ANTIBIOTICS RESISTANCE PATTERN OF *Escherichia coli* AND *Salmonella* species ISOLATED FROM FRESH MEAT SOLD IN SOME MARKETS IN BENIN METROPOLIS.

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(MICROBIOLOGY OPTION)
FACULTY OF SCIENCE
BENSON IDAHOSA UNIVERSITY
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A PROJECT WORK SUBMITTED TO THE DEPARTMENT OF BIOLOGICAL SCIENCES (MICROBIOLOGY OPTION),

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF A BACHELOR OF SCIENCE (B.Sc) DEGREE IN MICROBIOLOGY FACULTY OF SCIENCE,

BENSON IDAHOSA UNIVERSITY, BENIN CITY, EDO STATE

AUGUST, 2021.

CERTIFICATION

This is to certify that this project work was carried out by OMAMULI YALAJU with matriculation number BAS/MCB/170184, in the Department of Biological Science (Microbiology option), Faculty of Science, Benson Idahosa University, Benin City, Edo State.

Dr S. A. ENABULELE
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DEDICATION

I dedicate this project work to God Almighty and my parents Mr and Mrs Patrick Yalaju

ACKNOWLEDGMENT

I want to thank God Almighty for the strength to complete this project research. All thanks goes to my project supervisor; Dr S.A. Enabulele for his support throughout my research work. I want to thank the Dean of Faculty of Science; Prof. F.O.J. Oboh, the Head of Department, Dr (Mrs.) O. T. Okugbo and other lecturers in the Department for their support during my years of study at Benson Idahosa University. I also want to thank my course adviser; Dr O. Odigie and Mrs Yvonne Ibitoye for their advice and support.

I want to thank my parents; Mr and Mrs Patrick Yalaju for their unending love and support, and my friends Abagi Abigail, Alabi Glory, Osabohien Golden and Ewomazino Herbert thanking them for their support.

PLAGIARISM ATTESTATION

I affirm that this report is my own work, based on my personal research and that I have acknowledged all materials and sources used in its preparations, whether they be books, articles, journal reports, lecture notes, and all other documents, electronic or personal communication. I also attest that the report has not previously been submitted for assessment elsewhere and that I have not copied in part or whole or otherwise plagiarized the work of other students and/or persons.

NAME: **Yalaju Omamuli** **DATE:**

SIGN: _____

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ABSTRACT

Antibiotics resistance is an emerging problem worldwide which can develop as a result of antibiotics misuse by humans or overuse in animal feeding and treatment. This study was aimed at investigating the prevalence of *Escherichia coli* and *Salmonella* spp. and study the antibiotic resistance pattern of isolates. a total of 40 samples were collected from four different markets namely; Okha, Santana, Oba and Ekiosa markets in Benin City and were analysed using standard microbiological methods for the investigation. Results from the investigation showed that *E. coli* and *Salmonella* spp were present in almost all of the samples. The Total Aerobic Count showed that Santana market (5.97±0.53 cfu/g) had the highest average count whereas the lowest average count was observed in Ekiosa market (4.29±0.49cfu/g). The total *E. coli* count on Eosin-Methylene Blue Agar showed that the highest *E. coli* count was observed in samples taken from Ekiosa market (1.93±0.38 cfu/g), whereas, the lowest was observed in samples collected from Oba market (1.69±0.40 cfu/g). The Total *Salmonella* count on Salmonella-Shigella Agar showed that Oba market (2.502±0.32 cfu/g) had the highest count while Ekiosa market (1.073±0.22 cfu/g) had the lowest count. Based on the number of samples collected from each market the prevalence rate shows that *E. coli* was isolated from 25% of the samples collected while *Salmonella* was isolated from 45% of the samples collected. The antibiotics resistance pattern showed that all *E. coli* isolates were resistant to cefixime, augumentin, nitrofurantion and cefuroxime, while all *Salmonella* isolates were resistant to cefixime, augumentin, ceftazidime and cefuroxime. Monitoring of antimicrobial resistance in *E. coli* and *Salmonella* spp. isolates is valuable for epidemiological uses and for monitoring the increase of antimicrobial resistance among different microbial species.

CHAPTER ONE
INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Enterobacteriaceae, a large family of gram-negative, nonspore-forming, rod-shaped, facultative anaerobes capable of fermenting sugars to various end products, are important hygiene indicators for process verification in food production, recently replacing the poorly defined group of coliforms. Several members of this group, including some species of *Escherichia, Enterobacter, Klebsiella, Proteus, Citrobacter, Serratia, Salmonella, Shigella, and Yersinia,* are among the most important causes of serious hospital-acquired and community onset bacterial infections in humans (Jarzab *et al.,* 2011). Resistance to antimicrobial agents in Enterobacteriaceae has become an increasingly relevant problem. Because antibiotics are widely used in medical clinics and animal husbandry, high concentrations of antibiotics have appeared in aquacultures and other agricultural products, soil, water, and even food; consistent with this, antimicrobial-resistant Enterobacteriaceae have been appeared more and more frequently, and multiple drug-resistant strains have emerged (Capita and Alonso-Calleja, 2013; Laxminarayan *et al.,* 2013).

Bacterial resistance to antimicrobial agents is an increasing and globally occurring problem; therefore, monitoring this phenomenon and understanding its molecular basis is extremely important. Obtaining information about pathways of spreading of antimicrobial resistance-determining genes and their transmission between various components of the ecosystem will contribute to the development of new concepts to counteract this process (Angulo *et al.* 2004; Nunnery *et al.* 2006; Kadlec and Schwarz 2008). Moreover, transmission of zoonotic, bacterial risk factors poses a serious threat to public health (Ahmed *et al.* 2010).

The frequent use of similar antibiotics in the treatment of humans and animals is also a major problem, which makes it difficult to find an effective pharmacological agent in case of

infection. In addition, *Escherichia coli* is a commensal, opportunistic pathogen, constantly present both in the mammalian digestive tract and in the environment, e.g., in water or soil. This contributes to the transfer of genes between strains and to the increase in the drug resistance in environmental bacteria (Alekshun and Levy 2007; Scott 2008).

There are reports in the world literature on the spread of drug resistance genes in *Salmonella* spp. and *E. coli* isolated from animals (Sáenz *et al.* 2004; Dunowska *et al.* 2006; Vo *et al.* 2007; Ahmed *et al.* 2010).

Veterinarians report shows the medicinal methods used and drugs administered to slaughter animals; they are also required to keep records of animals that lost the status of slaughter animals. Such procedures are intended to minimize the risk of improper quality meat on the food market, which would be dangerous to consumers' health. But most veterinarians and their medicinal methods of drug administration to livestock animas and animal of meat origin are not always according to stipulated standards. This has been seen to greatly impact the spread of multi drug resistance in animals that are meat producers meant for human consumption. A serious problem arises while treating a animals not for slaughter, when the veterinarian can arbitrarily choose a medicine, based on the list of pharmaceuticals registered for treating horses, or in justified cases—outside of the list. Also, information about the method of treatment does not have to be recorded in the medical documentation of such animal. This is the case of most animals breed for meat production purposes and animals used in recreation and agriculture. It turns out that in the post slaughter collected meat samples, there may be residues of drugs. In addition, a significant problem is the falsification of meat products from animals belonging to other species by horse meat of unknown origin. Such activity is obviously illegal, and offered meat can endanger the health and life of consumers (Bryan *et al.,* 2010; Wróblewski and Wojtaszek, 2015). Apart from the risk associated with the consumption of meat that could have been treated with antibiotics, the use of

inappropriately processed manure derived from these multi drug resistance gene infected and contaminated animals as fertilizers is also problematic. In that case, we are dealing with environmental contamination by pathogenic microorganisms, often highly resistant to antibiotics, and with introduction of antibiotics and their metabolites into water and soil (Venglovsky *et al.,* 2009).

