UNIVERSITY FOR DEVELOPMENT STUDIES

SEASONSAL PREVALENCE AND ANTIBIOTIC RESISTANCE OF *ESCHERICHIA COLI* AND *SALMONELLA ENTERICA* ISOLATED FROM RAW MEATS IN BUIPE, GHANA

DIMONGSO OSMAN

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FACULTY OF AGRICULTURE, FOOD AND CONSUMER SCIENCES

DEPARTMENT OF ANIMAL SCIENCE

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BY

DIMONGSO OSMAN

(B.Sc. Agriculture Technology)

(UDS/MAN/0002/21)

THESIS SUBMITTED TO THE DEPARTMENT OF ANIMAL SCIENCE, FACULTY OF AGRICULTURE, FOOD AND CONSUMER SCIENCES, UNIVERSITY FOR DEVELOPMENT STUDIES, IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF PHILOSOPHY IN ANIMAL SCIENCE

MARCH, 2024

DECLARATION

Student

I hereby declare that this thesis is the result of my own original work and that no part of it has been presented for another degree in this University or elsewhere.

Candidate's Signature…  …Date……28/03/2024……

Name: Mr. Dimongso Osman

Supervisor

I hereby declare that the preparation and presentation of the thesis was supervised in accordance with the guidelines on supervision of thesis laid down by the University for Development Studies.

Principal Supervisor's Signature ……..  .....Date...05/04/2024

Prof. Frederick Adzitey (PhD)

(UDS, Ghana)

ABSTRACT

Meat is an important source of proteins and other nutrients for humans and its contamination with bacteria poses a threat to public health. The study was carried out to determine the seasonal prevalence and antibiotic resistance of *Escherichia coli* (*E. coli*) and *salmonella enterica* isolated from raw meats. A total of 180 samples, 30 each of beef, chevon and mutton were randomly collected from Buipe in both the dry and rainy seasons. *E. coli* and *Salmonella enterica* were isolated using the USA-FDA bacteriological analytical manual and antibiotic susceptibility test was performed using the disk diffusion method. *E. coli* recorded a prevalence of 30.0% for beef, 53.3% for chevon and 63.3% for mutton collected during the dry season; it was 6.7%, 16.6% and 16.6% for beef, chevon and mutton, respectively collected during the rainy season. Overall, 48.9% versus 13.3% of meat samples collected during the dry and rainy season, respectively were contaminated with *E. coli*. *Salmonella enterica* recorded a prevalence of 3.3% for beef, 13.3% for chevon and 0.0% for mutton collected during the dry season. *Salmonella enterica* was not detected during the rainy season. Overall, 5.6% versus 0.0% of meat samples collected during the dry and rainy season, respectively were contaminated with *Salmonella enterica*. Antibiotic resistance was highest for Amoxicillin (61.5%) in *E. coli* isolated during the dry season. Susceptibility was high for Azithromycin (84.6%), Ceftriaxone (80.8%), Chloramphenicol (80.8%), Ciprofloxacin (84.6%), Gentamicin (80.8%) and Trimethoprim/Sulfamethoxazole (73.1%) in *E. coli* isolated during the rainy season.

Antibiotic resistance was also highest for Amoxicillin (91.0%). Susceptibility was high for Ceftriaxone (82.0%), Ciprofloxacin (82.0%) and Gentamicin (82.0%) in *E. coli* isolated during the rainy season. Multidrug resistance (MAR) index ranged from 0.2 to 0.8 and 13 different resistant profiles were observed for *E. coli* isolated during the dry season, while MAR index ranged from 0.2 to 0.7 and 10 different resistant profiles were observed for *E. coli* isolated during the rainy season. The *Salmonella enterica* exhibited 40.0% intermediate resistance and 60.0% susceptibility to ceftriaxone. They were 100% susceptible to the rest of the antibiotics examined. All meat samples were contaminated with *E. coli* which were resistant to various antibiotics. Some meat samples were contaminated with *Salmonella enterica* which were susceptible to most antibiotics. Further research in the molecular characterization of *E. coli* and *Salmonella enterica* to reveal their genetic diversity, antimicrobial resistance genes and virulence genes is recommended.

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DEDICATION

I dedicate this work to my wife, Nyankpani Rukaya and our children; Osman Badegamsera Ishmael, Osman Mamboriba Izzideen, Osman Wunsumah Ilhaam and Osman Kuluag Hibatu, who in diverse ways gave me family love at home during my academic and research period.

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LIST OF ABBREVIATIONS/ACRONYMES

AI……. Avian Influenza

AMSA……..American Meat Science Association

CDC……..….Centres for Disease Control and Prevention

DNA…….……..Deoxyribonucleic Acid

FDA……Food and Drugs Authority

GIT…….Gastro-intestinal Tract

HACCP……..Hazard Analysis Critical Control Point

HAA…….…..Heterocyclic Aromatic Amines

HGT……Horizontal Gene Transfer

MUFA…….Mono-Unsaturated Fatty Acid

MAR index ……Multiple Antibiotic Resistance Index

PBPs……Penicillin-binding Proteins

PAH…… Polycyclic Aromatic Hydrocarbons

PCR…….Polymerase Chain Reaction

PUFA……..Polyunsaturated Fatty Acids

SSA……Sub-Saharan Africa

TBC……Total Bacterial Count

TCC…….Total Coliform Count

TPC…….Total Plate Count

TVC……Total Viable Count

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Meats including beef chevon and mutton and their related products are both significant sources of micronutrients for human sustenance and a good supply of high-value animal protein worldwide (Lukáová *et al*., 2014; Valsta *et al*., 2005). As families and individuals' incomes improve in Sub-Saharan Africa (SSA), so does the intake of meat and fish. According to Desiere *et al*. (2017), the trend of SSA consumers eating meat and fish is neither stabilizing nor slowing down. In the wealthy world, an adult can eat up to 80 kilograms of meat annually, compared to the developing world's annual average of 30 kg (Halweil, 2008). Foodborne illnesses can affect humans if they consume contaminated meat, meat products, milk, seafood or eggs that have been exposed to biological hazards like bacteria, viruses and parasites (Foley & Lynne, 2007; Hosseini & Haslberger, 2016). According to Newell e*t al*. (2010), the prevention, reduction and control of pathogens contamination on meat should begin at the farm level of the animal until slaughter at the abattoir to improve the wholesomeness of meat. Gastroenteritis, which is distinguished by abdominal discomfort, nausea, vomiting, diarrhea and headache, are unique signs and symptoms of salmonellosis in humans (Foley & Lynne, 2007; Nichols *et al*., 2022). Infection with pathogenic *E. coli* typically results in very bad diarrhea (Yang *et al*., 2017). Productivity in livestock reduces if they are diseased, leading to observed decreased feed conversion efficiency and growth rate which may result in higher fatality rates as well (Osei-Sekyere, 2014). Most bacteria like *E. coli* and *Salmonella* live in the gastro-intestinal tract (GIT) of humans and animals, especially in the stomach, small and large intestines (Adzitey *et al*., 2020; Yang *et al*., 2017). Bacteria end up contaminating the environment when contents of the GIT are released out of the animal through defecation, evisceration among others (Adzitey & Huda, 2021; Hosseini & Haslberger, 2016; Centers for Disease Control and Prevention (CDC), 2014).

A diverse number of bacterial prevalence including *Salmonella spp.*, *E. coli*, *Shigella spp.*, *Campylobacter spp.*, *Enterococcus spp.*, and *Pseudomonas spp.* have been isolated from carcasses and meat from slaughter houses at post-mortem, during dressing, evisceration and further processing of various meats. Contamination sources have been from hides and rectum of the animal, butchering equipment like knives, the environment including hands of butchers and other meat handlers, and the floor of the slaughter area (Adzitey et al., 2020a; Hosseini & Haslberger, 2016). *Salmonella* and *E. coli* infections when detected in humans are normally self-eliminating, but when the infection symptoms are diagnosed to be intense, antibiotics are given for curing purposes (Adzitey *et al*., 2020a; Foley & Lynne, 2007). Therefore, antibiotics are used in meta-phylaxis and prophylaxis conditions for the cure of *Salmonella* and *E. coli* infections in both humans and animals or sometimes as growth promoters. But this can sometimes lead to antibiotic resistance (Osei-Sekyere, 2014; Adzitey *et al*., 2019; Adzitey & Huda, 2021).

1.2 Problem statement

Food contamination with biological hazards is a serious public health challenge globally. Such foods can cause food poisoning in humans. Interestingly, raw meat has been reported to be a source of human food poisoning (Cohen *et al*., 2007). This can happen if meat is contaminated and cross-contaminated by biological hazards such as bacteria from slaughterhouses and abattoirs as a result of the unhygienic slaughtering, dressing and handling of animals and their carcasses at slaughter houses and abattoirs (Adzitey *et al*., 2015; Hosseini & Haslberger, 2016; Adzitey & Huda, 2021). Furthermore, bacterial contamination of meat and its products is of safety concern when it comes to the shelf life of meat during production and storage. Several studies have revealed that meat from abattoirs, slaughter houses and meat sales points have been contaminated with different microbes including *Salmonella*, *E. coli*, *Enterococcus*, *Pseudomonas*, *Klebsiella*, *Campylobacter*, *Yersinia*, *Listeria*, *Shigella*, *Brucella*, *Streptococcus* and *Staphylococcus*

species (Adzitey *et al*., 2015; Hosseini & Haslberger, 2016; Ekli, *et al.*, 2019). Both *Salmonella* and *E. coli* infections are self-limiting; they become life-threatening in the aged, infants, immunocompromised patients, patient receiving inadequate medical attention, or if microbial load in the host exceeds the immune system controllable level of the patient (Foley & Lynne, 2007; Schroeder & Hilbi, 2008; Yang *et al*., 2017). Antimicrobials like trimethoprim, ceftriaxone, azithromycin and others are used for the treatment of *Salmonella* and *E.coli* infections in humans (Schroeder & Hilbi, 2008; Dsani *et al*., 2020). However, the continuous and improper usage of these antibiotics enables bacteria to develop mechanisms of sresistance against multiple drugs over time (Osei-Sekyere, 2014; Dsani *et al*., 2020).

1.3 Justification

In literature, it has been observed that many research has been carried out on the prevalence and antimicrobial resistances of *Salmonella* and *E. coli* on raw meat and meat products in Ghana. For example, Adzitey *et al*. (2015) in Techiman; Adzitey *et al*. (2020) in Tamale and Ekli *et al*. (2020) in Wa, all worked on the prevalence and antimicrobial resistance of *Salmonella* isolated from raw meats in Ghana. Also, the prevalence of *E. coli* and their antibiotic resistances have been reported by Yaffeto *et al*. (2019) in Cape Coast, Adzitey *et al*. (2021) in the Upper East Region, and many more in other parts of Ghana as well. However, such data is unavailable in Buipe and its environs. Also, earlier researches in Ghana especially in the study location, considered no or little seasonal variation in the prevalence of the bacteria in meats and their resistance against antibiotics.. Therefore, this study seeks to report on the occurrence and antibiotic resistance of *E. coli* and *Salmonella* in meats both in the dry and rainy seasons in Buipe town in the Savannah Region of Ghana.

1.4 Importance of this study

Findings from this research will give consumers of meat and butchers from Buipe the contamination levels of *Salmonella* and *E. coli* in beef, chevon and mutton in order to prevent or reduce any possible foodborne diseases through meat consumption. It will equally give medical practitioners, patients and livestock farmers an informed decision for the choice of antibiotics which are not resistant to infections caused by *Salmonella* or *E. coli* from meats in Buipe for a more accurate prescription and to prevent possible antimicrobial resistance. It will also serve as literature for future and further investigations on meat related food issues from the area and for Ghana and the world at large. In the long term, help contribute to achieve Goal 3 [(Good health and well-being) - Ensure healthy lives and promote well-being for all at all ages)] of "The UN 17 Sustainable Development Goals (SDGs)".

1.5 Objectives of study

1. To determine the occurrence of *Salmonella enterica* and *E. coli* in raw beef, chevon and mutton in Buipe during the dry and rainy seasons.
2. To determine the antibiotic resistance of *Salmonella enterica* and *E. coli* isolated from the raw beef, chevon and mutton in Buipe during the dry and rainy seasons.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Meat

The American Meat Science Association (AMSA) refer to meat as the skeletal muscles and associated tissues that come from mammals, birds, fish, reptiles and amphibians which are often collected for human consumption (Boler & Woerner, 2017). In vitro, laboratory-produced, lab-grown or artificial meat coming from animal-derived satellite or stem cells may also be considered meat, and the first "lab-grown" hamburger was prepared and enjoyed by a group of Dutch researchers in 2013 (Boler & Woerner, 2017). According to Liu *et al*. (2017), in China meat refers to all edible parts of an animal consisting of skeletal muscle and fat suitable for human consumption as food. In South Africa, meat is said to be the parts of a slaughtered animal which are basically meant for human consumption and had not pass through any further process rather than quartering, deboning, mincing or cooling, that do not change the original characteristics of the meat (Erasmus & Hoffman, 2017). In Ghana, Ohene-Adjei & Bediako (2017) referred to meat as the muscles, skin, fat and other tissues and edible offal from vertebrates used as food for human. Edible non-carcass constituents such as offal, liver, kidneys, lungs, uterus, tongue and others which are tissues and organs, can also be referred to as meat (Boler & Woerner, 2017). Meat is well known globally to contain high levels of proteins, amino acids and vitamins B12 which is not found in plant sources (Yafetto *et al*., 2019). Meat also have essential amino acids like lysine and tryptophan; the essential fatty acid, linolenic acid, all of which plants sources do not have (Akorli, 2012).

2.2 Classification of meats by habitat of animal

Meat can be classified according to the habitat from which the animal meat is sourced into three (Ohene-Adjei & Bediako, 2017) as follows:

1. Home-grown meat: from domesticated animals such as beef from cattle, chevon from goat, chicken from domesticated fowl and guinea fowl, mutton from sheep and pork from pig
2. Bush-meat: from all wild animals like rats, crocodiles, monkeys, snakes, birds, etc.
3. Water-body-associated meat: from fishes.

2.3 Home-grown meat types in Ghana

This is any meat that come from a domestic animal. Such meat include beef, chevon, chicken, mutton, pork, etc. (Ohene-Adjei & Bediako, 2017).

2.3.1 Beef

Beef is the flesh or meat from a matured cattle whilst veel or veal is the flesh of a young cattle (Ndlovu, 2015; Őrsi, 2015). Beef is well known to contain many nutrients such as proteins, many minor nutrients like vitamins: A, B6, B12, D and E, and minerals like Fe, Zn and Se. It is also known to have some level of poly-unsaturated fatty acids (PUFA) good for human health (Scollan *et al*., 2006). According to Aikins-wilson *et al*. (2015) and Dsani *et al*. (2020), beef is the second most consumed meat after chicken, whilst chevon comes third then pork fourth, followed by mutton which is less consumed by Ghanaians. But other studies by Osei-Asare & Eghan (2014) and Abraham *et al*. (2022)s put beef as the most consumed type of meat in Ghana.

2.3.2 Chevon

According to Scollan *et al*. (2006), chevon, also called 'goat-meat', is meat from the animal species goat. A study by Lijalem *et al*. (2015) showed that the protein quality of goat meat is higher than that of beef and mutton. Reports from Abraham *et al*. (2022) indicated that chevon was the most preferred meat for consumption after beef to most of the people of the Northern Regions of Ghana. With more attention and improvements placed on good husbandry and production practices, a full potential on the meat, economical and other non-market benefits can be derived from the goat (Adams *et al*., 2021).

2.3.3 Mutton

Meat or flesh from a matured sheep is known as mutton (Őrsi, 2015). Meat from sheep is sometimes also referred to as lamb (Warriss, 2000). Mutton serve as a good source of animal protein, fat, vitamins and minerals and for that matter form important component of many people's meals (Scollan *et al*., 2006; Adams *et al*., 2021; Abraham *et al*., 2022). The sale of meat from sheep serve as a source of income to members of beneficiary families in the Ghanaian community (Adams *et al*., 2021). Mutton is among the popular mesats in Ghana and in particular in the Northern regions whose consumption is with little or no taboos serving as a good potential for livestock farmers to 'haverst' lagre income from for their livelihood (Adzitey, 2013; Adams *et al*., 2021).

2.3.4 Chicken

Meat from the domestic fowl (*Gallus gallus domesticus*), is referred to as chicken (Smith & Smith, 2012; Dikeman & Devine, 2014). The live domestic fowl is also

called chicken (Őrsi, 2015). Like any other meat types, chicken is a good source of protein, vitamins and minerals for human health (Ivanova *et al*., 2016; Haunshi *et al*., 2022). Compared with other meats like beef, lamb and pork, chicken happen to contain more and high-qaulity protein, low total fat and low saturated-fatty-acids (SFA). It is therefore regarded as having good nutritional qualities for the mantenance of good heatlth in human (Ivanova *et al*., 2016). A report from Aikins-wilson *et al*. (2015) puts chicken as the number one most consumed meat in Ghana.

2.3.5 Guinea fowl meat

It is meat from the poultry species, the domestic guinea fowl (*Numida meleagris*) (Teye and Adam, 2000; Moreki, 2009;). Guinea fowl meat has a gamey taste and cent than that of chicken and is therefore more favoured in consumption and attract higher price compared to chicken meat (Moreki & Seabo, 2012). Although guinea fowl meat is less tender than chicken (Haile, 2022), the meat has higher unsaturated fatty acids (mono-unsaturated fatty acids – MUFAs and poly-unsaturated fatty acids – PUFAs) and essential amino acids than chicken and good for a healthier coronary diet diseases prevention (Tlhong, 2008; Haile, 2022). Guinea fowl meat has low levels of total fat, high proteins, and high general mineral content compared to chicken (Tlhong, 2008). Conspiciously, the meat appear darker than that of chiken (Tlhong, 2008; Teye and Adam, 2000). Guinea fowl meat has both cultural and social benefits, seen as a delicacy by many Ghanaians, especially to people of the northern expedition who consume it most during traditional festivities like the fire festival and annual celebbrations including the two Islamic Eids and the Christian celebration, Chrismas (Issaka & Yeboah,

2016). Guinea fowl and other poultry production give both meat employment opportunities to a good number of rural household members in Ghana (Mensah-Bonsu & Rich, 2010).

