%) |
| **Tutu's Farm** | 102 | 92 | 23.2 | 6 | 1.5 | 4 | 1.0 |
| **Tartus' Farm** | 54 | 43 | 10.8 | 8 | 2.0 | 3 | 0.8 |
| **Bello's Farm** | 48 | 45 | 11.3 | 3 | 0.8 | 0 | 0.0 |
| **Ijaja's Farm** | 55 | 54 | 13.6 | 1 | 0.3 | 0 | 0 |
| **Baltic Farm, Jaikada-Fari** | 35 | 32 | 8.1 | 0 | 0 | 3 | 0.8 |
| **Baltic Farm, Sabon Line** | 32 | 29 | 7.3 | 1 | 0.3 | 2 | 0.5 |
| **Madam Fibi's Farm** | 71 | 70 | 17.6 | 1 | 0.3 | 0 | 0 |
| **Total** | **397** | **365** | **91.9** | **20** | **5.2** | **12** | **3.1** |

A total of 131 isolates of *E. coli* were obtained from broilers' farms. The frequencies of occurrence obtained from farm III, Farm IV and Farm V were 34.3%, 41.2% and 24.4% respectively. The highest frequency of occurrence (28.2%) of *E. coli* isolates in chickens was obtained from farm IV while the least (14.5%) was obtained from farm V. The highest frequency of occurrence (11.5%) of *E. coli* isolates in the chicken environment was obtained from farm IV while the least (7.6%) was obtained from farm V. The frequency rate of the *E. coli* isolates in farm III was 9.9%. No *E. coli* isolates was obtained from the chickens' rearers in farm III. The frequencies of occurrence of *E. coli* isolates among farm rearers in farm IV and farm V were 1.5% and 2.3% respectively.Meanwhile there is statistical difference($P<0.05$) in the frequency of occurence of *E. coli* in broilers' farms. This is presented in Table 4.10.

**Table 4.10: Frequency of occurrence of *E. coli* in broilers' Farms**

| | | **Bello's Farm** | | **Ijaja's Farm** | | **Baltic Farm Jaikada-fari** | |
|---|---|---|---|---|---|---|---|
| Sample | Number of Isolates | Number Isolated | Frequency of Isolates | Number Isolated | Frequency of Isolates | Number Isolated | Frequency of Isolate |
| Chicken s | 88 | 32 | 24.4 | 37 | 28.2 | 19 | 14.5 |
| Chicken | 38 | 13 | 9.9 | 15 | 11.5 | 10 | 7.6 |
| s'Environment | | | | | | | |
| Chickens | 5 | 0 | 0 | 2 | 1.5 | 3 | 2.3 |
| Rearers | | | | | | | |
| Total | 131 | 45 | 34.3 | 54 | 41.2 | 32 | 24.4 |

A total of 135 isolates of *E. coli* was obtained from layers' farms. The frequencies of occurrence in farm I and farm II were 68.2% and 31.9% respectively.The frequency of occurrence of*Escherichia coli* isolates from chicken in farm I and farm II were 58.5% and 21.5% respectively while that of the chicken environment was respectively 6.7% and 7.4%. The frequency of occurrence of *E. coli* from farm rearers in both farms was 3.0% each.However, there is no statistical difference (P>0.05) in the frequency of occurrence of *E.coli* from layers' farms as presented in Table 4.11.

**Table 4.11: Frequency of occurrence of *E. coli* in layers' farms**

| | | Tutu's Farm | | Tartius' Farm | |
|---|---|---|---|---|---|
| Sample | Number of Isolates | Number Isolated | Frequency of Isolate | Number of Isolated | Frequency of Isolate |
| Chickens | 108 | 79 | 58.5 | 29 | 21.5 |
| Chickens' Environment | 19 | 9 | 6.7 | 10 | 7.4 |
| Chickens Rearers | 8 | 4 | 3.0 | 4 | 3.0 |
| Total | 135 | 92 | 68.2 | 43 | 31.9 |

A total of 99 isolates of *E. coli* was obtained from cockerels' farms. The frequencies of occurrence of *E.coli* in farm VI and farm VII were 29.3% and 70.7% respectively. The highest frequency of occurrence (19.2%) was obtained from chickens while the least(4.0%)was obtained from chicken rearers in farm VI. In farm VII the highest frequency of occurrence (50.5%) was obtained from chickens while the least( 4.0%) was from chicken rearers. The frequency of occurrence of *E. coli* is in chickens' in farm VII is higher than that in farm VI in the ratio 50.5%:19.2%. The frequencies of occurrence of *E. coli* obtained from chicken rearers in farm VI was 4.0% while that in farm VII was 70.7%. Meanwhile there is no statistical difference ($P>0.05$) in the frequency of occurrence of *E.coli* in cockerels' farms as presented in Table 4.12.

**Table 4.12: Frequency of occurrence of *E. coli* in Cockerels' farms**

| | | ***Baltic Farm, Sabon line*** | | ***Madam Fibi's Farm*** | |
|---|---|---|---|---|---|
| ***Sample*** | ***Number of Isolate s*** | ***Number Isolated*** | ***Frequency of Isolate*** | ***Number Isolated*** | ***Frequency of Isolate*** |
| Chickens | 69 | 19 | 19.2 | 50 | 50.5 |
| Chicken s'Environment | 22 | 6 | 6.1 | 16 | 16.2 |
| Chickens Rearers | 8 | 4 | 4.0 | 4 | 4.0 |
| **Total** | **99** | **29** | **29.3** | **70** | **70.7** |

Three hundred and sixty five isolates of *E. coli* was obtained from the farms. The highest frequency of occurrence (72.6%) was obtained from the chickens while the least (5.8%) was obtained from the rearers. The frequencies of occurrence of *E. coli* in the three farms , broilers' layers' and cockerels' farms were 35.9%, 37.0% and 27.1% respectively as presented in Table 4.13.

**Table 4.13: Frequency rate of *E. coli* from Cockerels', Layers' and Broilers' farms**

| | | ***Chickens*** | | ***Chickens' Environment*** | | ***Chickens Rearers*** | |
|---|---|---|---|---|---|---|---|
| Sample | Number of Isolates | Number Isolated | Frequency of Isolate | Number Isolated | Frequency of Isolate | Number Isolated | Frequency of Isolate |
| Broilers' farms | 131 | 88 | 24.1 | 38 | 10.4 | 5 | 1.4 |
| Layers' farms | 135 | 108 | 29.6 | 19 | 5.2 | 8 | 2.2 |
| Cockerels' farms | 99 | 69 | 18.9 | 22 | 6.0 | 8 | 2.2 |
| **Total** | **365** | **265** | **72.6** | **79** | **21.6** | **21** | **5.8** |

A total of 397 isolates were obtained from the broilers' farms, layers' farms and cockerels' farms. In each of these farms, isolates were obtained from the chickens, the chicken environment and the chicken rearers. The occurrence of *K. pneumoniae* obtained from broilers, layers and cockerels farms were 1.0%, 3.5% and 0.5% respectively as shown in Figure 4.1.

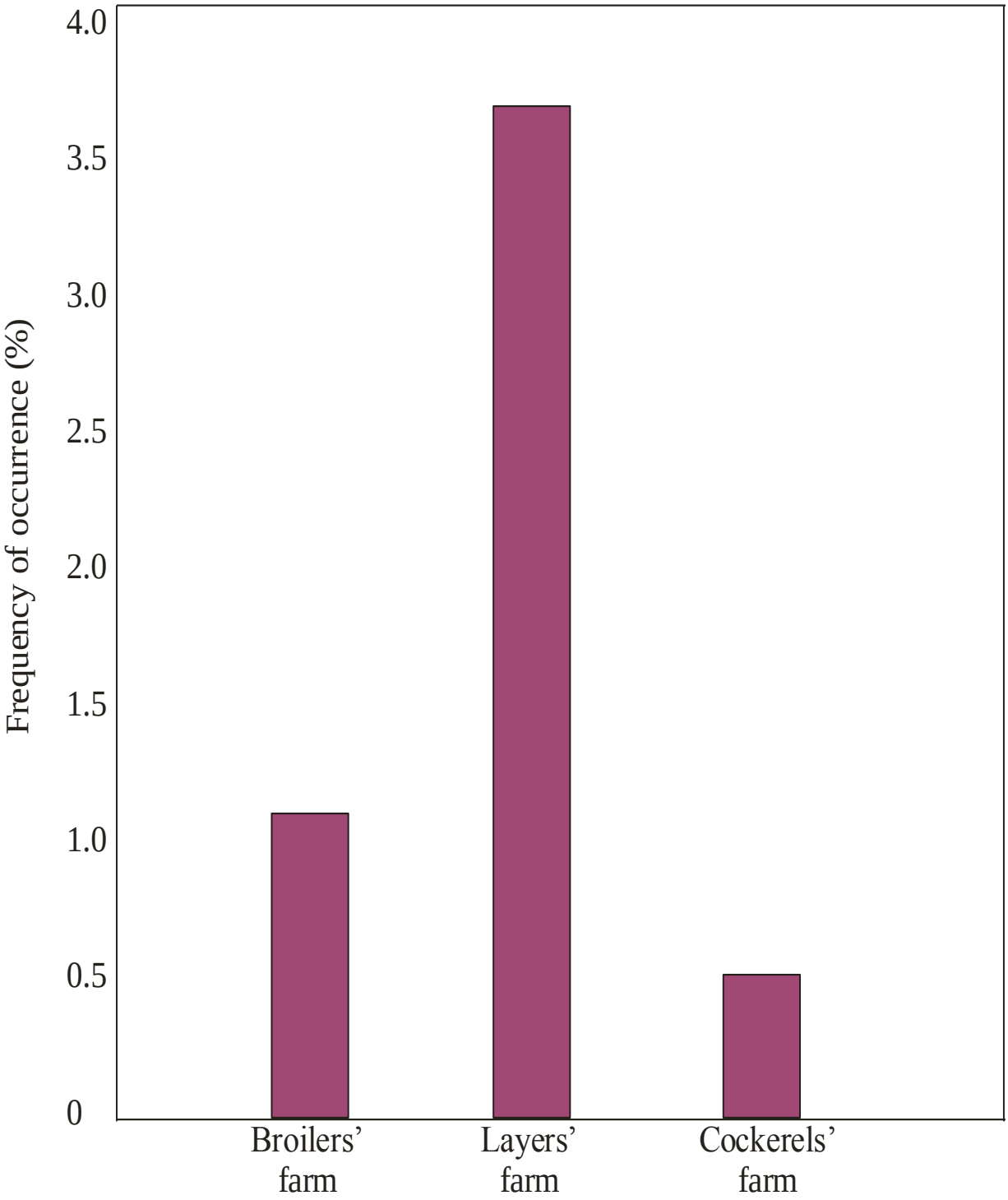


***Figure 4.1:*** **Frequency of occurrence of *K. pneumoniae* in Broilers', Layers' and Cockerels'**

A total of 397 isolates were obtained from broilers, layers and cockerels' farms. In each of these farms, isolates were obtained from chickens, chickens' environment and the rearers. The frequency of occurrence of *K.oxytoca* obtained from broilers, layers and cockerels' farms were 0.8%, 1.8% and 0.5% respectively as presented in figure 4.2.

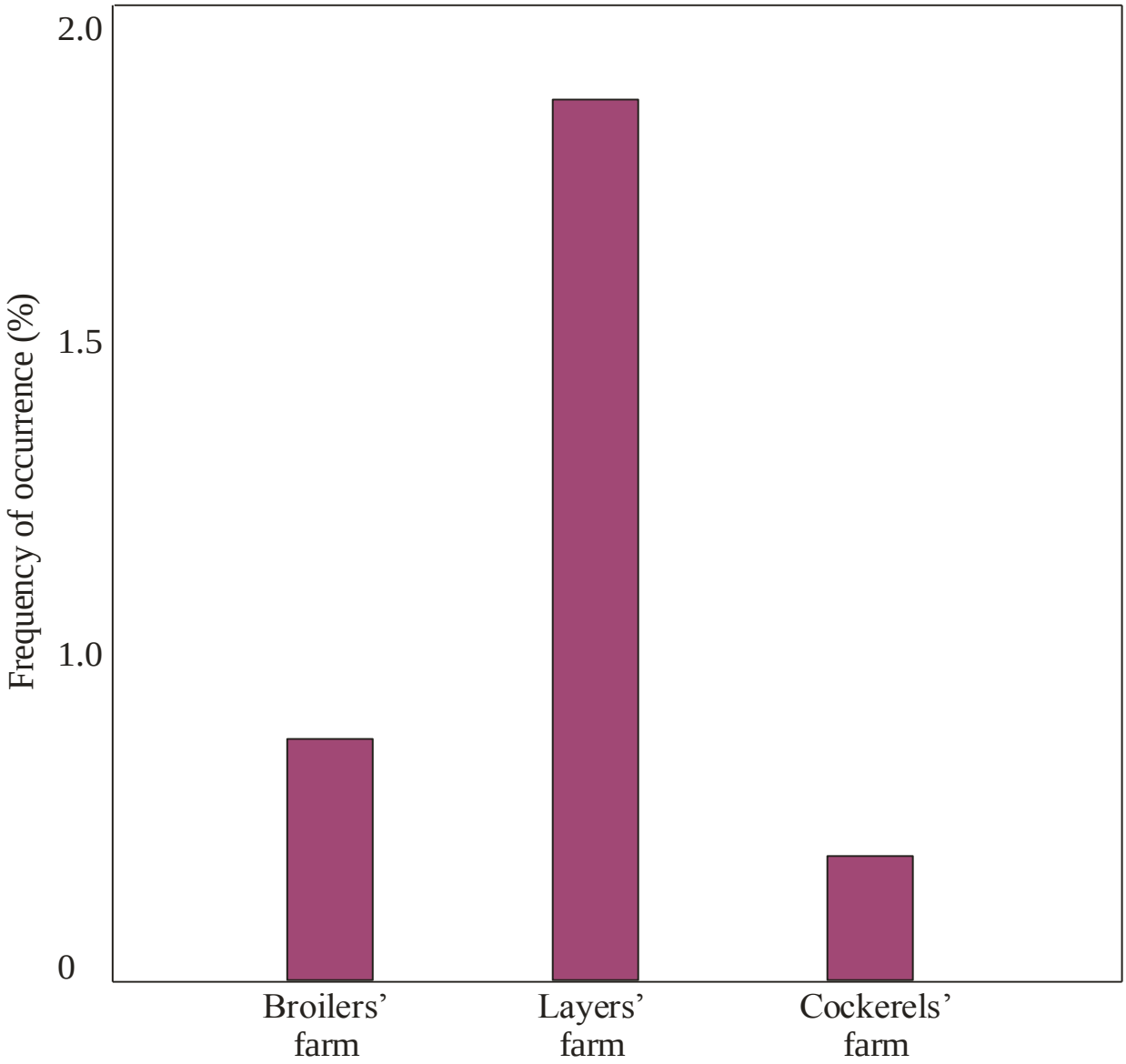


**Figure4.2: Frequency of occurrence of *K. oxytoca* in Broilers', Layers' and Cockerels' farms**.

The frequencies of occurrence $\beta - lactamase$ producing *E. coli, K. pneumoniae and K. oxytoca were* 46.9%, 2.0% and 0.75%respectively. The frequencies of occurrence of *E. coli, K. pneumoniae and K. oxytoca* in chickens were 35.3%, 1.5% and 0.25% respectively while that obtained from chickens' environment were respectively 9.3%, 0.5% and 0.25%. No *K. pneumoniae* was obtained from the chickens farmers while 2.3% isolates of *E. coli and* 0.25% isolate of *K. oxytoca* was obtained from the farmersMeanwhile there is no statisstical difference (P>0.05) in the frequency of occurrence of*β* –lactamase producing *E.coli, K. pneumonia*e and *K.oxytoca* from various samples. (P>0.05) as presented in Table 4.14.

***Table 4.14:*** **Frequency of occurrence β -lactamase producing *E.coli, K.pneumoniae* and *K.oxytoca*from various farm samples.**

| | **β -LACTAMASE PRODUCERS** | | | | | | |
|---|---|---|---|---|---|---|---|
| | | ***E.coli*** | | ***K. pneumoniae*** | | ***K.oxytoca*** | |
| Sample | Number of Isolates | Number Isolated | Frequency of Isolates | Number Isolated | Frequency of Isolates | Number Isolated | Frequency of Isolates |
| Chickens | 288 | 140 | 35.3 | 07 | 1.8 | 01 | 0.25 |
| Chickens' Environment | 86 | 37 | 9.3 | 02 | 0.5 | 01 | 0.25 |
| Poultry Farmers | 23 | 09 | 2.3 | 0 | 0 | 01 | 0.25 |
| **Total** | **397** | **186** | **46.9** | **09** | **2.3** | **03** | **0.75** |

Three hundred and ninety-seven isolates were obtained from the 7 farms. The frequencies of occurrence of beta lactamase producing *E.coli, K. pneumoniae and K. oxytoca*were 46.9%, 2.27% and 0.75% respectively. The highest frequency of occurrence (9.1%) of *E. coli*was obtained from farm VII while the least (4.5%) was obtained from Farm VI. Zero.76% of *K.pneumoniae*were separately obtained from Farm III and Farm IV. Zero.25% was individually obtained from Farm I, II and VII. No isolate of *K. pneumoniae* was obtained from Farm V and Farm VI. Zero.75 of *K. oxytoca* was obtained. Nevertheless, there is significance difference ($P<0.05$) in the frequency of occurence of $\beta - lactamase$ producers in the various farms. This is presented in the Table 4.15.

**Table 4.15: Frequency of occurrence β -lactamase producers in relation to site**

| | β -LACTAMASE PRODUCERS | | | | | | |
|---|---|---|---|---|---|---|---|
| | ***E. coli*** | ***K. pneumoniae*** | | ***K. oxytoca*** | | | |
| Study site | Number of Isolate | Number Isolated | Frequency of Isolates (%) | Number Isolated | Frequency of Isolates (%) | Number Isolated | Frequency of Isolates |
| Tutu's Farm | 102 | 28 | 7.1 | 1 | 0.25 | 0 | 0 |
| Tartius Farm | 54 | 23 | 5.8 | 1 | 0.25 | 0 | 0 |
| Bello's Farm | 48 | 27 | 6.8 | 3 | 0.76 | 0 | 0 |
| Ijaja's Farm | 55 | 32 | 8.1 | 3 | 0.76 | 0 | 0 |
| Baltic Farm, Jaikada-Fari | 35 | 22 | 5.5 | 0 | 0 | 2.0 | 0.5 |
| Baltic Farm, Sabon Line | 32 | 18 | 4.5 | 0 | 0 | 0 | 0 |
| Madam Fibi's Farm | 71 | 36 | 9.1 | 1 | 0.25 | 1 | 0.25 |
| **Total** | **397** | **186** | **46.9** | **09** | **2.27** | **3** | **0.75** |

A total of 138 isolates was obtained from the broilers' farms. The highest frequency of occurrence (23.1%) of the β – lactamase producing *E.coli*was from Farm IV, while 5.9% from Farm V was the least. The frequencies of occurrence of *K.pneumoniae* in Farm III and Farm IV were (2.2%) from each farm. Meanwhile there is no statistical difference ($P>0.05$) in the frequency of occurrence of beta –lactamase producers in broilers' farms as presented in Table 4.16.

**Table 4.16: Frequency of occurrence of Beta-lactamase Producers in Broilers' Farm**

| | **Beta- lactamase Producing Isolates.** | | | | | | |
|---|---|---|---|---|---|---|---|
| | | ***E. coli*** | | ***K. pneumoniae*** | | ***K. oxytoca*** | |
| Sample | Number of Isolates | Number Isolated | Frequency of Isolates (%) | Number Isolated | Frequency of Isolates (%) | Number Isolated | Frequency of Isolates (%) |
| Bello's Farm | 48 | 27 | 9.6 | 3 | 2.2 | 0 | 0 |
| Ijaja's Farm | 55 | 32 | 23.1 | 3 | 2.2 | 0 | 0 |
| Baltic Farm, Jaikada-Fari | 35 | 22 | 5.9 | 0 | 0 | 2 | 1.4 |
| **Total** | **138** | **81** | **38.6** | **06** | **4.4** | **02** | **1.4** |

A total of 103 isolates were obtained from cockerels' farms. The frequencies of occurrence of $\beta - lactamase$ producing *E. coli, K. pneumoniae and K. oxytoca*were 42.5%, 0.9% and 0.97% respectively. The highest frequency of occurrence (*35%)*$\beta -$ $lactamase$ producing *E. coli* was obtained from Farm VII while the least 7.5% was obtained from farm VI. The occurence rate of 0.97% was obtained from*K. Pneumonia* and *K. oxytoca* separately from farm VII. No *K. pneumoniae or K. oxytoca* was obtained from Farm VI while the frequency of occurence of *E. coli* obtained was 7.5%.However there is satastical difference (P=0.05) in the frequency of occurrence of beta –lactamase asproducing isolates in cockerels' farms. This is presented in Table 4.17.

**Table 4.17: Frequency of occurrence of Beta lactamase producing isolatesin cockerels' farms**

| | Beta lactamase producing isolates | | | | | | |
|---|---|---|---|---|---|---|---|
| | | ***E. coli*** | | ***K. pneumoniae*** | | ***K. oxytoca*** | |
| Sample | Number of Isolates | Number Isolated | Frequency of Isolates (%) | Number Isolated | Frequency of Isolates (%) | Number Isolated | Frequency of Isolates (%) |
| Baltic Farm, Sabon line | 32 | 18 | 7.5 | 0 | 0 | 0 | 0 |
| Madam Fibi's Farm | 71 | 36 | 35.0 | 1 | 0.97 | 1 | 0.97 |
| **Total** | **103** | **54** | **42.5** | **1** | **0.97** | **1** | **0.97** |

A total of 156 isolates was obtained from layers farms. The frequencies of occurrence, 22.69%, 1.28% and 0% were $\beta - lactamase$ producing *E. coli, K. pneumoniae and K. oxytoca* in the layers farms respectively.However, there is statistical significance difference ($P<0.05$) in the frequency of occurrence of beta –lactamase producing isolates in layers' farms. In farm II, 7.95% and 0.64% were the occurrence rates of *E.coli and K.pneumoniae* isolated from Farm I respectively. The frequency of occurrence of *K. pneumoniae and E. coli* were 0.64% and 14.74% respectively in farm II. No $\beta - lactamase$ producing *K. oxytoca* was obtained from layers' farms as presented in Table 4.18.

