UNIVERSITY FOR DEVELOPMENT STUDIES, TAMALE

KNOWLEDGE ON MEAT SAFETY, PREVALENCE AND ANTIBIOTIC SUSCEPTIBILITY OF *SALMONELLA ENTERICA* IN READY -TO-EAT (RTE) MEATS VENDED ON THE STREETS OF BOLGATANGA

ADUAH MARTIN

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BY

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

SEPTEMBER, 2020

DECLARATION

Candidate's 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 works of others cited in the text have been well referenced and duly acknowledged. Any assistance received in writing the thesis is as well recognized.

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

Supervisor's Signature: …….. Date: ……

Name: Prof. Frederick Adzitey (PhD)

ABSTRACT

All over the world, foodborne infection is a key challenge to public health which continuously threatens consumers through the consumption of contaminated foods including meat. This study was conducted to assess the knowledge and practices of meat safety by grilled ready-to-eat (RTE) meat vendors and consumers. The study also determined the microbial quality, prevalence and antibiotic susceptibility of *Salmonella enterica* in grilled ready-to-eat (RTE) meats vended on the streets of Bolgatanga, the Upper East Regional capital of Ghana. A descriptive survey design comprising semi-structured questionnaires was used to obtain information on the knowledge and practices of food safety from 300 grilled ready-to-eat (RTE) meat vendors and 382 RTE meat consumers, selected at random from the study area between October, 2019 and February, 2020. In addition, physical observations of the vending sites were made to appreciate the hygienic conditions under which RTE meats are grilled and sold to consumers. A total of three hundred grilled RTE meat swab samples were obtained from beef (50), chicken (50), chevon (50), guinea fowl (50), mutton (50) and pork (50) from the selected vender shops in Bolgatanga; and examined for the prevalence of *Salmonella enterica* and total aerobic bacteria according to the procedures in the USA-FDA Bacteriological Analytical Manual. Antibiotic susceptibility test was carried out using the disc diffusion method and the results interpreted as indicated in the Clinical and Laboratory Standards Institute (CLSI), (2008) guidelines. All data were analyzed using SPSS (version 20) and $P<0.05$ was considered significant. The results showed that almost all the vendors (97.7%) were males and majority aged between 21–40 years (77.3%) and were Muslims (63.0%) by religion. Also, 98.3% of the vendors heard about meat safety and 94.7% knew that it is necessary to refrigerate leftover meat. In addition, 37.0% of the vendors obtained their meat through backyard slaughter and most (48.0%) sold their meat on table with a wire mesh covering the meat. The results further revealed that vendors do not always wear gloves (68.0%), but are willing to adhere to food safety protocols (100.0%). For the RTE meat consumers, majority (71.7%) were males, had ages between 21 and 40

years (65.4%) and a greater proportion (69.6%) preferred grilled RTE guinea fowl when they go out with friends in the evening (86.9%). However, the study revealed that a good number of the consumers (76.6%) were not aware that eating, drinking and smoking by vending sites of RTE meat increases the risk of cross contamination. AlsoAlso, 94.0% of the respondents were aware that regular hand washing and the use of sterilized gloves by vendors reduces the risk of contamination and will want vendors to wear apron, gloves and mouth mask while preparing and selling the different meats. However, 46.3% of the consummers did not want the vendors to wear jewelries while handling RTE meat. This study revealed that the mean total plate count (TPC) of beef, chicken, chevon, guinea fowl meat, mutton and pork were 3.368 log_{10} cfu/cm^2, 2.526 log_{10} cfu/cm^2, 4.852 log_{10} cfu/cm^2, 4.057 log10 log_{10} cfu/cm^2, 4.171 log_{10} cfu/cm^2 and 4.02 log_{10} cfu/cm^2, respectively. It was revealed that chevon had the highest count of 4.852 log_{10} cfu/cm^2 whilst the least count of 2.526 log_{10} cfu/cm^2 for chicken. The results showed significant difference ($P<0.001$) among bacterial count of the various meat types. However, there were no significant difference ($P>0.05$) among guinea fowl meat, mutton and pork. Furthermore, the prevalence of *Salmonella enterica* in the RTE meats was 2% (6). Guinea fowl meat recorded the highest prevalence (4%) whilst tested beef was negative for of *Salmonella enterica*. There were no significant difference ($P>0.05$) among the prevalence of *Salmonella enterica* in all the grilled RTE meat types. Physical examination of the grilled RTE meats' environment revealed that the vendors largely engaged in good hygienic practices. The study also revealed that grilled RTE meats sold in Bolgatanga are generally safe from *Salmonella enterica* and had microbial load not above acceptable limit.

