UNIVERSITY FOR DEVELOPMENT STUDIES, TAMALE

ANTIBIOTIC RESIDUES AND PREVALENCE OF RESISTANT SALMONELLA SPECIES IN BEEF OBTAINED FROM WA ABATTOIR

EKLI REJOICE

2019

UNIVERSITY FOR DEVELOPMENT STUDIES, TAMALE

ANTIBIOTIC RESIDUES AND PREVALENCE OF RESISTANT SALMONELLA SPECIES IN BEEF OBTAINED FROM WA ABATTOIR

BY

EKLI REJOICE (BSc. Agriculture Technology)

(UDS/MAN/0004/17)

A THESIS SUBMITTED TO DEPARTMENT OF ANIMAL SCIENCE, FACULTY OF AGRICULTURE, UNIVERSITY FOR DEVELOPMENT STUDIES, IN PARTIAL FULFILMENT OF REQUIREMENTS FOR THE AWARD OF MASTER OF PHILOSOPHY (MPHIL) DEGREE IN ANIMAL SCIENCE (MEAT SCIENCE OPTION)

SEPTEMBER, 2019

DECLARATION

I hereby declare that this thesis has been authored by me and has neither been submitted for a degree nor any aspect published by another person elsewhere. However, all work of others cited in the text has been well referenced and any assistance received in writing the thesis is duly acknowledged.

Candidate' Signature…… Date……....

Name: Rejoice Ekli

Supervisors

We hereby declare that the preparation and presentation of this thesis are duly supervised in accordance to the guideline on supervision of thesis laid down by the University for Development Studies.

Principal Supervisor's Signature ……. Date……

Prof. Frederick Adzitey (PhD)

Co-Supervisor's Signature ……Date……

Dr. Anthony Amision Agbolosu (PhD)

ABSTRACT

Beef contamination with *Salmonella* and their resistance to antibiotics is a concern and a threat to the public health. This study determined the knowledge of ruminant farmers and veterinary officers in antibiotic usage, and the presence of antibiotic residues in beef samples in the Wa Municipality of Ghana. The microbiological quality and prevalence of resistant *Salmonella* spp. in the beef samples were also determined. Two hundred and fifty ruminant farmers and six veterinary officers were interviewed on their knowledge and usage of antibiotics administration in ruminant production using semi structured questionnaire. Snowball sampling was used to select the farmers for this research. Forty-eight meat samples from sixteen cattle were tested for antibiotic residues using Premi®Test Kit by following the manufacturer's instructions. One hundred and fifty beef swab samples, taken from Wa abattoir were examined for the prevalence of *Salmonella* spp. Isolation of *Salmonella*, and enumeration of coliforms and total aerobic bacteria were done according to the procedures in the USA-FDA Bacteriological Analytical Manual. Antibiotic susceptibility test was performed using the disc diffusion method and the results interpreted using the CLSI guidelines. The commonly used antibiotics were ciprofloxacin (32%), amoxicillin/clavulanic (27%), trimethoprim/ sulfamethoxazole (17.1%), azithromycin (5.4%), gentamicin (1.8%), ceftriaxone (0.9%), tetracycline (0.9%) and chloramphenicol (0.9%). Majority (63.6%) of the farmers had some knowledge on the antibiotics administered to their animals. They acquired the knowledge from veterinary officers (51%), colleague farmers (29%) and extension officers (20%). Majority (51%) also relied on veterinary officers to administer drugs to their animals. Out of the 48 meat samples examined, 14 (29.17%) were positive for antibiotic residues. The prevalence of antibiotic residues in the kidney, liver and muscles were 43.75%, 37.50% and 6.25%, respectively. And of 150 beef swab samples examined, 36 (24%) were positive for *Salmonella* spp. Total aerobic count was 3.57 logcfu/cm^2, 3.39logcfu/cm^2 and 3.23 logcfu/cm^2 for muscle, liver and kidney, respectively. Forty-two (42) *Salmonella* isolates were tested against 9 different antibiotics. The results revealed a high resistance to teicoplanin (97.62%). Resistant to azithromycin was 30.95%. The *Salmonella* isolates were highly susceptible to chloramphenicol (100%), ciprofloxacin (100%), suphamethoxazole/trimethoprim (100%), tetracycline (100%), ceftriaxone (95.24%), amoxycillin/clavulanic acid (90.48%) and gentamicin (78.57%). Out of 42 *Salmonella* isolates, 29 were resistant to one antibiotic, 6 were resistant to two antibiotics and 1 was resistant to four antibiotics Some of the beef samples in the area contained antimicrobial residue which were above acceptable daily intake. The result also revealed that beef samples in Wa municipality were contaminated with *Salmonella* spp some of which were resistant to some antibiotics. Therefore, consumers of beef in this municipality are at risk of harboring antibiotic residues and resistant *Salmonella* spp.

ACKNOWLEDGEMENTS

I am very grateful to the Almighty God for His protection and guidance from the beginning of my course to the end. I owe Him an unending gratitude for bringing me this far.

My appreciation goes to my project supervisors Prof. Frederick Adzitey and Dr. Anthony Amision Agbolosu for the fatherly love shown to me throughout this work which has brought the work to a successful end. I say God bless you all exceedingly abundantly above all that you can imagine.

I am also grateful to all the Lecturers in the Department of Animal Science for their encouragement and the knowledge imparted on me.

I would like to appreciate Mr. Samid, the veterinary officer in charge of Wa abattoir, for his generosity and kindness throughout the sample collection.

My appreciation goes to Mr. Halidu Agolisi Mamudu, Mr. Joseph and Mr. Rauf for the help they provided during data collection. I say Almighty Jehovah bless you.

I cannot forget of my lovely mother, madam Azaguh Grace and brother, Mr. Ekli William for their love and financial support. I greatly appreciate every bit of help I received from other family members either in kind or cash.

DEDICATION

This thesis is dedicated to my beloved mother, Madam Grace Azaguh and my brother Mr. Ekli William for their endless prayers and support in my education.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Foodborne disease outbreaks continue to be an important public health problem globally and most of the food safety hazards are caused by foods of animal sources (Maripandi and Al-Salamah, 2010). Bhandare *et al.* (2007) also indicated that contaminated raw meat has been recognized as one of the main sources of foodborne illnesses. Among the reasons for which meat and its products are

consumed include their high protein contents, available vitamins, minerals and lipids savory sensation. However, due to its high nutrient content which supports the growth of microorganisms, meat is classified among the most perishable foods products (Huda *et al.*, 2010). Adeyemo (2002) indicated that there are two sources for which microorganisms get into meat that is either they go in through the hide of the animal or through the abattoir where the animals are severed and processed. Foodborne pathogens including *Staphylococcus* spp., *Salmonella* spp., *Shigella* spp., *Bacillus* spp., *Listeria monocytogenes* and *Escherichia coli* have previously been isolated from meats in Ghana (Soyiri *et al*., 2008; Adzitey *et al*., 2015a; Adzitey *et al*., 2015; Anachinaba *et al*., 2015) and some of them have been subjected to antimicrobial susceptibility test (Adzitey *et al*., 2015b). According to Fratamico *et al*. (2005), most illnesses and deaths in developing countries are caused by foodborne diseases and these cost several dollars in medical treatment and social expenditures. Globally, *Salmonella* spp. are one of the pathogens that lead millions of enteric disease cases, thousands of people being hospitalized and some of them end up dying every year (Hur *et al*., 2011). CDC (2005) stated that 1.4

million estimated cases of foodborne illnesses are cause by *Salmonella* spp. and 500 and above perishes every year in the US. This pathogen is also responsible for food poisoning collectively with 65% of cases in France (Haeghebaert *et al*., 2003). The resistance of microorganism to antibiotics is a challenge to both veterinary and human practices globally (WHO, 2001). It is largely acknowledged that the antibiotic resistance increase is the core risk factor associated with the wide use of antibiotics. The use of antibiotics in animals are not only increasing the resistivity of animal pathogen, but also pathogens transferred from animal to humans (Molbak, 2004). The resistance of microbe to antibiotic is becoming a major challenge when treating serious infections with antibiotics and threatens unexpected effects on a greater range of medical actions. Researches have revealed that antibiotic use in animals mainly for food production could lead to antibiotic resistance in animal and human pathogens (Franklin, 2016). Doyle (2006) also emphasized that, antibiotic resistant bacteria could result in difficult to treat infection in human and also disturb normal human flora in the intestines. According to Tajick and Shohreh (2006), the main risk associated with antibiotics residue is the body's microflora becoming resistant to some antibiotics and these could cause severe problems when one is infected with microbes. Lee *et al*. (2001), stated that almost eighty percent of all food producing animals receive medication for part or most of their lives. According to Kozarova *et al.* (2001), several antibiotics have different periods to be eliminated from the body and this becomes a potential hazard to the health of human. When antibiotics are used in food producing animals, it could leave traces of antibiotics residues in meat and offal. Wasch *et al*. (1998) reported that, there are antibiotics residues in the muscle tissues of chickens and pork.

For some years now, the occurrences of antibiotic resistant *Salmonella* spp. have been a serious health challenge in the world. The frequent administration of antimicrobial agents in animal production especially those purposely for food and the monotonous practice of giving these antimicrobial agents to domestic animals as a means of treating and preventing ailments has also resulted in the development of *Salmonella* spp. that have decrease susceptibility to drugs (Angulo *et al*., 2000).

1.2 Objectives

1. To determine the knowledge of ruminant farmers on antibiotic usage in ruminant production in Wa Municipality.
2. To determine the prevalence of antibiotic residues in beef (muscle, liver and kidney) samples collected from abattoir in Wa.
3. To determine the microbial load of the beef samples collected from abattoir in Wa.
4. To determine the prevalence and antibiotic resistance of *Salmonella* spp isolated from beef in Wa.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Meat

According to Lawrie and Ledward (2006), the flesh of animal that is ingested as food is called meat. A report by Lawrie and Ledward (2006) and Food Standards Australia New Zealand (FSANZ) (2002) explained that meat is the skeletal muscle and it related fat and additional tissues which includes offal's, brain, liver, heart, pancreas, kidney, spleen, tongue, thymus and tripe. McArdle (2000) reported that meat is produced as a result of slaughtering and butchering animal, thus killing and cutting flesh out of the animal. According to Tutenel *et al. (*2003) the principal composition of meat is protein and water and this is mostly consumed together with other foods. Even though meat can be consumed in raw form, it is mostly consumed after successive cooking and processing in various ways. Meats that are untreated spoil within some few hours or days. Spoilage in general, is as a result of practically unavoidable contamination which lead to deterioration of meat by microorganisms and fungi that may be borne by the animal itself, the meat handlers and their instruments (Tutenel *et al.,* 2003). Depending on the myoglobin level in the myofibrils, meat could be broadly classified as "red" or "white". Myoglobin-rich meats appear reddish because when myoglobin in the meat exposes to oxygen, it reacts to oxygen and become oxy-myoglobin which is red. The red color of meat also depends on the age, species of animal and the type of myofibrils. Red meat has more slim myofibrils however, white meat has more fat myofibrils (Lawrie and Ledward, 2006). According to Williams (2007), the nutritional structure of red meats depend on the feed, breed, season and meat cut. Nevertheless, the protein content, essential vitamins and minerals are uniformly high in lean red meat. Sofos (2008), also emphases that meat is a whole protein food which

contained all the essential amino acids required for the human body. Meat includes beef, chevon, mutton, pork and chicken/poultry.

2.1.1 Beef

Bovine meat is called beef especially meat from cattle (*Bos primigenius*). Beef can be obtained from heifers, cows, steers and bulls. The tail, testicles, tongue and internal organs such as brain, liver, heart, stomach, pancreas and intestines are other portions that may be eaten. Beef from steers have more muscle with less fat than that of heifers. Globally, beef forms 25% of meat production and it is the third most extensively consumed meat next to pork (38%) and poultry (30%) (Raloff, 2003). Globally, the three largest Nations that consume beef includes United States, Chinaand Brazil (USDA, 2009). Furthermore, the United States, Australia, Brazil and India are the chief exporters of beef globally.

2.2 Meat Consumption and Related Health Issues

Speedy (2003) stated that meat consumption varies widely all over the world. According to FAO (2003) the overall meat consumption in the globe is increasing in the nations that are developed and U.S. is the premier consumer. Carrie *et al*. (2011) reported that in U.S. red meat is still the most consumed meat. The report further indicated that meat consumed in the U.S., only quarter of it is processed. Walker *et al.* (2005) and Speedy (2003) indicated that meat consumption in countries that are developing continues to advance as their production and consumption of meat increases with available income. A report by Cross *et al*. (2007), indicated that health risks related to meat consumption may vary depending on the animal the meat is obtained from and method of production, processing and preparation.

They further indicated in their report that colorectal, lung, esophagus and liver cancer can be due to high red meat consumption. A high dangers of lung cancer has been reported as a result of meat consumption; red meat (Alavanja *et al.* 2001), fried red meat (Sinha *et al*. 2000; Deneo-Pellegrini *et al*. 1996), well done red meat (Deneo-Pellegrini *et al*. 1996) and processed meat (Goodman *et al*. 1992). Larsson and Orsini (2013) indicated that high red meat consumption (processed meat) is associated with higher all-cause mortality. Globally those who mostly consumed meat that is processed and whole red meat had increased all-cause mortality of 23% and 29%, respectively, compared with those who consumed less (Larsson and Orsini, 2013).

2.3 Microorganisms Contaminating Meat

A report by Abaidoo and Obiri-Danso (2008), described microorganisms as minute living creatures found everywhere in nature including meat. They can be seen with the aid of microscope due to their small structure in nature. Some examples of microorganisms found in meat are bacteria, yeasts, molds and viruses. Some of these are pathogenic. Thus, they are capable of causing foodborne illnesses (Abaidoo and Obiri-Danso 2008). For this reason, Doyle (2007) indicated that meat should be stored in the coldest part of refrigerator or frozen, practicing good hygiene to prevent microbial contamination.

2.3.1 Bacteria of Health Concern in Meat

Meat can encourage the growth of a wide range of microorganisms if not appropriately handled, processed and preserved due to its high nutrients composition. The contact of the hide with carcass during slaughtering allows a multitude of microbes to contaminate the

carcasses. These contaminating microbes from the hide may be of fecal, feed, soil and water source (Bell, 1997). The greater number of these microbes exist in the intestinal tracts of animals and during slaughtering some of these could get into the carcass surfaces (Bell, 1997). *Salmonella, Staphylococcus aureus, Clostridia perfringens, Listeria monocytogenes, Escherichia coli*, *Yersinia enterocolitica,* and *Campylobacter jejuni* have been identified from raw meat samples. Some of these microbes are pathogenic and mostly cause foodborne disease (Bean *et al.*, 1990).

Table 1: Bacteria of health concern in meat and their sources

Organism	**Principal source(s)**
Staphylococcus aureus	Skin, mucous membranes of handlers
Clostridium perfringens	Soil, intestinal tract
Listeria monocytogenes	Soil, water, air or intestinal tract
Enteropathogenic Escherichia coli	Intestinal tract
Yersinia enterocolitica	Intestinal tract
Salmonella spp.	Intestinal tract

Source: Church and Wood (1992)

Foodborne pathogens contaminating carcasses is a major public health problem. Contamination of food by microbes decreases its shelf-life and promotes foodborne illness. Outbreaks of foodborne diseases have led to considerable illness and even death. In USA, 24 to 81 million cases of foodborne infections are dreporte yearly, out of which half (50%) are related to meat and poultry (Unnevehe, 2000).

2.4 Aerobic Plate Count, Coliforms and Enterobacteriaceae

Aerobic bacteria are those which utilize oxygen as a source of energy for metabolism and examples includes *Pseudomonas aeruginosa*, *Bacillus* spp, *Mycobacterium tuberculosis* and *Nocardia* spp. Coliform microorganisms are defined as rod-shaped Gram-negative non-spore forming and motile or non-motile bacteria that may ferment lactose to produce gas and acid when incubated at 35-37°C (Kanangire, 2013). They could be found naturally in the environs such as vegetation, soil, and also human and animal faces (Kanangire, 2013). The incidence of coliform bacteria in food and water give indication that further pathogenic microbes of fecal basis might be present and these may involve bacteria, protozoa, viruses and many multicellular parasites that cause disease (Kanangire, 2013). Examples of coliform bacteria are *Escherichia coli, Citrobacter, Klebsiella, Hafnia* and *Enterobacter*. Coliform bacteria are grouped into three and these are total coliforms, fecal coliforms and thermos-tolerant bacteria (WHO, 2011). The large group of diverse bacteria that live in the intestine is known as total coliform. Fecal coliform are group of bacteria found in feces for instance *Salmonella* and *E. coli.* Thermo-tolerant microbes also can be called fecal coliform microbes, which are a part of the total coliform bacteria coming from intestines and feces of humans or animals. Together with *E. coli*, thermos-tolerant bacteria are suggested as an indicator for fecal contamination. *Enterobacteriaceae* is a family of a huge, heterogeneous group of gram-negative rods whose regular environment is the alimentary canal of animals and humans (Abaidoo and Obiri-Danso, 2008). They may be found in soil and plant and these can be a source of contamination in the food chain and cause foodborne gastroenteritis.

They are regarded as indicators of fecal contamination when present in foods (Abaidoo and Obiri-Danso, 2008). *Enterobacteriaceae* are facultative aerobes and anaerobes that can ferment a different range of carbohydrates, possess a complex antigenic structure, and produce a variety of toxins and other virulence factors. The genera in this family include *Escherichia, Shigella, Salmonella, Yersinia, Enterobacter, Klebsiella, Serratia, Proteus* and others (Abaidoo and Obiri-Danso, 2008).

2.4.1 *Escherichia coli*

Some *Escherichia coli* strains are pathogenic and therefore produce an enterotoxin. Individuals can be infested through the consumption of food and water that are contaminated with *E. coli*. Raw beef might be a significant vehicle for *E. coli* transmission if not properly handled during slaughtering, processing or cross-contamination due to unhygienic food handling practices. The presence of *E. coli* in meat is mostly from fecal contamination (Publi Health Agency of Canada (PHAC), 2014; Abaidoo and Obiri-Danso, 2008). In Ghana, Adzitey (2015) reported 56% of *Escherichia coli* prevalence in beef in the Tamale Metropolis. Rotar *et al*. (2013) identified 34.46%, 45.83%, 15.38% and 24.75% of *E. coli* in minced meat, meat, meat products, and cheese from pasteurized milk, respectively. Ayla and Seza (2012) analyzed 168 samples of poultry meat (56), ground beef (56) and beef (56) and recorded 53.6% of all the samples contaminated with *E. coli*. They also reported 87.5%, 48.2%, and 25%, poultry meat, ground beef and beef samples contaminated with *E. coli*. Temellİ *et al*. (2012) assessed 52 ground meats and 20 chopped meats and reported the presence of *E. coli* to be 42.30% in ground meat and 25% in chopped meat.

2.4.2 *Salmonella* species

Ryan and Ray (2004) reported that *Salmonella* are generally dispersed in nature and are responsible for the following infections; food poisoning, typhoid fever and paratyphoid fever. According to CDC (2014), infants, aged, and those with weakened immune systems are severely affected by *Salmonella* infections. Animals could harbor the bacteria which make products obtained from them often implicated vehicles for *Salmonella* transmission. Hence, animal based foods are vehicles for salmonellosis (Institute of Food Technologists (IFT), 2004). Manure and litter may also be sources of *Salmonella* contamination, especially through soil and water which may end up contaminating products such as fruits and vegetables (IFT, 2004). Cross contamination at home or food service environment during food processing or food handling can also cause salmonellosis. The *Salmonella* bacteria may survive and contaminate foods that are not properly cooked. It is therefore common to have cross-contamination of foods after cooking. Food handlers may transfer *Salmonella* from raw products to cooked or other uncontaminated foods as a result of unsanitary practices such as poor hygiene (IFT, 2004).

2.5 Sources of Microbial Contamination of Beef Carcasses

Generally, carcass contamination by pathogens is related to a number of activities that occur during pre-slaughtering, slaughtering and post slaughtering operations. The slaughter stock is recognized as chief source of carcasses infection. The hide, alimentary canal and respiratory tracts of slaughtered animals are the main sources of carcass contamination since these places are the residents of pathogenic and spoilage bacteria (Sofos *et al.*, 1999). According to Anon (2002), slaughtering, dressing and evisceration processes of beef

carcass was identified as potential points of introducing major contaminant. Thus, when contact between carcass and the hide exist, several types of microorganisms may be introduced onto the carcass. These microbial contaminants are derived from the animals' pre-slaughter environs and could be of fecal, water, soil and feed source (Bell, 1997). According to Meat Technology Update (MTU), (2010) sanitation in the abattoirs, physical structures, personnel and their equipment also constitute a significant source of contamination.

2.5.1 Slaughter Stock

A significant source of carcasses contamination results from the animals themselves (Aberle *et al*., 2001). The hides, skins, fecal material, hooves and hairs of cattle are major sources of microorganisms. Contamination from hides surface has been found to range from 3.53 to 12.5 $\log_{10}$ cfu /cm² (MTU, 2010). Hayes (1985) found the bacteria counts on the hides of cattle to be 105 per cm^2. According to MTU (2010), microbial counts and prevalence of foodborne pathogens on hides is higher than intestinal contents and for this reason, carcasses from animal with wet hide contains more coliform count. According to Public Health Veterinarian (PHV), (2011) many pathogenic microbes especially *Salmonella*, *Campylobacter*, *E. coli* O157:H7 and others are located in the intestinal biota of livestock and poultry. During evisceration process the intestinal contents can contaminate carcasses if the gut ruptures (MTU, 2010).

2.5.2 Slaughterhouse and Equipment

A facility where animals are exsanguinated and processed into other meat products is called slaughterhouse. Developed countries have large slaughterhouse facilities where slaughtering is completely carried out in automated lines and carcasses move on a conveyor system from one point to another until the slaughtering process is completed. In developing countries including Ghana, there are limited slaughterhouse facilities. For instance in Ghana, the greater proportion of butchers use knives and machete as the main equipment for slaughtering (FAO, 1985; Adzitey *et al*., 2011). According to MTU (2010), the hides of animals are highly loaded with bacteria especially when it is dirty. The knife will become contaminated when it cuts through the skin and transfer the bacteria to the blood stream and finally spread through the body. Improper cleaning of equipment used in the operations has led to the outbreaks of foodborne diseases and it is therefore obvious that cleaning and disinfection procedures ought to be fully enforced and must be in accordance with standard regulations such as Standard Operating Procedures (SOPs) (Gill *et al*., 1999). Samelis and Metaxopoulos (1999), reported that the environments at which animals are processed are more implicated areas for *Listeria monocytogenes* than living animals. In addition, Gill *et al*. (2000) indicated that debris in equipment during deboning process may be the chief source of *E. coli* deposition on meat. Adzitey *et al*. (2011) reported that some butchers in Ghana dress their beef carcasses with unclean water on the bare floor in the abattoir and or unclean slaughter slabs which are always tarnished with rumen contents, blood and other waste materials from earlier operations. These practices increase the risk of carcass contamination.

2.5.3 Personnel

The health of workers in the meat industry is very essential. The body of humans is a receptacle for numerous pathogenic microbes. These microbes may be transferred to the meat which may end up causing disease to consumers (Gordon-Davis, 1998). Approximately $1x10^3$-$1x10^4$ viable microorganisms are shed per minute by human and food handlers without any symptoms of the related illness and are estimated to sheds around 109 pathogens per gram of faces (Forsythe, 2000). The report further stated that 107 counts of pathogenic microbes are present in the fingernails of food handlers. According to MTU (2010), the hands of food handlers may be loaded with *Staphylococcus* microbes due to the direct contact they have with their saliva and other body fluids during spitting, coughing and sneezing. It has been indicated that slaughterers in the northern parts of Ghana and Ashaiman observe hygiene inadequately. Thus, during meat processing, workers at the abattoir do not use or put on clean clothing, aprons, mesh gloves, hair cap and boots. Poor hygienic status during slaughtering and marketing process of meats is a major contributing factor to various pathogens being isolated from beef, chevon and mutton sold in various markets places (Sulley, 2006; Soyiri *et al*., 2008; Adzitey *et al*., 2011).

2.6 History, Classification and Nomenclature of *Salmonella*

Salmonella was first discovered in pigs in 1880 by Daniel Elmer Salmon, an American veterinary pathologist and Theobald Smith (Ziprin, 1994). In 1890, the organism was named after D.E. Salmon to honour him (Ziprin, 1994). Brenner *et al*. (2000) and Popoff *et al*. (2003) said that *Salmonella* comprises of two species thus *S. enterica* and *S. bongori* and these are further grouped into subspecies based on their genomic and biochemical

characteristics. The Kaufman-White typing system, classifies *Salmonella enterica* into six subspecies and each subspecies are further grouped into serovars. The serovar, is a type of classifying *Salmonella* to subspecies on the basis of the type of antigens that are located on the organism (Porwollik, 2011; Achtman *et al*., 2012). Over 2500 potential pathogens of *Salmonella* serovars have been reported (Bell and Kyriakides, 2002; Crum-Cianflone, 2008; Saroj *et al.*, 2009). Card (2009) provides an overview of the number of serovars of *Salmpnella* as shown in Table 2.

Table 2: Species, subspecies and serovars of *Salmonella* genus

Salmonella Species	Subspecies	Number of Serovars
S. enterica	enterica	1,478
	salamae	498
	arizonae	94
	diarizonae	327
	houteane	71
	indica	12
S. bongori		21
Total		2,501

Source: Card (2009)

The serotype of *Salmonella enterica* subspecie *enterica* (subspecies I), mostly cause human infections and also infects warm-blooded animals (Christenson, 2013). According to Molbak *et al*. (2006), *Salmonellae* subspecies *enterica* is the most important zoonotic

serotype and is found in the first subspecies, ssp. *enterica*. Three antigens, O, H, and Vi antigens characterize *Salmonella* strains serologically. Giannella (2002) explained that the O antigens are used to groupe *Salmonellae* and these is the outer polysaccharides of the cell wall of the organisms. H antigens are found on the flagella and help the bacterium to endure host immune response. There are two forms of H antigens, phases 1 and 2. The Vi (virulence) antigens are located in the capsular polysaccharide of some serovers like *S.* Dublin, *S.* Typhi and *S.* Paratyphi C.

Table 3: Antigenic formulae of some serotypes *Salmonella*

Serotype	Subgroup	Somatic Antigen O	Flagella Antigen H	
			Phase 1	Phase 2
S. Paratyphi A	A	1,2,12	a	(1,5)
S. Typhimurium	B	1,4, (5),12	i	1,2
*S.*Agona	B	4,12	f,g,s	-
S. Derby	B	1,4, (5),12	f,g	(1,2)
S. Typhi	D	9,12, (Vi)	c	1,2
S. Enteriditis	D	1,9,12	g,m	(1,7)

Source: Card (2009)

2.7 Characteristics of *Salmonella*

According to Joseph and Carlos (2012), *Salmonella* is a non-spore forming rod, gram-negative and facultative anaerobic bacteria that can ferment glucose. It is a member of the Enterobacteriaceae family. Most strains of these bacteria possess peritrichous flagellaand therefore are motile. They have ability to reduce nitrate to nitrite. The organism is

mesophilic with optimal temperature growth which range from 32 – 37°C. However, they are capable of growing within a temperature range of 6 – 46 °C (Joseph and Carlos, 2012; Bell and Kyriakides, 2002; Guthrie, 1991). Members of this genus generally use glucose for gas production. On triple-sugar iron agar they produce hydrogen sulfide and also grow on citrate as the only source of carbon. They are positive urease, indole, sucrose, salicin, inositol, and amygdalin-negative and lysine and ornithine decarboxylase activities (Molbak *et al*., 2006). The Table 4 shown the characteristics of *Salmonella* on different media as reported by (Pui *et al*., 2011).

