UNIVERSITY FOR DEVELOPMENT STUDIES, TAMALE

FARMERS KNOWLEDGE ON ANTIBIOTIC USAGE AND PREVALENCE OF ANTIBIOTIC RESISTANT ESCHERICHIA COLI IN READY-TO-EAT MEATS VENDED IN BOLGATANGA MUNICIPALITY OF GHANA

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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.

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Supervisor

I 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.

Supervisor's Signature ……. Date……

Prof. Frederick Adzitey (PhD)

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ABSTRACT

Fresh meat products may have a number of microorganisms that can make meat un-wholesome for human consumption when exposed to favorable growth conditions for these microoganisms. The presence of pathogenic and spoilage microorganisms in ready-to-eat (RTE) meats will have a significant impact on post-cooking handling activities, meat storage conditions and length at points of sale. The study investigated the prevalence of *E. coli* in RTE meats as well as the knowledge of livestock farmers on antibiotic usage. Data on demographics and knowledge of antibiotic usage was collected from livestock farmers through the administration of semi-structured questionnaires (n=376) using snowball sampling technique. The protocol in the USA-FDA Bacteriological Analytical Manual was used for *E. coli* identification. The disk diffusion method was used for antibiotic resistance tests. The findings revealed that majority of livestock farmers were male (74.5%), aged between 30-39 years (28.5%) and had tertiary education (30.3%). Sheep (65.7%) was the most reared livestock and tetracycline (36.7%) was the most used antibiotic to treat sick animals. Only 22.6% of the farmers received training on antibiotic usage but majority of the farmers had knowledge (56.1%) on antibiotic usage. Greater number of the farmers (40.2%) consume or sell unrecovered animals treated with antibiotics within a week and 23.9% consumed or sell after three weeks. About 56.6% of the farmers had knowledge on antibiotic residues in meat whereas 63.0% had knowledge of the effect of the antibiotic residue on human health. The microbial load of RTE meats ranged from 2.46 to 3.29 log cfu/cm^2 in chicken and pork, respectively. The prevalence of *E. coli* in RTE meats was lowest in pork (6%) and highest in chevon (20%). *E. coli* isolates from RTE meats

were highly resistant to teicoplanin (96.77%), followed by tetracycline (93.55%), amoxycillin/clavulanic (70.97%), and azithromycin (70.97%). For multidrug resistant, the multiple antibiotic resistance (MAR) index was found ranging from 0.22 to 0.78. Three isolates of *E. coli*, one each from chevon, pork and chicken were resistance to seven antibiotics, namely CipAmzAzmTecTeCSxt, AzmTecCnTeCCroSxt and AmcAzmTecCnTeCroSxt, respectively with MAR index of 0.78. One isolate each from chevon and mutton was not susceptible to two antibiotics with 0.22 MAR index. Therefore, in order to keep ready-to-eat meat safe for public consumption, vendors of RTE meats should take hygienic practices seriously.

DEDICATION

I dedicate this thesis to my wife, madam Alhassan Adam Hamdia and my children, Gufran Chalpang Abass and Nasara Abass for their patience and encouragement during my studies. Also to my father, Abdulai Ahamed and my mother, Fatima Dasaa for their endless prayers and support in my education.

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CHAPTER ONE

1.0 INTRODUCTION

Animal foods such as egg, meat and milk rich in proteins are important to the development and growth of the human body. Meat is a nutrient rich food that offers greater bioavailability of proteins, vitamins and minerals compared to other food sources (McAfee *et al*., 2010). However, most meats have high-water content that correlates with water activity of about 0.99 which is ideal for microbial growth (Rao *et al*., 2009). According to Chen *et al*. (2012) ready-to-eat meats (RTE) could harbor variety of microbes depending on the pH, texture, storage temperature and meat transport medium. These microbes in meats are often transmitted from food handling, polluted environment, equipment and utensils into meat and its products.

Micro-organisms such as moulds, viruses, bacteria including helminths are usually found in RTE meats. However, bacteria are often isolated from RTE meats and its products. Some bacteria species are now considered as high risk pathogens which were not recognized as a causative agent of foodborne infections (Lee Loir *et al*., 2003). For example, *E. coli, Campylobacter jejuni, Cyclospora cayetanesis and Listeria monocytogenes* are now considered high risk bacteria (Tauxe, 2002). *E. coli* strains are among the most dangerous and common foodborne pathogens responsible for food poisoning and food-related infections (Akbar and Anal, 2011). In developed nations, *E. coli* is responsible for 25% of childhood diarrhea (WHO, 2000).

E. coli is mostly the common species of facultative anaerobe of the family Enterobacteriaceae (Pitout, 2012). According to CDC (2014), *E. coli* are a large and

diverse group of bacteria. Some *E. coli* strains cause disease by producing toxins such as shiga toxin. The bacteria that make these toxins are called "Shiga toxin-producing *E. coli*" or STEC for short. STEC bacteria are usually transmitted to people when they eat foods contaminated with the bacteria (Fairbrother and Nadeau, 2006).

E. coli infections are mostly treated with antibiotics. In many countries, livestock farmers themsemselves engage in treating their animals against infections due to availability of drugs and its easy acquisition (Krecek and Waller, 2004) However, in some countries, it is illegal for farmers themselves to use drugs in animals (Acharya and Wilson, 2019). This is due to their inability to properly diagnose diseases in animals and use the right drugs for treatment (Walton and Currie, 2007). They also have little knowledge in site of drug administration, estimating accurate dosage and following withdrawal periods of antibiotics (Holmström *et al*., 2013).

According to Allen *et al.* (2013), antibiotics are not only used to treat and prevent animals from contracting diseases, but also to stimulate their growth and feed utilization efficiency. Abuse of of antibiotics in livestock has significant implications on public health because it encourages the production of antibiotic-resistant bacteria and genes that also become resistance to antibiotics can be transferred to people (Smith *et al*., 2002). This generally occurs through food intake, as well as through direct interaction with products such as food or by environmental mechanisms.

1.1 Problem statement

Most ready-to-eat meats are contaminated with foodborne pathogens thus becoming a possible source of foodborne infection (Tshipamba *et al.,* 2018). The processing of

meat after slaughter is associated with the contamination of meat by pathogens (Abdullahi *et al*., 2006). Therefore, failure to follow basic hygiene and sanitation practices can lead to meat contamination and subsequent foodborne infections.

Antimicrobial resistance is a major and increasing global healthcare problem (World Health Organization, 2012). A large number of bacteria have responded to antibiotic use with their ability to develop and spread antimicrobial resistance to other organisms since the introduction of penicillin (WHO, 2000). Increased consumption and improper use of antimicrobials are among factors that further accelerated this trend. Due to its outer membrane barrier, *E. coli* is intrinsically immune to therapeutic levels of penicillin G (Allocati *et al*., 2013). Different strains of *E. coli* also resist several different classes of antibiotics with distinct action mechanisms (WHO, 2000). The development and spread of multidrug-resistant strains of *E. coli* makes treatment for many severe infections difficult (Allocati, 2013). In hospitals and in the community, *E. coli* is the most common cause of infection (Ajiboye, 2009).

1.2 Justification

Several studies have been conducted and identified ready-to-eat food from various parts of Ghana contaminated with *E. coli*. For instance, *E. coli* was isolated from ready-to-eat food samples from Sunyani by Ofosu *et al*. (2014), in Accra by Mensah *et al*. (2002) and Agbodaze *et al*. (2005), and in Tamale by Abakari *et al.* (2008). In addition, Adzitey *et al*. (2015) identified only smoked guinea fowls contaminated with *E. coli* in Bolgatanga. However, there is little information on the occurrence of *E. coli* in ready-to-eat meats in the Bolgatanga municipality of Ghana. Hence, this study

assessed the prevalence and antibiotic resistance profile of *E. coli* isolated from ready-to-eat meats in Bolgatanga municipality. The study also sought to determine the knowledge of livestock farmers in the use of antibiotics.

1.3 Relevance of the study

At the end of the study, consumers of ready-to-eat meats should know that grilled meat sold by streets vendors contaminated with *E. coli* when meats are not properly handled. Also, antibiotics used by farmers to treat bacterial infections including *E. coli* infections in livestock identified. The study idenfied *E. coli* of ready-to-eat meats origin showed resistance to antibiotics used by farmers in livestock. In addition, this study could also add to scientific literature and serve as a basis for policy makers such government and non governmental organizations (NGO) to be able to improve public health.

1.4 Objectives of the study

1. To assess the knowledge of livestock farmers on antibiotic usage
2. To estimate the microbial load of ready-to-eat meats vended on the streets of Bolgatanga municipality
3. To determine the prevalence of *E. coli* in ready-to-eat meats vended on the streets of Bolgatanga municipality
4. To determine the antibiotic resistance profile of *E. coli* isolated from ready-to-eat meats.

CHAPTER TWO

2. 0 LITERATURE REVIEW

2.1 Ready-to-eat food (RTE)

These are foods which could be consumed without additional heating steps, because they have already undergone processes and making them healthy and prepared to be used (Muriana, 2002). A broad range of food items, such as fish, dairy products, meats and others, are ready-to-eat foods (Jofré *et al*. 2016). Several other foodstuffs, such as frozen dinners, ice cream and frozen fruit, are often referred to as ready-to-eat foods. Ready-to-eat foodstuffs are beneficial for travellers, so the market for such products is rising (Makinde *et al*., 2020). Mohammad *et al*. (2018) found that customer buying behaviour's surveys support the rise in ready-to-eat food intake. The demand for ready-to-eat foods is wide and rising (Hwang and Huang, 2010). Since ready-to-eat food items do not need any further processing before usage, the microbial hazards of these products are of interest to consumers (Mataragas, 2010). Some ready-to-eat foodstuff, such as sandwiches or meats, requires cutting or slicing, and handling that gives a chance for exposure with harmful bacteria (Todd and Notermans, 2011).

2.2 Sources of contamination of ready-to-eat meats

Mesophilic and psychrotrophic microorganisms normally contaminate raw meat. The initial number of microorganisms that can live, including pathogenic or sublethally harmed bacteria, spores and certain thermoduric bacteria such as Micrococci, Bacilli, Enterococci and Lactobacillili, will be substantially reduced by proper food preparation (Yousef and Balasubramaniam, 2012). Giraffa (2004) stated that, the

surviving microbial diversity contributes, qualitatively and quantitatively, to the overall microbial flora of the end product. Also, post-cooking handling practices, food supplies, meat storage conditions and length at points of sale can contribute significantly to the existence of microorganisms that are pathogenic and spoilable in ready-to-eat food (Henriques and Fraqueza, 2015). In most cases, ready-to-eat food such as beef, buns are processed in a central processing facility and then distributed to individual food stalls where they are heated up or packaged within a short span of time (Todd, 2007). However, the physical conditions in which such stalls are located lack sufficient facilities, such as clean drinking water and waste collection post treatment (Gowda, 2013). Because many vendors are congregated in overcrowded areas, liquid drainage or garbage disposal facilities are scarce (Howard and Bogh, 2002). These results in excessive waste disposal to local gutters, streets supplying excellent rodent habitats, fly breeding points and micro-organisms. Therefore, ready-to-eat meats found in open stalls have a greater chance of exposure to pathogenic microorganisms (Kotzekidou, 2016). Additionally, the use of unsanitize meat contact surfaces (cutting boards, knives, etc.) in food preparation and improper segregation of raw ingredients from finished meat can result in cross contamination with pathogenic and spoilage bacteria (Hui, 2012). Singh *et al*. (2011) found that the long-term state of ambient storage encourages the rise in microbial numbers to unsustainable levels, resulting in low quality meat with consequences. Faour-Klingbeil *et al*. (2015) found that staff in food handling plays a very important role in ensuring safety in the meat production, storage and preparation chain. A study found that a potential cause of *E. coli* and faecal

coliforms in ready-to-eat meat is the poorly washing hands of street food vendors (Faour-Klingbeil, 2017).

2.3 Pathogens in ready-to-eat meats

Clostridium perfringens, *Salmonella enterica* and *E. coli* are among the most prevalent harmful bacteria found in frozen ready-to-eat meat (Mor-Mur and Yuste, 2010). According to Nørrung (2009), *E. coli* and *Salmonella* are known to be natural part intestinal flora of animals and may lead to the detection of these pathogenic oganisms in meat due to faecal spoilage of ready-to-eat food. Podolak *et al.* (2010) reported that *E. coli* and *Salmonella* are highly resistant to pH and low humidity, enabling them to survive in food products. Also, a study by Wijnands *et al*. (2011) found *Clostridium perfringensis* is frequently found in meat and poultry. This pathogenic organism induces foodborne diseases and their existence in foods at levels that cause food poisoning in ready-to-eat meat or poultry products also results from inadequate cooling or cooking. Humans who ingest a meat contaminated with $>10^6$ viable *C. perfringens* vegetative cells, the pathogen will live and sporulate through the stomach and release enterotoxin into the intestines (McClane *et al*., 2012).

2.4 Foodborne diseases

Foodborne diseases are described as infectious or toxic diseases caused or perceived to have been caused by food or water intake (Newell, 2010). In advance economies, foodborne diseases are a significant public health concerns, as it includes a broad variety of diseases and accounts for a significant proportion of morbidities and deaths worldwide (Cohen, 2000). The World Health Organization's (WHO, 2015) estimates

of the global incidence of foodborne diseases report that, as many as 600 million, or around 1 in 10 people worldwide, fell ill in 2010. Epidemiological investigation in South Africa also revealed that 327 foodborne diseases outbreaks were reported with 11,155 people with the infections, 8,680 hospital visits recorded, 494 people hospitalized and 49 died (Shonhiwa *et al*., 2019). In Ghana, 420,000 patients suffer from foodborne diseases each year, with an approximate annual death rate of 65,000 (Ababio, 2015).

2.5 Food hygiene issues

According to WHO (2015), food protection refers to minimizing the occurrence of certain risks, whether chronic or acute, which may affect public health. Nyachuba (2010) reported that basic hygiene practice is the key to preventing food contamination and reducing foodborne diseases. Feglo and Sakyi (2012) found that food handling, processing and food hygiene practices were not experienced by suppliers. A study by Acheampong (2015) also revealed that 84.0% of food vendors used the same hands to serve and collect money from customers, 89% used their bare hands to serve or dish food, and 30.3% had not received a certificate for service. Also, chop bars, funerals, prepared meals, office canteens, parties, restaurants, street foods and school canteens were the predominant food source consumed by foodborne diseases patients (Todd, 2013). All these sources had fewer than 10 per cent of the reported cases, except for home meals and food sold on the market.

2.6 Impact of foodborne pathogens on public health

The public health impact of foodborne diseases is huge. Foodborne diseases (FBD) affect 6 to 80 million people each year in the United States, causing 9,000 deaths,

costing an estimated USD 5 billion, and are considered the world's largest cause of hospitalization and death. (Colavecchio *et al*., 2007). According to Nelluri and Thota (2018), the epidemiology of FBD is evolving rapidly as newly identified pathogens evolve and well-recognized pathogens increase in prevalence or associated with new food vehicles. Acute gastroenteritis can lead to chronic sequelae or impairment and several emerging foodborne diseases (Buzby and Roberts, 2009). For example, listeriosis may cause miscarriages in patients with chronic diseases or result in meningitis. Congenital malformation is a mainly caused by *E. coli*. Infection with *E. coli* O157: H7 is a leading cause of hemolytic uremic syndrome, the most serious cause of acute kidney failure in children in the USA (Scalise *et al*., 2019).

2.7 Controlling outbreaks of foodborne diseases

According to WHO (2012), by focussing on food preparation, in Africa, a significant reduction in the number of FBD cases can be achieved. In practice, only action at this level can control FBD caused by viral infections, toxins, *Clostridium perfringens*, Staphylococci and Bacillus (WHO, 2013). Although controls can lead to a decrease in Campylobacter and Salmonella infections in the food production sector, intervention in the public and private catering and household sectors can, also achieve the maximum decrease in these infections. Therefore, efforts should be focused on other infections (Griffith, 2000).

2.8 Meat

Meat is flesh like skeletal muscle plus any attached connective muscle tissue or fat that is ingested as food (Oddy *et al*., 2001). According to Leroy and Praet (2017) meats

are then produced as a result of slaughtering and butchering, thus killing and cutting flesh from animals. Tuntenel *et al*. (2003) stated that the main component of meat is protein and water, and this is often eaten along with other foods. While meat can be consumed in a raw form, it is often consumed in various ways after successive cooking and processing. Animal products are typically excellent protein sources that contain the basic amino acids which are well balanced and have high biological importance. (Biesalski, 2005). Also, meat is the most perishable of all staple foods, as it provides enough nutrients to help the production and growth of bacteria (Rawat, 2015). Bell *et al.* (2000) estimated that animal muscles are approximately 75% water, 20% protein, or 5% for fat, carbohydrates, and assorted proteins. Its protein content, available vitamins, minerals, lipids and savoury sensation are among the reasons why meat and meat products are consumed.