2.3.6 Pork

Pork refer to flesh from a pig, who is also known as swine, hog or *Sus scrofa* (Őrsi, 2015). Pork is globally known and consumed as a source of animal food rich in proteins, fats and other micronutrients essential for good human health (Zhang *et al*., 2022). The flavor and overall eating and acceptable quality of pork depends on its fat level; the higher the grade of pork, the more fat contained in the meat (Zhang *et al*., 2022). It is worthnoting that, higher levels of some PUFAs are negatively correlated to pork general flavor and acceptance to consumers whilst others (PUFAs) too are postively correlated to carcass flavor and as well its acceptability (Cameron *et al*., 2000; Zhang *et al*., 2022). It is the broadly eaten meat product globally (Banson *et al*., 2014) and the last but one consumed meat type in Ghana (Aikins-wilson *et al*., 2015). Consumers in Ghana do not only eat the skeletal meat of pigs but most also eat the offal (preferably the liver and stomach) as a delicacy (Weldam *et al*., 2015). Most offal consumers see such meat more as a delicacy and its nutritional value but not cost of that meat part (Weldam *et al*., 2015; Felix *et al*., 2016). In the report of Weldam *et al*. (2015), even though pork attract good market price, this is very low compared to other meats like beef, chicken and mutton due to a general dislike and religious taboos assocciated with the animal and its meat in Ghana. Tribally, Ashantis, Akans, Frafras and Fantes in that order are the majority of people who consume pork more than others in Ghana (Sekyere & Adu, 2015). The market of pork is affected mostly in overweighty or fatty pigs and prices also

fluctuate periodically due to frequent outbreak or incidences of the untreatable african swine (Sekyere & Adu, 2015).

2.4 Sources of contamination of raw meat

Tissues of healthy animals are typically sterile (Warriss, 2000). Many different bacteria or microbes can contaminate raw meats mainly at slaughter and dressing levels (Hosseini & Haslberger, 2016). Raw meat including beef, chevon, chicken and other related meat types displayed for sale at many retail shops and supermarkets are frequently contaminated with a diversity of microbes like *E. coli, Salmonella, Klebsiella, Listeria, Pseudomonas, Staphylococcus* among others (Ali *et al*., 2010; Iroha *et al*., 2011). Source of contaminations are usually on meat and meat surfaces, butchering equipement like knives, aprons, etc. and on surfaces of the slaughter surrounding environment like butchering tables, walls, etc. (Ali *et al*., 2010; Iroha *et al*., 2011; Hosseini & Haslberger, 2016). Air and aerosol droplets in less hygienic slaughter environments are also a source of meat contamination (Heuzenroeder, 2000; Warriss, 2000). Usually, contamination can occur horizontally between contaminated meat and that of wholesome meat especially in a filthy abattoir (Bughti *et al*., 2017). Studies indicated that the peripheral lymph nodes of infected meat animals can always habour a number of pathogenic bacteria like *Salmonella* (Koohmaraie *et al*., 2012; Olafson *et al*., 2016). Investigations also made on the blood of cattle exposed to infected biting insects revealed the prevalence of *Salmonella Senftenberg* (Olafson *et al*., 2016). Also, the hide of meat animals habour many biological hazards like *Salmonella* that can contaminate meat during slaughter and dressing of carcasses (Koohmaraie *et al*., 2012; Olafson *et al*., 2016). Again, animals droppings or waste contain high levels of bacteria which

contamite the environment, especially the slaughter and dressing floor or area of carcasses (Economou & Gousia, 2015; Adzitey *et al*., 2020). Unsterilized butchering equipment such as knives, utensils and weighing balances, unhygienic working dresses like aprons, hair gears and the hands of personnel including meat inspectors and butchers at abattoirs and meat shops are sources of pathogenic microbes that can contaminate meat (Hosseini & Haslberger, 2016; Adzitey & Huda, 2021). Infected and condemned carcasses and carcass parts or trimmings at the abattoir contains high levels of microbes and can be serious sources to cause contamination and cross contaminations of wholesome carcasses and meats if not proper handled or disposed (Roberts *et al*., 2009). Biofilms formed both in the air and on floors of slaughter houses and abattoirs are also considered hgh source of pathogenic bacterial contamination on meat (Ghidini *et al*., 2022). Water from unhygienic sources could also be a serious source of mircrobial contamination when used for washing carcass in the production line of meat in slaughter houses (Warriss, 2000). The soil serves as another source where some microbes like *Salmonella* habours in (Silva *et al*., 2014).

2.5 Pathogenic bacteria found on raw meat

There are many biological hazards particularly pathogenic bacteria that have been isolated from meats of all types from abattoirs and slaughter slabs or houses including but not limited to *Staphylococcus, Pseudomonas, Yersinia, Clostridium, Campylobacter, Streptococcus, Shigella, Salmonella, Listeria, Escherichia coli*, etc. (Adzitey *et al*., 2015; Lijalem *et al*., 2015; Hosseini & Haslberger, 2016; Ekli, *et al*., 2019; Yafetto *et al*., 2019; Dsani *et al*., 2020; Adzitey & Huda, 2021). Most of these microbes are normal flora or commensals haboring the intestinal tract of

the GIT of both human and animals. Such microbes include bacteria like *Proteous* and *E. coli* (Van Den Bogaard & Stobberingh, 2000; Yang *et al*., 2017). The pathogenic bacteria usually possess certain virulent properties like the production of toxins, spores formation, all in the case of *Clostridium perfringens*, and above all the ingestion of lethal doses by a host will cause infection in a healthy animal by invading the GIT (Li *et al*., 2016). They become pathogenic to human and animals epecially in the aged, younger ones and those whose immune system have been compromised (Foley & Lynne, 2007; Yang *et al*., 2017).

2.6 Foodborne diseases and zoonoses

A foodborne disease, also known as food poisoning or food illness, is any disease whose symptoms manifest from the eating of contaminated food containing biological pathogens like viruses, bacteria, protozoa and parasites, chemical substances like toxins and other heavy metals, and physical hazards (Adley & Ryan, 2016; Riemann & Cliver, 2006; Todd, 2014). Diseases that are caused by biological agents or pathogens from animals to human and the vice versa, either directly or indirectly, are referred to as zoonotic diseases (Adley & Ryan, 2016). According to Todd (2014), some zoonotic diseases can be acute or chronic in the manifestations of their signs and symptoms in the host, and can also be endemic, that is, infecting the host and occuring almost always in a particular or specific location; epidemic, occuring at many different locations; or pandemic if the disease prevalence is widespread across nations and continents, almost globally. Studies by Riemann and Cliver (2006), Todd (2014), Adley and Ryan (2016) and Addy *et al*. (2020) reported that zoonoses can be caused by bacteria including *Salmonella, Clostridium perfringens, Vibros, Escherichia coli. Campylobacter jejuni, Yersinia,*

Listeria, Clostridium botulinum, Staphylococcus, Bacillus cereus, Cronobacter, Mycobaterium, Brucella; viruses including Avian influenza (AI), Hepatitis A and E, NoV (Norwalk-like virus), Rotavirus, Poliovirus, Astrovirus; protozoa include *Cryptosporidium, Cyclospora, Toxoplasma, Entamoeba, Gardia*; parasites comprising helminths include nematodes (*Trichinella, Anisakis, Ascaris*), trematodes (*Opisthorchis, Paragonimus, Fasciola, Clonorchis*) and tapeworms or cestodes (*Echinococcus, Taenia, Diphyllobothrium*).

2.7 Food safety and hygiene

The requirements and precautions needed to guarantee the safety of food from manufacturing to consumption is known as food hygiene (Kamboj *et al*., 2020). According to Green and Kane (2014) and FDA-Ghana (2020), the concept of an effective Hazard Analysis Critical Control Point (HACCP) is required to achieve a good food safety and hygiene. Certain chemical contaminants like agrochemicals including pesticides and fertilizers in vegetables and fruits, antibiotic residues and other heavy metals like cadmium, mercury and lead in animal tissues during animal rearing (Nerín *et al*., 2016) or pathogenic microbial contaminants like bacteria in animal tissues from unhygienic husbandry practices during livestock production or at farm level (Beach *et al*., 2002; Gallo *et al*., 2020), all need to be detected at the raw material production stage with effective HACCP for possible elimination or minimal contamination to prevent their harm to man in final product as food (Nerín *et al*., 2016). Also, Beach *et al*. (2002) and Nerín *et al*. (2016) both reported that contamination of food (meat/carcass) can occur during transportation if the means of transport is contaminated with any sort of hazard. According to Botta *et al*. (2020), recommended, permitted and effective but not harmful levels of

cleaning and sanitizing agents and methods are employed in food processing to eliminate or control microbial contamination. Certain metals and glassware need to be avoided entirely as cleaning agents and/or equipment in the process of cleaning in food processing as they can leave harmful contaminants in the food (Nerín *et al.*, 2016). Apply only the amount of heat that is permissible in food processing to prevent the creation of certain dangerous substances such as chloropropanols, acrylamide, furans, polycyclic aromatic heterocycles (PAHs- which can be polycyclic aromatic hydrocarbons (PAH) or heterocyclic aromatic amines (HAA), nitrosamines, on food which are all deleterious to human (Nerín *et al*., 2016). Food packaging materials have been known to contain some levels of toxic migrants in such materials; about thirty (30) known different plastic materials contain varying degrees of these migrants which move out and mix up or contaminate with food packaged in them (Lau & Wong, 2000; Arvanitoyannis & Bosnea, 2004). As a result, the inclusion levels of such migrants which come in the form of monomers, additive plastics and oligomers of polymeric packaging materials of plastics are legislated and regulated by bodies like the USA (United States of America) FDA (Food and Drugs Administration) to assure consumers of high safety and a long shelf life of packaged food products (Lau & Wong, 2000). During storage, it is expected that the quality, safety and shelf life of a food product are maintained under legislated recommended conditions of temperature and humidity depending on properties of the packaging material and food type in order not to alter the organoleptic status of the product (Nerín *et al*., 2016).

2.8 Foodborne diseases' effects on public health

It is still hugely unclear the exact extent of havoc foodborne pathogen-related diseases have brought to man, and statistics on their burden is only available for some few industrialized nations and on a few pathogens as well (Käferstein, 2003; Newell *et al*., 2010). The situation is even worse in developing nations where data is scarcely taken and the public there only get to know how dreadful epidemics occur in the media (Käferstein, 2003). It is reported by Odeyemi (2016) that there have been more than 250 sources of foodborne illnesses known so far worldwide. It is reported by Hoffmann *et al*. (2015) that 1 person from every 6 people get infected with foodborne diseases in the USA annually. *Campylobacter* is said to be ranked as the number one leading cause of diseases by foodborne pathogens in the USA, with 17.8 cases in 100,000 people (Delahoy *et al*., 2023), whilst in the UK foodborne norovirus is reported to be the leading cause of foodborne illnesses (Holland *et al*., 2020). But in a worldwide view, norovirus cause the greatest number of foodborne diseases, then *Campylobacter, Salmonella* and *Listeria monocytogenes* in that order (Lee & Yoon, 2021). Globally, a reported 600 million cases of food contamination diseases occur resulting in about 420,000 deaths annually (Lee & Yoon, 2021). On the contrary, it had been stated by the USA that in the world, about 76 million cases, with 325,000 hospitalizations and 5,200 deaths from foodborne infections occur every year (Buzby & Roberts, 2009). In USA, the FoodNet (Foodborne Diseases Active Surveillance Network) reported in 2021 that foodborne illnesses from some 15 states for that year was 22,019 cases, resulting in 5,395 hospitalizations and 153 deaths (Delahoy *et al*., 2023), whilst in general in the USA, about 48 million people are infected, with 9.4 million of these cases been pathogen specific, resulting in 55,961 hospitalization and 1,351 deaths

due to foodborne infections only (Hoffmann *et al*., 2015). In the United Kingdom (UK) it is estimated that the annual human deaths due to foodborne infections stands at 180 deaths, with many of these recorded deaths in the elderly of over 75 years (Holland *et al*., 2020). Research on the financial impact of foodborne infections differ greatly, whilst certain studies focus on the impact of one disease causing agent, others seek to compute for many others within a nation (Buzby & Roberts, 2009). In 2013, it costed a total of US$15,534,647,106 trillion as economic burden from the infection of some 15 pathogen specific illnesses due to foodborne in the USA (Hoffmann *et al*., 2015).

2.9 Controlling the incidence of foodborne infections

To control foodborne illnesses, education, expertise in medical procedures, surveillance, laboratory research, hazard communication and handling of hazards are essential (Stein *et al*., 2010). After diagnosing the cause of disease outbreak, identifying the population at risk and doing a surrounding and further scientific analysis of the disease, execute the control and averting measures (Stein *et al*., 2010). Control measures for a confirmed disease outbreak include: control of the source of the food or premise causing the infection by taking away such food from the public domain, changing the method of such food manufacture or process, and banning or ceasing the location or food from operation or sales; control of the disease spread in situations the contaminated items cannot be withdrawn from the market but the disease spread can be reduced (Stein *et al*., 2010).

2.10 Coliform and Enterobacteriaceae

Coliform refers to a group of bacteria who are not-spore-producing, gram-negative bacilli and are capable of fermenting lactose to produce lactic acid and gas at incubation temperatures of 35±2ºC between 1 to 2 days (Parr, 1939; Halkman & Halkman, 2014). The first known members of this group of bacteria was *Klebsiella pneumoniae* (Parr, 1939). The group comprises the following four major genera: *Citrobacter, Enterobacter, Rauoltella* and *Klebsiella* (Halkman & Halkman, 2014; Li *et al*., 2014). They are found naturally everywhere in the environment including water, in soils, on plants, in foods, in human and animal wastes (Gordon, 2001). Some of these coliforms naturally inhabit the intestinal compartment of warm-blooded animals (Halkman & Halkman, 2014). Coliforms are usually used as indicators for lack of good hygiene or sanitation practices (Halkman & Halkman, 2014; Li *et al*., 2014; Niyoyitungiye *et al*., 2020). Water from drains and taps used for irrigating vegetable farms in the Accra Metropolis examined indicated the presence of coliforms *like Salmonella, Shigella, Pseudomonas, E. coli, Staphylococcus, Klebsiella* and many others (Mensah *et al*., 2001), but tap water which contain chlorinated-treated water indicated lower levels of fecal coliforms than drain or river water (Mensah *et al*., 2001; Niyoyitungiye *et al*., 2020). Fecal coliforms have been isolated from both vegetables at farms and markets, and from raw meats at slaughter slabs and meat retail shops (Mensah *et al*., 2001; Adjei *et al*., 2022). Many vegetable salad vended on streets have been reported to be contaminated by coliforms including *Bacillus, Shigella, E. coli* and *Salmonella* (Abakari *et al*., 2018). These coliforms are also found in ready-to-eat (RTE) foods, meat and drinking water, and on the hands of people handling these consumables (Adzitey *et al*., 2021; Dela *et al.*, 2023). Coliforms contaminants have also been

reportedly isolated form milk and dairy products (Adzitey *et al*., 2020a). *Enterobacteriaceae* is a family of bacteria which inhabits the intestinal floor of all warm-blooded animals, particularly, mammals, and the family is made up of about 53 genera (Unit *et al*., 2013). About 26 of these genera, including *Hafnia, Klebsiella, Escherichia, Proteus, Providencia, Salmonella, Serratia* among others, are considered to cause illnesses in man (Unit *et al*., 2013). Total viable count (TVC), total plate count (TPC), total bacterial count (TBC) or total coliform count (TCC) under any testing circumstances, provides a projection of the number of microbial cells in a substance (Halkman & Halkman, 2014; Rani & Mhlongo, 2023). Populations of microbes in food samples of the various micro-organisms are never the same as these are reliant on the properties and state/stage of the food type, the surrounding environmental conditions and the microbe involved (Halkman & Halkman, 2014). The infective level of microbial load of any type of microbe or pathogen on meat determines its infective dose to consumers when contaminated with meat (Rani & Mhlongo, 2023). It is known that TVC of pathogenic microbes cannot be avoided entirely on meat but can be minimized to acceptable limits or levels (Zweifel *et al*., 2005).

2.11 Microbial contamination of meat

Meat from the flesh of a healthy animal is always sterile (Warriss, 2000). Foodborne microbes isolated from contaminated meat in meat shops could many time be a result of horizontal or cross contamination from either the environment or other vertical animal carriers of these pathogens (Ali *et al*., 2010). The unhygienic handling of meat during the production of meat and meat products goes a long way to increase the level of microbial contamination (Iroha *et al*., 2011;

Akorli, 2012; Adzitey *et al*., 2019). According to Rani and Mhlongo (2023), meat stored for longer periods, and meat transported for longer distances all under unhygienic conditions increase the amount of microbial contamination in it. Meat also stored under refrigeration with fluctuating temperature conditions leads to high microbial contamination and growth (Rani & Mhlongo, 2023). A study by Adzitey *et al*. (2019),Yafetto *et al*. (2019), Adzitey *et al*. (2020a), Adzitey *et al*. (2021) and Adjei *et al*. (2022) from Ghana revealed the contamination of meat with total bacteria including *Staphylococcus spp, Klebsiella spp, Streptococcus spp, Salmonella spp, E. coli spp, Norcadia spp, Bacillus spp, Citrobacter spp, Pseudomonas spp, Enterococcus spp, Shigella spp, Proteus spp, Enterobacter spp, Campylobacter spp,* etc. All these bacteria cause FBDs including food poisoning (Warriss, 2000). Fungi isolated from raw meat include *Penicillium spp, Fusarium spp, Aspergillus spp, Candida spp, Rhodotorula spp*, (Yafetto et al., 2019). Viruses involved in meat contaminations mostly the RNA type, is the *Orthohepevirus* of the family *A/Hepeviridae,* also known as the Hepatitis E virus – HEV (Velebit *et al*., 2015). Aside HEV, Velebit *et al*. (2015) added other viruses like Rotaviruses, Rabies virus, Dengue virus among others as been of zoonotic importance. The prion responsible for Transmissible Spongiform Encephalopathy or Bovine Spongiform Encephalopathy infection in cattle (Hueston, 2013; Todd, 2014), whilst scrapie sheep and goats (Riemann & Cliver, 2006) have been contaminated in meat and bone meals (MBMs) and can zoonotically lead to the Creutzfeldt–Jakob Disease (CJD), Kuru, or Gertsmann-Straussler-Scheinker syndrome in human (Riemann & Cliver, 2006; Espinosa *et al.*, 2011; Hueston, 2013; Todd, 2014). Protozoal pathogenic microbes detected in contaminated raw meat which can cause deadly running stomach include *Cryptosporidium parvum, Toxoplasma*

gondii, Giardia lamblia, Entamoeba histolytica (Shaltout, 2000). Adult parasites and/or their eggs of zoonotic importance contaminated in meat include, *Taenia, Diphyllobothrium, Fasciola, Ecchinococcus, Trichinella, Ascaris, Anisakis, Opisthorchis, Paragonimus* and *Clonorchis* (Todd, 2014; Addy *et al*., 2020).