Table 4: Characteristics of *Salmonella* on the Various Media

	Salmonella enterica subsp.						
Characteristics	Enterica	Salamae	*Arizonae*	*Diarizonae*	*Houtenae*	*Indica*	*Salmonella*bongo
Classification	I	II	IIIa	IIIb	IV	VI	V (formerly)
Usual habitat	Warm-bl	Warm-blooded Animals	Cold-blooded animals & environmen	Cold-blooded animals & environmen	Cold-blooded animals & environmen	Cold-blooded animals & environmen	Cold-blooded animals & environment
Gram stain	-	-	-	-	-	-	-
Indole test	-	-	-	-	-	-	-
Potassium broth	c -	-	-	-	-	-	-
Urease	-	-	-	-	-	-	-
Voges-Proskauer	-	-	-	-	-	-	-
Glucose	+	+	+	+	+	+	+
Hydrogen sulfide	+	+	+	+	+	+	+
Lysine decarbox	+	+	+	+	+	+	+
Methyl red test	+	+	+	+	+	+	+

Source: Pui *et al*. (2011)

2.8 Pathology of *Salmonella* Infection

2.8.1 Mode of Transmission

Salmonellae infection occur through fecal-oral transmission and vehicle-borne (DOH, 2011). Taking in food and/ or water contaminated with human or animal feces, direct exposure to animals or their waste and foods handled in ways that speed up the growth of organisms may result in *Salmonella* infections (DOH, 2011). Christenson (2013) reported that eating fresh vegetables and fruits that have not been properly washed and getting close contact with infected animals like pigs, cattle, poultry, goats, cats and dogs are potential sources of infection. Circumstances that encourage gastric acids production reduce the *Salmonella* infectious dose suggesting that gastric acidity is a significant obstruction to infection. These means that *Salmonella* easily infect host with acidic environment as compare to alkaline host environments (Ohl and Miller, 2001)**.** Some *Salmonella* species are virulent so much that they are able to penetrate the intestine, travel into the lymphatic system and cause general infections. In this, the bacteria go through the capillaries of the small intestine through which fatty acids are transported into the lymphatic system and taken to the lymph nodes and they finally get access into the blood stream, resulting in a condition called septicemia (Tam, 2008)**.** *Salmonella* are thought first to settle in the intestine and later pierce the intestinal wall. They then attack the mucosa membrane of the intestine which may lead to an infected epithelial cells extruding into the lumen of the intestine and finally destroying microvilli leading to loss of absorptive surface. The invasion of epithelial cells elicits the production of the proinflammatory cytokines, which arouse the influx of polymorphonuclear leukocytes into the infected mucosa (Zhao, 2002)**.**

2.8.2 Infective Dose and Incubation

Salmonella are non-fastidious microorganism and they could thrive on the following foods; eggs, poultry, dairy products, processed meats and other substrate. About 10^5 *Salmonella* cells per gram of food can initiate infection (Jay *et al*., 2003). Burrows and Renner (1999) also reported that 10^4 *Salmonella* per litre of water is required to initiate an infection. According to Wannissorn (2001), the *Salmonella* inoculum needed for infection to occur depends on the type of strain and the physiological wellbeing of the host. For example those producing little gastric acids such as the aged and people who frequently use antacids could reduce the infective dose to 10^3 cells. However, for people who have been vaccinated against *Salmonella* infection, the infective dose can increase to 10^9 cells (Raffatellu *et al*.., 2006). Pui *et al*. (2011), stated that the typhoidal *Salmonella* infection has 7 to 14 days as its incubation period for typhoid fever and 1 to 10 days for paratyphoid fever. Christenson (2013) also reported that the incubation period for non-typhoidal *Salmonella* infections is 6 to 12 hours.

2.9.1 *Salmonella* Infections

Indiana State Department of Health (ISDH, 2009) stated that *Salmonella* is an organism that causes a disease called salmonellosis; the most common among them are *Salmonella* Typhimurium and Enteritidis. Symptoms of infection can be observed from 12 to 72 hours and these are diarrhea, fever, and abdominal cramps. It has been noted by Food Inspection Agency, Canada that most people are cured without seeking medical attention (ISDH, 2009). However, symptoms can be severe in some individuals which could lead to dehydration and as a result of this hospitalization may be necessary. Abscesses and

pneumonia can occur as well. Sometimes if complications are not taken care of with the suitable antibiotics, it may lead to death whiles others may develop Reiter's syndrome or even colitis in severe cases (ISDH, 2009). According to ISDH (2009), Reiter's syndrome is a disorder that develops in different parts of the body as a result of an infection. This can persist for months or years which may lead to chronic arthritis.

2.9.1 *Salmonella* Gastroenteritis

S*almonella* gastroenteritis (salmonellosis) is a disease mostly caused by non-typhoidal *Salmonella* serotypes, particularly *Salmonella* Enteritidis. Gastroenteritis usually starts with nausea, vomiting and later progresses to abdominal pain and diarrhea, which could be mild or severe and with or without blood (WHO/FAO, 2002; Darby and Sheorey, 2008). Salmonellosis normally takes some few days, self-limited and require no medications except in the case of very young and old (Christenson, 2013).

2.9.2 Enteric Fevers

According to Darby and Sheorey (2008), enteric fevers are another form of disease caused by *Salmonella* (*S*. Typhi and *S*. Paratyphi A, B and C). Typhoid fever is cause by *S*. Typhi whereas paratyphoid fever is cause by S.Paratyphi A, B and C (Jay *et al.*, 2003). Fever, vomiting, abdominal pains and distension abdominal, severe diarrhea, relative bradycardia, cough, rose spots and splenomegaly are normally observed as typical characteristic of typhoid and paratyphoid fever (Christenson, 2013).

2.9.3 Bacteremia

Bacteremia is common with *Salmonella* infections. The symptoms of Salmonellae bacteremia usually include chills, anorexia and high fever. *Salmonella* may lead to infections like endocarditis, urinary tract infections, meningitis, septic arthritis and osteomyelitis which are all life-threatening conditions (Hohmann, 2001 2001; Percival *et al*., 2004). Transplaccental disease of the foetus, abortion, foetal and maternal death may be as result of *Salmonella* infection (particularly *S.* Typhi) in pregnant women (Carroll and Williams, 2008). In Accra, Ghana, a report by Labi *et al*. (2014) showed higher prevalence of non-typhoidal *Salmonella* bacteremia (63.5%) than typhoidal *Salmonella* bacteremia (36.5%). The report further explained that non-typhoidal *Salmonella* bacteremia was highest in children below age five. In the study by (Wilkens *et al*. 1997) in Ghana, 24 (21.6%) children infected with *Salmonella* bacteremia, 59% (14) was as a result of *Salmonella* spp. out of which 25% (6) was due to *Salmonella* Typhi.

2.9.4 Asymptomatic Carriers

Giannella (2002) reported that after complete recovery of patients; about 3% of the typhoid infected and 0.1% of non-typhoid infected becomes chronic carriers. Almost 2-5% of individuals that recovers from typhoid fever become carriers either temporarily or permanently. The microbes are harbored in their biliary tract, gallbladder, or intestines (Vandepitte *et al*., 2003). According to Ul-Hassan *et al*. (2004) typhoid fever in many developing countries still remains endemic.

2.10 Food Poisoning by Microorganisms

Food products contamination with microorganisms is a challenge to the globe because of the faster growing and metabolism of these microbes that could cause severe food borne intoxications and speedy food products spoilage. The existence and nature of microbes in food product determine whether that food product will be acceptable and safe to consumers. Bacteria are the next principle cause of several types of food damage and foodborne intoxications apart from molds and yeasts (Blackburn, 2006). Pathogens may be introduced into food during production, processing, distribution and preparation stage by individuals handling the food. Therefore, these individuals play significant role in food safety (Green *et al*., 2005). The safety and quality of food is an important progressive public health issue. Currently, "food quality" and "food safety" are the major worries of food industries, because of increased globalization and complexity of the food supply chain. The main intention of "food safety" is to eliminate the health threats such as microbiological dangers, pesticide residues, food additives misuse, and other contaminating materials like biological toxins, chemicals and adulteration from foods for the consumer. According to FAO (2003), food quality include all characteristics of the food that stimulus its worth to the consumer. This includes both positive and negative attributes; some of the positive attributes are flavor, color, texture, the origin and the method of processing the food whiles the negative aspect of it includes discoloration, off-odors, spoilage and contamination with filth. Foodborne ailments are cause by agents that enter the body through the consumption of food. One person might transfer the disease to another person through food and any bacteria growth medium which can influence food poisoning. Globally, the incidence of foodborne disease is not easy to estimate. Nevertheless, in 2005 alone 1.8 million people

were reported to have died from diarrhea infections. Most of these cases were associated with the ingestion food and water that were contaminated. About 30% of the people in industrialized nations suffer from foodborne diseases annually (WHO, 2007). It is problematic to identify if food product is contaminated by pathogenic bacteria because they normally change not the odor, taste, texture and color of the food product. Bacteria are the cause of foodborne infection. They may continue their growth in the intestines and cause illness if they become numerous in the food eaten. Food intoxication results from the presence of toxins in foods which are produced as a by-product of bacteria growth in food. In this case the ailment is caused by the toxins and not the bacteria. Some examples of pathogenic bacteria species that cause food poisoning consist of *Eschericia coli*, *Salmonella* spp., *Staphylococcus* aureus (Tauxe, 2002). According to Gracey (1986) meat and meat products contain an excellent source of quality animal protein vitamins and iron. Fratamico *et al*. (2005) reported that meat handlers may transmit pathogenic microorganism to meat products throughout the processes of production packaging and marketing. Inappropriate cooking refrigeration or storage could lead to meat borne ailment. The chief sources of foodborne pathogens that cause food poisoning in humans are meat products. *Salmonella* spp and *E. coli* are the two most vital pathogenic bacteria associated with meat products (Borch *et al.*, 1996).

2.11 Epidemiology of *Salmonella*

2.11.1 Typhoidal *Salmonella* Infections

Globally, Enteric fever is the most common cause of death and disease. Bhutta (2006) estimated that 22 million cases of Enteric fever occur annually in the world, with 200,000

deaths as a result of that. It has been reported by Pui *et al*. (2011) that typhoid fever usually causes mortality of infected individual up to 30% in developing countries. According to Patela *et al.* (2006), *Salmonella* infections account for more than one million outbreaks which led to 500 deaths annually in the United States.

In Southeastern Asia, infants and children are mostly prone to *salmonella* infection (Crump *et al.*, 2004; Darton *et al*., 2014). In sub-Saharan Africa and countries in Southeastern Asia, typhoid fevers outbreaks are frequently reported (Muyembe-Tamfum *et al*., 2009). The sum total of typhoid fever cases reported in Ghana for the year 2011 was 103,353 with 793 (Case Fatality Rate = 0.77%) deaths (GHS, 2011).

Salmonella Typhi as a cause of indigenous infections was almost eliminated as a results of improvement in sanitation in the US and other developed nations (Molbak *et al*., 2006). According to CDC (2005), 36,184 cases of *Salmonellas* were documented and is the second cause of bacteria foodborne illness in Europe and *Campylobacter* being the first (Delhalle *et al.*, 2009). In the United States, it is estimated that 76 million people are infected annually with foodborne diseases (Hendriksen, 2010). If extrapolated, this result would be equal to one fourth of the people infected in the developed countries per year. However, this figure of *Salmonella* infection is expected to be much greater in the developing countries (Hendriksen, 2010).

2.11.2 Non –Typhoidal *Salmonella* Infections

Salmonella gastroenteritis is a public problem globally. The global concern is that 93.8 million people suffer from these illnesses with 155,000 deaths every year. The most

isolated subspecies is *Salmonella* Enteritidis which is responsible for 65% *Salmonella* infections, followed by *S*. Typhimurium at 12% (Christenson, 2013).

In countries that are developed non-typhoial *Salmonella* infections mostly exist as gastrointestinal infection and are rarely associated with systemic diseases, except in immunocompromised individuals (Ekdahl *et al*., 2005). Marks *et al*. (2010) also stated that these strains of *Salmonella* are major concerns in Sub-Sahara Africa since these are commonly isolate form blood of patients suffering from fevers. In Africa 2012, invasive non-typhoidal *Salmonella* infections were reported to have a case fatality rate of 20-25% (Feasey *et al*., 2012). In Ghana, a report by Labi *et al.* (2014) stated that the percentage prevalence of non-typhoidal *Salmonella* bacteremia is highern(63.5%) than that of typhoidal *Salmonella* bacteremia (36.5%). Wilkens *et al*. (1997) also indicated that in Ghanaian children, most of the *Salmonella* isolated from their blood culture were non-typhoidal *Salmonella* strains.

2.11.3 Age and Gender-Specificity of *Salmonella* Infections

Salmonella infections occur in two directions thus affecting both male and female with the highest occurring in children and the elderly. Several reports have indicated that the prevalence of *Salmonella* infection is highest in children under five years in South East Asia, with complications and hospitalization (Siddiqui *et al*., 2015; Ochiai *et al*., 2005). Additionally, Olsen *et al.* (2001) indicated that there is a greater occurrence of *Salmonella* infections in males than in females among the aged. They further added that middle-aged men were less infected than their female counterparts.

2.11.4 Risk Factors for *Salmonella* Infections

Christenson (2013) reported that infants, elderly and people who use antacids or proton pump inhibitors frequently are mostly prone to *Salmonella* infections. Moreover, any situations such as HIV/AIDS, malnutrition, corticosteroid therapy and post-transplantation immunosuppressive therapy that weaken the function of cell-mediated lymphocyte, are major risk factors. An overload of the reticuloendothelial system with iron or hemoglobin in patients with sickle cell anemia, thalassemia and malaria could increase the possibility of severe disease. The functions of reticuloendothelial system could also be impaired by leukemia and lymphoma (Christenson, 2013).

2.11.5 Seasonality of Occurrence

According to Mohanty *et al.* (2006), the epidemiology of *Salmonella* infections is influenced by seasonal variations. Labi *et al*. (2014) also reported that the occurrence and prevalence of *Salmonella* infections is seasonal in Ghana. They further stated that the infectious *Salmonella* Typhi follow the rainfall pattern with the highest occurring from March to August. The number of cases of non-typhoidal *Salmonella* infections was fairly scattered across the months (Labi *et al*., 2014). A study conducted by Kariuki *et al*. (2005) indicated a high number of Salmonella occurrences in May and June which is rainy seasons. They suggested that these may be as a result of poor sanitation during this time in homes and environment in which children live and playin homes and the environment in which children live and play (Kariuki *et al*., 2005). Mohanty *et al.* (2006) also observed that in the dry season in India the highest cases of typhoid fever occurred during April to June.

2.12 *Salmonella* Occurrence in Food

One of the greatest causes of foodborne diseases worldwide is *Salmonella* (Gómez-Aldapa *et al*., 2012). The elementary canals of both cold and warm blooded vertebrates are the reservoir of *Salmonella* microorganism, with many animals being asymptomatic. The fecal shedding by these asymptomatic animals contaminate the environment such as crops, plants, soil, rivers and lakes with *Salmonella* species (Gómez-Aldapa *et al*. 2012). Fruits, vegetables and animal products contaminated with sewage are the chief sources of *Salmonella* infections (ICMSF, 1996; Crum-cianflone, 2008 ; Jay *et al*., 2003). Globally, many foodborne diseases eruptions have been associated with *Salmonella* species. In France, according to Brouard *et al.* (2007) *Salmonella* Agona was associated with two outbreaks among infants consecutively, through the consumption of powdered infant formula. Nut butter was implicated with *Salmonella* Bredeney leading to its outbreak in New Mexico (Center for Disease Control (CDC), 2012). This outbreak occurred in twenty States as a result of that 10 out of 42 people that were infected were hospitalized (CDC, 2012). In 2002, a total of 145231 cases of human *Salmonella* were reported in EU and Norway (38 cases per 100 000 inhabitants). In human salmonellosis, *Salmonella* enteritidis was the dominant, which caused 67% of all noticed cases in Norway and EU. Seventeen percent (17%) of all the cases were caused by *Salmonella* Typhimurium. In addition *S*. Hadar, *S*. Infantis and *S*. Virchow are important types that were also identified. *Salmonella* infection also leads to economic losses apart from the health problems. A study conducted in England on the socio-economic impact of infectious intestinal disease and found that, the average cost per *Salmonella* treatment was £606 (Meerburg and Kijlstra, 2007).

2.12.1 Food Handlers

Salmonella infections may be transmitted by food handlers in the food chain through three different ways: as patient, as passive transmitter (not infected but passively transmitting the *Salmonella* from infected source such as poultry to food means of unwashed hands) and as a carrier (Cruickshank and Humphrey, 1987). According to Food Standard Agency, UK (FSA) (2004) food handler are individuals who directly touch open food as part of their work and therefore are the greatest source of contamination.

Several studies indicated that food handlers are carriers of *Salmonella* and this serve as a potential source of infection of enteric fever. Mensah *et al*. (1997) reported that out of 176 food vendors examined 3.2% prevalence of *Salmonella* was observed in Accra, Ghana. Feglo *et al.* (2004), also reported that in Kumasi, Ghana 2.3% of food vendors were *Salmonell*a carriers. Out of 53 stool samples of food handlers examined in Nigeria, 13.2% of *Salmonella* were isolated of which 5.7% were *S.* Typhi, 5.7% were *S.* Enteritidis and 1.8% was *S.* Choleraesuis (Smith *et al.*, 2009). According to Senthilkumar and Prabakaran (2005) in Namakkal India, out of the 35 stool samples examined from asymptomatic food handlers, 6 (17.1%) yielded positive for *Salmonella*. Of the total seventeen (17) isolates, five (5) of them were multidrug resistant strains. They further concluded that food handlers could be a source of drug resistant strains of these bacteria which is a serious health challenge to the public. Efforts were made to reduce *Salmonella* contamination and this has resulted in a drop in the total number of *Salmonella* infections noted in humans. Unusually however, this drop could completely be attributed to the reduction in serovar Typhimurium infections. Within this period infections caused by serovar Enteritidis increased dramatically (van de Giessen, 1996).

2.13 Media for Isolating Bacteria and their Classification

According to Andrew (2006), microbial culture are used to determine the type of microbes and their abundance in the samples being tested. The report further added that culture media contains nutrients and other physical growth parameters essential for microbial growth and all microorganisms can thrive not on a single culture medium. Media may be grouped into solid or liquid, synthetic or non-synthetic (Andrew, 2006) and base on use (basic, enrichment, non-selective and selective media) (Garrard, 2013).

2.14 Methods for *Salmonella* Isolation

According to Sandel *et al*. (2003) and Gracias and Mckillip (2004), enrichment of samples are done purposely to recover partially injured cells of bacteria due to heat, cold, acids or osmotic shock in a non-selective pre-enrichment media for example Buffered Peptone Water (BPW). Another purpose is to multiply the number of target cells (microorganism) as they are generally not uniformly distributed in the foods, occurs in small amount and might be in a mixed microbial population. Selenite Cysteine broth ,Rappaport Vasiliadis Soy broth, Tetrathionate broth or Muller-Kauffmann Tetrathionate-Novobiocin broth are some examples of primary enrichment media and Xylose lysine Deoxycholate agar, Bismuth Sulphite agar, Brilliant Green agar are some examples of selective media (Sandel *et al*., 2003; Gracias and Mckillip, 2004).

Table 5: Classification of bacterial counts based on International Standards

	Total Viable Count	
Category	AUS[a]	EU[b]
Excellent	< 3.0*	< 2.8
Good	3.0-4.0	-
Acceptable	-5.0	-
Marginal	> 5.0	> 4.3

Note. [a] Australian Standard (2002), [b] European Union (2002);* unit expressed in log CFU/g.

2.15 Definition of Resides

According to European Commission (EC) (2002) residues are well-defined as entire active elements of medical product or the metabolites of those elements that remain in meat and other animal products as a result of the animal receiving those medical products.The European Parliament and Council regulation Number 470/2009, defined residues as all pharmacological active substances, whether active ingredients, excipients or degradation products and their metabolites that remain in food made from animal (Codex Alimentarius Commission (CAC), 2011). Codex (2015) defined Maximum Residues Limits (MRLs) as the highest quantity of drug residue which is found in food substances that shall not be dangerous to the health of the individuals' and the amount of the drug residues that could

be consumed daily throughout life without any substantial health risk is called acceptable daily intake.

2.16 Occurrence of Antibiotic Residues in Foods

Antibiotics are chemicals that may be produced naturally by living organisms or synthetically created in the laboratory that are capable of killing or inhibiting the growth of other microorganisms (Aminov, 2010b). Avoidance and treatment of diseases in the livestock has increased antibiotics usage in the sector (Centre for Science and Environment Study (CSE), 2014). The treatment of microbial infections in humans with antibiotics has led to the introduction of antibiotics in the veterinary field. In animal rearing, the major utilization of antibiotics was for prevention, treatment and control of diseases. It is evident that antibiotics have been used to treat the following diseases: arthritis, mastitis, respiratory diseases, gastrointestinal infections and other infectious bacterial diseases (Draisci *et al.*, 2001). According to Debeuckelaere *et al.* (1998), antibiotics are generally used for three purposes in animals, thus therapeutic (treatment), prophylactic (prevention) and as agents of growth (to increase feed utilization and production for their growth promoting properties they are consistently used at sub-therapeutic level as animal feed additives). Antibiotics residues are found in animal products as a result of failure to observe safety instructions, and inability to identify treated animals as a result of improper record keeping (Sundlof, 1989). CAC (2001) stated that, the main reasons for prevalence of antimicrobial residues in meat and its products are: mismanagement, inappropriate treatment records, failure to note drug departure period, lack of supervision on withdrawal periods, lack or absence of implementation of restrictive law to antimicrobials usage, use of antimicrobial drugs

without label, difficult to identify treated animals, extended usage or unnecessary dosages of antimicrobials and laymen having access to antimicrobial drugs. Residues can also be transmitted to calves that consume milk from cattle on antimicrobials medication (Guest and Paige, 1991). Antibiotics residues can also be found in animal product through fecal recycling, where treated animals feces contaminate the feed of untreated (McCaughey *et al*., 1990). Contamination of animal feed with different type of compounds may also occur. The effect of this contamination rest on the pharmacodynamics of these compound and the animal species affected (McEvoy, 2002).

2.16.1 Occurrence of Antibiotic Residues in Beef

Babapour *et al*. (2012) screened 500 samples of beef and mutton collected from Iran for drug residues analysis and reported a prevalence rate of 22.8% and 14% for beef and mutton respectively. Donkor *et al*. (2011) in Ghana found out 30.8% antibiotic residues from a total of 156 beef samples. Abavelim (2014) indicated that there are antibiotic residues in beef samples collected from selected markets in Kumasi, Ghana. They further indicated that out of the total of 30 beef samples analyzed 24 (80%) showed chloramphenicol residues. It showed that 50% of the analyzed beef samples were positive for oxytetracycline residues. Morshdy *et al.* (2013) indicated a higher concentration of antibiotic residues in beef kidneys and livers compare to muscles in Egypt. Alla *et al*. (2011), reported only 3% of antibiotic residues in the muscle when they analyzed beef samples in Sudan. Mangsi *et al*. (2014) also emphasized that, 38.33% of beef samples were contaminated with antibiotic residues in Pakistan. They observed higher antibiotic residues at Karachi (48.33%), follow by Sukkur (41.67%), Hyderabad (36.67%), Mirpurkhas

(33.34%) and Larkana (31.67%). Ezenduka *et al*. (2011), recorded 54.44% antibiotic residue in beef meat in Nigeria. Muriuki *et al*. (2001) also reported in Kenya antibiotic residue of 45.6% in beef samples. Wahab *et al*. (2011), detected 17.33% of antibiotic residues in Sudanese beef. Birhan and Mulugojjam (2018) examined 250 beefsamples, and recorded antibiotic residues of (76.4%) in the liver and kidney, (43.6%) in the thigh muscle, and (42%) in fat. They further explained that the highest frequencies of these residues were shown in the liver and kidney (76.4%) while minimum frequency detected in fat (42%). According to Myllyniemi *et al.* (2008), beef samples collected from central parts of Ethiopia; Addis Ababa (93.8%), Debre Zeit (37.5%), and Nazareth abattoirs (82.1%) tested positive for oxytetracycline. Buket *et al*. (2011) stated that 57.7% of the beef samples examined were positive for quinolones. Abdelmoaty (2015) reported higher antibiotic residues of 47% in raw beef and 29% in processed beef. Gebre (2012) reported that tetracycline (28%) was the most predominant antibiotic residues in beef samples followed by sulfonamide (23%) and penicillin (20%).

2.16.2 Antibiotic Residues in Food in African Countries

In several Africa countries, antibiotics could be used inappropriately for the treatment of bacteria infections or feedstuff additives for animals reared domestically. The threat of antibiotic contamination is not a major challenge to Africa countries only but to human population globally (Cars *et al*., 2008). Antibiotics residues are rapidly spreading between countries regardless of their economical, legal or geographical differences (Harbarth and Samore, 2005). Abd El-Aty *et al*. (2001) in Egypt recorded high concentrations of ceftazidime residues in the liver, kidney, heart and muscle tissues of rabbits. Report by

Goudah *et al.* (2007) stated that, lactating ewe passed out *Erythromycin* rapidly from the blood to milk.