2.9 Types of meat

2.9.1 Chevon and mutton

Chevon and mutton are meat derived from goats and sheep respectively. Small ruminants are reared throughout Ghana. Their production is important in job creation, revenue generation and the provision of resources food (chevon and mutton) (Behera *et al*., 2019). Teye and Salifu (2006) reported that small ruminants are often slaughtered during Christmas, Islamic festivals, and months ahead of the peasant season. Therefore, chevon and mutton are common meats in Ghana, which most Ghanaians cherish. The bulk of these meats are cut into pieces, weighed in pounds and then sold to customers in the open markets. While chevon and mutton play a

significant role in the livelihood of most Ghanaians, due to poor meat handling conditions in some Ghanaian markets, they can be an important cause of human pathogenic microorganisms and, consequently, foodborne diseases (Dabassa, 2013).

2.9.2 Beef

The bovine carcass or meat is simply refered to as beef (Polkinghorne, 2008). According to Owusu-Sekyere (2014) beef is a ready source of animal protein to many Ghanaians as the three Northern regions of Ghana account for the majority of Ghana's cattle production, although some cattle are imported from neighboring countries into the country. The healthy living bovine muscle is practically sterile (Warriss, 2000). Beef can be contaminated by inaproperiate animal processing during pre- and post-slaughter handling (Węglarz, 2011).

2.9.3 Chicken

Domestic chickens are called Gallus domesticus, and belong to the family Phasianidae and with the genus Gallus (Nishibori *et al*., 2002). According to Mirkena *et al*. (2010), local chickens have adaptive characteristics which allow them to survive and reproduce under adverse environmental, nutritional and management conditions. The development of chicken in Africa has two categories: commercial (high input and high yield) and rural (village) (Alders and Pym, 2009). The commercial sector, according to Tegegne (2012), focuses on the intensive meat and egg production. They use high yield strains raised and supplied by international breeding organizations, while the village sector is known as the conventional, rural, scavenger, family, local or extensive production system. Local chickens' meat is portable, easily prepared, and low in fat

(Yang, 2010). They are also more appetizing than the exotic broiler breeds (Kolawole, 2010). Compared to the exotic breeds, local chicken has genetically poor carcass weight and egg production capacities (Busuulwa, 2009). Also, Zelizer (2017) found that 36% of local chicken were consumed in household, 33% are sold for money, 16% are used for ceremonies, 13% are donated as gifts and 2% are used for other purposes.

2.9.4 Guinea fowl

Raised in Africa, guinea fowls (*Numida meleagris*) were first domesticated by ancient Egyptians (Saina, 2005). Their development has risen rapidly all over the world. The bird is now raised commercially on a large scale in France, Belgium, Canada and Australia (Tharme, 2003), and sadly in Africa its development is in its infancy (Smith, 2000; Ligomela, 2000; Saina, 2001), since it was linked to small-scale farmers (Smith, 2000). In general, guinea fowls, unlike chicken and turkey, are resistant to common virulent diseases such as Newcastle disease, fowl pox, and gumboro which tend to wipe out the poultry population (Avornyo, 2016). According to Alders (2004), guinea fowls are highly priced in Africa because of its superior nutritional value compared to chicken commodity imported. Guinea fowl will grow to 1kg or more in weight, and their meat will be very tasty, almost pheasant-like (Roots, 2006). With the added advantage of having less fat and less calories, Guinea fowl meat is also richer in flavor than chicken meat (Soriano-Santos, 2010). The pure white guinea fowl tastes a little less gamey and the meat has a lighter colour (Zaraska, 2016). Patton (2011) stated that closer to a free-ranging chicken; guinea fowl meat is lean and high in vital fatty acids. The meat is low in calories and has less calories than turkey meat. Guinea fowl meat per 100 grams has about 134 kilocalories (kcal) and turkey meat has about 109 kcal.

2.9.5 Pork

According to Ruvinsky (2010), one of the species in the genus Sus, within the even-toed ungulate Suidae family, is a pig. Piglets are referred to as young pigs (D'Eath 2005). Pigs include domestic pigs and, along with other animals, their decendants, the popular Eurasian wild boar (Sus scrofa) (Owen *et al*., 2014). Pigs, like all suids, come from the continents of Eurasia and Africa, ranging from Europe to the Pacific islands (Jori and Bastos, 2009). Indonesia's Babirusa, Asia's Pygmy Hog, Africa's Warthog, and another genus of African pigs are distinct from pigs. Suids are a clade of Peccaries Sisters (Nidup, 2011). Pigs are social creatures and inherently intelligent (Joy, 2020). Pigs are similar to humans in genetics and are also often used for human clinical trials (Masson, 2004).

Pork is the meat of a domestic pig (Beattie *et al*., 2000). With evidence of pig husbandry dating back to 5000 BC, it is the most widely eaten meat worldwide (Mithen, 2011). Both freshly cooked and preserved, pork is eaten. Curing increases, the shelf life of items made from pork (Cassens, 2008). Van Esterik (2008) found that not only pork is one of the most common meats in the Western world and Central Europe, pork is also very common in India, Southeast Asia and in the Eastern and non-Muslim regions of Malaysia. According to Guo *et al*. (2016), pork is highly valued for its fat content and texture in Asian cuisines, particularly in China. Certain religions and societies forbid the eating of pork, especially Judaism and Islam (Easterbrook and Maddern, 2008). Kyriazakis (2006) stated that pigs are the world's most commonly consumed commodity, accounting for around 36% of the world's meat production.

2.10 Microbial contamination of meat

Meat is a highly perishable food and can become infected or spoiled and dangerous due to microbial growth and activities, unless properly stored, processed and distributed (Dave and Ghaly, 2011). All meat products especially fresh meats have a small number of microorganisms that can multiply to render meat unwholesome when exposed to favorable growth. Hughes *et al*. (2007) stated that poultry meat, red meat, deserts and egg are recognized as a major vector for pathogen transmission, such as *E. coli*. Because of its perishable nature, red meat and poultry are more likely to become pathogen infected during handling (Greer, 2005). Existence of pathogenic bacteria such as *E. coli* and others or their toxic products make meat unfit for human consumption and cause gastrointestinal diseases (Kuhnert, 2000). Newell *et al*. (2010) reported that, in the food industry particularly in the meat industry numerous food safety systems and procedures are available to decrease the threat of contamination and pathogen transmission.

Food hygiene micro-organisms of interest include helminthes, moulds, bacteria, and viruses (Kramer *et al*., 2013). Ninios *et al*. (2014) stated that bacteria play the most important role within these groups. Parasites are of no importance in meat which has passed post mortem inspection or where successful internal parasite control programs or measures are in place. The most widely known bacterial pathogens attributed to meat consumption are *Salmonella spp, Compylobacter spp, Staphylococcus aureus, E. coli, Listeria monocytogenes, Clostridium perfringens, Yersinia enterocolitica, Bacillus cereus* and *Vibrio parahaemolyticus* (Cho *et al*., 2008). In fresh meat and poultry, *Campylobacter spp, Salmonella spp,* and *E. coli* are the primary bacterial

pathogens that are present (Thormar and Bergsson, 2006). Minami *et al.* (2010) reported that in supermarket and meat shops, foodborne pathogens isolated from meat samples were *E. coli, Listeria spp, Salmonella enteritidis* and *Shigella* while Staphylococcus and Shigella spp were found in the meat handling equipment. *E. coli* and other bacteria such as *Bacillus cereus*, *Staphylococcus aureus* and *Clostridium perfringen* in meat sample were isolated (Borch and Arinder, 2010). Doyle (2007) therefore claimed that meat should be kept in the coldest part of the freezer or frozen, practicing good hygiene to avoid bacterial contamination.

2.11 Total coliform count and enterobacteriacea

Generally, coliforms are usually not dangerous; the presence of possible disease causing microorganisms is indicated by coliforms presence in meat samples (Cabral, 2010). Coliform micro-organisms are classified as gram-negative non-spore production in the shape of rods and motile or non-motile bacteria which can ferment lactose to produce gas and acid when incubated at 35-37°C (Prabhurajeshwar and Chandrakanth, 2017). In the surrounding areas such as trees, dirt, and even human and animal faces, aerobic coliform might naturally be found (Greub and Raoult, 2004). The prevalence of coliform bacteria in food and water suggests that additional fecal-based pathogenic microbes may be present, including bacteria, protozoa, viruses and several disease-causing multicellular parasites (Kanangire, 2013). Ekli (2019) reported that *E. coli*, Citrobacter, Klebsiella, Hafnia and Enterobacter are examples of coliform bacteria. Coliforms are classified as total coliforms, fecal coliforms and thermos-tolerant bacteria. Total coliform is classified as the large diverse bacteria that live in the gut (Espinosa *et al*., 2009). Fecal coliform is a category of bacteria present

in feces including *Salmonella* and *E. coli*. Fecal coliform microbes are the part of the total coliform bacteria from human or animal intestines and faeces, may also be considered thermo-tolerant microbes (Ashbolt, 2004). Thermos-tolerant bacteria are proposed, along with *E. coli*, as an indicator for fecal pollution (Svanevik *et al*., 2015). Enterobacteriaceae is a family of a broad, heterogeneous group of gram-negative rods (Ksoll, 2007). According to Verraes *et al*. (2013), they can be present in soil and on plants, and they can be a source of food chain contamination and cause gastroenteritis in foodstuffs. When found in foods, they are treated as sources of fecal contamination. Enterobacteriaceae are facultative aerobes and anaerobes capable of fermenting a distinct range of carbohydrates, possessing a complex antigenic structure, and generating a number of toxins and other factors of virulence (Kilian, 2015). Escherichia, Shigella, Salmonella, Yersinia, Enterobacter, Klebsiella, Serratia, Proteus and others belong to the genera in this family (Forsythe, 2012).

The Ghana Standard Authority (2013) recommends that fully prepared meat microbial contamination should be < 5log cfu/g. Kim and Yim (2016) observed average coliform counts for beef, pork, and chicken samples to be 2.90, 3.19, and 3.79log cfu/g, respectively ($P < 0.05$) in meat samples collected from Korea. Accra Central samples had TPC of 4.08 and Nima samples had TPCs of 4.80 log10 cfu/g of khebab as well as the findings gathered from Khebab samples at Osu recorded mean total plate count (TPC) of 5.02. Accra Central samples recorded the highest mean coliform count (5.12) with 4.41 and 3.70 log10 cfu/g respectively with samples purchased from Osu and Nima. Also, Accra Central samples again reported the highest faecal coliforms (4.4 log10 cfu/g) compared to 3.98 and 3.80 log10 cfu/g for samples purchased from Osu

and Nima, respectively (Agbodaze *et al*., 2005). According to Ampaw (2018) at the Banana Inn vending area, grilled street meat sold reported a microbial count of 5,929 ± 1,064 log10cfu/g while in the Tabora vending area the minimum count of 2,739 ± 0,370 log10cfu/g was recorded. Madina had the highest TVC load (7.267 log10 cfu/g) while Sowutuom had the lowest total viable count (TVC) of 4.732 log10 cfu/g in the vending region. In the Banana Inn sales area (6,394 log10 cfu/g), the total coliform count (TCC) was the highest, while the lowest count of 0.00 log10 cfu/g was in the Dome, Legon, North Kaneshie, and Tabora sales zones. Adzitey *et al*. (2019) noticed that the aerobic plate count for raw beef beef samples was 3.59 log cfu/cm2 and had the largest bacterial load, which was also significantly greater ($P < 0.05$) than grilled beef samples for 0 hours (2.94 log cfu/cm2 and 2.83 log cfu/cm2 for 1 hour 30 minutes).

2.12 *E. coli*

The genus of Escherichia is a facultative anaerobic gram-negative bacillus from the Enterobacteriaceae family and named after the German pediatrist Theodor Escherich (Torres *et al*. 2010). *E. coli* is widely spread in natureand constitute the predominantly facultative anaerobic bacteria found in large intestines of humans and other animals (Jang *et al*., 2017). There are hundreds of *E. coli* strains according Ochman and Davalos (2006). Some are harmless and reside in healthy human and animal intestines. Each group of *E. coli* strains has a distinct group of somatic (O) and flagella (H) antigens and has unique characteristics of virulence which are typically plasmid-mediated (Dulguer *et al*., 2003). *E. coli* has unique properties that are so easy to

control, the availability of the full genome sequence and its ability to grow in both aerobic and anaerobic environments, making it an important host organism in biotechnology (Alper and Stephanopoulos, 2009). It is used in a wide range of pharmaceutical and biological use, also, *E. coli* is the most widely used microorganism in recombinant DNA technology (Yoo *et al*., 2009).

2.12.1 Enterohemorrhagic *E. coli* (EHEC)

Hemorrhagic colitis is caused by *E. coli* 0157: H7 and less often by other *E. coli* (O26: H11) serotypes which produce cytotoxins similar to those found in *Shigella dysenteriae*, type 1. These toxins are called shiga, such as toxins and verotoxins (Engering and Slingenbergh, 2013). Enterohaemorrhagic *E. coli* has been linked to severe and bloody diarrhoea, as well as haemorrhagic colitis (HC) and haemorrhagic uraemic syndrome (HUS), particularly in children, over the last two decades (Verweyen *et al*., 2000). Global outbreaks of bloody diarrhea and HUS are caused by EHEC serotype O157:H7 (Karch *et al.*, 2005).

2.12.2 Enteroinvasive *E. coli* (EIEC)

These strains include unique *E. coli* serorypes (O28, O112, O115, O124, O136, O143, O144, O147, O152, O164 and O167) which differ from EPEC serotypes. The EIEC strains biochemically resemble Shigella and may invade the epithelial intestinal cells (Ud-Din and Wahid, 2014). Enteroinvasive *E. coli* is a pathogenic bacterium that causes a syndrome similar to shigellosis, with profuse diarrhea and a high fever (Chellapandi *et al*., 2017). EIEC are highly invasive, and they bind to and enter intestinal cells via adhesin proteins (Walsh *et al*., 2009). Dysentery caused by EIEC

usually occurs within 12 to 72 hours of consuming contaminated food. The illness is characterized by abdominal cramps, diarrhea, vomiting, fever, chills, and a generalized malaise (Bintsis, 2017).

2.12.3 Enteropathogenic *E. coli* (EPEC)

Traditionally, EPEC strains were identified as unique *E. coli* members. O44, O55, O86, O111, O114, O119, O125, O126, O127, O128, O142 and O158 are serotypes which were epidemiologically incriminated as triggers of childhood diarrhea (DuPont *et al*., 2009). More recently, the concept of EPEC was focused on distinct infectivity attributes. The strains of enteropathic *E. coli* adhere to the gastric tract and cause a distinctive gastrointestinal lesion. Enteropathogenic *E. coli* cause no enterotoxins and are not invasive (Ud-Din and Wahid, 2014). By definition, all EPEC lack the genes required to produce shiga toxin (*stx*) (Ferdous *et al*., 2015). *E. coli* strains with the phenotype *eae*, *+bfpA*, and *+stx* are classified as typical EPEC (tEPEC) (Abe *et al*., 2009). The majority of these strains belong to classic O:H serotypes and produce the localized adherence (LA) phenotype associated with BFP production (Trabulsi *et al*., 2002). *E. coli* strains that are *eae*, *+bfpA* and *stx*, on the other hand, are classified as atypical EPEC (aEPEC) and these strains exhibit patterns of localized-like (LAL), diffuse (DA), or aggregative adherence (AA) (Jafari *et al*., 2012). In aEPEC, the LAL pattern is associated with the *E. coli* common pilus and other known adhesins (Skaletsky *et al*., 2010). Among aEPEC strains, more than 200 O-serogroups have been identified; the majority do not belong to the classic EPEC O-serogroups, and many have been designated non-typeable (Ochoa and Contreras, 2011).

2.12.4 Enterotoxigenic *E. coli* (ETEC)

According to Robins-Browne and Hartland (2002), without reaching the intestinal tract, ETEC strains colonize and grow thermally enterotoxins. These strains are O6: H16, O8: H9 and O8: H, O15: H1 (Machado *et al*., 2000). Enterotoxigenic *E. coli* is a common cause of bacterial diarrhea (Fleckenstein *et al*., 2010). ETEC infection is the leading cause of travelers' diarrhea and a major cause of diarrheal disease in low-income countries, particularly among children (Khalil *et al*., 2018). ETEC is spread through contaminated food or water containing animal or human feces (Yang *et al*., 2017). Infection can be avoided by avoiding or safely preparing foods that may be contaminated with the bacteria, as well as by frequently washing hands with soap (Avery *et al*., 2019).