**Table 4.18: Frequency of occurrence of Beta-Lactamase Producing Isolates in Layers' Farms**

| | | ***E.coli*** | | ***K.pneumoniae*** | | ***K.oxytoca*** | |
|---|---|---|---|---|---|---|---|
| Sample | Number of isolates | Number Isolated | Frequency of Isolates (%) | Number Isolated | Frequency Isolates (%) | Number Isolated | Frequency of Isolates (%) |
| Tutu's Farm | 102 | 28 | 7.95 | 1 | 0.64 | 0 | 0 |
| Tartius Farm | 54 | 23 | 14.74 | 1 | 0.64 | 0 | 0 |
| **Total** | **156** | **51** | **22.69** | **2** | **1.28** | **0** | **0** |

The frequency of occurrence of beta lactamase producing *E.coli* from broilers', layers' and cockerels' farms respectively were 20.40%, 12.85% and 13.60%.However, there is statistical difference ($P<0.05$) in the frequency of occurrence of beta –lactamase producing isolates in cockerels' farms. The highest frequency of occurrence (1.51%) and the least (0.25%) were the beta – lactamase producing *K. pneumoniae* obtained from broilers farms and cockerels' farms respectively. The occurrence rates (0.5% and 0.75%) of *K. oxytoca* were obtained from broilers farms and cockerels' farms respectively. The frequency of occurence of *E.coli, K. pneumoniae* and *K.oxytoca* obtained from broilers farms were 20.40%, 1.51% and 0.50% respectively. The occurrence rates of *E. coli, K. pneumoniae* and *K. oxytoca* obtained from layers' farms were 0.5%, 0.0% and0.25% respectively. In cockerels' farms, 13.6%, 0.25% and 0.25% were respectively, the occurrence rates of *E.coli, K.pneumoniae* and *K.oxytoca*as presented in Table 4.19.

**Table 4.19: Frequency of occurrence Beta-lactamase Producing Isolates from Broilers' Layers' and Cockerels Farms**

| | Beta lactamase producing isolates | | | | | | |
|---|---|---|---|---|---|---|---|
| | ***E. coli*** | | ***K. pneumoniae*** | | ***K. oxytoca*** | | |
| Sample | Number of isolates | Number Isolated | Frequency of Isolates (%) | Number Isolated | Frequency Isolates (%) | Number Isolated | Frequency of Isolates (%) |
| Broilers' farms | 138 | 81 | 20.40 | 06 | 1.51 | 02 | 0.50 |
| Layers' farms | 156 | 51 | 12.85 | 02 | 0.50 | 0 | 0 |
| Cockerels farms | 103 | 54 | 13.60 | 01 | 0.25 | 01 | 0.25 |
| **Total** | **397** | **186** | **46.85** | **09** | **2.26** | **03** | **0.75** |

The occurrence rates of beta – lactamase producing isolates in broilers', layers'and cockerels' chickens were 16.1%, 9.8% and 11.1% respectively. From the chickens' environment, the occurrence rates of beta-lactamase producers in broilers' farms, layers farms and cockerels' farms were 5%, 3% and 2% respectively. The frequency of occurrence of beta – lactamase producing isolates from the chicken rearers was 0.8%, 0.8% and 1.0% in broilers, layers' and cockerels' respectively. In broilers farms, 16.1%, 5.0% and 0.8% were the frequencies of occurrence obtained from chickens, chickens' environment and chicken rearers respectively. The frequency of occurrence of $\beta - lactamase$ producers obtained in chickens, chickens' environment and chickens' rearers in layers' farms were respectively 9.8%, 3.0% and 0.8%. In cockerels' farms, the frequency rates were 11.1%, 2.0% and 1.0% respectively in chickens', chickens' environment and chickens' rearers. Meanwhile, the frequency of occurrence of $\beta - lactamase$ producing isolates in chickens, chicken environment and chicken rearers is not significant (P>0.05) as presented in Table 4.20.

***Table 4.20: Frequency of occurrence Beta-lactamase Producing Isolates in Chickens, Chickens' environment and chickens' Rearers***

| Sample | Number of isolates | **Broilers** | | **Layers** | | **Cockerels** | |
|---|---|---|---|---|---|---|---|
| | | Number Isolated | Frequency of Isolates (%) | Number Isolated | Frequency Isolates (%) | Number Isolated | Frequency of Isolates (%) |
| Chickens | 288 | 64 | 16.1 | 39 | 9.8 | 44 | 11.1 |
| Chickens' environment | 86 | 20 | 5.0 | 12 | 3.0 | 8 | 2.0 |
| Chickensrearers | 23 | 3 | 0.8 | 3 | 0.8 | 4 | 1.0 |
| **Total** | **397** | **87** | **21.9** | **54** | **13.6** | **56** | **14.1** |

A total of 365 *E. coli* isolates was obtained and the occurrence rate of β-lactamase producing *E. coli* obtained from chickens in broilers', layers' and cockerels' farms were 16.2%, 10.1% and 11.8% respectively while that from the chicken environment were respectively 5.2%, 3.0% and 2.2%.Also the occurrence rate of β-lactamase producing *E. coli* obtained from chicken rearers in broilers', layers' and cockerels' farms were each 0.8%as shown in Figure 4.3.

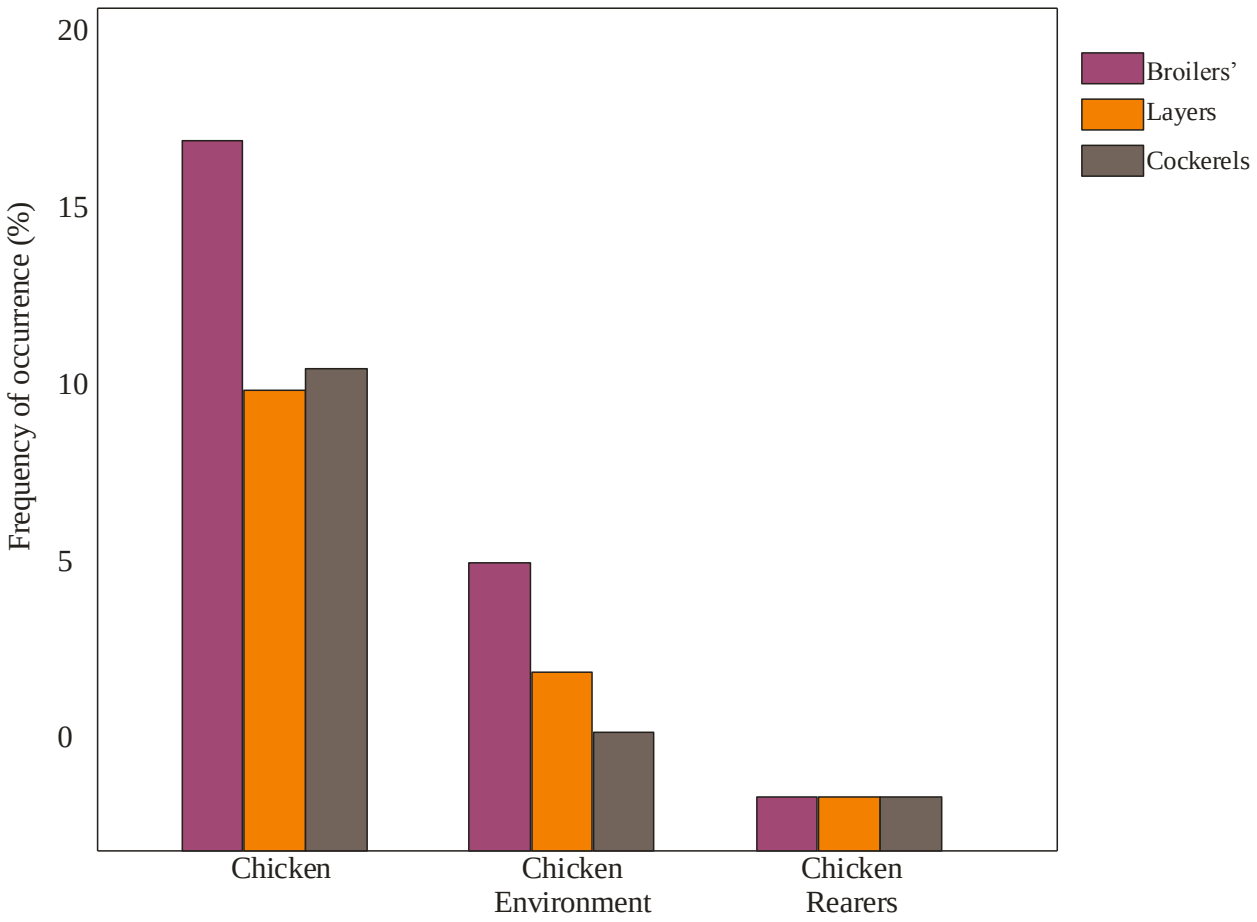


***Figure 4.3: Frequency of occurrence of Beta Lactamase Producing E. coli in Broilers, Layers and Cockerels Farms.***

A total of 397 isolates comprising of *E.coli*,*K. pneumoniae* and *K.oxytoca* was obtained. These isolates were obtained from broilers, layers and cockerels' farms. In each of the farms, bacterial isolates were obtained from the chickens, chicken environment and chicken rearers. The frequency of occurrence of beta-lactamase producing *K. pneumoniae* in broilers, layers and cockerels' farms were 1.5%, 0.5% and 0.3% as presented in Figure 4.4.

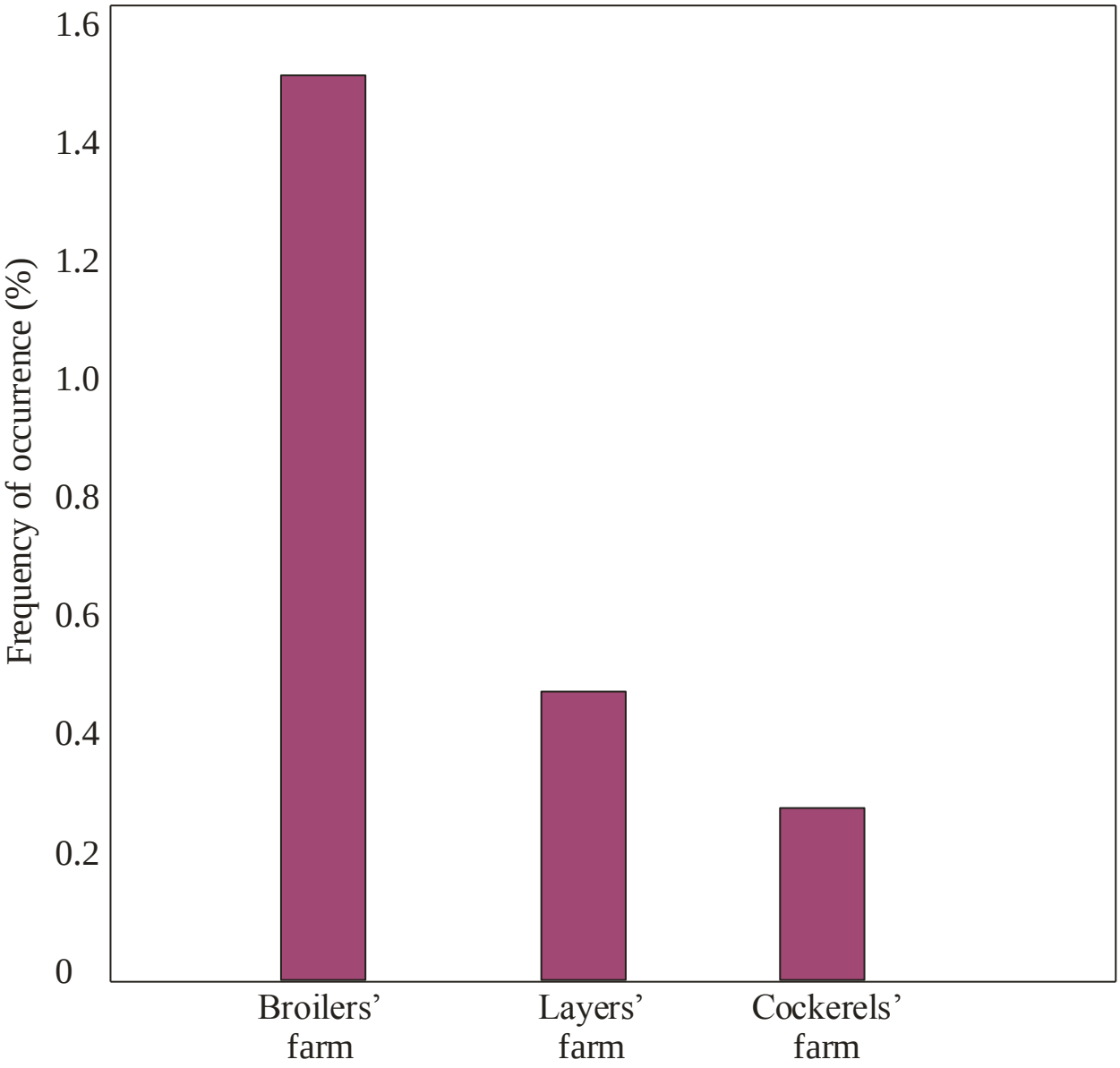


***Figure 4.4:*** **Frequency of occurrence of Beta Lactamase producing *K. pneumoniae* in Broilers, layers and cockerel's farms**

A total of 156 $\beta - lactamase$ producing isolates was obtained from chickens, chickens' environment and chickens rearers in layers farms. The frequencies of occurrence of beta lactamase producing isolates obtained from chickens in Tutu's farm and Tartuis farm were 16.7% and 7.7% respectively. From the chicken environment ,the occurrence rates were 0.6% and 7.1% in Tutu's and Tartius farms respectively while from the chicken rearers, the rates were 1.2% from each of the farms as presented in Figure 4.5.

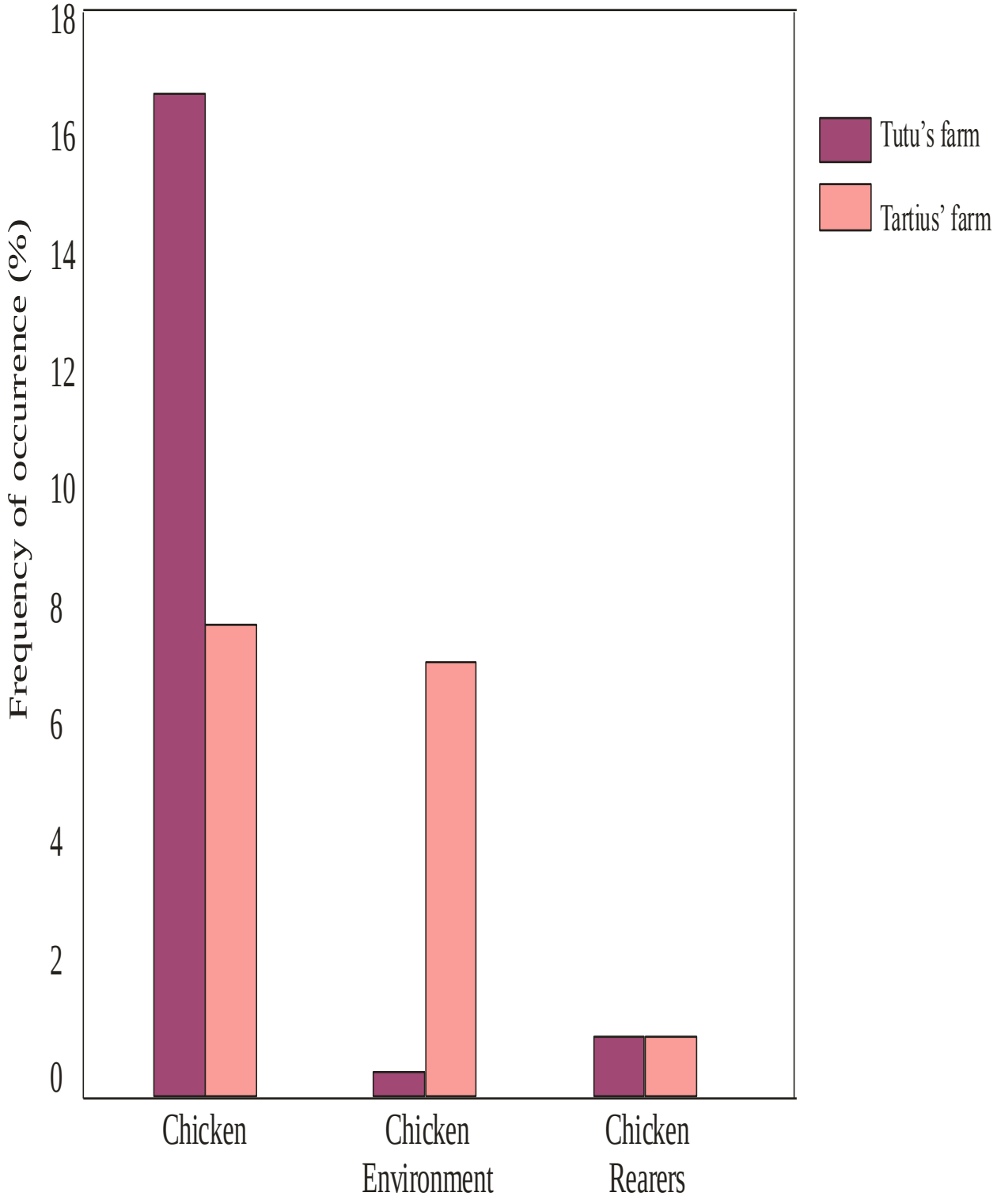


**Figure 4.5: Frequency of occurrence of Beta lactamase producing isolates in *chickens'*, chickens' environment and chickens' rearers in layers' farms.**

A total of 138 isolates was obtained from broilers' farms,the frequencies of occurrence of beta-lactamase producing isolates obtained from chickens in Bello's and Ijaja's farms and Baltic farm, Jaikada-fari were 16.7% 18.8% and 10.9% while from that chicken environment were 5.1%, 4.3% and 4.3% respectively. The frequency of occurrence of beta-lactamase producing isolates from chicken rearers in Bello's farm and Ijaja's farms and Baltic farm were 0%, 1.4% and 3.7% respectively as presented in Figure 4.6.

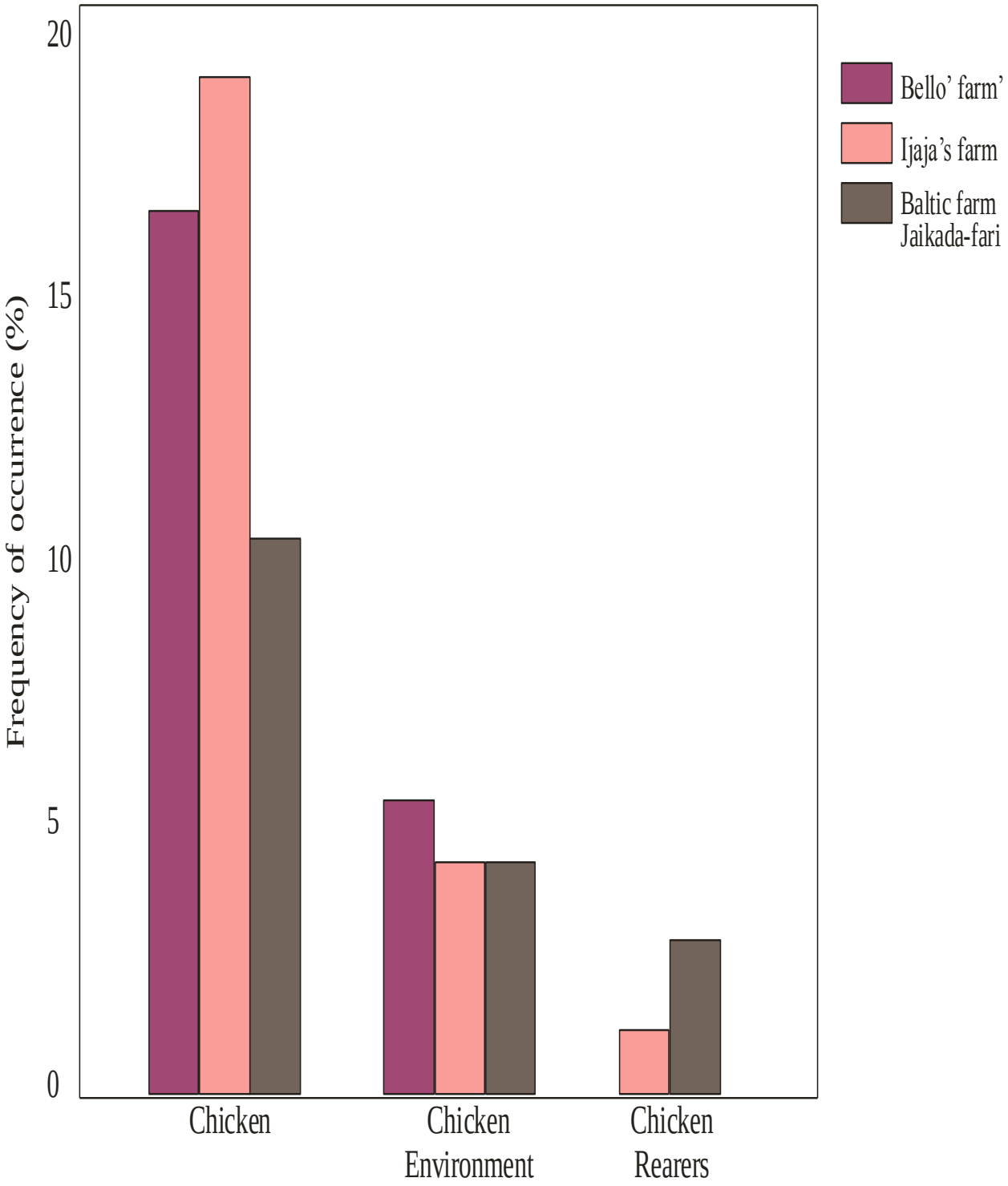


**Figure 4.6: Frequency of occurrence of Beta lactamase producing isolates in chickens', chickens' environment and chickens' rearers in boilers' farms.**

A total of 103 isolates was obtained from cockerels' farms. The frequencies of occurrence of beta-lactamase producing isolates from chickens in Baltic farm Sabon Line and Madam Fibi farm, were 16.5% and 26.2% respectively while from chicken rearers, they were 3.9% and 2.9% respectively. The occurrence of beta-lactamase producing isolates from chicken environment in Baltic farm, Sabon Line and Madam Fibis farm were 0% and 7.8% respectively as presented in Figure 4.7.