One of the most common mechanisms of resistance in *E. coli* and *Salmonella* spp. is their ability to produce multi drug resistance. The presence of *Salmonella enterica*, and *E. coli* multi drug resistance-producing strains has already been detected in feces of pigs, cattle, and horses (Wellington *et al.* 2013). Strains, in which the resistance mechanism has been found, are very dangerous from an epidemiological point of view, because they hydrolyze all penicillins, cephalosporins, and monobactams. In addition, they may exhibit cross resistance to trimethoprim/sulfamethoxazole and quinolones (Picozzi *et al.,* 2014). The multi-drug resistance-encoding genes are rapidly spreading, also among strains of different species, which is due to their location on conjugation plasmids (Marcade *et al.,* 2009; Rawat and Nair 2010). Hence, the high pathogenicity and antimicrobial resistance of multi drug-producing *E. coli* strains and *Salmonella* spp. Moreover, these isolates can be donors of resistance genes to many commonly used antibiotics, which hinder rational antibiotic therapy. This is why it is so important to understand, identify and monitor the phenomenon of antimicrobial resistance in the environment and animals especially those ones that serve as meat for human consumption

Previous studies have focused on investigating MDR in medical and veterinary clinics; however, relatively few reports have investigated ESBLs in foods (Reuland *et al.,* 2014; Ben Said *et al.,* 2015; Tekiner and Özpinar, 2016). Because of the wide use of broad-spectrum antibiotics in the livestock and animal husbandry industries, MDR-producing bacteria have evolved and shown increased incidence owing to mutations, selection, and the spread of drug-resistant genes in animal and food products of animal origin (Ojer-Usoz *et al.,* 2013;

Tekiner and Özpinar, 2016), as well as other types of foods, such as seafood (Nguyen *et al.,* 2016), raw vegetables (Reuland *et al.,* 2014; Ben Said *et al.,* 2015), ready-to-eat (RTE) foods (Campos *et al.,* 2015; Kim *et al.,* 2015), and milk (Odenthal *et al.,* 2016). Furthermore, these *E. coli* and *Salmonella* spp. bacteria can readily be transferred to humans through consumption of contaminated food, contributing to the spread and persistence of antibiotic-resistant bacteria in the general population and environment (Huijbers *et al.,* 2016).

Thus, in order to identify changes in antimicrobial resistance as early as possible and control the spread of MDR-producing strains in foods, implementing a system for monitoring the prevalence patterns of MDR-producing Enterobacteriaceae in foods from different areas in the state (Benin City, Edo State) and performing regular surveillance of the susceptibility of isolates to antimicrobial agents are necessary.

Hence this study is undertaken with the goal to provide new knowledge on the diversity of multi-drug producing enterbacteriaceae in foods most especially in fresh and raw meat samples collected from various markets and slaughter houses in the state so as to characterize their antibiotic resistance and determine the presence of multi drug resistance gene producers in the isolates recovered.

1.2 STATEMENT OF PROBLEM

Gram-negative bacteria are a leading cause of life-threatening infections and include nosocomial infections (NI), nosocomial pneumonia (NP), urinary tract infections (UTIs), intra-abdominal infections (IAIs), pediatric bacterial meningitis, septicaemia, neutropenia, community acquired infections (CAIs), and pelvic inflammatory diseases (Lamb *et al.,* 2002; Plosker *et al.,* 1998; Baughman, 2009). Since the discovery of antibiotics, many classes of antibiotics have been employed and derivatives of established antibiotics trialed to overcome increasing resistance (Coates *et al.,*2002).

Members of the family Enterobacteriaceae, which include important food-borne pathogens such as *Salmonella* enterica and Escherichia coli, are known to cause diverse types of infections ranging from wound infection to meningitis, and are also known agents of nosocomial infections. In recent years, the chemotherapeutic options for enterobacteria are becoming severely constricted owing to the development of resistance to multiple antibiotics, the most notable among these being resistance to _-lactam group of antibiotics such as cephalosporins and carbapenems (Arias, *et. al.,* 2009). Cephalosporin resistance is accomplished by the production of one or more types of _-lactamases called extended spectrum-_-lactamases (ESBLs) (Pitout & Laupland, 2008). ESBL-producing Gram-negative bacteria have become a severe challenge to chemotherapy (Jacoby & Bush, 2005, Paterson & Bonomo, 2005). ESBLs are classified into several groups, the prominent among them being TEM, SHV, and CTX-M types (Chong, Ito, Kamimura, 2005). ESBLs confer resistance to third generation cephalosporins (e.g., cefotaxime, ceftazidime) and monobactams (e.g., aztreonam), but cannot hydrolyze cephamycins (cefoxitin) or carbapenems (imipenem), and are inhibited by _-lactamase inhibitors such as clavulanic acid (Philippon *et al.,* 1989). Carbapenems are the antibiotics of choice against ESBL-producing bacteria, but the occurrence of carbapenem resistant enterobacteria (CRE) producing plasmid-encoded metallo-_-lactamases with carbapenemase activity have emerged worldwide (Nordmann *et al.,* 2011, Queenan & Bush, 2007). In 2008, a new _-lactamase, the New Delhi metallo-_-lactamase (blaNDM-1) capable of hydrolyzing all _-lactams with the exception of aztreonam, was identified in a clinical isolate of Klebsiella pneumoniae (Yong *et. al.,* 2009). Subsequently, the NDM-1 producing enterobacteria were isolated from different parts of the world and their rapid dissemination has become a global concern (Bonomo, 2011, Moellering, 2010). Studies from India and other countries have reported the occurrence of blaNDM-harboring bacteria in the environment (Isozumi, 2012, Zhang, 2014).

The presence of antibiotic-resistant bacteria in food and raw meat is not only a threat to human health, but also can result in the transfer of resistant determinants to other clinically important bacteria. The occurrence of human enteric pathogens such as *Escherichia coli* and *Salmonella* enterica in raw meat and food is due to the contamination of water bodies and environment from where the animals are reared or contaminations occurring at various stages of handling after rearing (Ponce, Khan, Cheng, 2008). The threat due to the presence of enteric pathogens in raw/fresh meat is more confounding when such bacteria are multi-drug resistant (MDR). The prevalence of MDR bacteria in raw meat has been reported in many studies in the recent past (Adesiji *et al.,* 2014, Boss *et al.,* 2016, Guo et. al., 2016, Le, *et. al.,* 2015, and Kumar, *et. al.,* 2005) but very little studies exist for it in Nigeria and no study or existing knowledge of its prevalence in Benin City, Edo State.

Multidrug resistance (MDR) is the ability of bacteria to resist different classes of antibiotics (three or more than three classes of antibiotics) which are structurally different and have different molecular targets (Nikaido, 2009). Antibiotic resistance is a result of antibiotic use. The greater the volume of antibiotics used, the greater will be the chances of arising antibiotic resistance population of bacteria (Gelband, 2015). There is growing evidence which revealed antibiotic resistance has been promoted by widespread use of non-therapeutic antibiotics in animals (Nhung, 2017). The misuse of antibiotic can lead to the development of bacterial resistance towards antibiotic, increases the burden of chronic disease, and increases costs of health services. Resistant bacteria are transmitted to human through direct contact with animal, by exposure to animal manure, through consumption of uncooked meat, and through contact with meat surfaces (Marshall and Levy, 2011)

The prevalence of MDR isolates and ESBL producing isolates is increasing in humans as well as animal. Fecal carriage of ESBL gene has been identified as the major reservoir in the environment. Bacterial species that carry ESBL genes are normal inhabitants of

gastrointestinal tract, and food is a potential source of them (Overdevest *et. al.,* 2011). Meat harbor different bacteria as an inherent contamination and are further contaminated during handling, improper dressing, cleaning, insanitary condition, and unhygienic practices of selling meat. Consumption of these unsafe meat arise public health hazards (Bhandari *et al.,* 2013; Rouger *et al.,* 2017). This study aims to find the prevalence of MDR and ESBL producing isolates from raw and fresh meat samples in Benin City, Edo State metropolis.

1.3 AIMS AND OBJECTIVE

The primary purpose of this study was to report on the prevalence of MDR producing isolates from raw and fresh meat samples in Benin City, Edo State metropolis in Nigeria. The study is also aimed to supplement the information on raw and fresh meat and the ESBL producing strains and prevalence in raw and fresh meat samples in the area and also to determine possible potential health risk on consumption of these ESBL contaminated fresh and raw meat.

The objectives of this research are:-

i. To isolate and identify *E. coli* and *Salmonella* spp. from fresh and raw meat samples collected from markets in Benin Metropolis.
ii. To determine the prevalence of *E. coli* and *Salmonella* spp. in fresh meats sold in some markets in Benin City.
iii. Determine the antimicrobial susceptibility pattern of the isolates using disc diffusion method.