2.12 *Salmonella* genus

The genus *Salmonella* is a bacillus Gram-negative facultative aerobic-anaerobic bacteria which grow better within a temperature range of 5 ºC-45 ºC and at an average temperature of 37ºC within a medium of pH ranging from 4.0 to 9.0, but with 7.0 as the optimum (Gast & Porter, 2020). The dimensions of *Salmonella* rods are 2.0µm-5.0µm and 0.7µm-1.5µm, respectively for their length and width (Dougnon *et al*., 2017). *Salmonella* have survived in slurry conditions of more than 5% solids with temperature less than 10ºC for close to 286 days (Heuzenroeder, 2000). With its nutritional needs being simple carbon and nitrogen, when cultured in simple media like nutrient agar, stab-inoculated and sealed, and kept at normal room temperature, can remain viable for longer periods (Bowden *et al*., 2014; Gast & Porter, 2020). *Salmonella* naturally inhabits the GIT of animals and man and they have been found in the environment as well as food or water is presumed to be contamination with feces (Meuten, 2002). Historically as a coliform belonging to the *Enterobacteriacae* family, *Salmonella* is reported to have contaminated many raw, processed and RTE foods leading to FBDs in humans and animals (Foley & Lynne, 2007; Riemann & Cliver, 2006). *Salmonella choleraesuiz* was the first ever of this organism to had been identified from swine by American Bacteriologists Daniel Elmer Salmon and Theobald Smith in 1886 (Meuten, 2002; Lamas *et al*., 2018). Whilst some serovars derived their names according to their

host specification (e.g. Gallinarum for fowl, Abortusovis for sheep), others were named based on the geographical location from which the earliest-most strain was discovered, e.g. *Salmonella* Dublin (Meuten, 2002). According to Brenner *et al*. (2000), Meuten (2002), Coburn *et al*. (2007) and Foley and Lynne (2007), some over 2,000-3,000 serovars of *Salmonella* are known. Historically, White's work in 1926 and that of Kauffmann in 1941 whose analyses both became the Kauffmann-White Scheme, use the relationship between the lipopolysaccharide layer (somatic or O antigen) and the filamentous flagella protein, flagellin, (H antigen) for the classification of *Salmonella* serovars (Brenner *et al*., 2000; Meuten, 2002; Foley & Lynne, 2007; Gast & Porter, 2020).

2.12.1 *Salmonella* species grouping based on serotyping

Genomically, *Salmonella* spp are clssified based on the DNA-DNA hybridization relatedness of strains being greater than or equal to 70% (Meuten, 2002). Based on the O and H antigens differences and the genomic relatedness of strains, some serovars share a great level of similarities compared to others thereby giving rise to the division into two broad species from the *Salmonella*; *Salmonella enterica* and *Salmonella bongori* (Meuten, 2002; Tindall *et al*., 2005; Foley & Lynne, 2007). But according to Su and Chiu (2006), a third and new species by name *Salmonella subterranean* was discovered in 2005 with the expectations that the CDC in future could add it to their system. Almost all (above 99%) the serovars are classified into the *Salmonella enterica* species in which all the pathogenic serovars to humans are contained (Foley & Lynne, 2007; Grimont & Weill, 2007). A collaborative body with WHO, Centre for Reference and Research on *Salmonella* of the Pasteur Institute in Paris France, charged for the updating of the Kauffmann-White Scheme

in 2004 indicated reports of 2,541 *Salmonella* serovars known in history (Su & Chiu, 2006). As *Salmonella ecterica* species have affirnity to humans and other warm-blooded animals like birds and terresterial mammals (Meuten, 2002; Lamas *et al*., 2018;), *Salmonella bongori* are mostly associated or found in cold-blooded species of animals like fishes and reptiles like snakes, lizards, tortises, etc. (Fookes *et al.*, 2011). *Salmonella bongori* and non-*enterica* subsp. usually do not invade man and other warm-blooded animals due to their lack of or altered disease causing features on their bodies (Lamas *et al*., 2018).

2.12.2 *Salnonella enterica* subspecies

Six distinct subscpecies named with roman numerals are known (Meuten, 2002; Lamas *et al*., 2018), which include;

- I for *Salmonella enterica* subsp. *enterica*
- II for *Salmonella enterica* subsp. *salamae*
- IIIa for *Salmonella enterica* subsp. *arizonae*
- IIIb for *Salmonella enterica* subsp. *diarizonae*
- IV for *Salmonella enterica* subsp. *houtenae*
- VI for *Salmonella enterica* subsp. *indica*

Subspecies IIIa and IIIb originated from III for *arizona.* Also, V is for *Salmonella bongori* which have few serotypes (about 22) and uncommon to human infections, usually infects infants of less than 3 years and immunocompromised people (Lamas *et al*., 2018) and the symbol V assumed to prevent possible disorder with serotypes epithets of *Salmonella enterica* subsp. *enterica* (Grimont & Weill, 2007). In terms of scientific naming, it is worth noting that serotypes are known not to be

species and for that matter do not have to be italicized, e.g., *Salmonella enterica* subsp. *enterica* serovar Dublin, in short *Salmonella* serovar Dublin. Again, as was legislated in 2005 by the Judicial Commission of the International Committee on the Systematics of Prokaryotes (Tindall et al., 2005), specifically, serovars of *Salmonella enterica* subsp. *enterica* which covers over 99.5% (about over 1,500 serotypes) of *Salmonella* isolates that cause over 99% of Salmonellosis in man (Lamas *et al*., 2018), are designated with names, mostly of geographical recognition (e.g. *Salmonella panama*); others rather than the subsp. *enterica* and *Salmonella bongori* are given the O:H ratios e.g. *Salmonella enterica* subsp. *diarizonae* serovar 61:(k):1 *Salmonella bongori* 12419 (Grimont & Weill, 2007; Meuten, 2002). Table 2.1 shows the differential characteristics of *Salmonella* species and subspecies and the number of serovars per species and subspecies as at 2007 is presented in Table 2.2.

Table 2.1: Differential characteristics of *Salmonella* species and subspecies

Species			***S. enterica***				***S. bongori***
Subspecies	*enterica*	*Salamae*	*Arizonae*	*diarizone*	*houtenae*	*indica*	
Characters							
Dulcitol	+	+	-	-	-	d	+
ONGP(2h)	-	-	+	+	-	d	+
Malonate	-	+	+	+	-	-	-
Gelatinase	-	+	+	+	+	+	-
Sorbitol	+	+	+	+	+	-	+
Growth with KCN	-	-	-	-	+	-	+
L (+) – tartrate [(a)]	+	-	-	-	-	-	-
Galacturonate	-	+	-	+	+	+	+
γ-glutamyltransferase	+[(*)]	+	-	+	+	+	+
β-glucuronidase	D	D	-	+	-	d	-
Mucate	+	+	+	-(70%)	-	+	+
Salicine	-	-	-	-	+	-	-
Lactose	-	-	-(75%)	+(75%)	-	d	-
Lysed by phage O1	+	+	-	+	-	+	d
Usual habitat	Warm-blooded animals		Cold-blooded animals and environment				

(a)= d-tartrate, (*) = Typhimurium d, Dublin-, + = 90% or more positive reactions, -= 90% or more negative reactions, d = different reactions given by different serovars.

Source: Grimont & Weill (2007).

Table 2.2: Number of serovars per species and subspecies as at 2007

Salmonella species	Numbers
Salmonella enterica	2,557
Salmonella enterica subsp. enterica	1,531
Salmonella enterica subsp. salamae	505
Salmonella enterica subsp. arizonae	99
Salmonella enterica subsp. diazizonae	336
Salmonella enterica subsp. houtenae	73
Salmonella enterica subsp. indica	13
Salmonella bongori	22
Total (genus *Salmonella*)	2,579

Source: Grimont & Weill (2007)

2.12.3 *Salmonella* infections in human and animals

According to Heuzenroeder (2000); Uzzau *et al*. (2000); Meuten (2002), Singh (2013) and Demirbilek (2018), on the basis of host, *Salmonellae* are classified into three categories based on their capacity to invade on a range of hosts. Such hosts groupings include;

1. Unrestricted hosts serovars: a wide number of hosts for serovars like *S.* Enteriditis or *S.* Typhimurium can infect such hosts, infecting almost all animals, leading to minor intestinal illnesses persisting with no serious signs.

2. Hosts restricted: they host serotypes like *S.* Abortusequi, *S.* Typhi and *S.* Gallinarum respectively for horses, human and poultry which are limited only

to a particular host, resulting in deadly systemic infections, with the capacity to multiply in fetuses in the hosts.

3. Host adapted: the group contains serovars including *S.* Cholerasuis and *S.* Dublin for porcine and bovine respectively which can accidentally be found in certain hosts rather than their usual and adapted hosts.

From the over 2,500 serotypes of *Salmonella* identified so far, just around 50 serovars are pathogenic to either man or animals, many of which come from the subsp. *enterica* (Uzzau *et al*., 2000). Some top ten ubiquitous *Salmonella* serovars isolated in the USA in the year 2005 by CDC for causing human infections are *S.* Heidelberg, *S.* Saint-Paul, *S.* Typhimurium, *S.* Javiana, *S.* Muenchen, *S.* Enteriditis, *S.* Braenderup, *S.* Montevideo, *S.* I 4,[5]12:i and *S.* Newport (Foley & Lynne, 2007). According to a review study by Coburn *et al*. (2007), *Salmonella* infection manifests in any of these four main syndromes: bacteremia or septicemia; enteric or typhoid fever; asymptomatic or chronic; and enterocolitis or diarrhea. Meanwhile Uzzau *et al.* (2000) indicated that the organism manifests its infection with three syndromes as septicemia, abortion and enteritis. Many serovars infections result in gastro-enteritis with diarrheal symptoms in very acute form as they colonize the intestinal region than the systemic form of the syndromes in both humans and animals (Uzzau *et al*., 2000; Coburn *et al*., 2007). A 25-year data from 1985-2009 of the Microbial Diseases Laboratory in California, USA, analyzed by Abbott *et al*. (2012) indicated that non-intestinal Salmonellosis by subspecies II-IV were most invasive causing diseases with the systemic or bacteremia syndrome, and isolates were from the blood, cervix, urine, cerebrospinal fluid, respiratory

tract, wounds, bile and abscesses of patients. *Salmonella enterica* subsp. *indica* (VI) and *Salmonella bongori* are normally less found in in humans (Abbott *et al*., 2012). While *Salmonella* Pullorum cause pullorum disease, a sudden systemic infection of young chicken or poults, *Salmonella* Gallinarum cause fowl typhoid, a chronic but can also be acute bacteremia infection in older birds, then *Salmonella* Enteriditis could systemically be transmitted into an egg during its formation in an infected hen, *Salmonella enterica* subsp. *arizonae* are also trans-ova transmitted resulting in the manifestation of neurological signs, reduced egg laying mostly in breeding turkeys infection (Demirbilek, 2018; Gast & Porter, 2020). Some top *Salmonella* serovars identified to cause infections in domestic animals are summarized in Table 2.3 by Heuzenroeder (2000), Foley and Lynne (2007) and Demirbilek (2018).

Table 2.3: *Salmonella* serovars identified to cause infections in domestic animals

S/N	Animal species	*Salmonella* serotypes
1	Domestic fowl	Pullorum, Gallinarum, Enteriditis, Infantis, Typhimurium, Kedougou, Montevideo, Senftenberg, Menston,Heidelberg, Kentucky, Hadar, some subsp. *arizonae*
2	Cattle	Dublin, Typhimurium, Mbandaka, Anatum, Cerro, Newport, Agona, Infantis, Montevideo, Muenster, Kentucky, Enteriditis
3	Pigs	Choleraesuis, Typhimurium, Derby, Enteriditis, Typhimurium-Copenhagen, Heidelberg, Agona, Senftenberg, Infantis, Mbandaka, Worthington, Anatum,
4	Sheep	Abortus ovis, Dublin, Typhimurium, Derby, Arizonae (O61: k:1,2,7), Montevideo.
5	Turkeys	Hadar, Senftenberg, Heidelberg, Saintpaul, Typhimurium, Agona, Montevideo, Muenster, Schwarzengrund, Worthington
6	Ducks	Typhimurium, Enteriditis, Livingstone, Nagoya, Hadar, Virchow, Give, London, Indiana, Wangata, Oregon, Lille, Infantis, Panama,
7	Horses	Abortus equi. Typhimurium, Anatum, Rostock, Hato, Meleagridis, Montevideo, Virchow, Good, Infantis, Newport, Vaertan, Derby
8	Cats and Dogs	Typhimurium, Enteriditis, Havana, Infantis, Derby, Javiana, Anatum, Agona, Haifa, Berta, Adelaide, II sofia, Thompson, Montevideo, Seftenberg, Saintpaul, Ohio, Gloucester, Cerro, Weslaco, Gaminara

2.13 *Escherichia coli*

A Bavarian microbiologist and pediatrician from Munich Germany, Theodor Escherich, in 1885 was working on some infants' mortalities when he first identified the microorganism, *Bacterium coli commune* in the feces of the neonates, in which the bacterium was later designated *Escherichia coli* (*E. coli*), in recognition of the founder (Taj *et al*., 2014; Méric *et al.*, 2016), and *coli* because it was found to be harboring the *colon* (Shulman *et al*., 2007; Tajs *et al*., 2014). A similar microbe which was known to be causing dysentery, *Bacillus dysentericus*, was in 1897 discovered by the Japanese bacteriologist, Kiyoshi Shiga, then renamed after him as *Shigella dysenteriae* (Blount, 2015), with four subspecies as *Shigella boydii, Shigella dysenteriae, Shigella flexneri* and *Shigella sonnei* (Belotserkovsky *et al*., 2018). It is a common commensal in the microbiota of the gut of almost all warm-blooded animals and invariably the most intensively studied and well understood microorganism on earth (Taj *et al*., 2014; Blount, 2015).

E. coli is a Gram-negative facultative and anaerobic non-spore forming rod-like bacteria belonging to the family Enterobacteriaceae, and can be flagellated or non-flagellated with fimbriae for adhesion to their host surfaces, together with a body dimensions of 0.25µm-1.0 µm width and around 2.0 µm long (Unit *et al*., 2013; Taj *et al*., 2014; Smith & Fratamico, 2021). They survive at optimum temperatures of 37^{o}C (Fotadar *et al*., 2005; Taj *et al.*, 2014), but some mutant pathotypes have been recorded to grow up to a maximum of 49^{o}C and can reproduce while those that grow at highest temperatures of 53^{o}C do not reproduce (Fotadar *et al*., 2005). At acidic environments of low pH values of below pH 2.0,

E. coli employ metabolic as well physiological mechanism of resistance to enable it survive (Kanjee & Houry, 2013). In a similar study by Hayes *et al*. (2006), *E. coli* multiplication rates are reduced at extreme pHs of 5-6 and 8-9 thereby activating defense mechanisms that sustain the pH balance within the bacteria. The bacteria survives good at an average pH of 7.6 (Hayes *et al*., 2006).

2.14 *E. coli* serotypes classifications

E. coli serotypes are grouped according to their O-antigens, which are considered as virulence factors, from their cell membrane lipopolysaccharides (LPS) (Debroy *et al*., 2011). Another method of classification was later realized to further subtype strains using a mixture of three main membrane antigens; O-antigen: capsule or Kapsel in German language, K-antigen: flagellar H-antigen (O:H:K), but due to labor demand and insufficient laboratories for dealing with the K-antigen, only the O:H combinations are now accepted as the 'gold standard' for their serotyping (Debroy *et al*., 2011). In the classification of *E. coli* and other prokaryotes genomically, the bacterial genomes are sequenced using the 16S rRNA amplicons by clustering; if the sequenced clusters amount to a similarity threshold of 97% or more, they are said to be from the same species; a 94% and above genes threshold similarity belong to the same genus (Batista *et al*., 2002; Lan *et al*., 2016). *E. coli* is said to be to be classified into six serogroups as Diffusely adherent *Escherichia coli* (DAEC), Enteroaggrasive *Escherichia coli* (EAEC), Enterohaemorrhagic *Escherichia coli* (EHEC), Enteroinvasive *Escherichia coli* (EIEC), Enteropathogenic *Escherichia coli* (EPEC) and Enterotoxigenic *Escherichia coli* (ETEC) (Croxen & Finlay, 2010; Jafari *et al*., 2012; Smith & Fratamico, 2021). They are also divided into T3SS-dependent serotypes (EPEC, EIEC and EHEC)

and non-T3SS dependent [DAEC, EAEC and ETEC (AIEC and STEAEC)] (Taylor *et al*., 2012).

2.14.1 Diffusely adherent *Escherichia coli* (DAEC)

They are so-called (DAEC) because they are known for having a peculiar ability of a diffuse attachment style with the aid of two adhesins, Afa/Dr DAEC and AIDA-I DAEC, on the epithelial surfaces of their hosts (Meza-segura & Estrada-garcia, 2016). As a diarrheagenic pathotype *Escherichia coli*, DAEC was known to be the sixth and last *E. coli* class in 1998 with varying serovars containing virulence genes, Afa/Dr strains, which are responsible for acute diarrhea in infants of less than six months recuring diarrhea in Mexican hospitals; in matured patients, they are identified to cause diarrhea in HIV-positive sufferers from Peru and other Latin Americans, but they could also cause any form of intestinal illness (Meza-segura & Estrada-garcia, 2016). Diarrhea from DAEC is usually watery and persistent especially from children (Cabrera-sosa & Ochoa, 2020); Afa/Dr DAEC strains are also known to cause urinary tract infections (UTIs) and health issues in gestating women (Servin & Servin, 2014).

2.14.2 Enteroaggrasive *Escherichia coli* (EAEC)

EAEC are defined by this name due to their peculiar ability of aggressively attaching to each other, even forcibly adding other different classes of *E. coli* into a biofilm in a design within the epithelial lining of the intestine (Elias & Navarro-garcia, 2016; Ekici & Dümen, 2019). EAEC is known to infect all age groups, from newborn babies to adults, especially those that are immunologically compromised like HIV positive patients in both developed and developing

countries (Taylor *et al*., 2012; Elias & Navarro-garcia, 2016; Ekici & Dümen, 2019). EAEC infection results in travelers' diarrhea and recuring slick stools which could sometimes be stained with blood or mucus of patients, and the bacteria is capable of burrowing through the thick mucus layer for attachment onto the intestinal surface with the aid of pAA, aggressive adherence fimbriae (AAF), AggR, SPATE Pic plasmids, flagella and other virulence factors (Croxen & Finlay, 2010; Smith & Fratamico, 2021). Generally, EAEC after establishing in the intestinal area leads to a build of biofilm with mucoid appearance, entero and cytotoxin production (Croxen & Finlay, 2010). There have been scanty data indicating that EAEC is an animal career pathogen consistently infecting human (Taylor *et al*., 2012).

2.14.3 Enterohaemorrhagic *Escherichia coli* (EHEC)

The EHEC is designated this name because their infections in humans results in profuse and hemorrhagic stools from patients, hemorrhagic colitis (HC) and as well hemolytic urinary syndrome (HUS) particularly in infants (Naylor *et al*., 2005). The bacteria is said to have the virulence plasmid factor, *vtx*, on it, enabling it to produce two types of verocytotoxins (VT); VT1 and VT2, in which VT1 is similar to that produced by *Shigella dysenteriae*, Shiga toxin (STX), and due to this the EHEC is sometimes referred to as Shiga toxin *E. coli* (STEC) (Naylor *et al*., 2005; Taylor *et al*., 2015). Coming with various variants including VTII, VTIIc, VTIId, VTIIe and VTIIf, the verocytotoxin V2, is noted for most infections of the bacteria to humans (Naylor *et al*., 2005). In the advanced world, toxins produced from bacteria are the basis for their classifications and not serotyping, thus they are usually referred to as verotoxin or shiga toxin generating *E. coli*

(VTEC or STEC) (Taylor *et al*., 2012). Cattle and other animals are said to be carriers or reservoirs of EHEC in many studies (Naylor *et al*., 2005; Aziz, 2018).