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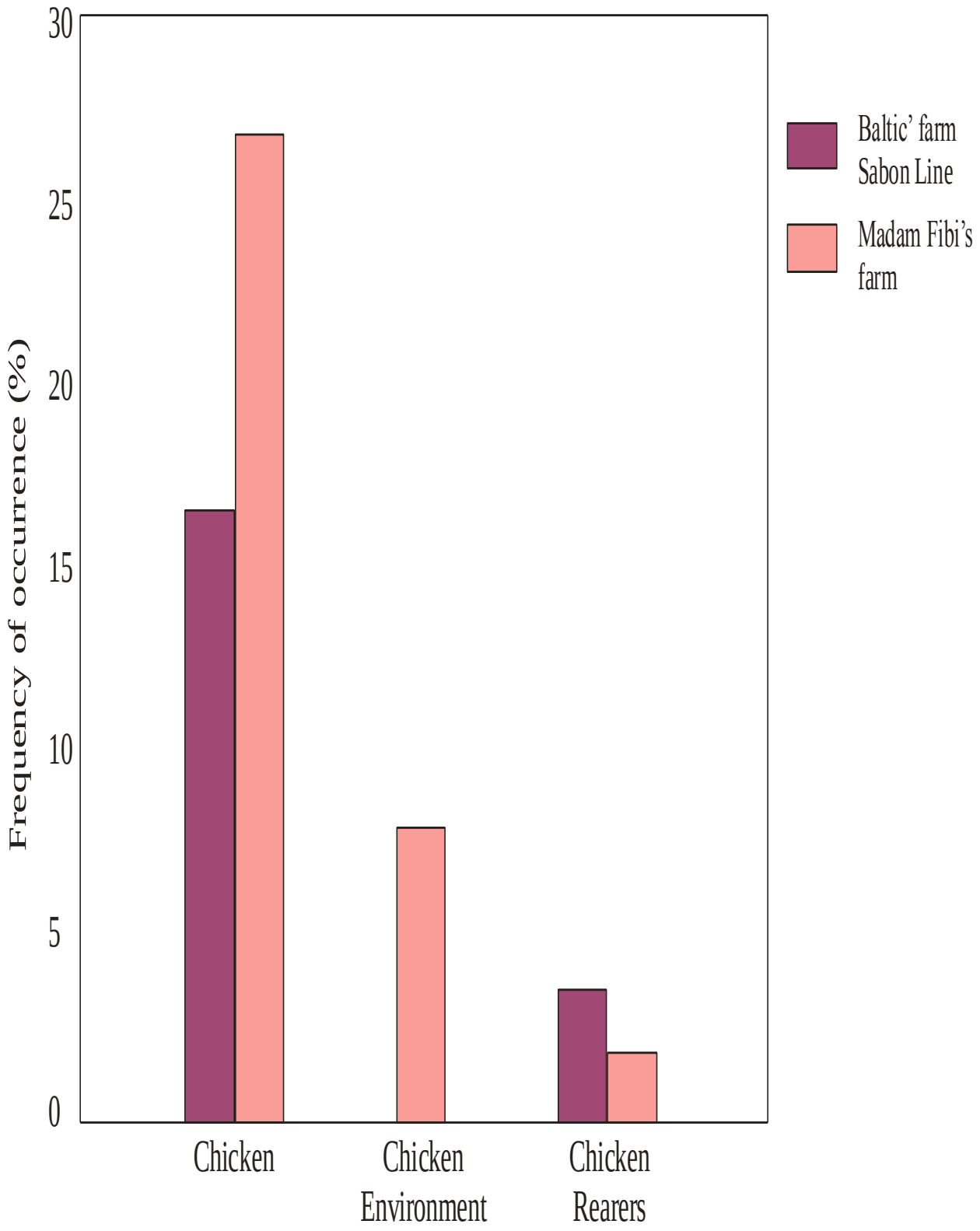


**Figure 4.7: Frequency of occurrence of Beta lactamase producing isolates in chickens', chickens' environment and chickens' rearers in Cockerels' farms.**

The zones of inhibition around antibiotics disks (1) ceftazidime and ceftriaxone (2) augumented towards the disc (4) containing clavulanic acid containing disk (augumentin) as presented in Plates 4.1a and 4.1b.

**a**


1- Ceftadime
2- Ceftriaxone
4- augumentin

**b**


***Plates 4.1a and 4.1b:***Synergy of clavulanic acid containing disk with ceftazidime and ceftriaxone in double disk synergy test (DDST) for *ESBL* phenotypic confirmation test.

The highest frequency of occurrence (15.6%) of ESBL producing *E.coli* was obtained from chicken while the least (1.5%) was obtained from chicken farmers. The frequency of occurrence (5.6%) was obtained from the chicken environment.Zero.5%. ESBL producing *K.pneumoniae* was obtained from chicken environment. No ESBL producing *K.oxytoca* was obtained from all the samples. Therefore, a total of (23.5%) of the isolates was ESBL producers while (76.5%) were non ESBL producers However there is no satastical difference (P>0.05) in the frequency of occurrence of ESBL positive *E. coli, K.pneumoniae* and *K. oxytoca* in various farm samples as presented in table 4.21 below.

**Table 4.21: Frequency of occurrence of ESBL Positive *E. coli,K. pneumoniae* and *K.oxytoca* in Various Farm Samples.**

| | ***ESBL POSITIVE ISOLATES*** | | | | | | |
|---|---|---|---|---|---|---|---|
| | | ***E. coli*** | | ***K. pneumoniae*** | | ***K. oxytoca*** | |
| Sample | Number of isolates | Number Isolated | Frequency of Isolates (%) | Number Isolated | Frequency Isolates (%) | Number Isolated | Frequency of Isolates (%) |
| Chickens | 288 | 62 | 15.6 | 0 | 0 | 0 | 0 |
| Chickens' Environment | 86 | 23 | 5.8 | 2 | 0.5 | 0 | 0 |
| Poultry Farmers | 23 | 06 | 1.5 | 0 | 0 | 0 | 0 |
| **Total** | **397** | **91** | **22.9** | **2** | **0.5** | **0** | **0** |

The highest frequency of occurrence (*4.8%*) of ESBL producing *E. coli* was obtained from farm V while the least (*2.0%*) was obtained from farm VII.However, there is no statistical significance difference (P>0.05) in the frequency of occurrence of ESBL positive isolates in relation to site. From farm II and farm III respectively was obtained*0.25* percent of *K. pneumoniae* each giving a total of 0.5%. No ESBL producing *K. oxytoca* was obtained from all the farm samples studied as presented in Table 4.22.

**Table 4.22: Frequency of occurrence of ESBL Positive Isolates in relation to Site**

| | ESBL POSITIVE ISOLATES | | | | | | |
|---|---|---|---|---|---|---|---|
| | | *E. coli* | | *K. pneumoniae* | | *sK.oxytoca* | |
| Study site | Number of Isolates | Number Isolated | Frequency of Isolates (%) | Number Isolated | Frequency of Isolated | Number Isolated | Frequency of Isolated (%) |
| **Tutu's Farm** | 102 | 13 | 3.3 | 0 | 0 | 0 | 0 |
| **Tartius Farm** | 54 | 12 | 2.8 | 1 | 0.25 | 0 | 0 |
| **Bello's Farm** | 48 | 11 | 2.8 | 1 | 0.25 | 0 | 0 |
| **Ijaja's Farm** | 55 | 17 | 4.3 | 0 | 0 | 0 | 0 |
| **Baltic Farm, Jaikada-Fari** | 35 | 19 | 4.8 | 0 | 0 | 0 | 0 |
| **Baltic Farm, Sabon Line** | 32 | 11 | 2.8 | 0 | 0 | 0 | 0 |
| **Madam Fibi's Farm** | 71 | 8 | 2.0 | 0 | 0 | 0 | 0 |
| **Total** | **397** | **91** | **22.9** | **2** | **0.5** | **0** | **0** |

The highest frequency of occurrence (13.8%) ESBL producing *E. coli* was obtained from farm V while the least frequency of occurrence was obtained from Farm III. Only 0.7% ESBL positive *K. pneumoniae* was obtained from farm III. No ESBL producing *K. oxytoca* was obtained from all the broilers' farms. However, there is significance difference ($P < 0.05$) in the frequency of occurrence of *ESBL* positive isolates in broilers' farms. This is presented in Table 4.23.

**Table 4.23: Frequency of occurrence of ESBL Positive Isolates in Broilers Farms**

| | ESBL POSITIVE ISOLATES | | | | | | |
|---|---|---|---|---|---|---|---|
| | *E. coli* | | | *K. pneumoniae* | | *K. oxytoca* | |
| Sample | Number of Isolates | Number Isolated | Frequency of Isolated (%) | Number Isolated | Frequency of Isolated (%) | Number Isolated | Frequency of Isolated (%) |
| **Bello's Farm** | 48 | 11 | 8.0 | 1 | 0.7 | 0 | 0 |
| **Ijaja's Farm** | 55 | 17 | 12.3 | 0 | 0 | 0 | 0 |
| **Baltic Farm, Jaikada-Fari** | 35 | 19 | 13.8 | 0 | 0 | 0 | 0 |
| **Total** | **138** | **47** | **34.1** | **1** | **0.7** | **0** | **0** |

The frequency occurrence of ESBL producing *E. coli* obtained from farm VI and farm VII were 10.7% and 7.8% respectively.However there is statistical significance difference ($P<0.05$) in the frequency of occurrence of ESBLproducing isolates from cockerels' farms. No ESBL producing *K. pneumoniae* and *K. oxytoca* was obtained from the farms. This is presented in Table 4.24.

**Table 4.24: Frequency of occurrence of ESBL Positive Isolates from Cockerels Farms**

| | ESBL POSITIVE ISOLATES | | | | | | |
|---|---|---|---|---|---|---|---|
| | ***E. coli*** | | | ***K. pneumoniae*** | | ***K. oxytoca*** | |
| Sample | Number of Isolates | Number Isolated | Frequency of Isolates (%) | Number of Isolates | Frequency of Isolates (%) | Number of Isolates | Frequency of Isolates (%) |
| **Baltic Farm Sabon Line** | 32 | 11 | 10.7 | 0 | 0 | 0 | 0 |
| **Madam Fibis's Farm** | 71 | 8 | 7.8 | 0 | 0 | 0 | 0 |
| **Total** | **103** | **19** | **18.5** | **0** | **0** | **0** | **0** |

The frequencies of occurrence of ESBL producing *E. coli* obtained from farm I and farm II were 8.3% and 7.7% respectively. No ESBL producing *K. pneumoniae* was obtained from farm I while (0.7%) was obtained from farm II. No ESBL producing *K. oxytoca* was obtained from the farm. However, there is significance difference ($P < 0.05$) in the frequency of occurrence of *ESBL* positive isolates in farms I and II. This is presented in Table 4.25.

**Table 4.25: Frequency of occurrence of ESBL Positive Isolates in Layers' Farms**

| | ESBL POSITIVE ISOLATES | | | | | | |
|---|---|---|---|---|---|---|---|
| | | ***E. coli*** | | ***K. pneumoniae*** | | ***K.oxytoca*** | |
| Sample | Number of Isolates | Number Isolated | Frequency of Isolates (%) | Number Isolated | Frequency of Isolates (%) | Number Isolated | Frequency of Isolates (%) |
| **Tutu's Farm** | 102 | 13 | 8.3 | 0 | 0 | 0 | 0 |
| **Tartius Farm** | 54 | 12 | 7.7 | 1 | 0.6 | 0 | 0 |
| **Total** | **156** | **25** | **16.0** | **1** | **0.6** | **0** | **0** |

From broilers farms was obtained the highest occurrence rate(11.8%) of ESBL producing *E. coli*. The least (4.8%) was obtained from cockerels' farms while 6.3% was obtained from layers farms. No ESBL producing *K. oxytoca* was obtained from all the farms while 0.25% ESBL producing *K. pneumoniae* was separately obtained from broilers farms and layers farms. Meanwhile, there is significance difference ($P < 0.05$) in the frequency of occurrence of *ESBL* positive isolates in cockerels, layers and broilers' farms. This is presented in Table 4.26.

**Table 4.26: Frequency of ocuurence ESBL Positive Isolates in Broilers', Layers' and Cockerels' Farms**

| Sample | ESBL POSITIVE ISOLATES | | | | | | |
|---|---|---|---|---|---|---|---|
| | | *E. coli* | | *K. pneumoniae* | | *K. oxytoca* | |
| | Number of isolates | Number Isolated | Frequency of Isolates (%) | Number Isolated | Frequency Isolates (%) | Number Isolated | Frequency of Isolates (%) |
| **BROILERS' FARMS** | 138 | 47 | 11.8 | 1 | 0.25 | 0 | 0 |
| **LAYERS' FARM** | 156 | 25 | 6.3 | 1 | 0.25 | 0 | 0 |
| **COCKERELS' FARMS** | 103 | 19 | 4.8 | 0 | 0 | 0 | 0 |
| **Total** | **397** | **91** | **22.9** | **2** | **0.5** | **0** | **0** |

A total of 156 isolates comprising of *E.coli*, *K.pneumoniae* and *K.oxytoca* were obtained in layers farms.The layers' farms were Tutu's and Tartius' farms. The isolates were obtained from the chickens, chicken environment and chicken rearers and the frequencies of occurrence of ESBL positive isolates were respectively 12.2%, 3.8% and 0.6% as presented in figure 4.8.

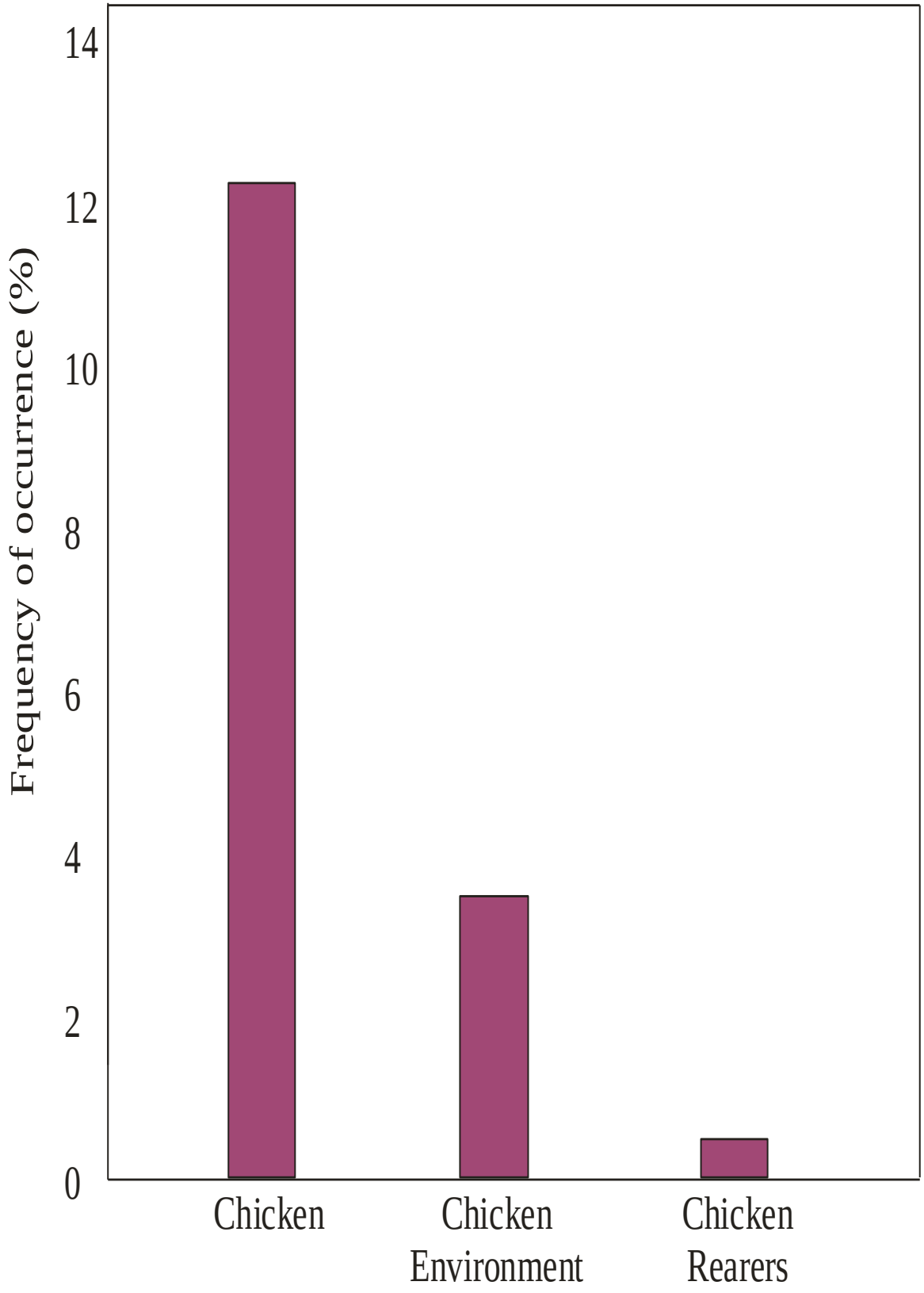


***Figure 4.8:*** **Frequency of occurrence of ESBL producing isolates in chickens', chickens' environment and chickens' rearers in layers'**

From broiler farms,which are Bello's and Ijaja'farms and Baltic farm Jaikada-fari, a total of 138 isolates were obtained from the chickens, chickens' environment and chickens' rearer and the frequencies of occurrence of ESBL producing isolates obtained were 21.7%, 10.1% and 2.9% respectively as presented in figure 4.9.

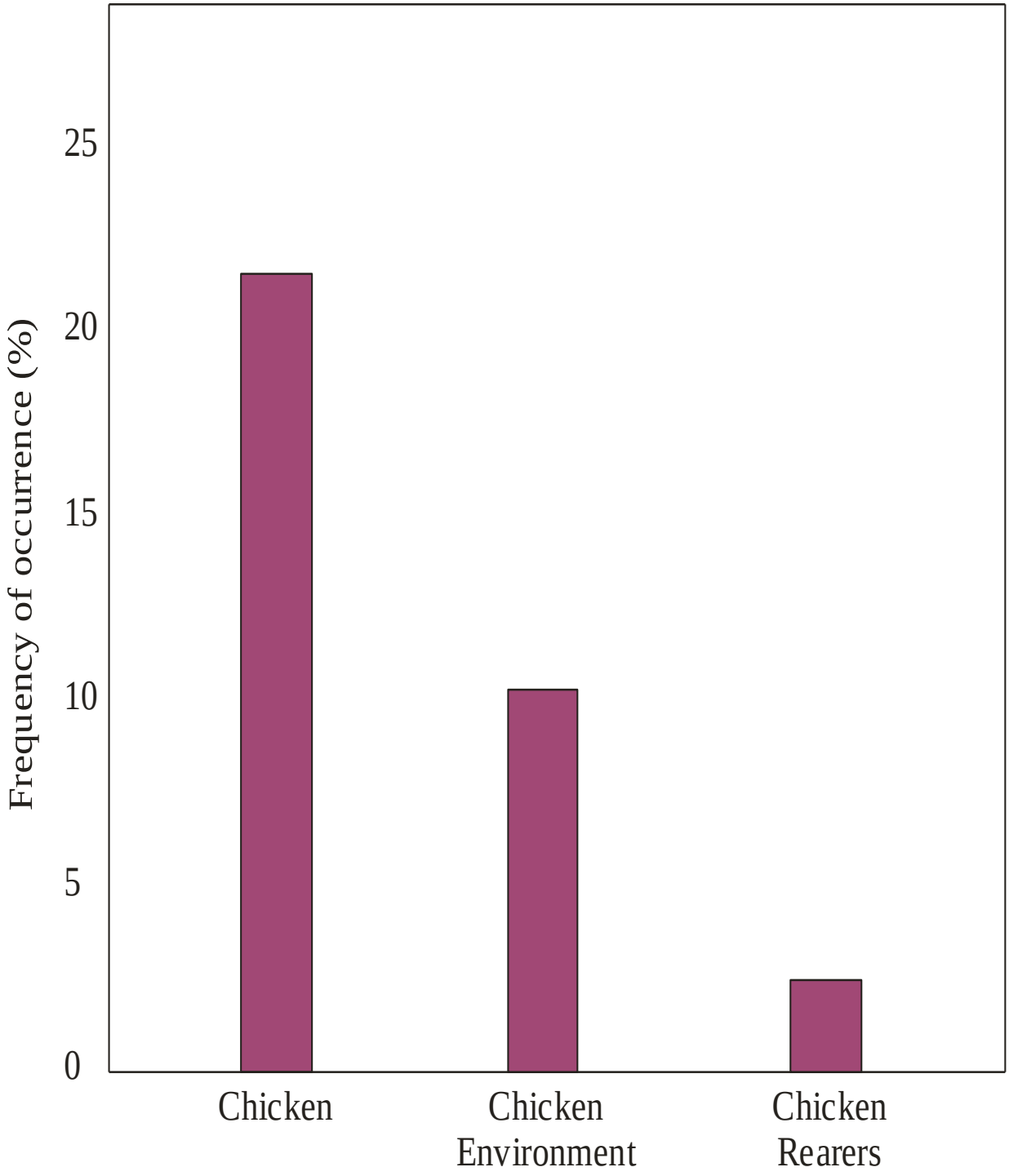


***Figure 4.9:*** **Frequency of occurrence of ESBL producing isolates in chickens', chickens' environment and chickens'rearers in broilers' farms.**

In cockerels farms which are Baltic farm, Sabon line and Madam Fibi's farm Nyamusala. A total of 103 isolates was obtained. The isolates were from the chickens, chickens' environment and chickens rearers and the occurrence rates of ESBL producing isolates were 12.6% 3.9% and 1.9% respectively as presented in figure 4.10.