2.12.5 Enteroaggregative *E. coli*

They are characterized in tissue-culture based assays by their characteristic adhesion pattern. All the strains display the "stacked brick" adhesion pattern on HEP-2 or HeLa cells, unlike EPEC, which demonstrates localized adherence (Aijuka, 2018). An extra band *E. coli* that adheres to these cell lines diffusely requires more explanation (Aijuka, 2018). EAEC is now recognized as a new enteric pathogen (Huang *et al*., 2004). EAEC are reported to be the second most common cause of traveler's diarrhea, second only to enterotoxigenic *E. coli*, and a common cause of diarrhea in pediatric populations (Adachi *et al*., 2001). A serious outbreak in Germany in 2011 raised awareness of EAEC, resulting in over 5000 cases and at least 50 fatalities (Meng *et al*., 2012). The pathogen was identified as an EAEC O104:H4 strain that was lysogenized by a Shiga toxin encoding phage (typically associated with Shiga toxin-

producing *E. coli*, which frequently encode the adhesin intimin) (Navarro-Garcia, 2014).

According to Allocati *et al.* (2013), the various pathogenic *E. coli* strains are presented in Table 2.1.

Table 2.1. Pathogenic *E. coli* strains

Pathotype	**Diseases**	**Symptoms**	**Virulence factors**
EnteroPathogenic *E. coli* (EPEC)	Diarrhoea in Children	Watery diarrhoea and vomiting	Bfp, Intimin, LEE
EnteroHaemorrhagic *E. coli* (EHEC)	Haemorrhagic Colitis, HUS	Bloody diarrheoa Shiga toxins	Intimin, Bfp
EnteroToxigenic *E. coli* (ETEC)	Traveler's diarrhoea	Watery diarrhoea and vomiting	Heat-labile and sheat-stable toxins, CFAs
EnteroAggregative *E. coli* (EAEC)	Diarrhoea in children	Diarrhoea with mucus and vomiting	AAFs, cytotoxins
Diffusely Adherent *E. coli* (DAEC)	Acute diarrhoea in children	Watery diarrhoea, recurring UTI	Daa, AIDA
EnteroInvasive *E. coli* (EIEC)	Shigellosis-like	Watery diarrhoea; dysentery Shiga toxin, hemolysin	Cellular invasion, Ipa
Adherent Invasive *E. coli* (AIEC)	Associated with Crohn disease	Persistent intestinal inflammation	Type 1 fimbriae, Cellular invasion
Extraintestinal E. coli (ExPEC)			
UroPathogenic *E. coli* (UPEC)	Lower UTI and systemic infections	Cystitis, pyelonephritis	Type 1 and

Neonatal Meningitis *E. coli* (NMEC)	Neonatal meningitis	Acute meningitis, sepsis	P fimbriae; AAFs, hemolysin S fimbrie; K1 capsule
Avian Pathogenic *E. coli* (APEC)	Probable source of food-borne disease	-	Type 1 and P fimbriae; K1 capsule

Bfp: Bundle-forming pili; LEE: Locus for enterocyte effacement; HUS: haemolytic-uraemic syndrome; CFA: colonization factor antigen; AAF: aggregative adherence fimbria; Daa: diffuse adhesin; AIDA: adhesion involved in diffuse adherence; Ipa: Invasion plasmid antigen.

2.13 Transmission of *E. coli*

E. coli normally occurs in healthy human and warm-blooded animal digestive system, and transmitted by oral-fecal route (Cabral, 2010). *E. coli* is almost entirely of faecal origin and spread through faecal-oral transmission. Also, contaminated water and by cross-contamination during food preparation or direct human contact with the *E. coli* can be transmitted (Nakhala, 2013). Meanwhile, contaminated foods such as raw or uncooked ground meat products, raw milk, and fresh produce tend to be the primary route of exposure (Gibson *et al*., 2019). Manning, (2010) reported that infections by *E. coli* involve undercooked or raw hamburgers, bovine livestock, pigs, goats, chickens, toys, lukewarm fresh meat, sproutings, dry-curated salamis, broccoli, cheese curds, unpasteurized milk or round milk, infected water and ice, and person-to- person spread.

2.14 Susceptibility of humans to *E. coli* infection

A specific concern is the intake of dangerous foods by more vulnerable individuals, such as children, and people with immune systems compromised by illness, or by the mechanism of pathological response itself (Gombart, 2020). Individual who work in slaughterhouses, fisheries, hospitals, nursing homes, kindergarten schools and food preparation facilities are more vulnerable to infection than other population (Carrasco *et al*., 2012).

2.15 Prevention and control of *E. coli*

Overall, the strategy for preventing and managing the transmission of *E. coli* should include; providing access to clean drinking water, effective food-contamination management procedures, hygiene initiatives, public education and vaccination (Michaels, 2004). A study by Abdulmumeen (2012) found that the primary goal for avoiding *E. coli* infections is access to clean water. Steps to protect food items from contamination require optimum temperatures for storage and boiling (Gomes-Neves *et al.,* 2007). To substantially reduce the bacterial load, techniques of food irradiation may be used in high-risk products. Hospital measures that reduce the likelihood of propagation of multi-resistant pathogens include the avoidance of cross-contamination by following basic hygienic quality guidelines and restricting the use of antibiotics (Capita and Alonso-Calleja, 2013). The key pathways for the transmission of pathogens are the hands and pharmaceutical products of healthcare personnel (Fisher and Shaffer, 2014). For cross-contamination prevention, good hand hygiene is important (Page *et al*., 2009).

It is important to use antibiotics for the prevention and control of *E. coli* infections in humans and animals (Van Den Bogaard and Stobberingh, 2000). It is widely agreed, however, that resistance to antimicrobials is correlated with the amount of antibiotic consumption (Van De Sande-Bruinsma, 2008). Underdose and abuse of antimicrobials increase resistance in pathogens both in normal human or animal bacterial flora (McEwen and Fedorka-Cray, 2002). Also, probiotics can be an alternative to the drug therapy of multiple infections with *E. coli* (Paton *et al.*, 2006).

Probiotics are stable and viable microorganisms that can colonize the gut tract and thus interact with pathogens, mainly Lactobacillus and Bifidobacterium. Several research on the potential use of probiotics to avoid or treat gastrointestinal infections have been conducted (Sanders, 2010). Rohde *et al*. (2009) stated that infectious diarrhoea chemotherapy with probiotics has shown positive benefits by reducing the incidence of diarrhoea. A vaccination may be the main human technique to defend against the most harmful strains, such as ETEC, UPEC and NMEC. However, no potent vaccine is presently capable of preventing these infections (Aliocati, 2013).

2.16 Prevalence of *E. coli* in ready-to-eat meats

The incidences of *E. coli* were 46.7%, 40% and 33.3% of the examined ready to eat meat hawawshi, kofta and shawerma samples respectively collected from different fast-food services in different districts in Menofia governorate (Hassanani *et al*. 2014). Ahamady *et al*. (2012) also found that only 5 samples (5.88%) out of 85 ready-to-eat meat and meat products found to be contaminated with *E. coli*. Shaltout *et al*. (2017) revealed that the incidence of *E. coli* in the examined ready to eat fast food samples

were 20%, 8%, 32% and 40% for sandwiches of kofta, shawerma, sausage and liver, respectively. The highest prevalence of *E. coli* was reported by Barua *et al*. (2007), in ready-to-eat meat products, was found to be 35.21%. Also, Ahmadi *et al*. (2012) revealed that out of 33 ready-to-eat meats and meat products, meat curry and non-veg momo recorded positive for *E. coli*. Furthermore, 212 chicken samples were identified by Zhao *et al*. (2001), 82 (38.7%) were contaminated with *E. coli*, while 40 (19.0%) of the 210 beef samples were positive for *E. coli*, 34 (16.3%) of the 209 pork samples and 23 (11.9%) of the 194 turkey samples were positive for *E. coli*. In addition, the total prevalence of *E. coli* in food of animal origin was 37.86%, 49.02% in chicken meat and 70% in beef in Bangladesh based on cultural, staining and biochemical characteristics (Rahaman *et al*., 2017). Another research in South Africa found that *E. coli* was most prevalent bacteria in meat samples with 67.5% (Tanih *et al.*, 2015).

2.17 Antibiotics

Early workers described antibiotics as any substance formed by a microorganism in a high dilution that is adversarial to the development of other high microbes (Dennis and Webster, 1971). Nevertheless, antimicrobials are naturally derived or process chemically capable of destroying or preventing the development of microorganisms (Pelaez, 2006). The word antibiotic is used as a similar term for antibacterial drugs used to treat bacterial diseases in both man and livestock (Defoirdt *et al*., 2007). They are popularly used for disease and infection prevention, monitoring and treatment (Ventola, 2015). Alanis (2005) stated that although, awareness of how antibiotics function has evolved in recent years, antibiotics have not been fresh for decades. Molds were used by Chinese and Central American Indians to treat infected wounds

by the ancient Egyptians, centuries ago, but they did not know how or why these molds were. Scientists started to classify antibiotics extracted from molds in the late 1880s and set a specific phase in health care (Levy, 2013). However, it was not until the 1940s that penicillin, which is extracted from a mold, became available. Murray *et al*. (2008) found that military personnel wounded or disabled during World War II were saved through its widespread use to treat infections. Throughout the 20th century, farmers have introduced antibiotics into their systematic animal husbandary practice, including clean water and nutritious food for their animals, heat and cold protection, vaccines and therapeutic care when required (Sudhi, 2020). Pankey and Sabath (2004) stated that antibiotics are categorized in practical ways that are called narrow if they only inhibit and gram positive and negative bacteria and broad spectrum if they kill bacteria that are both gram negative positive.

2.18 Benefits of antibiotics

Antibiotics save the lives of peoples by playing a key function in making most important advancements in medication and surgical treatment (Ventola, 2015). Infections that can infect patients seeking medical treatment, who have chronic diseases such diabetes, end-stage renal disease or rheumatoid arthritis, or who have undergone complicated procedures such as organ transplants, joint replacements, or cardiac surgery have also been successfully avoided or treated. Manyi-Loh *et al*. (2018) reported that antibiotics minimize death rate due to foodborne and other destitution infections. Worldwide, antibiotics have had similar beneficial effects.

2.19 Classes of antibiotics

It is possible to group antibiotics because of their molecular structures, means of action (bacteriostastic and bactericidal) and spectrum of operation (broad and narrow) (Hancock, 2005). Nicolau (2008) stated that methods of administration (oral and injection) are other ways to identify antibiotics. Whether they have the same molecular structure or class, the time limit, efficacy, and allergic effects of antibiotics are typically similar. Macrolides, quinolones, sulphonamides, beta lactams, aminoglycosides, tetracyclines, oxazolidinones and glycopeptides are any classification of antibiotics based on a chemical or molecular (Etebu and Arikekpar, 2016). According to Casadevall (2004), it is also possible to group veterinary drugs based on the type of disease causing the targeted organism or the types of disease they treat.

2.20 Common antibiotics used by livestock farmers

2.20.1 Gentamicin

According to Filazi *et al*. (2005) gentamicin is used primarily as an injection solution for pigs, cattle and horses and as an oral solution for poultry in veterinary medication. In human medication, it is often used, frequently as an injection solution for intramuscular administration. It is officially included in the World Health Organization's (WHO) list of essential medicines for human use (Tripathi, 2013). Mohammed *et al*. (2016) revealed that gentamicin is effective against the various pathogenic bacteria, including Pseudomonas, Proteus, *E. coli, Klebsiella pneumoniae, Enterobacter aerogenes*, Serratia, and Gram-positive Staphylococcus. The use of

gentamicin is to traeat infections of these susceptible bacteria in the respiratory tract, urinary tract infections, blood, bone, and soft tissue. Gentamicin is an antibiotic with aminoglycosides. Amikacin, tobramycin, neomycin, streptomycin, and kanamycin also include other aminoglycosides (Leclercq *et al*., 2013). Gentamicin is a broad-spectrum antibiotic that is bactericidal (except for streptococci and anaerobic bacteria). Its mechanism of action includes inhibition of bacterial protein synthesis by binding to 30S ribosomes (Arenz and Wilson, 2016). According to Arenz and Wilson (2016), for immediate, severe infections, such as those caused by gram-negative bacteria, gentamicin is used. Against resistant strains of bacteria, amikacin is more reliably active (Magnet and Blanchard, 2005).

2.20.2 Tetracycline

It is possible to access three natural tetracyclines (oxytetracycline, chlortetracycline and demethylchlortetracycline) and several semi-synthetic tetracyclines (tetracycline, rolitetracycline, methacycline, minocycline, doxycycline, lymecycline and others) (Adegboye, 2011). Further grouping into short-acting (tetracycline, oxytetracycline and chlortetracycline), intermediate-acting (demethylchlortetracycline and methacycline) and long-acting (doxycycline and minocycline) is allowed during elimination processes (Devi *et al*., 2019). Reversible binding to the bacterial 30S ribosomal subunit is reflected by the antibacterial activity of tetracyclines (Pommier *et al*., 2010). Tetracyclines are stronger and effective against multiplying microorganisms at a pH of 6 to 6.5 (Taylor-Robinson and Bebear, 1997). In general, tetracyclines are the medication of choice for rickettsiae and mycoplasma treatment Wolbachia, an intracellular rickettsial endosymbiont of nematodes, such as *Dirofilaria*

immitis, is among the susceptible species. As several pathogenic *E. coli* isolates are resistant, including *Pseudomonas aeruginosa*, Proteus, Serratia, Klebsiella, and *Trueperella spp* strains (Saidani *et al*., 2018). While there is general cross-resistance between tetracyclines, Staphylococci are typically resistant to doxycycline and minocycline (Ross *et al*., 2001). Giguère *et al*. (2013) advised that tetracyclines should not be given per os (PO) to ruminants at therapeutic concentrations because they absorbed poorly and can significantly suppress the microbial activity of ruminants.

2.20.3 Suphamethoxazole/trimethoprim

Sulfamethoxazole/trimethoprim is a possible antibiotic / antimicrobial sulfonamide administer for the treatment of ear infections, urinary tract infections, bronchitis, traveler's diarrhea, shigellosis and Pneumocystis jiroveci pneumonia, and has brand names such as Co-trimoxazole ®, Primsol ®, Bactrim ®, Sulfatrim ®, Novo-Trimel ®, and Septra ® (Gordon, 2008). Reygaert (2018) found that trimethoprim is a dihydrofolate reductase enzyme inhibitor which prevents bacteria from forming folate. This drug raises unconjugated bilirubin levels in the blood. During the first trimester, folic acid supplementation is required. Many microorganisms, including *Acinetobacter baumannii, Actinobacillus actinomycetemcomitans, Listeria monocytogenes, Enterobacter aerogenes, Salmonella* Typhi, *Streptococcus pneumonia, Escherichia coli, Yersinia enterocolitica, Yersinia pseudotuberculosis*, including various Mycobacteria and others, are susceptible to suphamethoxazole/trimethoprim (Rouli *et al*., 2015). According to Lim *et al*. (2012) this drug can be administered by oral, subcutaneous and through intravenous.

2.20.4 Amoxycillin/clavulanic acid

According Pozzi and Ben-David (2002) the chemical structure of clavulanic acid, formed by *Streptomyces clavuligenus*, is similar to some ßlactamines, e.g. penicillin. It binds in the presence of clavulanic acid of the bacteria extra-cellular space and inactivates ß-lactamases. Bacteria are therefore susceptible to ß-lactamine (Pozzi and Ben-David, 2002). Laxminarayan *et al*. (2013) stated that the mechanism of action of clavulanic acid forms an irreversible bond with ß-lactamase, clavulanic acid prevents ß-lactamase from inhibiting ß-lactamine bactericidal activity. Clavulanic acid is able to make ß-lactamase producing bacteria susceptible again to a significant number of penicillin and cephalosporin-class antibiotics due to its irreversible binding and inactivation of ß-lactamases (Pozzi and Ben-David, 2002). Zafar *et al.* (2017) found that as in human preparations, pharmacological preparations containing amoxyclav at a ratio of 4:1 or 2:1 completely ensure the amount of clavulanic acid required to inactivate all ß-lactamases responsible for inactivating ß-lactamine. The amoxi-clav combination is considered useful for intramuscular administration in animal food producers, in particular for infections of the lower respiratory tract in bovine animals caused by Actinobacillus, Haemophilus and by producers of Pasteurella ß-lactamase (Pozzi and Ben-David, 2002). Amoxi-clav formulations are used in food-producing animals to treat acute infections in the short term (a few days) (Battisti and Franco, 2006). Long-term treatment, by any path, is not suggested (Battisti and Franco, 2006). It is especially unlikely that this short-term use in individual animals would be chosen for resistance, because repeated and long-term treatment is not involved (Baym *et al*., 2016).