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DEDICATION

This thesis is dedicated to my beloved wife, Damwora Cecilia and kids; Remy, Phoebe and Zebedee.

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CHAPTER ONE

1.0 INTRODUCTION

Meat is consumed by many people worldwide because of its nutritive composition (Ahmad *et al*., 2018). Meat protein profile comprises of amino acids that have been described as excellent due to its requirement for body growth (Azage and Kibre, 2017). It also serves as a good medium for bacterial growth (Azage and Kibre, 2017). The major predominant food borne bacterial agents that have frequently been associated with food of animal origin include *Salmonella, Listeria monocytogenes, Escherichia coli* 0157:H7, *Bacillus cereus, Campylobacter, Staphylococcus aureus, Shigella,Vibro parahaemolyticus, Escherichia coli* and *Yersinia* spp. (Hur *et al.*, 2011; Centers for disease control and prevention (CDC), 2009). These organisms have been linked to a number of human illnesses (Mershal *et al*., 2010) and deaths annually (CDC, 2011). *Salmonella* species are the most frequently reported cause of foodborne illnesses (Birhaneselassie and Williams, 2013). These lead millions of cases of enteric diseases, thousands of hospitalizations as well as deaths world-wide each year (Hur *et al*., 2011; CDC, 2009). Rose *et al*. (2002) also indicated that *Salmonella* is the cause of large number of food borne disease outbreaks and fatalities from enteric pathogens. Various food products have been depicted as transporting agents of infection by *Salmonella* to humans, including beef, chicken, pork, eggs and seafood. *Salmonella* is also a persistent pathogen that is capable of surviving and proliferating in a variety of environmental conditions including food production and food processing plants (Mezal *et al*., 2013). Sofos (2005) indicated that the types of microorganisms and level of contamination present on the final

product are determined by sanitation measures, hygienic practices, food safety interventions applied, type of product handling, extent of product handling, method of processing coupled with the conditions of storage and distribution.

In recent times, the intensive use of common antimicrobials in human and veterinary medicine for different purposes such as therapeutics, prophylactics as well as growth promoters have increased the emergence and widespread of antibiotic resistant food borne bacteria (Zhang *et al*., 2018). Nowadays, the antimicrobial resistance (AMR) phenomenon is seen as one of the most worrisome public health concerns, with deleterious impact on the effectiveness of public health interventions (Zhang *et al*., 2018; European Center for Disease Prevention and Control (ECDPC), 2019). The European Union (EU) member states has made great efforts to establish harmonized inter-institutional strategies under a One Heaalth approach to combat AMR (Akbar and Anal, 2011). Foods contaminated with antibiotic-resistant bacteria are a major challenge to public health (Akbar and Anal, 2011). A study by Saleh and Mohammed (2019) revealed that majority of the participants (64.3%) know that antibiotics are effective against bacterial infections, while (46.8%) of participants believed that antibiotics can be used to treat viral infections. Gillespie *et al*. (2000), Fang *et al.* (2003) and Mahale *et al*. (2008) have likewise reported that, street vended foods are usually associated with food borne diseases.

Grilled RTE meats are animal products usually vended on the streets and easily accessible to most consumers (Agbodaze *et al*., 2005). The meats are often chevon, pork, mutton, beef, chicken, guinea fowl meat among others and may be potentially

hazardous to the health of consumers (Agbodaze *et al*., 2005). Tracking bacterial prevalence and their antibiotic resistance are very vital to appreciate the trends and degree of food associated pathogens required to plan an effective intervention (Fernandez *et al*., 2012). Epidemiological data associated with *Salmonella* incidence and its antimicrobial drug resistance pattern is essential in order to develop an efficient mechanism towards its control at every level of the food processing and production chain, to ensure food safety and public health (Angkititrakul *et al*., 2005). Proper sanitation, refrigeration and handling of the meat are of paramount concern, if contamination is to be minimized and microbial activity is to be curtailed (Agbdaze *et al*., 2005). Standardized, multi-regional data are also required to better understand the nature and burden of salmonellosis in Bolgatanga.