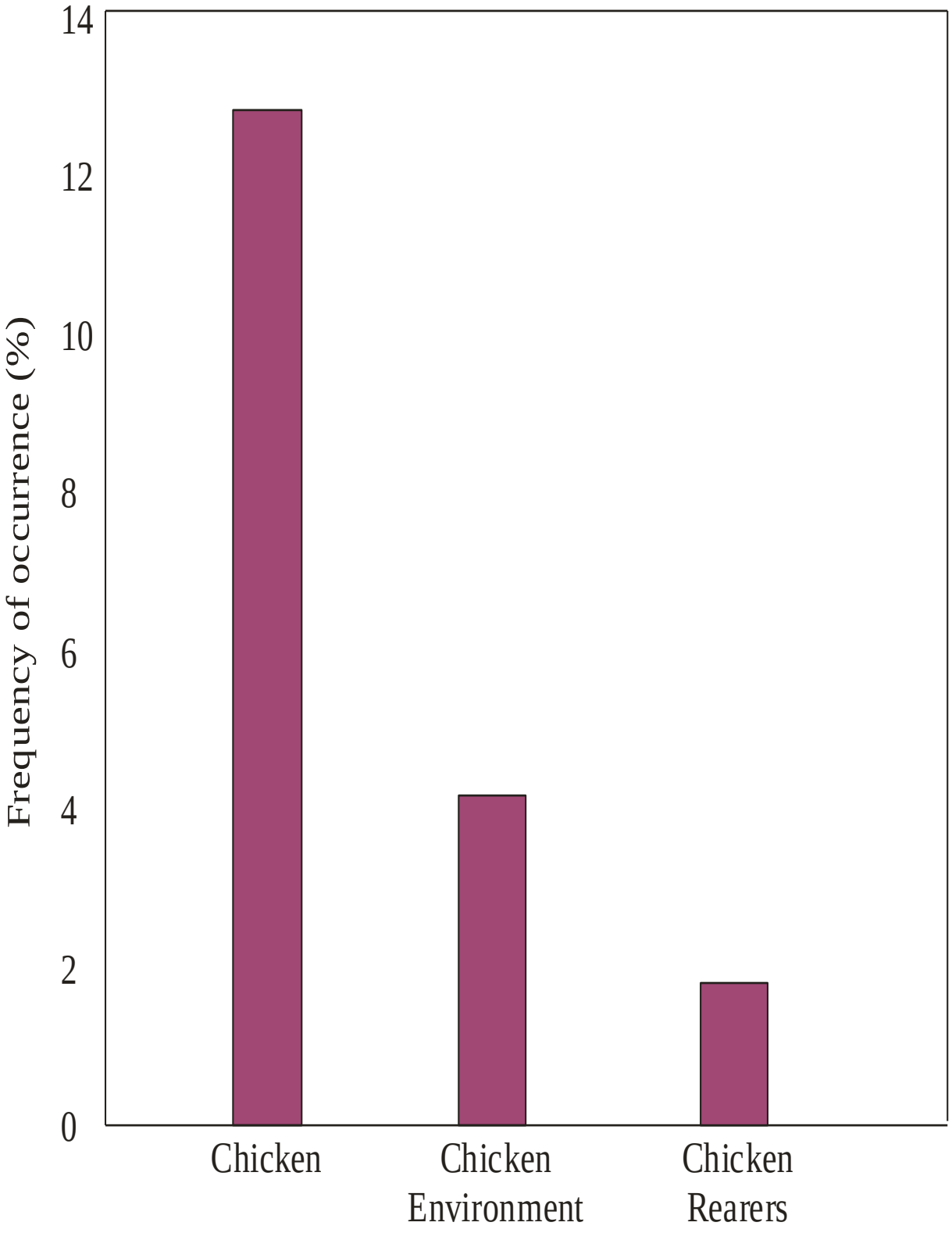


***Figure 4.10:*** **Frequency of occurrence of ESBL producing isolates in chickens', chickens' Environment and chickens rearers in Cockrerels' farms.**

A total 397 isolates was obtained from the chickens in broiler', layers' and cockerels' farms as well as from the chickens environment and the chicken rearers of the aforementioned farms. The frequency of occurrences of ESBL isolates in chickens, chickens' environment and chickens rearers were 15.6%, 6.0% and 1.8% respectively as presented in figure 4.11.

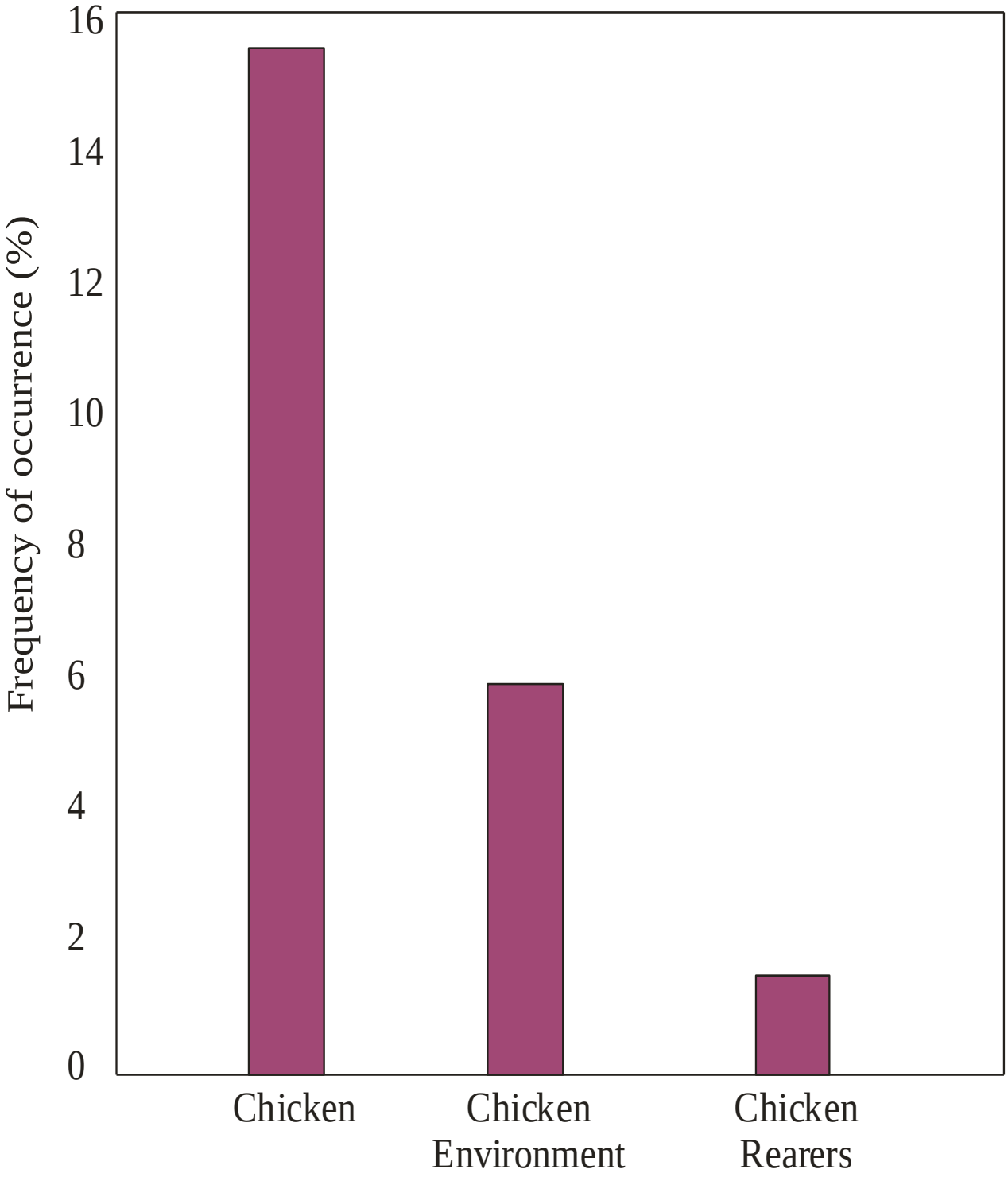


***Figure 4.11:*** **Occurrence of ESBL producing isolates in chickens', chickens' environment and chickens' rearers**

The ESBL producing *E. coli* and *K. pneumoniae* isolates were 100% resistant to ampicillin, ceftriaxone and ceftazidime. *Escherchia.coli* isolates were 47.2%, 70.3%, 2.2% 25.3% and 60.4% resistant to chloramphenicol ,ciprofloxacin, nitrofurantoin, gentamincin and tetracycline respectively. The *K. pneumoniae* isolates were 0%,100%,0%, 0%, and 100% resistant to chloramphenicol, nitrofurantoin, ciprofloxacin, gentamicin and tetracycline by respectively as presented in Table 4.27.

**Table 4.27: Antibiotics Susceptibility Profile of ESBL Positive Isolates**

| | | *Escherichia coli* | | | | | | *Klebsiella pneumoniae* | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **ANTIBIOTICS** | **(Ug/disc)** | **S** | **%** | **I** | **%** | **R** | **%** | **S** | **%** | **I** | **%** | **R** | **%** |
| Ampicillin | 10 | 0 | 0 | 0 | 0 | 91 | 100 | 0 | 0 | 0 | 0 | 2 | 100 |
| Chloramphenicol | 30 | 27 | 29.7 | 21 | 23.1 | 43 | 47.2 | 1 | 50 | 1 | 50 | 0 | 0 |
| Ciprofloxacin | 5 | 23 | 25.3 | 4 | 4.4 | 64 | 70.3 | 0 | 0 | 0 | 0 | 2 | 100 |
| Nitrofurantoin | 300 | 82 | 90.1 | 7 | 7.7 | 2 | 2.2 | 2 | 100 | 0 | 0 | 0 | 0 |
| Gentamicin | 10 | 61 | 67 | 7 | 7.7 | 23 | 25.3 | 2 | 100 | 0 | 0 | 0 | 0 |
| Tetracycline | 30 | 21 | 23.1 | 15 | 16.5 | 55 | 60.4 | 1 | 50 | 0 | 0 | 1 | 50 |
| Ceftriaxone | 30 | 0 | 0 | 0 | 0 | 91 | 100 | 0 | 0 | 0 | 0 | 2 | 100 |
| Ceftazidime | 30 | **0** | **0** | **0** | **0** | **91** | **100** | **0** | **0** | **0** | **0** | **2** | **100** |

Lanes 1 to 5 show the plasmid sizes of *ESBL* positive *E. coli* isolates from the farm environment. Lanes 6 to 7 show plasmid sizes of *ESBL* positive *K. pneumoniae* fromfarm environment. Lanes 8 to 12 show plasmid sizes of *ESBL* positive *E. coli* isolates from chicken cloacae. Lanes 13 to 17 show plasmid sizes of *ESBL* positive *E. coli* isolates from poultry farmers. Lanes 1 to 7, 9, 10, 12, 13 and 16 reveal single plasmid size of 23130bp each while lanes 8, 11, 14, 15 and 17 reveal double plasmid sizes of 23130bp and < 564bp as shown in figure4.12 below.

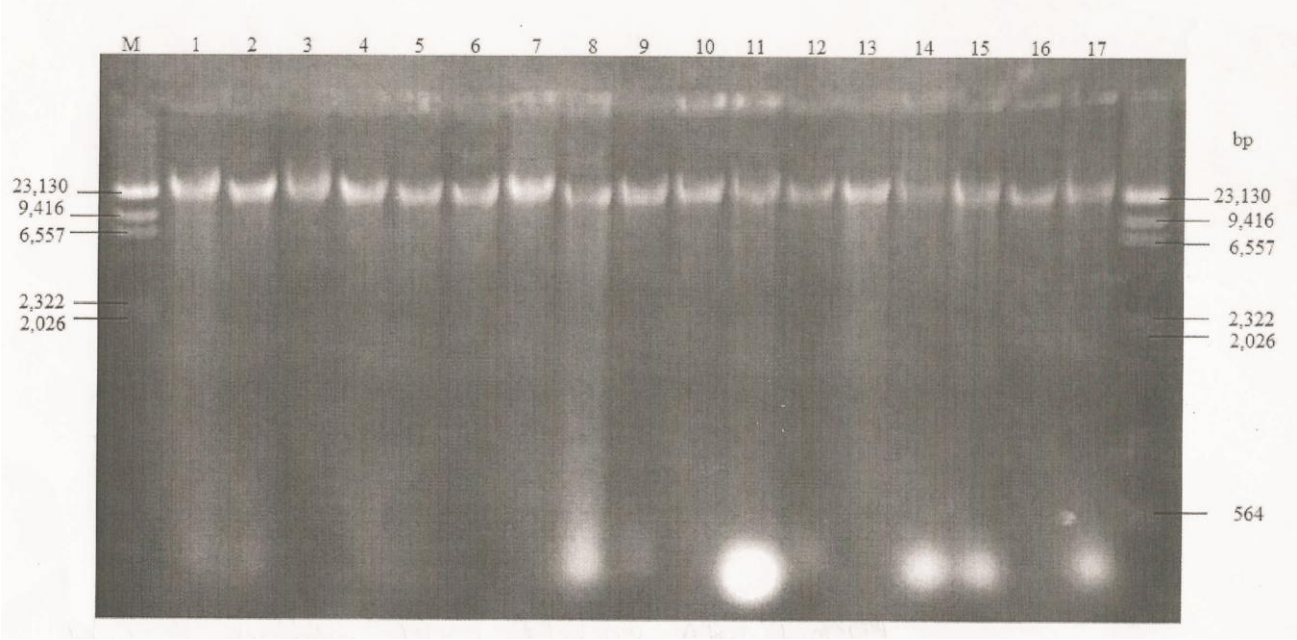


***Figure 4.12: Plasmid sizes of ESBL positive Isolates***

**lanes**

**1-5** ***E.coli*** **farm environment**

**6-7** ***K.pneumonae*****environment**

**8-12** ***E.coli*** **from chicken**

**13-17** ***E.coli*** **from chicken farmers**

Out of 17 isolates of *ESBL* positive isolates that their plasmids were analyzed, 5 harboured double plasmid sizes of 23130bp and < 564bp while 12 harboured only single plasmid size of 23130bp. Plasmid profiling was carried on, 5 *E. coli* isolates from chicken. Five of the isolates harboured plasmid size of 23130bp while two of the plasmids in addition also harboured a plasmid size < 564bp. The 5 *E. coli* isolates obtained from chicken rearers harboured plasmid size of 23130bp while 3 out of the 5 harboured double plasmid sizes of 23130bp and < 564bp. From chickens' environment, plasmid profiling was carried on 7 isolates. Two isolates of *K. pneumoniae* and 5 isolates of *E. coli.* All the *ESBL* producing isolates obtained in the chickens' environment, each harboured single plasmid size of 23130bp as presented in Table 4.28.

***Table 4.28: Plasmid sizes of ESBL Positive Isolates from different sources***

| ***Source*** | **Number of Isolates** | | **Number of Plasmid** | | **Number of isolates** |
|---|---|---|---|---|---|
| | ***E. coli*** | ***K. pneumonia*** | | **<564bp** | **23130bp** |
| Chickens | 5 | - | 7 | 2 | 5 |
| Chickens rearers | 5 | - | 8 | 3 | 5 |
| Chickens' environment | 5 | 2 | 7 | - | 7 |

The curing rate of the *E. coli* was 19.4% while that of *K. pneumoniae* was 0%.However, there is no statistical significance difference (P>0.05) in the plasmid curing rate of the ESBL producing *E.coli* and *K. pneumoniae* as presented in Table 4.29.

**Table 4.29: Plasmid curing rate of the ESBL producing *E. coli and K. pneumoniae*.**

| Isolate | Number of Isolates with plasmid | Number of isolates with no Plasmid After Curing | Percentage of Isolates cured. % |
|---|---|---|---|
| *E. coli* | 91 | 18 | 19.4 |
| *K. pneumonia* | 2 | 0 | 0 |
| **Total** | **93** | **18** | **19.4** |

The highest curing rate (4.3%) was individually obtained from farm I, farm II and farm V. No ESBL isolate from farm VI was cured while from farms III, VI and VII were observed the following curing rates respectively 1.1%, 3.2% and 2.2%.However, there is no statistical significance difference ($P>0.05$) in the plasmidcuring rate of the ESBL positive isolates in relation to site as presented in Table 4.30.

**Table 4.30: Plasmid Curing Rate of the ESBL Positive *Isolates in Relation to Site***

| Study Site | Number of Isolates with plasmid | Number of isolates without Plasmid After Curing | Percentage of Isolates cured. (%) |
|---|---|---|---|
| **Tutu's Farm** | 13 | 4 | 4.3 |
| **Tartius farm** | 13 | 4 | 4.3 |
| **Bello's Farm** | 12 | 1 | 1.1 |
| **Ijaja's Farm** | 17 | 3 | 3.2 |
| **Baltic Farm, Jaikada-Fari** | 19 | 4 | 4.3 |
| **Baltic Farm, Sabon Line** | 11 | 0 | 0.0 |
| **Madam Fibi's Farm** | 8 | 2 | 2.2 |
| **Total** | **93** | **18** | **19.4** |

The highest curing rate (8.6%) was obtained from chickens while the least curing rate (3.2%) was obtained from the rearers. The curing rate obtained from the chicken environment was 7.5%.However there is no statistical significance difference($P>0.05$) in the plasmid curing rate for the ESBL positive isolates in various samples as presented in Table 4.31.

**Table 4.31: Plasmid Curing Rate for the ESBL Positive *Isolates in Various Samples***

| Sample | Number of Isolates with plasmid | Number of isolates without Plasmid After Curing | frequency of Isolates cured. % |
|---|---|---|---|
| **Chicken** | 62 | 8 | 8.6 |
| **Environment** | 25 | 7 | 7.5 |
| **Rearers** | 06 | 3 | 3.2 |
| **Total** | **93** | **18** | **19.4** |

# CHAPTER FIVE

## 4.0 DISCUSSION

Extended spectrum beta lactamase (ESBL), a hydrolytic enzyme capable of conferring resistance to third generation cephalosporin which can be produced by Enterobacteriaceae and non Enterobacteriaceae.This study focuses on detecting their presencein chicken cloacal swabs, chickens environment (comprising of swabs from poultry floor, wall, drinker, feeder and poultry water and feed) and chicken rearers.Three hundred and ninety seven isolates comprising of 365 *E.coli 20 K.pneumoniae* and 12 *K. oxytoca* were isolated and used for this study. Other isolates are: - 107 *Enterobacter* spp., 22 *Salmonella spp.,* 24 *Shigella spp.,* 46 *Achromobacter spp. and* 92 *Pseudomonas aeruginosa*. This agrees with some of the isolates obtained by Oyinloye and Ezekiel, (2011) from chicken feed, which are *Enterobacter, Escherichia coli, Klebsiella, Salmonella and Yersinia.* Moreira and Moraes, (2002) reported the isolation of *Enterobacter, Serratia, Klebsiella, Kluyvera, Erwinia, Citrobacter, Pseudomonas and Aeromonas* from broiler chickens. Also, Bunkova *et.al, (2009)* reported having isolated *Aeromonas, Pseudomanas and isolates* of famil*y Enterobacteriaceae.* Ansari and Khatoon, *(1994),* isolated *Salmonella, Escherichia, Pseudomonas and Klebsiella* frompoultry.

The frequency of isolation of the isolates in Tutu's farm was studied and it was observed that the bacterial organism with the highest frequency of isolation was *E. coli* while *K. oxytoca* had the least. This could be because E.*coli* is a coliform and the water drank by these chickens as well as their feed may have been faecally contaminated by the workers (since it is a common practice in this part of the country to wash the buttocks with only water and hand after defecating).The 90.2% isolation frequency of *E. coli* obtained is this study is lower than 100% isolation rate obtained by Dadheech *et al.,* (2016).This is probably because Dadheech *et al.,* (2016) worked with clinically sick layer chickens.The isolation rate of *K.pneumoniae* was 5.9% and this agrees with 5.9% isolation frequency reported by Hassan *et. al.,* (2015).

In Tartius' farm, the observation made by the study of the frequency of isolates was that E. coli had the highest frequency of isolation while the least was *K. oxytoca*. The occurrence of *E. coli* was the highest probably because most of the specimens were faecal and *E. coli* is a coliform.The isolation rate, 14.8% of *Klebsiella pneumoniae* was higher than 5.8% reported by Hassan *et al.,* (2015) from dead laying hens. The dead layers did not die as a result of infection with *K. pneumoniae*.The reason for the difference is as a result of differences in hygienic measures observed in both farms.

The isolation rate (79.5%) of *E. coli* obtained from this farm is lower than 100% isolation rate reported by Dadheech *et al.,* (2016). This could be because the layer chickens studied by Dadheech *et al.,* (2016) were dead and because the chickens were heavily infected with *E.coli.* It can also be that the feed and water were faecally contaminated. Abiala *et al.,* (2016) reported 9% occurrence of *E. coli* isolated from layer chickens. This is higher than that obtained in this study probably because the management of this farm observed a much higher hygienic measure than those of the aforementioned farm.

The study on the frequency of isolation of isolates from Bello's farm revealed the rate of isolation of *E. coli* as the highest; no *K. oxytoca* was isolated while that of *K. pneumoniae* was 6.2%. The frequency of isolation of *E. coli* from the chickens is higher than from the environment. The reason could be that the chicken did not only get contaminated from the environment, there could be other sources like egg transmission of *E. coli* in fowls. The prevalence rate of *E.coli* (93.8%) obtained from this study is higher than 44% reported by Ashraf *et al.,* (2015) on imported chicks. The incidence in the imported chicks is lower may be because of the technological advancement and aseptic measures observed in handing the chickens in the country from where they were imported.