2.14.4 Enteroinvasive *Escherichia coli* (EIEC)

The EIEC, identified in 1971 (Smith & Fratamico, 2021) which is similar to the dysentery causing bacteria, *Shigella dysenteriae*, have certain plasmids, pINV (Jafari *et al*., 2012), like that of the Shigella enabling it to invade and multiply in the epithelia of the intestines leading to irritable infections to the GIT mucosa and submucosa within an incubation period of half a day (Grist *et al*., 2000). When infection occur, clinical manifestation includes temperature rise, stomach upsets, hemolytic and mucoid liquid diarrhea, painful micturition, differentiating EIEC infection to that of Shigella's dysentery, and all these symptoms are realized due to the interactions between the O antigens from both the EIEC and Shigella (Smith & Fratamico, 2021). Tentatively, patients that have diarrhea with white blood cells presence could be potential candidates of been infected with EIEC (Grist *et al*., 2000). Infections from EIEC reports are more in developing countries than the more industrialized world; only those who attend medical facilities taking care of patients with EIEC contract the pathogen from developed nations (Grist *et al*., 2000). Because of their relatedness, antibiotics used for the treatment of Shigella can also equally be used for the cure of EIEC infections as well (Jafari *et al*., 2012; Lääveri *et al*., 2018; Smith & Fratamico, 2021).

2.14.5 Enteropathogenic *Escherichia coli* (EPEC)

Being the first *diarrheagenic E. coli* (DEC) defined in 1982 (Mare *et al*., 2021), EPEC is a strain that have the capacity to attach and efface (A/E) the epithelial

surfaces of host intestines causing pathological damages but unable to generate Shiga toxins LT or ST enterotoxins (Mare *et al*., 2021; Smith & Fratamico, 2021). Their "hallmark of attaching-and-effacing" the host epithelial cells was first defined by Nataro and Kaper in 1998 (Nataro & Kaper, 1998; Mare *et al*., 2021). EPEC infection within some few hours results in sudden or prolonged slick diarrhea, vomiting, high body temperatures, loss of body moisture, notably in infants of less than 2 years and the infection can become complicated and persistent leading to hospitalization of patients. Reports indicate that EPEC is many times detected in mixed gastro-enteritis, and have been isolated in asymptomatic careers ae well (Mare *et al*., 2021). Clinical infections are normally self-eliminating but antimicrobials like fluoroquinolone, cephalosporins, penicillin and aminoglycosides are recommended in some cases for treating recuring stools and complications, in which many times there are reported incidences of EPEC exhibiting resistance against these antibiotics (Mare *et al*., 2021).

2.14.6 Enterotoxigenic *Escherichia coli* (ETEC)

ETEC been discovered in 1968 (Jafari *et al*., 2012), are known to possess virulence factor fimbriae, colonization factors (CF), enhanced by heat-liable (LT) or heat-stable (ST) enterotoxins to enable the bacteria establish in the GIT mucosa (Fleckenstein, 2013). The latest methods of naming indicates this feature as coli surface (CS) antigen even though earlier systems used to designate it as colonization factor antigen I (CFA/I) (Smith & Fratamico, 2021). ETEC is said to contain virulence characters including adhesins, fimbriae structures, CFs and other proteins to enhance its pathogenesis in the GIT and host particularity. ETEC is the major cause of diarrhea in infants and travelers' diarrhea in Asia and tropical

countries where there is limited access to health services and medications and low sanitary practices (Jafari *et al*., 2012; Taylor *et al.*, 2012). Yearly reported diseases from ETEC with stool relatedness is over 200m patients with nearly 75,000 mortalities basically infants from Sub-Saharan Africa and Latin America where unhygienic health practices are common at 3.5%-20.45% (Ferreira & Martinez, 2016).

2.15 Transmission of *Salmonella* and *E. coli*

Both *Salmonella* and *E. coli* are known to naturally be isolated from the GIT of human and animals and intensive relationships between animals and man go a long way to increase the transfer of these pathogens across species (Iovine *et al*., 2015), and their mode of transfer to human and animals infection is usually the fecal-oral (Cabral, 2010; Taj *et al*., 2014) even though Heuzenroeder (2000) and Warriss (2000) have reported transmission through the skin and nose respectively. According to Glaize *et al*. (2021), soil is a principal source from which *Salmonella* and *E. coli* can pollute fresh produce from plants and animals during production operations in the farm. *E. coli* and its moieties are used as indicators in the fecal pollution of water and the surrounding (Niyoyitungiye *et al*., 2020). Mensah *et al*. (2001) in their survey isolated *Salmonella* and other bacteria contaminating fresh vegetables and raw animal meat at Accra. Infections from *Salmonella* and *E. coli* are of public concern as they lead to many diseases including typhoid fever and urinary tract infections (Al-Zubaidy, 2020).

2.16 Human susceptibility to *Salmonella* and *E. coli* disease

People who stay in less hygienic environments and also the consumption of contaminated food products like meat, water, vegetables and other plant products make them very vulnerable to foodborne infections from *Salmonella, E. coli* and other microbes (Ekici & Dümen, 2019). Epidemiologically, both bacteria are known to have caused outbreaks of different infections like typhoid fever and hemolytic urinary syndrome (HUS) with varying magnitudes globally (Käferstein, 2003; Foley & Lynne, 2007; Taj *et al*., 2014). Infections from *Salmonella* (Foley & Lynne, 2007; Dougnon *et al*., 2017) and *E.coli* (Taylor *et al*., 2015; Aziz, 2018; Smith & Fratamico, 2021) are clinically observed in infants, the aged and immunologically suppressed patients globally with symptoms including diarrhea, vomiting, HUS or UTI, neurological problems, acute and chronic kidney incapacities, peritonitis etc.

2.17 *Salmonella* and *E. coli* occurrence in raw meat

Bacterial contaminations including *Salmonella* and *E. coli* in raw meats from different meat types have been reported (Ali *et al.*, 2010; Hosseini & Haslberger, 2016; Bughti *et al*., 2017) . According to a study by Cohen *et al*. (2007), meat at slaughter stages were contaminated with 1.6% *Salmonella,* 48.4% *E. coli*, 10.4% *Staphylococcus*, 7.2% *Clostridium perfringens* and 0.5% *Listeria monocytogenes*. A report by Adzitey *et al*. (2015) on 240 samples of beef and other similar products collected from Techiman, Ghana, indicated that, the prevalence of *Salmonella* was 57.08% and in a related survey, Adzitey *et al*. (2021) indicated that from 200 RTE meat samples examined from the Upper East region of Ghana, *E. coli* (80%) was detected in raw beef compared to 0% *E. coli* in some RTE meat.

An examination on the contamination of beef and mutton at slaughter houses by Hosseini and Haslberger (2016) at Tehran, Iran, showed that 28% beef and 13% mutton samples were *Salmonella*-contaminated whilst 30% beef and 14% mutton samples revealed to be *E. coli* positive.

2.18 *Salmonella* and *E. coli* prevention and control

According to Silva *et al*. (2014) and Adjei *et al*. (2022) , the one-health-concept is needed for the prevention of *Salmonella* and *E. coli* and other microbes from infecting human by reducing or restricting their transfer from food and related products, animals and the immediate surroundings. To produce safe meat for human consumption, there is the need to educate the public through meat handling professionals like veterinary personnel and other meat handlers (Adjei *et al*., 2022). For the prevention and control of food contamination and subsequent human infections from the two bacteria as well as others, an effective HACCP is also recommended (Green & Kane, 2014). For food contamination to be reduced or controlled, low storage temperatures are required, high temperatures are needed for hot foods; safe and standard packaging materials and recommended levels of chemical sterilizing and cleaning agents are recommended (Nerín *et al*., 2016). In an investigation at Mauritius, Heetun *et al*. (2015) indicated that raw poultry meat bought from open retail sources was highly contaminated with bacteria than meat that was gotten from cold meat channels. To prevent and/or control bacterial growth especially in meat, good hygienic practices need to be observed strictly; requiring standardized processing facilities and processing, use of sterilized meat processing equipment, use of high quality meat vans and other acceptable means for carting meat for storage, further processing and sale (Mensah *et al*., 2001;

Adzitey *et al*., 2020). The use of tap and other clean water sources which is devoid of microbial contamination for irrigating vegetables and other crops go a long way to prevent and control the level of *Salmonella, E. coli* and other bacterial infection through food in the long end (Mensah *et al*., 2001). Also, effective and the use of clean water and sanitizing chemicals for washing vegetables, the sale of vegetables in more sanitized environments in the market, availability of restrooms, improved personal hygiene of vegetable sellers/handlers all control and prevent *Salmonella* and *E. coli* transmission to man (Mensah *et al*., 2001). Vaccinating animals with lower doses of 10^3 bacterial etiological agents like *Salmonella* have caused immunity in birds and ruminants by the production of immunoglobulin (IgM) antibodies against any subsequent infections (Heuzenroeder, 2000). Antibiotics are used to prevent and control bacterial infections in both human and animals and animal products to promote public health but there have been increasing concerns on the resistance of these bacteria like *Salmonella* and *E. coli* to these antibiotics (Van Den Bogaard & Stobberingh, 2000).

2.19 Antibiotics

The start of the term 'antibiotic' was when Paul Vuillemin in 1890 found microbes producing their individual and varying metabolites, having opposing effects on other different microorganisms, *antibiose*, opposite to symbiosis, which could suppress the growth or cause death of the affected microorganism (Nicolaou & Rigol, 2017). Meanwhile, Maurer (2018) also indicated that 'antibiotic' is coined from Greek language to describe "*against life*". Depending on the antimicrobial effect, it could be *antibacterial*, acts on bacteria; *antifungal*, acts on fungi; *antiprotozoal*, acts on protozoa, etc. (Nicolaou & Rigol, 2017). It has been stated

by Singh *et al*. (2017) that ideally an antibiotic suppresses or destroys the multiplication of all detrimental microbes in a host no matter the predilection place of the microbe in the host, and not disturbing the microbiota in the GIT and the host own body cells. According to Maurer (2018), antibiotics act on the development of a microbe in 3 different forms*: bacteriolysis* – cause the death of the bacteria by breaking its cell wall; *bacteriostasis* – involves growth inhibition through protein limitation into the bacterium; and lastly, *bactericidal action* – cause bacterial death but not breaking that bacterium. According to Walsh (2003), some antibiotics act on Gram-positive whilst others act on Gram-negative bacteria, but some type act on both Gram-positive and Gram-negative bacteria; some also act on just a small number or class of bacteria and are known as narrow spectrum antibiotics, then those acting on many groups or classes of pathogenic bacteria are called broad spectrum antibiotics.

Antibiotics come in two distinct discovery lines; 'natural products', like tetracyclines, teicoplanin, penicillin, cephalosporins, erythromycin, aminoglycosides, which are all products of biological metabolites from microbes, and the second discovery line, 'synthetic products', are those that are not produced by nature but chemicals of synthetic origin, and such antibacterials include sulfa drugs, fluroquinolones, oxazolidinones, etc. (Walsh, 2003). Even though most of the 'natural products' are excess and by-products from bacteria and fungi, majority of antibiotics are metabolic waste from spiral-shaped fungi, actinomycetes and eubacteria, with actinomycetes generating many strong and different antibiotics (Maurer, 2018). Historically, many earlier scientists including Theodor Billroth (1829-1894), Sir John Scott Burden-Sanderson (1828-1905), John Tyndall (1820-

1893), Joseph Lister (1827-1912), Louis Pasteur (1822-1895) all made researches on discovering an antibiotic; but it was finally Alexander Fleming (1881-1955) who in 1929 was officially declared the discoverer of the first ever antibiotic known, Penicillin, and it was from the fungi, *Penicillium notatum*, afterwards known as *Penicillium chrysogenum* and now *Penicillium rubens* (Mohr, 2016). Other subsequent and notable 'natural product' antibiotics are listed in Table 2.4.

Table 2.4: Some antibiotics and their microbial source

Name of Antibiotic	Type of Microbe	Name of microbial source
Penicillin	Fungi	*Penicillium notatum*
Rugulin	Fungi	*Penicillium rugulosum*
Hirsutellone B	Fungi	*Hirsutella nivea*
Platensimycin	Fungi	*Streptomyces platensis*
Thiostrepton	Fungi	*Strep. (azureus, hawaiiensis, laurentii)*
Vancomycin	Fungi	*Nocardia orientalis*
Amphotericin B	Fungi	*Strep. nodosus*
Ansamycin/Rifamycin	Fungi	*Amycolatopsis rifamycinica*
Indanomycin (Antibiotic X-14547A)	Fungi	*Strep. antibioticus*
Efrotomycin	Fungi	*Nocardia lactamdurans*

Source: Nicolaou & Rigol (2017)

2.20 Benefits and purposes of antibiotics usage

Nicolaou and Rigol (2017) stated that antimicrobials application has evidently increased the life expectancy rate of people after their use against deadly microbial pathogens, even though many pathogenic microbial infections still persist. Antibiotic usage in veterinary practice serve as both a welfare for animals and as well increase meat production for human consumption (Durso & Cook, 2014). According to a survey conducted by Briyne *et al*. (2014) at 25 EU nations from a total of 3,004 veterinary professional as respondents, it was revealed that, among the five top classes of antibiotics commonly prescribed for treating various diseases in animals, 75% used penicillin, 50% used tetracycline, 49% prescribed fluroquinolones, 21% used $3^{rd}/4^{th}$ generation cephalosporins and 19% used lincosamides. Antibiotic-like substances, bacteriocins including sakacin, nisin, pentocin, enterocin, etc, which are produced by lactic acid bacteria (LAB) including *Enterococcus, Lactobacillus, Lactococcus*, etc., are known to have antibacterial effects by inhibiting pathogenic bacterial growth most especially on meat during preservation (Costa *et al*., 2019). In medical practices, antimicrobials, mostly β-lactams, usually of narrow spectrum in action to reduce resistance, are administered mostly as a single dose post-surgery as prophylactic agents to prevent secondary infections (Munckhof *et al*., 2005).

Orally dosed antibiotics like amoxicillin clavulanate cefixime, cefdinir and ciprofloxacin, or parenterally administered drugs including ampicillin, ceftriaxone, gentamicin, cefotaxime and cefepime are all used to treat urinary tract infections in human (Saadeh & Mattoo, 2011). The most commonly used antibiotics for the treatment of skin infections and acne are macrolides like roxithromycin,

erythromycin and azithromycin; tetracyclines including minocycline or doxycycline; lincosamides e.g., clindamycin, and other antibiotics such as dapsone, trimethoprim-sulfamethoxazole, rifampicin, metronidazole and levofloxacin (Xu & Li, 2019). A study by Ong *et al*. (2008) stated that tetracyclines, macrolides and penicillin can be prescribed for treating respiratory, UTIs and acute otitis media (AOM) as 'first-choice' or 'second-choice' antimicrobials. Instances of antibiotics and probiotics usage as growth promoters in livestock have been largely reported (Al-Khalaifah, 2018). The continuous, inappropriate and substandard use of antimicrobials in both human and veterinary medications is greatly causing the resistance of pathogenic microbes against such drugs thereby calling for reduction or an outright barn in their applications (Allen *et al*., 2014). Even though veterinary antibiotics are applied for the health and productions of livestock, the subsequent excretion of these antibiotics in excess through animal droppings into soils for agricultural use alters the soil microbiota or agroecosystem leading to interference in the recycling of nitrogen and carbon and a consequential low yield of agricultural produce (Wepking *et al*., 2019). Due to increasing reports of resistance of deadly microbes to antibiotics usage, it is necessary to find substitutes like bacteriocin, unconventional bacteria and bacteriophages to avert resistance against target pathogens (Allen *et al.*, 2014).

2.21 Classification of antibiotics

According to Calderón and Sabundayo (2007) and MacGowan *et al*. (2016), antibiotics are also classified on the basis of their molecular structures indicated in Table 2.5.

Table 2.5: Classifications of antibiotics

Antibiotic class	Chemical structure	Examples	Suffix
Amphenicols	p-nitrophenyl, N-dichloroacetyl, 1,3-propanediol	Chloramphenicol, Fluoramphenicol, Thiamphenicol	-col
Tetracyclines (1^{st}-3^{rd}) generation	4 Hydrocarbon rings (4-tetracyclic ring)	Tetracycline, Chlortetracycline, Minocycline,	-cycline
Glycylcycline	9-dimethylglycylamido (DMG) group	Tigecycline	-cycline
Fluroquinolones/ Quinolones (1^{st}-4^{th} generations)	Nalidixic acid	Ciprofloxacin, Ofloxacin, levofloxacin, temafloxacin	-oxacin
Aminoglycosides	3-amino sugars – glycosidic bonds	Gentamicin, Tobramycin Neomycin, Amikacin	-micin
Oxazolidinones	Linezolid, tedizolid phosphate	Cadazolid, Radezolid, Posizolid, Sutezolid Linezolid, Tedizolid	-zolid
Glycopeptides	Heptapeptide linkages	Vancomycin, Balhimycin, Chloroeremomycin Teicoplanin	
Streptogramins	Polyunsaturated macrolactones: Hexadepsipeptides	Pristinamycin, Madumycin, Virginiamycin (Staphylomycin)	
Cyclic lipopeptides	Fatty acid – oligopeptide macrocyclic ring	Daptomycin (Cubicin), Fruilimicin, Ramoplanin, Empedopeptin, Polymyxin	
Sulphonamides/ Sulfonamides	Sulfanilamide	Sulfisoxazole, Sulfapyridine, Sulfadoxine, Sulfadiazine,	
Macrolides	Macrocyclic lactose ring	Azithromycin, Erythromycin, clarithromycin	-mycin
Ketolides	Erythronolide ring-ketone bond	Telithromycin (ABT-773, HMR-3004, HMR-3562, HMR-3787)	
Beta-Lactams (3-Carbon, 1-Nitrogen ring, beta-lactam ring)			
Penicillin	6-aminopenicillanic acid	Penicillin G, Piperacillin, Ticarcillin, Amoxicillin, Oxacillin, Bacampicillin	-cillin
Cephalosporin (1^{st} – 5^{th}) generations	7-aminocephalosporanic acid	Cephradine, Cefuroxime, Ceftriaxone, Cefepime	
Monobactam	Monocyclic beta-lactam	Aztreonam, Carumonam, Tigemonam	
Carbapenems	Clavulanic acid	Imipenem, Meropenem, Ertapenem	

2.22 Antibiotics commonly used for human and animal treatment

According to Almeida *et al*. (2014), in Portugal the cumulative and comparative yearly use of antibiotics for both human and animals' disease treatment were ranked as "65% penicillin, 13% quinolones, 7% macrolides, 6% cephalosporins and 5% sulphonamides. In a similar study by Amponsah *et al*. (2021) in Ghana, where total antimicrobial use for medication in hospitals were observed to be 60.5% "penicillin, 48.7%, cephalosporins 23.5%, fluoroquinolones 17.4%, lincosamides 4.4%, aminoglycosides 2.6%, macrolides 1.7% and nitroimidazoles 1.7% ". Also from Ghana, Adeapena *et al*. (2021) indicated in their 5years (2013-2019) of data from the Kintampo North Municipal Veterinary Clinic that antibiotics used for treating 513 animals (71.9% dogs, 13.1% goats, 11.1% sheep, 1.2% cats, 1% cattle, 0.6% chicken, 0.6% pigs, 0.4% rabbits and 0.2% monkeys), tetracycline use was 99.6% and penicillin 0.4%. Another study on types of antibiotics used among pig farms in the Ashanti Region of Ghana documented the various antibiotics used by farms and farmers as follows: tetracyclines (59%), penicillin-streptomycin (44%), sulfadimidine (29%), enrofloxacin (+ norfloxacin) (9%), metronidazole (6%), tylosin (6%), erythromycin (5%), gentamicin (4%), trimethoprim (4%) and amoxicillin (2%) for treating skin rashes, watery feces and coughs in pigs (Osei-Sekyere, 2014).