2.20.5 Chloramphenicol

A studied by Moffa and Brook (2015) identified chloramphenicol is active against many distinct bacterial diseases as a broad-spectrum antimicrobial, including those induced by anaerobic bacteria and Rickettsia. Auerbach *et al*. (2002) reported that by binding to the 50S subunit of the 70S ribosome and inhibiting the function of peptidyl transferase, phenicoles suppress microbial protein synthesis. Peptides do not elongate because peptide-bond formation is hindered (Wang and Hayashi, 2021). Usually their action is bacteriostatic, but chloramphenicol can be bactericidal for some bacteria at high concentrations (Panky and Sabath, 2004). In both prokaryotic and eucaryotic (mitochondrial) ribosomes, protein synthesis is inhibited (Pozzi and Ben-David, 2002). It is also possible to give chloramphenicol orally, and it's reasonably inexpensive. Unfortunately, this substance can also be very toxic to the animals and human being who come into contact with it (Levy, 2013). Snyder (2008) found chloramphenicol to induce suppression of the bone marrow, where red and white blood cells are formed, in some animals. This is more of an issue for long-term use, but if the bone marrow is suppressed, the problem is normally overcome by stopping treatment with chloramphenicol. Chloramphenicol is banned from being used in all food animals in many countries (Landoni and Albarellos, 2015). It may not be used in many other species, but in cats it may be used (Schwarz *et al*., 2004).

2.20.6 Ciprofloxacin

The broad-spectrum antibiotic used to treat infections is ciprofloxacin (Ali *et al*., 2010). This medication is not licensed by the Food and Drug Administration for use in animals, but is legally administered as an extra-label drug by veterinarians (Giguère

et al., 2013). In general, DNA synthesis inhibitors include ciprofloxacin and other fluoroquinolones, as well as quinolones (Hooper and Jacoby, 2016). Pommier *et al*. (2010) stated that during bacterial DNA replication, they primarily bind to the DNA gyrase enzyme (or topoisomerase), and this binding prevents the enzyme from performing its biological function of cutting, repairing and coiling DNA molecules during bacterial DNA replication. The role of DNA gyrase in DNA replication will be inhibited as soon as ciprofloxacin binds to the enzyme topoisomerase (Tran *et al*., 2005). Since inhibition of DNA synthesis in bacteria prevents cell division, the target pathogenic bacteria ultimately die, and this obstructs the organism's other significant cellular activities (Bansal *et al*., 2017). Ciprofloxacin is used to treat bacterial infections that are gram-negative and gram-positive, and is also used in combination with other antibacterial agents. Fluoroquinolones in combination with ciprofloxacin are used for anthrax therapy (Oliphant and Green, 2002). And they are used clinically for the prevention of UTIs, respiratory tract infections, skin infections, anaerobic bacteria and infections with Chlamydia (Oliphant and Green, 2002). Quinolones (e.g. nalidixic acid) are primarily used against Gram-negative bacteria and are primarily used for the treatment of urinary tract infections (UTIs). Typically, fluoroquinolone and quinolone bacterial resistance is due to mutations in the chromosome of the organism, rendering them less susceptible to the drug (Poole, 2000).

2.20.7Azithromycin

Azithromycin is an antibiotic derived from a semi-synthetic macrolide called erythromycin (Topp *et al*., 2016). It is used to take care of infections caused by bacteria (Fernandes *et al*., 2017). In the treatment of dogs and cats, azithromycin is more

generally an option than erythromycin because it has a higher half-life and both animals utilize it faster (Giguère, 2013). Among many macrolides, Azithromycin is a bacteriostatic antibiotic that links to ribosome 50s by suppressing protein synthesis (Giguère, 2013). This treatment has been proven to be successful against a lot of different pathogens of Gram-positive and Gram-negative bacteria, but has some efficacy against anaerobic bacteria (Poelstra *et al*., 2001). Lappin *et al*. (2019) has shown that azithromycin is active against rickettsia, spirochetes, and protozoa (e.g. *Toxoplasma gondii, Giardia spp.,* and *Cryptosporidium spp*.) in addition to its effectiveness against bacteria. After per os (PO) injection in dogs, azithromycin is extremely bioavailable (97%), and only moderately bioavailable in cats (58%) and humans (37%). In dogs, tissue levels which were proportional to the dose were generated at a single oral dose of 10 to 40 mg/kg (Davis *et al*., 2006). Four to seven-fold increases in tissue concentration were recorded in dogs after treatment of 20 mg/kg for 7 days (Almeida *et al*., 2014).

2.20.8 Ceftriaxone

Like other third generation cephalosporins, ceftriaxone is active against strains of Haemophilus and Neisseria (Lamb *et al*., 2012). Ceftriaxone, however, has no beneficial activity against *Pseudomonas aeruginosa*, unlike ceftazidime and cefoperazone (Bush, 2010). Jacoby (2009) stated that as a general rule, against Enterobacter species, ceftriaxone is not effective and its use in the treatment of Enterobacter infections should be avoided, even though, due to the emergence of resistance, the isolate appears susceptible. By forming cephalosporinases (these enzymes hydrolyze cephalosporins and make them inactive), certain pathogens, such

as Providencia and Citrobacter, have the potential to become immune (Page, 2004). Ceftriaxone is a β-lactam (cephalosporin / cephamycin) broad-spectrum antibiotics that demonstrates in vitro experiments with gram-positive and gram-negative aerobic and anaerobic bacteria (Bassetti *et al*., 2008). Ceftriaxone's bactericidal activity stems from inhibition of the cell wall synthesis and is mediated through ceftriaxone binding to proteins binding to penicillin (Matzneller *et al*., 2016). Ceftriaxone is often used to treat pneumonia in combination with macrolide antibiotics and aminoglycoside antibiotics (Cahyaningsih *et al*., 2019). It is also the medication of choice for bacterial meningitis therapy (Rivera and Boucher, 2011).

2.20.9 Teicoplanin

Teicoplanin is a glycopeptide antibiotic that is almost equivalent to vancomycin with respect to its antibacterial scope of action (Khamesipour *et al*., 2015). It is approved for use in the treatment of several infections that are gram-positive (Srinivasan *et al*., 2002). Teicoplanin has been accepted for the treatment of severe Staphylococcal or Streptococcal infections which cannot be treated with less toxic antibiotics in a satisfactory manner (Brink *et al*., 2008). Teicoplanin can, therefore, be used in cases of osteomyelitis, septic arthritis and septicemia (Brink *et al*., 2008). Parenti *et al*. (2000) stated that it should be known to clinicians that teicoplanin may be less active than vancomycin against some staphylococci, such as *Staphylococcus haemolyticus*. Teicoplanin binds to the terminal dipeptide peptidoglycan sequence (amino D alanyl D alanine residue) and prevents interaction and cross-linkage of peptidoglycan, thus inhibiting the synthesis of the cell wall (Lambert *et al*., 2002) Teicoplanin is well

absorbed as parenterally administered and widely distributed throughout the body as a plasma protein bound form and as well excreted through urine (Wilson, 2000).

2.21 Knowledge of farmers on antibiotic usage

2.21.1 Demorgraphics information

According to Ferdous *et al.* (2019) the educational status of farmers in Bangladish ranged from primary to tertiary; majority (56%) had secondary education. Another study found that the vast majority of the participants were male (98.33%), primary school graduates (61.94%), at the age of 48 years or more (43.33%, and were engaged with animal breeding for at least 11 years (73.88%) (Ozturk *et al*., 2019). Phares *et al*. (2020) revealed that out of 600 respondents, the majority (71%) were male. The age group was between 21 and 63 years with a majority greater than 30 years (80.5%). Most (57.0%) farmers were in the animal husbandry sector for less than 10 years. Large number (31%) respondents were illiterate and majority (70%) raised poultry.

2.21.2 Sources where livestock farmers obtained antibiotics

There are many sources where livestock farmers can get antibiotics for use. According to Landfried *et al*. (2018), most farmers depend on veterinarians for the particular drugs they used on their goats, and also felt assured that they would get antibiotics in a feed store or online. Half of the farmers stated that they were using only the medicines recommended by the veterinarians. Phares *et al.* (2020) revealed that most (84.2%) farmers bought over-the-counter non-prescription, 10.0% bought on veterinary recommendation, 3.9% from agro-dealers without recommendation, and 1.9% from fellow farmers without recommendation on how to buy antibiotics for

farmers. According to Katakweba *et al*. (2012) farmers source antibiotics from agriculture and veterinary implements shops (29%), livestock markets (19%), veterinary drug shops (12%), veterinarians (18%), livestock field officers (9%), neighbors (5%) and exhibition areas (8%). In another report, antibiotics were purchased mainly from veterinary stores (91.4%) and only a few were purchased from local suppliers (8.6%) (Oluwasile *et al*., 2014).

2.21.3 Purpose of using antibiotics

The common uses of animal antibiotics are for treatment of sick animals, prophylaxis and growth promoters, which are important for a safe and efficient animal industry (McEwen, 2006). Consequently, the utilization in animals of such antibiotic drugs, particularly food animals, can result in the selection of resistant strains of bacteria, which in effect, can cause both animals and humans to become infected (Singer *et al*., 2003). Molecular identification of genes of antibiotic resistance and mobile elements of resistance to antibiotics showed that bacteria that colonize both animals and humans have similar elements, suggesting that natural foods play a role in the transmission of resistant bacteria and genes of resistance to humans through the food chain (Heur *et al*., 2011). For instance, the use of fluoroquinolones in animals that provide food contributed to the proliferation of *E. coli* and other ciprofloxacin-resistant bacteria, which have caused human infections that are hard to treat (Collignon *et al*., 2016). Casewell (2003) stated that the most frequent uses of antibiotics by weight were those for the treatment and prevention of diseases in poultry and pigs and as growth promoters. In another study, in South Africa, Rasmussen *et al*. (2015) observed that tylosin, one of four growth promoters, was the most commonly marketed antibiotic,

followed by tetracyclines, sulphonamides and penicillins. A surveyed conducted by Ferdous *et al*. (2019) found that antibiotics were used by farmers for therapeutic purposes (34.16%), prophylaxis purposes (14.17%) and for both therapeutic and prophylaxis purposes (40.83%). Phares *et al*. (2020) reported that the greater number (8%) of farmers administered antibiotics to prevent and treat diseases, whilst 13.0% said it was done in Ghana to facilitate growth and prophylaxis (0.7%). Also, a study by Oluwasile *et al*. (2014) revealed that poultry farmers was using antibiotics both for medicinal purposes (36.2%), for prophylactic purposes (29.3%), and for prophylaxis (32.8%), while antibiotics were used as growth promoters (6.9%).

2.21.4 Antibiotic drug administration and prescription

According to Phares *et al*. (2020), merely 8.5% of the administration of antibiotics was handled out by veterinary officers, while 65.3% was performed by farm employees and 23.2% by farm managers. In another study, 50% of poultry farmers reported that veterinary doctors administered antibiotics (43.1%), self-medication procedures and (6.9%) antibiotics prescribed by animal health staff (Oluwasile *et al*., 2014).

2.21.5 Antibiotic used by farmers in livestock production

A study by Adesokan *et al*. (2015) found that the majority of antimicrobials used in animal production in south-western Nigeria were tetracyclines (33.6%), fluoroquinolones (26.5%) and beta-lactams/aminoglycosides (20.4%). Also, ciprofloxacin (22.5%), followed by enrofloxacin (17.5%), amoxicillin (16.66%), oxytetracycline (10.83%), sulfa (3.33%), and norfloxacinin, were the most popular antibiotics in the farms surveyed (1.66%). Fluoroquinolones are classically mainly

antibiotics, followed by tetracyclines, aminopenicillin, and polymyxin, and the remainders are in the macrolides and sulfa classes (Ferdous *et al*., 2019). Katakweba *et al*. (2012) recorded tetracycline (61%), sulphadimidine (23%), penicillin-streptomycin (2%) and gentamycin (1%) as among the most commonly used and frequently reported antibiotics among livestock keepers in Tanzania. In addition, the most common class of antibiotics that were used in poultry farms were quinolones. The popular antibiotics used were neoceryl (a commercially prepared broad-spectrum antibiotic consisting of neomycin, erythromycin, oxytetracycline, streptomycin, and colistin) (36.2%), enrofloxacin (27.6%), and furazolidone (20.0%) (Oluwasile *et al*., 2014). In another study Sekyere (2014) stated that tetracyclines (oxytetracycline, doxycycline, remacycline and tetracycline) were very frequent in districts in the Ashanti region of Ghana (64 out of 110 farms), followed by strep-tomycin (48/110), penicillins (48/110), and sulphadimidine (31/110) among pig farms. There was very limited use of fluoroquinolones, macrolides, and aminoglycosides.

2.22 Antibiotic resistance

Abraham and Chain (1940) first identified antimicrobial resistance in *Bacillus coli* (now known as *E. coli*) in 1940, shortly before the introduction of the use of penicillin in the same year to treat human infectious diseases (Zaffiri *et al*., 2012). To facilitate the proliferation, selection and dissemination of antibiotic resistance microorganisms, antibiotic use is considered the most significant factor in both veterinary and human medicine (Economou and Gousia, 2015). Alanis (2005) reported that resistance to antibiotics occurs when bacteria respond to the use of these medicines. Bacteria, not people or animals, are antibiotic-resistant. Such bacteria can infect humans and

animals, and it is difficult to treat the infections. When the antibiotics used to treat them become less effective, it becomes harder to treat increasing numbers of bacterial infections, such as pneumonia, tuberculosis, gonorrhea, and salmonellosis (Morehead and Scarbrough, 2018) The planet is in desperate need of improvement the manner in which it prescribes and uses antibiotics (Hulscher *et al.*, 2010). Even if new medicines are created, without a change in behaviour, antibiotic resistance will remain a major problem (Laxminaravan *et al*., 2013). Lifestyle changes must also include steps to reduce the spread of diseases through vaccination, hand washing, safe sex conduct and healthy food safety (WHO, 2015). According to Spellberg and Gilbert (2014) the fast spread of resistant bacteria is taking place throughout the world, hampering the potency of chemotherapy-changing antibiotics that millions of live were saved. Bacterial infections have become a threat again many decades after antibiotics were treated in the first patients (Spellberg and Gilbert, 2014). The problem of antibiotic resistance has been linked to the excessive usage and abuse of these drugs, as well as the healthcare industry's lack of new drug production due to limited financial incentives and difficult regulatory standards (Rather *et al*., 2017) In livestock, the indiscriminate usage of antibiotics in products resulting in resistance to microorganisms (Serrano, 2005). In order to present immediate, extreme, and related risks, a number of bacteria have been detected, most of which are now capable of putting a major financial and clinical burden on the U.S. health care system, patients and their families (Rossolini *et al*., 2014). Gould and Bal (2013) stated that there is a great need for collective action in order to enact new strategies, undertake research efforts and take measures to manage the crisis. Bacteria which are known to

metabolize antimicrobials and utilize them as a form of nutrients have been found to show multidrug resistance (Simoes *et al*., 2009).

2.23 Mechanisms of antibiotic resistance

The ways in which microorganisms resist antimicrobial agents are many. According to Lewis (2013) bacteria generate enzymes that kill the antimicrobial agents, for example, Beta lactamase enzyme hydrolyses beta lactam drugs that establish resistance hit their targets. Gram negative bacteria may also become immune to Beta lactam antibiotics by creating a permeability barrier for impermeable cells for antimicrobial drugs (Zgurskaya *et al*., 2015). Ribosome methylation of ribosomal RNA, for example, develops macrolide resistant mutation (Garza-Ramos *et al*., 2001) In fact, before it can meet its targets, the bacterial efflux pump expels antimicrobial drugs from the cell (Luthra *et al*., 2018). Tenover (2006) reported that in bacteria, unique metabolic pathways are genetically engineered so that antibacterial agents may not have an effect.

2.24 Antibiotic resistance indicators in epidemiology studies

According to Volkova (2013), antimicrobial resistance was studied using bacterial indicators in different body tracts that are commensal habitats. The native condition of the gastrointestinal tract is *E. coli* and *Enterococci spp*, while *S. aureus* is that of humans and other warm-blooded animals in the respiratory tract (Newell *et al*., 2010). Economou and Gousia (2015) stated that popular human and animal commensals and pathogens are these species of bacteria and are considered to be an important source of antimicrobial resistance determinants. These bacteria are being used as markers of

bacteria to assess the amount of antibiotic resistance in a population leading to permanent indwelling in the gut and respiratory tract (Parsek and Singh, 2003). Witte (2004) found that these bacteria may develop and disseminate resistance that might be passed on to infective or zoonotic bacteria.