Also studied was the frequency of isolation of the isolates in Ijaja's farm. *Escherihia coli* had the highest occurrence followed by *K. pneumoniae*, no *K. oxytoca* was isolated. Ashraf*et al.,* (2015) reported a prevalence of 5.7% and this is lower than (98.2%) observed in this study. The reason could be because the broilers Ashraf *et al.,* (2015) reported on were confirmed healthy broilers while the ones used in this study were not. The frequency of isolation of the isolates obtained from Baltic farm, Jaikada-fari was studied. Only *E.coli* and *K.oxytoca* isolates were obtained. The occurrence of *E.coli* was

higher than that of *K. oxytoca*. The occurrence rate, 91.5%, of *E. coli* obtained from this farm is higher than 44% reported by Ashrat *et al.,* (2015). This could be because of the difference in the level of hygiene of the workers in both farms as well as in the level of cleanliness observed in the farms. Nwakaeze *et al.,* (2013) reported having isolated 141 E. *coli* isolates from 200 faecal and cloacal swab samples of broilers from Abakaliki. The occurrence, 70.5% is higher than 54.3% which was obtained from broiler chickens in this study. This could be because the chickens used in this study were confined to a place and therefore, were not exposed to much contaminants

A study of frequency of isolation of isolates from Baltic farm, Sabonline was done.The occurrence of *E.coli* was the highest while that of *K.oxytoca* was the least.No *Klebsiella* species was obtained from the chickens while 59.4% prevalence of *E.coli* was obtained from the chickens. From the chickens' environment, the occurrence rate of *E.coli* obtained was higher than that of *K. oxytoca* while no *K. pneumoniae* was obtained. From the rearers, *E.coli* also had the highest frequency of occurrence while that of *K. pneumoniae* and *K.oxytoca* were equal. There is no previous study on the occurrence/prevalence of *E.coli/Klebsiella* species on cockerels to the best of the knowledge of the researcher.

The frequency of isolation of isolates from Madam Fibi's farm was studied and it was observed that the isolate with the highest frequency of occurrence was *E. coli*, no *K. oxytoca* was obtained. Meanwhile, the frequency of occurrence of *K. pneumoniae* was 1.4% from the chickens. The frequency of occurrence of *E.coli* was higher than that of *K. pneumoniae.* From the farm environment, only *E. coli* isolates were obtained, no *Klebsiella* species was isolated. This is probably the first research on the occurrence of E. coli/Klebsiella species in cockerels to the best of the knowledge of the researcher

The frequency of isolation of isolates in relation to site was studied .The isolate that had the highest frequency of occurrence (91.9%) was *E.coli* while the least (3.1%) was *K.oxytoca*. Younis *et al.,* (2016) reported the prevalence rate of 73.3% of *K. pneumoniae* isolates obtained from diseased chicken organs. This is higher than 5.2% occurrence rate obtained in this study. This difference could be because the chickens studied by Younis *et al.,* (2016) were diseased. Also Davies *et al.,* (2016) reported 25% frequency of

occurrence of *K. pneumoniae* from passerine and psittacine birds. This is also higher than that obtained in this study. This difference can be attributed to differences in farm management and hygiene measures observed in these farms. The frequency of occurrence of *K.oxytoca* reported by Younis *et al.*, (2016) in diseased chicken organs was 26.6% and this is higher than that obtained from this study and this could be because the chickens were diseased. Ojo *et al.*, (2012) reported prevalence rate of 56.5% in *E.coli* isolates obtained from free range chickens and this is lower than 91.1% prevalence rate obtained in this study probably because the level of hygiene observed by the rearers of the chicken used in this study wass not high.

The frequency of occurrence of *E.coli* in broilers' farms was investigated. The frequencyof occurrence of *E.coli* was highest in Ijaja's farm, followed by Bello's farm. The least frequency of occurrence was obtained in Baltic farm, Jaikada-fari. Ijaja's farm had the highest frequency of occurrence because the drinking water of the chickens was not clean enough. The drinker, feeders including the walls and floors were very dirty. The feed were not hygienically prepared. The feed was mixed factory prepared and self prepared feed.In Bello's farm, the floor and the walls of the poultry farms were much cleaner than those of the Ijaja's farm. The water used in this farm for the chickens were not very clean and again, they use mixed factory prepared and self prepared chickens' feed . The measure of aseptic techniques observed in making self prepared feed is not likely to be very high. In Baltic farm, Jaikadas-fari, only factory prepared feed were used. Antibiotics were added to the water provided for the chickens. The floor and walls of the poultry farms were not very dirty though not very clean. Therefore, Ijaja's farm was the most contaminated, followed by Bello's farm and the least was Baltics farm, Jaikada-fari.

In layers farm, the frequency of isolation of *E.coli* from Tutu's farm is higher than from Tartius' farm. That of Tutu's farm is higher because some of the chickens studied looked sick or rather looked as if they were diseased.

The frequency of occurrence of *E.coli* in cockerels' farms was studied. The occurrence of *E. coli* in chickens in Madam Fibi's farm was higher than that from Baltic farm; this could be because of egg transmission. Also, the frequency of occurrence of *E. coli* isolated from the chicken environment in Madam Fibi's farm is higher than in Baltic

farm, Sabo line. This could be as a result of the contamination of the floor of the poultry farm by the faeces of the chickens.

The frequency of occurrence of *E.coli* in cockerels',layers' and broilers' farms was studied.The highest frequency of occurrence of *E.coli* isolated from chickens was obtained from layers farm while the least was obtained from cockerels' farm. The reason could be because some of the layer chickens investigated seemed to be diseased. In the chicken environment, the highest frequency of occurrence was obtained from broilers' farm and the least was obtained from layers farm. The reason could be attributed to the fact that mixed self prepared feed and factory prepared feed were used in two of the three broilers' farms investigated. In one of the broilers' farm, there was no caution on how people enter the farm. Also, the drinking water of the chickens in two of the broilers farms was not clean.

The frequency of occurrence of *E.coli* isolated from broilers was 24.1%. This is lower than 42% prevalence reported by Hiroi *et al.,* (2011). This difference can be linked to differences in the cleanliness of the farm environment, personal hygiene of the farm rearers and also on the health conditions of the chickens. Also, Hiroi *et al.,* (2011) reported the occurrence rate of 48% of *E.coli* in layers chicken. This is also higher than 29.6% occurrence observed in this study. This could be because the farm environments used in this study were cleaner and the rearers also observed a better personal hygiene. Also, the chickens may have been healthier than those studied by Hiroi *et al.,* (2011).The occurrence rate of *E.coli* isolated from cockerels' farms was 18.9%. This is probably the first research on the occurrence of *E. coli* in cockerels' farms to the best of the knowledge of the researcher.The frequency of occurrence of *E.coli* obtained from broilers' chicken rearers was 1.4%. This is lower than 27.6% also obtained from broiler farmers as reported by Bogard *et al.,* (2001). The difference could be because the farmers investigated in this study were in good health and take several measures like washing their hands with antiseptic/disinfectant immediately after leaving the farm.

The frequency of occurrence of *E.coli* isolates obtained from layers chickens farmers was 2.2%. This is also lower than 29.6% obtained from layers farmers according to the study

by Bogard *et al.,* (2001). This can also be attributed to the hygiene measures observed by the layers farmers investigated in this study.

The frequency of occurrence *E.coli* obtained from cockerels' farmers was 2.2%. To the best of the knowledge of the researcher, this is most probably the first research on the occurrence of *E. coli* in cockerels' farmers.

The frequency of occurrence of *K.pneumoniae* in broilers',layers', and cocker els'chickens were studied.The highest frequency (3.5%) of occurrence *of K. pneumoniae* was obtained from layers' farms while the least (0.5%) was obtained from cocker els' farms. The subspecies of the chicken could have affected the occurrence *of K. pneumoniae*. The occurrence of 5% of *K. pneumoniae*islower than 73.33% obtained by Younis *et al*., (2016) because the chickens studied by *Younis et al*., (2016) were diseased. Davies *et al.,* (2016) reported a higher occurrence (25%), *of K. pneumoniae* from passerine and psitacines birds. This is also higher than that obtained from this study. The reason could be that the chicken rearers handling the chickens investigated in this study observed a higher level of hygiene than the later.

The frequency of occurrence *of K. oxytoca* in broilers, layers'and cockerels' farms was investigated. The highest occurrence was obtained in layers' farms and the least in cockerels' farms. The occurrence rate of 26.67% reported by Younis *et al*., (2016) is higher than 3.1% obtained in this study. This could probably be because the chickens investigated by Younis *et al.,* (2016) were diseased.

The frequency of occurrence of beta-lactamase producing *E.coli, K.pneumoniae* and *K.oxytoca* were studied.The frequency of occurrence of *E.coli* obtained from the chickens was the highest while the least obtained was *K. oxytoca*. The occurrence of 24.3% of β-lactamase producing *E.coli* was obtained from chickens according to the study by Hiroi *et al.,* (2011). This is lower than 35.3% obtained from the study because the level of contamination of the farms investigated in this study was probably higher than that of the chickens studied by Hiroi *et al.,* (2011). Chah and Obogbulem, (2007) reported a prevalence rate of 98.8% which is higher than 35.3% obtained in this study. The reason

could be because only Ampicllin resistant *E. coli* was investigated while in this study both Ampicillin resistant and non-Ampicillin resistant *E.coli* were investigated.

The occurrence of β-lactamase producing isolates in the various farms was studied. The highest frequency of occurrence (9.1%) of *E. coli* was obtained from Madam Fibi's farm while the lowest (4.5%) was obtained from Baltic's farm, Sabon line. The highest occurrence rate was obtained in Madam Fibi's farm probable because of horizontal genetic transfers and egg transmission since the chicken were only 2 days old.

Also, the occurrence rates of β -lactamase producing isolates in broilers' farms were studied. The highest occurrence rate of *E.coli* was obtained in Ijaja's farm, while the least was obtained in Baltic's farm, Jaikada-fari. The reason can only be attributed to the level of hygiene observed in both farms. The occurrence of *K. pneumoniae* observed in Bello's and Ijaja's farms were same, no *K. pneumoniae* was isolated from Baltic farm .*Klebsiella oxytoca* was not obtained from Bello's and Ijaja's farm. The occurrence of 13.8% of *E. col*i was obtained from a study made by Hiroi *et al.,* (2011). This is lower than 38.6% obtained in this study probably because in this study cloacae swabs were investigated, while in the later, the meat was investigated and the cloacal swab will likely have a heavier load of *E.col*i than chicken meat. Laube *et al.,* (2013) reported 85.7% of occurrence of *E.coli*. This is higher than 38.6% obtained in this study because the rearers investigated in this study observed a higher level of hygiene and most of them do not add antibiotics to the feed and water of the chickens. They do not abuse drugs.

The occurrence of β-lactamase producing isolates in cockerels' farms was studied and it was observed that a higher frequency of occurrence of *E.coli* was obtained from Madam Fibi's farm than in Baltic's farm. The reason could be because of the difference in the age of the chickens. In Madam Fibi's farm, the high occurrence rate (35%) most probably would be because of horizontal genetic transfer and egg transmission. *Klebsiella*

*pneumoniae* and *K. oxytoca* were not obtained from Baltic's farm but one isolate of each was obtained from Madam Fibi's farm. To the best of the knowledge of the researcher, this study is most probably the first in the occurrence of β-lactamase producing *E.coli* and *K. pneumoniae* species in cockerels' farms.

The frequency of occurrence of beta-lactamase producing isolates was also studied in layers' farms. The occurrence rate of E. *coli* obtained from Tartius' farm was higher than that obtained from Tutu's farm. The reasons could be because there are two different subspecies of layers. Also, a factor could be the differences on how hygienic both farms were. The occurrence of *K. pneumoniae* in both farms were equal.No *K.oxytoca* was obtained from layers' farms.From the study by Hiroi *et.al*., (2011), 10.5% occurrence rate was obtained and this is lower than 22.69% obtained in this study. The reason again could be because Hiroi *et al.,* (2011) investigated only chicken meat while in this study, cloacae swabs as well as stool specimen (which usually have heavy load of bacteria which includes *E. coli*, and chicken environment were studied.

The researcher also studied the occurrence of beta-lactamase producing isolates in broilers, layers and cockerels' farms. The frequency of occurrence of *E. coli* in broilers farm was the highest (20.40%) while the least (12.85%) was in cockerel's farm. The reason for this difference could be because the chickens were of different subspecies though the level of cleanliness of the farms and the chicken rearers might have contributed to this.

The researcher studied also the occurrence of beta-lactamase producing isolates in chicken, chicken environment and chicken rearers. In chickens, the occurrence of beta-lactamase producing isolates obtained was highest (16.1%) in broilers and the least (9.8%) in layers. The reason, again, can be attributed to the fact that the chicken (broilers and cockerels) are of different subspecies. In the chickens' environment, the highest (5%)

frequency of occurrence was also obtained from the broilers' farm while the least (2.0%) was obtained from the cockerel's farm probably because the broilers' farm environment must have been more contaminated with beta-lactamase producing bacteria in the faeces of the chickens than in layers and cockerels' farm environment.

The frequency of beta-lactamase producing *E.coli* in broilers', layers' and cockerels' farms was also studied. The occurrence of *E.coli* obtained in chickens was highest in broilers farms and least in layers' farm. This could be because they are of different subspecies of chicken. The occurrence of *E. coli* is also highest in the broilers chicken environment. The reason could also be because the faeces of the chickens contaminated the floors, the drinkers and the feeders. The occurrence of *E.coli* in all the rearers was same.

The researcher also studied the occurrence of beta-lactamase producing

*K. pneumoniae* in broilers', layers', and cockerels' farm and observed that the highest frequency of occurrence (1.5%) was obtained from the broilers' farms while the least (0.3%) was obtained from the cockerels' farms. The occurrence rate of 0.5% was obtained from the layers' farms. The most probable reason for this difference is because they all belong to different subspecies and to the best of the knowledge of the researcher, this is the first research on the occurrence of beta lactamase producing*K. pneumoniae* in broilers', layers' and cockerels' farms.

The occurrence of beta-lactamase producing isolates in chickens, chickens environment and chicken rearers in layers' farm was studied. In the chickens, the occurrence rate was higher in Tutu's farm than in Tartius farm. This could be because they are of different subspecies of layers and also the chickens investigated in Tartuis' farm, were of better health condition than in Tutu's farm. The frequency of occurrence obtained from the chickens' environment was higher in Tartuis' farm than in Tutu's farm. The reason is because in Tutu's farm, for anyone to enter the farm, the person must wear a special pair

of slippers and must step on disinfectants soaked towel before entering the farm and therefore should have less contaminants than in Tartuis' farm. The frequency of occurrence obtained from the rearers' of both farms was same.

In broilers farms, the frequencies of occurrence beta-lactamase producing isolates were also studied. The highest frequency (18.8%) was obtained from Ijaja's farmwhile the least (10.9%) was obtained from Baltic farm. The occurrence of beta-lactamase producing isolates obtained in Bello's farm was (16.7%). Since chickens studied in Bello's farm and Ijaja's farm were almost the same age, and the difference in the frequency of occurrence obtained from these two farms were minimal while the difference in occurrence obtained from Baltic farm and Bello's farm and from Ijaja's farm was much. Therefore, it can be inferred that age of the chickens affected the frequency of occurrence of the isolates. In the chicken's environment, the highest frequency of occurrence was obtained from Bello's farm and it is surprising that no isolate was obtained from the rearers. Therefore, it is believed that the environment was most probably contaminated by the bacteria in the faeces of the chickens. The occurrence rates obtained from the Ijaja's and Baltic farms chicken environment were same.Though, it is expected that the occurrence rate obtained from Ijaja's farm should be higher. This was not so, because in Ijaja's farm, disinfectants were always used by the rearers to wash their hands before and after touching the chickens and therefore, no or minimal contaminants gets to the chicken environment (feed, water, drinkers, feeder wall) from the rearers.

The frequency of occurrence of beta-lactamase producing isolates in chickens, chickens' environment and chickens rearers in cockerels' farms were studied. The frequency of occurrence obtained in Madam Fibi's farm is higher than in Baltic farm. This could be because of the differences in the ages of the chickens. In Baltic' farm, no isolates was obtained from the chicken environment while in Madam Fibi's farm, 7.8%, occurrence rate was obtained.This could be because, the floor, drinker, and feeder were contaminated by the beta-lactamase producing isolates in the faeces of the chicken. A higher occurence rate was obtained from the chicken rearers in Baltic's farm than in Madam Fibi's farm.

This could be because the chicken rearers in Madam Fibi's farm observed a higher level of personal hygiene than the chicken rearers in Baltic's farm.

The synergy of clavulanic acid containing disk with ceftazadime and ceftriaxone in double disk synergy test (DDST) for ESBL phenotyptic confirmation was investigated. The inhibition zone around ceftazidime disk and ceftriaxone disk (2) augmented towards the disk (4) containing clavulanic acid (augmentin). This is because clavulanic acid inhibited the action of ESBL. Therefore, the bacterial species containing ESBL were made susceptible; therefore, the zone of inhibition close to the disc containing calvulanic acid is larger than the zone of inhibition close to the disk that contained no calvulanic acid. This synergy as a result of the presence of clavulanic has also been reported by Adeyankinnu *et al.*, (2014). Also, Oyinloye and Ezekiel (2011) reported a synergistic effect as a result of using together of a third generation cephalosporin and clavulanic acid.

The occurrence of ESBL positive *E.coli, K. pneumoniae* and*K.oxytoca* in various farm samples was studied. The occurrence 22.9% ESBL producing *E.coli* was obtained from all the observed farm samples but this is lower than 65.9% observed by Hiroi *et al.*, (2012). Probably because in this study, the reaers observed a higher level of hygiene and also most of the chickens were in good health. All the rearers were also healthy. The occurrence rate of *K. pneumoniae* (0.5%), is lower than (3.6%) reported by Tekinar and Ozpinar, 2016 because the rearers of the chickens investigated in this study were enlightened and they observed a higher level of personal hygiene.

The observation made from the study of the frequency of occurrence of ESBL positive isolates in relation to site showed that the highest occurrence (4.8%) of *E.coli* was obtained from Baltic's farm Jaikada-fari while the least (2.0%) was obtained from Madam Fibi's farm. The differences in the frequency of occurrence of *E.coli* observed in

the various farms could be because of the differences in the age of the chicken, the hygienic measures observed in the farms and on the rate of abuse of drugs.

The frequency of occurrence of ESBL positive isolates in broilers' farms was studiedand it was observed that the number of isolates from boilers farms was 138. Out of the 138 isolates, 47 *E. coli* isolates were ESBL producers while only one *K. pneumoniae* was an ESBL producer.

Therefore, $^{48}/_{138}$ (34.8%) prevalence obtained from this study is higher than 14.2% obtained by Nwakaeze *et al,* (2013) from cloacae specimen of broilers in Abakaliki metropolis, Ebonyi state of Nigeria. The difference could be as a result of differences in the sources of water and the personal hygiene of the rearers. Also, in Baltic farm, Jaikada-fari antibiotics were added to the chickens' drinking water and this must have contributed to that which made the occurrence of ESBL producing isolates obtained in this study to be high. Dierikx *et al.,* (2012) reported a high prevalence (75% $\pm$ 10%) of ESBL producers from Dutch broilers studied. Hiroi *et al.,* (2012) reported having isolated one ESBL producing *K. pneumonia* from faecal samples of broilers analyzed in Japan. This agrees with the observation made from this research where only one ESBL producing *K. pneumoniae* was detected from broilers' farms.

The researcher also studied the frequency of occurrence of ESBL positive isolates from cockerels'farm andobserved that 103 isolates were obtained from cockerels' farms. The frequency of occurrence of ESBL producing strain of *E. coli* was 18.5%. No strain of K. *pneumoniae* was obtained. This low frequency of occurrence could be because no antibiotic was used in the chickens'feed and water. This research on the prevalence/occurrence of ESBL producing *E. coli* in cockerels' farms was most probably the first to the best of the knowledge of the researcher

The frequency of occurrence of ESBL positive isolates in Layers' farms was also studied and it was observed that 156 isolates were obtained, 26 of which were ESBL producing isolates and it gave a prevalence of 16.6%. This is lower than 65% prevalence detected by Blaak *et al*., (2015) in Netherland. The reason was that the Netherlands is one of the highest users of antimicrobial agents in animal food production which leads to drug resistance in these animals (Grave *et. al.,* 2010). According to the report by Hiroi *et al*., (2012) 5.9% prevalence rate of ESBL producing *E.coli* was detected from rectal samples of layers in a study conducted in Japan. This is lower than 16.0% prevalence rate obtained in this study. This could be as a result of differences in handling and hygiene measures observed in the farms

The frequency of occurrence of ESBL positive isolates in broilers', layers'and cockerels'farms was studied.The study revealed the occurrence rate of ESBL producing *E. coli* obtained to be the highest in broilers farm and least in cockerels' farm. This could be because one of the broilers farms included antibiotics in the chickens' water. The occurrence of ESBL producing *E. coli* studied was 22.9% and this is lower than 79.8% prevalence obtained in chicken meat in Netherland according to Overdevest *et al.,* (2011). This could be because Netherlands is one of the highest users of antibiotics in animal food production according to Grave *et al.,* (2010).