2.22.1 Amoxicillin

Known to be a broad spectrum antibiotic for treating many different diseases, Kaur *et al.* (2011) indicated that, amoxicillin is among the class of beta-lactams belonging to the penicillin groups, and as a member of the aminopenicillins they were the first

known members to kill both Gram+ and G- bacteria like *Staphylococcus pyogenes*, *Clostridium spp., E. coli, Salmonella spp.* and *Shigella spp.* (Calderón & Sabundayo, 2007). Amoxicillin is indicated for the treatment of acute otitis media (AOM), respiratory infections like pneumonia, bronchitis, laryngitis, UTIs and endocarditis (Kaur *et al*., 2011). Although amoxicillin is indicated for the treatment of varying infections, some moderate harm or adverse effects from its use like skin rashes, diarrhea, abdominal aches, etc. have been reported (Loke & Mattishent, 2014).

2.22.2 Amoxicillin-clavulanic acid

This antibiotic, which is a semi-synthetic type, is an amoxicillin proportionally formulated with clavulanic acid in the ratio of amoxicillin 2:1 clavulanic acid (Ball, 2007). As a result of merging with the clavulanic acid, amoxicillin-clavulanate has a broader spectrum of its antibacterial activity especially against beta-lactamase-producing enzymes such as *E. coli*, *Moraxella catarrhalis*, *Neisseria gonorrhea*, *Proteus mirabilis*, *Streptococcus pneumoniae*, *Hemophilus influenza*, etc. compared to amoxicillin alone (Ball, 2007). Amoxicillin-clavulanate is indicated for the treatment of UTIs, respiratory tract infections (RTIs), skin and soft tissues infections (SSTIs), prophylaxis of post-surgical operations, gynecological infections, abdominal infections, etc. (Ball, 2007; Calderón & Sabundayo, 2007; Geddes *et al*., 2007). It is worth noting that the use of amoxicillin-clavulanic acid could come with adverse effects such as a feeling of throwing-up, vomiting, watery stool, anaphylactic shock, skin rashes, allergy, improper circulatory functioning, nervous reactions like

hallucinations, but which can be restored following stop in medication (Ball, 2007; Calderón & Sabundayo, 2007).

2.22.3 Azithromycin

As a semi-synthetic antibiotic with other members like clarithromycin, dirthromycin, erythromycin, erythromycin estolate, azithromycin is a macrolide which is structurally made of a macrocyclic lactose ring (Calderón & Sabundayo, 2007). As a way of exerting its antimicrobial activity, azithromycin interfere with the gathering and binding of 50S ribosomal units and peptide chains (Echeverría-esnal *et al*., 2020), in a bacteriostatic effect, but could also be bactericidal depending on the microbe and concentration of the antibiotic (Calderón & Sabundayo, 2007). Azithromycin is very effective against certain Gram-negative microbes including *E. coli*, *Moraxella catarrhalis, Shigella, Hemophilus pneumoniae, Salmonella*, etc. but weak against Gram+ prokaryotes (Calderón & Sabundayo, 2007; Parnham *et al*., 2014). It has also been active against protozoa like *Toxoplasma gondii, Plasmodium spp.*, *Cryptosporidium spp.*, and *Chlamydia* such as *Chlamydia trachomatis* (Bakheit & Al-hadiya, 2014). Azithromycin is indicated for the treatment of RTIs, ear infections, SSTIs, STDs, *Helicobacter pylori*-related duodenal ulcer infection, mild typhoid, prophylaxis of dental surgeries patients who have historical heart-related challenges (Calderón & Sabundayo, 2007; Bakheit & Al-hadiya, 2014). Side effects of azithromycin include diarrhea, vomiting, dizziness, tongue color change, stomach ache, insomnia, interstitial nephritis, disruption in olfaction and taste, hepatitis, etc. (Calderón & Sabundayo, 2007; Bakheit & Al-hadiya, 2014).

2.22.4 Ceftriaxone

Ceftriaxone is a half-synthetic cephalosporin against a wide range of both Gram+ and Gram- bacteria including *Staphylococcus aureus, Streptococcus pyogenes, E. coli, Neisseria gonorrhoeae, Klebsiella pneumoniae, Neisseria meningitidis* etc. (Khan *et al*., 2017). Because ceftriaxone have similar characteristic of antibacterial activity to other 3rd and 4th generation cephalosporins as being able to go deep into the CNS, it has been indicated in multiple occasions as the best antibiotic of choice for treating meningitis, both in children and adults (Lamb *et al*., 2002; Calderón & Sabundayo, 2007; Khan *et al*., 2017). It has also been favored for the treatment of "community-acquired pneumonia (CAP)", SSTIs, AOM, STDs like gonorrhea, acute pyelonephritis notably in pregnant ladies, typhoid fever, as a prophylactic medication in wounds post any surgical operation (Lamb *et al*., 2002; Khan *et al*., 2017). Adverse effects from the use of ceftriaxone are rare but moderately reported symptoms include diarrhea and GIT disturbances, nausea, rashes, head ache, candidiasis, dizziness, anemia, leucopenia, phlebitis, etc. (Lamb *et al*., 2002).

2.22.5 Chloramphenicol

It is a synthetic broad spectrum antibiotic and was discovered in 1947 from the fungus *Streptomyces venezuelae* and it is predominantly a bacteriostatic agent (Balbi & Balbi, 2014; Hanekamp & Bast, 2015). Chloramphenicol is an amphenicol with a chemical composition of p-nitrophenyl, N-dichloroacetyl, 1,3-propanediol and is active against a wide range of Gram+ and Gram- bacteria like *Streptococcus pneumoniae, Actinobacillus pleuropneumoniae, Hemophilus influenzae, Salmonella spp., Neisseria*

meningitidis, Histophilus somnus, etc.; rickettsia, spirochetes and chlamydia (Balbi & Balbi, 2014). Adverse side effects from chloramphenicol use are bone marrow suppression, hemolytic anemia, cardiovascular collapse or "gray baby syndrome", head ache, mental confusion/depression, glossitis, stomatitis, nausea, diarrhea, hypersensitivity and ototoxicity (Balbi & Balbi, 2014; Hanekamp & Bast, 2015).

2.22.6 Ciprofloxacin

Discovered around 1980s among other similar antibiotics like enoxacin, lomefloxacin, temafloxacin, etc. as "second generation" fluoroquinolones/quinolones, ciprofloxacin is chemically made of nalidixic acid (Calderón & Sabundayo, 2007). According to Conley *et al*. (2018), ciprofloxacin and as well other quinolones mechanism of antibacterial activity is by disrupting the enzymes "DNA gyrase and DNA topoisomerase" of bacteria like *Mycobacteria spp.* from linking two DNA strands for the bacterium metabolism, hence seizing its growth. Ciprofloxacin comparatively among other quinolones has been active (Calderón & Sabundayo, 2007) against a wide range of some Gram+ and Gram- bacteria like *Pseudomonas aeruginosa, Neisseria gonnorhoeae, Moraxella catarrhalis, Acinetobacter baumanii, Hemophilus influenzae, E. coli, Stenophotromonas maltophilia*, etc. (Calderón & Sabundayo, 2007; Sharma *et al*., 2010; Vidyavathi & Srividya, 2018). Ciprofloxacin is effective for the treatment of "Crohn's Disease" (inflammatory bowel disease with abdominal pain, diarrhea, emaciation, weight loss) (Arnold *et al*., 2002), UTIs, skin and skin structure infections (SSSIs), typhoid fever, bone and joint infections, infectious diarrhea, acute sinusitis, chronic bacterial infections, urethral gonorrhea, acute uncomplicated cystitis

in females, pyelonephritis and diverticulitis or colon infections (Sharma *et al*., 2010; Conley *et al*., 2018; Vidyavathi & Srividya, 2018). The use of ciprofloxacin come with the following side effects: CNS (confusion, dizziness, hallucinations, nightmares, insomnia; Eye (blurred vision, irritation, redness of eyes, sensitivity to light); GIT (taste perversion, nausea, constipation, flatulence, stomach ache, dysphagia, pseudomembranous colitis); Skin (rashes, "Stevens-Johnson syndrome', erythema, exfoliative dermatitis); Musculoskeletal system (tendon rupture, myalgia, tendinitis); Hepatic system (hepatic necrosis, jaundice), etc. (Arnold *et al*., 2002; Sharma *et al*., 2010; Conley *et al*., 2018; Vidyavathi & Srividya, 2018).

2.22.7 Gentamicin

It is a semisynthetic broad-spectrum antibiotic of the aminoglycoside group of antibacterials and a precursor (Gentamicin B) for isepamicin (Ban *et al*., 2019). Gentamicin and other aminoglycosides application against Gram-negative bacteria data is extensively available while that of Gram+ bacteria data is scanty, and in particular, gentamicin has shown varying and encouraging "killing profiles" against *Staphylococcus aureus* and *Pseudomonas aeruginosa* indicating an emphatic activity against staphylococcal diseases in particular (Tam *et al*., 2006). It is also effective against infections caused by Gram-negative bacilli such as *E. coli, Salmonella spp.* and *Shigella spp.* (MacGowan *et al*., 2016). Due to the less resistance threshold of community and hospital acquired Gram-negative infectious bacteria, gentamicin is the drug of choice against many Gram-negative pathogenic agents (Moulds *et al*., 2010). Having a synergy with beta-lactams medication, gentamicin like any other

aminoglycoside is indicated for treating cystic fibrosis, upper respiratory tract diseases, glandular tularemia, “Meniere’s Disease or vertigo”, cell and tissue culture (Kumar & Dubois, 2008). Even though gentamicin is known to be good therapeutically, its overdose or long-term use could lead to ototoxicity or nephrotoxicity (Kumar & Dubois, 2008; Ban *et al*., 2019).

2.22.8 Sulfamethoxazole/trimethoprim

Sulfamethoxazole and it’s other members including sulfadiazine, sulfapyridine, sulfisoxazole and sulfadoxine, is a synthetic antimicrobial agent belonging to the sulfonamide class of antibiotics which chemically contain the active ingredient sulfanilamide (Calderón & Sabundayo, 2007; MacGowan *et al*., 2016). Sulfamethoxazole or trimethoprim individually suppresses the growth of bacteria but become lethal when synergically used together by Sulfamethoxazole disrupting the enzyme, inserting para-aminobenzoic acid (PABA) inside the pro-folic acid, dihydrofolic acid, while trimethoprim suppresses the enzyme, dihydrofolate reductase, which converts folic acid to folinic acid during prokaryotic and a mammals’ DNA construction (Calderón & Sabundayo, 2007). Cotrimoxazole have been effective against a broad-spectrum of either Gram-negative/positive bacteria such as many *Enterobacteriaceae* (including *E. coli, Salmonella, Klebsiella, etc*.), *Nocardia spp., Chlamydia trachomatis, Burkholderia spp., Pseudomonas spp.* excluding *P. aeruginosa, Staphylococcus aureus, Listeria spp., Shigella spp., Stenotrophomonas maltophilia, Hemophilus influenzae,* and some protozoa like *Toxoplasma gondii* (Calderón & Sabundayo, 2007; Livermore *et al*., 2013). Cotrimoxazole is

recommended for the treatment of SSTIs notably of group A Streptococcus (GAP) or *Staphylococcus aureus*, impetigo (a contagious skin infection of infants, reddish sores on face, nose, feet and hands), septicemia, purulent cellulitis, bacillary dysentery, some UTIs, tonsilitis, and abscesses (MacGowan *et al*., 2016; Bowen *et al*., 2017). It has been indicated that Sulfamethoxazole and other sulfonamides are active against some tumorous cells (Vullo & Supuran, 2013; MacGowan *et al*., 2016). Sulfamethoxazole-Trimethoprim also treats infections of the upper respiratory tract like otitis, bronchitis and infections of the GIT (Calderón & Sabundayo, 2007). A study by Karpman and Kurzrock (2004) showed that side effects of cotrimoxazole use for treatment is more evidently observed in the aged already having comorbidities; infants scarcely exhibit any adverse effect, and the common side effects symptoms are abdominal disturbances, blood-related toxicity, rashes.

2.22.9 Tetracycline

Tetracyclines are effective against a wide array of both Gram-positive and Gram-negative bacteria like *Bartonella quintana, Bacillus anthracis, Francisella tularensis, E. coli, Yersinia pestis, Brucella spp., etc.; chlamydia* such as *Chlamydia trachomatis, C. pneumoniae, C. psittaci; mycoplasmas* including *Mycoplasma pneumoniae, etc; rickettsia* such as *Rickettsia monteiroi, R. andeaneae, R. bellii, R. canadensis, etc.* and *protozoan parasites* like *Plasmodium falciparum, Toxoplasma gondii, Gardia lamblia* and *Trichomonas vaginalis* (Roberts, 2003). Tetracycline and its analogs like omadacycline, eravacycline, tigecycline, minocycline, etc., have been indicated for treating complicated SSTIs, "community acquired bacteria pneumonia (CABP)",

intricate GIT infections, “diabetic foot infections” and hospital acquired pneumonia (Roberts, 2003; Grossman, 2016). Note that tigecycline is restricted to only intravenous route of administration (Grossman, 2016). They are also used for the treatment of malaria in humans and for prophylaxis or growth promoters in animals (Roberts, 2003). According to Briyne *et al*. (2014), in Europe, tetracycline was prescribed for treating 19% of respiratory diseases, 16% of uterine infections, 4% of other diseases conditions and 24% of prophylaxis in bovine; 47% of respiratory infections and 12% of other infections in porcine; 7% of respiratory infections, 5% of skin infections, 16% of unidentified source of fever, 10% of movement disorder in equine, and 50% of respiratory infections in feline. A study conducted by Hamilton and Guarascio (2019) indicated that among all the tetracyclines, minocycline has the most reported adverse side effects upon therapeutic dosage; but the general side effects of all tetracyclines are rashes on face, mouth, and tongue; erythema, pruritic rash on penis, rashes on body extremities including fingers and feet, edema, balanitis, convulsion, dizziness, tachycardia, hives, generalized urticaria, wheezing, dyspnea and fever.

2.23 Places antimicrobials are obtained for human and animal use and their types

According to Aslam *et al*. (2020), many of the public from low and middle income countries who indulge in self-medication of antibiotics (SMA) usage obtain their drugs from pharmacists, drug retailors, family members, friends, left-over of previously effective medications, private health facilities, corner stores and street vendors. In a

survey by Russom *et al*. (2021), the study population who had their prescription from a certified healthcare practitioner were 88.1%; those who practice self-medication/treatment without a health practitioner's prescription obtained their antibiotics from drug retail outlets (83.6%), left-over drugs (5.2%), family/friends (4.5%), abroad (3.0%), health facility (2.9%) and 1.1% cannot remember their source of medication. A survey report by Afari-Asiedu *et al*. (2020) from Kintampo North and South Districts of Ghana health professionals comprising medical doctors, pharmacists, physician assistants, nurses/midwives, dispensing technicians from both public and private healthcare facilities, licensed/over-the-counter chemical sellers, all indicated the misuse and self-medication of antibiotics by the public for treating infections like boils, diarrhea, abdominal aches, gonorrhea and head pains. Antibiotics commonly prescribed by these healthcare practitioners for treatments were amoxicillin, chloramphenicol, tetracycline and metronidazole (Afari-Asiedu *et a*l., 2020). The source farmers obtain antibiotics for treating their animals include veterinarians (50.7%), veterinary drug outlets (20.8%), family/friends (14.4%), open market areas (14.3%), left-over drugs (5.7%) and others (1.0%) (Russom *et al*., 2021). Antibiotics used for treating animals were amoxicillin (44.1%), oxytetracycline (36.2%) then penicillin (16.3%) (Russom *et al*., 2021). In a similar study from the Kintampo Municipal Veterinary clinic, where only certified professionals operate, tetracycline was 99.6% used whilst penicillin use was 0.4% for treatment of animals (Adeapena *et al*., 2021).

2.24 Antibiotic resistance

Antibiotics exert their effect on bacterial cell membranes or cell walls (glycopeptides & beta-lactams); protein synthesis with ribosomes (aminoglycosides, chloramphenicol, lincozids, macrolides and tetracyclines); nucleic acid synthesis (fluoroquinolones) and metabolic process (sulfonamides) preventing the bacterial growth, or total death (Sengupta *et al*., 2013). However, microbes develop strategies or assays of genes (plasmids), proteins or enzymes to invade (e.g. inactivate, modify, protect its enzymes) these antibiotics "by weakening the interaction between the antibiotic and the microorganism" and this phenomenon is known as "antibiotic resistance" (Costa *et al*., 2011). The resistance of antibiotics to some bacteria could also be due to gene mutation of the microbe, like GenB1 amplifying the DNA of *Micromonospora echinospora* against gentamicin (Ban *et al*., 2019). According to Larsson (2022), antibiotics resistance is been considered a worldwide concern with microbes and their genes moving between living things and their surroundings, and antibiotic resistance control is even getting worse as microbes obtain novel resistance elements from other species they interact with. Maurer (2018) stated that the prominence of antibiotics resistance in the bacterial community is bringing about serious health and monitory impact to both the public and the livestock industry. It has been found that the deletion of G942 from *E. coli* numbering leads to tetracycline resistance (Grossman, 2016). The production of enzymes especially by some Gram-negative bacteria like *E. coli* and *Klebsiella pneumoniae* producing NDM-1 (New Delhi metallo-beta-lactamase) is known to confer multi-drug resistance to every beta-lactam antibiotic known. The beta-lactamases: penicillinase, from Gram-positive bacteria *Gonococcus spp., Staphylococcus spp.,* and *Hemophillus influenzae*;

cephalosporinase from Gram-positive bacteria like *Enterococcus spp., Staphylococcus spp.* and *Streptococcus spp.*, and some Gram-negative bacteria such as *Proteus mirabilis, E. coli* and *Klebsiella pneumoniae* are both known to cause antibiotic resistance in penicillin-resistant and cephalosporin-resistant bacteria, respectively (Calderón & Sabundayo, 2007).