CHAPTER TWO
LITERATURE REVIEW

2.1 ANTIBIOTIC RESISTANCE

In 1928, Sir Alexander Fleming serendipitously discovered a new drug with remarkable properties; penicillin. Subsequently, related compounds were developed with even broader antimicrobial spectrum – the cephalosporins, the carbapenems, and the monobactams. The Greek words *anti-* (means against) and *bios* (means life) are the source of the word antibiotic. Antibiotics are the substances that can kill or inhibit the growth of bacteria. Antibiotics can be chemical agents such as sulfa compounds or can be derived from natural origin with a chemical modification such as *Penicillium notatum*.

Micro-organisms capable of evading antibiotic action, commonly known as antibiotic resistance, is according to the World Health Organization (WHO) one of the greatest threats to human health. Estimates suggest that due to multidrug-resistant bacterial infections around 25,000 people are dying each year and the cost of treatment is about €1.5 billion annually in EU countries (Davies *et al.,* 2017). Over 2 million people are infected annually with antibiotic-resistant bacteria in the United States and 23,000 people are dying due to resistant bacterial infections (World Health Organization, 2013; Hampton, 2016). It has been estimated that ten million people would die annually in 2050 if the development of resistance continues as now (O'Neill, 2016).

2.2 MECHANISMS OF ANTIBIOTIC RESISTANCE

Bacteria exhibit antibiotic resistance intrinsically or they can acquire it. Resistance mechanisms to some common antibiotics are listed in (Kumar, 2013). Intrinsic resistance is the ability of a bacterium to resist antibiotic action by its inherent structural or functional

characteristics. In comparison to Gram-positive bacteria, intrinsic resistance to a variety of compunds is rather common in Gram-negative due to:

i) differences in the composition of the cytoplasmic membrane; presence of anionic phospholipids is low in the cytoplasm of Gram-negative bacteria

ii) ii) absence of susceptible targets and iii) inability of the antibiotics to cross outer membrane of Gram-negative bacteria. For example, vancomycin is active against Gram-positive bacteria by inhibiting peptidoglycan crosslinking by binding to D-Ala-D-Ala peptides, but it is not active against Gram-negative bacteria as it cannot reach to peptides in periplasm (Blair *et al.,* 2015).

Gram-negative pathogens can also acquire resistance through gene mutations or horizontal gene transfer. The mechanisms of acquired resistance can be classified in four main classes;

(i) antibiotic modification/inactivation

(ii) antibiotic target alteration

(iii) increased antibiotic efflux

(iv) reduced antibiotic uptake (Arzanlou *et al.,* 2017).

Antibiotic inactivation can occur by hydrolysis of β-lactam antibiotics. The four membered β-lactam ring is hydrolyzed by β-lactamase enzymes (Figure 2A). Different groups of β-lactamases have been reported, which can inactivate different groups of antibiotics including, penicillin, cephalosporins and carbapenems (Yong *et al.,* 2009). Some enzymes such as aminoglycoside *N*-acetyltransferases (AACs), aminoglycoside *O*-nucleotidetransferases (ANTs) and aminoglycoside *O*-phosphotransferases (APHs) can modify aminoglycoside antibiotics to exhibit resistance against aminoglycoside antibiotics (Garneau-Tsodikova and Labby, 2016). Target alteration is another common mechanism of resistance against several classes of antibiotics where the target of antibiotic is changed. One example of such target alteration is DNA-topoisomerase complex. Mutation of the DNA gyrase encoding gene (*gyrA*)

can alter the protein structure which reduces fluoroquinolone-binding affinity leading to resistance (Aldred *et al.,* 2014). Modification of specific nucleotides in the aminoglycoside binding site of 16S rRNA by 16S rRNA methylases can confer high-level aminoglycoside resistance (Usui *et al.,* 2019). Bacteria use efflux pumps to force out antibiotics from the cell so that they can lower the antibiotic concentration to sub-lethal levels. This is a first line defence and it can force out different compounds, depending on the specificity of the efflux pump. Using efflux pumps, bacteria can develop multidrug resistance, as they can simultaneously export drugs of different drug classes (Poole, 2004).

Gram-negative bacteria can reduce uptake of antibiotics by altering outer membrane permeability. The outer membrane of Gram-negative bacteria is a barrier, but porins in the outer membrane facilitate the diffusion of small hydrophilic antibiotics such as β-lactam antibiotics. Gram-negative bacteria can acquire resistance through:

(i) down-regulation the expression of porins by mutations (e.g. loss of OmpF in *Escherichia coli* exhibits resistance to β-lactam antibiotics)

(ii) (ii) substitution of smaller channel size porin with a large channel size porin (e.g. *Klebsiella. pneumoniae* isolates exhibit resistance against β-lactam antibiotics by substitution of OmpK35 with a smaller channel size OmpK36)

(iii)(iii) impaired porin function by mutation (e.g. permeation of benzylpenicillin is reduced in PenB porin of *Neisseria gonorrhoeae* by addition of two negatively charged amino acids in the channel-constricting loop 3) (Nikaido, 2003; Delcour, 2009; Pagès *et al.,* 2008).

2.3 RESISTANCE TO BETA-LACTAM ANTIBIOTICS

The beta-lactam group of antibiotics is the largest and most commonly used group globally. It consists mainly of penicillins, cephalosporins, monobactams, carbapenems, and cephamycins,

which are semi-synthetic compounds originating from fungi and bacteria (Walsh, 2003). All drugs in the group possess a beta-lactam ring, the functional part of the antibiotic.

There are four major ways for a bacterium to develop resistance to betalactams:

(1) production of beta-lactamases (enzymes that hydrolyse the beta17 lactam ring), rendering the antibiotic inactive

(2) altered penicillin-binding proteins (PBPs) with low affinity for beta-lactams

(3) lowered or lacking expression of porins in the outer membrane of Gram-negative bacteria,

(4) efflux (a pumping mechanism)

(5) leads to difficulty for the beta-lactams to access the PBPs (Babic, 2006).

Beta-lactamases are enzymes located in the periplasm on the outer surface of the inner membrane of the cell envelope in Gram-negative bacteria (Ghuysen, 1991). The *Enterobacteriaceae* family mainly produces betalactamases to deactivate beta-lactam antibiotics, and the first described resistance to β-lactams was the production of penicillinase by *E. coli* (Medeiros, 1997; Bush, 2001; Bush, 2013). To date, over 1300 betalactamases have been reported (Bush, 2013). The genes encoding them (*bla* genes) are located on either the bacterial chromosome, plasmids, transposons or integrons (Wright, 2005; Babic, 2006; Drawz, 2010).

There are four classes of beta-lactamases (A–D) based upon DNA sequence similarity. Classes A, C, and D utilize serine on the active site, while the class-B enzymes (MBLs) usually utilize zinc. The updated classification system includes group 1 (class C) cephalosporinases; group 2 (classes A and D) broad-spectrum, inhibitor-resistant beta-lactamases, ESBLs and serine carbapenemases; and group 3 (class B) MBLs.

2.4 Escherichia coli

Escherichia coli are non-sporulating, motile, lactose-fermenting, rod-shaped bacteria. They are also important member of the family Enterobacteriaceae of Gram negative bacteria. They

are found in the normal microbiota of the digestive tracts of humans and animals. *E. coli* have 171 somatic (O), 55 flagellar (H) and 80 capsular (K) antigens. Until today, there are over 160 serological types of *E. coli*.

Escherichia coli is a Gram-negative bacteria of the Enterobacteriaceae family (Center for Disease Control and Prevention (CDC), 2020). *Escherichia coli* live in the gastrointestinal tracts (GIT) of humans and animals (Center for Disease Control and Prevention (CDC), 2020, Feng *et al.,* 2020). They commonly end up in the environment due to the close association of animals and humans to their environment (Center for Disease Control and Prevention (CDC), 2020; Founou *et al.,* 2018). The interaction between animals and humans also paves the way for transfer of these bacteria between them (Jang *et al.,* 2017). Although most *E. coli* strains are noted to be commensals, pathogenic *E. coli* exists (Feng, *et al.,* 2020). Pathogenic *E. coli* strains have been responsible for the cause of food poisoning, pneumonia and urinary tract infections (Center for Disease Control and Prevention (CDC), 2020).