The occurrence rate of ESBL producing isolates in chickens',chickens'environment and chicken rearers in layers farms was studied.The study revealed that, the occurrence of ESBL positive isolates in chickens was the highest while that in chicken rearers was the least. The frequency of occurrence of ESBL positive isolates in chickens was 12.2%. This is higher than 5.9% occurrence rate of ESBL producing *E. coli* obtained by Hiroi *et al.,* (2012), probably because there was less contaminants in the farm and may be the rearers observed a high measure of hygiene s. Blaak *et al.,* (2015) reported an occurrence rate of 6.5% of ESBL producing *E. coli* obtained from laying hens farms.This is higher than 16.6% obtained in layers' farm in this study and this could be because the farms

investigated in this study reared more healthy chickens, the environment which comprises of the floor, wall, drinkers, feeders, feed and water were cleaner. And the rearers investigated in this study may have been in a better health condition and most probably observed a higher level of hygiene than those studied by Blaak *et al.,* (2015). Also, Netherland is one of the highest users of antibiotics it animal food production according to the report of Grave *et al.,* (2010).The frequency of occurrence of ESBL positive isolates in the chickens' environment was 3.8%. This is lower than 81% from rinse water and run-off water, 6% in barn air as reported by Blaak *et al*., (2015) in Netherland, because the Netherlands is one of the highest users of antibiotics in animal food production. Therefore, the ESBL positive strains of *E.coli* from the chickens might have contaminated the environment.

The frequency of occurrence of ESBL producing isolates in chickens', chickens'environment and chicken rearers in broilers'farms was studied. It was observed that the highest frequency of occurrence of ESBL positive isolates was obtained from chickens while the least was from the chicken rearers.

The frequency of occurrence of ESBL positive isolates in chicken environment was 10.1% and this is lower than 57% obtained from surface water adjacent to farm and 55% from soil as reported by Blaak *et.al.,* (2015) probable because the Netherlands (where Blaak *et. al.,* 2015 studied) are one of the highest users of antimicrobial in animal food production. Therefore, the ESBL positive isolates from the chickens contaminated the soil and the water adjacent to the soil. Also, this study observed an occurrence rate of 2.9% of ESBL positive isolates in chicken rearers. This is lower than 10.9% observed by Dalims *et.al*., 2015 from broiler farm workers probably because, the farmers investigated in this study observed a higher level of personal hygiene.The frequency of occurrence of ESBL producing isolates obtained from chickens was 21.7% and this is lower than 60% occurrence reported by Hiroi *et. al*., 2012 probably because of high health status of the rearers investigated in this study. Also, the chicken environment might not have been as contaminated as that studied by Hiroi *et al., (*2012). Laube *et al.,* reported occurrence of 54.2% of ESBL positive *E.coli* from broilers' chicken farm environment. This is higher

than 10.1% observed in this study. This is likely going to be because the chicken environment investigated in this study was less contaminated probably because the chicken reaers observed a higher level of hygiene. The chickens reared in these farms may have been healthier than those in the farm investigated by Laube *et al.,* 2013.

The frequency of occurrence of ESBL producing isolates in chickens, chickens' environment and chicken rearers in cockerels' farms was studied. It was observed that the frequency of occurrence of ESBL producing isolates obtained from chickens was the highest while the least was obtained from the rearers.This is most probably the first research on occurrence of ESBL producing isolates obtained from the rearers in cockerels' farm.

The frequency of occurrence of ESBL producing isolates in chickens',chickens'environment and chicken rearers in broilers ,layers' and cockerel' farms was studied,and it was observed that the frequency of occurrence of ESBL producing isolates was the highest in the chickens while it was the least in the rearers. In the chickens, the frequency of occurrence of EBSL producing isolates was 15.6% and this is lower than 84.5% reported by Overdevest, (2011) in Netherland, the reason is most likely because Netherlands is one of the highest users of antimicrobial in animal food production. Also Chah and Oboegbulem (2007) reported the occurrence of 9.4%. This is lower than 15.6% obtained in this study, probably because the level of hygiene observed in the farms investigated in this study were likely not as high as that observed in the farms studied by Chah and Oboegbulem (2007). Also, the level of personal hygiene of the rearers observed in the farms investigated in this study might not have been as high as that of the rearers investigated by Chah and Oboegbulem (2007).Also Gao *et al.,* (2014) reported the occurrence of 10.7% which is lower than 15.6% observed in this study. This difference could be because one of the farms investigated in this study added antibiotics to the drinking water of the chickens

.From the chickens' environment the occurrence rate of 6.0% ESBL positive isolates was obtained. Gao *et al.,* (2014) reported occurrence of 3.7% and 14.8% from upstream and downstream waters close to a poultry (chicken) farm. The 3.7% occurrence rates from upstream water is lower than 6.0% obtained in this study probably because new and fresh water always flow out from the source and also contain less contaminant from the chicken environment and the river banks. While the 14.8% occurrence obtained in downstream water is higher than 6.0% obtained in this study because the downstream water contain contaminants from the upstream water, from the chicken environment as well as from the river banks.

The antibiotic susceptibility profile of ESBL positive isolates was studied; the observation made from this study showed that ESBL producing *E.coli* and *K. pneumoniae* were 100% resistance to ceftriaxone, ampicillin and ceftazidime. The ceftriaxone and ceftazidime contain β lactam ring (7 amino cephalosporanic acid) while penicillin has 6 amino penicillanic acid. These compounds can be hydrolyzed by ESBL producing strains of *E.coli* and *K. pneumoniae* (Brooks *et al,* 1998). Emergence of ESBL resistant has been associated with the use of third generation cephalosporin (particularly ceftiofur) in chickens.Tetracycline group of antibiotics, though snot used not now for treatment of human infections, *E.coli* was highly resistant to tetracycline. As observed in this study, ESBL producing *E.coli* and *K. pneumoniae* showed 60.4% and 100% resistance to tetracycline. Gundogan and Avci, 2003 reported high resistance (77.8% and 69.8%) to tetracycline by ESBL positive *E.coli* and *K. pneumoniae* respectively. The observed resistance to gentamicin by *E. coli* was high (60.4%). Sabir *et al.,* (2014) also reported a high resistance (59.4%). Also, Adeyankinnu *et al*., (2014) reported a high resistance (54%) to gentamicin by ESBL producing *E coli* strains. One of the reasons for this high resistance can be co-expressed resistance mechanisms in ESBL producing *E.coli* and *K. pneumoniae strains. Escherichia coli* and *K. pneumoniae* show high (70.3% and 100% respectively) resistance to ciprofloxacin. Afunwa *et al*, (2011) reported also a high resistance (40%) of ESBL producing isolate to ciprofloxacin. Also Motayo *et al*, (2013) reported a resistance of 52.6% to oflaxocin (quinolone). Nakamura *et al*, (2012) reported

70% of ESBL producing *E.coli* strains resistant to ciprofloxacin. The high resistance the ESBL producing isolates have against this class of antibiotics is because of the therapeutic use of such antibiotics in animals and widespread addition of it to the animal feed. ESBL producing isolates are highly susceptible to nitrofuratoin and this agrees with the observation made in this study that ESBL producing *E. coli* and *K. pneumoniae* show 90.1% and 100% susceptibility to nitrofuratoin. Also, Shaikh *et al*., (2015) reported minimal resistance to nitrofurantoin. A high resistance (47.2%) to chloramphenicol was observed in this study. Tadesse *et al*., 2002 reported 35.6% chloramphenicol resistant strains. The observations could be explained by co-selection of mobile resistance elements or by unknown substrates for the chloramphenicol resistance determinant that serve as a selection pressure.

The plasmid sizes of ESBL positive isolates were studied.asll the isolates (both ESBL positive *E. coli and K. pneumoniae*) from the chicken environment harboured single plasmid size of 23,130bp.Two of the isolates from the chicken cloacae revealed double plasmid sizes of 23,130bp and <564bp. The other 3 isolates harboured single plasmid size of 23,130bp. The observation made from the plasmid profiling of the ESBL positive *E. coli* isolates from chicken farmers revealed that 3 of the isolates harboured doubleplasmid sizes of 23,130bp and <564bp. The other two revealed only single plasmid size of 23,130bp. In a report by Adeyankinnu *et al.,* (2014), the highest average weight of 24.3kbp and 22.1kbp were the plasmid sizes of ESBL producing *E. coli* and *K. pneumoniae* respectively. This agrees with the large plasmid size of 23.1kbp observed in this study. It was also observed that some of the isolates harboured double plasmids. This also agrees with the study by Odeyemi *et al*., (2013).

The plasmid curing rate of ESBL producing *E.coli* and *K. pneumoniae* was investigated and it was observed that out of 93 ESBL producing isolates obtained from the poultry farms, only 18 could be cured by subjecting them to acridine orange and this gave a curing rate of 19.4%. This is higher than the 13.5% curing rate reported by Adeyankinnu

*et al.,* (2014) and 0% curing rate reported by Iroha *et al.,* (2009). Since some of the ESBL producing strains were cured, it implied that they expressed beta lactamase genes from plasmids while those that were not cured most probably had the beta lactamase gene borne within the chromosomes. This observation showed that resistant property is borne not only within the plasmid but also within the chromosomes which is in accordance with the study by Iroha *et al.,* (2009). It also coincided with the report by Bradford, (2001) that many species of gram negative bacteria possess naturally occurring chromosomally mediated beta-lactamase enzyme. It was observed that out of 93 ESBL producing isolates obtained from the poultry farms, only 18 could be cured by subjection their to acridineorange and this gave a curing rate of 19.4%. This is higher than the 13.5% curing rate reported by Adeyankinmu *et al.,* (2014) and 0% curing rate reported by Iroha *et al*. (2009). Since some of the ESBL producing strains were cured, it implied that they expressed beta-lactamase genes from plasmids while those that were not cured most probably had the beta-lactamase gene borne within the chromosomes. This observation should that resistant property is borne not only within the plasmid but also within the chromosomes which is accordance with the study by Iroha *et al*., (2009).It also coincided with the report my Bradford, (2001) that many species of gram-negative bacteria possess natuarally occurring chromosomally mediated beta-lactamse enzymes.

All the isolates (both ESBL positive E. *coli* and *K. pneumonia*) from the chicken environment harboured simple plasmid size of 23,130bp. Two of the isolates from the chicken cloacae revealed double plasmid size of 23,130bp. Two of the isolates from the chicken cloacae revealed double plasmid sizes of 23,130bp and <564bp. The other 3 isolate harboured single plasmid size of 23,130bp. The observation made from the plasmid profiting of the ESBL positive *E. coli* isolates from chicken farmers revealed that 3 of 23,130bp and <564bp. The other two revealed only single plasmid size of 23,130bp. In a report by Adeyankinmu *et al*., (2014), the highest average weight of 24.3kbp and 22.1kbp were the plasmid sizes of ESBL producing *E. coli* and *K. pneumonia* respectively. This agrees with the large plasmid size of 23.1kbp observed in this study. It was also observed that some of the isolates harboured double plasmids. This also agrees

with the study by Odeyemi *et al*., (2013). It was observed that out of 93 ESBL producing isolates obtained from the poultry farms, only 18 could be cured by subjecting them to acidine orange and this gave a curing rate of 19.4%. This is higher than the 13.5% curing rate 19.4%. This is higher than the 13.5% curing rate reported by Adeyankinmu *et al.*, (2014) and 0% curing rate reported by Iroha *et al*., (2009). Since some of the ESBL producing strains were cured, it implied that they expressed beta lactamase genes from plasmids while those that were not cured most probably had the beta lactamase gene borne within the chromosomes. This observation showed that resistant property is borne not only within the plasmid but also within the chromosomes which is in accordance with the study Iroha *et al*. (2009). It also coincided with the report by Bradford, (2001) that many species of gram negative bacteria possess naturally occurring chromosomally mediated beta-lactamase enzymed.

The plasmid curing rate of the ESBL positive isolates in relation tosite was studied and it was observed that the highest plasmid curing rate was separately obtained from Tutu's and Tartius' farms and Baltic farm Jaikada-fari.This indicates that these farms were reservoirs of ESBL positive *E.coli* and *K. pneumoniae* strains that harbour both plasmid and chromosome mediated resistant genes. None of the ESBL producing isolates obtained from Baltic farm, Sabon line was cured this could be because all the resistant genes harboured by the bacterial isolates in this farm were borne within the chromosome.

Also studied, was the plasmid curing rate of ESBL positive isolates in various samples.The highest plasmid curing rate was observed among the ESBL producing isolates obtained from chickens while the least was from the chicken rearers.The curing rate (8.6%) obtained from ESBL positive isolates from chickens is high than 0% curing rate reported by Duru *et al*., 2013 this is because all the isolates investigated by the later had the resistant genes borne within the chromosomes.

## 5.1 CONCLUSION

From the observations made in this study, it can be concluded that:

ESBL producing *E.coli and K.pneumoniae* can be isolated from chickens, chickens' environment and chicken rearers.

In broilers, age is a factor that affects the occurrence of *E.coli* as well as the occurrence of betalactamase producing and ESBL producing *E. coli* and *Klebsiella pneumoniae*.

The subspecies of the chicken affects the occurrence of *E coli, K. pneumoniae* and

*K. oxytoca*. It also affects the occurrence of beta-lactamase and ESBL producing *E.coli* and *K. pneumoniae*in chickens.

In layers, the subspecies of the chicken affects the occurrence of *E.coli* as well as the occurrence beta lactamase producing and ESBL producing *E.coli* and *Klebsiella pneunoniae*.

In cockerels, age is a factor that determines the occurrence of *E.coli* and *K.pneumoniae*. The subspecies of the chicken affects the occurrence of *E.coli, K.pneumoniae* and *K.oxytoca*. It also affects the occurrence of beta-lactamase producing and ESBL producing *E.coli* and *K. pneumoniae* in chickens.

ESBL producing *E.coli* and *K.pneumoniae* are resistant to antibiotics including third generation cephalosporins.

Some ESBL positive *E. coli strains* have the resistant gene borne on the plasmids while most including *K. pneumoniae* have theirs borne within the chromosomes.

Anti-bacterial activity of third generation cephalosporin against ESBL producing *E.coli* and *K.pneumoniae* can be increased by combining the drugs with ESBL inhibitors like clavulanic acid.

## 5.2 RECOMMENDATION

From the observations made from this study, it is recommended that:

1. Poultry farmers should observe a good personal hygiene.
2. Poultry farmers should avoid indiscriminate use of antibiotics (especially cephalosporins).
3. Third generation cephalosporins should be co-administered together with an ESBL inhibitor like clavulanic acid to prevent the emergence of ESBL resistant strain of bacterial species.

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**APPENDIX I**

**Equipment, Media and Reagents**

**Equipment**

Petri dishes

Measuring Cylinder

Conical flask

Cotton wool

Aluminium foil

Masking tape

Bijou bottles

Filter paper

Antibiotic discs

Wire loop

Incubator

Bunsen burner

Refrigerator

Generator

Ice pack

Hand gloves

Face mask

Weighing balance

pH paper and colour chart

**MEDIA**

Mac Conkey agar

Nutrient agar

Mueller Hinton agar

Mueller Hinton broth

Nutrient broth

Peptone water

EMB agar

Urease agar

Blood agar

TSI agar

Potassium hydroxide

Normal saline

Methyl red

Acridine orange

Distilled water

Crystal violet iodine

Safranin

Alcohol

Crystalline penicillin bromocresol purple

Benzylpenicillin

Bromocresol purple

Phosphate buffer

Bromocresol purple

Sodium dihydrogen phosphate

Disodium hydrogen phosphate

Concentrated tetraoxosulphate (VI) acid

Barium chloride

# APPENDIX II

## INTERPRETATIVE CHART OF ZONE SIZES

**Interpretative chart of zone sizes** – The values given in the table below refer to the Kirby-Bauer technique

| *Antimicrobial agent* | *Disc potency* | *Diameter of zone of inhibition (mm)* | | |
|---|---|---|---|---|
| | | *Susceptible* | *Intermediate* | *Resistant* |
| **β-Lactams** | | | | |
| • ampicillin when testing: | | | | |
| enterococci | 10 µg | ≥ 17 | – | ≤ 16 |
| Gram-negative organisms | 10 µg | ≥ 17 | 14–16 | ≤ 13 |
| *Haemophilus* spp. | 10 µg | ≥ 22 | 19–21 | ≤ 18 |
| • benzylpenicillin when testing: | | | | |
| *N. gonorrhoeae*[1,2] | 10 IU | ≥ 47 | 27–46 | ≤ 26 |
| staphylococci | 10 IU | ≥ 29 | – | ≤ 28 |
| • cefazolin[3] | 30 µg | ≥ 18 | 15–17 | ≤ 14 |
| • cefotaxime[3] | 30 µg | ≥ 23 | 15–22 | ≤ 14 |
| • ceftazidime[3] | 30 µg | ≥ 18 | 15–17 | ≤ 14 |
| • ceftriaxone[3] when testing *N. gonorrhoeae*[4,5] | 30 µg | ≥ 35 | – | – |
| • oxacillin[6] when testing: | | | | |
| *Streptococcus pneumoniae* for penicillin susceptibility[4] | 1 µg | ≥ 20 | – | – |
| staphylococci | 1 µg | ≥ 13 | 11–12 | ≤ 10 |
| • piperacillin when testing *P. aeruginosa* | 100 µg | ≥ 18 | – | ≤ 17 |
| **Quinolones** | | | | |
| • ciprofloxacin when testing: | | | | |
| Gram-negative enteric bacilli | 5 µg | ≥ 21 | 16–20 | ≤ 15 |
| *N. gonorrhoeae*[4] | 5 µg | ≥ 41 | 28–40 | ≤ 27 |
| • nalidixic acid | 30 µg | ≥ 19 | 14–18 | ≤ 13 |
| **Other drugs** | | | | |
| • chloramphenicol when testing: | | | | |
| Gram-negative enteric bacilli | 30 µg | ≥ 18 | 13–17 | ≤ 12 |
| *Haemophilus* spp.[4] | 30 µg | ≥ 29 | 26–28 | ≤ 25 |
| *S. pneumoniae*[4,7] | 30 µg | ≥ 21 | – | ≤ 20 |
| • erythromycin | 15 µg | ≥ 23 | 14–22 | ≤ 13 |
| • gentamicin[8] | 10 µg | ≥ 15 | 13–14 | ≤ 12 |
| • nitrofurantoin[9] | 300 µg | ≥ 17 | 15–16 | ≤ 14 |
| • sulfonamides | 300 µg | ≥ 17 | 13–16 | ≤ 12 |
| • tetracycline when testing: | | | | |
| Gram-negative enteric bacilli | 30 µg | ≥ 19 | 15–18 | ≤ 14 |
| *N. gonorrhoeae*[1,2] | 30 µg | ≥ 38 | 31–37 | ≤ 30 |
| • trimethoprim | 5 µg | ≥ 16 | 11–15 | ≤ 10 |
| • trimethoprim + sulfamethoxazole (co-trimoxazole) | 1.25 µg + 23.75 µg | ≥ 16 | 11–15 | ≤ 10 |
| • vancomycin when testing: | | | | |
| enterococci | 30 µg | ≥ 17 | 15–16 | ≤ 14 |
| other Gram-positive organisms[4] | 30 µg | ≥ 15 | – | – |

Notes

1. An intermediate category for *N. gonorrhoeae* indicates a lower cure rate (85–95%) among infected patients compared to more than 95% cure rates for susceptible strains.
2. Gonococci with 10 IU penicillin disc zones of ≤ 19 mm are likely to be β-lactamase producers. With tetracycline 30-µg discs, zone diameters of ≤ 19 mm usually indicate a plasmid-mediated tetracycline-resistant *N. gonorrhoeae* (TRNG) strain (MIC correlate ≥ 16 mg/l).
3. Choices for cephalosporin surveillance testing: cephalothin represents the group cephalothin, cephalexin and cefadroxil, while cefazolin represents cefazolin and cefaclor. Ceftazidime maximizes recognition of extended spectrum β-lactamase-mediated resistance; cefotaxime is used for testing against salmonellae; and ceftriaxone is a reserve antimicrobial used for gonococcal testing only.

1. Interpret your findings with the aid of the table below. (The instructor may substitute other antibiotics and indicate their zone diameter data.) Compare the patterns of susceptibility and resistance with respect to gram reaction, potential pathogenicity and natural habitat of each organism.

Figure 10-2 lists many common antibiotics that are used in the disk sensitivity test and their zones of clearing.

**Figure 10-2 Zones of clearing for various antibiotics**

| Antibiotic (and disc identifier) | Disk potency | Inhibition zone diameter to nearest mm | | |
|---|---|---|---|---|
| | | Resistant | Intermediate | Susceptible |
| Ampicillin (AM10) Gram-negative rods and enterococci | 10 µg | 11 | 12-13 | 14 |
| Ampicillin (AM10) Staphylococci and highly penicillin-sensitive organisms | 10 µg | 20 | 21-28 | 29 |
| Bacitracin (B) | 10 units | 8 or less | 9 to 12 | 13 or more |
| Chloramphenicol (C) | 30 µg | 12 or less | 13 to 17 | 18 or more |
| Ciprofloxacin (CIP) | 5 µg | 15 or less | 16 to 20 | 21 or more |
| Colistin (CL) | 10 µg | 8 or less | 09 to 10 | 11 or more |
| Doxycycline (D) | 30 µg | 12 | 13-15 | 16 |
| Erythromycin (E) | 15 µg | 13 or less | 14 to 17 | 18 or more |
| Gentamycin (G) | 10 µg | 12 | 13-14 | 15 |
| Kanamycin (K30) | 30 µg | 13 | 14-17 | 18 |
| Methicillin (ME5) | 5 µg | 9 | 10-13 | 14 |
| Nalidixic Acid (NA) | 30 µg | 13 or less | 14 to 18 | 19 or more |
| Oxacillin | 1 µg | 17 | 18-24 | 25 |
| Penicillin G (P) | 10 units | | | |
| staphylococci | | 28 or less | - | 29 or more |
| most others | | 11 or less | 12 to 21 | 22 or more |
| Polymyxin B (PB) | 300 units | 8 or less | 9 to 11 | 12 or more |
| Streptomycin (S) | 10 µg | 11 or less | 12 to 14 | 15 or more |
| Sulfadiazine (SD) * | 300 µg | 12 or less | 13 to 16 | 17 or more |
| Sulfpoxizole (G300) | 300 µg | 12 or less | 13-16 | 17 or more |
| Sulfisoxazole (G25) * | 25 µg | 12 or less | 13 to 16 | 17 or more |
| Tetracycline (TE) | 30 µg | 14 or less | 15 to 18 | 19 or more |

**Appendix III**

**Preparation of reagents**

**Preparation of Turbidity Standard Suspension**

1% V/V solution of tetraoxosulphate (VI) acid ($H_2SO_4$) was prepared by adding 1ml of concentrated $H_2SO_4$ to 99ml of distilled water. The solution was well mixed

1% W/v solution of barium chloride was prepared by dissolving 0.5g of dihydrate barium chloride ($Bacl_{2.}\ 2H_2O$) in 50ml of distilled water.