2.25 *E. coli* bacteria as antibiotic resistant indicator

The sub-therapeutic use of antibiotics has facilitated and supported the prevalence of multiple antibiotic resistant *E. coli* in the faecal environment of these animals in the mass production of poultry, eggs and pork (Fairbrother *et al*., 2005). Multiple antibiotic resistance *E. coli* have been developed by the widespread use and abuse of antibiotics in human therapy in the faeces of humans (Lekshmi *et al*., 2017) In these large reservoirs of human enteric disease, these practices led to the peaceful coexistence of multi antibiotic resistance *E. coli*, according to Radhouani *et al*. (2014). *E. coli* is considered as a bacterial indicator in swines, birds and large ruminants in Spain (Colomer-Lluch *et al*., 2011). A study found diarrheagenic *E. coli* prevalence and antimicrobial susceptibility in children from birth to five years of age in Ifakara, Tanzania (Moyo *et al*., 2007). White (2002) conducted susceptibility studies in the years 1985 to 2000 on isolates of *E. coli* derived from man, large ruminants, pigs and foods from different countries. It was resistant to tetracycline, sulfamethoxazole, and cephalothin ampicillin resistant isolates. Among pig isolates, the highest frequencies of resistance were. Antimicrobial resistance is widespread among humans and food animals living with some strains of *E. coli* such as O26, O103, O111, O128 and O145 (Sayah *et al*., 2005). By bacterial conjugation, transduction or transformation, *E. coli*

and associated bacteria are capable of transmitting DNA, allowing genetic material to spread horizontally across an existing population.

2.26 Prevalence of antibiotic resistance

In recent years, the prevalence of antimicrobial resistance within foodborne pathogens has increased (Van *et al*., 2007). According to Ahamadi *et al*. (2012) *E. coli* isolates showed maximum resistance to ampicilin followed by ceftazidime, nalidixic acid, nitrofurantoin, tetracycline and cu-trimuxazole. Also, isolates of *E. coli* were strongly teicoplanin teicoplannin-resistant (97.78%) from beef samples in Wa abattoir, but amoxycillin/clavulanic, ceftriaxone chloramphenicol, ciprofloxacin, gentamicin, and suphamethoxazole/trimethoprim were susceptible (80%) (Adzitey, 2020). A study by Dsani (2019) in the Greater Accra region revealed that *E. coli* isolates from beef, mutton and chevon were resistance to ampicillin (57%), tetracycline (44%), cefuroxime (21%) and sulphamethoxazole/trimethoprim (17%). Highly susceptibility was observed for meropenem (100%). Significant susceptibility rates have been observed for ceftriaxone (99%), cefotaxime (98%), chloramphenicol (97%), gentamycin (97%), ciprofloxacin (92%) and amikacin (92%). *E. coli* was isolated from beef and its associated sample in Techiman municipal of Ghana was found to be resistant to vancomycin (88.89%) and erythromycin (68.89%), but susceptible to ciprofloxacin (95.56%), amoxycillin/clavulanic acid (86.67%), suphamethoxazole/trimethoprim (82.22%) and gentamicin (75.56%) (Adzitey, 2015).

2.27 Multidrug resistant of *E. coli*

In bacteria, multidrug resistance is mostly characterized by the increase of genes on R plasmids, each of which codes for resistance to a particular drug (Nikaido, 2009). Mechanisms given by transposons, integrons, and iron-sulfur cluster regulator (ISCR) elements accomplish the assembly of resistance genes on a single R-plasmid (He *et al*., 2015). For example, integrons are extremely potent in generating multidrug resistance, as they combine multiple resistance genes in the correct position to provide an efficient regulator for their expression (Pal *et al*., 2016). According to Nikaido (2009), the resistance gene is labelled once incorporated into an integron, so that it can quickly form one of some other integron. Resistance genes seem to have their biological origins in microbes generating antibiotics (Baquero *et al*., 2008). Some even emerge from environmental species, especially microorganism from soil (Davies and Davies, 2010). The recent selection of mutants with generalized resistance genes is a disturbing pattern. R-plasmids are exceptionally well preserved and are also easily moved from cell to cell (Gama *et al*., 2018). In gram-negative bacteria, the resistance nodulation division (RND) super-family pumps are particularly important because they are normally coded by chromosomal genes and can easily be over-expressed (Fodor *et al*., 2018).

Multidrug resistance is a severe medical issue and poses a significant barrier to the treatment of disease and the production of novel therapeutics (Li *et al*., 2016). A study conducted by Adzitey (2020) found that the MAR's index ranged from 0.11 to 0.56. The most frequent was the Tec (resistant to only teicoplanin) resistance pattern and 21 isolates were found.

This was accompanied by AzmTec (azithromycin-teicoplanin showing 6 isolates and TecTeSxt (teicoplanin-tetracycline-suphamethoxazole/trimethoprim indicating 5 isolates) resistance trends. Ali *et al*. (2014) found that *E. coli* isolates were not susceptible to at least 3 antibiotics, commonly shown to Gram-negative bacteria, in all poultry meat. In nine (9) *E. coli* isolates, resistance to almost 4 antibiotics was seen, while in 15 isolates, resistance to 5 antibiotics was detected. The highest resistance of 1 isolate of *E. coli* to 7 different antibiotics (ampicillin, tetracycline, sulfamethoxazole/trimethoprim, gentamicin, chloramphenicol, nalidixic acid and kanamycin) was observed out of 9 used in the study.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study area

This research was conducted in Bolgatanga municipality. The Bolgatanga municipality is the regional capital of the Upper East region of Ghana. The Municipality is located across latitudes 10 ° 30 'and 10 ° 50' North and longitudes 0 ° 30 'and 1 ° 00' North, roughly (Anonymous, 2015). Bolgatanga bordered by Bongo District to the north, the Talensi and Nabdam Districts to the south and east, and the Kassena-Nankana Municipality to the west (Fig 3.1). A total land area of 729 square kilometres covered (Abi-Atingah 2018).

The Municipality of Bolgatanga has a population of 131,550, representing 12.6% of the population of the Upper East Region (Aovare, 2017). The municipality has a male population of 62783 (47.7%), and female population of 68767 (52%) (Ghana Statistical Service, 2013). While Bolgatanga municipality is rapidly catching up with urbanization, the rural population still accounts for half (50.2%) of the population (Awimba, 2018).

The farmers in the municipality practice four kinds of agricultural operations, namely crop farming, tree growing, livestock rearing and fish farming. Around 90.0% of farming households are employed in crop farming, while 80.0% are also actively involved in rearing livestock (Ghana Statistical Service, 2013). According to Ghana Statistical Service (2013), following crop production, livestock rearing is the second most important agricultural activity in the Municipality. According to Kyere *et al.*

(2017) the average number per holder varies from 8-20 for the bird type, chicken and guinea fowl.

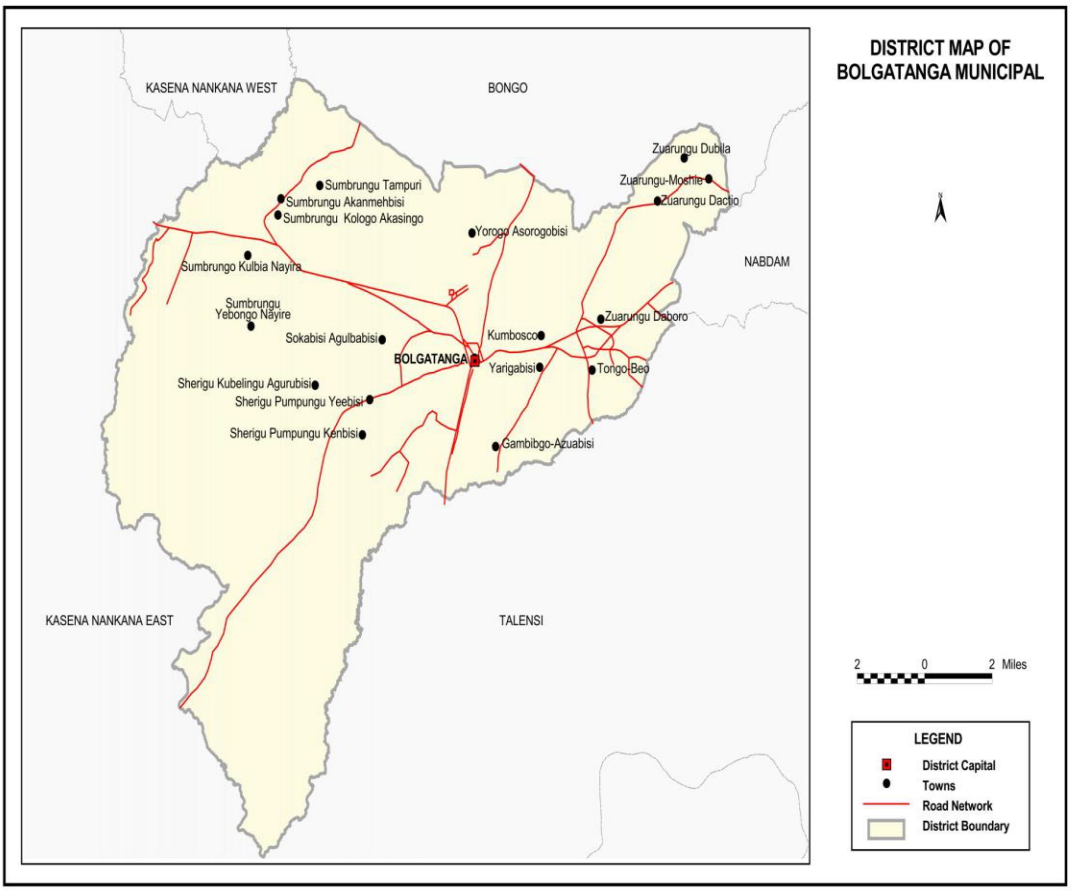

Fig. 3.1: Map of Bolgatanga Municipal (Abdulai *et al*., 2015).

3.2 Study design

Mixed approach method was used to obtain data for this study. Descriptive survey was used for the first part to obtain data using semi-structured questionnaire from the livestock farmers. The other part was a cross sectional study to get data on the microbial contamination of the grilled RTE meats using laboratory investigation techniques.

The survey was conducted to gather information on the knowledge of livestock farmers on antibiotic usage. The laboratory analysis involved the isolation and identification of *E. coli* on grilled RTE meats in all the 300 ramdomly selected vendor shops in Bolgatanga.

3.2.1 Survey of livestock farmers' knowledge on antibiotic usage

3.2.1.1 Study population, sample size and sampling methods

The study populations were the ruminant and non-ruminants' farmers in the Municipality who were willing to take part in the study by answering the questionnaires. A sample size of 376 livestock farmers was interviewed by employing snow ball sampling technique to select the farmers for the interview for the period between June and August 2020. The sample size was obtained by evaluating the population of livestock farmers in the sample size calculator. Secondary data on the population of livestock farmers was taken from the municipal Animal Production Officer and the active livestock farmers in the Bolgatantang municipal was 15,000 as at 2019. The livestock farmer's population of 15000 and confidence interval of 5 was

entered into the sample size calculator (www.surveysystem.com) and a sample size of 376 was obtained at a confidence level of 95%.

3.2.1.2 Structure of questionnaire

The research employed structured questionnaires which entailed both open and close ended questions developed to gather information from the livestock farmers. The first part of the questionaires sought information on the demographic characteristics such as the gender, age, highest educational level and the livestock reared. The second part of the questionaires dealt with the information on farmers' knowledge on antibiotics usage in livestock such as antibiotics use by farmers, purpose of antibiotic usage, sources of antibiotics acquisition, antibiotic administration and others.

3.2.1.3 Questionaires administration

Five veterinary technical officers and two agricultural extension officer working in the Bolgatanga municipality and understand the farmers' language were recruited to administer the questions. The administration of the questionnaire was done from April to July 2020 and was not pretested.

3.3 Laboratory microbiological investigation

3.3.1 Sampling

A total of 300 swabs sample from ready-to-eat meats made of 50 mutton, 50 beef, 50 chevon, 50 pork, 50 chicken and 50 guinea fowl meats were randomly collected from street vendors to examine for the presences of *E. coli*. Sterile swabs were aseptically used to swab the surface of meat ($10cm^2$) and immediately placed in its cap. The

samples were labeled and placed in an ice chest with ice block to maintain the cold chain. The meat samples were transported to the Spanish laboratory at the Nyankpala campus of the University for Development Studies for analysis.

3.3.2 Isolation of *E. coli*

Bacteriological analysis of *E. coli* was conducted according to the Food and Drug Administration-Bacteriological Analysis Manual (FDA-BAM) (Feng *et al*., 2002). The swabs from the meat samples were dispensed into universal bottles containing the 9ml buffered peptone water and incubated at 37 ℃ for 24 hours. A loopful of the culture from the buffered peptone water was then transferred onto an levine eosine methylene blue (LEMB) agar plate by streaking, and then incubated at 37 ℃ for 24 hours.

3.3.3 Confirmation of *E. coli*

Presumptive *E. coli* on the LEMB agar that apppeared red-pink with or without metallic sheen was streaked onto tryptic soy agar and incubated at 37 ℃ for 24 hours. The identification and confirmation of pure cultures were done by using gram staining, growth on brilliant green bile (BGB) broth with Durham tubes and latex agglutination test for *E. coli*.

3.3.3.1 Gram Stain

Gram staining was done according to James *et al.* (2011) method. Suspected *E. coli* colony was smeared on a clean microscope slide. The smear was air dried and heat fixed. Crystal violet was used to stain the dried smear for 2 minutes and water washed. Lugol's iodine was used to stain the smear and allowed for 1 minute before washing

with water. It was decolorized with acetone alcohol for 30 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 *E. coli*.

3.3.3.2 Growth of *E. coli* in brilliant green bile broth

The presumptive *E. coli* in tryptic soy agar was transferred into tubes of brilliant green bile broth, loosely capped and incubated at 35 °C for 24 hours. The tubes were then examined for turbidity and gas production. The pH, colour, depth, and sterility were checked and there was turbid with gas in the durham tubes.

3.3.3.3 Latex agglutination

A drop of the test latexon was mixed with complete loop of pure culture of suspected *E. coli* colonies in a clean slide for 10-15 seconds and emulsified. The suspension was applied with one drop of the Oxoid *E coli* test kit and thoroughly mixed. There was a rotation of the slide and the result was read in 2 minutes. Visible clumps indicate positive agglutination whilest no clumping within 2 minutes suggest a negative outcome.

3.3.4 Determination of total coliform

Total coliform was determined according to modified procedures of Maturin and Peeler (2001) and Adzitey *et al*. (2019). Ready-to-eat meat swabs were added to 9ml of 1% sterile peptone water. Serial dilution from 10^{-1} to 10^{-5} was done, spread plated on MacConkey agar and incubated at 37 °C for 24 hours. the colonies were measured as colony forming unit per cm^2 (cfu/cm^2) using the formula:

$N = \sum C / [(1 * n1) + (0.1 * n2) \ldots] * (d)$

Where N = Number of colonies per cm^2; $\sum C$ = Sum of all colonies on all plates counted; n1 = Number of plates in first dilution counted; n2 = Number of plates in second dilution counted; d = dilution from which the first counts were obtained.

3.3.5 Antimicrobial susceptibility test

The antibiotic discs' diffusion method (Kirby–Bauer) by Bauer *et al*. (1966) was used for the antibiotic susceptibility test. A total of nine antibiotic discs (Oxoid®, United Kingdom) such as streptomycin (10 µg), amoxicillin (AMX), 10µg, chloramphenicol (C), 30µg, trimetophrim (5 µg), sulfamethoxazole), 30µg tetracycline (TE), ciprofloxacin (CIP), 30µg, azithromycin (15µg), 30 µg teicoplanin (TEC), 30ug ceftriaxone (CRO) and 10ug gentamicin (CN) were used to check against *E. coli* isolates.

Pure cultures of E. coli were grown overnight in tryptic soy broth (TSB) at 37°C. One hundred microlitre of the culture was then spread onto Mueller Hinton agar (Oxoid-UK) using sterile cotton swabs. Antibiotic dics were placed on the surface of the agar plate at a distance (10cm) to avoid overlapping of inhibition zones. The plates were incubated at 37 °C for 16-18 hours. Inhibition zones were measured and interpreted as sensitive, intermediate, or resistant according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2005).

3.4 Analysis of data

The laboratory results and questionnaires were entered in Microsoft Excel. The SPSS version 16.0 was used to analyze data from questionnaire and the prevalence data from *E. coli* in the meat, GenStat 12.1 edition was also used to analyze coliforms 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 Survey on farmers' knowledge on antibiotic usage

4.1.1 Demographic characteristics of livestock farmers (respondents)

The demographic characteristics of the livestock farmers are presented in Table 4.1. Majority of the farmers were male (74.5%) with age ranges between 30-39 years (28.5%). Educational status of farmers was non-formal to tertiary; most had education up to tertiary (30.3%). Most of the farmers have engaged in the livestock business between 6-10 years (31.6%). The farmers were mainly Grune by tribe (71.3%). Other major tribes were Dagomba, Namdam and Dagaati. However, Morshi and Ewe formed the minor tribesof the farmers (Figure 4.1).