Table 6: Antibiotic Residues in Various Foodstuffs of Animal Source in African

Country	Antibiotic	Foodstuff	Reference
Egypt	Tetracyclines	Chicken meat	Salama *et al*. (2011)
		Bovine carsasses	Morshdy *et al*. (2013)
	β-Lactams	Eggs	Khattab *et al*. (2010)
	Cephalosporines	Rabbit meat, liver and kidne	AbdEl-Aty *et al*. (2001)
	Macrolides	Milk	Goudah *et al*. (2007)
Sudan	Quinolones Tetracy	Animal derived foods	El-tayeb *et al*. (2012)
Kenya	Tetracyclines	Beef, liver and kidney	Murinki *et al*. (2001)
	β-Lactams	Milk	Shitandi and Sternyo, (20(
Ethiopia	Tetracyclines	Edible tissues	Myllyniemi *et al*. (2000)
Ghana	Tetracyclines	Milk	Addo *et al*. (2011)
Nigeria	Tetracyclines	Meat	Olufemi and Agboola, (20
	Tetracyclines	Eggs	Ezenduka *et al*. (2011)
	Nitrofurans	Animal derived foods	
	Chloramphenicol	Eggs	Omeiza *et al*. (2012)
	β-Lactams	Beef	Ibrahim *et al*. (2010)
Tanzania	Tetracyclines	Milk	Kurwijila *et al*. (2006)
South Africa	Chloramphenicol	Egg	
	Tetracyclines	Milk	Bester and Lombard, (197

Source: (Abavelim, 2014)

Khattab *et al.* (2010) carried out a study to detect the residues of amoxicillin in laying chicken and commercial eggs. Amoxicillin residues were identified in both egg yolks and whites in different levels for the six sequential days after last exposure to the drugs. They further assessed the effects of boiling and storage on amoxicillin impurity in the eggs. Results revealed that eggs stored at 37°C and 4°C did not destroy amoxicillin impurity up to the seventh day after drug administration. Amoxicillin residues in eggs were not destroyed when the eggs were boiled for ten minutes. According to Salama *et al.* (2011), tetracycline residues (tetracycline, oxytetracycline, doxycycline and chlorotetracycline) were found in breast (42%), liver (52%) and thigh (38%) of fresh chicken samples collected from Cairo in retail shops over a one year period. These residues were above the maximum residue limits and 7%, 8% and 13% were recorded from thigh, breast and liver respectively. Tetracycline residues were above the maximum residue limits. The residues were higher in the liver samples than those from breast or thigh. Also, 600 samples of bovine carcasses made of kidney, liver and muscle from the abattoir of Mansoura (Dakahlia Province, Egypt) were examined for oxytetracycline residues and two percent of these samples tested positive of oxytetracycline residues. Of this two percent, the maximum limits was exceeded by 1.3% (Morshdy *et al.,* 2013). In Nairobi Kenya, 250 beef samples were obtained from five abattoirs and 114 (45.6%) tetracycline residues were detected. Out of the 114 sample that were positive for tetracycline residues, 19 (7.6%) were from muscle, 35 (14%) from kidney and 60 (24%) from liver (Muriuki *et al*., 2001). Shitandi and Sternesjo (2001) indicated that, a higher prevalence of penicillin-G residues was found in milk samples sold in Nakuru. Ekuttan *et al.* (2007) recorded higher (9-16%) concentration of antibiotic residues in marketed milk in Dagoritti division, Nairobi. Kurwwijila *et al*. (2006) in

Tanzania detected 36% of antibiotic residues in marketed milk samples. Nonga *et al*. (2010) detected the residues of the following; chloramphenicol, oxytetracycline, chlortetracycline, doxycycline and flumeguine antibiotics from chicken eggs in the Morogoro municipality between January and February 2007. In Ethiopia, the total samples analyzed for the presence of tetracycline residues, 71.3% shows oxytetracycline residues (Myllyniemi *et al.*, 2000). Oxytetracycline residues found in the kidney were higher than those found in the muscle and 48% were above the recommended maximum limits (Myllyniemi *et al*., 2000). In Nigeria, antimicrobial residues were found in eggs from farms and retail outlet (Ezenduka *et al.,* 2011). Chloramphenicol residues were found in eggs sampled from farm that used human chloramphenicol (Omeiza *et al*., 2012). Ramos *et al.* (2003) also reported a similar result that chloramphenicol residues were persistently found in the tissue of both poultry and cattle. In addition to this oxytetracycline residues were also found in the muscles and tissues of slaughtered cattle at Akure metropolitan within January to June 2008. The residue levels were above WHO/FAO recommendations (Olufemi and Ehinmowo, 2009; WHO, 1999). Higher levels of penicillin residues were also detected in slaughtered cattle in the Sokoto metropolitan abattoir. However, tetracycline and streptomycin were detected in low levels (Ibrahim *et al.,* 2010). A report by Aning *et al*. (2007) in Ghana indicated that 35% of the marketed raw milk in Accra and Kumasi were contaminated with residues of antibiotics. Of this 35%, 33.1% of these raw milk samples were above the maximum residue limit of the European Union. Addo *et al*. (2011), also indicated the presence of beta-lactans, macrolides, aminoglycosides, sulphonamides and tetracyclines residues in raw milk samples.

In South Africa, antibiotic residues were found in raw and pasteurized milk sold in the Pretoria markets (Bester and Lombard, 1979). From the above, it is clear that most African countries have observed antibiotic residues in foods of animal source and some of these exceeded the maximum residue limits according to World Health Organization (WHO) recommendation (Bester and Lombard, 1979).

2.17 Health Implication of Antibiotic Residues in Foods

Antimicrobials drugs use in animals' production has led to problems of antibiotics residues in foods of animal source. Antibiotics can either have primary or secondary effect on human health. Thus through consumption of foods of animal source contaminated with antibiotics residues or spread through human pathogen by the selection of antimicrobial resistance determinant (Paige *et al.*, 2000). Allergic responses in sensitive person, toxicity and carcinogenic effect are some of the human health problems associated with exposure to antibiotics residues. Antibiotics belonging to beta-lactam family, especially penicillin, could cause allergies in penicillin allergic person; when they consume milk with high concentration of penicillin residues. Also, according to Phillips *et al.* (2000), the yellowing colour of teeth in children could be as a result of tetracycline residue in food. An allergic reaction could activate antimicrobial residues in individual who were formerly sensitized. Nisha (2008) indicated that the main pathological effects created by antibiotic deposits in food of animal origin include the transfer of antibiotic resistant bacteria to human beings. Generally, β-lactams are non-toxic in nature however, they appear to be accountable to the allergic reactions of human to antimicrobials (WHO, 1999). Tetracyclines, aminoglycosides and sulphonamides, could also initiate allergic reactions (Paige *et al.*,

1997). Liver injury and hepatic cells are caused by certain macrolides (Dewdney *et al*., 1991). However, Raison-Peyron *et al*. (2001) reported that only minor situations of hypersensitivity were attributed to exposure of residues in meat. Penicillin in beef and pork is responsible for anaphylactic reactions. Tinkelman and Bock (1984) also stated that, only one case that anaphylaxis was attributed to streptomycin residues. Angioneurotic tightness and edema in the chest could be attributed to the residues of penicillin in meat consumed (Schwartz and Sher, 1984). Improper administration of antimicrobials in animal rearing has triggered the evolution of resistant bacteria which is transmitted to human being via food, environment or direct contact with the affected meat. It is therefore compulsory to observe the withdrawal days for antibiotic when using in animal rearing to ensure that public health is safeguarded (CSE, 2014). In recent years, antimicrobials deposits in animal products have created a major health hazard due to the increased microbial resistance (Butaye *et al.,* 2001). Low dosage of antibiotics may not cause contamination or create risk to public health but extensive use of these might increase the hazardous effect of residues on the consumer plus development of antibiotic resistance bacteria and hypersensitivity.

2.17.1 Drug Resistance

Resistant bacteria that are from animal may get into human via direct or indirect contact to meat, egg and milk to colonize human endogenous flora or superimpose and added to the resistance genes presence in man. There is indication of animal to human transfer of antibiotic resistance bacteria. Antibiotics use in animals rearing has led to human developing antibiotic resistance (Landers *et al*., 2012 and Beyene, 2016). According to

Landers *et al.* (2012) and Beyene (2016), evidence have shown that human have developed resistant to *Campylobacter*, *Salmonella* and *Staphylococcus* drugs through animal product consumption. Bacteria from foods of animal source have developed resistant to fluoroquinolones and avaoparin. Obviously, antibiotic use in livestock rearing has been associated with the development of human antibiotic resistance (Beyene, 2016). Food producing animals may have resistant bacteria isolates when they are continually fed with low level of antibiotic at prophylactic stage. The resistivity of microorganisms, escalating from sub therapeutic uses of sulfa, tetracyclines, and penicillin drugs in agriculture is recommended by WHO (World Health Organization) to be a priority issue (Beyene, 2016). World Health Organization (WHO) has raise concern about penicillin, tetracyclines and sulfa drugs resistance in agriculture National Research Council (NRC, 1991). Antibiotics could boost the spread of bacteria resistant to antibiotics in humans during infection. Hence it has been suggested that antibiotics used in human medication shall not be use in veterinary filed (Carlet *et al*., 2012). Bacterial resistance has increased in animal production due to prevalent utilization of antibiotics. Resistant strains might lead to failure of antimicrobial treatment in clinical situations in future (Nisha, 2008). Consumption of animal products containing antibiotic residues could trigger the development of direct and indirect toxicity, hypersensitivity, liver damage, teeth discoloration and disorder of the gastrointestinal in human beings (FAO, 2002; Jing *et al*., 2009). Alteration of microflora due to low dosage of antimicrobial exposure, and antimicrobial resistant pathogen transfer through the food chain is also a major concern (FAO, 2002).

2.17.2 Drug Hypersensitivity

Riedl and Cassilas (2003) said that medicines are foreign particles, but their molecular weight is normally not big to cause immunogenic reaction, they act as haptens which need to combine with already sensitize person to be immunogenic and would elicit antibody formation. Drug hypersensitivity is an immune arbitrated reaction in a sensitized patient to a drug agent. Some allergic reaction like serum sickness, anaphylaxis, cutaneous reaction and delay hypersensitivity reaction to medicine is normally associated with the antibiotics, such as penicillin. Almost half of the human population has been considered hypersensitive to a number of substances including penicillin (Dewdney *et al*., 1991; McDonald, 1998). A report by Kanny *et al*. (1994) and Raison-Peyron *et al*. (2001) explained that there are few incidence of hypersensitivity as a result of exposure to antibiotic residues in meat. The residues of penicillin in meat can cause tightness in the chest and angioneurotic edema (Muriuki *et al*., 2001). Certain macrolides cause liver injuries which are activated by a particular allergic reaction to macrolide modified hepatic cell. Chloramphenicol residues in foods could cause serious blood dyscrasia in individuals (Settepani, 1984). According to Paige *et al*. (1997) tetracycline,

aminoglycosides and sulphonamides may also cause allergic reactions.

2.17.3 Carcinogenic Effect

A carcinogenic effect is defined as an effect produced by a drug which has carcinogenic or cancer producing activity. Nitrofurans, nitromidazoles and quinoxaline are some examples of carcinogenic veterinary drugs which are used in many countries and these are received by human through food from animal source as antimicrobial residues (Aiello *et al.*, 2005).

The great fear of carcinogenic residues is their capability to collaborate or covalently bind to several intracellular compounds like proteins, RNA (ribonucleic acid), DNA (deoxyribonucleic acid), phospholipids, glycogenand glutathione and glutathione (Beyene, 2016). Chloramphenicol causes cancer (Nisha, 2008). According to Anon (2002) the Expert Committee on Food Additives indicated that chloramphenicol may cause cancer due to its genotoxic effect.

2.1.4 Intestinal Flora Disruption

In the intestines the bacteria living acts as a barrier to stop incoming pathogen which can cause diseases. Large scale utilization of antibiotics may reduce the bacteria number or selectively kill some significant species (Myllyniemi *et al*., 2000; Beyene, 2016). Intestinal flora and gastrointestinal may be affected negatively due to wide usage of broad-spectrum antimicrobials drugs such as flunixin, tylosin and streptomycin in animals and in addition the use of metronidazole, nitroimidazole and vancomycin, in humans (Beyene, 2016).

2.17.5 Mutagenic Effect

Mutagens are any chemical or physical agents that could cause permanent change in the DNA molecule or damage the genetic component of an organism. Various chemicals of DNA bases such as alkalizing agents and analogous have shown mutagenic activities. The public concern is that drugs may present threat to the human population by causing mutation in chromosome that will negatively affect human fertility (Beyene, 2016).

2.17.6 Teratogenic Effect

Teratogen refers to the deadly effect in the foetus or embryo throughout a perilous phase of conception chemical or drug. As a result, a congenital abnormality, that affects the functional and structural integrity of the organism is produced (Gorbach, 1993). Anthelmintic and benzimidazole have teratogenic effect on the embryo when it is given during initial phase of pregnancy. Adding to embryo fatality together with teratogenicity, benzimidazole drug from oxfendazole has mutagenic effect (Nisha, 2008). Furazolidone, sulphamethazine oxytetracycline and Gentamicin are other antimicrobial residues that can be transfer to human through meat and its products (Settepani, 1984).

2.18 Antibiotics for Prophylactic and Therapeutic Purposes

Treatment of bacterial ailments in human beings with antibiotic was the genesis of antibiotic practice in veterinary field. In animal production, antibiotics are usually use for prevention, control and treatment of diseases such as respiratory disorders, mastitis, gastrointestinal diseases, arthritis and other and other communicable bacterial diseases (Draisci *et al*., 2001). According to Goetting *et al.* (2011), antibiotics are purposely use as therapeutic (higher dosages of antibiotics given to animal for a short periods), prophylactic (animals exposed to reasonable amount of antibiotics for a longer period), and growth promoters (giving antibiotics sub therapeutic doses) (Marshall and Levy, 2011; Chowdhury *et al.*, 2009). Antibiotics are administered intravenously or parenterally, orally and topically (Lawal *et al*., 2015; Adel *et al.*, 2016). The antibiotics inhibit the following functions DNA replication, protein and RNA synthesis, cell differentiation, development and division. They also interrupt cell membrane and wall synthesis of the organisms liable

for distribution of infections and target folic acid metabolism (Kohanski *et al.*, 2010; Diarra and Malouin, 2014). Antibiotics usage in both humans and animals is common in the developing Nations. The most frequently used antibiotics are: tetracycline (Zakeri and Wright, 2008), gentamicin (Filazi *et al.*, 2013), neomycin, tyrosine, erythromycin (Alhendi *et al.*, 2000). Ceftiofur, bacitracin and virginiamycin are helpful in the prevention and reduction of necrotic enteritis infections and respiratory diseases (Sarkozy, 2001; Soni, 2012). Quinolone or flouroquinolones antibioticsare used for treating skin or soft tissue and gastroenteritis diseases (Sarkozy, 2001; Soni, 2012). Sulfonamide antibiotics are administered as chemotherapeutic and preventive agents against fowl typhoid, pullorun, coccidiosis and coryza disease (Soni, 2012; Kolaczek *et al.*, 2014).

2.19 Types of Antibiotics

Antibiotics may be grouped based on their molecular structures, mechanism of action (bacteriostatic and bactericidal) and range of activity (broad and narrow) (Calderon and Sabundayo, 2007; Aminov, 2010). Method of administration (oral and injection) is other forms of classifying antibiotics. Generally, the deadliness, efficiency and allergic effects of antibiotics are similar if they have the same molecular structure or class. Some antibiotics classification on the bases of chemical or molecular structure are macrolides, quinolones, sulphonamides, beta lactams, aminoglycosides, tetracyclines, oxazolidinones and glycopeptides (van Hoek *et al*. 2011; Frank and Tacconelli 2012; Adzitey, 2015). Veterinary drugs may also be grouped base on the type of disease causing organism targeted or types of disease they cure (Abebew, 2001).

2.19.1 Macrolides

According to Moore (2015) a metabolic product was the first antibiotic discovered from *Macrolide**s*** and isolated by J.M. McGuire in 1952 as soil inhabiting fungus (*Saccharopolyspora erythraea*). They are formerly called *Streptomyces erythraeus* which belongs to the genus *Saccharopolyspora* of actinomycete bacteria. Macrolides are categorized by 14, 15 or 16 membered macrocyclic lactose rings with unusal dexoxy sugars L-cladinose and D-desosamine attached (Moore, 2015). They are mostly given to penicillin allergic patients since they have broader spectrum of antibiotic activity than Penicillin (Moore, 2015). According to him macrolides may either inhibit or kill microbes by powerfully inhibiting the bacterial protein production (Moore, 2015). The liver is able to reutilize macrolides therefore they have the tendency to build up in the body. They also cause inflammation in humans (Moore, 2015).

2.19.2 Quinolones (Ciprofloxacin or a Fluoroquinolone)

The search for antimalarial drugs led to the discovery of this class of by scientists as nalidixic acid. Throughout the development of quinine the early sixties, nalidixic acid was discovered as an impurity (Domagala, 1994). These antibiotics are capable of interfering with DNA replication and transcription in the bacteria. Quinolones and naphthyridones are derivative of ciproxacin, cinoxacin, norfloxacin, enoxacin, temafloxacin, ofloxacin, sparfloxacin, nalidixic acid, etc. (Domagala, 1994). Previously quinolones antibiotics consist of two ring structure, later, in order to extend their spectrum in fighting bacterial infection additional ring structure was added to improve their effectiveness (Domagala, 1994). The effectiveness of this antibiotic is due to the numerous changes that have been

made to its parent structure, which enhance their performance in the treatment of countless forms of diseases like urinary, systemic and respiratory tract infections (Domagala, 1994). Ciprofloxacin, floxacin and levofloxacin are some of the examples of this class of antibiotic (Domagala, 1994). Aside these notable achievements, there is still safety concerns regarding these class of antibioticsthat has resulted in the withdrawal of sparfloxacin, grepafloxacin, trovafloxacin, temafloxacin, etc. belonging to the family of quinolones from the market (Domagala, 1994).

2.19.3 Sulphonamides

Sulphonamides is the first class of antibiotics used in therapeutic medication and still plays significant role in veterinary practice and human medicine (Eyssen *et al*., 1971). Sulphonamides antibiotic impede the growth of both Gram-positive and Gram-negative bacteria for instant *Salmonella, E. coli, Nocardia, Klebsiella*, *Enterobacter* and *Shigella*. Sulphonamides are also used extensively in the treatment of several infections like dysentery, tonsillitis ,bacillary, septicemia, meningococcal meningitis, and some urinary tract diseases (Eyssen *et al*., 1971). Research have revealed that Sulphoamides are capable of impeding cancerous cell agents (Stawinski *et al*., 2013; Xu *et al*., 2014). Henry (1943) stated that the original antimicrobial Sulphonamides are artificial antimicrobial agents that contain the the Sulphonamides group. Sulphonamides were generally considered to be bacteriostatic rather than bactericidal but Henry (1943) in his research stated that sulphonamides could be bactericidal if its concentration is adequately high or if its concentration is accompanied by other environmental situations unfavorable to the bacteria which may include toxic proteolytic product, adverse temperature, poor cultural

conditions, antibodies, etc. According to Slatore and Tilles (2004) Sulphanamides are good and effective in the treatment of several diseases and infections. However, due to their deadliness and side effects such as urinary tract infections, porphyria, hemolytic anemia and hypersensitivity reactions, so they are administered with caution.

2.19.4 Beta-lactams

Antibiotics belonging to this class has three carbon and one nitrogen ring that is very reactive (Heesemann, 1993). They restrict synthesis of essential proteins of the bacteria cell wall which may impede the growth of bacteria or kill it (Heesemann, 1993). The cross linked peptide units during production of peptidoglycan are due to penicillin –binding protein which is a bacterial enzyme. Beta-lactam antibiotics usually bind with penicillin-binding protein (PBP) enzymes and gradually interfere with the synthesis of peptidoglycan causing lysis and cell death (Heesemann, 1993). Cell wall synthesis and cell division are carried out by these enzymes (PBP) and as penicillin bind with this enzyme it causes the internal osmotic pressure to go high leading to the rupture of the cell Wanamaker and Boyce (2000). The most commonly used beta-lactam classes are the Penicillins, Cephalosporins, Monobactams, Carbapenems, Ampicillin, Amoxicillin, Cloxacillin, and Dicloxacillin (Heesemann, 1993).

2.19.5 Penicillin

According to McGeer *et al*. (2001), penicillin was discovered in 1929 by Alexander Fleming, which was the first antibiotic and afterward was found to be part of other antibiotic compound known as penicillins. Boundless (2016) reported some examples of

penicillin to be penicillin G, ampicillin, nafcillin, oxacillin (dicloxacillin), methicillin, penicillin V, piperacillin, mezlocillin, amoxicillin, carbenicilin, and ticarcillin.

For effective control of bacteria, augmentin are mixed with non-anibiotic compound that are capable of inhibiting the activities of bacterial penicillinase enzyme (Poirel *et al*., 2005). Augmentin composes of antibiotic (amoxicillin) and non-antibiotic (clavulanic acid) a non-antibiotic compound (Poirel *et al*., 2005). Clavulanic acid is also capable of inhibiting beta-lactamase enzyme leading to prolong antimicrobial action of the amoxicillin component of the augmentin even in the midst of penicillinase producing bacteria (Poirel *et al*., 2005).

2.19.6 Cephalosporin

In terms of structure and mode of action, these groups of antibiotics are related to penicillin (Talaro and Chess, 2008). According to Talaro and Chess (2008) these antibiotic are the most generally recommended and account for one third of all antibiotics recommended and used in the United Kingdom (Talaro and Chess, 2008). Guiseppe Brotzu was the first to isolate this antibiotic from fungus *Cephalosporium acremonium* in 1945 (Pegler and Healy, 2007). However, Edward Abraham was able to extract the compound and so got the patent right (Pegler and Healy, 2007). Cephalosporins have 7-anminocephalosporaniic acid nucleus and side chain of 3, 6-dihydro-2 H-1, 3-thiazane rings. Cephalosporins are divided into first to fifth generations according to their target microorganism but later forms are more effective against Gram-negative pathogens. They are able to attach to various penicillin binding proteins, due to the several side chains. Which enable them to avoid

blood brain barrier, resist the breakdown of penicillinase producing bacteria and ionize too aid entry into Gram-negative bacterial cells (Abraham,1987).

2.19.7 Monobactams

This antibiotic was derived from the bacterium *Chromobacterium violaceumm* which was part of beta-lactam compounds. However, the ring of monobactams is not bonded to another ring unlike most other beta-lactams, it stands alone (Bonner and Sykes, 1984; Sykes and Bonner, 1985). Aztreonam is an example of monobactam antibiotic which is in use commercially with a narrow spectrum. It is used for treating septicemia, urinary tract infection and pneumonia which was affected by these groups of bacteria (Sykes *et al.*, 1981). This antibiotic is only active in fighting Neisseria and Pseudomonas bacteria. The monobactams are effective against Gram-negative bacteria or aerobic but not active in fighting Gram positive bacteria or anaerobes (Sykes *et al.*, 1981). They are used as inhalers and injectable (Sykes *et al*., 1981).

2.19.8 Carbapenems

Carbapenems antibiotics were discovered in 1976 (Papp-Wallace *et al*., 2011). Before this time the efficacy of penicillin was seriously threatened in the late 1960s because of the emergence of beta-lactammase in the bacteria and this made bacteria to develop resistance to penicillin (Papp-Wallace *et al*., 2011). This situation pushed scientist to search for beta-lactamase inhibitors. In 1976 olivanic acids was discovered as a result of their efforts (Brown *et al*., 1976; Butterworth *et al*., 1979). *Streptomyces clavuligerus*, as Gram-positive bacterium produce olivanic acid which impede beta-lactamase (Brown *et al*., 1976;

Butterworth *et al*., 1976). Regrettably, olivanic acids could not easily penetrate the bacterial cell and so became unstable. These challenges affected the development and further research on the olivanic acids (Reading and Farmer, 1984). This challenge led to the discovery of two superior beta-lactamase inhibitors clavulanic acid which is obtained from *S. clavuligerus* (Brown *et al.*, 1976). According to Papp-Wallace *et al*. (2011) thienamycin serves as the standard for other carbapenem and is considered as the first "carbapenem". When it comes to combating bacterial infection carbapenems is very effective in this regard due to their ability to resist hydrolytic actions of beta-lactamase enzyme (Torres *et al.*, 2007). Carbapenems is broad spectrum in action and is very efficient against Gram-negative and Gram-positive bacteria among most of the known beta- lactams (Torres *et al.*, 2007). Hence, they are usually known as "antibiotics of last resort" and are administered to patients who have developed resistant to most bacteria infections (Torres *et al.*, 2007). Some examples are imipenem, ertapenem and meropenem (Brink *et al*., 2004).According to Livermore *et al*., (2011) and Patel and Bonomo (2011), bacterial has developed resistance to carbapenems antibiotic in recent days.

2.19.9 Aminoglycosides

According to Mahajan and Balachandran (2012), streptomycin is the first antibiotic identified among aminoglycosides family in 1943. Peterson (2008) reported that streptomycin has been used to combat tuberculosis in human. The report further explained that aminoglycoside are broad spectrum antibiotic and are very efficient in controlling Gram-negative rods and some Gram-positive bacteria. According to a report by Gilbert (2000), although streptomycin was very effective against a wide range of infections, its

side effect was highly deadly. This has prompted researchers to look for new members of this family that will still be effective but less harmful to human health. This resulted in the discoveries of amikacin, neomycin tobramycin and gentamicin. Gentamicin is less toxic as compared to streptomycin and is mostly used to treat infectious diseases caused by *Shigella*, *Pseudomonas*, *Escherichia coli* and *Salmonella*.

2.19.10 Tetracyclines (Chlortetracycline, Oxytetracycline, Tetracycline)

According to Sanchez *et al*., (2004), in 1945 tetracycline was discovered by Benjamin Duggar from a bacteria in the soil in the *Streptomyces* genus. According to Fuoco (2012) Chlorotetracycline (Aureomycin) was the first among this class discovered. Generally, this group of antibiotics is classed into various generations on bases of the method at which they are synthezed. The first generation is those obtained by biosynthesis which includes tetracycline, chlortetecycline, oxytetracycline and demeclocycline whereas meclocycline, doxycycline, methacycline, lymecycline, rolitetracycline and minocycline are known to be second generation since they are products of semi-synthesis. And the third generation originate from total synthesis such as tigecyclines (Fuoco, 2012). According to Medical News Today (MNT) (2015), this antibiotic attack the ribosome of the bacteria and it work by interfering amino acids addition to polypeptide chain throughout protein production in the bacteria organelle. For better absorption, tetracyclines are taken two hours earlier to or after meal at least. Due to discoloration of teeth among patients, all tetracyclines are prescribed for patients above eight years and could be used to treat elephantiasis, malaria, rickettisia and amoebic parasites (Sanchez *et al*., 2004). In recent days, bacteria have

developed resistance to these antibiotics unlike the past, they were the best antibiotics used for treating several infections due to their wide spectrum (Chopra and Roberts, 2001).

2.19.11 Oxazolidinoes

They are artificial antibiotics permitted for use recently. Linezolid was the first synthesized and was accepted for clinical use only in 2000 (Etebu and Arikekpar, 2016). Bozdogan and Appelbaum (2004) stated that oxazolidinones impede protein production. They further emphazed that this antibiotic is broad spectrum in action against vancomycin and methicillin-resistant *Staphylococci*, vancomycin-resistant *Enterococci*, penicillin-resistant-*Pneumococci* and anacrobes. Moellering (2003) indicated that linezolid is used for treating infections of the respiratory tract and the skin usually caused by Gram-positive pathogenic bacteria. According to Bozdogan and Appelbaum (2004) when it comes to surgical infections oxazolidinones are the best drug since they easily infiltrate and store in the tissues like hematoma, bone, lungs and cerebrospinal fluid. Kuter and Tillotson (2001) reported that although the standard procedures for administering linezolid are usually safe, myelosuppression which result to anemia and thrombocytopenia are the side effects when treatment is prolonged.

2.19.12 Glycopeptides

According to Kahne *et al*. (2005) glycopeptide antibiotics (GPAs) are derived from natural products however, for the past 20 years the semi-synthetic derivatives have been developed which have improved activity and pharmacokinetic properties. Kang and Park (2015) reported that glycopeptides are naturally cyclic peptide of seven amino acids with which

two sugars are bounded. According to Allen and Nicas (2003) most glycopeptides antibiotic attack the target organism through the formation of 5 hydrogen bonds with the peptididc backbone of the drug. At times during the production of this drug extra chlorine and sugar are attached to the backbone of the drug and this drug bind to the target organism more effectively (Allen and Nicas, 2003). Likewise, a lipophilic side chain antibacterial effectiveness and lengthen half-life of glycopeptides.

2.20 Antibiotic Residues Test

According to Biopharm (2016) Premi® test kit is a bacteriological screening kit for the recognition of antibiotic residues in food. This kit is centered on the growth inhibition of *Bacillus stearothermophilus*, which is a thermophilic bacterium that is extremely sensitive to several antibiotics and sulfonamide compounds. The kit identifies drug residues when they are above the drug specific amount. Thus, only detect the antibiotic residue that are higher than the standard acceptable daily intake concentration level (Codex, 2015). The analyze results are ready within four hours (Biopharm, 2016). The detection limits for different animal products is shown in Table 7.

Table 7: The Premi® Test Kit Detection Limits in Various Animal Food Products.