All these types are involved in causing different infections in human including, urinary tract infections (UTI), hospital-acquired pneumonia (HAP), sepsis, surgical site infection (SSI), gastrointestinal tract infections, hemolytic-uremic syndrome (HUS), meningitis and inflammation of the meninges, bloodstream infections (BSI), abdominal infections and pneumonia (Alkeskas *et al.,* 2015). Most *E. coli* are members of the commensal microbiota with low pathogenicity. In the mucus layer of the colon, *E. coli* are the most frequent facultative anaerobes with a quantity of 107-109 cfu/gram of feces (Tenaillon *et al.,* 2010).

Phylogenetic analysis of *E. coli* discloses eight phylogroups; A, B1, B2, C, D, E, F and clade I. Strains of the different phylogroups behave differently. Strains of A and B are mainly commensal bacteria, whereas strains of B and D are more virulent and pathogenic bacteria mostly associated with causing extra-intestinal infections (ExPEC) (Clermont *et al.,* 2000); Clermont *et al.,* 2019). Virulence factors are important for a pathogen to attach with the

mucosal surface of the host and to overcome immunologic response. The main virulence factors of uropathogenic *E. coli* include adhesins (e.g. pili associated with pyelonephritis (*pap*) encoding P-fimbriae and type 1 fimbriae encoded by *fimH*), toxins (e.g. alpha-hemolysin, cytotoxic necrotizing factor 1, auto-transporter toxins), iron/heme acquisition systems and iron ion transporter, capsule production (K1/K2/K5) (Sarowska *et al.,* 2019).

E. coli infections can also lead to hospitalizations, loss of manpower and even death (Center for Disease Control and Prevention (CDC), 2010), Center for Disease Control and Prevention (CDC), 2020). For instance, in the USA, the Center for Control and Disease Prevention (CDC) (2020) reported an *E. coli* infection from an unknown food that caused 16 people to be ill, with eight hospitalizations resulting in one developing hemolytic uremic syndrome and one death.

Outbreaks of *E. coli* infections in Europe have also been noted (World Health Organization, 2020). The contribution of some strains of *E. coli* to travelers' diarrhea, especially among children in lower income countries, including Ghana, cannot be overemphasized, although official reports are limited (Center for Disease Control and Prevention (CDC), 2020; Malm *et al.,* 2015).

Escherichia coli infections in humans normally result from the consumption of contaminated foods that are present in the food chain. Foods of animal origin (meat and meat products), in particular, have played a major role in the spread of *E. coli* infections in humans. For example, beef, chevon, pork, poultry and/or their products have been implicated in the cause of foodborne infections in the USA and Europe (Center for Disease Control and Prevention (CDC), 2010), Center for Disease Control and Prevention (CDC), 2020, European Food Safety Authority, 2018). These meat and meat products, to a lesser extent, also serve as sources for which *E. coli* can cross contaminate other foods and the environment. The European Food Safety Authority (EFSA) (2018) reported that meat and meat products were

the food category that were tested the most for *E. coli* linked to human infections in a five-year period in Europe. The occurrence of *E. coli* in raw meats, ready-to-eat (RTE) meats, humans, working tools and/or the environment have been reported in America (Center for Disease Control and Prevention (CDC), 2010), Center for Disease Control and Prevention (CDC), 2020; Zhao *et al.,* 2012), Europe (European Food Safety Authority, 2018; Ciekure *et al.,* 2016), Asia (Altalhi *et al.,* 2010; Yulistiani *et al.,* 2017), Parvin, Talukder, Ali, Chowdhury, Rahman & Islam, 2020), and Africa Bekele *et al.,* 2014, Gwida *et al.,* 2014, Adzitey *et al.,* 2015). *Escherichia coli* infections are usually self-limiting; however, when treatments are required, antimicrobials are used (Gill and Hamer, 2001).

Antimicrobials are agents used to curtail the growth of microorganisms or are used to kill them (Fair and Tor, 2014; Purssell, 2019). Antimicrobial resistance occurs when bacteria resist antimicrobials that are meant to destroy them. The unabated use of antimicrobials in farm animals for growth promotion, treatment of sick animals or as prophylactics has been the major cause of the spread of antimicrobial resistances in animals. This can spread to humans via consumption of meat and meat products from farm animals containing resistance bacteria.

Humans and animals can cross contaminate other foods and the environment with resistant bacteria when they come into contact with them. There is also the possibility of resistant bacteria to spread across the food chain, humans and animals from the environment. The resistance of bacteria, including *E. coli*, to antimicrobials has received much attention in recent times due to the emergence of multidrug resistance and the difficulty in destroying such bacteria when they are involved in infections (Gill and Hamer, 2001, Fair and Tor, 2014). The use of antimicrobials in animal production in Ghana is not strictly regulated and monitored. As such farmers and farm attenders sometimes treat their animals with antimicrobials without prescription or adherence to instructed dosage and withdrawal periods

(Akansale *et al.,* 2019; Ekli,*et al.,* 2020). In addition, farmers did not demonstrate in-depth knowledge of the antimicrobials used (Akansale *et al.,* 2019; Ekli *et al.,* 2020). These practices pose the risk of bacteria developing resistance to antimicrobials. Furthermore, studies have shown the presence of antimicrobial resistance *E. coli* in meats and meats products in Ghana (Dsani *et al.,* 2020; Eibach, Dekker *et al.,* 2018). Dsani *et al.* (2020) reported that *E. coli* isolated from meat sources were resistant to cefuroxime (17%), sulfamethoxazole-trimethoprim (21%), tetracycline (45%) and ampicillin (57%). Eibach *et al.* (2018) found that *E. coli* isolates from poultry meat were resistant to ciprofloxacin (21%) and sulfamethoxazole-trimethoprim (32%), and harbored resistance genes such as BlaSHV, blaCTX-M and blaTEM. Resistance of *E. coli* isolated from beef to tetracycline, azithromycin and sulfamethoxazole-trimethoprim was 40%, 33% and 20%, respectively (Eibach, *et al.,* 2018). They also reported that some of the *E. coli* isolates were resistant to multiple antimicrobials and/or harbored BlaSHV, blaCTX-M, blaTEM, tet (A), aph (3")-Ib, apha (6), dfrA14 and mdf (A) resistance genes (Dsani, *et al.,* 2020, Eibach, *et al.,* 2018, Adzitey, 2020). Thus, meats and meat products in Ghana are potential sources of antimicrobial-resistant bacteria.

Moreover, studies on the spread of *E. coli a*mong meats, humans and their environment in Ghana are limited. Studies have also shown that the manner in which animals are handled prior to slaughter, during slaughter and post-slaughter makes meat and their related samples potential sources of foodborne pathogens (Agbodaze *et al.,* 2015; Twum, 2016; Adzitey *et al.,* 2019; Yafetto *et al.,* 2019; Adzitey *et al.,* 2020, Adzitey, 2015). In addition, the sale of meats and meat products is sometimes conducted in open markets and by roadsides without adherence to strict hygiene (Adzitey *et al.,* 2018; Sulleyman *et al.,* 2018; Adzitey *et al.,* 2020). RTE meats in particular are consumed without prior heating (Abass *et al.,* 2020). With all these handling activities, meat and meat products expose consumers to foodborne infections

and antimicrobial resistance bacteria. Therefore, this study is aimed at determining whether raw meats, RTE meats and their related samples (hands of meat sellers, knives for cutting meat, tables on which meats are placed for cutting and for sale, utensils used by meat sellers) are contaminated by antimicrobial-resistant *E. coli*.

2.5 Salmonella Spp.

Avian *Salmonella* infections are important causes of clinical disease in poultry and a potential source of foodborne transmission of *Salmonella* in human (Shivaprasad, 1991). About 95.0% of salmonellosis cases were estimated to originate from food materials (Murray, 2010) and the colonization of *Salmonella* covers humans and animals including livestock, poultry, rodents and birds (Carramiñana *et al.,* 2004; Soultosb *et al,,* 2003). The adaptive ability of pathogen itself, the changing characteristics of the population, the increasing globalization of the food trade, and the changes in industrial structure, poor hygienic environment, improper storage or cooking, cross-contamination, infected stocks contribute to the development of *Salmonella* in poultry and poultry products leading to the major source of human foodborne illness and loss of product shelf-life (Hald, 2013; Hirsh *et al., 2*004).