Table 4.1: Demographic characteristics of livestock farmers

Variable	Frequency	Percentage (%)
Gender		
Male	280	74.5
Female	96	25.5
Total	376	100
Age		
20-29	48	12.8
30-39	107	28.5
40-49	103	27.4
50-60	84	22.3
61 and above	34	9.0
Total	376	100.0
Religion		
Christianity	183	48.7
Islamic	87	23.1
Traditionalist	106	28.2
Total	376	100.0
Educational status		
Non formal	89	23.7
Primary	60	16.0
Junior High School	54	14.4
Senior High School	50	13.3
Tertiary	114	30.3
Other form of worship	9	2.4
Total	376	100.0
Year in livestock business		
0-11 Months	24	6.4
1-2 Years	61	16.2
Between 3-5 Years	101	26.9
6-10 Years	119	31.6
Above 10 Years	71	18.9
Total	376	100.0

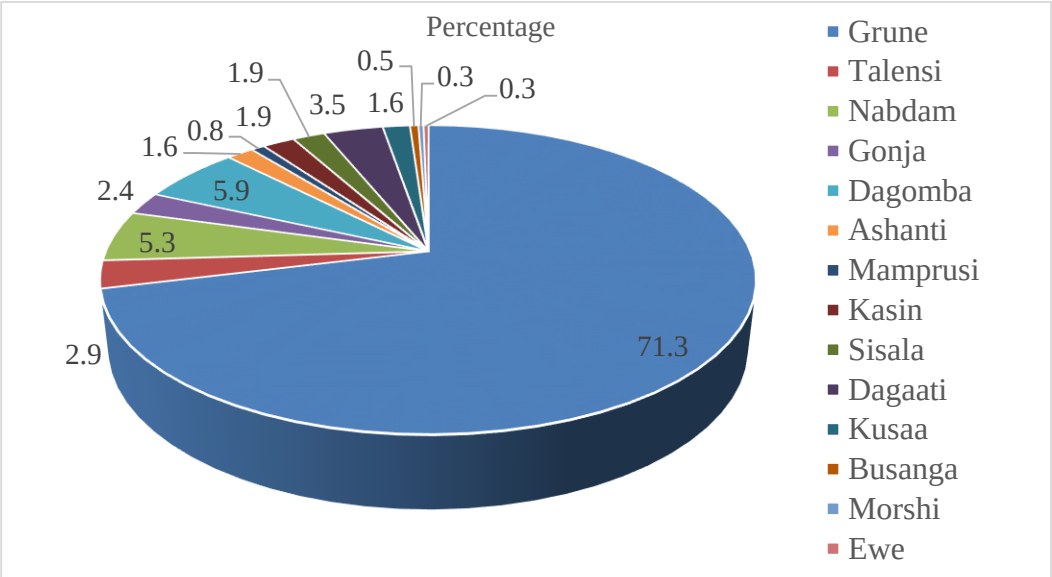

Figure 4.1: Tribes of livestock farmers

4.1.2 Species of livestock animals reared and their populations

Sheep farmers were the highest with 65.7% followed, by farmers who reared fowls with 65.4% and pig farmers were the least with 24.7%. Few farmers engaged in pigs rearing (24.7%) and slightly above half of the farmers were not engaged in guinea fowl production (53.5%). The result is presented in Figure 4.2. Fowls had the highest population followed by guinea fowls and the least were pigs. Farmers reared between one to two thousand fowls, between one to two hundred and twenty-five guinea fowl and the least reared between one to fifty goats (Table 4.2).

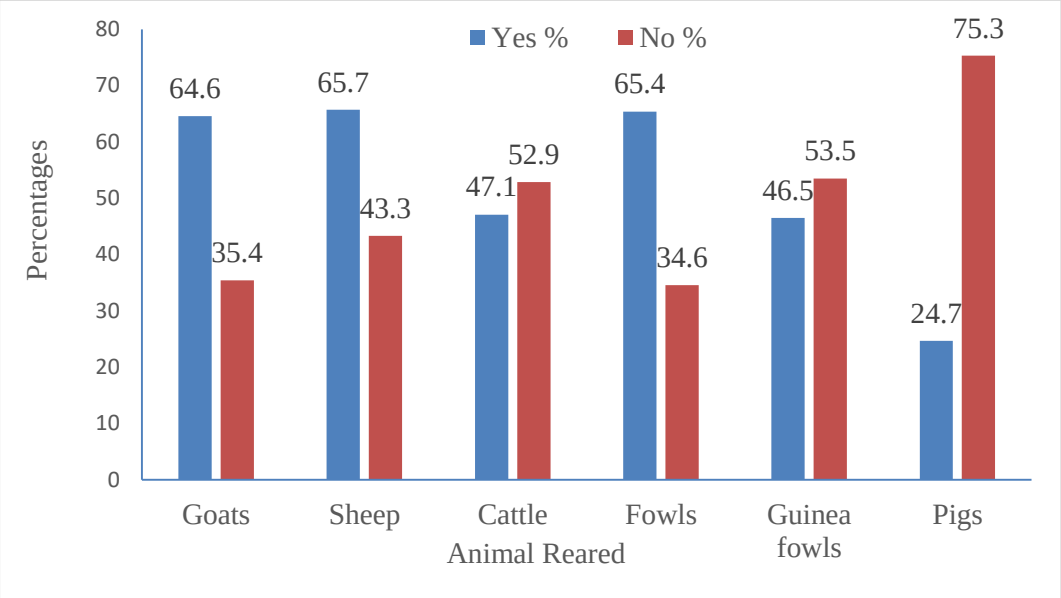

Figure 4.2: livestock reared by farmers

Table 4.2: The population of the various animal types reared by respondents

Specie of Animal	Minimum	Maximum	Sum	Mean	Number of respondents
Goats	1	50	2,796	7	376
Sheep	1	77	3,802	10	376
Cattle	1	150	3,479	9	376
Fowls	1	2,000	7,707	20	376
Guinea Fowl	1	225	5,907	15	376
Pigs	1	150	1,577	4	376

4.2.3 The livestock farmer's knowledge on antibiotics

From Table 4.3, most farmers had infections (92.8%) in their farm and mostly consulted veterinary officers (77.7%) for advise. Chemo-therapeutic (70.2%) is the main medication adopted which include mainly antibiotics (81.1%). The farmers'

educational status had no influence on their knowledge of the usage of antibiotics (X^2=5.651a, df=10, P=0.844), yet had an influence on the type of antibiotics used (X^2=32.158a, df=5, P=0.000). The years of experience in livestock production did not influence their knowledge of the use of antibiotics (X^2=8.460a, df=8, P=0.390) and the type of antibiotics used (X^2=5.701a, df=4, P=0.223). Many farmers (67.6%) involved in treating their animals and as well observed safety and dosage instruction (72.1%). The main antibiotic used was tetracycline (27.7%) and the least were penicillin (4.3%) and erythromycin (4.3%). Only 22.6% had training on antibiotic usage but more than half of the farmers had knowledge on antibiotic usage (56.1%). Farmers got information on antibiotic usage from several sources including colleague farmers (32.7%), veterinary staff (21.8%), extension officers (14.9%) and NGOs (12.0%) (Figure 4.3). Antibiotics prescription or recommendation was by colleague farmers (35.1%), veterinarians (31.6%) and drug sellers (18.1%). Most of the farmers only use antibiotic when the animals are sick, and also as prophylaxis in every three months (Figure 4.4). Antibiotics administration is mostly done by the veterinary officers (33%), farmers themselves (25.5%) and their colleague farmers (2.9%). Majority of the farmers used antibiotics purposely for sick animals' treatments (36.7%) and only few used it for both prophylactic and growth purposes (2.1%) (Figure 4.5). Many farmers observed safety dosage instructions (72.1%). Majority of farmers sold their unrecovered animals after treatment to butchers (29.0%) whilst those who never experienced that situation (0.5%) not engaged in such practices (Figure 4.6).

Most farmers consume or sell unrecovered animals treated with antibiotics for a period of one week (40.2%) and the least also consumed or sell after three weeks period (23.9%). Majority of the farmers had knowledge on antibiotic residues in meat (56.6%) and their effect to human health (63.0%).

Table 4.3: The knowledge of farmers on antibiotic usage

Variable	Frequency	Percentage (%)
Infections encountered on the farm		
Yes	349	92.8
No	27	7.2
Total	360	100
Did you consult veterinarian?		
Yes	292	77.7
No	84	22.3
Total	376	100
Kind of medication recommended		
Ethno veterinary	67	17.8
Chemo-therapeutic	264	70.2
Both	45	12.0
Total	376	100.0
Antibiotic medication		
Yes	305	81.1
No	71	18.9
Total	376	100.0
Farmer treating animals		
Yes	254	67.6
No	122	32.4
Total	376	100.0
Antibiotics used		
Gentamicin	27	7.2
Tetracycline	104	27.7
Amoxicillin/clavulanic acid	70	18.6
Trimethoprim/sulfamethoxazole	44	11.7
Ciprofloxacin	29	7.7
Erythromycin	16	4.3
Chloramphenicol	37	9.8
Penicillin	16	4.3
Herbal drug	33	8.8
Total	376	100.0

Table 4.3 continue: The knowledge of farmers on antibiotic usage

Antibiotics prescribe by	Frequency	Percentage (%)
Colleagues farmers	132	35.1
Veterinarian	119	31.6
Drug sellers	68	18.1
Farmers and veterinarian	14	3.7
Farmers and drug seller	26	6.9
Veterinarian and drugs seller	8	2.1
Colleague farmer, veterinarian and drug seller	9	2.4
Total	376	100.0
Training received on antibiotic usage		
Yes	85	22.6
No	291	77.4
Total	376	100.0
Knowledge on antibiotics usage		
Yes	211	56.1
No	165	43.9
Total	376	100.0
Who administer antibiotic?		
Self	96	25.5
Veterinary officers	124	33.0
Both self and veterinary officer	145	38.6
Colleague farmer	11	2.9
Total	376	100.0
Observation of safety and dosage instructions		
Yes	271	72.1
No	105	27.9
Total	376	100.0
Knowledge on antibiotics withdrawal period		
Yes	234	62.2
No	142	37.8
Total	376	100.0
Do you follow withdrawal instruction on the leaflet?		
Yes	247	65.7
No	129	34.3
Total	376	100.0
Knowledge on antibiotics expiring date		
Yes	342	91.0
No	34	9.0

Total	376	100.0
How long does it take to consume/sell unrecovered animals?		
1-7days	151	40.2
8-14 days	79	21.0
15-21 days	56	14.9
21-28 days	56	14.9
28 and above	34	9.0
Total	376	100.0
Knowledge on residues in meat		
Yes	213	56.6
No	163	43.4
Total	376	100.0
Knowledge on antibiotic residues in meat and its effects on man		
Yes	237	63.0
No	139	37.0
Total	376	100.0