Substances	Chicken	Pork	Beef	Eggs	Shrimp
β-lactams					
Amoxicillin	5	5	5	5	15
Ampicillin	5	5	5	5	
Penicillin-G	2.5	2.5	2.5	2.5	5
Cloxacillin		>100		100	
Oxacillin		100			
Dicloxacillin					
Cephalosporins					
Cefquinome	75	100	100		
Ceftiofur	100	200	100	400	
Macrolide					
Tylosin	50	25-50	50	50	
Erythromycin	100	100	100	50	100
Lincomycin	100	100	100		
Tilmicosin	50	50	50		
Spiramycin	1000	1000	1000		
Tetracyclines					
Chlortetracycline	100	100	100	600	1000
Oxytetracycline	100	100	100	400	100
Doxycycline	100	100	100	200	
Tetracycline		50		200	
Demeclocycline		50			

Table 7 cont.: Premi® Test Kit Detection Limits in Various Animal Food Products

Substance	Chicken	Pork	beef	Eggs	Shrimp
Sulfonamides					
Sulfamethazine	75	50-100	100	25	
Suldiazine	75	50-75	75	25	50
Sulfamethizole		5-10			
Sulfguanidine	<200	150	<200		
Sulfadimethoxine		25-50	<100		
Sulfapyridine	<50	50	<100		
Sulfamethoxypyridine		25			
Sulfisoxazole	<100	25			
Sulfathiazole	<100	25			
Sulfachloropyridazine	<100	25			
Sulfmerazine	<100	25	<100		
Sulfanilamidee	<100	150			
Sulfaquinoxaline	<100	50	<50		
Sulfametiozole	<100		<50		
Sulfamethoxazole				25	
Aminoglycosides					
Gentamicin	100	100	100	100	
Streptomycin	1500	1500	3000	1000	200
Neomycin	300	300	300	300	
Spectinomycin				5000	

Table 7 Cont.: Premi® Test Kit Detection Limits in Various Animal food Products

Substances	Chicken	Pork	Beef	Eggs	Shrimp
Quinolones					
Enrofloxacin	>600	>600	>600		
Flumequine	>100	>100	>100		
Polypepptide					
Virginiamycin	500	500	500		
Bacitracin	500	500	500		
Zn-bactracin	1250				
Colistin	>1000				
Ionophores					
Sainomycin	1000				
Monensin	1250				
Lasalocid	10000				
Oligosaccharides					
Avilamycin	>50000				
Andere					
Florfenicol	100	100	100		5000
Chloranphinicol	2500	2500	2500	2500	
Trimethosprim	50				
Narasin	1250				
Amprolium	>2000				
Phosphomycine	.1500				
Ronidazoe					>5000
Furazolidone	>1500				

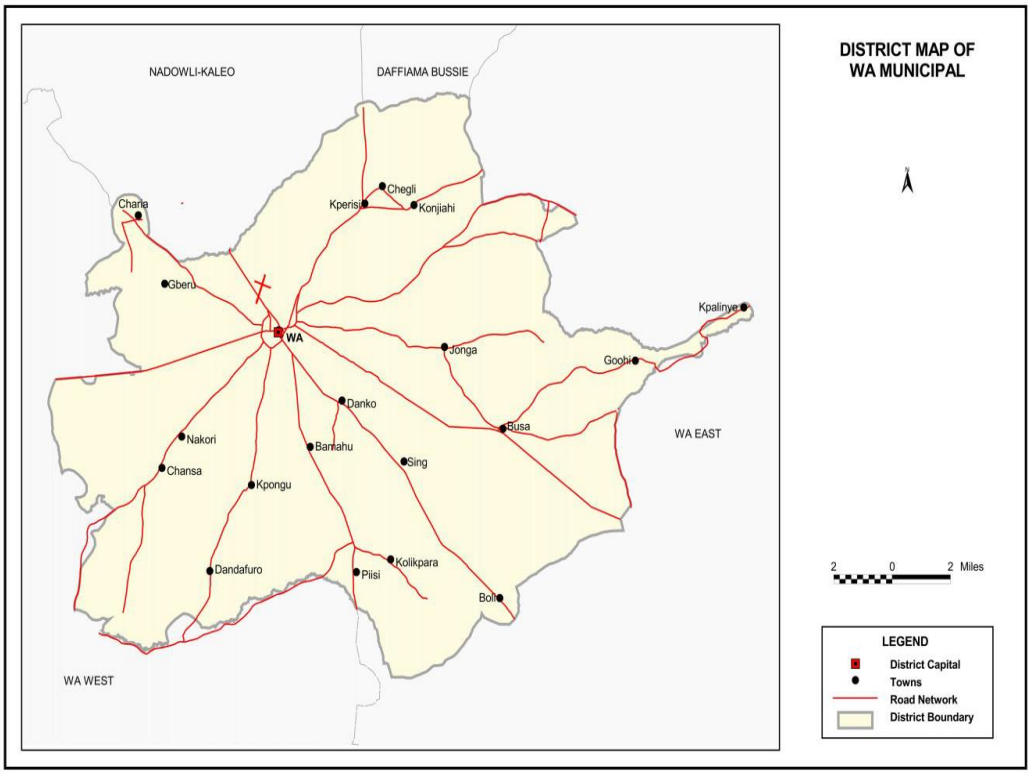
Source: Biopharm (2016)

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Location

The study was conducted in Wa Municipality in the Upper West Region of Ghana. The Municipality has its capital as Wa, which represent the Reginal capital of Upper West (Figure 1). Wa has a total land area of about 579.86 square kilometres. That is around 6.4% of the total land area of the region (Ghana Statistical Services (GSS), (2014)). The Municipality shares administrative boundaries with Nadowli District to the north, Wa East to the east and Wa West to the west and south. It is found on the latitudes 1º40'N to 2º45'N and longitudes 9º32'W to 10º20'W (GSS, 2014).



Source: GSS (2014)

Figure 1: Map of Wa Municipality

The population of the Municipality is 107, 214 which is 15.3% of the total population of the region (GSS, 2014). Males are lesser (49.7%) than females (50.6%). Out of this, about 34% of the population lives in the rural areas. Roughly 30.9% of the households are into agriculture (GSS, 2014). Approximately58% of the households in the rural areas are into agriculture whiles 20.3% of the households on the urban areas are binto agriculture (GSS, 2014). Most (82.9%) of the agricultural households in the Municipality are crop farming. Poultry (chicken) is the dominant animal (29.5% of all animals) kept by the highest proportion (27.3%) of households in the Municipality (GSS, 2014).

3.2 Survey on Antibiotics use in Ruminants Production

3.2.1 Questionnaire Administration

3.2.2 Enrollment of Participants

Permission was sought from veterinary staff at veterinary clinic to help identify farmers. Permission was also sought from the sanitation inspector and the veterinary officers in charge of the slaughterhouse in the municipality to help explain the objective of the research to the butchers.

3.2.3 Study Population, Sample Size and Sampling Method for the Survey

The study population consisted of ruminant farmers and veterinary staff in the municipality who accepted to take part in the study by answering the questionnaires. Sample size of 250 livestock farmers and 6 veterinary staffs were selected for the study. Snow ball sampling technique was employed to select the farmers for the interview.

Plate 1: One of the farmers' being interviewed

3.2.4 Structure of Questionnaire

The research employed structured questionnaires which entailed both open and close ended questions developed to gather information from farmers and veterinary officers. The antibiotics that were used as prophylactic treatment to improve the health of animals, knowledge of farmers on antibiotics withdrawal period and drug residues were assessed from August September, 2018.

3.3 Laboratory Investigation

3.3.1 Beef Meat Sampling

Three different parts of beef samples (muscle, liver and kidney) were collected between October and December 2018 in Wa Municipality for antibiotic residue test. In total forty-eight meat samples (muscle 16, liver 16 and kidney 16) were collected from Wa abattoir using random sampling methods. The samples were kept in an ice chest with ice block immediately after collection and conveyed to the Spanish laboratory of University for Development Studies Nyankpala campus. The samples were frozen before analyses due to the extraction process used.

3.3.1.1 Sample Extraction

The raw frozen meat (beef) was allowed to thaw at room temperature for thirty (30) minute and the extract collected for the analysis.

3.3.1.2 Antibiotic Residues Test

The Premi®Test Kit was used for antibiotic residues testing. This kit was used to determine qualitatively the antibiotic residues in meat samples by following the manufacturer's instructions. Approximately 2 cm^2 of raw beef (muscle, liver and kidney) were used. The samples were frozen and thawed to obtain their extract/juice. About hundred microliter extract were pipetted and transferred onto the agar inside the ampoule of the kit and incubated at 37 °C for 20 minutes for pre diffusion. The extract was carefully flushed out by cleaning the test tubes two times with demineralized water. The test ampoule was closed with foil to avoid evaporation during incubation. The test ampoules were incubated in water bath at 64 °C till the negative control change colour from purple to yellow. Colour

change in the negative control was observed within 4 hours. After the color of the negative control has changed, the results were read using color chart. If antibiotic residues are not in the extracted samples, the bacteria in the agar would grow and produce acids. This reduces it pH and causes the agar to change colour from purple to yellow and this is marked as negative. If antibiotics were inside the extracted sample, they would kill the bacteria in the agar or suppress it growth as a result, the entire or part of the agar in the tube remains purple and this is marked as positive for antibiotic residue.

Plate 2: Premi®Test Kit Result

3.3.2 Sampling

A total of 150 swab samples made up of 50 muscles, 50 livers and 50 kidneys from 50 cattle were randomly collected from October to December, 2018 at Wa Abattoir and examined for the presence of *Salmonella* spp. The samples were taken immediately after slaughter (dressing) in the abattoir hell before the carcasses were taken to the market and

thirty (30) samples were collected per Month. With gloves worn, sterile swabs were used to swab the surface of meat ($10mm^{2)}$ and immediately placed in its cap. The samples were properly labeled and placed in an ice chest with ice block. The samples were transported to Spanish laboratory of University for Development Study, Nyankpala campus for instant analysis.

3.3.2.1 Total Viable Count (TVC) and Coliforms

Buffered peptone water (BPW) at 0.1% was prepared and 9 ml were transferred into universal bottles. Swabs (kidney, liver and muscle swabs) were put in the universal bottle containing BPW and serial dilutions of up to 10^4 were prepared. Hundred microliter aliquots were transferred from each of the dilutions and spread unto the plate count agar (total viable count) and MacConkey agar (coliforms). Afterward they were incubated at 37 °C for 24 hours and colony counter was then used for counting.

3.3.2.2 Isolation and confirmation of *Salmonella* spp

3.3.2.3 Non Selective Pre-Enrichment

To allow recovery and growth of any stressed organism, each swab was placed in 9 ml buffered peptone water and incubated at a temperature of 37°C for 18-24 hours.

3.3.2.4 Selective Enrichment

Rappaport-Vassiliadis (RV) and Selenite broths were the two selective enrichment media used and these encourage growth of *Salmonella* spp. while inhibiting other microorganisms. After non-selective pre-enrichment, 0.1ml was transferred into RV broth

and inoculated at 42 °C for 18 to 24 hours while 1 ml was transferred to Selenite broth and incubated at 37 °C for 18 to 24 hours.

3.3.2.5 Isolation

Selective enrichment media were streaked unto Xylose Lysine Deoxycholate agar and Brilliant Green agar containing one or more agents that inhibit non-*Salmonella* organisms. Xylose Lysine Deoxycholate and Brilliant Green Agar plate were incubated after the streaking at 37 °C for 18-24 hours. Colonies with a slightly clear zone of reddish/ pinkish color with or without a black center on Xylose Lysine Deoxycholate plates; and grey to reddish/pink and somewhat convex colonies that caused the colors of the media to be red/pink in Brilliant Green Agar plates were suspected as *Salmonella*.

The suspected *salmonella* colonies were inoculated on XLD agar for pure colonies. The pure colonies were transferred onto Trypticase Soy broth for sensitivity test and the pure colonies were also subcultured on Trypticase Soy Agar plates for biochemical confirmation.

3.3.2.6 Confirmation of *Salmonella* species

Presumptive *Salmonella* spp. were subcultured onto Trypticase Soy agar and incubated at 37^{o}C for 24 hours, to get pure colonies. They were then subjected to gram staining, biochemical {LIA (Lysine iron agar) and TSI (triple sugar iron agar)} and serological (using *Salmonella* Latex Agglutination Kit) tests.

Plate 3: *Salmonella* on XLD

3.3.2.7 Gram Stain

Gram staining was done to identify the bacteria as gram positive or negative. The suspected *Salmonella* colony was smeared on a clean microscope slide. The smear was air dried and heat fixed by passing it through fire three times. Crystal violet was used to stain the dried smear for 2 minutes and water washed. For one minute Lugol's iodine was flooded on the smear and washed with water. It was decolorized with acetone alcohol for some few seconds, washed and finally counter stained with neutral red for 1-2 minutes. The slide was washed, air dried and examined with 100X in oil immersion under a light microscope for gram negative rods which is a common features of *Salmonella* (James *et al.*, 2011).

3.3.2.8 Triple Sugar Iron (TSI)

Sterilized loops were used to pick 2-3 well isolated suspected *Salmonella* colonies and carefully incubated it on TSI Agar inside a tube (bottle). The loop was first stabbed through the center of the medium (TSI) before streaking was done on the top of the slant and incubated at 37 ºC for 18 to 24 hours. *Salmonella* mostly displays alkaline slant (red) with an acid butt (yellow). Depending on the *Salmonella* species isolated, H_2S and gas could be produced (Hohmann, 2001).

3.3.2.9 Latex Agglutination

A full loop of pure culture of suspected *Salmonella* colonies was mixed a drop of the test latex on a clean slide for 10- 15 seconds and emulsified. One drop of the Oxoid *Salmonella* test kit was added to the suspension and mixed thoroughly. The slide was rotated and the result was read in two minutes. Positive result shows rapid agglutination in a form of visible clumps. No agglutination within 2 minutes is a negative result.

3.3.2.10 Lysine Iron Agar (LIA)

Pure colonies of presumptive *Salmonella* spp. were inoculated on LIA slant by piercing the butt twice before streaking the slant and then incubated at 37°C for 24 hours. *Salmonella* in LIA usually shows an alkaline (purple) slants and alkaline butts.

3.3.2.11 Antimicrobial Susceptibility test

The susceptibility test of *Salmonella* isolates was checked against some commonly used antibiotics such as Amoxycillin/clavulanic acid (30µg), Azithromycin (15µg), Ceftriaxone

(30 µg), Chloramphenicol (30 µg), Ciprofloxacin (5 µg), Gentamicin (10 µg), Teicoplanin (30 µg), Tetracycline (30 µg) and Suphamethoxazole/trimethoprim using Kirby-Bauer antibiotic discs diffusion method (Buaer *et al*., 1966).

Using a sterile loop, 2-3 *Salmonella* colonies isolated from each other were picked and inoculated in a tube of trypticase soy broth and the turbidity of the inoculum was adjusted to 0.5 McFarland standards. Mueller-Hinton agar was inoculated by dipping a sterile cotton swab into the inoculum and swabbed completely on the surface of the agar plate. A sterile forceps was used to pick antibiotic disks which were placed on the inoculated agar plate and incubated at 37 ºC for 18-24 hours (Bauer *et al.,* 1966). The results were interpreted following the guideline of Clinical and Laboratory Standards Institute (CLSI), (2008) as sensitive, intermediate, or resistant. All media, reagents and antibiotic disc used in this research were purchased from Oxoid, Basingstoke, UK.

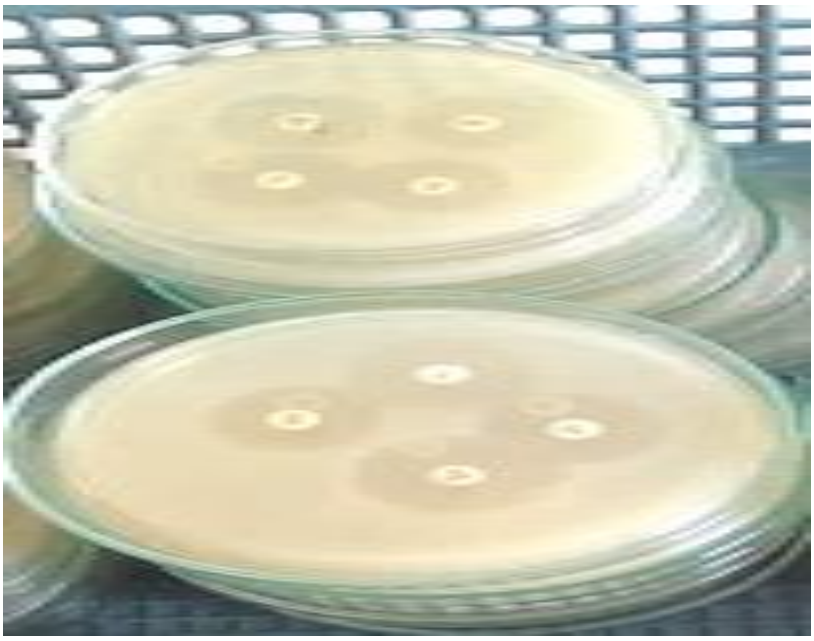


Plate 4: A picture showing antibiotic discs on plates with their inhibition zones

3.4 Analysis of Data

Laboratory results and questionnaires were entered in Microsoft Excel The SPSS version 16.0 was used to analyzed data from questionnaire, the presence or absence of antibiotic residues and *Salmonella* spp in the meat samples. GenStat 12.1 edition was used to analyze total aerobic plate count.

The data were presented in summary tables and graphs. Data presented as categorical proportions were compared by the chi-square (X^2) test. Significant differences between proportions were set at 0.05.

CHAPTER FOUR

4.0 Results

4.1 Demographic characteristics of Veterinary Staffs

Table 8 shows the demographic characteristics of the veterinary staffs. From this Table, the greater portion respondents were males (66.7%) and aged between 30-39 years (36%). They were all married (100%) and have tertiary education (100%) but half of them had worked for 6-10years (50%).

Table 8: Demographic characteristics of respondents (veterinary staffs)

Variable	Frequer	Percenta
Gender		
Male	4	66.7
Female	2	33.3
Total	6	100.0
Age		
30-39	5	83.3
40-49	1	16.7
Total	6	100.0
Marital status		
Married	6	100.0
Total	6	100.0
Education Status		
Tertiary	6	100.0
Total	6	100.0
Work Experience		
1-5 years	2	33.3
6-10 years	3	50.0
11-15 years	1	16.7
Total	6	100.0

4.2 Knowledge of Veterinary Staffs on Antibiotics Administration to Ruminants.

Table 9 shows the bacteria suspected among ruminants and the antibiotics mostly administered. All the bacteria (*Salmonella, E. coli, Clostridia and Brucilla*) have mostly been suspected in these species (cattle, sheep and goat) and same antibiotics were administered among all the species.

Table 9: Bacterial suspected and antibiotic administered

Species	Bacteria	Treatment	Type of Antibiotic	Reasons
Cattle	*Salmonella*	Antibiotics	Kepro	Therapeutic
	Brucilla	Antibiotics	Sulpha	Therapeutic
	Clostridia	Antibiotics	Tyloson	Therapeutic
	E. coli	Antibiotics	MulxyVit	Therapeutic
Goat	*Salmonella*	Antibiotics	Kepro	Therapeutic
	Brucilla	Antibiotics	Sulpha	Therapeutic
	Clostridia	Antibiotics	Tyloson	Therapeutic
	E. coli	Antibiotics	MulxyVit	Therapeutic
Sheep	*Salmonella*	Antibiotics	Kepro	Therapeutic
	Brucilla	Antibiotics	Sulpha	Therapeutic
	Clostridia	Antibiotics	Tyloson	Therapeutic
	E. coli	Antibiotics	MulxyVit	Therapeutic

4.2.1 Dosage recommended by manufacturer and dosage administered

The veterinary staffs administer the following antibiotics (Kepro, Sulpha,Tyloson, Mulxyvet and Penstrep) as recommended by the manufacturer and this is based on the weight of the animal. All the antibiotics have the same withdrawal period (21 days).

Table 10: Antibiotics dosages recommended by manufacture and administered by veterinary staff.

Antibiotics	Manufacturer recommended D	Dosage Admin	Withdrawal p	Reasons for below and recommended dosage
Kepro	1ml-10g	1ml-10g	21 days	Overdose burden the liv
Sulpha	1ml-6kg	1ml-6kg	21days	bacteria will not die
Tyloson	1ml-25kg	1ml-25kg	21 days	underdoes but rather will
Mulxyvet	1ml-10kg	1ml-10kg	21 days	into different form and th
Penstrep	1ml-25kg	1ml-25kg	21 days	antibiotic cannot cure.

4.2.2 Demographic characteristics of livestock farmers

Table 11 shows the demographic characteristics of the livestock farmers. From Table 11, majority of the farmers were males (93.6%) and aged between 40-49 years (36%). Most of them have been in the production of livestock between 6-10years (60.4%) and are married (91.2%). Islamic is the dominating religion (93.6) and Wala was the main language spoken (82.4) but, do not have formal education (36.4%).

Table 11: Demographic characteristics of respondents (farmers)

Variable	Frequer	Percenta
Gender		
Male	234	93.6
Female	16	6.4
Total	250	100.0
Age		
20-29	30	12
30-39	46	18.4
40-49	90	36
50-60	66	26.4
61 and above	18	7.2
Work Experience		
1-2 years	26	10.4
3-5 years	20	8
6-10years	151	60.4
Above 10 years	53	21.2
Total	250	100.0
Marital status		
Married	228	91.2
Single	16	6.4
Others	6	2.4
Total	250	100.0
Religion		
Christianity	14	5.6
Islamic	234	93.6
Traditional	2	.8
Total	250	100.0
Tribe of farmers		
Waala	206	82.4
Damkaba	44	17.6
Total	250	100.0
Education Status		
Non Formal Education	91	36.4
Primary School	34	13.6
Junior High School	69	27.6
Senior High School	20	8
Tertiary Education	10	4
Others	26	10.4
Total	250	100.0

4.2.3 Distribution of Antibiotic Used by Farmers

Table 12 represents responses from ruminant farmers. The most commonly used antibiotic was ciprofloxacin (32%). Other antibiotics used were 14%, these represent those antibiotics that farmers used but did not know their names. Twenty-eight farmers did not answer this aspect.

Table 12: Distribution of Antibiotic Used by Farmers

Antibiotic	Frequency/ percentage (%)
Quinolones	
Ciprofloxacin	
Yes	**71(32)**
No	151 (68)
Beta-lactams (Penicillin)	
Amoxicillin/Clavulanic	
Yes	60 (27)
No	162(73)
Sulfonamides	
Trimethoprim/Sulfamethoxazole	
Yes	38 (17.1)
No	174 (82.9)
Macrolides	
Azithromycin	
Yes	12 (5.4)
No	210 (94.6)
Aminoglycosides	
Gentamicin	
Yes	4 (1.8)
No	218 (98.2)
Cephalosporin	
Ceftriaxone	
Yes	2 (0.9)
No	220 (99.1)
Chloramphenicol	
Chloramphenicol	
Yes	2 (0.9)
No	220 (99.1)
Tetracycline	
Tetracycline	
Yes	2 (0.9)
No	220 (99.1)
Others	
Yes	31 (14)
No	191 (96)

4.2.4 Reasons for using the above antibiotics

The table 13 indicated the ideal behind the use of those antibiotics mentioned. The greater portion of the respondents used them due to their effectiveness (84.6%) and the rest used them due to their colleagues recommendation (6.1%), less cost (3.7 %), easy to use (2.8%) and others (2.8%).

Table 13: Reasons for using the above antibiotics

Reasons	Frequency	Percent
It is effective	181	84.6
It is less costly	8	3.7
It is easy to use	6	2.8
Colleagues advice	13	6.1
Other	6	2.8
Total	214	100.0

4.2.5 Knowledge of Farmers on Antibiotics usage

All the farmers confirmed that they have ever encountered infections in their livestock (Table 14). Majority consulted veterinary officers (96%) and had ever used antibiotics as medication (96.8%). More than half of the farmers (63.6%) have some knowledge on the antibiotics they administered to their animals and 50.9% of them had the knowledge from veterinary staffs. The majority of the farmers (50.8%) invite veterinary staffs to administer antibiotics to their animals. More than half of the farmers observed safety and dosage instructions (88.2%) but do not follow the withdrawal period (73.2%).

Table 14: Knowledge of Farmers on Antibiotics usage

Variable	Frequency	Percentage
Ever encountered infection in the farm		
Yes	250	250
No	0	0
Total	250	100.0
Consult veterinary officers		
Yes	240	96
No	10	4
Total	250	100.0
Antibiotic medication		
Yes	242	96.8
No	8	3.2
Total	250	100.0
Knowledge on antibiotic usage		
Yes	159	63.6
No	91	36.4
Total	250	100.0
Source of information		
Extension Officers	32	20.1
Colleague Farmers	46	28.9
Veterinary Staff	81	50.9
Total	159	100.0
Source of antibiotics		
Veterinary Clinic/Shops	152	62.8
Friends	10	4.1
Market	22	9.1
Others	58	24.0
Total	242	100.0
Administers of antibiotics		
Self	43	17.8
Veterinary staff	123	50.8
Both	76	31.4
Total response	242	100.0
Observation of safety and dosage instructions		
Yes	105	88.2
No	14	11.8
Total response	119	100.0
Observe withdrawal period		
Yes	67	26.8
No	183	73.2
Total	250	100.0

4.2.3 Dosages and Withdrawal Periods According to Farmers

From Table 14, out of 73 farmers who answered this portion, 45.2% administered 2-5ml, 35.6 % also administered 11-15 ml per treatment while the lowest was 19.2% representing those who administered 6-10 ml. This means that 177 farmers did not respond to this question. Regarding the withdrawal period, most farmers said that they allow for 3-7 days (55.2%), 23.9% observed it for 14 days, and 20.9% observed it for 21 days.

Table 15: Dosages and Withdrawal Periods of Antibiotics by farmers

Dosage	Frequency	Percentage (%)
2-5 ml	33	45.2
6-10 ml	14	19.2
11-15 ml	26	35.6
Total	**73**	**100.0**
Withdrawal Periods		
3-7days	37	55.2
14 days	16	23.9
21 days	14	20.9
Total	**67**	**100.0**

4.2.4 Educational Level of Farmers * Knowledge on Antibiotics

The Table shows the effect of education on the farmer's knowledge on antibiotics usage. From this it can be deduced that, education have no impact on the knowledge of farmers on antibiotic usage since there was no clear path.

Table 16: Educational Level of Farmers * Knowledge on Antibiotics

Educational level		Knowledge on the antibi		Total
		Yes	No	
Non-formal	Count	61	30	91
	%within knowledge antibiotics	38.4%	33.0%	36.4%
Primary school	Count	24	10	34
	%within knowledge antibiotics	15.1%	11.0%	13.6%
JHS	Count	38	31	69
	%within knowledge antibiotics	23.9%	34.1%	27.6%
SHS	Count	10	10	20
	%within knowledge antibiotics	6.3%	11.0%	8.0%
Tertiary	Count	10	0	10
	%within knowledge antibiotics	6.3%	.0%	4.0%
Others	Count	16	10	26
	%within knowledge antibiotics	10.1%	11.0%	10.4%
Total	Count	159	91	250
	%within knowledge antibiotics	100.0%	100.0%	100.0%

4.3 Prevalence of Antibiotic Residues in Beef (Kidney, Liver and Muscle)

Figure 2 shows the prevalence of antibiotic residues in the kidney, liver and meat muscle of the beef samples. The prevalence of antibiotic residues in kidney (43.75%), liver (37.5%) and meat muscle (6.25%) and with overall percentage been 29.17%.