Then 0.6ml of the barium chloride solution was added to 99.4ml of the $H_2SO_4$ solution and mixed.

A small volume of the turbid solution was transferred to a test tube of the same type as used for preparing the test inoculum.

**Preparation 0.5 Mc Farland Turbidity Standard Test Suspension**

Using a sterile wire loop, 11 well isolated colonies of similar appearance were touched and emulsified in 0.4ml of sterile physiological saline.

In a good light, the turbidity of the test suspension to the turbidity standard were matched. A printed card was used to compare the turbidities of the two suspensions. When the turbidities of the two suspensions were same, that gave 0.5 McFarland turbidity standard for the test suspension.

**Preparation of Crystalline Penicillin Bromoscresol Purple Solution**

Benzylpencillin(20g) was dissolved in the phosphate butter (pH8). Then 0.8ml of bromocresol purple indicator solution was added and the solution mixed well.

**Preparation of Bromocresol Indicator Solution**

Bromocresol purple(1g) was dissolved in 500ml of distilled water.

**Preparation of Phosphate Buffer(pH8)**

**Preparation of Stock Phosphate Solution A**

Sodium dihydrogen phosphate ($NaH_2P0_4$)(13.8g) was weighed and dissolved in 500ml of distilled water and mixed well. The bottle was labeled 'stock phosphate solution A' and stored at $4^0$C.

**Preparation of Stock Phosphate Solution B**

Disodium hydrogen phosphate (28.40g) was weighed and dissolved in litre of distilled water and properly mixed. The bottle containing the solution was labeled 'stock solution B'. The solution was stored at $4^o$C.

**Preparation of Phosphate Buffer (pH 8)**

'Stock solution A'(53ml )was measured and mixed with 947ml of 'stock solution B' .The pH was checked using pHpaper.

**Preparation of 0.1ng/ml of Acridine Orange**

Acridine orange(1g) was accurately weighed and dissolved in 100ml of sterile distilled water.

1g/100ml = 1000mg/100ml = 10mg/ml…….. solution K 1

1;100 dilution was done with the stock solution K until $1:10^{10}$ was obtained giving 0.1ng.

## Appendix IV

### Preparation of The Antimicrobial Discs

Discs 6mm in diameter were punched out from a sheet of good quality filter paper (eg. whatman No. 1). The discs were marked with numbers for identification. The discs were placed in a petridish leaving 3mm in between and then sterilized in hot air oven at $160^{o}$C for one hour. A solution of the antimicrobial in the desired strength was prepared. On each disc, the desired amount of the solution of the antimicrobial was pippetted on each of the cooled discs. The discs were dried in an incubator at $37^{0}$C for one hour and then kept in a labeled airtight container and stored in the refrigerator.

### Preparation of Antibiotics Disc of The Desired Strength

The dilution procedur

**For Antibiotics With 250mg**

(Chloramphericol and tetracycline) per tablet.

To get 30*u*g/ disc,

250mg of the antibiotics was dissolved in 25ml of sterile distilled water

= 250mg/25ml = 10mg/ml…….solution A

A. 3:10 dilution was done by mixing 3ml of solution A and 7ml of sterile distilled water

30mg/10ml = 3mg/1ml

= 3000*u*g/ml

1ml was pipetted and dropped on 100 discs = 3000*u*g/100discs = 30*u*g/disc

**For Antibiotics With 250mg (Amplicillin) Strenth Per Tablet.**

To get 10*u*g/disc

Dissolve 250mg of the antibiotic in 25ml of sterile distilled water

= 250mg /25ml = 10mg/ml ……solution B

Do 1:10 dilution, by mixing 1ml of solution B and 9ml of sterile distilled water

=10mg /10ml

=1mg/ 1ml

= 1000*u*g/lml

Pipette 1ml and drop on 100 discs

= 1000*u*g/ 100disc =10*u*g/disc

**For The Antibiotics with 500mg (Ciprofloxacin) Strength per Tablet.**

**To get 5*u*g/disc**

Dissolve 500mg antibiotic in 100ml of sterile distilled water.

= 500mg/100ml = 5mg/ml……..solution C

Do 1:10 dilution by mixing 1ml of solution C and 9ml of sterile distilled water, to get

= 5mg/10ml = 0.5mg/ml = 500*u*g/ml

Pipette 1ml and drop on 100 discs

= 500*u*g/100discs

= 5*u*g/disc

**For The Antibiotics(Nitrofurantoin) With 100mg Strength**

**To get 300*u*g**

300mg of the antibiotics was dissolved in 10ml sterile distilled water

= 300mg/10ml = 30mg/ml

= 30,000*u*g/1ml

=30,000*ug*/ml

1ml was pippetted and dropped on 100 discs,giving

30,000*u*g /100discs = 300*u*g/disc

**For The Antibiotics (Gentamicin With 80 Mg/2ml Ampoule)**

**To obtain 10*u*g/disc**

1 containing 40mg/ml was pippetted- ……..solution D

1:10 dilution was done by mixing 1ml of solution D and 9ml of sterile distilled water, to get

40mg/10ml = 4mg/ml……..E

1:4 dilution was done by pippetting 1ml of solution E and mixing it with 3ml of sterile distilled water to get

4mg/4ml = 1mg/ml = 1000*u*g/ml

1ml was pippeted and dropped on 100 discs,giving

1000*u*g/100discs =10*u*g/disc.

**For the antibiotics (Ceftriaxone and Ceftazidime) with 1g strength per vial**

**<u>To obtain 30*u*g/disc</u>**

1g = 1000mg

1000mg of the antibiotics was dissolved in 100ml of sterile distilled water

= 1000mg/100ml = 10mg/ml ……solution F

3:10 dilution was done by pipetting 3ml of solution F and mixing it with 7ml of sterile distilled water to get 30mg/10ml

=3mg/1ml

=3000*u*g/1ml

1ml was pippeted and dropped on 100 discs, giving

3000*u*g/100discs = 30*u*g/disc

**For the antibiotics (augmentin) with 375g strength per tablet.co-amoxiclav 250/125 amoxicillin 250mg, clavulanic acid 125mg.**

**<u>To get 30*u*g (amoxicillin 20*u*g and clavulanic acid 10*u*g)</u>.**

375mg of augmentin was dissolved in 37.5ml of sterile distilled water

to get 375mg /37.5m1…… solution G

3:10 dilution was done by mixing 3ml of solution G and 7m1 of sterile distilled water to get 30mg/10ml = 3mg/ml = 3000*u*g/1ml

Then 1m1 was pipetted and dropped on 100 discs, giving

3000*u*g/100discs

=30*u*g/1disc

**Appendix V**

Table 4.2

```
CROSST
ABS
  /TABLES=Location BY Organisms
  /FORMAT=AVALUE TABLES
  /STATISTICS=CHISQ
  /CELLS=COUNT
  /COUNT ROUND CELL.
```

**Sources from where the samples were collected * Organisms isolated in farm I Crosstabulation**

Count

| | | Organisms isolated in farm I | | | Total |
|---|---|---|---|---|---|
| | | E. coli | K. pneumonia | K. oxytoca | |
| Sources from where the samples were collected | Chicken | 79 | 6 | 4 | 89 |
| | Chicken environment | 9 | 0 | 0 | 9 |
| | Chicken rearers | 4 | 0 | 0 | 4 |
| Total | | 92 | 6 | 4 | 102 |

**Chi-Square Tests**

| | Value | df | Asymp. Sig. (2-sided) |
|---|---|---|---|
| Pearson Chi-Square | 1.619[a] | 4 | .805 |
| Likelihood Ratio | 2.881 | 4 | .578 |
| Linear-by-Linear Association | 1.236 | 1 | .266 |
| N of Valid Cases | 102 | | |

a. 6 cells (66.7%) have expected count less than 5. The minimum expected count is .16.

Table 4.3

```
CROSSTABS
  /TABLES=Location BY Organisms
  /FORMAT=AVALUE TABLES
  /STATISTICS=CHISQ
  /CELLS=COUNT
  /COUNT ROUND CELL.
```

**Sources from where the samples were collected * Organisms isolated in farm II Crosstabulation**

Count

| | | Organisms isolated in farm II | | | Total |
|---|---|---|---|---|---|
| | | E. coli | K. pneumonia | K. oxytoca | |
| Sources from where the samples were collected | Chicken | 29 | 4 | 3 | 36 |
| | Chicken environment | 10 | 3 | 0 | 13 |
| | Chicken rearers | 4 | 1 | 0 | 5 |
| Total | | 43 | 8 | 3 | 54 |

**Chi-Square Tests**

| | Value | df | Asymp. Sig. (2-sided) |
|---|---|---|---|
| Pearson Chi-Square | 2.539[a] | 4 | .638 |
| Likelihood Ratio | 3.407 | 4 | .492 |
| Linear-by-Linear Association | .129 | 1 | .719 |
| N of Valid Cases | 54 | | |

a. 6 cells (66.7%) have expected count less than 5. The minimum expected count is .28.

Table 4.4

```
CROSSTABS
  /TABLES=Location BY Organisms
  /FORMAT=AVALUE TABLES
  /STATISTICS=CHISQ
```

```
  /CELLS=COUNT
  /COUNT ROUND CELL.
```

**Sources from where the samples were collected * Organisms isolated in farm III Crosstabulation**

Count

| | | Organisms isolated in farm III | | Total |
|---|---|---|---|---|
| | | E. coli | K. pneumonia | |
| Sources from where the samples were collected | Chicken | 32 | 2 | 34 |
| | Chicken environment | 13 | 1 | 14 |
| Total | | 45 | 3 | 48 |

**Chi-Square Tests**

| | Value | df | Asymp. Sig. (2-sided) | Exact Sig. (2-sided) | Exact Sig. (1-sided) |
|---|---|---|---|---|---|
| Pearson Chi-Square | .027[a] | 1 | .870 | | |
| Continuity Correction[b] | .000 | 1 | 1.000 | | |
| Likelihood Ratio | .026 | 1 | .871 | | |
| Fisher's Exact Test | | | | 1.000 | .654 |
| Linear-by-Linear Association | .026 | 1 | .871 | | |
| N of Valid Cases | 48 | | | | |

a. 2 cells (50.0%) have expected count less than 5. The minimum expected count is .88.

b. Computed only for a 2x2 table

Table 4.5

```
CROSSTABS

  /TABLES=Location BY Organisms
  /FORMAT=AVALUE TABLES
  /STATISTICS=CHISQ
  /CELLS=COUNT
  /COUNT ROUND CELL.
```

```
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```

**Sources from where the samples were collected * Organisms isolated in farm IV Crosstabulation**

Count

| | | Organisms isolated in farm IV | | Total |
|---|---|---|---|---|
| | | E. coli | K. pneumonia | |
| Sources from where the samples were collected | Chicken | 37 | 1 | 38 |
| | Chicken environment | 15 | 0 | 15 |
| | Chicken rearers | 2 | 0 | 2 |
| Total | | 54 | 1 | 55 |

**Chi-Square Tests**

| | Value | df | Asymp. Sig. (2-sided) |
|---|---|---|---|
| Pearson Chi-Square | .456[a] | 2 | .796 |
| Likelihood Ratio | .748 | 2 | .688 |
| Linear-by-Linear Association | .399 | 1 | .527 |
| N of Valid Cases | 55 | | |

a. 4 cells (66.7%) have expected count less than 5. The minimum expected count is .04.

Table 4.6

```
CROSSTABS
  /TABLES=Location BY Organisms
  /FORMAT=AVALUE TABLES
  /STATISTICS=CHISQ
  /CELLS=COUNT
  /COUNT ROUND CELL.
```

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**Sources from where the samples were collected * Organisms isolated in farm V Crosstabulation**

Count

| | | Organisms isolated in farm IV | | Total |
|---|---|---|---|---|
| | | E. coli | K. oxytoca | |
| Sources from where the samples were collected | Chicken | 19 | 1 | 20 |
| | Chicken environment | 10 | 2 | 12 |
| | Chicken rearers | 3 | 0 | 3 |
| Total | | 32 | 3 | 35 |

**Chi-Square Tests**

| | Value | df | Asymp. Sig. (2-sided) |
|---|---|---|---|
| Pearson Chi-Square | 1.610[a] | 2 | .447 |
| Likelihood Ratio | 1.722 | 2 | .423 |
| Linear-by-Linear Association | .176 | 1 | .675 |
| N of Valid Cases | 35 | | |

a. 4 cells (66.7%) have expected count less than 5. The minimum expected count is .26.

Table 4.7

```
CROSSTABS
  /TABLES=Location BY Organisms
  /FORMAT=AVALUE TABLES
  /STATISTICS=CHISQ
  /CELLS=COUNT
  /COUNT ROUND CELL.
```

```
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```

**Sources from where the samples were collected * Organisms isolated in farm VI Crosstabulation**

Count

| | | Organisms isolated in farm VI | | | Total |
|---|---|---|---|---|---|
| | | E. coli | K. pneumonia | K. oxytoca | |
| Sources from where the samples were collected | Chicken | 19 | 0 | 0 | 19 |
| | Chicken environment | 6 | 0 | 1 | 7 |
| | Chicken rearers | 4 | 1 | 1 | 6 |
| Total | | 29 | 1 | 2 | 32 |

**Chi-Square Tests**

| | Value | df | Asymp. Sig. (2-sided) |
|---|---|---|---|
| Pearson Chi-Square | 7.869[a] | 4 | .097 |
| Likelihood Ratio | 7.579 | 4 | .108 |
| Linear-by-Linear Association | 4.842 | 1 | .028 |
| N of Valid Cases | 32 | | |

a. 6 cells (66.7%) have expected count less than 5. The minimum expected count is .19.

Table 4.8

```
CROSSTABS
  /TABLES=Location BY Organisms
  /FORMAT=AVALUE TABLES
  /STATISTICS=CHISQ
  /CELLS=COUNT
  /COUNT ROUND CELL.

[DataSet1] C:\Users\Chukwudi\Documents\Mba modesta suppl.sav
```

**Sources from where the samples were collected * Organisms isolated in farm VII Crosstabulation**

Count

| | | Organisms isolated in farm VII | | Total |
|---|---|---|---|---|
| | | E. coli | K. pneumonia | |
| Sources from where the samples were collected | Chicken | 50 | 1 | 51 |
| | Chicken environment | 16 | 0 | 16 |
| | Chicken rearers | 4 | 0 | 4 |
| Total | | 70 | 1 | 71 |

**Chi-Square Tests**

| | Value | df | Asymp. Sig. (2-sided) |
|---|---|---|---|
| Pearson Chi-Square | .398[a] | 2 | .820 |
| Likelihood Ratio | .667 | 2 | .716 |
| Linear-by-Linear Association | .340 | 1 | .560 |
| N of Valid Cases | 71 | | |

a. 4 cells (66.7%) have expected count less than 5. The minimum expected count is .06.

Table 4.9

```
CROSSTABS
  /TABLES=Farms BY Organisms
  /FORMAT=AVALUE TABLES
  /STATISTICS=CHISQ
  /CELLS=COUNT
  /COUNT ROUND CELL.

[DataSet1] C:\Users\Chukwudi\Documents\Mba modesta
suppl.sav
```

**Different farms where the samples were collected * Organisms isolated Crosstabulation**

Count

| | | Organisms isolated | | | Total |
|---|---|---|---|---|---|
| | | E. coli | K. pneumonia | K. oxytoca | |
| Different farms where the samples were collected | Farm I | 92 | 6 | 4 | 102 |
| | Farm II | 43 | 8 | 3 | 54 |
| | Farm III | 45 | 3 | 0 | 48 |
| | Farm IV | 54 | 1 | 0 | 55 |
| | Farm V | 32 | 0 | 3 | 35 |
| | Farm VI | 29 | 1 | 2 | 32 |
| | Farm VII | 70 | 1 | 0 | 71 |
| Total | | 365 | 20 | 12 | 397 |

**Chi-Square Tests**

| | Value | df | Asymp. Sig. (2-sided) |
|---|---|---|---|
| Pearson Chi-Square | 28.383[a] | 12 | .005 |
| Likelihood Ratio | 31.317 | 12 | .002 |
| Linear-by-Linear Association | 4.547 | 1 | .033 |
| N of Valid Cases | 397 | | |

a. 13 cells (61.9%) have expected count less than 5. The minimum expected count is .97.

Table 4.10

```
ONEWAY Frequency BY location
  /MISSING ANALYSIS
  /POSTHOC=DUNCAN ALPHA(0.05).
```

**ANOVA**

Frequency rate of E. coli in broilers

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 1224.222 | 2 | 612.111 | 16.445 | .004 |
| Within Groups | 223.333 | 6 | 37.222 | | |
| Total | 1447.556 | 8 | | | |

**Post Hoc Tests**

**Homogeneous Subsets**

**Frequency rate of E. coli in broilers**

Duncan

| sources of collection of E. coli in broiler farms | N | Subset for alpha = 0.05 | |
|---|---|---|---|
| | | 1 | 2 |
| Chicken rearers | 3 | 1.6667 | |
| Chicken environment | 3 | 12.6667 | |
| Chicken | 3 | | 30.0000 |
| Sig. | | .069 | 1.000 |

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Table 4.11

```
ONEWAY Frequency BY location
  /MISSING ANALYSIS
  /POSTHOC=DUNCAN ALPHA(0.05).
```

**ANOVA**

Frequency rate of E. coli in layers farm

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between | 3007.000 | 2 | 1503.500 | 3.607 | .159 |

| | | | | | |
|---|---|---|---|---|---|
| Groups | | | | | |
| Within Groups | 1250.500 | 3 | 416.833 | | |
| Total | 4257.500 | 5 | | | |

**Post Hoc Tests**
**Homogeneous Subsets**

**Frequency rate of E. coli in layers farm**

Duncan

| sources of collection of E. coli in layers farms | N | Subset for alpha = 0.05 |
|---|---|---|
| | | 1 |
| Chicken rearers | 2 | 4.0000 |
| Chicken environment | 2 | 9.5000 |
| Chicken | 2 | 54.0000 |
| Sig. | | .092 |

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Table 4.12

```
ONEWAY Frequency BY location
  /MISSING ANALYSIS
  /POSTHOC=DUNCAN ALPHA(0.05).
```

**ANOVA**

Frequency rate of E. coli in cockerel

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 1021.000 | 2 | 510.500 | 2.887 | .200 |
| Within Groups | 530.500 | 3 | 176.833 | | |

| | | | | | |
|---|---|---|---|---|---|
| Total | 1551.500 | 5 | | | |

**Post Hoc Tests**

**Homogeneous Subsets**

**Frequency rate of E. coli in cockerel**

Duncan

| sources of collection of E. coli in cockerel farms | N | Subset for alpha = 0.05 |
|---|---|---|
| | | 1 |
| Chicken rearers | 2 | 4.0000 |
| Chicken environment | 2 | 11.0000 |
| Chicken | 2 | 34.5000 |
| Sig. | | .106 |

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Table 4.14

```
CROSSTABS
  /TABLES=Sample BY BLactamateproduction
  /FORMAT=AVALUE TABLES
  /STATISTICS=CHISQ
  /CELLS=COUNT
  /COUNT ROUND CELL.
```

**Sources from where the samples were collected * Frequency rate of B-lactamase producing *E. coli*, *K. pneumoniae* and *K. oxytoca* in various farm samples Crosstabulation**

Count

| | | Frequency rate of B-lactamase producing E. coli, K. pnemonia and K. oxytoca in various farm samples | | Total |
|---|---|---|---|---|
| | | Number positive | Number negative | |
| Sources from where | Chicken | 147 | 141 | 288 |

| | | | | |
|---|---|---|---|---|
| the samples were collected | Chicken environment | 40 | 46 | 86 |
| | Poultry farmer | 10 | 13 | 23 |
| Total | | 197 | 200 | 397 |

**Chi-Square Tests**

| | Value | df | Asymp. Sig. (2-sided) |
|---|---|---|---|
| Pearson Chi-Square | .912[a] | 2 | .634 |
| Likelihood Ratio | .914 | 2 | .633 |
| Linear-by-Linear Association | .900 | 1 | .343 |
| N of Valid Cases | 397 | | |

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 11.41.

Table 4.15

```
CROSSTABS
  /TABLES=Farms BY BLactamateproduction
  /FORMAT=AVALUE TABLES
  /STATISTICS=CHISQ
  /CELLS=COUNT
  /COUNT ROUND CELL.
```

**Different farms from where the samples were collected * Frequency rate of B- lactamase producing *E. coli, K. pneumoniae* and *K. oxytoca* in various farm samples Crosstabulation**

Count

| | | Frequency rate of B-lactamase producing *E. coli, K. pneumoniae* and *K. oxytoca* in various farm samples | | Total |
|---|---|---|---|---|
| | | Number positive | Number negative | |
| Different farms from | Farm I | 29 | 73 | 102 |

| | | | | |
|---|---|---|---|---|
| where the samples were collected | Farm II | 24 | 30 | 54 |
| | Farm III | 30 | 18 | 48 |
| | Farm IV | 35 | 20 | 55 |
| | Farm V | 23 | 12 | 35 |
| | Farm VI | 18 | 14 | 32 |
| | Farm VII | 38 | 33 | 71 |
| Total | | 197 | 200 | 397 |

**Chi-Square Tests**

| | Value | df | Asymp. Sig. (2-sided) |
|---|---|---|---|
| Pearson Chi-Square | 31.026[a] | 6 | .000 |
| Likelihood Ratio | 31.809 | 6 | .000 |
| Linear-by-Linear Association | 14.068 | 1 | .000 |
| N of Valid Cases | 397 | | |

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 15.88.