Poultry products have always topped the incidence of salmonellosis in India, Egypt, Brazil, Zimbabwe, Nepal and other developing countries (Henson, 2003; Shrestha *et al.,* 2017) and are the most seriously perceived food risks in chicken meat, even in the developed countries (Yeung and Morris J, 2001). The incidence of human salmonellosis has increased greatly over the past 20 years and this can mostly be attributed to epidemics of *S. enteritidis* in poultry in numerous countries (Guard-Petter, 2001). *Salmonella* serotypes differ significantly in their pathogenic potentials and a study suggested the confirmed cases of *Salmonella* sp. in the surveillance network Food Net from the period 1996–2006 (Jones *et al.,* 2008). Chicken liver is an important low-cost source of animal protein, rich in nutrients, phosphorus, others minerals, and B-complex vitamins (FAO, 2010); however, the presence of MDR resistant

Salmonella sp. in chicken livers have become the solemn concern of food safety and one of the major public health problems (Nair *et al.,* 2018; Varma *et al* 2003). Different food items have been documented as a reservoir of ESBL producing bacteria and such food items are probable sources for the acquirement of beta-lactamase producing bacteria. The frequency of isolation of *Salmonella* strains resistant to several antimicrobial agents has increased in several countries worldwide (Pui *et al., 2*011; Yoke-Kqueen *et al.,* 2008; Shrestha *et al.,* 2017).

2.6 ANTIMICROBIAL RESISTANCE IN ENTEROBACTERIACEAE

Infections caused by Enterobacteriaceae are usually treated with β-lactam antibiotics. These are broad spectrum antibiotics consisting of molecular ring-shaped structure, the β-lactam ring. This group include penicillins, cephalosporins, monobactams and carbapenems. Hydrolysis of β-lactam structure mediated by β-lactamases is the most common resistant mechanism for inactivation of β-lactam antibiotics. There are mainly four β-lactamases groups which can be identified based on substrate specificities:

penicillinases, AmpC-type cephalosporinases, extended-spectrum β-lactamases (ESBLs), and carbapenemases (Nordmann, Dortet and Poirel, 2012). Decreased porin function or increased efflux are other types of β-lactam resistance mechanism exhibited by Enterobacteriaceae. Porin alterations and β-lactamase production can synergize in increasing antibiotic resistance in many pathogens of Enterobacteriaceae (Blair *et al.,* 2015).

CHAPTER THREE
MATERIALS AND METHODS

3.1 Study Area

The study was conducted in Benin City, Edo State, Nigeria. Raw meat from cow was purchased from some markets in Benin metropolis. The markets are Okha market, Santana market, Oba market, and Ekiosa market.

3.2 Sample Collection from Meat

Ten samples each of cow meat were collected from the markets listed above. The samples were aseptically transported on ice to the Microbiology laboratory of Benson Idahosa University for immediate analysis. Samples not used immediately were stored in the refrigerator at freezing temperature. Sampling was conducted between March and April, 2021. In all, 40 samples were randomly selected and examined for the presence of *E. coli* and *Salmonella* spp.

3.3 Isolation and Identification of *Escherichia coli* and *Salmonella* spp. in Raw Meats

Five (5) gram each of the samples was transferred into a sterile conical flask containing 45ml of buffered peptone water and left to incubate at room temperature for 1hr. This was labeled stock sample. Serial dilution was done from the stock sample for all collected samples until the sixth dilution. One (1) ml each of the diluters was then plated on Nutrient Agar (NA), *Salmonella* Shigella Agar (SSA) and Levine's Eosin-Methylene Blue Agar (EMB) using pour plate method. All the plates were incubated at 37^{o}C for 24hrs.

3.4 Total Aerobic Counts (After incubation)

After incubation for 24hrs, all discrete colonies on the various petri dishes were appropriately counted and recorded. Growth on nutrient agar was used to calculate the total aerobic count, while that on EMB and SSA were used to calculate the total *E. coli* and *Salmonella* count

respectively.

3.5 Identification of the isolates

Identification of the isolates was done using the cultural, morphological and cultural characteristics of the isolates according to the methods of Holt *et al.,* (1994).

3.5.1 Gram staining

The isolates were smeared on a glass slide and stained with crystal violet (primary stain), iodine (mordant), acetone (decolourizer) and safranin (secondary stain) for 30sec, 60sec, 3sec and 30sec respectively. Using a microscope under 100 x magnifications, Gram positive isolates appeared purple while Gram negative isolates appeared pink.

3.5.2 Biochemical Identification

Pure colonies were selected and sub-cultured on nutrient agar and were further processed for biochemical tests. Well known traditional biochemical test and selective media for bacteria identification were employed (Eddouaouda *et al.,* 2012). The isolates were identified based on their growth characteristics on Nutrient Agar, *Salmonella*-Shigella Agar and Eosin Methylene Blue Agar, and their Biochemical test such as Catalase, Citrate Utilization test, Indole test and Urease test. The identified bacteria species were kept in 20% glycerol/medium mixture at refrigeration temperature at the Microbiology Laboratory until needed for further analysis.

3.6 Antibiotic Susceptibility Test (AST)

Pure colonies were picked and emulsified in normal saline. Mueller-Hinton agar antibiotic diffusion technique (Kirby-Bauer NCCLS modified disc diffusion technique) was used for antibiotic susceptibility (Pitout and Laupland, 2008). Antibiotic discs were used to test for susceptibility of the isolated bacteria to the commonly used antibiotics, some of which are; ceftazidime (30µg), cefuroxime (30µg), gentamicin (10µg), cefixime (5µ g), ofloxacin (5 µg), augumentin (30 µg), nitrofurantion (300µg) and ciprofloxacin (5µg). The dried Mueller

Hinton agar plate was plated with the isolated microbes. The appropriate antimicrobial impregnated disks were placed on the surface of the agar using sterilized forceps and incubated at 35^{o}C overnight. The antibiotic inhibition zones were measured to the nearest millimetre (mm) from the center of the disk to a point on the circumference of the zone where a distinct edge was seen using a rule.

CHAPTER FOUR

RESULT

4.1 TOTAL BACTERIAL COUNTS OF SAMPLES

Result of the total bacterial count shows that samples taken from Okha market, Santana market, Oba market and Ekiosa market had a mean count of 5.11±0.65 cfu/g, 5.97±0.53 cfu/g, 4.71±0.83cfu/g and 4.29±0.49cfu/g respectively as shown in Table 4.1. Interestingly, the highest bacterial count was observed in samples taken from Santana market (6.82) in sample E whereas the lowest bacterial count was observed in samples collected from Oba market (3.21) in sample F. Although, all samples had bacteria growth.

Table 4.1: Total Aerobic Count of Fresh Meat Sold in Some Markets in Benin City.

	Counts x 10^7 cfu/g			
Sample	**Okha Market**	**Santana Market**	**Oba Market**	**Ekiosa Market**
A	5.85	6.6	5.13	4.15
B	4.31	6.20	5.57	4.23
C	5.26	5.98	5.92	4.61
D	5.00	5.44	4.11	3.28
E	4.21	6.82	4.73	3.91
F	5.71	5.10	3.21	4.26
G	6.17	6.00	5.03	5.01
H	4.62	6.29	5.27	4.61
I	4.81	5.52	4.17	4.66
J	5.11	5.79	4.00	4.60
Standard Deviation	5.11±0.65	5.97±0.53	4.71±0.83	4.29±0.49

4.2 TOTAL *E. coli* COUNTS OF SAMPLES

Table 4.2 shows the total *E. coli* counts for the forty (40) samples collected from the four markets used in this study. The highest *E. coli* count was observed in samples taken from Okha market (2.5×10^7cfu/g) in sample D and the lowest *E.coli* count was observed in samples collected from the same market in sample F (1.26×10^7cfu/g). Where the highest total *E. coli* count for all samples collected from each market put together came from the total *E. coli* count for Ekiosa market with (19.31×10^7cfu/g) and the lowest total *E. coli* count of all collected sample in a particular market came from Oba market with (16.86×10^7cfu/g).