1.2 Objectives

1. To assess the knowledge, attitude and practices of RTE meat venders on microbiological safety of meat.
2. To assess the knowledge, attitudes and practices of meat consumers on the microbiological safety of RTE meats vended on the streets of Bolgatanga.
3. To determine the microbial load of grilled RTE meats vended on the streets of Bolgatanga.
4. To determine the prevalence of resistant *Salmonella enterica* in ready-to-eat (RTE) meats vended on the streets of Bolgatanga.
5. To determine the antibiotic resistance *Salmonella* spp. isolated from RTE meats vended on the streets of Bolgatanga.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Nutritional Value of Meat

A report by Lawrie and Ledward (2006) defined meat as the skeletal muscle and it related fat and additional tissues which includes offals, brain, liver, heart, pancreas, kidney, spleen, tongue, thymus and tripe. McArdle (2000) indicated that meat is produced as a result of slaughtering and butchering animal, thus killing and cutting flesh out of the animal. According to Tutenel *et al.* (2003) the primary composition of meat is protein and water and it is mostly consumed together with other foods. Meat tissues serve as ready source of nutrients required for the growth of microorganisms and consist of 0.2% glucose and 0.4% amino acids; which are metabolized by microflora (Sofos, 2008; Dainty and Mackey, 1992). Meat and meat products are of great importance since they consist of all B- vitamins such as thiamin (B1), riboflavin (B2), niacin (B3), pantothenic acid (B5), pyridoxine (B6), biotin (B7), folate (B9) and cobalamin (B12) (Sofos, 2008). Meat is also a good source of some minerals such as iron (Fe), copper (Cu), znc (Zn) and manganese (Mn) (Sofos, 2008). Meat also plays a vital role in mitigating iron and zinc deficiency (Dainty and Mackey *et al.*, 1990).

Even though meat can be consumed in raw form, it is mostly consumed after successive cooking and processing in various ways. Meats that are unprocessed spoil within short periods (Tutenel et al., 2003). Spoilage in general, is due to practically unavoidable contamination which lead to deterioration of meat by microorganisms and fungi that may be borne by the animal itself and or the meat handlers and their

instruments (Tutenel *et al.*, 2003). Depending on the myoglobin level in the myofibrils, meat can be largely categorized as "red" or "white". Meats with higher myoglobin content appear reddish because when myoglobin in the meat exposes to oxygen, it reacts with oxygen and become oxy-myoglobin which is red (Lawrie and Ledward, 2006). The red color of meat is also determined by the age, species of animal and the type of myofibrils. Red meat has more slim myofibrils whereas white meat has more fat myofibrils (Lawrie and Ledward, 2006). Williams (2007) indicated that the nutritional structure of red meats depends on the feed, breed, season and meat cut. Nevertheless, the protein content, essential vitamins and minerals are uniformly high in lean red meat. Sofos (2008), also indicated that meat is a complete protein food which contains all the essential amino acids required for the proper growth and development of the human body.

2.2 Consumption of Meat and Related Health Problems

Cross *et al*. (2007) reported that health risks related to meat consumption may differ based on the meat type and the method of production, processing and preparation. They further indicated in their report that colorectal, lung, esophagus and liver cancer can be due to high red meat consumption. A high dangers of lung cancer has also been reported to be associated with meat consumption; red meat (Alavanja *et al*. 2001), fried red meat (Sinha *et al*. 2000). Larsson and Orsini (2013) indicated that high consumption of processed red meat is associated with higher mortality rates compared with those who consume less red meat.

2.3 Microbiological Food Safety and Foodborne Disease

Food safety describes preparation, storage and handling of food in ways that prevent foodborne illness (WHO, 2002). Food provide energy and nutrients required by human for survival, however, it may serve as a channel and a medium for transportation and growth of pathogens respectively (Ababio and Adi, 2012). These pathogens are responsible for foodborne illness in humans and animals (Adzitey *et al*., 2014). Flu-like gastrointestinal symptoms such as diarrhea and vomiting are just a few of the symptoms associated with foodborne illness in humans (CDC, 2013). Economic losses and death which still affect all countries worldwide are associated with foodborne diseases (Ababio and Adi, 2012). According to Iyer *et al*. (2013), in developing countries, foodborne pathogens are known to be the most cause of illness and death with approximately1.8 million killed annually. The Centers for Disease Control and Prevention (CDC) has previously forecasted 76 million cases of foodborne illness each year, with 325,000 hospitalizations resulting in 5,000 deaths (CDC, 2009).