2.25 Mechanisms of antibiotics resistances

Bacteria have developed a number of mechanisms to enable them counter threats in and around their surroundings for their survival and existence (Munita & Arias, 2016). While some bacteria mostly produce enzymes (e.g. beta-lactamases from Gram-negatives), others modify binding sites [e.g. penicillin-binding proteins (PBPs) of Gram-positive beta-lactams] of genes for resistance to occur (Munita & Arias, 2016). The occurrence of antibiotics resistance is alarming; for instance, from some nations in Europe, a study by Grossman (2016) on tetracycline resistance alone was 66.9% against ESBL-producing *E.coli*.

To achieve resistance against antimicrobials, bacteria adjust to two methods of combat: horizontal gene transfer (HGT) of resistant DNA for the bacteria; and bacterial genes mutations (Munita & Arias, 2016). From the three ways of HGT (transformation, transduction and conjugation), transformation, the easiest and seen in only few bacteria (Munita & Arias, 2016), involves the use of integrons [being mobile genetic elements (MGEs)] which are plasmids on a bacterial chromosome, are responsible for the insertions and deletions of resistant genes in bacteria (Foley & Lynne, 2007). Biofilm is also another good source of DNA material for antimicrobial

resistance of many pathogenic bacteria (Davies, 2003). In the transduction of HGT, the genes which may be resistant are transferred through "bacterial viruses" like bacteriophages; from the "donor bacterial cell to the recipient cell". According to documentations from, Frost *et al.* (2005), Foley and Lynne (2007) and Munita and Arias (2016), HGT conjugation is achieved through the "cell-to-cell" "sexing" (by the aid of pilus- 'brush-like' appendage on the DNA surface) of transconjugants or a donor and a recipient by a conjugative transposons which then pump the resistant MGE from one side (the donor) into the other (the recipient).

Enzyme production by most Gram-negative and Gram-positive bacteria makes changes or modifications, or total destruction of the antibiotic stopping it from reaching its target site of action, which in many cases is at the ribosome where protein synthesis occurs (Wilson, 2014). Enzymes are capable of destroying the antibiotic compound by breaking the bonds as seen in beta-lactam rings by beta-lactamase like penicillinase (Poirel *et al*., 2007). Some Gram-negative bacteria like *Enterobacter cloacae, E. coli, Salmonella* Typhimurium and Enteritidis, *Klebsiella pneumoniae, Pseudomonas aeruginosa, Shigella dysenteriae*, etc., contain in their outer membrane a protein, porin (a water-loaded unobstructed path around the external membrane), which regulates the influx of water-loving substances including some quinolones, tetracyclines, beta-lactams into the cell leading to the bacteria resistance against such antibiotics (Munita & Arias, 2016; Pagès *et al*., 2008). The characterization of porins is on the basis of their regulation and expression, their activity (specific, non-specific or selective) and their functional structure (trimeric or monomeric); *E. coli* in

particular have three main trimeric porins as *OmpF* (outer membrane protein F), *OmpC* then *PhoE,* with *OmpC* and *OmpF* members having little affinity for cations whilst PhoE prefer inorganic phosphate anions (Pagès *et al*., 2008). Some bacteria have developed efflux-mediated resistance systems to extrude poisonous compounds including antibiotics like carbapenems, quinolones, tetracyclines, polymyxins, and disinfectants, bile salts, dyes, etc. out of the bacteria cell cytoplasm (Poole, 2005; Munita & Arias, 2016). The first ever efflux-mediated mechanism of ressistance was seen on *E. coli* showing resistance to tetracycline, *tet* genes of the MF family, many of these genes, *tet(A-E), G, H, I, J, Z,* all from Gram-negative bacteria and only *tet(K)* and *tet(L)* found in Gram-positive bacteria (Poole, 2005).

2.26 *Salmonella* and *E. coli* as antibiotic resistance indicators

Samples from nine broiler chicken farms in British Colombia indicated the prevalence of antibiotic resistance indicator genes $bla_{\text{CMY-2}}$ in both *E. coli* (80.0%) and *Salmonella* (81.5%) isolates; *tetA* and *tetB* genes, showing resistance against tetracyclines; class 1 integrons (with *aadA1* gene cassette contain resistance against spectinomycin and streptomycin); *qacEΔ1-SulI,* a multi-antibiotic resistant gene; were all found in some *E. coli* isolates (Diarrassouba *et al*., 2007). Both *E. coli* and *Salmonella* isolates exhibited resistance against clindamycin, erythromycin, penicillin, novobiocin and tylosin with many of the *tetB* resistant *E. coli* isolates being from feed with salinomycin pre-included as growth promoter antibiotic (Diarrassouba *et al*., 2007). *Salmonella* isolates collected from humans, animals and the environment comprising 35 phenotypes revealed that with the exception of only five (5) serovars,

the remaining (30 strains) exhibited some level of antibiotics resistance to the nine (9) antimicrobials used; tetracycline, gentamicin, streptomycin, chloramphenicol, sulfadiazine, kanamycin, trimethoprim, spectinomycin and ampicillin (Randall *et al*., 2004). From the work of Hassan (2014) on poultry layers at a farm in Bangladesh, it was reported that all 13 *E. coli* isolates showed resistance against Ciprofloxacin (100%), Enrofloxacin (100%), Pefloxacin (100%), Tetracycline (100%), Amoxicillin (85%), Kanamycin (69%), Doxycycline (54%), Colistin (54%), Neomycin (23%) and Gentamicin (0%); whilst all 8 *Salmonella* isolates were resistant against Amoxicillin (100%), Tetracycline (100%), Ciprofloxacin (88%), Enrofloxacin (88%), Pefloxacin (88%), Colistin (50%), Doxycycline (50%), Kanamycin (50%), Gentamicin (0%) and Neomycin (0%).

2.27 Antibiotics resistance prevalence in *E. coli* and *Salmonella*

Isolates of *E. coli* and *Salmonella* from broilers in Malaysia showed in totality that, 51.8% and 6.5% respectively of *E. coli* and *Salmonella* were resistant against at least nine (9) antibiotics, but individual *E. coli* and *Salmonella* were correspondingly resistant against ampicillin (87.0% and 47.7%), cephalothin (11.0% and 0.0%), chloramphenicol (84.5% and 76.2%), ciprofloxacin (23.8% and 4.8%), colistin sulphate (0.0% and 0.0%), erythromycin (100.0% and 100.0%), gentamicin (20.2% and 0.0%), kanamycin (57.0% and 28.6%), nalidixic acid (60.7% and 9.6%), streptomycin (66.0% and 19.0%), sulfamethoxazole/trimethoprim (83.3% and 42.9%) and tetracycline (94.6% and 62.0%) (Ibrahim *et al*., 2021). From Thailand, isolates of *E. coli* and *Salmonella* from abattoirs, poultry and pig farms, and human excreta

indicated that these bacteria (*E. coli* and *Salmonella*), respectively exhibited resistance against the following antibiotics: ampicillin (61.6% and 13.6%); ceftiofur (4.9% and 3.4%); ceftriaxone (1.5% and 0.0%); ciprofloxacin (12.5% and 0.0%); enrofloxacin (1.5% and 0.0%); florfenicol (51.8% and 18.6%); nalidixic acid (67.4% and 27.1%) and tetracycline (91.5% and 84.7%) (Hanson *et al.*, 2002). In Egypt, isolates of *E. coli* and *Salmonella* from fresh beef and chicken from some slaughter slabs and meat retail outlets revealed respectively that *E. coli* and *Salmonella* exhibited in a reverse and complexity of resistance percentages against ampicillin (71.4% and 86.7%), cefotaxime (33.3% and 80.0%), cefpodoxime (23.8% and 60.0%), streptomycin (61.9% and 26.0%), sulphamethoxazole-trimethoprim (61.9% and 53.3%) and tetracycline (80.9% and 40.0%) (Moawad *et al*., 2017).

2.28 Multidrug resistance of *E. coli* and *Salmonella*

Multidrug resistance occur when bacteria become resistant to more than one antibiotic aided primarily by the buildup of many resistant genes (plasmids) on a single bacterial cell and secondly with efflux pump genes which expel a spectrum of antimicrobial agents and other elements out of the bacterial cell cytoplasm (Nikaido, 2009). The sources for these genes or plasmids (R) causing this multiple drug resistance are from many cases the soil, decomposing organic materials and antibiotic-resistant microorganisms (Nikaido, 2009; Munita & Arias, 2016), and are usually transferred into the bacteria through any of the three methods; transduction, conjugation and transformation (Munita & Arias, 2016).

In the work of antibiotic resistance of bacterial isolates from beef and chicken from Egypt, it was observed that *E. coli* exhibited multidrug resistance to twelve (12) antibiotics namely AMC-AMP-CAZ-CHL-CIP-CRO-CTX-ENR-NAL-STR-TET-S/T; that of *Salmonella* resistance profile was AMC,-AMP-CAZ-CHL-CRO-CTX-ENR-NAL-STR-TET-S/T (Moawad et al., 2017). When *E. coli* and *Salmonella* were isolated from vegetables in Tamale, Ghana, the *E. coli* isolates exhibited some resistance against at least 3 (three) of the 9 (nine) antibiotics, an *E. coli* isolate was resistant to 6 (six) antibiotics with the profile CroAmpTeOfxEC, with a multidrug resistance (MDR) index of 0.67; as for *Salmonella*, a resistant isolate showed resistance against at least 2 (two) and at most 7 (seven) antibiotics with the longest pattern being CroAmpTeOfxECSxt (Ceftriaxone, Ampicillin, Tetracycline, Ofloxacin, Erythromycin, Chloramphenicol and Sulphamethoxazole-trimethoprim), and MDR index of 0.78 (Adzitey, 2018). Also, *E. coli* and *Salmonella* isolates from broiler farms in Malaysia indicated that the *E. coli* were resistant to at least three (3) and a maximum of ten (10) antibiotics; *Salmonella* isolates were resistant to at least three (3) or at most resistant to eight (8) of the twelve (12) antibiotics used (Ibrahim *et al*., 2021). In Accra Ghana, *E. coli* isolates from raw meat revealed that an isolate could be multiple resistant to as low as three (3) or as high as five (5) when eleven (11) antibiotics were used in the antibiotic's resistance survey (Dsani *et al*., 2020), while in a similar study with isolates of *Salmonella* obtained from veterinary and human laboratories in the UK stated that a *Salmonella* isolate could exhibit multidrug resistance to at least two (2) or maximum seven (7) from the nine (9) antibiotics screened (Randall *et al*., 2004).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area

This research was conducted in Buipe, the administrative capital of the Central Gonja District Assembly in the Savannah Region of Ghana. According to Ghana Statistical Service (2023), the district covers a land size of 8,353km^2 and has a population of 142,762, with 71,635 (50.2%) being males and 71,127 (49.8%) being females. The district lies between longitude 1º 5’ and 2 º58’ West and latitude 8º32’ and 10º2’ North, and shares boundaries to the north with the Tamale Metropolitan Assembly, to the south with the Kintampo North Municipal Assembly, to the West with the West Gonja District Assembly, to the North East with North Gonja District Assembly, and to the East with the North East Gonja District Assembly (Ghana Statistical Service, 2014; Ghana Statistical Service, 2023). Figure 3.1 shows the map of Central Gonga District indicating Buipe where this study was carried out.

Figure 3.1: Map of Central Gonja

Source: Ghana Statistical Service (GSS, 2014)

3.2 Sample collection for isolation of *Salmonella enterica* and *E. coli*

A total of 180 (90/season) raw meat swab samples comprising thirty (n=30) each of beef, chevon and mutton were collected from five each of the three different meat type butchers (15 butchers in all) in the dry and rainy seasons. Dry season samples were taken between February-April 2023, while that of rainy season were collected from August-October 2023. For each season, meat swab samples were taken repeatedly from the same butchers for three consecutive months, twice from every randomly selected butcher for any meat type, every month at two weeks interval. Purposive

sampling was used to select the butchers; however, simple random sampling was employed to collect meat swab samples from the butchers.

The samples were transported on ice in an ice chest containing ice block to the Bruce Hunter Microbiology Laboratory at the University for Development Studies, Nyankpala campus where microbiological analyses were carried out for *Salmonella enterica* and *Escherichia coli* immediately upon reaching the laboratory.

3.3 Isolation of *Salmonella enterica*

This was carried out according to the method of Wallace and Hammack (2013). Sterile cotton swabs were used to swab an area of 10 cm^2 of the various meats. The swabs were pre-enriched in 10 ml Buffered Peptone Water (BPW) and incubated at 37 ºC for 24 hours. After which, 0.1 ml of the BPW aliquots were transferred into 10 ml Selenite Cystine (SC) and Rappaport Vassilidis (RV) broths. The samples in SC broths were incubated at 37 ºC for 24 - 48 hours while those in RV broths were incubated at 41 ºC for 24 - 48 hours. Thereafter, samples (10 µl) from both RV and SC broths were streaked onto Xylose Lysine Deoxycholate (XLD) and Brilliant Green (BG) agars, and incubated at 37 ºC for 24 - 48 hours. Presumptive *Salmonella* species appeared as red colonies on XLD agar with or without black centers, while it appeared as pinkish-white or red colonies surrounded by a red halo in the medium in BG. Presumptive *Salmonella* colonies were streaked on Trypticase Soy Agar and incubated at 37 ºC for 24 hours and confirmed using Gram stain (Gram negative rod shaped), *Salmonella* latex agglutination test kit (by coagulation) and polymerase chain reaction.

All the media used were purchased from Oxoid Limited, Basingstoke, UK and all incubations were done under aerobic conditions.

3.4 Isolation of *Escherichia coli* (*E. coli*)

This was carried out according to the method of Feng *et al*. (2020). Sterile cotton swabs were used to swab an area of 10 cm^2 of the various meats. The swabs were pre-enriched in 10 ml Buffered Peptone Water (BPW) and incubated at 37 ºC for 24 hours. After which they were streaked on Levine's Eosin-methylene Blue Agar and incubated at 37 ºC for another 24 hours. Presumptive *E. coli* colonies appeared as dark centered and flat, with or without metallic sheen. Presumptive *E. coli* were streaked unto Trypticase Soy Agar and incubated at 37 ºC for 24 hours. They were then identified and initially confirmed using Gram stain (Gram negative rod shaped), *E. coli* latex agglutination test kit (by coagulation) and polymerase chain reaction. All the media used were purchased from Oxoid Limited, Basingstoke, UK and all incubations were done under aerobic conditions.

3.5 Polymerase chain reaction (PCR) for the confirmation of *Salmonella* and *E. coli*

3.5.1 Extraction of deoxyribonucleic acid (DNA)

Deoxyribonucleic acid (DNA) was extracted from *Salmonella* and *E. coli* colonies that were freshly grown. The freshly grown colonies transferred into 30 µl Dnase/Rnase Free Water and incubated at 99 °C for 10 min in peqSTAR 96X Universal thermal cycler (VWR Prelab, UK). The incubated lysates were used as DNA template for the PCR.

3.5.2 Confirmation of *Salmonella* and *Escherichia coli* isolates by PCR

The confirmation of *Salmonella* was done using a slightly modified method of Bej *et al*. (1991), while that of *E. coli* was done using a slightly modified method of Upadhyay *et al*. (2010). The same PCR mixture (20 µl) was used for both *Salmonella* and *E. coli*. The mixture was made up of 10 µM each of primers as shown in Table 3.1, 22 mM NH_4Cl, 20 mM Tris-HCl (pH 8.9 at 25 °C), 1.8 mM $MgCl_2$, .06 % IGEPAL® CA-630, 5 % glycerol, 0.2 mM dNTPs, xylene Cyanol FF, 00.05 % Tween-20, Tartrazine, 0.25U One *Taq*® DNA polymerase (New England Biolabs® Inc) and 2 µl lysate as template. The temperature cycles used varied for *Salmonella* and *Escherichia coli* as shown in Table 3.1.

Table 3.1: Primers and cycling conditions for PCR-based assays

Organism	Primer/Sequences (5' – 3')	Cycling Conditions *(Expected Fragment Size)*
Salmonella	*invA-F* GTGAAATTATCGCCACGTTCGGGCAA *invA-R* TCATCGCACCGTCAAAGGAACC	94 °C for 5 min, 40 cycles of 94 °C for 40s, 64 °C for 30s 72 °C for 30s and a final extension at 72 °C for 7 min.
Escherichia coli	*uidA-F* AAAACGGCAAGAAAAAGCAG *uidA-R* ACGCGTGGTTAACAGTCTTGCG	95 °C for 5 min, 40 cycles of 95 °C for 30s, 57 °C for 30s 72 °C for 30s and a final extension at 72 °C for 5 min.

3.5.3 Gel electrophoresis

Gel electrophoresis was performed on 2% agarose containing 2.5µl ethidium bromide. The PCR products (7 µl) were mixed with 1µl of 6X loading dye and loaded into the gels. They were electrophorized at 80 V for 30 minutes and observed under UV light using UV Transilluminator and images captured with microDOC (Cleaver Scientific Company, UK). The size of the fragment was determined using FastRuler™ Middle Range DNA Ladder.

3.6 Antibiotic resistance of *Salmonella enterica* and *Escherichia coli* from meat swab samples

The antibiotic resistance test was conducted using the disc diffusion method of Bauer *et al*. (1966). Confirmed colonies were inoculated in 10 ml Trypticase Soy Broth (TSB) and incubated at 37 ºC for 15 hours. Afterward, the turbidity was adjusted with sterile TSB to a 0.5 McFarland solution and spread plated on Müller Hinton Agar (MHA). Four to five antibiotic discs comprising of Amoxicillin (AML 30µg), Amoxicillin-clavulanic acid (AUG 30 µg), Azithromycin (AZM 15 µg), Ceftriaxone (CRO 30 µg), Chloramphenicol (C 30 µg), Ciprofloxacin (CIP 5 µg), Gentamicin (CN 10 µg), Tetracycline (TE 30 µg) or Sulfamethoxazole-Trimethoprim (SXT 25 µg) were placed on the MHA plates and incubated at 37 ºC for 24 hours. Inhibition zones were measured with a ruler and the results were interpreted using the Clinical Laboratory Standard Institute (CLSI) (2017). Multiple antibiotic index (MAR) was computed using the formula; a/b, where ‘a’ represents the number of antibiotics to which a particular isolate was resistant to and ‘b’ the total number of antibiotics

examined (Krumperman, 1983). All the media used were purchased from Oxoid Limited, Basingstoke, UK and all incubations were done under aerobic conditions.

3.7 Data analysis

Data from prevalence of *Salmonella enterica* and *Escherichia coli* were analyzed using descriptive statistic of Excel 2021, Office 365, version (v16.0) and the results were presented in Figures and Tables.

CHAPTER FOUR

4.0 RESULTS

4.1 Prevalence of *E. coli* isolates from raw meat swab samples during the dry and rainy seasons

4.1.1 Prevalence of *E. coli* isolates from raw meat swab samples during the dry season

The prevalence of *E. coli* in the various meat swab samples (beef, chevon and mutton) in the dry season is presented in Figure 4.1. The prevalence was 63.3%, 53.3%, and 30.0% for mutton, chevon and beef, respectively.