Table 4.2: Total *E. coli* count Eosin Methylene Blue Agar on fresh meat samples sold in some markets in Benin City

	Counts x cfu/g			
Sample	**Okha Market**	**Santana Market**	**Oba Market**	**Ekiosa Market**
A	2.04	1.72	1.56	1.77
B	2.13	1.52	1.83	1.87
C	2.21	1.86	1.41	2.37
D	2.50	2.47	1.16	1.52
E	1.91	1.62	1.90	1.67
F	1.26	1.79	2.36	1.93
G	1.32	1.32	2.15	2.46
H	1.63	1.49	1.29	1.41
I	2.00	2.16	1.30	2.48
J	2.14	2.43	1.90	1.83
MEAN±SD	1.91±0.40	1.84±0.40	1.69±0.40	1.93±0.38

SD= Standard Deviation

4.3 TOTAL *Salmonella* COUNTS OF SAMPLES

The total *Salmonella* spp counts for the forty (40) samples of fresh meat collected from the four markets in this study is shown in Table 4.3. The highest *Salmonella* count was observed in samples taken from Oba market (2.9×10^7cfu/g) in sample B while the lowest count was observed in samples collected from Ekiosa market in sample C (0.83×10^7cfu/g). The highest total *Salmonella* count for all samples collected from each market put together was in Oba market (2.502×10^7cfu/g) and the lowest was in Ekiosa market with (1.073×10^7cfu/g).

Table 4.3: Total *Salmonella* spp. Count on *Salmonella Shigella Agar (SSA)* on fresh meat samples sold in some markets in Benin City.

	Counts x 10^7cfu/g			
Sample	**Okha Market**	**Santana Market**	**Oba Market**	**Ekiosa Market**
A	1.56	1.45	2.60	0.83
B	1.82	1.49	2.90	0.93
C	1.76	1.57	2.88	1.12
D	1.44	1.55	2.62	1.18
E	1.43	1.44	2.26	1.26
F	1.59	1.69	2.29	1.47
G	1.60	1.72	2.70	1.29
H	1.37	1.39	2.00	0.87
I	1.36	1.30	2.67	0.93
J	0.97	1.33	2.10	0.85
MEAN±SD	1.49±0.24	1.49±0.14	2.502±0.32	1.073±0.22

SD= Stand Deviation

4.4 CHARACTERIZATION OF BACTERIAL ISOLATES

The isolates collected from the fresh meat samples were screened for their cultural and morphological characteristics as shown in Table 4.4. Through gram staining, 43 gram negative rod shaped bacteria of both *E. coli* and *Salmonella* spp. were identified. Forty-eight (48) of the isolates where observed to be catalase positive, whereas the citrate test revealed thirty (33) that were positive. The indole test detected fifteen (18) isolates that were positive to the test and finally the urease test detected forty six (46) gram negative isolates.

Table 4.4: Cultural Morphological Characteristic of Bacterial Isolates

ISOLATES	GRAM STAIN	COLOR ON MEDIA SOLUTION	CATALASE	CITRATE	INDOLE	UREASE	PROBABLE ORGANISM
1	+ve Cocci	Yellow	+ve	+ve	-ve	+ve	***Staphyloccous*** **spp**
2	+ve Cocci	White	-ve	-ve	-ve	-ve	***Streptococcus*** **spp**
3	-ve Rods	White	+ve	+ve	-ve	-ve	***Salmonella*** **spp**
4	-ve Rods	White	+ve	+ve	-ve	-ve	***Salmonella*** **spp**
5	-ve Rods	White	+ve	+ve	-ve	-ve	***Salmonella*** **spp**
6	-ve Rods	White	+ve	-ve	+ve	-ve	***E. coli***
7	-ve Rods	White	+ve	-ve	+ve	-ve	***E. coli***
8	-ve Rods	White	+ve	+ve	-ve	-ve	***Salmonella*** **spp**
9	-ve Rods	White	+ve	+ve	-ve	-ve	***Salmonella*** **spp**
10	-ve Rods	White	+ve	-ve	+ve	-ve	***E. coli***
11	+ve Cocci	Yellow	+ve	+ve	-ve	+ve	***Staphyloccous*** **spp**
12	-ve Rods	White	+ve	+ve	-ve	-ve	***Salmonella*** **spp**
13	-ve Rods	White	+ve	+ve	-ve	-ve	***Salmonella*** **spp**
14	-ve Rods	White	+ve	+ve	-ve	-ve	***Salmonella*** **spp**
15	-ve Rods	White	+ve	+ve	-ve	-ve	***Salmonella*** **spp**
16	+ve Cocci	Yellow	+ve	+ve	-ve	+ve	**Staphylococcus spp**
17	-ve Rods	White	+ve	-ve	+ve	-ve	***E. coli***
18	+ve Cocci	Yellow	+ve	+ve	-ve	-ve	**Staphylococcus spp**
19	+ve Cocci	Yellow	+ve	+ve	-ve	-ve	**Staphylococcus spp**
20	-ve Rods	White	+ve	+ve	-ve	-ve	***Salmonella*** **spp**
21	-ve Rods	White	+ve	-ve	+ve	-ve	***E. coli***
22	-ve Rods	White	+ve	-ve	+ve	-ve	***E. coli***
23	-ve Rods	White	+ve	-ve	+ve	-ve	***E. coli***
24	-ve Rods	White	+ve	-ve	+ve	-ve	***E. coli***

25	**-ve Rods**	**colourless**	**+ve**	**+ve**	**-ve**	**-ve**	***Salmonella* spp**
26	**-ve Rods**	**Black**	**+ve**	**+ve**	**-ve**	**-ve**	***Salmonella* spp**
27	**-ve Rods**	**Black**	**+ve**	**+ve**	**-ve**	**-ve**	***Salmonella* spp**
28	**-ve Rods**	**Black**	**+ve**	**+ve**	**-ve**	**-ve**	***Salmonella* spp**
29	**-ve Rods**	**colourless**	**+ve**	**+ve**	**-ve**	**-ve**	***Salmonella* spp**
30	**-ve Rods**	**Black**	**+ve**	**+ve**	**-ve**	**-ve**	***Salmonella* spp**
31	**-ve Rods**	**Black**	**+ve**	**+ve**	**-ve**	**-ve**	***Salmonella* spp**
32	**-ve Rods**	**Black**	**+ve**	**+ve**	**-ve**	**-ve**	***Salmonella* spp**
33	**-ve Rods**	**Black**	**+ve**	**+ve**	**-ve**	**-ve**	***Salmonella* spp**
34	**-ve Rods**	**colourless**	**+ve**	**+ve**	**-ve**	**-ve**	***Salmonella* spp**
35	**-ve Rods**	**colourless**	**+ve**	**+ve**	**-ve**	**-ve**	***Salmonella* spp**
36	**-ve Rods**	**colourless**	**+ve**	**+ve**	**-ve**	**-ve**	***Salmonella* spp**
37	**-ve Rods**	**Black**	**+ve**	**+ve**	**-ve**	**-ve**	***Salmonella* spp**
38	**-ve Rods**	**Black**	**+ve**	**+ve**	**-ve**	**-ve**	***Salmonella* spp**
39	**-ve Rods**	**Black**	**+ve**	**+ve**	**-ve**	**-ve**	***Salmonella* spp**
40	**-ve Rods**	**Black**	**+ve**	**+ve**	**-ve**	**-ve**	***Salmonella* spp**
41	**-ve Rods**	**Black**	**+ve**	**+ve**	**-ve**	**-ve**	***Salmonella* spp**
42	**-ve Rods**	**Black**	**+ve**	**+ve**	**-ve**	**-ve**	***Salmonella* spp**
43	**-ve Rods**	**Green**	**+ve**	**-ve**	**+ve**	**-ve**	***E. coli***
44	**-ve Rods**	**Green**	**+ve**	**-ve**	**+ve**	**-ve**	***E. coli***
45	**-ve Rods**	**Green**	**+ve**	**-ve**	**+ve**	**-ve**	***E. coli***
46	**-ve Rods**	**Green**	**+ve**	**-ve**	**+ve**	**-ve**	***E. coli***
47	**-ve Rods**	**Green**	**+ve**	**-ve**	**+ve**	**-ve**	***E. coli***
48	**-ve Rods**	**Green**	**+ve**	**-ve**	**+ve**	**-ve**	***E. coli***
49	**-ve Rods**	**Green**	**+ve**	**-ve**	**+ve**	**-ve**	***E. coli***
50	**-ve Rods**	**Green**	**+ve**	**-ve**	**+ve**	**-ve**	***E. coli***
51	**-ve Rods**	**Green**	**+ve**	**-ve**	**+ve**	**-ve**	***E. coli***
52	**-ve Rods**	**Green**	**+ve**	**-ve**	**+ve**	**-ve**	***E. coli***