WHO (2002) indicated that food safety must consistently be one of the highest needs of the sustenance of the food industry worldwide. Gilling *et al*. (2001) suggested that microbiological safety of food can be achieved if both the handlers and the foods are continually monitored.

2.4 Microbiological Food Hazards

The very common means by which commercially processed foods are contaminated from the factory environment is the post-process contamination (Kornacki, 2000; Allan *et al.*, 2004; Reij and Den Aantrekker, 2004). The post cook handling practices, food ingredients, condition and the duration of the food storage at selling points can significantly contribute to growth of pathogenic and spoilage microorganisms in ready-to-eat (RTE) food (Khairuzzaman *et al.*, 2014). Food workers frequently mishandle food by subjecting them to unsanitary conditions usually on the street (Agbodaze *et al.*, 2005; Muinde and Kuria, 2005; Ghosh *et al.*, 2007). Nutritious foods, such as meat, provide the favourable inherent condition to support the colonization of contaminating pathogenic and spoilage microorganisms (Clarence *et al.*, 2009). Animal products have been identified as the major vehicle for foodborne pathogens such as *Salmonella* spp., *Escherichia coli* 0157:H7, *Listeria monocytogenes*, *Camphylobacter jejuni*, *Clostridium perfringens*, and *Staphylococcus aureus* (Clarence *et al.*, 2009). The use of contaminated equipment and food ingredients can also serve as major source of foodborne pathogens (Medeiros *et al.*, 2001; Beumer and Kusumaningrum, 2003; Redmond and Grifith, 2003).

2.5 Contamination of Meat by Microorganisms

Abaidoo and Obiri-Danso (2008) described microorganisms as minute living creatures that can be found everywhere in nature including meat. They are microscopic and some examples of microorganisms found in meat are bacteria,

yeasts, molds and viruses. Many of these are disease-causing (pathogenic); they are capable of causing foodborne illnesses (Abaidoo and Obiri-Danso 2008). Due to this, Doyle (2007) suggested that meat should be frozen and good hygiene practices observed to prevent microbial contamination.

2.6 Major Bacteria of Health Concern in Meat

Meat serves as a good medium for bacterial growth and a major contributor to foodborne diseases (Bintsis, 2017; Ashwathi, 2020). The contact of the hide with carcass during slaughtering allows a multitude of microbes to contaminate the carcasses. These contaminating microbes from the hide may be of fecal, feed, soil or water source (Church and Wood, 1992) (Table 2.1). Majority of these microbes exist in the intestinal tracts of animals and during slaughtering, could get into the carcass surfaces (Church and Wood, 1992). *Salmonella, Staphylococcus aureus, Clostridia perfringens, Listeria monocytogenes, Escherichia coli*, *Yersinia enterocolitica,* and *Campylobacter jejuni* have been identified to be associated with raw meat samples (Church and Wood, 1992).

Table 2.1: Bacteria of health concern in meat and their sources

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

Source: Church and Wood (1992)

2.7.0 Salmonellosis

Salmonellosis is endemic in most countries and causes heavy economic social losses (Agbaje *et al*., 2011).

According to Lillehoj *et al*. (2003), human salmonellosis includes several syndromes such as enteric fever, gastroenteritis, septicaemia, focal infections and, in the case of some typhoidal strains, an asymptomatic carrier state. Bacillary white diarrhoea (Pullorum disease) and fowl typhoid, caused by *Salmonella* Gallinarum, biovars Pullorum and Gallinarum, respectively, are important among various forms of poultry salmonellosis Agbaje *et al*. (2011).

Salmonella enterica is one of the prominent causes of enteric diseases worldwide (Akbar *et al*., 2013). It causes great number of illness and substantial economic losses in both developing and developed countries (Akbar *et al*., 2013). *Salmonella*

enterica infections are more often associated with foodborne illness (Fernandez *et al*., 2012) and human gastroenteritis (Skov *et al*., 2007). Consumption of animal food products are major cause of the *Salmonella* outbreaks (Thai *et al*., 2012). *Salmonella* spp. Are responsible for 1,722 outbreaks of foodborne infections in the European countries in 2009 (Fernandez *et al*., 2012).