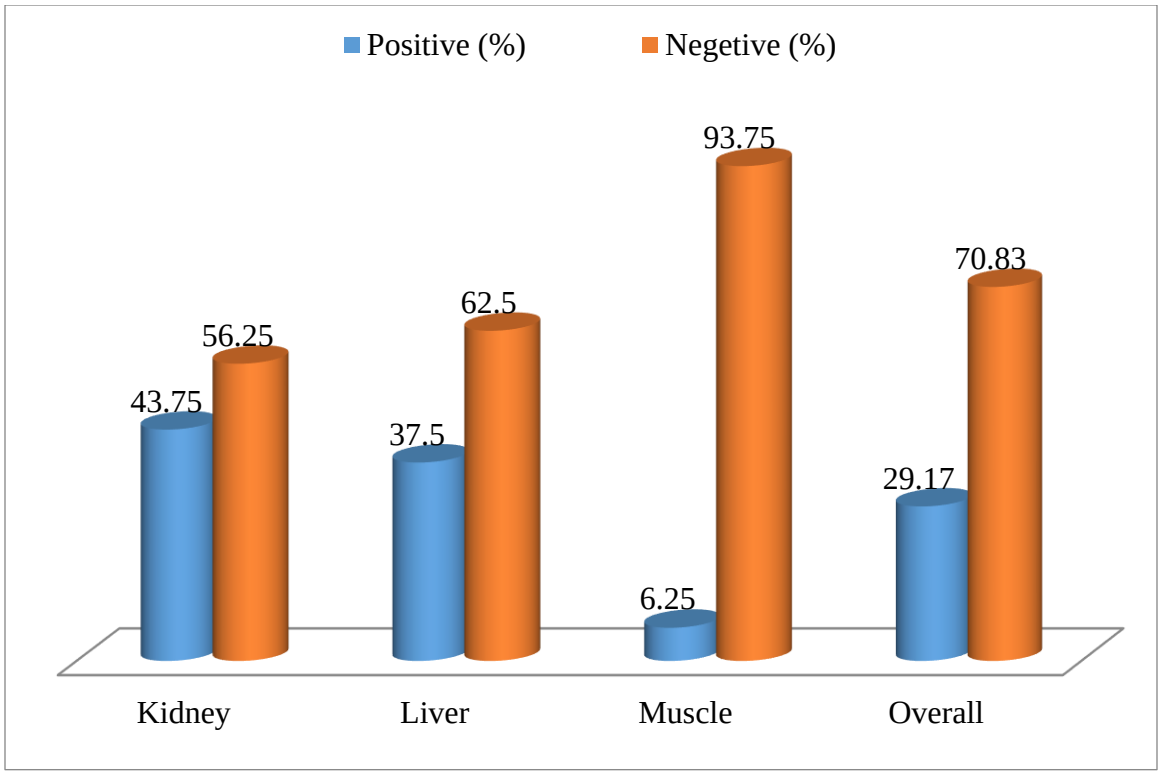


Figure 2: Prevalence of antibiotic residues in beef.

4.3.1 Aerobic Bacteria Count of beef Samples.

The total aerobic bacteria count is shown in Table 17. There were no significant difference (P>0.05) in the meat samples. However, muscle (3.57 log cfu/cm^2) tended to have the highest load, followed by liver (3.39 log cfu/cm^2) and kidney (3.32log cfu/cm^2).

Table 17: Total aerobic bacteria plate count of beef samples

Source	Bacteria load (log cfu/cm^2)
Kidney	3.32
Liver	3.39
Muscle	3.57
Sed	1.196
p-value	0.959

4.3.2 Prevalence of *Salmonella* spp in Kidney, Liver and Muscle

The prevalence of *Salmonella* spp. in muscles, kidneys and liver of cattle is shown in Table 18. From Table 18, liver (32%) was the most contaminated source, followed by muscle (30%) and kidney (10%).

Table 18: Prevalence of *Salmonella* spp in Kidney, Liver and Muscle

Source	Tested	Positive	Prevalence
Kidney	50	5	10
Liver	50	16	32
Muscle	50	15	30
Total	150	36	24

4.3.3 Pairwise Comparisons of *Salmonella* spp in Kidney, Liver and Muscle

Table 19 indicates the significant of the Salmonella spp isolated from the various parts of the beef carcasses. There was significant different between the *Salmonella* isolated from kidney and liver ($P<0.05$), kidney and muscle ($P<0.05$) but liver and muscle, there were no significant different ($P>0.05$).

Table 19 : Pairwise Comparisons of *Salmonella* spp in Kidney, Liver and Muscle

(I) Source	(J) Source	Mean Difference (I J)	Std. Error	df	Sig.	
Kidney	Liver	.22a	0.078	1	0.005	Yes
	Muscle	.20a	0.077	1	0.01	Yes
Liver	Kidney	-.22a	0.078	1	0.005	Yes
	Muscle	-0.02	0.092	1	0.829	No
Muscle	Kidney	-.20a	0.077	1	0.01	Yes
	Liver	0.02	0.092	1	0.829	No

4.3.3 Antimicrobial Susceptibility of *Salmonella* spp. Isolated from Meat Samples

Salmonella spp. were all susceptible to chloramphenicol, ciprofloxacin, tetracycline and suphamethoxazole/trimethoprim. They were highly resistant to teicoplanin (97.62%). Some

intermediate resistance was observed for amoxycillin/clavulanic acid (7.14%), azithromycin (9.52%), ceftriaxone (4.76%) and gentamicin (7.14%).

Table 20: Antimicrobial Susceptibility of *Salmonella* spp.

Antimicrobial	R (%)	I (%)	S (%)
Amoxycillin/clavulanic acid 30µg (AMC)	2.38	7.14	90.48
Azithromycin 15µg (AZM)	30.95	9.52	59.52
Ceftriaxone 30 µg (CRO)	0.00	4.76	95.24
Chloramphenicol 30 µg (C)	0.00	0.00	100.00
Ciprofloxacin 5 µg (CIP)	0.00	0.00	100.00
Gentamicin10 µg (CN)	14.29	7.14	78.57
Teicoplanin 30 µg (TEC)	97.62	0.00	2.38
Tetracycline 30 µg TE	0.00	0.00	100.00
Sulphamethoxazole/trimethoprim (SXT)	0.00	0.00	100.00

S, susceptible; I, intermediate; R, resistant

4.3.4 Antibiotic Resistant Profile and Multiple Antibiotic Resistant Index of Individual *Salmonella* Species

The antimicrobial resistant profile and MAR index of the *Salmonella* spp. isolates were showed in Table 20. The *Salmonella* spp. exhibited 6 antibiotic resistant patterns and the resistant pattern to Tec (teicoplanin, MAR index = 0.11) was the most common. One isolate was resistant to four different antbiotics AmcAzmTecCn. Resistant to 3 different antibiotics AmcAzmTec (MAR index=0.33), and AzmTecCn (MAR index=0.33) were observed in 2 and 4 isolates respectively. Thus, 14.3% (6/42) of the *Salmonella* spp. exhibited multidrug resistant.

Table 21: Antibiotic Resistant Profile and Multiple Antibiotic Resistant Index of Individual *Salmonella* Species

Code	No. of Antibiotics	Antibiotic resistant profile	MAR index
K10-1	4	AmcAzmTecCn	0.44
K10-2	3	AmcAzmTec	0.33
L18	3	AmcAzmTec	0.33
K30-1	3	AzmTecCn	0.33
L14	3	AzmTecCn	0.33
M11	3	AzmTecCn	0.33
M28-2	3	AzmTecCn	0.33
M8	2	AzmCn	0.22
K30-4	2	AzmTec	0.22
L15	2	AzmTec	0.22
M9	2	AzmTec	0.22
M36-2	2	AzmTec	0.22
M36-3	2	AzmTec	0.22
K6-1	1	Tec	0.11
K6-2	1	Tec	0.11
K6-3	1	Tec	0.11
K30-2	1	Tec	0.11
K30-3	1	Tec	0.11
L1	1	Tec	0.11
L2	1	Tec	0.11
L7	1	Tec	0.11
L16	1	Tec	0.11
L20	1	Tec	0.11

Table 20: cont.

L35	1	Tec	0.11
L36-1	1	Tec	0.11
L36-2	1	Tec	0.11
L42-1	1	Tec	0.11
L42-2	1	Tec	0.11
L42-3	1	Tec	0.11
L43	1	Tec	0.11
L43-1	1	Tec	0.11
L49-1	1	Tec	0.11
L49-2	1	Tec	0.11
M4	1	Tec	0.11
M6	1	Tec	0.11
M21	1	Tec	0.11
M23	1	Tec	0.11
M27	1	Tec	0.11
M28-1	1	Tec	0.11
M36-1	1	Tec	0.11
M48-1	1	Tec	0.11
M48-2	1	Tec	0.11

4.3.5 Multidrug resistant of individual *Salmonella* spp isolate

Table 21 shows the individual Salmonella isolates that were multidrug resistant. From this table, about seven isolates (16.7%) were multidrug resistant since they were resistant to more than two antibiotics.

Table 22: **Multidrug resistant of individual *Salmonella* spp isolates.**

Number of Antibiotic (s)	Number of Resistant Isolates (%)
1	29 (69.0)
2	6 (14.3)
3	6 (14.3)
4	1 (2.4)
Total	42 (100)

CHAPTER FIVE

5.0 DISCUSSIONS

5.1 Demographic characteristics of farmers

Table 11 shows the demographic characteristics of the livestock farmers. From Table 11, majority of the farmers were males (93.6%) and aged between 40-49 years (36%). Most of them have been in the production of livestock between 6-10years (60.4%), but, do not have formal education (36.4%). The majority of the livestock farmers being males can be associated with the fact that, males are normally the heads of the family and perform all roles associated with their position, including taking possession of things owned by women. This agrees with a study by Rupa *et al*. (2018) who reported that males are directly involved in livestock production than females due to their responsibility as family heads; which indicates that livestock production is a male dominated job. This study revealed that a higher portion of those involved in livestock production were the middle age. This collaborates with work by Olafadehan *et al*. (2014) who reported that 42.7% of ruminant farmers were within the ages of 40-49 years. Majority of farmers in the Wa municipality have been in livestock farming for long and might have gathered lots of experience. A study conducted by Olafadehan *et al*. (2014) recorded that only 9.8% of farmers had no formal education, which is lower the current study. The high percentage of non-formal education among farmers could have negative impact on adoption of new knowledge in antibiotic usage and livestock production as a whole.

5.2 Antibiotics Usage by Farmers

A total of 250 ruminant farmers were interviewed and out of this, 222 farmers used more than one antibiotic in their farms. This study show that the commonly used antibiotics by farmers were Quinolones (Ciprofloxacin) (32%), Beta-lactams (Penicillin-Amoxicillin/Clavulanic) (27%), Sulfonamides (Trimethoprim/Sulfamethoxazole) (17.1%), Macrolides (Azithromycin) (5.4%), Aminoglycosides (Gentamicin) (1.8%), Cephalosporin (Ceftriaxone) (0.9 %), Chloramphenicol (Chloramphenicol) (0.9 %), and Tetracycline (Tetracycline) (0.9%). Thus, Quinolones (Ciprofloxacin) was the most widely used antibiotic by farmers to treat bacterial infections in this area which agree with Er *et al*. (2013) who also reported that quinolones (ciprofloxacin, nalidixic acid etc) have been used widely in animal production for the treatment and prevention of diseases. The high usage may be due to its effectiveness as reported by Sultan (2014) that ciprofloxacin is very effective in combating microorganisms even those that are resistant to other class of antibiotics such as aminoglycosides, tetracyclines, macrolides, and beta-lactams etc.

Beta-lactams were the second antibiotics that were mostly used by farmers to treat bacterial infections in their farms which were 27%. This result is lower than the 48.84% of beta lactams usage by beef farmers recorded by Birhan and Mulugojjam (2018) in Ethiopia. However, this was higher than other findings of Ezenduka *et al*. (2011) who recorded 14% penicillin usage in Nigeria. In addition, this percentage was higher than the 18% reported by Darwish *et al*. (2013) as the average use of Beta-lactams for Africa counties. Sulfonamides are the third most frequently used antibiotics by farmers in this study. The percentage recorded for sulfonamide in the present study was higher than the 9.30% usage recorded by Birhan and Mulugojjam (2018) in beef farms. The higher prevalence of

antibiotic usage in this present study was attributed to their effectiveness, easy to administer, less costly and their availability when the need arises. In addition, it could be attributed to the fact that, there are a lot of bacterial infections in the study area that accounted for it, especially during the wet season.

5.3 Farmers Compliance to Antibiotics Withdrawal Period

The results in the present study revealed that majority of farmers have some knowledge on the antibiotics they administered to the animals whereas few knew nothing about the antibiotics they administered to their animals. Work done by Birhan and Mulugojjam (2018) indicated that 86% of the farmers had no knowledge on antibiotics usage which resulted in poor application. In addition to this, a survey conducted by Beyene (2015) showed that 67.6% of beef farmers in Central Ethiopia did not have any knowledge on antibiotics that were administered to their animals. However, in the present study, only 36.4% of the farmers do not have knowledge on the antibiotic they use. This means that the majority of farmers in the Wa Municipality have at least little knowledge on antibiotic usage and this can be attributed to the fact that the veterinary and extension staffs are extensively educating the farmers in this area.

Majority of farmers did not know the correct withdrawal period for the antibiotics administered to their animals. Only 67 out of 250 farmers were able to say something which most were wrong, based on veterinary staff perception. Also, some farmers administered the drugs by themselves. This could possibly lead to wrong administration and development of antibiotic residues or resistance pathogens to these antibiotics. This agrees with a study by Birhan and Mulugojjam (2018) who reported 100% of beef cattle farmers did not respect

the drug withdrawal period and 48.84% of beef farms were using nonprofessionals to administer drug to animals. In addition, Beyene *et al*. (2015) also reported that 67.6% of the dairy farmers interviewed were not aware of drug withdrawal period in Bishoftu and Modjo, Central Ethiopia.

For drug administration, most (88%) farmers who administer the antibiotic by themselves reported by following manufacturers' safety instructions on drugs before using it while 12% did not observe safety instructions before administering the drug to the animal**.** Even though the 88% claimed they followed the manufactures' instructions, they could not indicate the correct withdrawal period of the drugs they administer which contradicts their earlier claim of following safety instructions. These people may introduce a lot of antibiotic residues into the meat as a result of giving overdose to the animal and selling animals having antibiotic residues which may lead to eating meat containing antibiotic residues and finally the microbes may develop resistance to these antibiotics in humans. Administering under dose of the drug to animal is also risky in the sense that the animals' normal microflora in the gastrointestinal tract may develop resistance to those antibiotics and on the subsequent treatment, they may not respond to treatment.

5.4 Farmers Source of Knowledge on Antibiotic Usage

The result of the study showed that farmers acquired their knowledge from veterinary staff, extension officers and colleague farmers. This means that most farmers are trained by professionals on antibiotics usage. This could be attributed to the limited number of veterinary staff in the area of study so the staff trained most of the farmers in order to handle minor issues in absence of the veterinary staffs.

5.5 Antibiotic Residues in Meat (muscles, kidney and liver) of Cattle

Of the 48 samples (16 livers, 16 kidneys and 16 muscles) examined for the presence of antimicrobial residue, 29.17% (14/48) were positive for the presence of antibiotic residues. The overall antibiotic residues prevalence was similar to a report by Babapour *et al*. (2012) who screened 500 samples of beef and mutton collected from Iran for drug residues and reported prevalence rate of 22.8% and 14% for beef and mutton respectively. The current result is also in line with that of Donkor *et al*. (2011) who reported 30.8% of antibiotic residues from a total of 156 beef samples in Ghana. The research revealed the presence of antibiotic residues in the meat samples tested. This was in agreement with Abavelim (2014) who reported that beef samples collected from selected markets in Kumasi, Ghana contained drug residues. The prevalence of antibiotic residues in kidney and liver was higher as compare to those found in the muscles. The liver and kidneys are the major organs involved in the metabolism and elimination of drugs in the body. For this reason much of the drug residues are found there. The result of this experiment is in line with that of Morshdy *et al.* (2013), who also reported kidneys and livers showing higher concentration of the antibiotic residue compare to muscles in Egypt. This result also agree with Alla *et al*. (2011), who reported only 0.3% of the muscles containing antibiotic residue when they analyzed beef samples in Sudan. Mangsi *et al*. (2014) also emphasized that 38.33% of beef samples were contaminated with antibiotic residues in Pakistan. They observed 48.33% at Karachi, 41.6% at Sukkur, 36.67% at Hyderabad, 33.34% at Mirpurkhas and 31.67% at Larkana. The antibiotic residues found in this current study was much lower than what Ezenduka *et al.* (2011) recorded in beef (54.44%) in Nigeria and what Muriuki *et al*. (2001) recorded in beef (45.6%) in Kenya. The presence of antimicrobial drugs / residues in the beef samples

examined may be attributed to non-observance of withdrawal periods to antibiotics, self-administration of drugs (since the veterinary staff said that they trained farmers to handle minor issues but not antibiotic administration), giving overdose of these drugs to animals and animal feed being contaminated with the excreta of treated animals**.** The use of antibiotic in animal production is mainly responsible for the dissemination of antibiotic resistance bacteria isolates (EFSA, 2016).

5.6 Microorganism

5.6.1 Bacterial Load in Beef

Total aerobic plate count or total viable count (expressed in log cfu/cm^2) was highest in muscle, followed by kidney and the liver. The microbial loads obtained from the study were all below the limit stated in the International Guideline for raw beef (Table 5). The average load for muscle, liver and kidney were below the acceptable limit according to the Australian guideline which is 5 log cfu/g (Australian Standard (AS), 2002). The lower load could be attributed to the betters compliance to inspection officers recommendations which is in agreement with CAC (2005) report. In addition, the rumen content was not emptied within the abattoir hall, these has reduced the number of flies in the hall. The result also agrees with Raji (2006) who reported 3.5 cfu/g count on dried slide beef in Nigeria but lower than 5.35 cfu/g reported by Ahmad *et al.* (2013) in Tanzania and Twum (2015) in Ghana who found high microbial counts of 5.37 to 5.62 log cfu/g in fresh beef carcasses. According to Okonko *et al*. (2008), food substances may be contaminated with microorganisms as a result of "sneezing" and "coughing" by food handlers. Koffi-Nevry *et al*. (2011) also indicated that, "sneezing and coughing carelessly among butchers can also introduce

microbes to the products". The contamination of meat by microbes may occur during slaughtering, processing and transport as reported by MTU (2010); unclean water, knives, slaughter slabs and slaughter floor as reported by Adzitey *et al*. (2011) and abattoir workers as reported by Forsythe (2000). The microbial presence in meat is a challenge to the meat industry (Komba *et al*., 2012).

5.6.2 Prevalence of *Salmonella* spp in Meat of Cattle (muscles, kidneys and liver)

The prevalence of *Salmonella* spp in muscles, kidneys and liver of cattle in the Wa, Municipality of Ghana is shown in Table 18. Of one hundred and fifty beef samples tested for *Salmonella*, thirty-six of these samples were *Salmonella* positive. The overall prevalence in this case which was 24% is a little bit similar to Adzitey *et al*. (2015) who reported an overall prevalence of *Salmonella* spp. in beef samples to be 31% (22/70) in the Tamale Metropolis. Thus the muscles, kidneys and liver samples were contaminated with *Salmonella* spp. Contamination of meat samples happens when maximum care is not taken during slaughtering and dressing of animals. Comparing the *Salmonella* contamination among muscle, kidney and liver the higher load was from the liver, followed by muscle and the least was kidney. There were no significant ($P>0.05$) difference in the *Salmonella* contamination of muscles and liver, but between these two and kidney, significant difference ($P<0.05$) existed. The contamination of *Salmonella* with the muscle and liver could be due to slaughtering floor, hides and knives since these parts of the carcasses were placed on the floor after slaughtering and dressing, which was observed during sample collection. The floor hides and knives are mostly contaminated with the content of gastrointestinal tract which is the reservoir for these microbes, this could have accounted

for the higher contamination of liver and muscle. The contamination of *Salmonella* in kidney may be from the knives since knives were used to divide the kidney before taking the swabs and also the kidneys were inside the dressed hot carcass whiles taking the swabs. Ed and Frans (1990), reported *Salmonella* Dublin isolates in the following percentages in liver (53%) kidneys (33%) and muscle (27%) which agree with the current study in the sense that the kidney was lower than the liver. In addition, Biswas *et al*. (2011) reported that instruments used in slaughtering and dressing animals such as knives, saws and cleavers may act as sources of contamination which agree with the current result. Adzitey *et al.* (2015a) who reported 60% of *Salmonella* spp prevalence on knives used in slaughtering cattle (beef) in Techiman Municipality. According to Anachinaba *et al*. (2015) meat (beef) sold at Bolgatanga Municipality were contaminated with *Salmonella* spp. The contamination of various meat types such as beef (Adzitey, 2015b; Adzitey *et al*. 2015; Anachinaba *et al*., 2015), guinea fowl meat (Adzitey *et al*., 2015), and pork (Anachinaba *et al.*, 2015) by *Salmonella* spp. in Ghana have been reported. In Nigeria, Adesiji *et al*. (2011) did not find *Salmonella* spp. in retail raw chicken, beef and goat meat but found out that 8% (6) of pork was positive for *Salmonella* spp.

5.6.3 Antibiotic Resistance of *Salmonella* spp Isolated from Meat (muscles, liver and kidney) of Cattle

The *Salmonella* spp. was mostly resistant to teicoplanin followed by azithromycin (30.95%). The *Salmonella* isolates were highly susceptible to (100-78.57%) chloramphenicol, ciprofloxacin, tetracycline, suphamethoxazole/trimethoprim, ceftriaxone, amoxycillin/clavulanic acid and gentamicin. This result confirmed the reason why most of

the farmers use ciprofloxacin in treating their animals. Thus 32% uses ciprofloxacin, 27% use amoxycillin/clavulanic acid and 17% use Suphamethoxazole/trimethoprim. All these antibiotics are effective as the farmers reported in this study. Danikuu (2004) reported that *Salmonella* spp. isolated from farm animals in the Kumasi Metropolis, Ghana were all resistance to tetracycline but susceptible to ciprofloxacin, which agrees with the current study. Azithromycin also shows some degree of intermediate resistance (9.52%). According to Adzitey *et al*. (2015) an intermediate resistance means that those isolates are not obviously susceptible or resistance and those isolates have the propensity to easily become resistant. Veterinary staff said that most bacterial infections that they usually treat in this area, includes those suspected to be caused by *Salmonella*. In this study, 14.3% (6/42) of the *Salmonella* spp. were resistant to two antibiotics whereas 69% were resistant to one. In this study, 16.7% (7/42) of the isolates were multidrug resistant. This result agrees with Adzitey *et al.*. (2015) who found out that some of the *Salmonella* spp. were resistant to more than two and exhibited multidrug resistance. They also found that the resistant pattern Eva was the commonest and was exhibited by 9 different *Salmonella* spp. isolated from beef in Techiman.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusion

- Ruminant farmers have inadequate knowledge about antibiotics usage especially concerning antibiotic withdrawal period.
- Some of the beef samples harbor antibiotic residues, with the greater portion found in the liver and kidney.
- The microbial loads of the sample are within the acceptable limit.
- Some of the samples were contaminated with *Salmonella* spp which few of the isolate were multidrug resistant.

6.2 Recommendations

- Ruminant farmers should be educated on the proper utilization of antimicrobials and the side effects of not observing the withdrawal periods. They should also be taught why they must rely on veterinary service for treatment of their animals especially when using antibiotics. In addition, veterinary drug usage should be regulated to prevent or reduce the level of drug residues in beef meat.
- Consumers of meat should try their best to know the sources (health status) of meat they buy
- All players in the livestock chain should be trained to follow standard hygienic practices thus personal and general hygiene necessary to improve consumers' safety. Another research should be carrying out, to find the specific antibiotic residue in the meat samples quantitatively in the study area.

REFERENCES

Abaidoo, R., and Obiri-Danso, K. (2008). *Biology 503: Environmental Microbiology. KNUST, IDL (MSC Environmental Science).*

Abavelim, D. A. (2014). *Determination of Antibiotics Residues in Beef and Mutton from Some Selected Markets in Kumasi –Ghana*. Kwame Nkrumah University of Science and Technology, Kumasi, Ghana College.

Abd El-Aty, A. M., Goudah, A., and Abo El Sooud, K. (2001). Pharmacokinetics, intramuscular bioavailability and tissue residue profiles of ceftazidime in a rabbit model‖. *Dtsch Tierarztl Wochenschr, 108* (4), 168-71.

Abdelmoaty, D. A. (2015). Antibiotic residues in beef and poultry meat, 2805–28974. https://doi.org/10.13140/RG.2.1.5018.8645.

Abebew, D. (2001). Detection and determination of Oxytetracycline and Penicillin antibiotic residue levels in bovine bulk milk from Debrezeit and Nazareth Dairy Farms. *In Analytica Chimica Acta (Addis Ababa.*, *432*, 325–346).

Aberle, E. D., Forrest, J. C., Gerrard, D. E., and Mills, E. W. (2001). *Principles of meat science (4th edition). USA: Kendall/Hunt Publishing Company.*

Abraham, E. (1987). Cephalosporins. *Drugs.*, *4* (2), 1-4.

Achtman, M. Wain, J. Weill, F. X. Nair, S. Zhou, Z. Sangal, V. Krauland, M. G. Hale, J. L. Harbottle, H. Uesbeck, A. Dougan, G. Harrison, L. H. and Brisse, S. . (2012). "Multilocus sequence typing as a replacement for serotyping in *Salmonella* enterica".Enterica, S. MLST Study Group. PLoS Pathogens. e1002776. *Journal. Ppat.1002776.*, *8*(6). https://doi.org/10.1371

ACMSF (Advisory Committee on the Microbiological safety of Food). (2005). *Measures*

to Prevent Campylobacter Contamination of Chicken Meat in Scandinavia. Second Report on Campylobacter. Accessed June 2019. http://www.food.gov.uk/multimedia/ pdfs/acmsfcampylobacter.pdf.

Addo, K. K., Mensah, G. I., Aning, K. G., Nartey, N., Nipah, G. K., Bonsu, C. Akyeh, M. L and Smits, H. L. (2011). Microbiological quality and antibiotic residues in informally marketed raw cow milk within the coastal savannah zone of Ghana. *Tropical Medicine and International Health, 16* (2), 22-32.

Adel, M., Dadar, M., and Conti, G. O. (2016). Antibiotics and Malachite Green Residues in Farmed Rainbow Trout (*Oncorhynchus mykiss*) from the Iranian Markets: A Risk Assessment. *International Journal of Food Properties*. https://doi.org/2016. DOI:10.1080/10942912.2016.1163577.

Adesiji, Y. O., Alli, O. T., Adekanle, M. A., and Jolayemi, J. B. (2011). Prevalence of *Arcobacter, Escherichia coli, Staphylococcus aureus* and *Salmonella* species in retail raw chicken, pork, beef and goat meat in Osogbo, Nigeria. *Sierra Leone Journal of Biomedical, 3*, 8-12.

Adeyemo, O. K. (2002). Unhygienic operation of a city abattoir in South Western Nigeria: Environmental Implication. *African Journal of Environmental and Applied Microbiology, 4* (1), 23-28.

Adzitey, F. (2015). Prevalence of *Escherichia coli* and *Salmonella* spp. in beef samples sold at Tamale Metropolis, Ghana. *International Journal of Meat Science, 5* (1), 8-13.