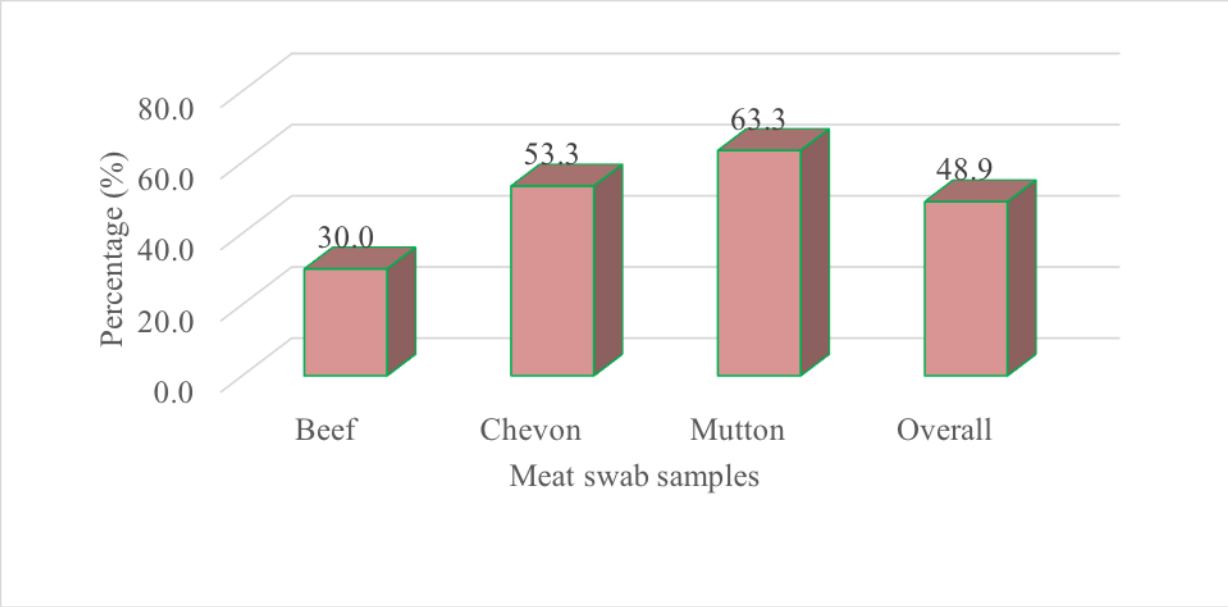

Figure 4.1: Prevalence of *E. coli* isolates from raw meat swab samples during the dry season

4.1.2 Prevalence of *E. coli* isolates from raw meat swabs samples during the rainy season

The prevalence of *E. coli* in the various meat samples (beef, chevon and mutton) in the rainy season is presented in Figure 4.2. The prevalence was 16.7%, 16.7%, and 6.7% for mutton, chevon and beef, respectively.

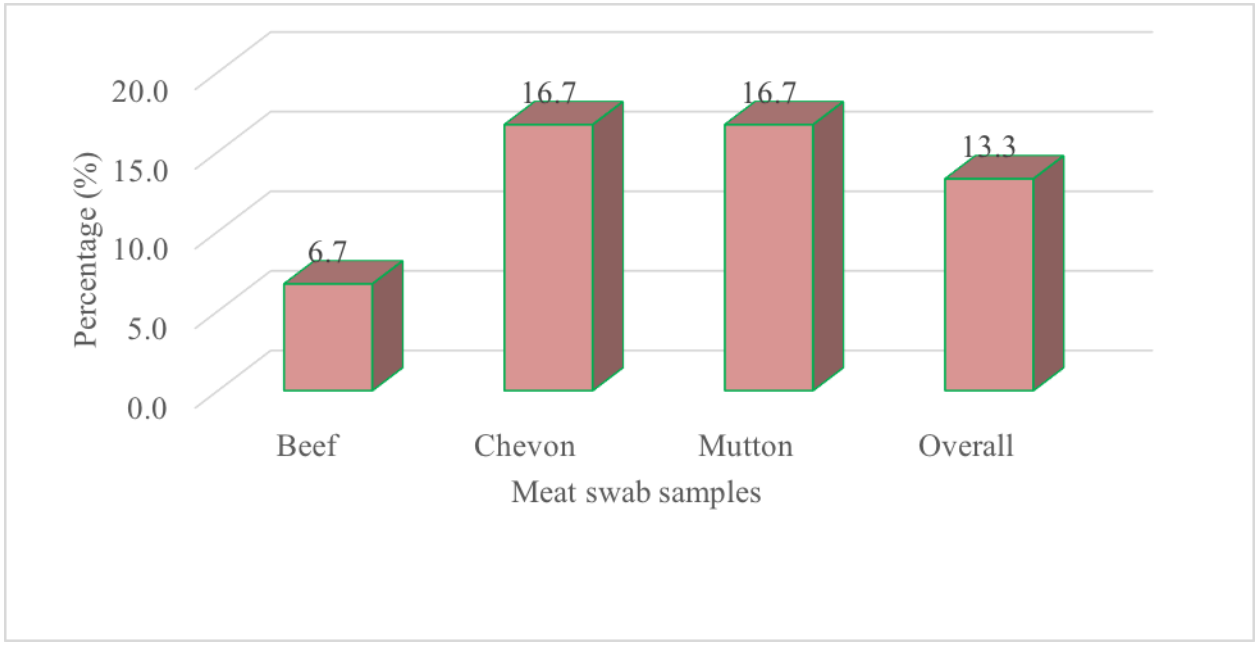

Figure 4.2: Prevalence of *E. coli* isolates from raw meat swab samples during the rainy season

4.2 Prevalence of *Salmonella enterica* isolates from raw meat swab samples during the dry and rainy seasons

4.2.1 Prevalence of *Salmonella enterica* isolates from raw meat swab samples during the dry season

The prevalence of *Salmonella enterica* in the various meat samples (beef, chevon and mutton) in the dry season is presented in Figure 4.3. The prevalence was 13.3.7%, 3.3%, and 0.0% for chevon, beef, and mutton, respectively.

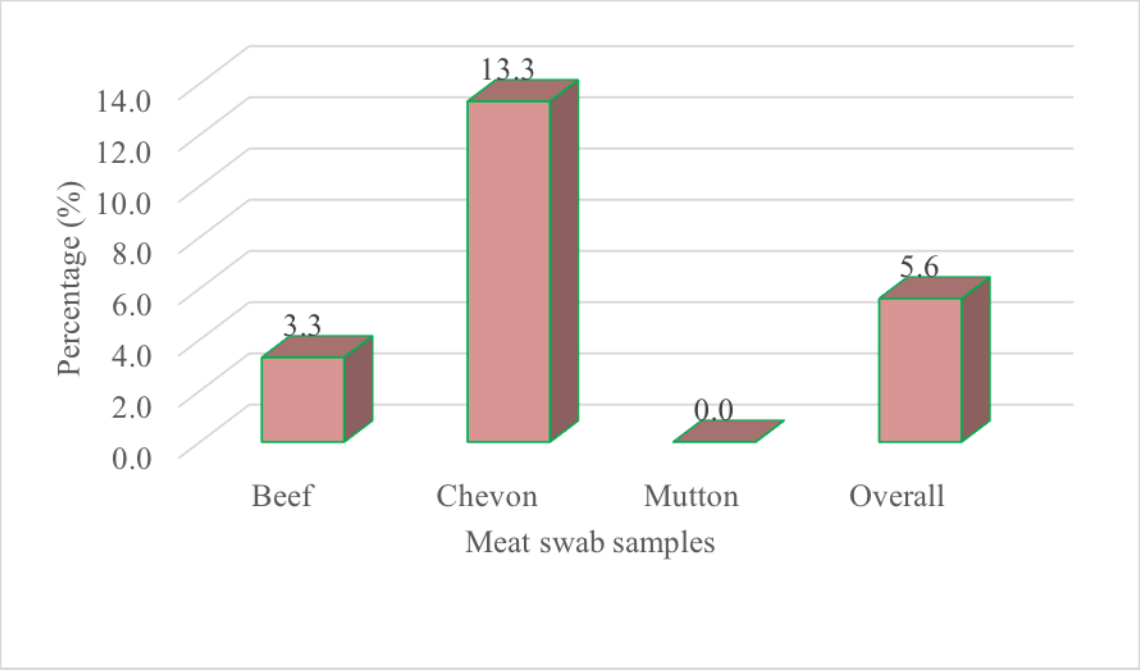

Figure 4.3: Prevalence of *Salmonella enterica* isolates from raw meat samples during the dry season

4.2.2 Prevalence of *Salmonella enterica* isolates from raw meat samples during the rainy season

Salmonella enterica isolates were not isolated from the various meat samples.

4.3 Polymerase chain reaction for the confirmation of *E. coli* and *Salmonella enterica* isolates from raw meat swab samples

4.3.1 Polymerase chain reaction (PCR) for the confirmation of *Escherichia coli* isolates

Results for polymerase chain reaction to confirm selected *E. coli* isolates is presented in Figure 4.4 The PCR amplification of the partial fragment *uidA* gene of the isolates, and separation of DNA successfully yielded a band of ~147 bp fragment (Figure 4.4) and confirms that the isolates were *Escherichia coli*.

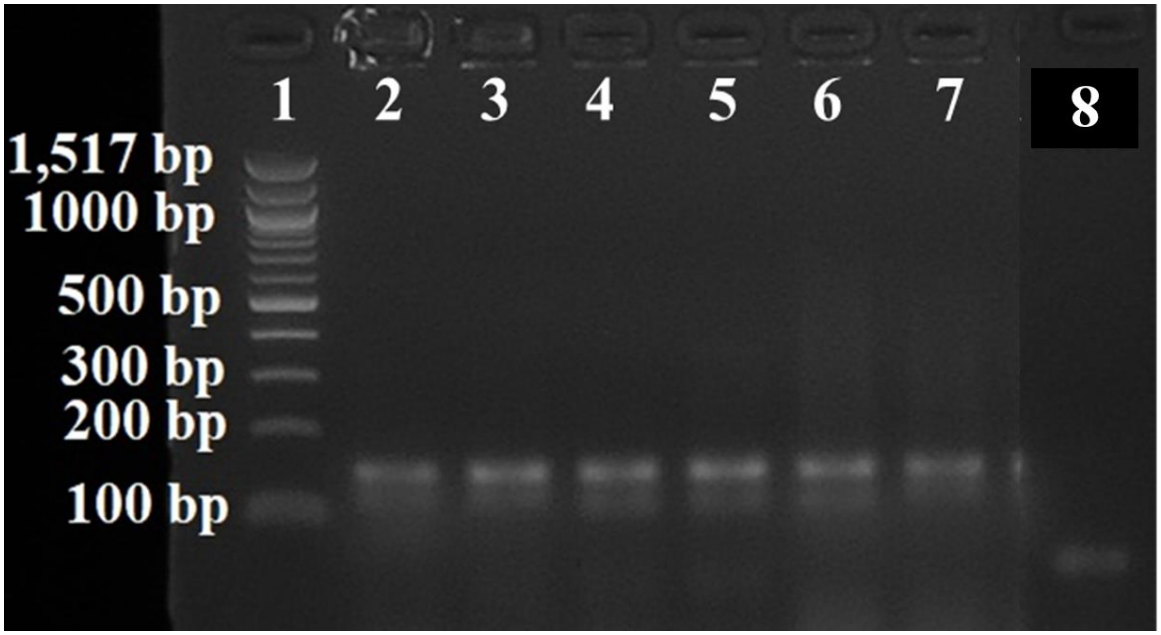

Figure 4.4: Polymerase chain reaction products for the confirmation of *Escherichia coli* isolates. Lane 1: Quick-Load® Purple 100 bp DNA Ladder (New England Biolabs); lanes 2 to 6 *Escherichia coli* isolates from various meat samples (~147 bp fragment); lane 7, positive control and lane 8, negative control.

4.3.2 Polymerase chain reaction (PCR) for the confirmation of *Salmonella enterica isolates*

Results for polymerase chain reaction to confirm selected *E. coli* isolates is presented in Figure 4.5 The PCR amplification of the partial fragment *InvA* gene of the isolates, and separation of DNA successfully yielded a band of ~284 bp fragment (Figure 4.5) and confirms that the isolates were *Salmonella enterica*.

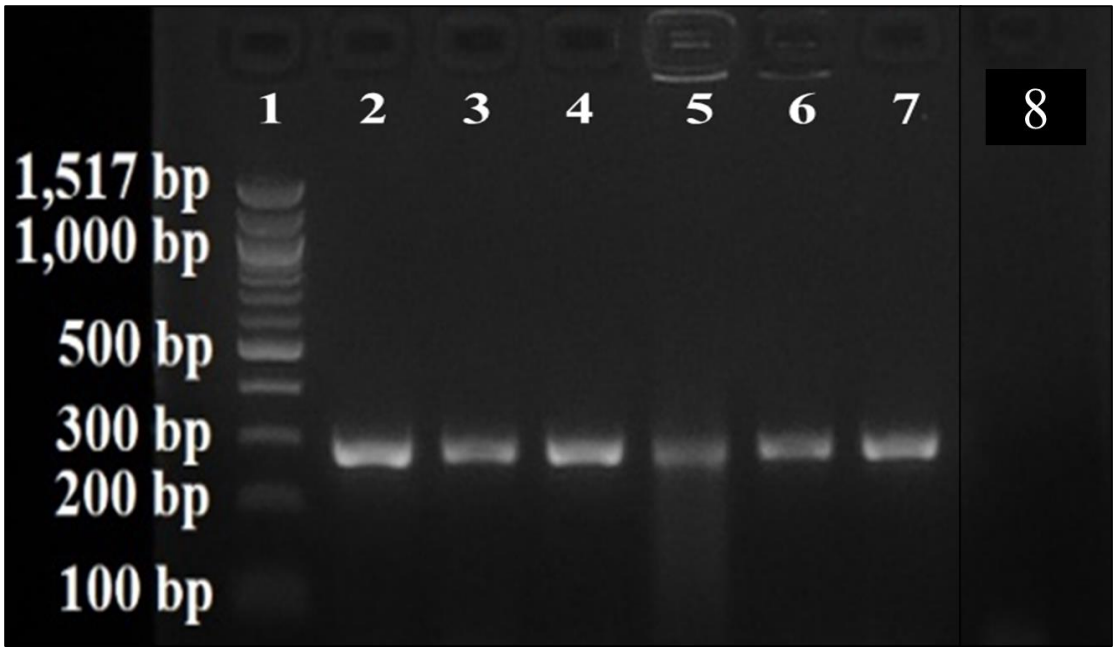

Figure 4.5: Polymerase chain reaction products for the confirmation of *Salmonella enterica* isolates. Lane 1: Quick-Load® Purple 100 bp DNA Ladder (New England Biolabs); lanes 2 to 6 *Salmonella enterica* isolates from various meat samples (~284 bp fragment), lane 7, positive control and lane 8, negative control.

4.4 Antibiotic resistance of *E. coli* isolates from raw meat swab samples during the dry and rainy seasons

4.4.1 Antibiotic resistance of *E. coli* isolates from raw meat swab samples during the dry season

The antibiotic resistance of *E. coli* isolated from the various meat swab samples (raw beef, chevon and mutton) in the dry season is shown in Table 4.1. The highest resistance was observed for Amoxicillin (61.5%), followed by Tetracycline (50%) however, susceptibility was high for Azithromycin (84.6%), Ceftriaxone (80.8%), Chloramphenicol (80.8%), Ciprofloxacin (84.6%), Gentamicin (80.8%) and

Sulfamethoxazole/Trimethoprim (73.1%). Intermediate resistance was relatively high for Amoxycillin/clavulanic acid (34.6%).

Table 4.1: Antibiotic resistance of *E. coli* isolated from raw meat samples in the dry season

Antibiotic	Resistance	Intermediate	Susceptibility
Amoxicillin (AML) 30µg	61.5	19.2	19.2
Amoxycillin/clavulanic acid (AUG) 30µg	11.5	34.6	53.9
Azithromycin (AZM) 15µg	0.0	15.4	84.6
Ceftriaxone (CRO) 30µg	11.5	7.7	80.8
Chloramphenicol (C) 30µg	15.4	3.8	80.8
Ciprofloxacin (CIP) 5µg	15.4	0.0	84.6
Gentamicin (CN) 10µg	3.8	15.4	80.8
Tetracycline (TE) 30µg	50.0	11.5	38.5
Sulfamethoxazole/Trimethoprim(SXT)25 µg	26.9	0.0	73.1
Overall	21.8	12.0	66.3

4.4.2 Antibiotic resistance of *E. coli* isolates from raw meat samples during the rainy season

The antibiotic resistance of *E. coli* isolated from the various meat samples (raw beef, chevon and mutton) in the rainy season is shown in Table 4.2. The highest resistance was observed for Amoxicillin (91.0%), followed by Tetracycline (64.0%) and

Trimethoprim/Sulfamethoxazole (64.0%). Susceptibility was high for Ceftriaxone (82.0%), Ciprofloxacin (82.0%), and Gentamicin (82.0%).

Table 4.2: Antibiotic resistance of *E. coli* isolated from raw meat swab samples in the rainy season

Antibiotic	Resistance	Intermediate	Susceptibility
Amoxicillin (AML) 30µg	91.0	0.0	9.0
Amoxycillin/clavulanic acid (AUG) 30µg	45.0	0.0	55.0
Azithromycin (AZM) 15µg	27.0	18.0	55.0
Ceftriaxone (CRO) 30µg	18.0	0.0	82.0
Chloramphenicol (C) 30µg	18.0	18.0	64.0
Ciprofloxacin (CIP) 5µg	9.0	9.0	82.0
Gentamicin (CN) 10µg	0.0	18.0	82.0
Tetracycline (TE) 30µg	64.0	18.0	18.0
Sulfamethoxazole/Trimethoprim(SXT)25 µg	64.0	18.0	18.0
Overall	37.3	11.0	51.7

4.4.3 Multiple antibiotic resistance (MAR) index and resistance profile of individual *E. coli* isolates from raw meat swab samples in the dry season

The MAR index and resistance profile of individual *E. coli* isolates from raw meat swab samples in the dry season is presented in Table 4.3. The MAR index of *E. coli* ranged from 0.2 to 0.8. Thirteen (13) different resistant profiles were observed. Six

(42.9%) of the *E. coli* isolates were resistant to two (2) antibiotics, four (28.6%) were resistant to three (3) antibiotics, two (14.3%) were resistant to four (4) antibiotics and one each (7.1%) was resistant to five (5) and seven (7) antibiotics, respectively.