2.7.1 Gastroenteritis

Salmonella gastroenteritis (salmonellosis) is a disease mostly caused by non-typhoidal *Salmonella* serotypes, particularly *Salmonella Enteritidis*. Gastroenteritis usually starts with nausea, vomiting and later progresses to abdominal pain and diarrhea, which could be mild or severe and with or without blood (WHO/FAO, 2002; Darby and Sheorey, 2008). Salmonellosis normally takes some few days symptoms to show, it can be self-limited and the patient may not require medications except in patients that a very young or immunecompromised (Christenson, 2013).

2.7.2 Enteric Fever

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

2.7.3 Bacteremia

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

2.7.4 Asymptomatic Carriers

Giannella (2002) stated that 3% of recovered patients infected with typhoidal and 0.1% of non-typhoidal Salnmonella became chronic carriers. Close to 2-5% of individuals who recovered from typhoid fever become carriers either temporarily or permanently. The microorganisms are usually harbored in their biliary tract,

gallbladder, or intestines (Vandepitte *et al*., 2003). According to Ul-Hassan *et al*. (2004), typhoid fever in many developing countries still remains endemic.

2.8 Treatments for Salmonellosis

Non-typhoidal *Salmonella* infections mostly occur with mild-to-moderate self-limiting gastroenteritis and antimicrobial treatments are only required in severe cases in immune- compromised patients or invasive infections (Andoh *et al*., 2017). Fluoroquinolones are one of the alternatives in the treatment of invasive infections caused by *Salmonella* with multi-resistance to traditional antibiotics (Chen *et al*., 2013). However, resistance to fluoroquinolones has been frequently reported in many countries (Chen *et al*., 2013). Extended-spectrum cephalosporins are also used for treatment of invasive infections caused by multidrug-resistant Salmonella (Chen *et al*., 2013).

In Ghana, Andoh *et al*. (2017) reported that the Ghana Health Service recommends the use of chloramphenicol and ciprofloxacin as first-line drugs of choice for the treatment of invasive Salmonella infections, but in areas with multi-antimicrobial resistance (MAR), third-generation cephalosporins like cefotaxime or ceftriaxone as well as chloramphenicol or a fluoroquinolone are recommended. Lately, treatment with fluoroquinolones or third-generation cephalosporins has become common practice since ampicillin and cotrimoxazole have become ineffective due to resistance (Andoh *et al*., 2017).

2.9 Clinical Importance of *Salmonella* Spp.

Salmonella causes approximately 1.4 million human infections each year in the United States, resulting in 116,000 hospitalizations and 600 deaths (Centers for Disease and Prevention Control (CDC), 2011). Most Salmonella infection is limited to uncomplicated gastroenteritis that seldom requires antimicrobial treatment (Haeusler and Curtis, 2013). In fact, antimicrobial treatment does not reduce the duration or severity of gastroenteritis and instead may result in prolonged fecal excretion and emergence of resistant strains (Su and Chiu, 2007). However, severe sequelae, such as bacteremia or meningitis may develop in an approximately 5-10% of individuals infected with non-typhoid Salmonella (Su and Chiu, 2007). Invasive *Salmonella* infections can be fatal and antimicrobial treatment is essential in these circumstances (Crump *et al*., 2015). Those at risk of development of complications of extra-intestinal salmonellosis include patients at the two age extremes and those with immune suppression, or accompanying severe infections, such as meningitis, septic arthritis and osteomyelitis (Vugia *et al*., 2004). All *Salmonella* can cause extra-intestinal infections, but *S*. Typhi, *S*. Paratyphi, *S*. Choleraesuis and *S*. Dublin are the major serotypes which cause invasive salmonellosis in humans (Birhaneselassie and Williams, 2013). The other serotypes, such as *S*. Typhimurium, *S*. Enteritidis and *S*. Heidelberg, are associated with a relatively low proportion of invasive infections (Birhaneselassie and Williams, 2013). However, the total number of invasive cases caused by these serotypes appears to be high, because they are relatively prevalent among the whole *Salmonella* population (Birhaneselassie and Williams, 2013). Among the invasive non-typhoid *Salmonella* serotypes, *S*.

Choleraesuis is particularly rampant in Asian countries, including Taiwan, while *S.* Dublin is much more prevalent in western countries (Su and Chiu, 2007).