Adzitey, F., Nsoah, J. K., and Teye, G. (2015). Prevalence and antibiotic susceptibility of *Salmonella* species isolated from beef and its related samples in Techiman Municipality of Ghana. *Turkish Journal of Agriculture - Food Science and Technology, 3* (8), 644-650.

Adzitey, F., Teye, G. A., and Anachinaba, I. A. (2015). Microbial quality of fresh and smoked guinea fowl meat sold in the Bolgatanga Municipality, Ghana. Asian. *Journal of Poultry Science, 9*, 165-171.

Adzitey, F., Teye, G. A., and Dinko, M. M. (2011). Pre and post-slaughter animal handling by butchers in the Bawku Municipality of the Upper East Region of Ghana. *Livestock Research for Rural Development., 23*, 39.

Adzitey, F. (2015). Antibiotic classes and antibiotic susceptibility of bacterial isolates from selected poultry; a mini review. *World Veterinary Journal, 5* (3), 36-41.

Ahmad, M. D., Sawar, A., Najeeb, M. I., Nawaz, M., Anjum, A. A., Ali, M., and Mansur, A. (2013). Assessment of microbial load of raw meat at abattoir and retail shops. *Journal of Animal and Plant Sciences*, *23*, 745–748.

Ahmed, H. Hariharan, H. and Yason, C. (1992). Drug resistance and toxigenic properties of *Campylobacter jejuni* from broiler chickens. In *Abstract. CVMA 44th Annual Convention.* (p. 469).

Aiello, S. E., Lines, P. R., and Kehn, C. M. (2005). Anthelmintics In: The Merck Veterinary Manual. *9th Edition.USA*, 2111–2124.

Alavanja, M. C., Field, R. W., Sinha, R., Brus, C. P, and Shavers V. L. (2001). Lung cancer risk and red meat consumption among Iowa women. *Lung Cancer*, *34*, 37–46.

Alhendi, A. B., Homeida, A. A. M., and Galli, E. S. (2000). Drug Residues in Broiler Chicken Fed with Antibiotics in Ration. *Veterinarski Arhiv*, *10*, 199–205.

Ali, H. (2009). *"Clostridial species and related organisms in meat and meat products". (Meat Hygiene), Thesis, Faculty of Veterinary Medicine, Benha University* .

Alla, M. B. W., Mohamed, T. E., and Abdelgadir, A. E. (2011). Detection of antibiotics

residues in beef in Ghanawa Slaughterhouse, Khartoum State, Sudan. *African Journal of Food Science.*, *15* (10), 574–580.

Allen, N. E., and Nicas, T. I. (2003). Mechanism of action of oritavancin and relatedglycopeptide antibiotics. *FEMS Microbiology Reviews*, *26* (5), 511--532.

Aminov, R. (2010). A brief history of the antibiotic era: lessons learned and challenges for the future. *Fronter Microbiology*, *8*, 1:134.

Anachinaba, I. A., Adzitey, F., and Teye, G. A. (2015). Assessment of the microbial quality of locally produced meat (beef and pork) in Bolgatanga Municipal of Ghana. *International Journal of Food Safety*, *1*, 17.

Andrew, S. (2006). Bacterial Cell Culture Methods. *Journal of Cell Biology*, *33* (2), 267–281.

Andrews, W. (1995). Microbial methods. In Official Methods of analysis of AOAC International, 16th ed. AOAC. *International. Arlington, VA.*, 1–1199.

Angulo, F., Johnson, K., Tauxe, R., and Cohen, M. (2000). Origins and consequences of antimicrobial-resistant nontyphoidal *Salmonella*: implications for the use of fluoroquinolones in food animals. *Microbial Drug Resistance*, *6* (1), 77–83.

Aning, K. G., Donkor, E. S., Omore, A., Nurah, G. K., Osafo, E. L. K., and Staal, S. (2007). Risk of exposure to marketed milk with antimicrobial drug residues in Ghana. *The Open Food Science Journal*, *1*, 1–5.

Anon. (2002). USFDA improvises testing for chloramphenicol in seafood. *Seafood Export Journal*, *10*, (5).

AS, (Australian Standard). (2002). Meat Standards Committee Microbiological testing for process monitoring in the meat industry guideline. *Retrieved from*

Https://Www.Primesafe.Vic.Gov.Au/Uploads/Victorian%20Standards/Microbiological%20Guidelines_Meat.Pdf.

Ayla, Eyý. G., and Seza, A. (2012). Prevalence of *Escherichia coli* in retail poultry meat ground beef and beef. *Medycyna weterynaryjna, 68* (4), 237.

Babapour, A., Azami, L., and Fartashmehr, J. (2012). Overview of Antibiotic Residues in Beef and Mutton in Ardebil, North West of Iran, 19 (10), 1417–1422. https://doi.org/10.5829/idosi.wasj.2012.19.10.1802

Barnes, E. (1985). "Isolation methods for anaerobes in foods". *International Journal of Food Microbiology*, *2* (1–2), 81.

Bauer, A. W., Kirby, W. M. M., Sherris, J. C., and Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology*, *45*, 493-496.

Baynes, R. E., Dedonder, K., Kissell, L., Mzyk, D., Marmulak, T., Smith, G. Tell, L. Gehring, R. Davis, J. and Riviere, J. E. (2016). Health concerns and management of select veterinary drug residues. *Food and Chemical Toxiicology*, 88, 112-122.

Bean, N. H., Griffin, P. M., Goulding, J. S., and Ivey, C. B. (1990). Foodborne disease outbreaks, 5 years summary, 1983 -1987. *Journal of Food Protection*, *53*, 711-728.

Bell, C., and Kyriakides, A. (2002). *Salmonella*: A practical approach to the organism and its control in foods. *Blackwell Science, Oxford*.

Bell, R. G. (1997). Distribution and sources of microbial contamination on beef carcasses. *Journal of Applied Microbiology*, *88*, 292–300.

Bester, B. H., and Lombard, S. H. (1979). The effect of the dye-marking of mastitis remedies on the incidence of antibiotic residues in Pretoria's market milk supplies. *Journal*

of South African Veterinary Association 50 (3), 151-3.

Beyene, T, Kemal, A., Jibat, T., Tadese, F., Ayana, D., and Feyisa, A. (2015). Assessment on Chemicals and Drugs Residue in Dairy and Poultry Products in Bishoftu and Modjo, Central Ethiopia. *Journal of Nutrition and Food Sciences*, *13*, 3.

Beyene, T. (2016). Veterinary Drug Residues in Food-animal Products : Its Risk Factors and Potential Effects on Public Health. *Journal of Veterinary Science and Technology, 7* (1), https://doi.org/10.4172/2157-7579.1000285.

Bhandare, S. G., Sherikar, A. T., Paturkar, A. M.. S., Waskar, V. S., and Zende, R. J. (2007). A comparison of microbial contamination of sheep/goat carcasses in a modern Indian abattoir and traditional meat shops. *Food Control*, *18*, 854-858.

Bhutta, Z. (2006). Current concepts in the diagnosis and treatment of typhoid fever. *British Medical Journal, 333* (7558), 78–82.

Biopharm, *Germany*. (2016). Premi®test Microbial Inhibition Test for Broad Spectrum Screening of Food. *Available from: http://www.r-biopharm.com.* Last accessed on Feb. 2019

Birhan, A., and Mulugojjam, A. (2018). Antimicrobial residue occurrence and its public health risk of beef meat. *Veterinary World, EISSN:* 2231-0916. *Available at www.veterinaryworld.org.* 11, 902–908. *https://doi.org/*10.14202.

Biswas, A. K., Kondaiah, A. K., Anjaneyulu, A. S., and Mandal, P. K. (2011). Causes, concerns, consequences and conrol of microbial contaminats in meat. *International Journal of Meat Science*, *1*, 27-35.

Blackburn, C. D. W. (2006). "Food spoilage microorganisms." *Woodhead Publishing.*

Bonner, D. P., and Sykes, R. B. (1984). Structure activity relationships among the

monobactams. *Journal of Antimicrobial Chemotherapy*, *14*, 313-327.

Borch, E., Nesbakken, T., and Christenes, H. (1996). Hazard identification in swine slaughter with respect to foodborne bacteria. *International Journal of Food Microbiology, 30*, 9-25.

Boundless.(2016). Antibiotic Classifications. Boundless microbiology. https://www.boundless.com/microbiology/textbooks/boundless-microbiology-textbook/antimicrobial-drugs-13/overview-of-antimicrobial-therapy-153/antibiotic-classifications-775-4905/.

Bozdogan, B., and Appelbaum, P. C. (2004). Oxazolidinones: activity, mode of action, and mechanism of resistance. *Ineternational Journal of Antimicrobiall Agents*, *25* (5), 113--119.

Brenner, F., Villar, R., Angulo, F., Tauxe, R., and Swaminthan, B. (2000). *Salmonella* Nomenclature. *Journal of Clinical Microbiology*. 38, 2465-2467.

Brink, A. J., Feldman, C., Grolman, D. C., Muckart, D., Pretorius, J., Richards, G. A., and Sieling, W. (2004). Appropriate use of the carbapenems. *South African Medical Journal*, *94* (10), 857--861.

Brouard, C., Espie, E., Weill, F. X., Kerouanton, A., Brisabois, A., Orgue, A. M., Vaillant, V. and de Valk, H. (2007). Two consecutive large outbreaks of *Salmonella* enterica serotype Agona infections in infants linked to the consumption of powdered infant formula. *Pediatric Infectious Disease Journal*, *26*, 148–152.

Brown, A. G., Butterworth, D., Cole, M., Hanscomb, G., Hood, J. D., Reading, C., and Rolinson, G. N. (1976). Naturally-occurring beta-lactamase inhibitors with antibacterial activity. *Journal of Antibiotics (Tokyo)*, 29 (6), 668--669.

Buket, E., Fatma, Kaynak Onurdağ Burak, D., Selda, Ö. Ö., Aysel, B. Ö., and Ufuk, A. (2011). Screening of quinolone antibiotic residues in chicken meat and beef sold in the markets of Ankara, Turkey. *Poultry Science*, 92, 2212–2215.

https://doi.org/ht.org/ 10.3382/ps.2013-03072

Buncic, S. (2006). Integrated Food Safety and Veterinary Public Health. Cromwell Press, Trowbridge, Wiltshire, UK. Devore, J. L., and R. Peck. 1996. Statistics: The Exploration and Analysis of Data. *Brooks/Cole Publishing Company, London, UK.*

Burrows, D. W., and Renner, E. S. (1999). Biological Warfare Agents as Threats to Potable Water. *Environmental Health Perspectives*, *107* (12), 975–984.

Butaye, P., Devriese, L. A., and Haesebrouck, F. (2001). Differences in antibacterial resistance patterns of Enterococcus faecalis and Enterococcus faecium strains isolated from farm and pet animals. Antimicrobial *Agents Chemotherapy.*, *45*, 374-378.

Butterworth, D., Cole, M., Hanscomb, G., and Rolinson, G. N. (1979). Olivanic acids, a family of beta-lactam antibiotics with beta-lactamase inhibitory properties produced by *Streptomyces* species. Detection, properties and fermentation studies. *Journal of Antibiotics (Tokyo).*, 32, 287–294.

CAC. (2001). Codex Alimentarius Commission. Committee on residues of veterinary drugs in foods, document control of veterinary drug residues in milk and milk products. Joint Food and Agriculture Organization of the United Nations /World Health Organization Food Standa.

CAC. (2011). Codex Alimentarius Commission (2011): Maximum residue limits for veterinary drugs in food. In *CCAC Rome*, (p. 36.).

CAC, (Codex Alimentarius Commission). (2005). Code of hygienic practice for meat.

CAC/RCP.

Calderon, C. B., and Sabundayo, B. P. (2007). Antimicrobial classifications: Drugs for bugs. In: Schwalbe R, Steele-Moore L and Goodwin AC (eds). Antimicrobial susceptibility testing protocols. *CRC Press, Taylor and Frances Group.*, ISBN 978--0--8247--4100--6.

Card, R. (2009). Microarrays-closing the gap between research and diagnostic tools. *Microbiologist*, 10, 30–33.

Carlet, J., Jarlier, V., Harbarth, S., Voss, A., Goossens, H., and Didier Pittet, D. (2012). Ready for a world without antibiotics? The Pensières Antibiotic Resistance Call to Action. *Antimicrobial Resistance and Infection Contol*, 1, 11.

Carrie, R., Daniel, A. J., Cross, C. K., and Rashmi Sinha. (2011). Trends in meat consumption in the United States. Public Health Nutrition. *National Institute of Health Journal*, 575–583.

Carroll, I. D., and Williams, D. C. (2008). Pre-travel vaccination and medical prophylaxis in the pregnant traveler.*Travel Medicine and Infectious Disease*, *6*, 259-275.

Cars, O., Hogberg, L. D., Murray, M., Nordberg, O., Sivaraman, S., Lundborg, C. S., So, A. D., and… Tomson, G. (2008). Meeting the challenge of antibiotic resistance. *British Medical Journal*, *337*, 14--38.

Casalinuovo, F., Ciambrone, L., Cacia, A., and Rippa, P. (2016). Contamination of bovine , sheep and goat meat with Brucella spp . *Italian Journal of Food Safety*, *5*, 5913. https://doi.org/10.4081/ijfs.2016.5913

CDC. (2005). Food borne illness report;, 1–13.

CDC. (Centre for Disease Control). (2012). Summary of notifiable diseases. United States, 2010. *Morbidity and Mortality Weekly Report*, *59* (53), 1–111.

CDC (Center for Disease Control and Prevention). (2014) . An Atlas of *Salmonella* in the United States, 1968-2011.

Chopra, I., and Roberts, M. (2001). Tetracycline antibiotics: Mode of action, application, molecular biology, and epidemiology of bacterial resistance. *Microbiology reviews 65* (2), 232–260.

Chowdhury, R., Haque, M. N., Islam, K. M. S., and Khaleduzzaman, A. B. M. (2009). A review on Antibiotics in an Animal Feed. . *Bangladesh Journal of Animal Science*, *38*, 22–32.

Christenson, J. C. (2013). *Salmonella* Infections. *Pediatrics in Review*, *34* (9), 375-383.

Church, P. N., and Wood, J. M. (1992). The manual of manufacturing meat quality. Compiled at the Leatherhead Food Research Association. *London: Elsevier Applied Science*.

CLSI. (2008). Clinical and Laboratory Standards Institute Development of in vitro susceptibility testing criteria and quality control parameters; approved guideline, *CLSI document M23-A , 20083rd ed.Wayne, PAClinical and Laboratory Standards Institute*.

Codex. (2015). Standards Alimentarius International Food Maximum Residue Limits and Risk Management Recommendation for Residue of Veterinary Drugs in Foods. *In 38th Secession, 3-39. Available from: http://www.codex alimentarius. org. Last accessed on 11-09-2016.*

Cross, A. J., Leitzmann, M. F., and Gail, M. H. (2007). A prospective study of red and processed meat intake in relation to cancer risk. *PLOS Medicine.*, *4*, 325.

Cruickshank, J. K., and Humphrey, T. J. (1987). The carrier food handler and Non-typhoidal Salmonellosis. *Epidemiology and Infection*, *98*, 223–230.

Crum-Cianflone, N. (2008). Salmonellosis and the gastrointestinal tract: more than just peanut butter. *Current Gastroenterology Report, 10*, 424–431.

Crump, J. A., Luby, S. P., and Mintz, E. D. (2004). The global burden of typhoid fever. *Bull World Health Organization, 82*, 346-353.

CSE, (Centre for Science and Environment Study). (2014). Antibiotics in chicken meat *(36 p.). New Delhi: Pollution Monitoring Laboratory*.

Dachet, F. Prouzet-Mauléon, V. Oleastro, M. Mégraud, F., and Ménard, A. (2004). Identification par PCR en temps réel et FRET des Campylobacters les plus fréquents. *In: 6th National Congress of the French Society of Microbiology, Bordeaux, No. 444.* (pp. 10–12).

Danikuu, M. (2004). Isolation and serotyping of *Salmonella* from slaughtered food animals in the Kumasi Metropolis. *Available at: http://ir.knust.edu.gh/handle/123456789/1936 [Accessed Feb. 2019].*

Darby, J.,and Sheorey, H. (2008). Searching for *Salmonella*. Australian Family Physician, *37* (10), 806–810.

Darton, T., Blohmke, C., and Pollard, A. (2014). Typhoid epidemiology, diagnostics and the human challenge model. *Curr Opin Gastroenterol., 30*, 7–17.

Darwish, W. S., Eldaly, E. A., El-Abbasy, M. T., Ikenaka, Y., and Nakayama, S. (2013). Antibiotic residues in food: The African scenario. *Japanese Journal of Veterinary Research, , 61*, 13-22.

Debeuckelaere, W., Okerman, L., and Van Hoof, J. (1998). Evaluation of the European four plate test as a tool for screening antibiotic residues in meat samples from retail outlets. *Journal -Association of Official Analytical Chemist, 81*, 51-56.

Delhalle, L., Saegerman, C., Farnir, F., Korsak, N., Maesd, D., Messens, W., and… Daube, G. (2009). "*Salmonella* surveillance and control at post-harvest in the Belgian pork meat chain". *Food Microbiology, 26*, 265–271.

Deming, M. S. Tauxe, R.V. Blake, P. A. Dixon, S. E. F., and B. S. Jones, T. S. Lockamy, E. A. Patton, C. M.Sikes, R. (1987). Campylobacter enteritis at a university: transmission from eating chicken and from cats. *American Journal of Epidemiology*, *126*, 526–534.

Deneo-Pellegrini, H., De, S. E., Ronco, A., Mendilaharsu, M., and Carzoglio, J. Meat consumption and risk of lung cancer; a case-control study from Uruguay. Lung Cancer (1996).

Dewdney, J. M., Maes, L., Raynaud, J. P., Blanc, F., Scheid, J. P., and Jackson, T. (1991). Risk assessment of antibacterial residues of $β$-lactams and macrolides in food products with regard to their immuno allergic potential. *Food Chemotherapy Toxicology.*, *29*, 477–483.

Diarra, M. S., and Malouin, F. (2014). Antibiotics in Canadian Poultry Productions and Anticipated Alternatives. *Frontiers Microbiology*, *5*, 1–15.

Diaz-Aparicio, E. (2013). Epidemiology of brucellosis in domestic animals caused by Brucella melitensis, Brucella suis and Brucella abortus. *Revue Scientifique et Technique, 32*, 53-60.

DOH. (2011). nontyphoidal Salmonella"[Online].Retrieved on 14th of October,2012. Retrievedfrom:http://www.doh.wa.gov/Portals/1/Documents/5100/420-035-Guidelin Salmonellosis.pdf).

Domagala, J. M. (1994). Structure-activity and structure-side-effect relationships for the quinolone antibacterials. *Journal of Antimicrobial Chemotherapy*, *33*, 685–706.

Donkor, E., Newman, M. J., Tay, S. C. K., Dayie, N. T. K. D., Bannerman, E., and Olu-Taiwo., M. (2011). Investigation into the risk of exposure to antibiotic residues contaminating meat and egg in Ghana. *Food Control*, *22*, 869-873.

Doyle, MP. Buchanan, R. (ed). (2013). Food microbiology: fundamentals and frontiers, 4th ed. *ASM Press, WashingSton, DC.* https://doi.org/https://doi.org/ 10.1128/9781555818463.

Doyle, M. E. (2006). Veterinary Drug Residues in Processed Meats-Potential Health Risk. FRI Briefings; Food Research Institute, University of Visconsin: Madison, WI, USA, pp. 148–196.

Doyle, M. P. (2007). Microbial Food Spoilage -Losses and Control Strategies, (A Brief Review of the Literature), Fri Briefings (www.wisc.edu/fri/).

Draisci, R., delli Quadri, F., Achene, L., Volpe, G., Palleschi, L., and Palleschi, G. (2001). A new electrochemical enzyme-linked immunosorbent assay for the screening of macrolide antibiotic residues in bovine meat. *Analyst*, *126*, 1942–1946.

EC, (European Commission). (2010). Commission Regulation (EU) No 37/2010 of 22 December 2009 on Pharmacologically Active Substances and their Classification Regarding Maximum Residue Limits in Foodstuffs of Animal Origin. *Official Journal of the European Union*, *15*, 1-72.

EC, (European Commission). (2002). Food law regulations No. 178/2002 *Journal of European Communities*, *221*, 8–36. *Retrieved from Https://Www.Fsai.Ie/UploadedFiles/Legislation/Food_Legisation_Links/General_ Principles_of_Food_Law/Reg178_2002.Pdf.*

Ed, G. M. van K., and Frans, J. M. S. (1990). A comparison of different enrichment media

for the isolation of *Salmonella* dublin from livers , kidneys and muscles of *Salmonella*-positive veal calves. *International Journal of Food Microbiology, 10*, 177–182.

EFSA. (2016). European Food Safety Authority and European Centre for Disease Prevention and Control (ECDC). The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2015. *EFSA Journal, 14*, 4634–4865.

EFSA, (European Food Safety Authority). (2012). The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2010. *Journal of EFSA, 10* (3).

Ekdahl, K., De Jong, B., Wollin, R., and Andersson, Y. (2005). Travel-associated non-typhoidal salmonellosis: geographical and seasonal differences and serotype distribution. *Clinical Microbiology and Infection, 11,138-144*

https://doi.org/10.1111/j.1469-0691.2004.01045.x.

Ekuttan, C. E., Kang'ethe, E. K., Kimani, V. N., and Randolph, T. F. (2007). Investigation on the prevalence of antimicrobial residues in milk obtained from urban small holder dairy and non dairy farming households in Dagoretti Division, Nairobi, Kenya. *East African Medical Journal, 84* (11), 87 91.

El-melegy, A. (2015). "Microbial status of meat and chicken received to university student hostel". M. V. Sc. Thesis (Meat hygiene), Faculty of Veterinary Medicine, Benha University.

Eman, M. (2007). "Enterotoxigincity and typing of C. Perfringens isolated from poultry products in Egypt". *Journal of Applied Sciences Research, 12* (3), 1804–1808.

Etebu, E., and Arikekpar, I. (2016). Antibiotics : Classification and mechanisms of action

with emphasis on molecular perspectives. *International Journal of Applied Microbiology and Biotechnology Research*, *4*, 90–101.

Retrieved from www.bluepenjournals.org/ijambr

Eyssen, H. J., Van den Bosch, J. F., Janssen, G. A., and Vanderhaeghe, H. (1971). Specific inhibition of cholesterol absorption by sulfaguanidine. *Atherosclerosis.*, *14* (2), 181--192.

Ezenduka, E. V., Oboegbulem, S. I., Nwanta, J. A., and Onunkwo, J. I. (2011). Prevalence of antimicrobial residues in raw table eggs from farms and retail outlets in Enugu State, Nigeria. *Tropical Animal Health and Production*, *43* (3), 557-9.

Fahim, A. S., Islam, M. O., Enas, A. K., and Amira, K. A.-A. (2017). Isolation of Clostridium perfringens from Meat Samples Obtained from the University Students' Hostel. *Ec Nutrition*, *3*.

FAO/WHO, (Food and Agriculture Organization/ World Health Organization). (1986). *Sixth report of the joint FAO/WHO Expert Committee on brucellosis. World Health Organization, Geneva, Switzerland.*

FAO. (1985). Agriculture and consumer protection paper 53. Slaughterhouse cleaning and sanitation.

FAO. (2002). Evaluation of certain veterinary drug residues in food: fifty eighth report of the Joint FAO/WHO Expert Committee on Food Additives, WHO technical report series, No. 911. *FAO, Rome.*

FAO. (2003). "Assuring food safety and quality: guidelines for strengthening national food control systems." *FAO Food and Nutrition Paper*, *76*, ,0254-4725.

Feasey, N. A., Archer, B. N., and Heyderman, R. S. (2010). Typhoid fever and invasive nontyphoid salmonellosis, Malawi and South Africa.Eerging infectious Diseases, *16*,

1448–1451.

Feasey, N., Dougan, G., Kingsley, R., Heyderman, R., and Gordon, M. (2012). Invasive non-typhoidal salmonella disease: an emerging and neglected tropical disease in Africa. *Lancet, 10*, 2489-2499. https://doi.org/10.1016/S0140-6736 (11)61752-2.

Feglo, P., Frimpong, E., and Essel-Ahun, M. (2004). Salmonellae carrier status of food vendors in Kumasi, Ghana. *East African Medical Journal, 81*, 358-361.

Filazi, A., Sireli, U. T., Pehlivanlar-Onen, S., Cadirci, O., and Aksoy, A. (2013). Comparative Pharmacokinetics of Gentamicin in Laying Hens. Kafkas University. *Veterinar Fakultesi Dergisi*, *19* (3), 495–498.

Food and Drug Administration, (FDA). (2012). Bad bug book: Foodborne pathogenic microorganisms and natural toxins handbook, 2nd ed. US Food and Drug Administration, *Silver Spring,*

Food Standard Agency, U. K. (2004). Regulatory Guidance and Best Practice Advice for Food Business Operators. Food Handlers*: Fitness to work*.

Forsythe, S. J. (2000). The microbiology of safe food. Oxford: *Blackwell Science*.

Foster, G., Osterman, B., Godfroid, J., Jacques, I., and Cloeckaert, A. (2007). *Brucella ceti* sp. nov and *Brucella pinnipedialis* sp. nov. for Brucella strains with cetaceans and seals as their preferred hosts. *International Journal of Systematic and Evolutionary Microbiology, 57*, 2688–2693.

Frank, U., and Tacconelli, E. (2012). The Daschner Guide to In-Hopsital Antibiotic Therapy. European standards. *Available Online at: Http://Www.Springer.Com/978-3-642-18401-7. 300p.*

Franklin, A. M., Aga, D. S., Cytryn, E., Durso, L. M., McLain, J. E., Pruden, A., Roberts, M. C, Rothrock, M. J., Snow, D. D., Watson, J.E., and Dungan, R. S. (2016). Antibiotics in agroecosystems: introduction to the special section. *Journal of Environmental Quality, 45*, 377–393. https://doi.org/https://doi.org/10.2134/jeq2016.01.0023.

Fratamico, P. M., Bhunia, A. K., and Smith, J. L. (2005). Foodborne pathogens in Microbiology and Molecular Biology. *Caister Academic Press, Wymondham Norfolk, UK.*, 270–275.

Friedman, C.R. Neimann, J. Wegener, H.C. and Tauxe, R. V. (2000). Epidemiology of Campylobacter jejuni infections in the United States and other industrialized countries. In: Nachamkin I., Blaser M.J. (eds.): Campylobacter. 2nd ed. *ASM Press, Washington, D.C., U.S.A.*

FSANZ. (2002). Food Standards Code. *Canberra Information Australia, 2*.

Fuoco, D. (2012). Classification framework and chemical biology of tetracycline-structure-based drugs. *Antibiotics.*, 1, 1–13.

Garrard, J. (2013). Health sciences literature review made easy. *Journal of Nutrition and Health Sciences.*, *24*, 115–128.

Gebre, B. A. (2012). Qualitative screening of antibiotic residues and identification of antibiotic resistant salmonella from raw and ready to eat meat in Thailand. *International Journal of Advanced Life Sciences, 5* (1), 51–64.

GHS, (Ghana Health Service). (2011). Annual Report: Surveillance and control of priority diseases. Accra Ghana.

Giannella, R. A. (2002). Salmonella. In Baron, S.(ed.). *Medical Microbiology*, 4th ed.

Gilbert, D. (2000). Aminoglycosides. In: Mandell G. L., Bennett J. E. and Dolin R, (eds.)