Table 4.16

```
CROSSTABS
  /TABLES=Broiler BY BLactamateproduction
  /FORMAT=AVALUE TABLES
  /STATISTICS=CHISQ
  /CELLS=COUNT
  /COUNT ROUND CELL.
```

**Different broiler farms sampled * Frequency rate of B-lactamase producing E. coli, *K. pneumoniae* and *K. oxytoca* in various broiler farms Crosstabulation**

Count

| | | Frequency rate of B-lactamase producing *E. coli, K. pneumoniae* and *K. oxytoca* in various broiler farms | | Total |
|---|---|---|---|---|
| | | Number positive | Number negative | |
| Different broiler farms sampled | Farm III | 30 | 18 | 48 |
| | Farm IV | 35 | 20 | 55 |
| | Farm V | 24 | 11 | 35 |
| Total | | 89 | 49 | 138 |

**Chi-Square Tests**

| | Value | df | Asymp. Sig. (2-sided) |
|---|---|---|---|
| Pearson Chi-Square | .355[a] | 2 | .837 |
| Likelihood Ratio | .359 | 2 | .836 |
| Linear-by-Linear Association | .301 | 1 | .583 |
| N of Valid Cases | 138 | | |

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 12.43.

Table 4.17

```
CROSSTABS
  /TABLES=Cockerel BY BLactamateproduction
  /FORMAT=AVALUE TABLES
  /STATISTICS=CHISQ
  /CELLS=COUNT
  /COUNT ROUND CELL.
```

**Different cockerel farms sampled * Frequency rate of B-lactamase producing *E. coli*, *K. pneumoniae* and *K. oxytoca* in various cockerel farms Crosstabulation**

Count

| | | Frequency rate of B-lactamase producing *E. coli, K. pneumoniae* and *K. oxytoca* in various cockerel farms | | Total |
|---|---|---|---|---|
| | | Number positive | Number negative | |
| Different cockerel farms sampled | Farm VI | 17 | 15 | 32 |
| | Farm VII | 57 | 14 | 71 |
| Total | | 74 | 29 | 103 |

**Chi-Square Tests**

| | Value | df | Asymp. Sig. (2-sided) | Exact Sig. (2-sided) | Exact Sig. (1-sided) |
|---|---|---|---|---|---|
| Pearson Chi-Square | 8.042[a] | 1 | .005 | | |
| Continuity Correction[b] | 6.756 | 1 | .009 | | |
| Likelihood Ratio | 7.714 | 1 | .005 | | |
| Fisher's Exact Test | | | | .008 | .005 |
| Linear-by-Linear Association | 7.964 | 1 | .005 | | |
| N of Valid Cases | 103 | | | | |

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 9.01.

b. Computed only for a 2x2 table

Table 4.18

```
CROSSTABS
  /TABLES=Layers BY BLactamateproduction
  /FORMAT=AVALUE TABLES
```

```
/STATISTICS=CHISQ
/CELLS=COUNT
/COUNT ROUND CELL.
```

**Different layers farms sampled * Frequency rate of B-lactamase producing *E. coli,K. pneumoniae* and *K. oxytoca* in various layers farms Crosstabulation**

Count

| | | Frequency rate of B-lactamase producing E. coli, K. pnemonia and K. oxytoca in various layers farms | | Total |
|---|---|---|---|---|
| | | Number positive | Number negative | |
| Different layers farms sampled | Farm VI | 29 | 73 | 102 |
| | Farm VII | 24 | 30 | 54 |
| Total | | 53 | 103 | 156 |

**Chi-Square Tests**

| | Value | df | Asymp. Sig. (2-sided) | Exact Sig. (2-sided) | Exact Sig. (1-sided) |
|---|---|---|---|---|---|
| Pearson Chi-Square | 4.036[a] | 1 | .045 | | |
| Continuity Correction[b] | 3.354 | 1 | .067 | | |
| Likelihood Ratio | 3.974 | 1 | .046 | | |
| Fisher's Exact Test | | | | .052 | .034 |
| Linear-by-Linear Association | 4.010 | 1 | .045 | | |
| N of Valid Cases | 156 | | | | |

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 18.35.

b. Computed only for a 2x2 table

Table 4.19

```
CROSSTABS
  /TABLES=Farms BY BLactamateproduction
  /FORMAT=AVALUE TABLES
  /STATISTICS=CHISQ
  /CELLS=COUNT
  /COUNT ROUND CELL.
```

**Different categories of farms * Frequency rate of B- lactamase producing *E. coli, K. pnemoniae* and *K. oxytoca i*n various categories of farms Crosstabulation**

Count

| | | Frequency rate of B-lactamase producing *E. coli, K. pneumoniae and K. oxytoca* in various categories of farms | | Total |
|---|---|---|---|---|
| | | Number positive | Number negative | |
| Different categories of farms | Broiler farm | 89 | 49 | 138 |
| | Layer farm | 53 | 103 | 156 |
| | Cockerel farm | 56 | 47 | 103 |
| Total | | 198 | 199 | 397 |

**Chi-Square Tests**

| | Value | df | Asymp. Sig. (2-sided) |
|---|---|---|---|
| Pearson Chi-Square | 28.404[a] | 2 | .000 |
| Likelihood Ratio | 28.859 | 2 | .000 |
| Linear-by-Linear Association | 4.052 | 1 | .044 |
| N of Valid Cases | 397 | | |

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 51.37.

Table 4.20

```
CROSSTABS
  /TABLES=Farms BY Location
  /FORMAT=AVALUE TABLES
  /STATISTICS=CHISQ
  /CELLS=COUNT
  /COUNT ROUND CELL.

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```

**Different categories of farms * site of collection of B- lactamase producing *E. coli, K. pneumoniae and K. oxytoca* in various categories of farms Crosstabulation**

Count

| | | site of collection of B- lactamase producing *E. coli, K. pneumoniae and K. oxytoca* in various categories of farms | | | Total |
|---|---|---|---|---|---|
| | | Chicken | Chicken environment | Chicken rearers | |
| Different categories of farms | Broiler farm | 64 | 20 | 3 | 87 |
| | Layer farm | 39 | 12 | 3 | 54 |
| | Cockerel farm | 44 | 8 | 4 | 56 |
| Total | | 147 | 40 | 10 | 197 |

**Chi-Square Tests**

| | Value | df | Asymp. Sig. (2-sided) |
|---|---|---|---|
| Pearson Chi-Square | 2.527[a] | 4 | .640 |
| Likelihood Ratio | 2.631 | 4 | .621 |
| Linear-by-Linear Association | .007 | 1 | .933 |

| | | | |
|---|---|---|---|
| N of Valid Cases | 197 | | |

a. 3 cells (33.3%) have expected count less than 5. The minimum expected count is 2.74.

Table

```
CROSSTABS
  /TABLES=Farms BY Location
  /FORMAT=AVALUE TABLES
  /STATISTICS=CHISQ
  /CELLS=COUNT
  /COUNT ROUND CELL.

[DataSet1] C:\Users\Chukwudi\Documents\Mba modesta
suppl.sav
```

**Different categories of farms * site of collection ESBL producing isolates in various categories of farms Crosstabulation**

Count

| | | site of collection of ESBL producing isolates in various categories of farms | | | Total |
|---|---|---|---|---|---|
| | | Chicken | Chicken environment | Chicken rearers | |
| Different categories of farms | Broiler farm | 30 | 13 | 4 | 47 |
| | Layer farm | 19 | 1 | 1 | 21 |
| | Cockerel farm | 13 | 4 | 2 | 19 |
| Total | | 62 | 18 | 7 | 87 |

**Chi-Square Tests**

| | Value | df | Asymp. Sig. (2-sided) |
|---|---|---|---|
| Pearson Chi-Square | 5.593[a] | 4 | .232 |
| Likelihood Ratio | 6.626 | 4 | .157 |
| Linear-by-Linear | .322 | 1 | .571 |

| | | | |
|---|---|---|---|
| Association | | | |
| N of Valid Cases | 87 | | |

a. 5 cells (55.6%) have expected count less than 5. The minimum expected count is 1.53.

Table 4.21

```
CROSSTABS
  /TABLES=Sourceofsample BY Isolates
  /FORMAT=AVALUE TABLES
  /STATISTICS=CHISQ
  /CELLS=COUNT
  /COUNT ROUND CELL.
```

**Different sources from where the samples were obtained * Status of the isolates Crosstabulation**

Count

| | | Status of the isolates | | Total |
|---|---|---|---|---|
| | | ESBL positive | ESBL negative | |
| Different sources from where the samples were obtained | Chicken | 62 | 226 | 288 |
| | Chicken environment | 23 | 63 | 86 |
| | Chicken rearers | 6 | 17 | 23 |
| Total | | 91 | 306 | 397 |

**Chi-Square Tests**

| | Value | df | Asymp. Sig. (2-sided) |
|---|---|---|---|
| Pearson Chi-Square | 1.158[a] | 2 | .560 |
| Likelihood Ratio | 1.134 | 2 | .567 |
| Linear-by-Linear Association | .947 | 1 | .330 |
| N of Valid Cases | 397 | | |

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 5.27.

Table 4.22a

```
CROSSTABS
```

```
  /TABLES=Farm BY ESBL
  /FORMAT=AVALUE TABLES
  /STATISTICS=CHISQ
  /CELLS=COUNT
  /COUNT ROUND CELL.

[DataSet0] C:\Users\Chukwudi\Documents\Mba Modesta.sav
```

**Different farms visited * Frequency of ESBL positive isolates Crosstabulation**

Count

| | | Frequency of ESBL positive isolates | | Total |
|---|---|---|---|---|
| | | ESBL positive isolate absent | ESBL positive isolate present | |
| Different farms visited | Farm I | 89 | 13 | 102 |
| | Farm II | 41 | 13 | 54 |
| | Farm III | 36 | 12 | 48 |
| | Farm IV | 38 | 17 | 55 |
| | Farm V | 16 | 19 | 35 |
| | Farm VI | 21 | 11 | 32 |
| | Farm VII | 63 | 8 | 71 |
| Total | | 304 | 93 | 397 |

**Chi-Square Tests**

| | Value | df | Asymp. Sig. (2-sided) |
|---|---|---|---|
| Pearson Chi-Square | 34.854[a] | 6 | .000 |
| Likelihood Ratio | 33.346 | 6 | .000 |
| Linear-by-Linear Association | 1.545 | 1 | .214 |
| N of Valid Cases | 397 | | |

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 7.50.

Table 4.22b

```
ONEWAY ESBL BY Farm
  /MISSING ANALYSIS
  /POSTHOC=DUNCAN ALPHA (0.05).


[DataSet0] C:\Users\Chukwudi\Documents\Mba Modesta.sav
```

**ANOVA**

Frequency of ESBL positive isolates

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 50.929 | 2 | 25.464 | 3.340 | .140 |
| Within Groups | 30.500 | 4 | 7.625 | | |
| Total | 81.429 | 6 | | | |

Table 4.23

```
ONEWAY Broilers BY Organisms
  /MISSING ANALYSIS
  /POSTHOC=DUNCAN ALPHA (0.05).


[DataSet0] C:\Users\Chukwudi\Documents\Mba Modesta.sav
```

**ANOVA**

Frequency rate of ESBL positive isolates in broiler farms

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 352.667 | 1 | 352.667 | 39.925 | .003 |
| Within Groups | 35.333 | 4 | 8.833 | | |
| Total | 388.000 | 5 | | | |

Table 4.24

```
ONEWAY Cockerel BY Organisms
  /MISSING ANALYSIS
  /POSTHOC=DUNCAN ALPHA (0.05).


[DataSet0] C:\Users\Chukwudi\Documents\Mba Modesta.sav
```

**ANOVA**

Frequency rate of ESBL positive isolates in cockerel farms

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 90.250 | 1 | 90.250 | 40.111 | .024 |
| Within Groups | 4.500 | 2 | 2.250 | | |
| Total | 94.750 | 3 | | | |

Table 4.25

```
ONEWAY Layers BY Organisms
  /MISSING ANALYSIS
  /POSTHOC=DUNCAN ALPHA (0.05).

[DataSet0] C:\Users\Chukwudi\Documents\Mba Modesta.sav
```

**ANOVA**

Frequency rate of ESBL positive isolates in layers farms

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 144.000 | 1 | 144.000 | 288.000 | .003 |
| Within Groups | 1.000 | 2 | .500 | | |
| Total | 145.000 | 3 | | | |

Table 4.26a

```
ONEWAY E. coli BY Farms
  /MISSING ANALYSIS
  /POSTHOC=DUNCAN ALPHA(0.05).
```

```
[DataSet0] C:\Users\Chukwudi\Documents\Mba Modesta.sav
```

**ANOVA**

Frequency rate of ESBL positive *E. coli*in different farms

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 46.333 | 2 | 23.167 | 2.336 | .213 |
| Within Groups | 39.667 | 4 | 9.917 | | |
| Total | 86.000 | 6 | | | |

Table 4.26b

```
ONEWAY K. pneumoniae BY Farms
  /MISSING ANALYSIS
  /POSTHOC=DUNCAN ALPHA (0.05).

[DataSet0] C:\Users\Chukwudi\Documents\Mba Modesta.sav
```

**ANOVA**

Frequency rate of ESBL positive *K. pneumoniae* different farms

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | .262 | 2 | .131 | .449 | .667 |
| Within Groups | 1.167 | 4 | .292 | | |
| Total | 1.429 | 6 | | | |

Table 4.29

```
CROSSTABS
  /TABLES=Organisms BY Plasmid
  /FORMAT=AVALUE TABLES
  /STATISTICS=CHISQ
  /CELLS=COUNT
  /COUNT ROUND CELL.

[DataSet0] C:\Users\Chukwudi\Documents\Mba Modesta.sav
```

**ESBL producing organisms * Plasmid curing rate for the ESBL producing organisms Crosstabulation**

Count

| | | Plasmid curing rate for the ESBL producing organisms | | Total |
|---|---|---|---|---|
| | | Plasmid present after curing | Plasmid absent after curing | |
| ESBL producing organisms | *E. coli* | 73 | 18 | 91 |
| | *K.pneumoniae* | 2 | 0 | 2 |
| Total | | 75 | 18 | 93 |

**Chi-Square Tests**

| | Value | df | Asymp. Sig. (2-sided) | Exact Sig. (2-sided) | Exact Sig. (1-sided) |
|---|---|---|---|---|---|
| Pearson Chi-Square | .491[a] | 1 | .484 | | |
| Continuity Correction[b] | .000 | 1 | 1.000 | | |
| Likelihood Ratio | .871 | 1 | .351 | | |
| Fisher's Exact Test | | | | 1.000 | .649 |
| Linear-by-Linear Association | .485 | 1 | .486 | | |
| N of Valid Cases | 93 | | | | |

a. 2 cells (50.0%) have expected count less than 5. The minimum expected count is .39.

b. Computed only for a 2x2 table

Table 4.30

```
CROSSTABS
  /TABLES=Farms BY Plasmid
  /FORMAT=AVALUE TABLES
  /STATISTICS=CHISQ
  /CELLS=COUNT
  /COUNT ROUND CELL.


[DataSet0] C:\Users\Chukwudi\Documents\Mba Modesta.sav
```

**Different farms visited * Plasmid curing rate for the ESBL producing organisms Crosstabulation**

Count

| | | Plasmid curing rate for the ESBL producing organisms | | Total |
|---|---|---|---|---|
| | | Plasmid present after curing | Plasmid absent after curing | |
| Different farms visited | Farm I | 9 | 4 | 13 |
| | Farm II | 9 | 4 | 13 |
| | Farm III | 11 | 1 | 12 |
| | Farm IV | 14 | 3 | 17 |
| | Farm V | 15 | 4 | 19 |
| | Farm VI | 11 | 0 | 11 |
| | Farm VII | 6 | 2 | 8 |
| Total | | 75 | 18 | 93 |

**Chi-Square Tests**

| | Value | df | Asymp. Sig. (2-sided) |
|---|---|---|---|
| Pearson Chi-Square | 5.974[a] | 6 | .426 |
| Likelihood Ratio | 8.008 | 6 | .238 |
| Linear-by-Linear Association | 1.518 | 1 | .218 |
| N of Valid Cases | 93 | | |

a. 7 cells (50.0%) have expected count less than 5. The minimum expected count is 1.55.

Table 4.31

```
CROSSTABS
  /TABLES=Sample sources BY Plasmid
  /FORMAT=AVALUE TABLES
  /STATISTICS=CHISQ
  /CELLS=COUNT
  /COUNT ROUND CELL.

[DataSet0] C:\Users\Chukwudi\Documents\Mba Modesta.sav
```

**Different sources from where samples were collected * Plasmid curing rate for the ESBL producing organisms Crosstabulation**

Count

| | | Plasmid curing rate for the ESBL producing organisms | | Total |
|---|---|---|---|---|
| | | Plasmid present after curing | Plasmid absent after curing | |
| Different sources from where samples were collected | Chicken | 54 | 8 | 62 |
| | Chicken environment | 18 | 7 | 25 |
| | Chicken rearers | 3 | 3 | 6 |
| Total | | 75 | 18 | 93 |

**Chi-Square Tests**

| | Value | df | Asymp. Sig. (2-sided) |
|---|---|---|---|
| Pearson Chi-Square | 6.460[a] | 2 | .040 |
| Likelihood Ratio | 5.738 | 2 | .057 |
| Linear-by-Linear Association | 6.303 | 1 | .012 |
| N of Valid Cases | 93 | | |

a. 3 cells (50.0%) have expected count less than 5. The minimum expected count is 1.16.

**Comparing *E. coli and K. pneumoniae* in chicken**

```
ONEWAY Frequency BY Organisms
  /MISSING ANALYSIS
  /POSTHOC=DUNCAN ALPHA(0.05).


[DataSet0] C:\Users\Chukwudi\Documents\Mba Modesta.sav
```

**ANOVA**

Number of isolates

| | Sum of Squares | Df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 6123.714 | 2 | 3061.857 | 20.318 | .000 |
| Within Groups | 2712.571 | 18 | 150.698 | | |
| Total | 8836.286 | 20 | | | |

**Post Hoc Tests**

**Number of isolates**

Duncan

| Different organisms isolated in chickens | N | Subset for alpha = 0.05 | |
|---|---|---|---|
| | | 1 | 2 |
| *K. oxytoca* | 7 | 1.1429 | |
| *K. pneumonia* | 7 | 2.1429 | |
| *E. coli* | 7 | | 37.8571 |
| Sig. | | .881 | 1.000 |

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 7.000.

**Comparing *E. coli* and *K. pneumoniae* in chicken environment**

```
ONEWAY Frequency BY Organisms
  /MISSING ANALYSIS
  /POSTHOC=DUNCAN ALPHA(0.05).
[DataSet0] C:\Users\Chukwudi\Documents\Mba Modesta.sav
```

**ANOVA**

Number of isolates

| | Sum of Squares | Df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 550.095 | 2 | 275.048 | 57.000 | .000 |
| Within Groups | 86.857 | 18 | 4.825 | | |
| Total | 636.952 | 20 | | | |

**Post Hoc Tests**

**Number of isolates**

Duncan

| Different organisms isolated in chicken environment | N | Subset for alpha = 0.05 | |
|---|---|---|---|
| | | 1 | 2 |
| *K. pneumonia* | 7 | .4286 | |
| *K. oxytoca* | 7 | .4286 | |
| *E. coli* | 7 | | 11.2857 |
| Sig. | | 1.000 | 1.000 |

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 7.000.

**Comparing *E. coli and K. pnseumoniae*in chickenrearers**

```
ONEWAY Frequency BY Organisms
  /MISSING ANALYSIS
  /POSTHOC=DUNCAN ALPHA(0.05).

[DataSet0] C:\Users\Chukwudi\Documents\Mba Modesta.sav
```

**ANOVA**

Number of isolates

| | Sum of Squares | Df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 36.286 | 2 | 18.143 | 20.053 | .000 |
| Within Groups | 16.286 | 18 | .905 | | |

| | | | | | |
|---|---|---|---|---|---|
| Total | 52.571 | 20 | | | |

**Post Hoc Tests**

**Number of isolates**

Duncan

| Different organisms isolated in chicken rearers | N | Subset for alpha = 0.05 | |
|---|---|---|---|
| | | 1 | 2 |
| *K. oxytoca* | 7 | .1429 | |
| *K. pneumonia* | 7 | .2857 | |
| *E. coli* | 7 | | 3.0000 |
| Sig. | | .782 | 1.000 |

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 7.000.

**Comparing different sources for *E. coli***

```
ONEWAY E. coli BY Samplesources
  /MISSING ANALYSIS
  /POSTHOC=DUNCAN ALPHA (0.05).

[DataSet0] C:\Users\Chukwudi\Documents\Mba Modesta.sav
```

**ANOVA**

Number of E. coli isolated

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 4642.667 | 2 | 2321.333 | 15.171 | .000 |
| Within Groups | 2754.286 | 18 | 153.016 | | |
| Total | 7396.952 | 20 | | | |

**Post Hoc Tests**

**Number of *E. coli* isolated**

Duncan

| Different sources where samples were collected from | N | Subset for alpha = 0.05 | |
|---|---|---|---|
| | | 1 | 2 |
| Chicken rearers | 7 | 3.0000 | |
| Chicken environment | 7 | 11.2857 | |
| Chicken | 7 | | 37.8571 |
| Sig. | | .226 | 1.000 |

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 7.000.

**Comparing different sources for *K. pneumoniae***

```
ONEWAY K. pneumoniae BY Samplesources
  /MISSING ANALYSIS
  /POSTHOC=DUNCAN ALPHA (0.05).

[DataSet0] C:\Users\Chukwudi\Documents\Mba Modesta.sav
```

**ANOVA**

Number of *K. pneumoniae* isolated

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 14.952 | 2 | 7.476 | 3.364 | .057 |
| Within Groups | 40.000 | 18 | 2.222 | | |
| Total | 54.952 | 20 | | | |

**Post Hoc Tests**

**Number of *K. pneumoniae* isolated**

Duncan

| Different sources where samples were collected from | N | Subset for alpha = 0.05 | |
|---|---|---|---|
| | | 1 | 2 |
| Chicken rearers | 7 | .2857 | |
| Chicken environment | 7 | .4286 | |
| Chicken | 7 | | 2.1429 |
| Sig. | | .860 | 1.000 |

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 7.000.