2.10 Transmission and Pathogenesis of *Salmonella* Spp.

In addition to faecal contamination, cross-contamination of foods by *Salmonella* during food preparation can be an important source of foodborne illnesses (Odumeru and León-Velarde, 2012). The most common clinical manifestation of salmonellosis is diarrhoea but in certain instances, septicemia can occur depending on the host factors; the and strain type (Nataro *et al.*, 2011). Host factors include age, immune status, concurrent disease and composition of normal flora which provide resistance to colonization (Nataro *et al.*, 2011).

Salmonella can disseminate and multiply within the phagocytic cells (macrophages mainly) in phagosomes (Haraga *et al.* 2008) as shown in Figures 1a and Figures 1b. Following systemic dissemination, septicemia and endotoxic shock may develop (Haraga *et al.*, 2008). Uncontrolled multiplication of the organism eventually results in endotoxemia, severe vascular damage and death (Haraga *et al.*, 2008).

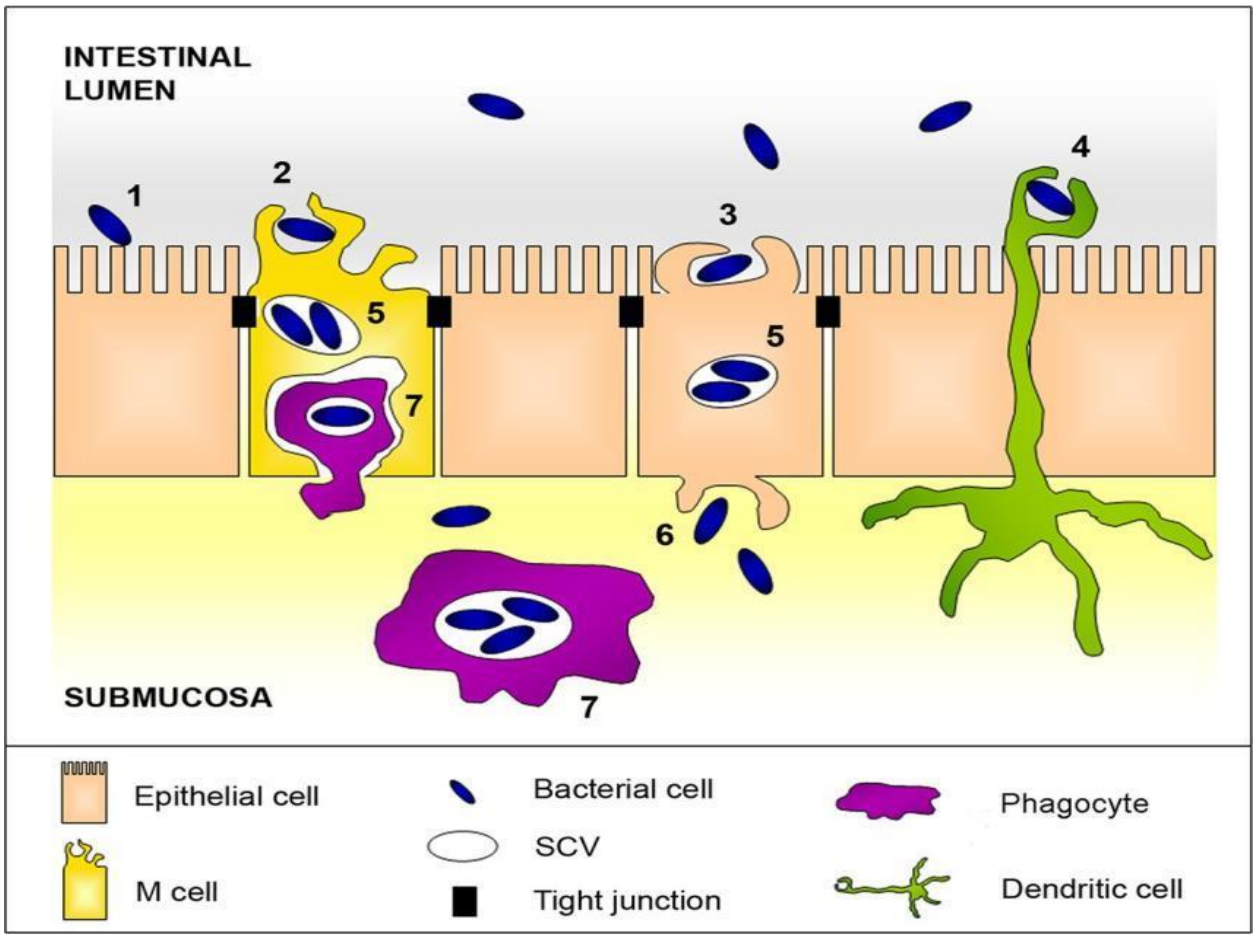

Figure 1a: The pathogenesis procedure of Salmonellosis (Haraga *et al*., 2008)