Mandell, Douglas, and Bennett's principles and practice of infectious diseases. 5th ed. Philadelphia*: Churchill Livingstone.*

Gill, C. O., Badoni, M., and McGinnis, J. C. (1999). Assessment of the adequacy of cleaning of equipment used for breaking beef carcasses. *International Journal of Food Microbiology, 46*, 1–8.

Gill, C. O., Bryant, J., and Brereton, D. A. (2000). Microbiological conditions of sheep carcasses from conventional or inverted dressing processes. *Journal of Food Protection.*, *63* (9), 1291-1294.

Goetting, V., Lee, K. A., and Tell, L. A. (2011). Pharmacokinetics of Veterinary Drugs in Laying Hens and Residues in Eggs: A Review of the Literature. *Journal of Veterinary Pharmacology and Therapeutics*, *34*, 521–556.

Gómez-Aldapa, C. A., Torres-Vitela, M. R., Villarruel-López, A., and Castro-Rosas, J. (2012). The Role of Foods in Salmonella Infections. *Research Gate*.

https://doi.org/10.5772/28316

Goodman, M., Hankin, J., Wilkens, L., and Kolonel, L. (1992). High-fat foods and the risk of lung cancer. *Epidemiology*, *3*, 288–299.

Gorbach, S. (1993). Perturbation of intestinal microbiota. *Veterinary Human Toxicology.*, *35*, 15–23.

Gordon-Davis, L. (1998). The hospitality industry handbook on hygiene and safety for South African students and practitioners. *Kenwyn: Juta*.

Goudah, A., Sher Shah, S., Shin, H. C., Shim, J. H., and Abd El-Aty, A. M. (2007). Pharmacokinetics and mammary residual depletion of erythromycin in healthy lactating ewes. *Journal of veterinary medicine, Physiology, Pathology, clinical medicine,* 54 (10),

607-11

Gracey, J. . (1986). Meat Hygiene. 8th Edn., The English long Book Sic. And Baillier, Tindall, London.

Gracias, K. S., and Mckillip, J. L. (2004). Review / Synthèse. A review of conventional detection and enumeration methods for pathogenic bacteria in food. *Canadian Journal of Microbiology*, (June 2014). https://doi.org/10.1139/w04-080

Gravani, R. B. (1987). The cause and costs of foodborne diseases. *Dairy Food Saint*, *7*, 20-25.

Green, L., Selman, C., Banerjee, A., Marcus, R., Medus, R., Angulo, F., and Buchanan, S. (2005). Food service workers self reported food preparation practices: an EHS – Net study. *International Journal of Hygiene Environmental Health*, *67*, 27–35.

GSS. (2014). 2010 Population and Housing Census.

Guest, G. B., and Paige, J. C. (1991). The magnitude of the tissue residue problem with regard to consumer needs. Journal of the American Veterinary Medical Association, 198, 805–808.

Guthrie, R. (1991). Taxonomy and Grouping of the Salmonella, pp. *Chemical Rubber Company*, 23–40.

Haeghebaert, S., Sulem, P., Deroudille, L., Vanneroy-Adenot, E., Bagnis, O., Bouvet, P., Grimont, F., Brisabois, A., Le Querrec, F., Hervy, C., Espie, E., de Valk, H., and Vailant, V. (2003). Two outbreaks of Salmonella Enteritidis phage type 8 linked to the consumption of Cantal cheese made with raw milk, France, 2001. *Eurosurveillance*, *8*, 151–156.

Harbarth, S., and Samore, M. H. (2005). Antimicrobial resistance determinants and future control. Emerging Infectious Diseases, 11, 794–801.

Hassan Ali, N., Farooqui, A., Khan, A., Yahya Khan, A., and Kazmi, S. (2010). Microbial contamination of raw meat and its environment in retail shops in Karachi, Pakistan. *Journal of Infection in Developing Countries*, *4* (6), 382–388.

Hayes, P. R. (1985). Food microbiology and hygiene. London: *Elsevier Applied Science Publishers*.

Heesemann, J. (1993). Mechanisms of resistance to beta-lactam antibiotics. *Infection.*, *21* (1), S4--9.

Hendriksen, R. S. (2010). Global epidemiology of non-typhoidal Salmonella infections in humans. Kgs. Lyngby, Denmark: Technical University of Denmark (DTU)

Henry, R. J. (1943). The mode of action of sulfonamides. *Bacteriological Reviews*, 7 (4), 175--262.

Hohmann, E. L. (2001). Nontyphoidal salmonellosis. Clinical Infectious Diseases, *32*, 263–269. Retrieved from http://www.rapidmicrobiology.com/test-method/salmonella-detection-and-identification-methods. Accessed on 15th May, 2016.

Huda, N., Shen, Y. H., Huey, Y. L., Ahmad, R., and Mardiah, A. (2010). Evaluation of Physico-Chemical Properties of Malaysian Commercial Beef Meatballs. *American Journal of Food Technology*, *5* (1), 13–21.

Hur, J., Jawale, C., and Lee, J. H. (2011). Antimicrobial resistance of *Salmonella* isolated from food animals: A review: *Food Research International*, *45* (2), 819 – 830.

Hussein, A. Z., and Farrag, I. (1981). "Incidence of clostridium perfringens in beef". *Journal of the Egyptian Veterinary Medical Association*, *41*, 57.

Ibrahim, A. I., Junaidu, A. U., and Garba, M. K. (2010). Multiple antibiotic residues in meat from slaughtered cattle in Nigeria. *Internet Journal of Veterinary Medicine*, 8: 1-DOI: 10.5580/1fcd.

ICMSF. (1996). Salmonellae. Ch 14 In: Microorganisms in food 5: Microbiological specifications of food pathogens. *Blackie Academic and Professional, London,* 217–264.

IFT, (Institute of Food Technologists). (2004). Scientific Status Summary of Bacteria.

Indiana State Department of Health. (2009). Retrieved from http://www.state.in.us/isdh/files/-diseases.pdf.

Indiana State Department of Health. (2009). http://www.state.in.us/isdh/files/-diseases.pdf. Retrieved from http://www.state.in.us/isdh/files/-diseases.pdf.

James, V., Carroll, K. C., Funke, G., Jorgensen, J. H., and Marie Louise Landry, D. W. W. (2011). *In Manual of Clinical Microbiology. Vol 1. 10th edition, Edited by J Versalovic. Washington DC, ASM Press,* (Vol. 1).

Jay, S., Davos, D., Dundas, M., Frankish, E., and Lightfoot, D. (2003). *Salmonella*. In Hocking, A.D. (Ed.) Foodborne Microorganisms of Public Health Significance. *Australian Institute of Food Science and Technology, Waterloo.*, 207–266.

Jing, T., Gaol, X. D., Wang, P., Wang, Y., Lin, Y. F., and Hu, X. Z. (2009). Determination of trace tetracycline antibacterials in foodstuffs by liquid chromatography tandem mass spectrometry coupled with selective molecular-imprinted solid-phase extraction. *Analytical and Bioanalytical Chemistry., 393*.

Joseph, A. O., and Carlos, G. L.-V. (2012). Detection Methods for Food and Food Ingredients, *Salmonella* - A Dangerous Foodborne Pathogen, Dr. Dr. Barakat S M Mahmoud (Ed.), (ISBN: 978-953-307-782-6,).

Kahne, D., Leimkuhler, C., Lu, W., and Walsh, C. (2005). Glycopeptide and lipoglycopeptide antibiotics. *Chemical Reviews* 105 (2), 425–448.

Kanangire, O. (2013). Laboratory validation of low-cost methods for the measurement of E.coli and total coliforms. *Unesco-IHE [in Undetermined]*.

Kanarat, S. Sriharach, S. and Vecharangsan, P. (2004). Surveillance of Campylobacter jejuni and Campylo-bacter coli contamination in chicken rearing industry. In The 19th Livestock Conference. *Chalermprakiat Museum, Prathumthani, Thailand. [CD-ROM]*.

Kang, H.-K., and Park, Y. (2015). Glycopeptide antibiotics: Structure and mechanism of action. *Journal of Bacteriology and Virology*, *45* (2), 67--78.

Kanny, G., Puygrenier, J., Beaudoin, E., and Moneret-Vautrin, D. A. (1994). Choc anaphylactique alimentaire: implication des résidus de pénicilline. Journal of Allergy and Clinical Immunology, *26*, 181-183.

Kariuki, S., Revathi, G., Muyodi, J., Mwituria, J., Munyalo, A., Kagendo, D., Murungi, L. and Hart, C. A. (2005). Increasing prevalence of multidrug-resistant non-typhoidal salmonellae, Kenya, 1994–2003. *International Journal of Antimicrobial Agents*, *25*, 39–45.

Khattab, W. O., Elderea, H. B., Salem, E. G., and Gomaa, N. F. (2010). Transmission of Administered Amoxicillin Drug Residues from Laying Chicken to their Commercial Eggs. *Egyptian Public Health Association.*, *85* (5–6), 297-316.

Koffi-Nevry, R., Koussemon, M., and Coulibaly, S. O. (2011). Bacteriological quality of beef offered for retail sale in Cote d'ivoire. *American Journal of Food Technology.*, *6* (9), 835–842.

Kohanski, M. A., Dwyer, D. J., and Collins, J. J. (2010). How Antibiotics Kill Bacteria:

From Targets to Networks. *Nature Reviews Microbiology*, *6* (8), 423–435.

Kolaczek, A., Fusiarz, I., Lawecka, J., and Branowska, D. (2014). Biological Activity and Synthesis of Sulfonamide Derivatives: A Brief Review. *Chemik*, *68* (7), 620–628.

Komba, E. V. G., Mkupasi, E. M., A. O., M., Mshamu, S., Luwumbra, D., Busagwe, Z., and Mzula, A. (2012). Sanitary practices and occurrence of zoonotic conditions in cattle at slaughter in Morogoro Municipality, Tanzania: Implications for Public Health. *Tanzania Journal of Health Research*,14 (2)

https://doi.org/Ttp://Dx.Doi.Org/10.4314/Thrb.V14i2.6

Kozarova, L., Mate, D., and Cabadaj, R. (2001). light inactivated antibiotic. Journal of Medicinal Chemistry, 43 (2): 128-132. Strbske Pleso, High Tatras, SR., 159-163.

Kropp, H., Kahan, J. S., Kahan, F. M., Sandolf, J., Darland, G., and Birnbaum, J. (1976). Abstract on 16th Interscientific conference on antimicrobial agents and chemotherapy. In *American Society for Microbiology*, Abstract (p. 228).

Kurwijila, L. R., Omore, A., Staal, S., and Mdoe, N. S. Y. (2006). Investigation of the risk of exposure to antimicrobial residues present in marketed milk in Tanzania. *Journal of Food Protection*, *69* (10), 2487-2492.

Kuter, D. J., and Tillotson, G. S. (2001). Hematologic effects of antimicrobials: focus on the oxazolidinone linezolid. *Pharmacotherapy.*, *21*, 1010-1013.

Labi, A. K., Noah, O. N., Naa, O. A., and Eric, S. D. (2014). *Salmonella* blood stream infections in a tertiary care setting in Ghana. *BMC Infectious Diseases*, *14*, 3857. https://doi.org/DOI 10.1186/s12879-014-0697-7

Lampel, K., and Maurelli, A. (2003). *Shigella species. Ch 11 In: Miliotis MD, Bier JW (eds) International handbook of foodborne pathogens. Marcel Dekker, New York, p.*

Landers, T. F., Cohen, B., Wittum, T. E., and Larson, E. L. (2012). A review of antibiotic use in food animals: perspective, policy, and potential. *Public Health. Reports.*, 127: 4-22.

Larsson, S. C., and Orsini, N. (2013). Systematic Reviews and Meta- and Pooled Analyses: Red Meat and Processed Meat Consumption and All-Cause Mortality, 179 (3), 282–289. https://doi.org/10.1093/aje/kwt261

Lawal, J. R., Jajere, S. M., Geidam, Y. A., Bello, A. M., Wakil, Y., and Mustapha, M. (2015). Antibiotic Residues in Edible Poultry Tissues and Products in Nigeria: A Potential Public Health Hazard. International. *Journal of Animal and Veterinary Advances*, 7 (3), 55–61.

Lawrie, R. A., and Ledward, A. D. (2006). Lawrie"s meat science (7th Ed.). *Cambridge: Woodhead Publishing Limited. ISBN*, 978-1-84569-159–2.

Lee, H. J., Lee, M. H., and Ruy, P. D. (2001). Public health risks: chemical and antibiotic residues. Asian Australasian Journal of Animal Sciences, 14, 402–413.

Levine, M., Kotloff, K., Barry, E., Pasetti, M., and Sztein, M. (2007). Clinical trials of Shigella vaccines: Two steps forward and one step back on a long, hard road. *Nature Reviews Microbiology*, *5*, 540–553.

Lightfoot, D. (2003). *Shigella. Ch 17 In: Hocking AD (ed) Foodborne microorganisms of public health significance. 6th ed, Australian Institute of Food Science and Technology (NSW Branch),. Sydney*.

Lim, E., Lopez, L., Borman, A., Cressey, P., and Pirie, R. (2012). Annual report concerning foodborne disease in New Zealand 2011. *Ministry for Primary Industry, New Zealand.Http://Www.Foodsafety.Govt.Nz/Science-Risk/Human-Health-Surveillance/Foodborne-Disease-Annual-Reports.Htm. Accessed 11 April 2013*.

Livermore, D. M., Warner, M., Mushtaq, S., Doumith, M., Zhang, J., and Woodford, N. (2011). What remains against carbapenem-resistant Enterobacteriaceae? Evaluation of chloramphenicol, ciprofloxacin, colistin, fosfomycin, minocycline, nitrofurantoin, temocillin and tigecycline. *International Journal of Antimicrobial. Agents*, *37*, 415–419.

Mahajan, G. B., and Balachandran, L. (2012). Antibacterial agents from actinomycetes - a review. Frontiers in Bioscience (Elite Ed)., 4, 240–253.

Malik, G. (1997). A clinical study of brucellosis in adults in the Asir region of southern Saudi Arabia. *American Journal of Tropical Medicine and Hygiene*, *56*, 375–377.

Mangsi, A. S., Khaskheli, M., Soomro, A., Muhammad, H., and Shah, G. (2014). Antibiotic Residues Detection in Raw Beef Meat Sold for Human Consumption in Sindh,. *Pakistan International Journal of Research in Applied, Natural and Social Sciences*, *2* (7), 15–20.

Mantur, BG. and Amarnath, S. (2008). Brucellosis in India: a review. *Journal of Biosciences*, *33*, 539–547.

Maripandi, A., and Al-Salamah, A. (2010). Multiple antibiotic resistance and plasmid profiles of S. enteritidis isolated fromretail chicken meeats. *American Journal of Food Science and Technology*, *5*, 260-268.

Marks, F., Adu-Sarkodie, Y., Hunger, F., Sarpong, N., Ekuban, S., Agyekum, A., and… May, J. (2010). Typhoid fever among children, Ghana. *Emerging Infectious Diseases*, *16*, 1796–1797.

Marshall, B. M., and Levy, S. B. (2011). Food Animals and Antimicrobials: Impacts on Human Health. *Clinical Microbiology Reviews*, *24* (4), 718–733.

McArdle, J. (2000). Humans are omnivorous. *Vegetarian Resource Group. Retrieved October 6, 2013*.

McCaughey, W. J., Elliott, C. T., and Crooks, S. R. H. (1990). Carry-over of sulphadimidine in the faeces and urine of pigs fed medicated feed. Veterinary Record, *126*, 351–354.

McClane, BA. Uzal, FA. Miyakawa, MEF. Lyerly, D. and Wilkins, T. (2006). The enterotoxic clostridia. In Dworkin M, Falkow S, Rosenberg E, Schleifer K-H, and Stackebrandt E (ed), The prokaryotes, 3rd ed, vol 4. *Springer, New York, NY, 4*, 698–752.

McDonald, L. E. (1998). Veterinary pharmacology and therapeutics, 6th edition. *Iowa state University press.* Ames, *USA.*

McEvoy, J. D. G. (2002). Contamination of animal feeding stuffs as a cause of residues in food: a review of regulatory aspects, incidence and control. *Analytica Chemica Acta*, 473: 3--26.

McGeer, A., Fleming, C. A., Gree, K., and Low, D. E. (2001). Antimicrobial resistance in Ontario: Are we making progress? *Laboratory Proficiency Testing Program Newsletter*, 293:1--2.

MDH, (Minnesota Department of Health). (2013). Fact sheet. Causes and symptoms of Staphylococcus aureus. Revised February, (2013).

Mead, P. S., Slutsker, L., Dietz, V., McCaig, L. F., Bresee, J. S., Shapiro, C., Griffin, P. M. and Tauxe, R. V. (1999). Food-related illness and death in the United States. *Emerging Infectious Diseases*, *5*, 607–625.

Meerburg, B., and Kijlstra, A. (2007). Role of rodents transmission of Salmonella and Campylobacter". *Journal of The Science of Food and Agriculture, 87*, 2774–2781.

Mensah, P., Owusu-Darko, K., Yeboah-Manu, D., Ablordey, A., Nkrumah, F., and Kamiya, H. (1997). The role of street food vendors in the transmission of enteric pathogens

in Accra. *Ghana Medical Journal*, 33,19-29.

MNT, (Medical News Today). (2015). Antibiotics: How do antibiotics work? MediLexicon International Ltd. Bexhill-on-Sea UK.

Moellering, R. C. (2003). Linezolid: The first oxazolidinone antimicrobial. Annals of Internal Medicine, 138, 135--142.

Mohanty, S., Renuka, K., Sood, S., Das, B., and Kapil, A. (2006). Antibiogram pattern and seasonality of Salmonella serotypes in a North Indian tertiary care hospital. *Epidemiology and Infection*, 134,961-966.

https://doi.org/10.1017/S0950268805005844.

Molbak, K. (2004). Spead of resistant bacteria and resistance antibiotic genes from animals to humans concrn. *Journal of Veterinary Medicine, B 51*, 364–369.

Molbak, K., Olsen, J., and Wegener, H. (2006). Salmonella Infections. In H. Reimann, D. Cliver (eds.), Foodborne Infections and Intoxications. *Academic Press*, 55-155.

Montville, T., and Matthews, K. (2005). Food microbiology: An introduction. *ASM Press, Washington D.C.*

Moore, D. (2015). Antibiotic Classification and Mechanism. htt://www.orthobullets.com /basic-science/9059/antibiotic-classification-and-mechanism. Accessed on September, 1 2016.

Morshdy, A. E., El-Atabany, A. I., Hussein, M. A., and Wageh, S. D. W. S. (2013). Oxytetracycline residues in bovine carcasses slaughtered at Mansoura Abattoir, Egypt. *Japanese Journal of Veterinary Research, In Press*.

MTU, (Meat Technology Update). (2010). *Sources of contamination on beef carcasses during dressing.*

Muriuki, F. K., Ogara, W. O., Njeruh, F. M., and Mitema, E. S. (2001). Tetracycline residue levels in cattle meat from Nairobi slaughterhouse in Kenya. *Journal of Veterinary Science, 2*, 97–101.

Muyembe-Tamfum, J. J., Veyi, J., Kaswa, M., Lunguya, O., Verhaegen, J., and Boelaert, M. (2009). An outbreak of peritonitis caused by multidrug-resistant *Salmonella* Typhi in Kinshasa, Democratic Republic of Congo. *Travel Medicine and Infectious Disease, 7*, 40-43.

Myllyniemi, A.L. Rannikko, R. Lindfors, E. Niemi, A. and Bäckman, C. (2000). Microbiological and chemical detection of incurred penicillin G, oxytetracycline, enrofloxacin and ciprofloxacin residues in bovine and porcine tissues. *Food Additives and Contaminants, 17*, 991–1000.

NACMCF, (National Advisory Committee on Microbiological Criteria for Foods). (1993). Generic HACCP for raw food. U.S. Department for Agriculture,

Food Microbiology 10, 449-488.

Newell, D.G. and Wagenaar, J. A. (2000). Poultry infections and their control at the farm level. In: Nachamkin I., Blaser M.J. (eds.): *Campylobacter*. 2nd ed. *ASM Press, Washington, D.C., U.S.A.*

Nisha, A. R. (2008). Antibiotics residues-A global health hazard. *Veterinary World*, 1 (12), 375-377.

NNDSS, (National Notifiable Disease Surveillance System). (2013). Notifications for all disease by State and Territory. Department of Health and Ageing, Canberra. *Http://Www9.Health.Gov.Au/Cda/Source/Cda-Index.Cfm. Accessed March,2019*.

Nonga, H. E., Simon, C., Karimuribo, E. D., and Mdegela, R. H. (2010). Assessment of

antimicrobial usage and residues in commercial chicken eggs from smallholder poultry keepers in Morogoro municipality, Tanzania. *Zoonoses Public Health, 57* (5), 339-44.

Noormohamed, A., and Fakhr, M. K. (2013). A Higher Prevalence Rate of *Campylobacter* in Retail Beef Livers Compared to Other Beef and Pork Meat Cuts, 2058–2068. https://doi.org/10.3390/ijerph10052058

NRC (National Research Council), (1991). The use of drugs in food animal benefits and risks. *The National Academies Press.*, 253-284.

Nygren, B., Schilling, K., Blanton, E., Silk, B., Cole, D., and Mintz, E. (2012). Foodborne outbreaks of shigellosis in the USA, 1998-2008. *Epidemiology and Infection*, *14* (2), 233–241.

Ochiai, R. L., Wang, X., von Seidlein, L., Yang, J., Bhutta, Z. A., Bhattacharya, S. K., Agtini, M., Deen, J. L., Wain, J., Kim, D.R., Ali, M., Acosta, C.J., Jodar, L., Clemens, J. D. (2005). *Salmonella* paratyphi A rates, Asia. *Emerging Infectious Disease*, 11, 1764-1766.

Ohl, M. E., and Miller, S. I. (2001). *Salmonella*: a model for bacterial pathogenesis. *Annual Review of Medicine, 52*, 259-274.

Okonko, I. O., Ukut, I. O. E., Ikpoh, I. S., Nkang, A. O., Udeze, A. O., Babalola, T. A., Mejeha, O. K. and Fajobi, E. A. (2008). Assessment of bacteriological quality of fresh meats sold in Calabar Metropolis, Nigeria. *Electronic Journal of Environmental, Agricultural and Food Chemistry.*, *9* (1), 89-100.

Olsen, S., Bishop, R., Brenner, F., Roels, T., Bean, N., Tauxe, R., and Slutsker, L. (2001). The changing epidemiology of *Salmonella*: trends in serotypes isolated from humans in the United States, 1987–1997. *Journal of Infectious Disease*, *183*, 753–761.

Olufemi, O. I., and Ehinmowo Adebayo Agboola, E. A. (2009). Oxytetracycline Residues in Edible Tissues of Cattle Slaughtered in Akure, Nigeria. *Internet Journal of Food Safety*, 11, 62-66.

Omeiza, G. K., Kabir, J., Mamman, M., Ibrahim, H., and Fagbamila, I. O. (2012). Response of Nigerian farmers to a questionnaire on chloramphenicol application in commercial layers. *Veterinary Italiana*, 48 (1), 87–93.

Oyarzabal, O. A. Macklin, K. S. Barbaree, J. M. and Miller, R. S. (2005). Evaluation of agar plates for direct enumeration of Campylobacter spp. from poultry carcass rinses. *Applied and Environmental Microbiology*, 71, 3351–3354.

OzFoodNet. (2012). Monitoring the incidence and causes of diseases potentially transmitted by food in Australia: *Annual Report of the OzFoodNet Network, 2010. Communicable Diseases Intelligence, 36* (6), E213–E241.

Padungtod, P., and Kodahira, M. Hill, G. (2008). Livestock production and foodborne diseases from food animals in Thailand. *Journal of Veterinary Medicical Science*, 70, 873–879.

Paige, J. C., Sundlof, S. F., and Fernandez, A. H. (2000). Antimicrobial drug residues in food producing animals. In: Prescott, J. F., Baggot, J. D. and Walker, R. D. (eds.), Antimicrobial Therapy in Veterinary Medicine, 3rd ed. *Ames, Iowa, USA*, 744-759.

Paige, J. C., Tollefson, L., and Miller, M. (1997). Public health impact on drug residues in animal tissues. *Veterinary and Human Toxicology* 39, 162--169.

Papp-Wallace, K., Endimiani, A., Taracila, M., and Bonomo, R. (2011). Carbapenems: past, present, and future. *Antimicrobial Agents Chemotherapy.*, *55* (11), 4943--4960.

Pappas, G. Akritidis, N. Bosilkovski, M. T., and E. (2005). Brucellosis. New England

Journal of Medicine, *352*, 2325–2336.

Pappas, G. Panagopoulou, P. Christou, L., and Akritidis, N. (2006). Brucella as a biological weapon. Cellular and Molecular Life Sciences, *63*, 2229–2236.

Patel, G., and Bonomo, R. A. (2011). Status report on carbapenemases: challenges and prospects. *Expert review of Anti-infective Therapy*, 9, 555--570.

prospects. *Expert review of Anti-infective Therapy*, 9, 555--570.

Patela, J. R., Bhagwatb, A. A., Sanglaya, G. C., and Solomon, M. B. (2006). Detection of Salmonella from hydrodynamic pressure-treated poultry using molecular beacon real-time PCR. *Food Microbiology*, *23*, 39–46.

Pegler, S., and Healy, B. (2007). In patients allergic to penicillin, consider second and third generation cephalosporins for life threatening infections. *British Medical Journal*, 335 (7627), 991.

Pepin, M. Russo, P. and Pardon, P. (1997). Public health hazards from small ruminant meat products in Europe. *Revue scientifique et technique, 16*, 415–425.

Percival, S. L., Chalmers, R. M., Embrey, M., Hunter, P. R., and Sellwood, J. (2004). Microbiology of Waterborne Diseases, *Elsevier Academic Press, San Diego, California, USA.*

Peterson, L. R. (2008). Currently available antimicrobial agents and their potential for use as monotherapy. *Clinical Microbiology and Infection*, 14 (6), 30-45.

Phillips, E., Lovie, M., Knowles, R., and Simor, K. (2000). A cost effectiveness analysis of six strategies for cardio vascular surgery prophylaxis in patient labeled penicillin allergic residues review. *Asian-Australasian Journal of Animal Sciences, 14*, 402–413.

PHV, (Public Health Veterinarian). (2011). Entry training for Public Health Veterinarian;

Overview of Food Microbiology., 1–37.

Poirel, L., Brinas, L., Verlinde, A., Ide, L., and Nordmann, P. (2005). BEL-1, a novel clavulanic acid-inhibited extended-spectrum beta-lactamase, and the class 1 integron In120 in *Pseudomonas aeruginosa*. *Antimicrobial Agents Chemotherapy*, 49 (9), 3743–3748.

Popoff, M., Bockemuhl, J., and Gheesling, L. (2003). Supplement 2001 (no. 45) to the Kauffmann-White scheme. *Research in Microbiology*, *154*, 173–174.

Porwollik, S. ed. (2011). *Salmonella*: From Genome to Function. *Caister Academic Press. ISBN*, 978-1-904455-73-8.

Public Health Agency of Canada (PHAC). (2014). *Food safety fact sheet. E. coli www.publichealth.gc.ca*.

Pui, C. F., Wong, W. C., Chai, L. C., Tunung, R., Jeyaletchumi, P., Noor Hidayah, M. S., Ubong, A. Farinazleen, M. G. Cheah, Y. K. and Son, R. (2011). *Salmonella*.A foodborne pathogen. *International Food Research Journal*, 18, 465-473.