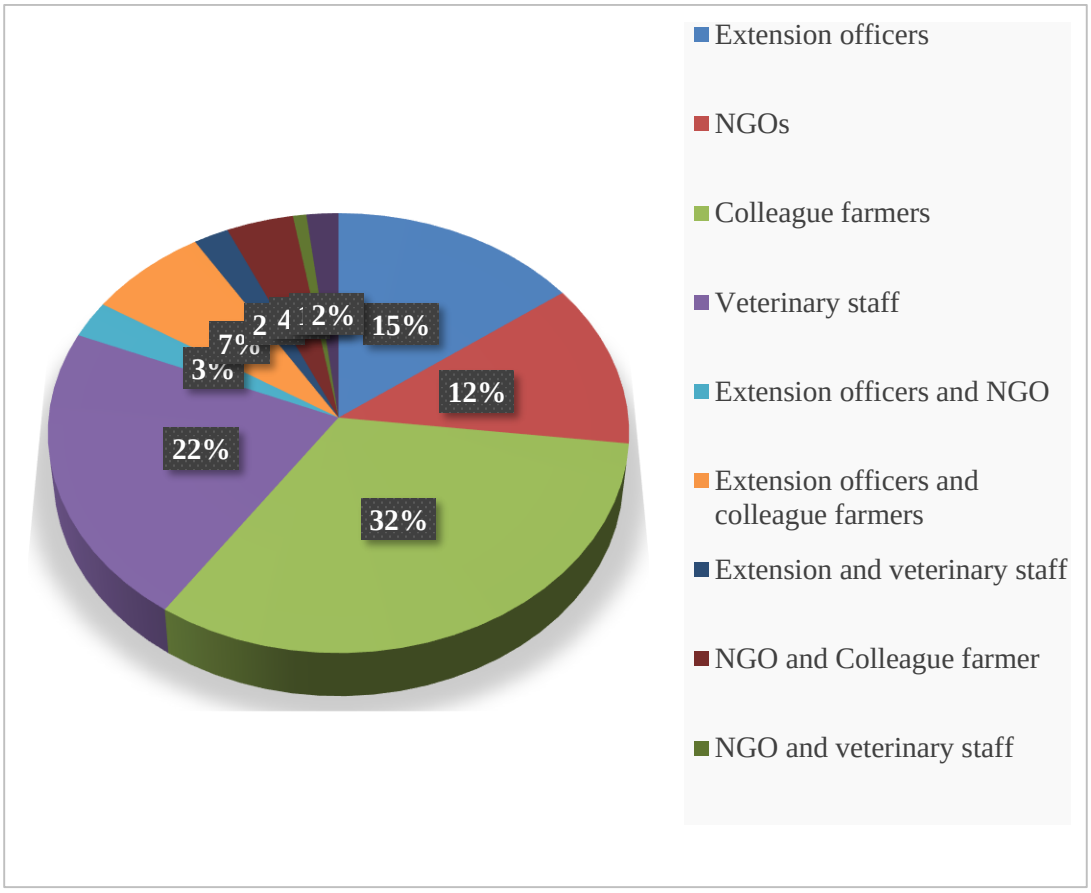

Figure 4.3: Farmers source of knowledge on antibiotic usage

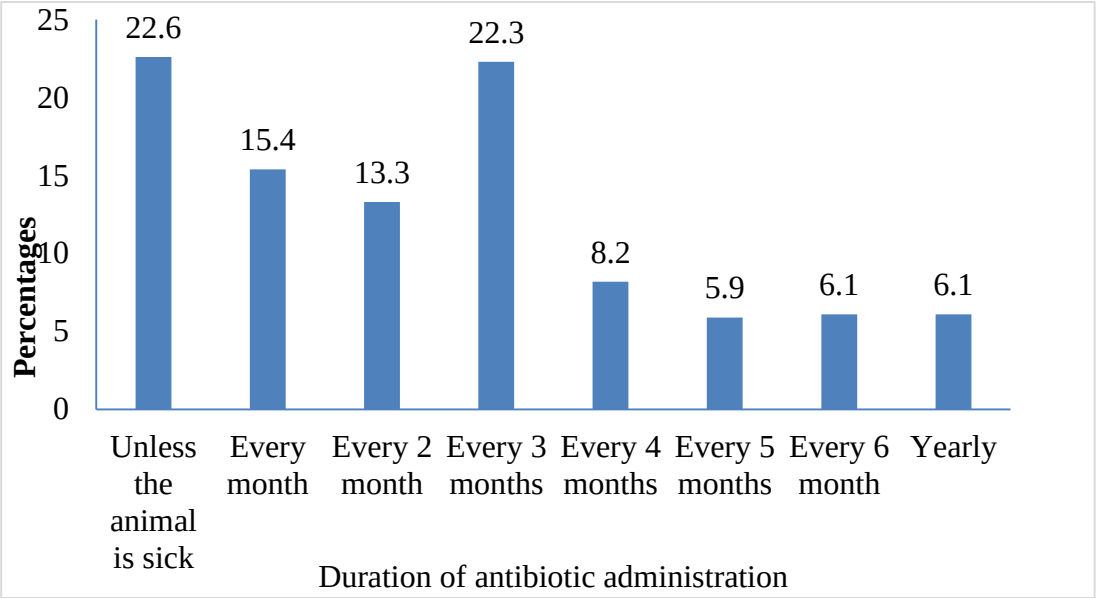

Figure 4.4: Number of times farmers treat animals with antibiotics

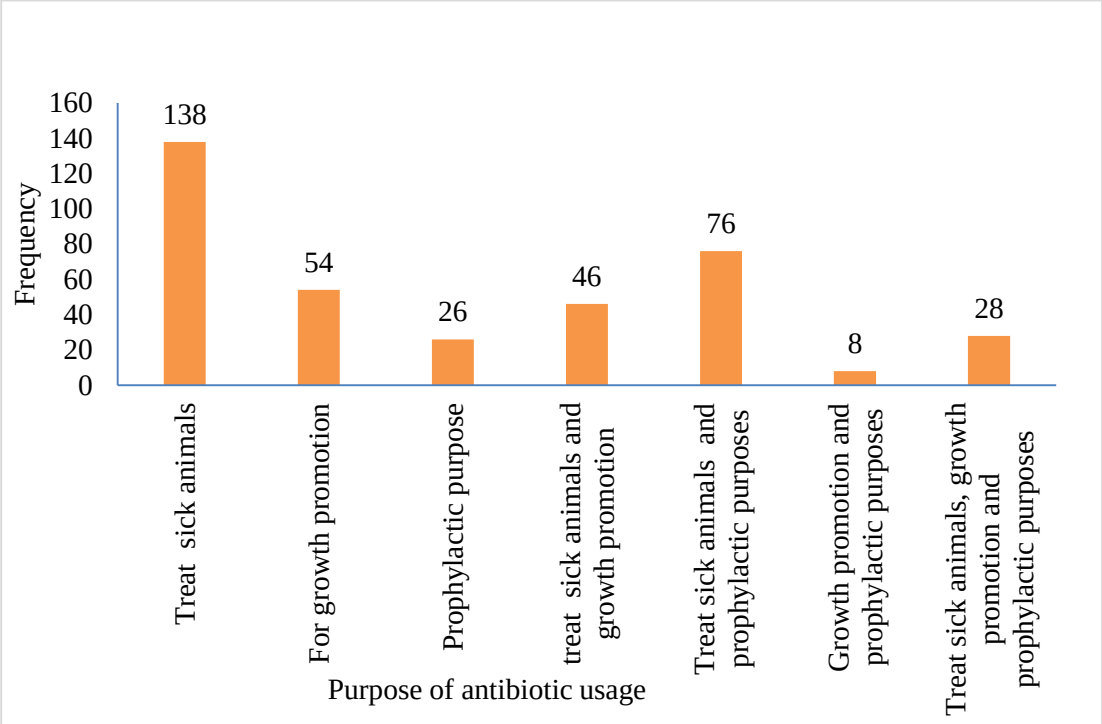

Figure 4.5: Purpose of antibiotic usage

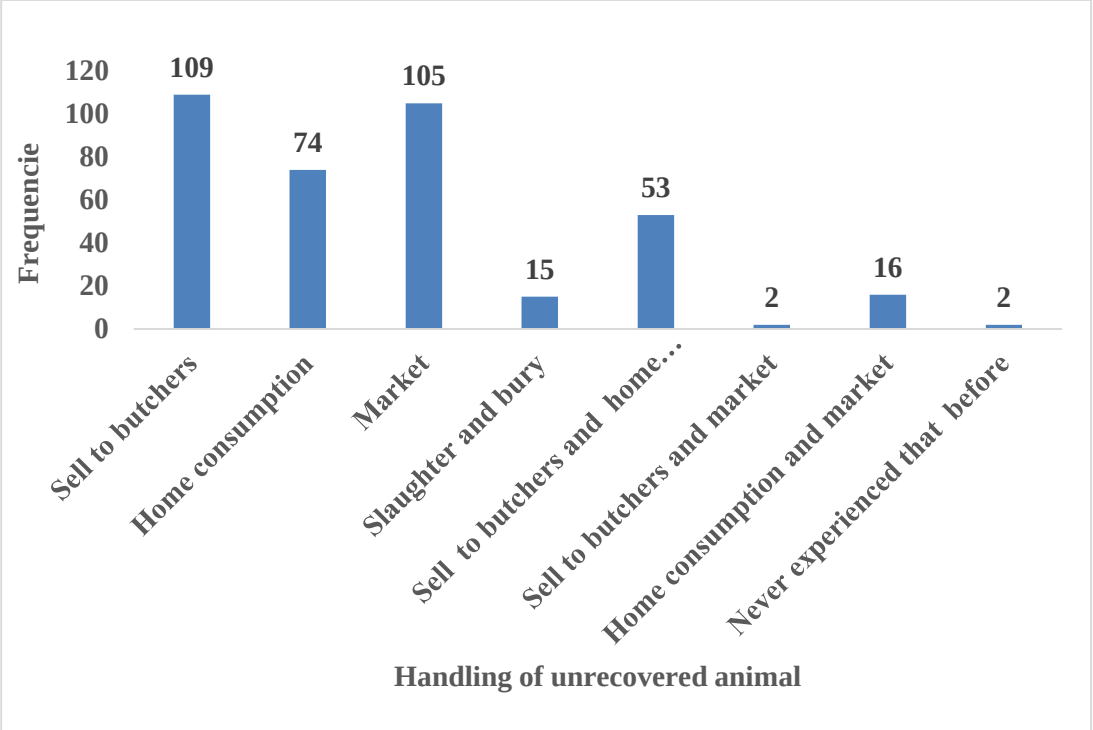

Figure 4.6: Handling of unrecovered animals after antibiotic treatment

4.2 Antibiotic resistance profile of *E. coli* isolated from ready-to-meats

4.2.1 Coliform counts of ready-to-eat meat samples

Pork has the highest coliform counts with 3.29 logcfu/cm^2 followed by beef (3.19 logcfu/cm^2), and the chicken had the least with 2.4 logcfu/cm^2.

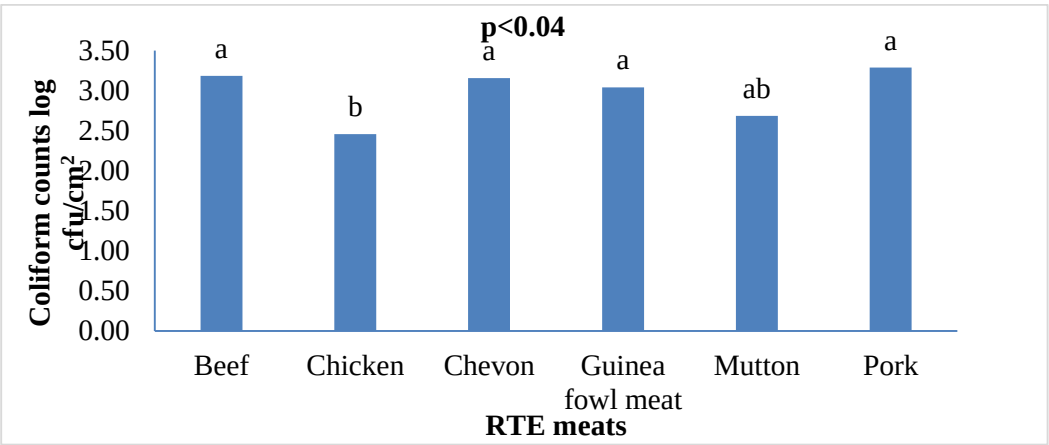

Figure 4.7: Coliform counts of RTE meats samples

4.2.2 Prevalence of *E. coli* in RTE meats

The overall prevalence rate of *E. coli* was 12.6% of the RTE meats samples examined. Chevon had the highest contamination rate with *E. coli* (10) and pork (3) had the least prevalence rate. This is shown in Figure 4.8.

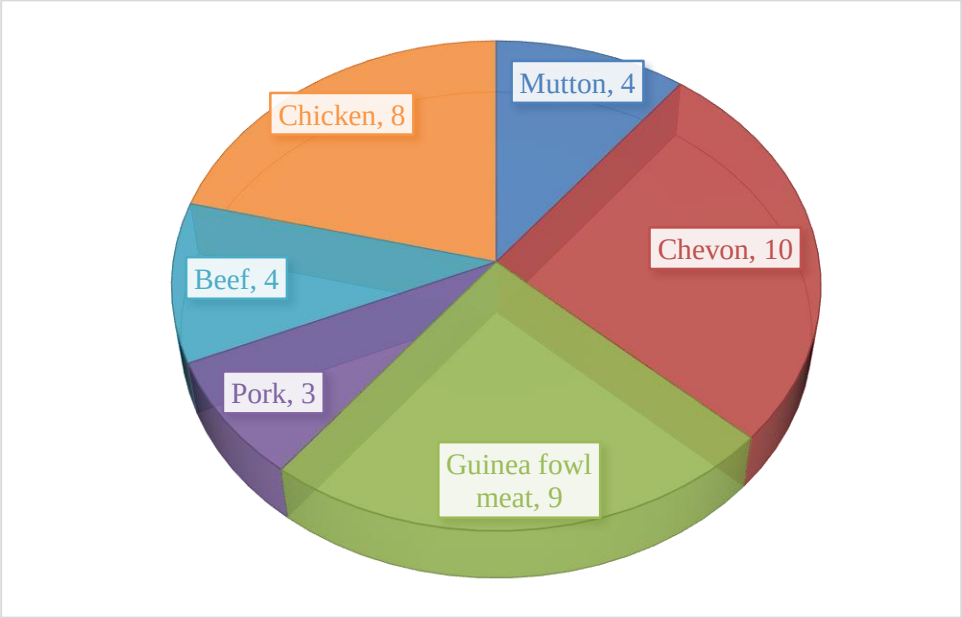

Figure 4.8: Prevalence of *E. coli* in RTE meat

4.2.3 Antibiotic resistant of *E. coli* isolated from ready-to-eat meats samples

From Table 4.4, *E. coli* was resistance to teicoplanin (96.77%), tetracycline (93.55%) and amoxycillin/clavulanic acid and azithromycin (70.97%). Some intermediate resistance was observed for gentamicin (38.71%), suphamethoxazole/trimethoprim (25%), ceftriaxone (22.58%) and azithromycin and ciprofloxacin (16.13%). *E. coli* was susceptible to chloramphenicol (93.55%). Also, 0% susceptibility to tetracycline was recorded.

Table 4.4: Antibiotic resistance of *E. coli* isolated from meat samples

Antibiotic	R%	I%	S%
Amoxycillin/clavulanic acid 30µg	70.97	0.00	29.03
Azithromycin 15µg (AZM)	70.97	16.13	12.90
Ceftriaxone 30µg (CRO)	19.35	22.58	58.06
Chloramphenicol 30µg(C)	6.45	0	93.55
Ciprofloxacin 5µg (CIP)	22.58	16.13	61.29
Gentamicin10µg (CN)	22.58	38.71	38.71
Teicoplanin 30 µg (TEC)	96.77	0.00	3.23
Tetracycline 30µg TE	93.55	6.45	0.00
Suphamethoxazole/trimethoprim (SXT)	58.06	25.81	16.13
Overall%	51.25	13.98	34.77

S, susceptible; I, intermediate; R, resistant

4.2.4 Multidrug resistant of individual *E. coli* isolates

The multiple antibiotic resistant (MAR) index ranged from 0.22 (resistant to 2 antibiotics) to 0.78 (resistant to 7 antibiotics). Out of 32 isolates, 2, 5, 7, 9 and 4 isolates were resistant to 2, 3, 4, 5 and 6 respectively. Three isolates of *E. coli* from chevon, pork and chicken were resistance to seven antibiotics CipAmzAzmTecTeCSxt, AzmTecCnTeCCroSxt and AmcAzmTecCnTeCroSxt respectively Mutton and chevon had the lowest resistant profile with resistant to 2 antibiotics (TecTe) in Table 4.5.

Table 4.5: **Antibiotic resistance profile and multiple antibiotic resistance index of individual E. coli isolates**

Codes	Source	No. of Antibiotics	Antibiotic resistant profile	MAR index
GO18	Chevon	7	CipAmcAzmTecTeCSxt	0.78
P12	Pork	7	AzmTecCnTeCCroSxt	0.78
C17	Chicken	7	AmcAzmTecCnTeCroSxt	0.78
GO4	Chevon	6	AmcAzmTecTeCroSxt	0.67
P5	Pork	6	CipAmcTecCnTeSxt	0.67
B7	Beef	6	CipAmcAzmTecTeSxt	0.67
C19	Chicken	6	AmcAzmTecCnTeSxt	0.67
GO1	Chevon	5	CipAmcTecTeCro	0.56
GO16	Chevon	5	AmcAzmTecCnTe	0.56
S26	Mutton	5	AzmTecTeCroSxt	0.56
G33	Guinea fowl meat	5	AmcTecTeCroSxt	0.56
B37	Beef	5	AmcAzmTecTeSxt	0.56
C7	Chevon	5	AmcTecCnTeSxt	0.56
C13	Chicken	5	AmcAzmTecTeSxt	0.56
C18	Chicken	5	AmcAzmTecTeSxt	0.56
C20	Chicken	5	AmcAzmTecTeSxt	0.56
C23	Chicken	5	CipAmcAzmTecTe	0.56
GO30	Chevon	4	AzmTecTeSxt	0.44
GO40	Chevon	4	AmcAzmTecTe	0.44
S11	Mutton	4	AzmTecTeSxt	0.44
G11	Pork	4	CipAmcAzmTec	0.44
B4	Beef	4	AmcAzmTecSxt	0.44
B49	Beef	4	CipAmcAzmTe	0.44
C15	Chicken	4	AmcAzmTecTe	0.44
G046	Chevon	3	AmcAzmSxt	0.33
GO49	Chevon	3	AzmTecTe	0.33
P3	Pork	3	TecTeSxt	0.33
G14	Giunea fowl meat	3	AzmTecTe	0.33
G30	Giunea fowl meat	3	TecCnTe	0.33
GO6	Chevon	2	TecTe	0.22
S3	Mutton	2	TecTe	0.22

CHAPTER FIVE

5.0 DISCUSSION

5.1 Demorgraphics characteristics of farmers

The study on farmers' knowledge on antibiotic usage on their livestocks revealed that male farmers were majority and with age ranging between 30 to 39 years. Most of the farmers were educated with majority reaching tertiary level. Also, most farmers have engaged in the livestock business between 6-10 years. The observed majority of male livestock farmers could be attributed to the fact that in the area, men are usually the heads of the family and perform all duties related to their status, including taking care of women's belongings. This study isin agreement with Ozturk *et al*. (2019) reported that 98.33% of the farmers were males. Also, Phares *et al*. (2020) found that majority of the respondents were male (71%) which also agrees with this study. This shows that livestock production is a business predominated by men. This study found that most of the farmers are youthful. This study is in tandem with the findings of Phares *et al*. (2020) who found majority of the farmers to be greater than 30 years (80.5%). However, this study disagrees with Ozturk *et al*. (2019) who found majority of the farmers to be at age of 48 year or more (43.33%). Compared to non-formal education, a higher percentage of the farmers had tertiary education and had considerable years of work experience in livestock business. This influenced and related to their antibiotic knowledge and its usage, as most of them consulted veterinarians when encountering infections on their farm. Majority said they had knowledge on the use of antibiotics and observed safety precautions and dosage instructions when administering

antibiotics on their own. This finding disagrees with Ferdous *et al*. (2019) who revealed that, most of the farmers had their education up to secondary school, whilst Phares *et al*. (2020) found large number of respondents were illiterate (31%).

5.2 Species of livestock animals reared by farmers

Out of 376 farmers, majority reared sheep followed by fowl, goat, cattle, with guinea fowl and pig being the least. The high number of sheep farmers could probaby be due to the usefulness of sheep for religious purposes and the readness its market and demand. The pig farmers were few due to some religious restrictions. For instance, Islamic religion forbids Muslims from keeping and consuming pigs and pork respectively.

5.3 Knowledge of farmers on antibiotic usage

This study revealed that few of the farmers did not have infections in their farms as presented in Table 4.2. These were farmers who had just started the livestock husbandry and probable acquired animals from disease free farms. Also, few did not consult veterinarian which could be attributed to the absence of diseases in their animals or they practicing ethno veterinary medication on their animals (Table 4.3). Most farmers use therapeutics (70.2%) recommended by the veterinarian to treat their animals and is probably due to availability of both veterinary drug shops and chemical shops within their locality.

5.4 Farmers sources of knowledge on antibiotic usage

The outcome of the study showed that farmers gained their experience from colleague farmers, veterinary staff, extension officers and NGOs as seen in Fig 4.3. This ensures

that most farmers are educated in the use of antibiotics. This may be due to the low number of veterinary staff, such that the veterinary staff trained most farmers known as community livestock workers (CLW) to deal with minor problems. This CLW engage in passing technical information to their colleague farmers to practice.

5.5 Antibiotic prescription and training

According to Oluwasile *et al*. (2014), prescriptions of antibiotics were done by veterinary doctors followed by practices of self-prescription and prescription by animal health workers. However, in this current study, colleague farmers were mostly engaged in prescriping antibiotics to farmers (35.1%), veterinarians (31.6%) and drug sellers (18.1%). This could be attributed to the limited number of veterinary staff, their delay or failure to attend to farmers' early as well as expensive veterinary service charges that compel farmers to resort to their colleagues for such service. Most farmers (Table 4.3) had no training on antibiotic usage. This finding is in tandem with the work of Ozturk *et al*. (2019) who found that 91.1% of the farmers have not received training on any subject relating to antibiotics usage.