KEY: +ve : Positive; -ve : Negative

4.5 PREVALENCE RATE OF *E. coli* AND *Salmonella* spp ISOLATED FROM SAMPLES.

Table 4.5 shows the prevalence rate of both *E. coli* and *Salmonella* spp isolated from samples collected from markets in Benin City. The table confirmed that in Okha market the prevalence of *E. coli* was 0% as there were no *E. coli* detected from the samples collected from the market while *Salmonella* spp. was very prevalent with a 70% prevalence as the samples collected from Okha market were detected for *Salmonella* spp. while in Santana market no bacterial was detected in both samples or isolates from the market as they both showed 0% prevalence. While in Oba market there was a high prevalence of about 70% of *E. coli* contamination of samples collected from the market and a low prevalence of 30% *Salmonella* spp. contamination of the isolates collected from the market. Finally Ekiosa market shows a low prevalence rate of 30% for *E. coli* and 80% for *Salmonella* samples collected from the market.

Table 4.5: Prevalence Rate of *E. coli* And *Samonella* species Isolated from Fresh Meat Based on the Numbers of Samples collected.

Market	**Number Of Sample**	***E. Coli* Number (%)**	***Salmonella* Spp. Number (%)**
Okha	10	0(00)	7(70)
Santana	10	0(00)	0(00)
Oba	10	7(70)	3(30)
Ekiosa	10	3(30)	8(80)
Total	40	10(25)	18(45)

4.6 ANTIBIOTIC RESISTANCE PATTERN OF *E. coli* ISOLATED FROM FRESH MEATS SOLD IN SOME MARKETS IN BENIN CITY.

Table 4.6 shows the antibiotics resistance profile of the *E. coli* isolates from both Ekiosa and Oba markets against eight (8) antibiotcs; ceftazidime (30µg), cefuroxime (30µg), gentamicin (10µg), cefixime (5µ g), ofloxacin (5 µg), augumentin (30 µg), nitrofurantion (300µg) and ciprofloxacin (5µg). Out of the 10 isolates collected from Oba market , 85.7% (6) were resistant to CAZ, 100% (7) to CRX, 100% (7) to CXM, 100% (7) to AUG, 100% (7) to NIT, 28.57% (2) to CPR, 28.57% (2) was slightly resistant to GEN and 14.28% was mildly resistant to OFL. For the *E. coli* isolates collected from Ekiosa market, 100% (3) were resistant to CAZ, 100% (3) to CRX, 100% (3) to GEN, 100% (3) to CXM, 100% (3) to OFL, 100% (3) to AUG, 100% (3) to NIT and 66% (2) to CPR.

Table 4.6: Antibiotic Resistance Pattern of *E. coli* Isolated from Fresh meats sold in some markets in Benin City.

Antibiotics Resistance Pattern (%)			
Antibiotics	**Oba** **N=(7)**	**Ekiosa** **N=(3)**	**Total Resistance** **N=10**
CAZ	6(85.7)	3(100)	9(92.85)
CRX	7(100)	3(100)	10(100)
GEN	2(28.57)	3(100)	5(64.28)
CXM	7(100)	3(100)	10(100)
OFL	1(14.28)	3(100)	4(57.14)
AUG	7(100)	3(100)	10(100)
NIT	7(100)	3(100)	10(100)
CPR	2(28.57)	2(66.00)	4(47.28)

Key: N: Number of Isolates tested; CAZ: Ceftazidime; CRX: Cefuroxine; GEN: Gentamicin; CXM: Cefixime; OFL: Ofloxacin; AU: Augumentin; NIT: Nitrofurantoin; CPR: Ciprofloxacin.

4.7: ANTIBIOTIC RESISTANCE PATTERN OF *Salmonella* ISOLATED FROM FRESH MEATS SOLD IN SOME MARKETS IN BENIN CITY.

The antibiotic resistance profile of the *Salmonella* spp isolates collected from both Ekiosa, Okha and Oba markets, against eight (8) antibotics; ceftazidime (30µg), cefuroxime (30µg), gentamicin (10µg), cefixime (5µ g), ofloxacin (5 µg), augumentin (30 µg), nitrofurantion (300µg) and ciprofloxacin (5µg), is shown in Table 4.7. Out of the 18 isolates collected from all three markets ,100% (18) were resistant to CAZ, 100% (18) to CRX, 100% (18) to CXM, 100% (18) to AUG, 59.09% (9) to NIT, 76.7% (14) to CPR with 56.52% (10) resistant to GEN and 35.09% (5) was slightly resistant to OFL.

Table 4.7: Antibiotic Resistance Pattern of *Salmonella* spp Isolated from Fresh meats sold in some markets in Benin City.

	Antibiotics Resistance Pattern (%)			
Antibiotics	**Okha N=(7)**	**Oba N=(3)**	**Ekiosa N=(8)**	**Total Resistance N=18**
CAZ	(7)100	(3)100	(8)100	18(100)
CRX	(7)100	(3)100	(8)100	18(100)
GEN	(2) 28.57	(2)66	(6)75	10(56.52)
CXM	(7)100	(3)100	8(100)	18(100)
OFL	(1) 14.28	(2)66	2(25)	5(35.09)
AUG	(7)100	(3)100	8(100)	18(100)
NIT	(1) 14.28	(3)100	5(63)	9(59.09)
CPR	(7)100	(2)67	5(63)	14(76.7)

Key: N: Number of Isolates Tested; CAZ: Ceftazidime; CRX: Cefuroxine; GEN: Gentamicin; CXM: Cefixime; OFL: Ofloxacin; AU: Augumentin; NIT: Nitrofurantion; CPR: Ciprofloxacin.

CHAPTER FIVE
DISCUSSION AND CONCLUSION

5.1 DISCUSSION

Contaminated food is a major source of gastrointestinal pathogens and has caused numerous foodborne disease outbreaks in the developing world. Because of the wide use of third- and fourth-generation cephalosporin antibiotics, the prevalence of multidrug-resistant Enterobacteriaceae has increased yearly, around the globe. In this study, we investigated the antibiotics resistance pattern of *E. coli* and *Salmonella specie* isolated from fresh meat sold in some markets in Benin City, Edo State, Nigeria. A total of forty (40) samples were taken. Samples taken from Okha market, Santana market, Oba market and Ekiosa market had a mean count of 5.11±0.65 cfu/g, 5.97±0.53 cfu/g, 4.71±0.83cfu/g and 4.29±0.49cfu/g respectively. The highest bacterial count was observed in samples taken from Santana market (6.82) in sample E whereas the lowest bacterial count was observed in samples collected from Oba market (3.21) in sample F. Based on morphological and biochemical characteristics, the presumptive organisms isolated in this study were *E. coli and Salmonella spp.* In agreement with the observation of this study, *E. coli and Salmonella* spp. were reportedly isolated from meat sources and human epithelial cells (Schierack *et al.,* 2011) and in poultry animals and sources (Sackey *et al.,* 2001, Saeed *et al.,* 2010)

Human infections of *E. coli and Salmonella spp.* have mostly been recognized to be from food products with animal origin (Jo *et al.,* 2004). Domestic ruminants, mainly cattle, sheep, and goats, have been established as major natural reservoirs for *E. coli and Salmonella spp.* bacteria's and play a significant role in the epidemiology of human infections (Kim *et al.,* 2015). Several recent reports have clearly identified (Espie *et al.,* 2006; La Ragione *et al.,* 2008) or implicated (Chapman *et al.,* 2000; Pritchard *et al.,* 2000; Rey *et al.,* 2006) goats and cow meat sources as sources of *E. coli and Salmonella* infection. Not only can these animals

be colonized with *E. coli and Salmonella spp.*, but their innately inquisitive behavior means that they are much more likely to be in regular direct contact with humans, consequently increasing the risk of the direct faecal–oral transmission of zoonotic infection (La Ragione *et al.,* 2008).