Raffatellu, M., Chessa, D., Wilson, R. P., Tukel, C., Akcelik, M., and Baumler, A. J. (2006). Capsule-mediated immune evasion: a new hypothesis explaining aspects of typhoid fever pathogenesis. *Infection and Immunity*, 74, 19–27.

Raison-Peyron, N., Messaad, D., Bousquet, J., and Demoly, P. (2001). Anaphylaxis to beef in penicillin-allergic patient. *Allergy*, *56*, 796-797.

Raji, A. I. (2006). Bacteriological Quality of Dried Sliced beef (kilishi) sold in Ilorin Metropolis. *Journal of Applied Sciences and Environmental Management*, *10*, 97–100.

Raloff, J. (2003). Food For Thought: Global Food Trends. *Science News Online.*

Ramos, M. A., Arnanda, A., Pozuelo, M. M., and Reuvers, T. (2003). Chloramphenicol residues in food samples, their analysis and stability during storage. *Journal of Chromatography and Separation Technique*, 26, 2535-2449.

Reading, C., and (Farmer, T. (1984). The inhibition of periplasmic βlactamase in Escherichia coli by clavulanic acid and other -lactamase inhibitors. *McGraw-Hill, New York.*

Riedl, M. A., and Cassilas, A. M. (2003). Adverse drug reactions types and treatment options. *American Journal of Physics*, 68, 1781–1790.

Rollins, D. M. (1991). Potential for reduction in colonization of poultry by Campylobacter from environmental sources. In: Blankenship L.C. (ed.): Colonization Control of Human Bacterial Enteropathogens in Poultry. *Academic Press, San Diego, California, U.S.A.*

Rotar, A. M., Semeniuc, C. A., Apostu, S., Pop, C., Duma, M., Suharoschi, R., and Giura, L. (2013). Identification and Prevalence of *Escherichia coli* and *Escherichia coli* O157 : H7 in Foods. *Bulletin UASVM Food Science and Technology*, 70 (2), 139–140.

Ryan, K. J., and Ray, C. G. (2004). Sherris Medical Microbiology (4th Ed.). *McGraw Hill.*, 362–8.

Salama, N. A., Abou-Raya, S. H., Shalaby, A. R., Emam, W. H., and Mehaya, F. M. (2011). Incidence of tetracycline residues in chicken meat and liver retailed to consumers. *Food Addititves and Contaminants*, *4* (2), 88-93.

Samelis, J. and Metaxopoulos, J. (1999). Incidence and principle sources of *Listeria* spp. and *Listeria monocytogenes* contamination in processed meats and a meat processing plant. *Food Microbiology*, *16*, 465–477.

Sanchez, A. R., Rogers, R. S., and Sheridan, P. J. (2004). Tetracycline and other tetracycline-derivative staining of the teeth and oral cavity. *International Journal of Dermatology*, 43 (10), 709-715.

Sandel, M. K., Wu, Y. G., and Mckillip, J. L. (2003). Detection and recovery of sublethally-injured enterotoxigenic Staphylococcus aureus, 90-94.

Sarkozy, G. (2001). Quinolones: A Class of Antimicrobial Agents. *Veterinary Medicine-Czech*, *46*, 257-274.

Saroj, S., Shashidhar, R., and Bandekar, J. (2009). Gamma Radiation Used as Hygienization Technique for Foods Does Not Induce Viable but No Culturable State (VBNC) in *Salmonella* enterica subsp. enterica serovar Typhimurium. *Current Microbiology*, *59*, 420–424.

Schnéeider, RC. Santos, MD. Lunardi, M. B., AH. Camargo, LM. Freitas, SH. Negreiro, R., and Costa, D. (2013). Prevalence of brucellosis and risk factors associated with its transmission to slaughterhouse employees in the Cuiaba metropolitan area in the state of Mato Grosso. *Cienc Agrar*, *34*, 2367–2374.

Scholz, HC. Nockler, K. Gollner, C. Bahn, P., Vergnaud, G. Tomaso, H. Al Dahouk, S., Kampfer, P. Cloeckaert, A. Maquart, M., Zygmunt, MS. Whatmore, AM. Pfeffer, M., and Huber, B. Busse, HJ. De, B. (2010). Brucellainopinata sp. nov., isolated from a breast implant infection. *International Journal of Systematic and Evolutionary Microbiology*, 60, 801-8.

Schwartz, M. H., and Sher, T. H. (1984). Anaphylaxis to penicillin in a frozen dinner. Sekuloski, P. (2008). Znaaj Brucella melitensis za bezbednost hrane. *Vet Glasnik*, *62*, 289-99.

Senthilkumar, B., and Prabakaran, B. (2005). Multidrug Resistant *Salmonella* typhi in Asymptomatic Typhoid Carriers among Food Handlers in Namakkal District, Tamil Nadu. *Indian Journal of Medical Microbiology, 23*, 92–94.

Settepani, J. A. (1984). The hazard of using chloramphenicol in food animals. *Journal of American Veterinary Medicine Association., 184*, 30--39.

Shitandi, A., and Sternesjo, A. (2001). Detection of antimicrobial drug residues in Kenyan milk. *Journal of Food Safety*, *21*, 205-268.

Siddiqui, T. R. S. B., Muhammad, A. M., and Sobiya, M. A. A. K. (2015). High prevalence of typhoidal Salmonella enterica serovars excreting food handlers in Karachi-Pakistan: a probable factor for regional typhoid endemicity. *Journal of Health, Population and Nutrition*, *33*, 27. https://doi.org/10.1186/s41043-015-0037-6

Sinha, R., Kulldorff, M., Swanson, C., Curtin, J., and Brownson, RC . (2000). Dietary heterocyclic amines and the risk of lung cancer among Missouri women. *Cancer Research, 60*, 3753–3756.

Slatore, C. G., and Tilles, S. A. (2004). Sulfonamide hypersensitivity. Immunol. *Allergy Clinics North America.*, *24* (3), 477--490.

Smith, S. I., Bamidele, M., Goodluck, H. A., Rowora, M. N. Omonigbehn, E. A., Opere, B. O., and Aboaba, O. O. (2009). Antimicrobial susceptibilities of Salmonellae isolated from foodhandlers and cattle in Lagos, Nigeria. *International journal of Health Research, 2*, 189-193.

Sofos, J. N. (2008). Challenges to meat safety in the 21st century. *Meat Science, 18*, 3-13.

Sofos, J. N., Kochevar, S. L., Bellinger, G. R., Buege, D. R., Hancock, D. D., Ingham, S. C., Morrga, Reagan J. O., and Smith, G. C. (1999). Sources and extent of

microbiological contamination of beef carcasses in seven United States slaughtering plants. *Journal of Food Protection, 62* (2), 140–145.

Soni, K. (2012). Fluoroquinolones: Chemistry and Action: A Review. *Indo Global Journal of Pharmaceutical Sciences, 2* (1), 43-53.

Soyiri, I. N., Agbogli, H. K., and Dongdem, J. T. (2008). A pilot microbial assessment of beef sold in the Ashaiman market, a suburb of Accra, Ghana. *African Journal of Food, Agriculture, Nutrition and Development, 8*, 91–103.

Speedy, A. W. (2003). Global production and consumption of animal source foods. *Journal of Nutrition., 133*, 4048S–4053.

Sprenger, R. A. (1995). Food for thought: Approaches for steering successful meat business into the next century. *Die Fleischerei*, *6*, Vii-Xi.

Stawinski, J., Szafranski, K., Vullo, D., and Supuran, C. T. (2013). Carbonic anhydrase inhibitors. Synthesis of heterocyclic 4-substituted pyridine- 3-sulfonamide derivatives and their inhibition of the human cytosolic isozymes I and II and transmembrane tumor-associated isozymes IX and XII. *European Journal of Medicinal Chemistry*, 69, 701-710.

Strachan, N.J.C. MacRae, M. Thomson, A. Rotariu, O. Ogden, I.D. ans Forbes, K. J. (2012). Source attribution, prevalence and enumeration of *Campylobacter* spp. from retail liver. *International Journal of Food Microbiology, 153*, 234–236.

Sulley, M. S. (2006). The hygienic standard of meat handling in the Tamale Metropolis. Bsc. Dissertation, University for Development Studies, Tamale, p 44.

Sundlof, S. F. (1989). Drug and chemical residues in livestock. *Clinical Toxicology*, *5*, 411–449.

Sykes R. B., Cimarusti C. M., Bonner D. P., Bush K., Floyd D. M., Georgopapadakou, N. H. Koster, W. H., Liu, W. C., Parker, W. L., Principe, P. A., Rathnum, M. L., Slusarchyk, W. A., Trejo, W. H., and Wells, J. S. (1981). Monocyclic β-lactam antibiotics produced by bacteria. *Nature.*, 291, 489-491.

Sykes, R. B., and Bonner, D. P. (1985). Discovery and development of the monobactams. *Clinical Infectious Disease*, *7* (4), S579--S593.

Tajick, M. A., and Shohreh, B. (2006). Detection of antibiotics residue in chicken meat using TLC. *International Journal of Poultry Science*, *5* (7), 611-612. https://doi.org/10.3923/ijps.2006.611.612

Talaro, K. P., and Chess, B. (2008). Foundations in microbiology. 8th Ed. *McGraw Hill, New York.*

Tam, R. C. (2008). Salmonella_description, pathogenesis, symptoms.

Tauxe, R. V. (2002). Emerging foodborne pathogens. *International Journal of Food Microbiology*, *78* (1–2), 31–41.

Temellİ, S., Eyİgör, A., and Anar, Ş. (2012). Prevalence of *Escherichia coli* O157 in red meat and meat products determined by VIDAS ECPT and LightCycler PCR, *36* (3), 305–310. https://doi.org/10.3906/vet-1107-38

Tinkelman, D. G., and Bock, S. A. (1984). Anaphylaxis presumed to be caused by beef containing streptomycin. *Annals of Allergy*, *53*, 243--244.

Torres, J. A., Villegas, M. V, and Quinn, J. P. (. (2007). Current concepts in antibiotic-resistant gram-negative bacteria. *Expert Review of Anti-infective Therapy*, *5*, 833–843.

Tutenel, A. V., Pierard, D., Van Hoof, J., Cornelis, M., and De Zutter, L. (2003). Isolation and molecular characterization of Escherichia coli O157 isolated from cattle, pigs and

chickens at slaughter. *International Journal of Food Microbiology*, *84* (1), 63-9.

Twum, E. (2015). *Microbial Quality of Fresh Beef Sold in the Birim North District of the Eastern Region of Ghana Thesis*. Kwame Nkrumah University of Science and Technology, Kumasi.

Ul-Hassan, S. R., Verma, V., and Qazi, G. N. (2004). Rapid detection of Salmonella by polymerase chain reaction". *Molecular and Cellular Probes, 18*, 333–339.

Unneveher L. J., (2000). Food safety issues and fresh food product exports from LDCS, *Agricultural Economics,* 23, 231– 240

USDA. (2009). Livestock and poultry; World markets and trade.

van de Giessen, A. (1996). Epidemiology and control of *Salmonella* enteritidis and *Campylobacter* spp. in poultry flocks. *Thesis*.

van Hoek, A. H. A. M., Mevius, D., Guerra, B., Mullany, P., Roberts, A. P., and Aarts, H. J. M. (2011). Acquired antibiotic resistance genes: An overview. *FAO Animal Production and Health Paper*, 135, Rome.ront. Microbiol. 2:203 doi: 10.3389/fmic.

Vandepitte, J., Verhaegen, J., Engbaek, K., Rohner, P., Piot, P., and Heuck, C. (2003). Basic laboratory procedures in clinical bacteriology, WHO, 2nd ed, Geneva.

Ul-Hassan, S. R., Verma, V., and Qazi, G. N. (2004). Rapid detection of Salmonella by polymerase chain reaction". *Molecular and Cellular Probes, 18*, 333–339.

Unneveher L. J., (2000). Food safety issues and fresh food product exports from LDCS, *Agricultural Economics,* 23, 231– 240

USDA. (2009). Livestock and poultry; World markets and trade.

van de Giessen, A. (1996). Epidemiology and control of Salmonella enteritidis and

Campylobacter spp. in poultry flocks. *Thesis*.

van Hoek, A. H. A. M., Mevius, D., Guerra, B., Mullany, P., Roberts, A. P., and Aarts, H. J. M. (2011). Acquired antibiotic resistance genes: An overview. *FAO Animal Production and Health Paper*, 135, Rome.ront. Microbiol. 2:203 doi: 10.3389/fmic.

Vandepitte, J., Verhaegen, J., Engbaek, K., Rohner, P., Piot, P., and Heuck, C. (2003). Basic laboratory procedures in clinical bacteriology, WHO, 2nd ed, Geneva.

Warren, B., Parish, M., and Schneider, K. (2006). Shigella as a foodborne pathogen and current methods for detection in food. *Critical Reviews in Food Science and Nutrition*, *46*, 551–567.

Wasch, D., Okerman, L., Croubels, S., De Brabander, H., Van Hoof, J. and De Backer, P. (1998). Detection of residues of tetracycline antibiotics in pork and chicken meat: correlation between results of screening and confirmatory tests. *Analyst, Trakia, 123* (12), 2737–2744.

WHO/FAO. (2002). Risk assessments of Salmonella in eggs and broiler chickens. World Health Organization and Food and Agriculture Organization of the United Nations, Geneva.

WHO. (1999). Evaluation of Certain Veterinary Drug Residues in Food. *WHO Technical Report Series (15th Report of the Joint FAO/WHO Expert Committee on Food Additives). No.888.*

WHO. (2001). Monitoring antimicrobial usage in food Protection of human health. Report of a WHO consultation Oslo, Norway 10-13 September.

WHO. (2006). Brucellosis in human and animals. World Health Organization, Geneva, Switzerland. *Available from: www.who.int/csr/resources/publications/B rucellosis.pdf*.

WHO. (2007). Fact Sheet Number 237: “Food safety and foodborne illness.” World Health Organization (WHO), . *Retrieved from www.who.int/mediacentre/factsheets/fs237 /en/*

WHO. (2011). Guidelines for drinking-water quality World Health Organization.

Wilkens, J., Newman, M., Commey, J., and Seifert, H. (1997). Salmonella bloodstream infection in Ghanaian children. *Clinical Microbiology and Infection*, 1469–0691. https://doi.org/doi: 10.1111/j.1469-0691.1997.tb00467.x.

Williams, P. G. (2007). Nutrient composition of red meat. *http:// ro. uow .edu .au / hbspapers/48*.

Xu, F., Xu, H., Wang, X., Zhang, L., Wen, Q., Zhang, Y., and Xu, W. (2014). Discovery of N-(3-(7H-purin-6-yl)thio)-4-hydroxynaphthalen-1-yl)- sulfonamide derivatives as novel protein kinase and angiogenesis inhibitors for the treatment of cancer: synthesis and biological evaluation. Part III. *Bioorganic and Medicinal Chemistry*, *22* (4), 1487--1495.

Zakeri, B., and Wright, G. D. (2008). Chemical Biology of Tetracycline Antibiotics. *Biochemistry and Cell Biology*, *86*, 124–136.

Zhao, Y. (2002). Virulence factors of *Salmonella* enterica serovar Enteritidis. *Utrecht University*.

Ziprin, R. L., In Hui, Y. H., Gorham, J. R., Murrell, K. , and Cliver, K. D. (eds.). (1994). Foodborne Disease Handbook: Diseases Caused by Bacteria, Marcel Dekker,. *Inc. New York. Pp. 613.*, *1*, pp.253-318.

APPENDIX I A

UNIVERSITY FOR DEVELOPMENT STUDIES

FACULTY OF AGRICULTURE

DEPARTMENT OF ANIMAL SCIENCE

A SURVEY ON ANTIBIOTICS USAGE IN RUMINANT PRODUCTION IN THE WA MUNICIPAL ASSEMBLY

This study is to identify the most frequently used antibiotics, their dose, time of use and the withdrawal times prior to market or slaughter. Please, information given will be treated with high level of confidentiality.

Please fill the questions below as best as you can. Tick where appropriate [√]

A. PERSONAL DATA

1. Gender: Male [ ] Female [ ]
2. Age group. a. 20-29 [ ] b. 30-39 [ ] c. 40-49 [ ] d. 50-60 [ ] e. 61& above [ ]
3. Marital status: a. Married [ ] b. Single [ ] c. Divorced [ ] d. Others [ ]
4. Religion a. Christianity [ ] b. Islamic [ ] c. Traditional [ ] d. Others [ ]
5. Tribe ……..
6. Educational level. a. Non formal [ ] b. Primary school [ ] c. Junior high school [ ] d. Senior High school [ ] e. Tertiary [ ] f. Others [ ]

B. FARMER

7. What type of ruminants do you rear? a. Cattle [ ] b. Goat [ ] c. Sheep [ ]

8. How many years have you been in this business? a. 1-2 years [ ] b. Between 3 - 5 years [ ] c. 6-10 years [ ] d. above 10 years
9. Have you ever encountered any infection in the animals on your farm? a. Yes [ ] b. No [ ]
10. If yes, did you consult veterinary officers to know what kind of infection it was? a. Yes [ ] b. No [ ]
11. If No, Give reason why? …….

    ……..
12. If yes, what kind of medication did he/she recommend for you to treat the animals?

    a. Ethno veterinary medication b. Therapeutic drugs c. Others specify
13. Did the medication include antibiotics? a. Yes b. No
14. If yes, what type of antibiotic did you use? a. Gentamicin [ ] b. Tetracycline [ ] c. Azithromycin [ ] d. Amoxycillin/Clavulanic [ ] e. Trimethoprim/Sulfamethoxazole [ ] f. Ciprofloxacin [ ] g. Ceftriaxone [ ] h. Chloramphenicol [ ] i. Teicoplanin [ ] j. Others specify……..……
15. Why do you prefer this/these antibiotic/s? a. it is effective [ ] b. it is less costly [ ] c. it is easy to use [ ] d. easily accessible [ ] e. colleague advice [ ] e. other [ ]
16. Do you have knowledge on the antibiotic you use? a. Yes [ ] b. No [ ]
17. If yes, who/where did you get the information? a. extension officers [ ] b. NGOs [ ] c. colleague Farmers [ ] d. veterinary staff e. others [ ]
18. Where do you buy the antibiotics from? a. Veterinary clinic/Shops [ ] b. Friends [ ] c. Market [ ] d. Others (specify)

    ……

19. How often do you treat your animals with antibiotics? ……

……

20. Who administers the antibiotic to the animals? a. self [ ] b. veterinary officer [ ] c. both. d. Others specify

……

21. If self or both do you observe safety and dosage instructions for the antibiotic? a. Yes [ ] b. No [ ]

22. If No, why? …….

23. If Yes, what are the dosages and withdrawal periods of the antibiotics you have ever used for treating ruminants?

Species	**Antibiotic Name**	**Dosage**	**Withdrawal periods**

24. In case the treated animal is not recovering, what do you do to the animal? a. sell to butchers [ ] b. home consumption [ ] c. market [ ] d. others

specify……

25. In case you are going to sell or consume the unrecovered animal how long does it take from the time of treatment to the time of sale or consumption?

.....

APPENDIXES IB

UNIVERSITY FOR DEVELOPMENT STUDIES

FACULTY OF AGRICULTURE

DEPARTMENT OF ANIMAL SCIENCE

A SURVEY ON ANTIBIOTICS USAGE IN RUMINANT PRODUCTION IN THE WA MUNICIPAL ASSEMBLY

This study is to identify the most frequently used antibiotics, their dose, time of use and the withdrawal times prior to market or slaughter. Please, information given will be treated with high level of confidentiality

Please fill the questions below as best as you can. Tick where appropriate [√]

A. Personal Data

1. Gender: a. Male [ ] b. Female [ ]

2. Age group (Years). a. 20-29 [ ] b. 30-39 [ ] c. 40-49 [ ] d. 50-60 [ ] e. 61& above [ ]

3. Marital status: a. Married [ ] b. Single [ ] c. Divorced [ ] d. Others [ ]

4. Household size. a. 1-4 [ ] b. 5-9 [ ] c. 10-14 [ ] d. 15 and above [ ]

5. Educational level. a. Non formal [ ] b. Primary school [ ] c. Junior/Senior High school [ ] d. Tertiary [ ] e. Others specify ……

B. Veterinary officers

6. How many years have you been in this work?.....

7. Do you encounter bacterial infections in animals you have ever treated? a. Yes b. No

8. If Yes in which animal species do/did you encountered this? a. Cattle b. Sheep c. Goats

d. All

9. In every species that you encountered the bacterial infection indicate the causal agent suspected, the kind of treatment provided and antibiotic/antibiotics used

ies	Bacteria suspected	Treatment Provided	Type/types of antibiotic used	Reasons

10. Where do you buy your antibiotics from? ……

……

What are the dosages of the five most common antibiotics used for treating ruminants?

Ar : Name	Dosage Recommended by Manufacturer	Dosage Administered	What are the withdrawal periods for these antibiotics?	Reason for Below or Above recommended dosage

14. Do you advice farmers on residual effect of antibiotics? a. Yes [ ] b. No [ ]

15. If Yes, what are their responses?

. ……..

16. If No, Give reasons why? .....

17. What are the constraints you encounter when advising the farmers?

……..……

……..……..……

18. Do you have Community Livestock workers operating in your area of jurisdiction? a. Yes [ ] b. No [ ]

19. If Yes, do they get training regarding the administration of antibiotics? a. Yes [ ] b. No [ ]

20. If No Give reasons why? .....

21. Do you encounter untrained personnel offering veterinary services on the field? a. Yes [ ] b. No [ ]

22. If Yes, what action did you take? ……

……

23. Do you have any way of tracking animals which have just been treated with antibiotics but are being sent to be slaughtered? a. Yes [ ] b. No [ ]

24 If No why give reasons?

……

25: If Yes, how is that done and how is the success rate?

……..……

26. What action do/did you take against farmers selling a treated animal whose withdrawal period has not elapsed?

……..……

APPENDIX II

Analysis of Salmonella Using SPSS Version 18

GET

Generalized Linear Models

Case Processing Summary

	N	Percent
Included	150	100.0%
Excluded	0	0.0%
Total	150	100.0%

Categorical Variable Information

			N	Percent
Dependent Variable	Bacteria	.0	114	76.0%
		1.0	36	24.0%
		Total	150	100.0%
Factor	Source	Kidney	50	33.3%
		Liver	50	33.3%
		Muscle	50	33.3%
		Total	150	100.0%

Tests of Model Effects

Source	Type III		
	Wald Chi-Square	Df	Sig.
(Intercept)	35.242	1	.000
Source	7.307	2	.026

Dependent Variable: Bacteria

Model: (Intercept), Source

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test	
			Lower	Upper	Wald Chi-Square	df
(Intercept)	.847	.3086	.242	1.452	7.538	1
[Source=Kidney]	1.350	.5634	.246	2.454	5.740	1
[Source=Liver]	-.094	.4326	-.941	.754	.047	1
[Source=Muscle]	0[a]	.	.	.	.	.
(Scale)	1[b]					

Estimated Marginal Means: Source

Estimates

Source	Mean	Std. Error	95% Wald Confidence Interval	
			Lower	Upper
Kidney	.90	.042	.78	.96
Liver	.68	.066	.54	.79
Muscle	.70	.065	.56	.81

Pairwise Comparisons

(I) Source	(J) Source	Mean Difference (I-J)	Std. Error	df	Sig.	95% Wald Confidence Interval for Difference	
						Lower	
Kidney	Liver	.22[a]	.078	1	.005	.07	
	Muscle	.20[a]	.077	1	.010	.05	
Liver	Kidney	-.22[a]	.078	1	.005	-.37	
	Muscle	-.02	.092	1	.829	-.20	
Muscle	Kidney	-.20[a]	.077	1	.010	-.35	
	Liver	.02	.092	1	.829	-.16	

Pairwise comparisons of estimated marginal means based on the original scale of dependent variable Bacteria

a. The mean difference is significant at the .05 level.

Overall Test Results

Wald Chi-Square	df	Sig.
11.228	2	.004

The Wald chi-square tests the effect of Source. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

Salmonella

Pairwise Comparisons

(I) Source	(J) Source	Mean Difference (I-J)	Std. Error	df	Sig.	
Kidney	Liver	.22a	0.078	1	0.005	Yes
	Muscle	.20a	0.077	1	0.01	Yes
Liver	Kidney	-.22a	0.078	1	0.005	Yes
	Muscle	-0.02	0.092	1	0.829	No
Muscle	Kidney	-.20a	0.077	1	0.01	Yes
	Liver	0.02	0.092	1	0.829	No

APPENDIX IV

Analysis of Total Aerobic Count Using GenStat Version 12.1

GenStat Release 12.1 (PC/Windows Vista) 28 December 2018 02:48:58

Registered to: The NULL Corporation

GenStat Twelfth Edition

GenStat Procedure Library Release PL20.1

Data imported from Excel file: C:\Users\FREDs OWN\Desktop\Rejoice Analysis\Copy%20of%20Antibiotic%20rejoice%202.xlsx

on: 28-Dec-2018 2:49:25

taken from sheet ""Salmonella PCA log"", cells A2:B10

Identifier	Values	Missing	Levels
Source	9	0	3

Identifier	Minimum	Mean	Maximum	Values	Missing
logcfu_cm2	1.996	3.396	5.653	9	0

Analysis of variance

Variate: logcfu_cm2

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Source	2	0.181	0.090	0.04	0.959
Residual	6	12.868	2.145		
Total	8	13.049			

Information summary

All terms orthogonal, none aliased.

Message: the following units have large residuals.

units 1 2.43 s.e. 1.20

Tables of means

Variate: logcfu_cm2

Grand mean 3.40

Source	Kidney	Liver	Muscle
	3.23	3.39	3.57

Standard errors of differences of means

Table	Source
rep.	3
d.f.	6
s.e.d.	1.196

[DataSet1] K:\Rejoice_2.sav

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
educational leve of farmers * knowledge on the antibiotics	250	100.0%	0	.0%	250	100.0%

educational leve of farmers * knowledge on the antibiotics Crosstabulation

			knowledge on the antibiotics		
			yes	No	Total
educational leve of farmers	non-formal	Count	61	30	91
		% within knowledge on the antibiotics	38.4%	33.0%	36.4%
	primary school	Count	24	10	34
		% within knowledge on the antibiotics	15.1%	11.0%	13.6%
	JSH	Count	38	31	69
		% within knowledge on the antibiotics	23.9%	34.1%	27.6%
	SHS	Count	10	10	20
		% within knowledge on the antibiotics	6.3%	11.0%	8.0%
	Tertiary	Count	10	0	10
		% within knowledge on the antibiotics	6.3%	.0%	4.0%
	others	Count	16	10	26

	% within knowledge on the antibiotics	10.1%	11.0%	10.4%
Total	Count	159	91	250
	% within knowledge on the antibiotics	100.0%	100.0%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)	Point Probability
Pearson Chi-Square	10.717[a]	5	.057	.055		
Likelihood Ratio	13.955	5	.016	.020		
Fisher's Exact Test	11.364			.042		
Linear-by-Linear Association	.212[b]	1	.645	.656	.336	.029
N of Valid Cases	250					

a. 1 cells (8.3%) have expected count less than 5. The minimum expected count is 3.64.

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)	Point Probability
Pearson Chi-Square	10.717[a]	5	.057	.055		
Likelihood Ratio	13.955	5	.016	.020		
Fisher's Exact Test	11.364			.042		
Linear-by-Linear Association	.212[b]	1	.645	.656	.336	.029
N of Valid Cases	250					

b. The standardized statistic is .460.