Key:1- Bacterial cell, 2- Bacteria cell engulfed by the M cell, 3- Bacteria cell engulfed macrophage, 4- Bacteria cell ingested by dendritic cell, 5- Bacteria cell endocytosed by Salmonella-containing vacuole (SCV), 6- Bacteria cell escape lysis from macrophage, 7- Bacteria cell engulfed by phagocyte

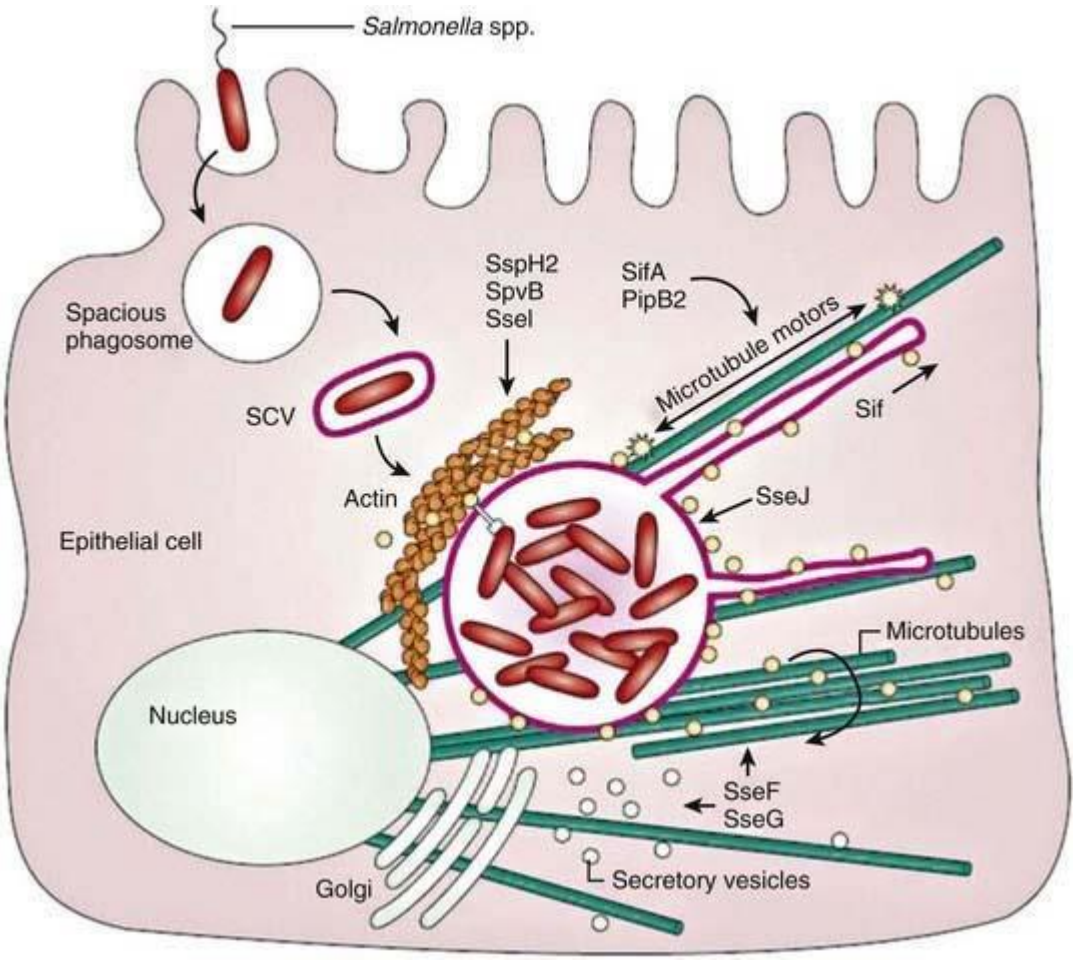

Figure 1b: Pathogenesis procedure of Salmonellosis (Haraga *et al*., 2008)

2.11 Occurrence of *Salmonella* Spp. Worldwide

World Health Organization (2020) estimated that 600 million people fall ill after eating contaminated food and 420,000 die every year as a result. This is particularly encountered in tropical countries including India, South and Central America and Africa, where they constitute serious source of morbidity and mortality with rapid population growth, increased urbanization, limited safe water and health infrastructure problems (WHO, 2006). In Europe, *Salmonella* was the second most reported cause of food borne diseases in humans with 160,649 people suffering from

Salmonella infections in 2006, approximately 35 people in every 10,000 people (European Food Safety Authority, EFSA, 2007).