Table 4.3: Multiple antibiotic resistance (MAR) index and resistance profile of individual *E. coli* isolates from raw meat samples in the dry season

Code	Source	Number of antibiotics	MAR profile	MAR index
CY3	Chevon	7	AML-C-CIP-CN-CRO-SXT-TE	0.8
MH3	Mutton	5	AML-CIP-CRO-SXT-TE	0.6
BA1	Beef	4	AML-CIP-SXT-TE	0.4
ME1	Mutton	4	AML-C-CRO-SXT	0.4
BA6	Beef	3	AML-AUG-TE	0.3
CA5	Chevon	3	AML-C-TE	0.3
CK1	Chevon	3	AML-SXT-TE	0.3
MY6	Mutton	3	AML- CIP-TE	0.3
BB2	Beef	2	AML-C	0.2
BB6	Beef	2	AML-AUG	0.2
BG3	Beef	2	AML-TE	0.2
CA1	Chevon	2	AML-SXT	0.2
MA1	Mutton	2	AML-SXT	0.2
MY5	Mutton	2	AML-AUG	0.2

4.4.4 Multiple antibiotic resistance (MAR) index and resistance profile of individual *E. coli* isolates from raw meat samples in the rainy season

The MAR index and resistance profile of individual *E. coli* isolates from raw meat swab samples in the rainy season is presented in Table 4.4. The MAR index of *E. coli* ranged from 0.2 to 0.7. Ten (10) different resistant profiles were observed. Three (30.0%) of the *E. coli* isolates were resistant to two (2) antibiotics, two (20.0%) were resistant to three (3) antibiotics, two (20.0%) were resistant to four (4) antibiotics and one (7.1%) was resistant to six (6) antibiotics.

Table 4.4: Multiple antibiotic resistance (MAR) index and resistance profile of individual *E. coli* isolates from raw meat samples in the rainy season

Code	Source	Number of antibiotics	MAR profile	MAR index
BG2	Beef	6	AML-AUG-C-CRO-SXT-TE	0.7
BA4	Beef	5	AML-AUG-C-CRO-SXT	0.6
MA4	Mutton	5	AML-AUG-AZM-SXT-TE	0.6
CB3	Chevon	4	AML-AUG-SXT-TE	0.4
CR6	Chevon	4	AML-AZM-SXT-TE	0.4
CK3	Chevon	3	AML-SXT-TE	0.3
CY1	Chevon	3	AML-AUG-TE	0.3
CK4	Chevon	2	AML-SXT	0.2
MA1	Mutton	2	AML-TE	0.2
MY1	Mutton	2	AML-CIP	0.2

4.5 Antibiotic resistance of *Salmonella enterica* isolates from raw meat swab samples during the dry and rainy seasons

4.5.1 Antibiotic resistance of *Salmonella enterica* isolates from raw meat swab samples during the dry season

The antibiotic resistance of *Salmonella enterica* isolated from the various meat swab samples (raw beef, chevon and mutton) in the dry season is shown in Table 4.5. None of the *Salmonella enterica* isolates was resistant to the antibiotics examined. They were all susceptible to the antibiotics examined except for Ceftriaxone (60.0%). Intermediate resistance was also relatively high for Ceftriaxone (40.0%).

Table 4.5: Antibiotic resistance of Salmonella isolated from raw meat swab samples in the dry season

Antibiotic	Resistance	Intermediate	Susceptibility
Amoxicillin (AML) 30µg	0.0	0.0	100.0
Amoxycillin/clavulanic acid (AUG) 30µg	0.0	0.0	100.0
Azithromycin (AZM) 15µg	0.0	0.0	100.0
Ceftriaxone (CRO) 30µg	0.0	40.0	60.0
Chloramphenicol (C) 30µg	0.0	0.0	100.0
Ciprofloxacin (CIP) 5µg	0.0	0.0	100.0
Gentamicin (CN) 10µg	0.0	0.0	100.0
Tetracycline (TE) 30µg	0.0	0.0	100.0
Sulfamethoxazole/Trimethoprim (SXT) 25 µg	0.0	0.0	100.0
Overall	0.0	4.4	95.6

4.5.2 Antibiotics resistance of *Salmonella enterica* isolates from raw meat swab samples in the rainy season

The various meat samples (beef, chevon and mutton) examined during the rainy season were negative for *Salmonella enterica*, therefore, antibiotic resistance test was not done.

4.5.3 Multiple antibiotic resistance (MAR) index and resistance profile of individual *Salmonella enterica* isolates from raw meat swab samples in the dry season

The *Salmonella enterica* isolates detected were resistant to none of the antibiotics except for an intermediate resistant that was observed for ceftriaxone.

4.5.4 Multiple antibiotic resistance (MAR) index and resistance profile of individual *Salmonella enterica* isolates from raw meat swab samples in the rainy season

This was not done since *Salmonella enterica* was not detected in the various meat swab samples examined during the rainy season.

CHAPTER FIVE

5.0 DISCUSSION

5.1 Prevalence of *E. coli* isolates from raw meat swab samples during the dry and rainy seasons

Strains of *E. coli* is among the pathogens implicated in foodborne illnesses and diseases. Centers for Disease Control and Prevention (2022) reported an outbreak of *E. coli* from the consumption of contaminated ground beef which caused 7 illnesses, 6 hospitalizations and zero deaths. *E. coli* can cause infections with symptoms such as diarrhea (often bloody) and vomiting (Centers for Disease Control and Prevention, 2024a). Some strains of *E. coli* can cause severe infections including urinary tract infections, respiratory illnesses and pneumonia, and even death. Furthermore, season can influence the presence of pathogens in an environment (European Food Safety Authority Panel on Biological Hazards, 2016; Kritzberg and Bååth, 2022). Bacteria have optimum growth range within which they grow. However, most of them prefer to grow at warm temperatures (Government of Western Australia Department of Health, 2022).

In this study, *E. coli* was detected in all meat samples analyzed during the dry and rainy seasons. The presence of *E. coli* was higher during the dry season (48.9%) than the rainy season (13.3%). Among the meat samples, mutton (63.3%) obtained during the dry season was the most contaminated sources, followed by chevon (53.3%) and beef (30.0%) obtained during the dry season, and chevon and mutton (16.7% each) obtained during the rainy season. The least contaminated source was beef (6.7%)

obtained during the rainy season. The higher prevalence of *E. coli* in the dry season than the rainy season may be due to the relatively higher temperature in the dry season that support the growth of most bacteria including *E. coli*. Feng *et al*. (2020) reported that the growth range for *E. coli* is between 35^{o}C and 44.5^{o}C, which is within the temperature range found in the study area during the dry season. Also, the presence of *E. coli* in raw meats suggests faulty processing or cross contamination during slaughter and processing of cattle as Warriss (2000) indicated that the tissues of healthy animals are typically sterile. Sources for cross contamination of meat by bacteria are said to include the knives used for cutting meats, the tables meats are placed on, aprons worn by butchers, hands of butchers/meat sellers and surfaces of the slaughterhouse environment like the floor and walls (Ali *et al*., 2010; Iroha *et al*., 2011; Hosseini & Haslberger, 2016).

The results of this study are comparable to others such as Adzitey *et al*. (2020a) who reported that mutton (88.9%), beef (86.7%) and chevon (75.6%) collected from Tamale metropolis of Ghana were contaminated with *E. coli*, which were higher than what was observed in this study. An earlier study by Adzitey (2015) found that 56.0% of beef samples were contaminated with *E. coli* which was quite similar to the prevalence observed for chevon in the dry season but not the rest of the meat samples and the seasons they were analyzed. Adjei *et al*. (2022) sampled raw beef samples from Ashaiman, Ghana and reported that 29.0% were contaminated with *E. coli*., which is similar to the 30.0% reported for beef collected during the dry season. In Egypt, 11.7% of beef samples collected from slaughterhouses and markets were

contaminated with *E. coli* (Moawad *et al*., 2017), which was lower than that reported in the current study except the 6.7% found for beef obtained during the rainy season.

5.1 Prevalence of *Salmonella enterica* isolates from raw meat samples during the dry and rainy seasons

Salmonella enterica are among the most important foodborne pathogens of public health significance. They cause salmonellosis with symptoms such as diarrhea, fever and stomach cramps, which can be self-limiting (Centers for Disease Control and Prevention, 2024b). Centers for Disease Control and Prevention (2023) reported an outbreak of *Salmonella* infection from the consumption of contaminated ground beef which caused 18 illnesses, 7 hospitalizations and zero deaths. Nonetheless, the consumption of foods contaminated with *Salmonella* can cause death especially in immune-compromised individuals (Centers for Disease Control and Prevention, 2024c). Therefore, the presence of *Salmonella enterica* in raw meats will be a worrying situation that require proper attention via education, proper cooking and avoidance of cross contamination prior to consumption of meat.

In this study *Salmonella enterica* was detected in chevon (13.3%) and mutton (3.3%) samples collected during the dry season, but not the rest of the samples in both the dry and rainy seasons. Therefore, chevon collected during the dry season was the most contaminated sample by *Salmonella enterica*, followed by mutton collected during the dry season and the rest of the samples were negative for *Salmonella enterica*. Similarly, to the results of *E. coli* in the various meat samples, the prevalence of *Salmonella enterica* was higher in the dry season. The absence of *Salmonella enterica*

in the beef, chevon and mutton during the rainy season and beef in the dry season is good and recommended. The flesh of a healthy cattle meant for slaughter is expected to be free from bacteria except if the animal is suffering from bacteremia. Thus, careful and hygienic handling, slaughtering and marketing of meats will ensure that meats are safe for human consumption. Veterinary officers also inspect cattle to ensure that they are healthy prior to slaughter and inspects carcasses after slaughter to ensure that only wholesome meats are passed for consumption. Nonetheless, *Salmonella* harbors the gastrointestinal tract of animals and cross contaminate meats due to poor hygienic slaughter environment and practices during slaughtering and marketing of meats by meat processers, butchers and meat sellers.

Other works have reported on the prevalence of *Salmonella* in various meats. Adzitey (2015) found a prevalence rate of *Salmonella enterica* to be 31.0% for beef samples collected from different locations in Ghana. A later study by Adzitey *et al*. (2020b) reported a prevalence of 42.2%, 48.9% and 73.3% for raw beef, chevon and mutton, respectively in Tamale metropolis. Again, Ekli *et al*. (2019) reported that, 30.0% of beef samples collected from Wa, Ghana was contaminated with *Salmonella enterica*. These prevalence rates were higher than what was recorded in this study. *Salmonella enterica* was found in 7.5% of raw beef samples collected from Ashaiman, Ghana (Adjei *et al*., 2022). In Egypt, Moawad *et al*. (2017) reported that 8.3% of raw beef samples were contaminated with *Salmonella enterica*. In Ethiopia and USA, a prevalence of 12.0% (Ejo, *et al* 2016) and 4.2% (Bosilevac *et al*., 2009), respectively was reported for *Salmonella enterica* in beef samples. The findings of Adjei et al.

(2022), Moawad *et al*. (2017), Ejo *et al*. (2016) and Bosilevac *et al*. (2009) are within the range (13.3% and 3.3%) reported by this study.

5.3 Antibiotic resistance of *E. coli* isolates from raw meat samples during the dry and rainy seasons

Antibiotics are used in animal production for treatment of animals, as prophylactics and sometimes as growth promotors (Roberts 2003; Al-Khalaifah, 2018). For instance, Ekli *et al*. (2020) reported that farmers in the Wa, municipality of Ghana use antibiotics such as ciprofloxacin (32.0%), sulphamethoxazole/trimethoprim (17.1%), gentamicin (1.8%), ceftriaxone (0.9%), chloramphenicol (0.9%) and tetracycline (0.9%) as prophylactics or to treat animal diseases. They also reported that 73.2% of the farmers did not observe withdrawal periods when they administer, prior to sale and slaughter of animals. The use of antibiotics, particularly for the treatment of sick animals is unavoidable and the use of antibiotics in animal production has links with the development of resistances by bacteria in farm animals. Economou and Gousia (2015) indicated that, agriculture and food animals have been reported to be sources of antimicrobial resistance bacteria.

In this study, the *E. coli* isolated from the various meats in the dry season exhibited higher resistances to Amoxicillin (61.5%) and Tetracycline (50%), but susceptible to Azithromycin (84.6%), Ceftriaxone (80.8%), Chloramphenicol (80.8%), Ciprofloxacin (84.6%), Gentamicin (80.8%) and Sulfamethoxazole/Trimethoprim (73.1%). Quite similarly, *E. coli* isolated in the rainy season exhibited higher resistances to Amoxicillin (91.0%), Tetracycline (64.0%) and Sulfamethoxazole/Trimethoprim

(64.0%), but susceptible to Ceftriaxone (82.0%), Ciprofloxacin (82.0%), and Gentamicin (82.0%). The MAR index ranged from 0.2 (resistant to 2 antibiotics) to 0.8 (resistant to 7 antibiotics) and 0.2 (resistant to 2 antibiotics) to 0.7 (resistant to 6 antibiotics) for *E. coli* isolated from dry and rainy season, respectively. Furthermore, thirteen (13) different resistant profiles and 57.1% multidrug resistance occurred for *E. coli* isolated during the dry season while, ten (10) different resistant profiles and 70% multidrug resistance occurred for *E. coli* isolated during the rainy season.

All *E. coli* isolates resistant to an antibiotic was resistant to at least Amoxicillin. *E. coli* isolates, that is, CAI from chevon and MAI from mutton isolated in the same season, but different meat type exhibited the same resistant profile of AML-SXT (resistant to Amoxicillin and Sulfamethoxazole/Trimethoprim). Some *E. coli* isolates from dry and rainy seasons from the same or different meat types exhibited the same resistant profile. For example, CA1 from chevon and C4 from chevon isolated from *E. coli* in the dry and rainy season exhibited the resistant pattern, AML-SXT. Also, BA6 isolated from beef and CY1 isolated from chevon in the dry and rainy seasons, respectively exhibited the same resistant profile of AML-AUG-TE (resistant to Amoxicillin, Amoxycillin/clavulanic acid and Tetracycline). Overall resistance resistant of 21.8% versus 37.3%, intermediate resistant of 12.0% versus 11.0% and susceptibility of 66.3% and 51.7%, respectively was recorded for the *E. coli* isolated from the dry and rainy season, respectively, which were relatively similar.

A study by Adzitey et al. (2020a) found that *E. coli* isolates exhibited higher resistances to tetracycline (73.33%), but susceptible to gentamicin (88.33%), ciprofloxacin (85.00%), sulphamethoxazole/trimethoprim (85.00%), chloramphenicol (83.33%) and ceftriaxone (80.00%), which are comparable to this study. MAR index ranged from 0.13 to 1 and 23 antimicrobial resistance profile was observed. This study found fewer antimicrobial resistance profiles, that is 13 for dry season *E. coli* isolates and 10 for rainy season *E. coli* isolates and MAR index ranged from 0.2 to 0.8. Resistant to TeAmpE (tetracycline-ampicillin-erythromycin) was the most common and multidrug resistance was 68.3% (Adzitey *et al*., 2020a). Multidrug resistance was lower for *E. coli* isolates from the dry season but not the rainy season. According to Moawad *et al*. (2017), *E. coli* isolated from meat sources were resistant to tetracycline (80.9%), ampicillin (71.4%), streptomycin, sulphamethoxazole/trimethoprim (61.9% for each) and cefotaxime (33.3%). Resistance to tetracycline was higher in the study of Moawad *et al*. (2017) compared to this study, while that of sulphamethoxazole/trimethoprim varied (lower for dry season and higher for rainy season). Bacteria uses different mechanisms including the production of enzymes (e.g., beta-lactamases from Gram-negatives) and modification of binding sites (e.g., penicillin-binding proteins of Gram-positive beta-lactams) to increase their resistance (Munita & Arias, 2016). Also, the role of transformation, transduction and conjugation in the transfer of antibiotic resistance genes cannot be over-emphasized (Munita & Arias, 2016).

5.4 Antibiotic resistance of *Salmonella enterica* isolates from raw meat swab samples during the dry and rainy seasons

The wide spread usage and misuse of antibiotics in livestock farming makes a significant contribution to the spread of antibiotic resistances among bacteria associated with farm animals.

In this study, the *Salmonella enterica* isolates were 40.0% intermediately resistant and 60.0% susceptible to ceftriaxone. The *Salmonella enterica* was 100.0% susceptible to Amoxicillin, Amoxycillin/clavulanic acid, Azithromycin, Chloramphenicol, Ciprofloxacin, Gentamicin, Tetracycline and Sulfamethoxazole/Trimethoprim and exhibited no resistance.

Ekli *et al*. (2019) found that *Salmonella enterica* from beef samples were highly resistant to teicoplanin (96.77%) but susceptible to chloramphenicol (100%), ciprofloxacin (100%), tetracycline (100%), suphamethoxazole/trimethoprim (100%), amoxycillin/clavulanic acid (93.55%), ceftriaxone (93.55%) and gentamicin (83.87%). Susceptibility to chloramphenicol, ciprofloxacin, tetracycline and suphamethoxazole/trimethoprim were similar to this study. *Salmonella enterica* from meat showed 5 different resistance profiles and MAR index ranged from 0.11 to 0.33 (Ekli et al., 2019), these were absent in the current study. Adzitey et al. (2020b) found that *Salmonella enterica* isolates from various meat sources were susceptible to ciprofloxacin (97.73%), chloramphenicol (93.18%), gentamicin (79.55%), suplfamethoxazole/trimethoprim (90.91%) and tetracycline (84.09%), which contradicts the findings of this study which found 100% susceptibility throughout.

According to Moawad *et al*. (2017), *Salmonella enterica* isolates from meat sources were resistant to ampicillin (86.7%), cefotaxime (80.0%), cefpodoxime (60.0%), sulphamethoxazole/trimethoprim (53.3%) and tetracycline (40.0%). In this study resistance to sulphamethoxazole/trimethoprim and tetracycline was not found.

Antimicrobial resistances result from the use antibiotics in animal production which is sometimes easily accessible without strict regulations. Anachinaba *et al*. (2022) indicated that farmers heard about antibiotic resistance from extension officers (53%), veterinary officers (27%), media (11%) and colleague farmers/schools (9.0%), and used antibiotics for treatment of sick animals (45%), as growth promoters (35%), as prophylactics (12%) and a combination of the three (9%). They also engaged veterinary officers (15%), administer antibiotics themselves (35%), by colleague farmers (41%) and a combination of the three (9%).

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

6.1 Conclusion

The results of this study showed that all raw meats were contaminated with *E. coli* and *Salmonella enterica* with highest prevalences during the dry season and mutton been the contaminated whilst beef was the least contaminated.

The highest antibiotics resistance was observed for Amoxicillin followed by Tetracycline for both seasons and an *E. coli* isolate was resistant to a maximum of seven antibiotics and a minimum of two antibiotics with susceptibility greater than 80.0% against Azithromycin, Ceftriaxone, Chloramphenicol, Ciprofloxacin and Gentamicin. *Salmonella enterica* was susceptible to all the antibiotics except ceftriaxone in which 40.0% intermediate resistance occurred. Multidrug resistance was not observed in the isolated *Salmonella enterica*.

6.2 Recommendations

- The condition of the slaughter slab in Buipe needs general restructuring and expansion by the District Assembly to minimize contaminations and cross contaminations during animal handling, slaughtering and processing of meats.
- Butchers and other meat handlers need basic training on hygienic meat handling by the appropriate authorities such as Veterinary Services or Meat Science Professionals to reduce meat contaminations at the slaughter and throughout the meat production value chain to prevent possible zoonoses.
- Further work by researchers is recommended for demographic characterization and knowledge on hygienic practices among butchers and meat sellers.

- Further studies are required on the molecular characterization of the foodborne bacteria by researchers and relevant stakeholders to identify antimicrobial resistance genes, virulence genes and genetic diversity of other foodborne microbes in Buipe meats.

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