The most pressing safety issues in the food industry are caused by the presence of *E. coli* and *Salmonella* spp in raw meat and poultry products (Sperber, 2005). In this present study, presence of *E. coli* and *Salmonella* spp on raw meat samples collected from the various markets suggests high bacterial contamination of our meat sources. This contamination could be due to the transfer of faecal material onto the sterile carcass during the slaughtering process, which may suggest that currently available dressing procedures at abattoirs and markets cannot be relied upon to prevent faecal contamination of our food meat sources during slaughter.

In this study, the prevalence rate of both *E. coli* and *Salmonella* spp isolated from samples collected from markets in Benin City confirmed that in Okha market, the prevalence of *E. coli* was 0% as there were no *E. coli* detected from the samples while *Salmonella* spp was very prevalent with a 70% prevalence. In Santana market, no bacteria was detected in both samples or isolates from the market as they both showed 0% prevalence which indicates that fresh meat from Santana market are more safe for consumption as they are free from bacteria of the likes of *E. coli* and *Salmonella spp.* While in Oba market there was a high prevalence rate of about 70% of *E. coli* contamination of samples collected from the market and a low prevalence of 30% *Salmomella* spp. Finally Ekiosa market showed a low prevalence rate of 30% for *E. coli* and 80% for *Salmonella* samples collected from the market. The findings in the study do not differ greatly from those that reported the isolation of this bacteria from fresh and raw meat of goats and cows in other countries. This has already been reported in various studies, 3% by Mersha *et al.* (2009) in Modjo and 2 % by Hiko *et al.* (2008) in Debre Zeit

and Modjo towns of Ethiopia. Moreover, the prevalence rate reported for carcass and faeces in my study was consistent with reports from other parts of the world such as 2.7% from United States (Jacob *et al.,* 2013), 1.7% from Iran (Rahimi *et al.,* 2012a) 1.2% from Greece (Dontorou *et al.,* 2004), 2.5% from Nigeria (Akanbi *et al.,* 2011, Isibor *et al.,* 2013). On the contrary, 50% prevalence was documented for goat meat in India (Gomashe *et al.* 2011), while 9.1% prevalence was noted in goats in Bangladesh (Islam *et al.,* 2008).

The observed differences in the results of this present study from those of other authors could be due to differences in husbandry practices and prevailing climatic conditions which may account for the varied prevalence of *E. coli* and *Salmonella* spp from one geographical region to another. The methods and techniques used in the laboratory identification of these bacteria in this study could also be responsible (Ojo *et al.,* 2010).

Emergence and dissemination of antimicrobial resistance is on the increase among enteric bacteria (Sawant *et al.,* 2007). Antimicrobial resistance may arise either spontaneously by selective pressure or due to antimicrobial misuse by humans or overuse in feeding or treatment of animals by farmers (Schroeder *et al.,* 2002). Resistance development also might be related to exchange of resistance factors between related bacteria (Tenover, 2006).

All the *E. coli* and *Salmonella* spp. isolated in this study exhibited resistance to two or more antibiotics. In Ethiopian situation, two studies were reported on the antimicrobial susceptibility of *E. coli* O157:H7 isolated from cattle, sheep and goat. The first study showed that the isolated pathogen is highly sensitive to amikacin, chloramphenicol, gentamicin, kanamycin, nalidixic acid, norfloxacin, polymyxin B and trimethoprim-sulfamethoxazole and highly resistant to streptomycin, cephlaothin, tetracycline, ampicillin and trimethoprim (Hiko *et al.,* 2008). The second study revealed that all beef isolates were found susceptible to kanamycin, chloramphenicol and spectinomycin and 100% resistance to ampicillin and amoxicillin and 33.33% resistance to tetracycline (Taye *et al.,* 2013). In this study, the

antibiotic resistance of the *E. coli* isolates collected from both Ekiosa and Oba markets showed that, out of the 10 isolates collected from Oba market, 85.7% (6) were resistant to CAZ, 100% (7) to CRX, 100% (7) to CXM, 100% (7) to AUG, 100% (7) to NIT, 28.57% (2) to CPR with 28.57% (2) to slightly resistant to GEN and 14.28% (1) was mildly resistant to OFL. While for the *E. coli* isolates collected from Ekiosa market, 100% (3) were resistant to CAZ, 100% (3) to CRX, 100% (3) to GEN, 100% (3) to CXM, 100% (3) to OFL, 100% (3) to AUG, 100% (3) to NIT and 66% (2) to CPR. This study also showed the antibiotic resistance of the *Salmonella* spp isolates collected from both Ekiosa, Okha and Oba markets, where out of the 18 isolates collected from all three markets, 100% (18) were resistant to CAZ, 100% (18) to CRX, 100% (18) to CXM, 100% (18) to AUG, 59.09% (9) to NIT, 76.7% (14) to CPR with 56.52% (10) resistant to GEN and 35.09% (5) slightly resistant to OFL.

The resistance of all *E. coli* and *Salmonella* spp. to ceftazidime, cefuroxine, cefixime, augumentin**,** comes in agreement with the results of Harakeh *et al.* (2005) and Osaili *et al.* (2013). Furthermore, our results showed that high proportion of *E. coli* and *Salmonella* spp isolates were resistant to the nitrofurantion and other antimicrobial agents. This observation contradicts Hiko *et al.* (2008) who reported 100% susceptibility for trimethoprim-sulfamethoxazole in *E. coli* O157:H7 isolates from bovine, sheep and goat meat. Although ofloxacin, had moderate resistance in this study, it is one of the most commonly available for use as routine chemoprophylaxis among livestock in Nigeria. They are readily available in different dosage forms and in combination with other antibiotics and vitamins. The increasing developing multi-drug resistant bacteria is signaling a serious alarm from treatment point of view or the possible transforming of resistance genes to other related pathogens (Osaili *et al.,* 2013). In this study, multiple antimicrobial resistance is also noted among *E. coli* and *Salmonella* spp. Isolates which was in agreement with Schroeder *et al.* (2002) and Zhao *et al.*

(2001) report in the USA. They found that out of the twenty nine tested *E. coli* and *Salmonella* spp, four (4) isolates showed multiple resistance to five antimicrobials: tetracycline, ampicillin, streptomycin, kanamycin, and sulfamethoxazole. Two isolates originated from cattle, and two isolates were from human and ground beef.

The public health significance of this finding is that antimicrobial resistant bacteria from food animals may colonize the human population via the food chain, contact through occupational exposure, or waste runoff from meat production facilities to the neighbor-hood. It is essential to keep up with isolate characteristics for any global changes in isolate distribution and similarities and prevalence of common virulence factors. Also, it is essential to track the resistance pattern recorded globally to follow changes in antimicrobial sensitivity patterns that may require a reassessment of zoonotic control strategy. Monitoring of antimicrobial resistance in *E. coli* and *Salmonella* spp. isolates is valuable for epidemiological uses and for monitoring the increase of antimicrobial resistance among different microbial species (Osaili *et al.,* 2013).

5.2 CONCLUSION

This study investigated the antibiotics resistance pattern of *E. coli* and *Salmonella Specie* isolated from fresh meat sold in some markets in Benin City, Edo State. The study showed that a higher isolation rate of *E. coli* and *Salmonella* spp. in fresh meat destined for human consumption in the studied area with some antimicrobial resistance pattern. In addition, the results showed the risk of this pathogen to consumers due to unhygienic meat processing most commonly practiced in Benin City markets, abattoirs and butchering houses and the contributions to global epidemiology of bacterial resistance. The presence of *E. coli* and *Salmonella* spp. is being reported for all collected meat samples and markets, the study confirmed a need for preventative approach to control *E. coli* and *Salmonella* spp. in fresh meat sales, marketing and production processes and chain. This study has also attempted to

cast light on features about the knowledge, attitudes and practices of fresh meat sellers and abattoir slaughter staff pertaining food safety and general hygiene. The results indicated that there were poor personal and general hygiene measures in place and that the workers not focus on hygienic practice. Generally, this study provides an initial baseline data on the occurrence of *E. coli* and *Salmonella* spp. in markets studied.

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