5.6 Antibiotic medication

About 81.1% of the farmers used antibiotics for medication in this present study. Tetracycline was the most commonly used antibiotic by farmers in this current study followed by amoxycilin/clavulanic acid, trimethoprim/sulfamethoxazole, chloramphenicol, herbal drug, ciprofloxacin and gentamicin with erythromycin and penicillin being the least. The misuse of such antibiotics has a greater potential for antimicrobial resistance (WHO, 2017). This study is comparable to the work of

Adesokan *et al*. (2015) who revealed that tetracyclines (33.6%), fluoroquinolones (26.5%) and beta-lactams/aminoglycosides (20.4%) constituted the majority of antimicrobials used in livestock animal production in south-western Nigeria and also agrees with Katakweba *et al*. (2012) who identified tetracycline (61%), sulphadimidines (23%), penicillin-streptomycin (2%) and gentamycin (1%) as the most used antibiotic among livestock keepers in Tanzania. In general, tetracyclines are the medication of choice for rickettsiae and mycoplasma treatment (Eliopoulos *et al*., 2003). However, several pathogenic *E. coli* isolates are resistance to tetracycline (Hoerauf *et al*., 1999) probably due to misuse in animal production by farmers.

5.7 Purpose of antibiotic usage

The common uses of antibiotics are for treatment of sick animals, prophylaxis and growth promoters, which are important for a safe and efficient animal industry (McEwen, 2006). The above finding is in tandem with this work where majority of the farmers use antibiotic for treatment of sick animals (36.7%) only, for both treatments of sick animals and prophylactic purposes (20.2%), for growth purposes (14.4%) and the least use was for growth and prophylactic purposes. The current findings also agree with the work of Oluwasile *et al*. (2014) who stated that antibiotics were used by poultry farmers for therapeutic purpose (36.2%), prophylactic purpose (29.3%), both therapeutics and prophylactics (32.8%) and as growth promoters (6.9%). Also, a surveyed conducted by Ferdous *et al*. (2019) found that antibiotics were used by farmers for therapeutic purposes (34.16%), prophylaxis purposes (14.17%) and for both therapeutic and prophylaxis purposes (40.83%). Another study also reported that

the most frequent uses of antibiotics were those for the prevention and treatment of diseases as well as growth promoters in poultry and pigs (Casewell, 2003).

5.8 Observation of safety and dosage instructions and management of unrecovered animals

This study showed that few farmers did not follow safety dosage (28.9%), however, majority of the farmers observed the safety and dosage instruction (72.1%). This practice is good and must be encouraged to prevent the under dose and over dose administration of drugs that contribute to antimicrobial resistance. Also, a greater number of farmers sell their unrecovered animals after antibiotic treatment to butchers (29.0%) and some farmers send those animals to market for sell (27.9%). Animals treated with antibiotics but not recovered and sold to butchers and those that are taken to markets may end up in abattoirs to be processed for meat which may contain the antibiotic residues. This may not be good for human consumption especially when the withdrawal period is not overdue. The practice where farmers slaughter and bury unrecovered animals after antibiotic treatment must be encouraged in order to prevent public from consuming meat with drug residues.

5.9 Coliform count in RTE meats

Pork had the highest coliform counts followed by beef, and chicken had the least. This was an indicative of microbial presence in the RTE meat samples analyzed as found in Figure 4.7. Ready-to-eat meats are cooked using sufficient heat that can kill all microbes. However, due to poor personal hygiene and handling after preparation, cross contamination is likely. The Ghana Standard Authority recommends microbial load of

less than 5logcfu/cm for microbial contamination of grilled meat. This means that the appropriate limit put out by the Ghana Standard Authority was met for all RTE meats. The microbial contamination contained in this study is also satisfactory. Microbial contamination of 4.732-7.267 log cfu/g was recorded by Ampaw (2018) in 'Khebabs' sold on Accra Lane, Ghana. Microbial contamination of 5.02 log cfu/g for grilled RTE meat samples purchased from Osu, Nima and Accra Central was also reported by Agbodaze *et al*. (2015) and attributed to poor hygiene and sanitary measures adapted by RTE meat vendors.

5.10 Prevalence of *E. coli* in RTE meats

This study found that 32 isolates of *E. coli* had been found which indicated that the RTE meats were directly or indirectly contaminated with faecal materials. Chevon recorded the greater prevalence rate of *E. coli* followed by guinea fowl and chicken. However, mutton and beef had the same prevalent rate and the least was pork as presented in Figure 8. The most widely identified carriers of foodborne pathogens were red meat and poultry (Akbar and Anal, 2011). The overall prevalence rate of *E. coli* was 12.6% of the RTE meats samples examined. The contamination rate may be as a results of poor post-cooking handling practices, food supplies, meat storage conditions and length at points of sale can contribute significantly to the pathogenic and spoilage microorganisms' presence in RTE (Henriques and Fraqueza, 2015). This finding concord with Zhao *et al*. (2001) who identified chicken to have the highest contamination rate with *E. coli*, followed by beef, pork and the least was turkey. The study is also comparable to the Hassanani *et al*. (2014) findings where the incidences of *E. coli* was highest in hawawshi RTE meat, kofta and shawerma collected from

different fast-food services in different districts in Menofia governorate county. Also, Ahmadi *et al*. (2012) revealed that out of 33 ready-to-eat meats and meat products such as meat curry and 25 samples of non-veg momo, 4 (12.12%) and 1(4.0%) were found to be positive for *E. coli* respectively.

5.11 Antimicrobial resistance

Susceptibility test of *E. coli* was examined against nine (9) antimicrobial agents and the results are presented in Table 6. *E. coli* was highly susceptible to chloramphenicol, ciprofloxacin and ceftriaxone. However, the result found higher resistance to teicoplanin, tetracycline and equal resistance rate to amoxycillin/clavulanic acid and azithromycin. From Table 4.3 the farmers mostly used tetracycline, amoxycillin/clavulanic and suphamethoxazole/trimethoprim. These drugs were resistance to *E. coli* isolates. This may be ascribed to the abuse of these antibiotics medications, as well as the absence of new drug development by the pharmaceutical industry due to restricted economic enticement and difficult regulatory conditions (Capital and Alonso-Calleja, 2013). This finding is in agreement with Adzitey (2020) but differs from the study by Dsani (2019). Intermediate resistance occurred for gentamicin, suphamethoxazole/trimethoprim, ceftriaxone and ciprofloxacin. Intermediate resistance suggests potential resistance and when they are involved in infections, those isolates are hard to handle or must be destroyed (Weinstein and Fridkin, 2001).

5.12 Multiple antibiotic resistance (MAR)

E. coli isolates from chevon, pork and chicken had the highest antibiotic resistant profile with resistance to 7 antibiotics each, with MAR index 0.78. Mutton and chevon had the lowest resistant profile (TecTe) with resistant to 2 antibiotics with MAR index of 0.22. In a related study, Akbar *et al*. (2014) identified that *E. coli* isolates were not susceptible to at least 3 antibiotics, often used against Gram-negative bacteria, in all poultry meat. Almost nine (9) *E. coli* isolates were not susceptible to 4 antibiotics, while resistance to 5 antibiotics was observed in 15 isolates. The highest resistance of one *E. coli* isolate was observed in 7 antibiotics (ampicillin, tetracycline, sulfamethoxazole/trimethoprim, gentamicin, chloramphenicol, nalidixic acid, and kanamycin) out of nine included in the study. These disparities and similarities are due to the degree of use of antibiotics for animal farming and human therapeutic purposes (Schroeder *et al*., 2002; Carson *et al*., 2008).

CHAPTER SIX

6.0 CONCLUSIONS

The livestock farmers in the Bolgatanga municipal had some knowledge on antibiotic usage in animals even though majority of them had not received training on antibiotic usage. In RTE meats, *E. coli* contamination was low and was within the acceptable limit of 25g. *E. coli* isolates were resistance to tetracycline which was the main drug use by farmers in the study area on their animals. However, *E. coli* isolates were highly susceptible to chloramphenicol.

CHAPTER SEVEN

7.0 RECOMMENDATIONS

1. Livestock farmers need training to enhance their knowledge on antibiotic usage to mitigate the spread of antibiotic resistance.
2. Also, RTE meats sellers must take hygienic practice seriously to keep the meat safe and wholesome for public consumption.
3. Further research should investigate the presence of resistance genes and genetic characterization of the *E. coli* isolates.

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APPENDIX I

Questionaire for livestock farmers

UNIVERSITY FOR DEVELOMENT STUDEIS
FACULTY OF AGRICULTURE
DEPARTMENT OF ANIMAL SCIENCE

A SURVEY ON ANTIBIOTICS USAGE IN LIVESTOCK IN BOLGATANGA MUNICIPAL

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 [√]

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. Religion a. Christianity [ ] b. Islamic [ ] c. Traditional [ ] d. Others [ ]
4. Tribe ……..
5. Educational level. a. Non formal [ ] b. Primary school [ ] c. Junior high school [ ] d. Senior High school [ ] e. Tertiary [ ] f. Others [ ]

THE FARMER'S KNOWLEDGE ON ANTIBIOTICS AND THEIR USAGE

6. What type of animals do you rear? a. Cattle [ ] b. Goat [ ] c. Sheep [ ] d. Pig [ ] e. Guinea fowl [ ] f. Fowl [ ] e. Others [ ]
7. State the number of each species of animals you rear.? a. Cattle …… b. Goat …….c. Sheep …….d. Pig …….. e. Guinea fowl …… f. Fowl …… e. Others……
8. How many years have you been in this business? a. 0-11 months b. 1-2 years [ ] c Between 3 - 5 years [ ] d. 6-10 years [ ] e. 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/technicians/animal health 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. Chemo-therapeutic medication c. Others specify……..
13. Did the medication include antibiotics? a. Yes[ ] b. No[ ]
14. Have you ever treated your animals by yourself? a. Yes[ ] b. No[ ]
15. What type of antibiotic did you use? a. Gentamicin [ ] b. Tetracycline [ ]
    c. Amoxycillin/Clavulanic [ ] d. Trimethoprim/Sulfamethoxazole [ ] e. Ciprofloxacin [ ] f. Erytromycin [ ] g. Chloramphenicol [ ] h. Others specify……
16. Who recommended the antibiotics for you? a. Colleagues farmer [ ] b. Veterinarian [ ] c. Drug seller d. others ……
17. Have you received training on antibiotic usage? a. Yes [ ] b. No [ ]
18. If No, do you have knowledge on the antibiotic you used? a. Yes [ ] b. No [ ]
19. If yes, who/where did you get the information from? a. extension officers [ ] b. NGOs [ ] c. colleague farmers [ ] d. veterinary staff e. others [ ]
20. Where doyou buy the antibiotics from? a. Veterinary clinic/Shops [ ] b. Friends [ ] c. Chemical shops [ ] d. Others (specify)……
    …
21. How often do you treat your animals with antibiotics? ……
22. For what purpose do you use antibiotics? A. treat sick animals [ ] b. for growth promotion [ ] c. prophylactic purposes [ ] d others specify …….

23Who administers the antibiotic to the animals? a. self [ ] b. veterinary officer [ ] c. both. d. Others specify……

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

25 If No, why? ……

26 Do you know that antibiotics have withdrawal periods? a. Yes [ ] b. No [ ]

27 Do you go by the withdrawal period the manufacturer stated on the drug leaflet?a. Yes [ ] b. No [ ]

28 Do you know that antibiotics have expiring date? a. Yes [ ] b. No [ ]

29 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……

30 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?
.....

31 Do you know that antibiotics used can leave residues in meat? a Yes [ ] b No [ ]

32 Do you know that meatwith antibiotic residues may affect human healthwhen consumed? a Yes [ ]b No [ ]

APPENDIX II

Analysis of *E. coli* using SPSS version 18

Generalised linear model

Case Processing Summary

	N	%
Included	300	100.0%
Excluded	0	0.0%
Total	300	100.0%

Categorical Variable Information

			N	%
Dependent Variable	Escherichiacoli	0	260	86.7%
		1	40	13.3%
		Total	300	100.0%
Factor	Meattype	Beef	50	16.7%
		Chevon	50	16.7%
		Chiken	50	16.7%
		Guinea fowl meat	50	16.7%
		Mutton	50	16.7%
		Pork	50	16.7%
		Total	300	100.0%

Tests of Model Effects

Source	Type III		
	Wald Chi-Square	df	Sig.
(Intercept)	110.767	1	.000
Meattype	9.482	5	.091

Dependent Variable: Escherichiacoli

Model: (Intercept), Meattype

Parameter Estimates

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test
			Lower	Upper	Wald Chi-Square
(Intercept)	2.752	.5955	1.584	3.919	21.350
[Meattype=Beef]	-.309	.7914	-1.860	1.242	.153
[Meattype=Chevon]	-1.486	.6864	-2.831	-.141	4.686
[Meattype=Chiken]	-1.093	.7095	-2.484	.297	2.374
[Meattype=Guinea fowl meat]	-1.365	.6925	-2.723	-.008	3.886
[Meattype=Mutton]	-.309	.7914	-1.860	1.242	.153

[Meattype=Pork]	0[a]	.	.	.	.	
(Scale)	1[b]					

Parameter Estimates

Parameter	Hypothesis Test	
	df	Sig.
(Intercept)	1	.000
[Meattype=Beef]	1	.696
[Meattype=Chevon]	1	.030
[Meattype=Chiken]	1	.123
[Meattype=Guinea fowl meat]	1	.049
[Meattype=Mutton]	1	.696
[Meattype=Pork]	.[a]	.
(Scale)		

Dependent Variable: Escherichiacoli

Model: (Intercept), Meattype

a. Set to zero because this parameter is redundant.

b. Fixed at the displayed value.

Estimated Marginal Means: Meat type

Estimates

Meattype	Mean	Std. Error	95% Wald Confidence Interval	
			Lower	Upper
Beef	.92	.038	.81	.97
Chevon	.78	.059	.64	.87
Chiken	.84	.052	.71	.92
Guinea fowl meat	.80	.057	.67	.89
Mutton	.92	.038	.81	.97
Pork	.94	.034	.83	.98

Pairwise Comparisons

(I) Meattype	(J) Meattype	Mean Difference (I-J)	Std. Error	df	Sig.	95% Wald Confidence Interval for Difference
						Lower
Beef	Chevon	.14[a]	.070	1	.046	.00
	Chiken	.08	.064	1	.215	-.05
	Guinea fowl meat	.12	.068	1	.079	-.01
	Mutton	.00	.054	1	1.000	-.11
	Pork	-.02	.051	1	.695	-.12
Chevon	Beef	-.14[a]	.070	1	.046	-.28
	Chiken	-.06	.078	1	.443	-.21

	Guinea fowl meat	-.02	.081	1	.806	-.18
	Mutton	-.14[a]	.070	1	.046	-.28
	Pork	-.16[a]	.068	1	.018	-.29
	Beef	-.08	.064	1	.215	-.21
	Chevon	.06	.078	1	.443	-.09
Chiken	Guinea fowl meat	.04	.077	1	.602	-.11
	Mutton	-.08	.064	1	.215	-.21
	Pork	-.10	.062	1	.105	-.22
	Beef	-.12	.068	1	.079	-.25
	Chevon	.02	.081	1	.806	-.14
Guinea fowl meat	Chiken	-.04	.077	1	.602	-.19
	Mutton	-.12	.068	1	.079	-.25
	Pork	-.14[a]	.066	1	.033	-.27
	Beef	.00	.054	1	1.000	-.11
	Chevon	.14[a]	.070	1	.046	.00
Mutton	Chiken	.08	.064	1	.215	-.05
	Guinea fowl meat	.12	.068	1	.079	-.01
	Pork	-.02	.051	1	.695	-.12
	Beef	.02	.051	1	.695	-.08
Pork	Chevon	.16[a]	.068	1	.018	.03
	Chiken	.10	.062	1	.105	-.02

	Guinea fowl meat	.14[a]	.066	1	.033	.01
	Mutton	.02	.051	1	.695	-.08

Pairwise Comparisons

(I) Meattype	(J) Meattype	95% Wald Confidence Interval for Difference
		Upper
Beef	Chevon	.28[a]
	Chiken	.21
	Guinea fowl meat	.25
	Mutton	.11
	Pork	.08
Chevon	Beef	.00[a]
	Chiken	.09
	Guinea fowl meat	.14
	Mutton	.00[a]
	Pork	-.03[a]
Chiken	Beef	.05
	Chevon	.21
	Guinea fowl meat	.19
	Mutton	.05
	Pork	.02
Guinea fowl meat	Beef	.01

	Chevon	.18
	Chiken	.11
	Mutton	.01
	Pork	-.01[a]
	Beef	.11
	Chevon	.28[a]
Mutton	Chiken	.21
	Guinea fowl meat	.25
	Pork	.08
	Beef	.12
	Chevon	.29[a]
Pork	Chiken	.22
	Guinea fowl meat	.27[a]
	Mutton	.12

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

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

Overall Test Results

Wald Chi-Square	df	Sig.
10.414	5	.064

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