Salmonellosis is endemic to rural and urban Sub-Saharan Africa (Njenga, 2014). In rural Mozambique, the incidence of salmonellosis is 120 cases per 100,000 people annually (Crump and Mintz, 2010). The true incidence of salmonellosis is likely to be 2–3 times this figure, because the incidence of bacteremia among patients who die before reaching the district hospital has not been ascertained in either study (Crump and Mintz, 2010; Cox and Pavic, 2010). In Uganda, occurrence of salmonellosisis was 500 cases per 100,000 people per year (Njenga 2014). In rural Kenya, the estimated minimum incidence of salmonellosis is 88 cases per 100,000 people per year (Kariuki *et al*., 2015).

In Ghana, typhoid fever ranks among the leading 20 causes of outpatient illness, accounting for 0.92% of hospital admissions (Sory, 2009).

2.12 Symptoms of Salmonellosis

CDC (2013) indicated that the symptoms of salmonellosis in healthy persons among others are fever, diarrhoea, nausea, abdominal pain and vomiting. The FAO and WHO (2017) also stated that the symptoms of salmonellosis include, headache, high fever, lethargy, gastrointestinal symptoms, muscle pains, loss of appetite and in some cases typhoid fever manifests itself by pink spots on the skin. In immune-compromised individuals, elderly and infants, septicaemia, reactive arthritis as well as neurological and neuromuscular illnesses can occur (European Food Safety

Authority (EFSA), (2014); Forshell and Wierup, 2006). According to Healthdirect (2019), the signs and symptoms of salmonella infection include fever, diarrhea, loss of appetite, headache, stomach cramps, nausea and vomiting. Sometimes there may be blood or mucus in the faeces. Dehydration is a serious complication. The illness may be particularly severe in young children, the elderly and people with immune suppression (EFSA 2014).

2.13 Grilled Ready-To-Eat (RTE) Meat

Meat in Ghana havebeen reported to be contaminated with foodborne pathogens (Adzitey *et al*., 2011). Nutrients provided by meat are ready source of nutrients for the microorganisms such as *Staphylococcus* spp., *Aspergillus* spp., *Salmonella* spp., *Enterococcus* spp., *Streptococcus* spp., and *Escherichia coli* (Jay *et al*., 2005). These micro-organisms have all been implicated in meat contamination with high levels recorded for microbes like Salmonella *spp.*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli* (Jay *et al*., 2005). According to Dyckman and Lansburg (2002), poorly cooked meat may affect the health of consumers and hence it is considered as the most important safety hazard attributed to poultry and meat. Grilled ready-to-eat (RTE) meats are a very popular street vended food and may therefore affect the health of many consumers if not well grilled (Adeyemo, 2002). The methods involved in the grilling process can also cause contamination by microorganisms (Adeyemo, 2002).

2.14 Sources of Contamination Of Grilled RTE Meat

The highest significant food safety hazard involves foods from animals (Maripandi and Al-Salamah, 2010). Possible sources of contamination are through slaughtering of unhealthy animals, butchers using dirty water to wash meat, flies contamination as processing is done in unhygienic environments, transport of meat through rickety vehicles, and using contaminated equipment such as knives and chopping tables (Koussemon *et al*., 2008). Contamination of grilled RTE meat may also be due to improper handling and improper hygiene thereby affecting health of consumers (Koussemon *et al*., 2008). Cross contamination of the food (meat) products after the heat treatment is possible and may subsequently lead to the growth of pathogens (Buncic *et al*., 1990).

2.15 Aerobic Bacteria Load Of Ready to Eat Meat

According to Maturin and Peeler (2001), aerobic plate count (APC) gives an indication of the level of microorganism(s) in a product. Ramsubhag *et al*. (2013) stated that the acceptable limit of aerobic bacteria count for food products is 10^5 cfu/g. Also, the Italian guidelines for the microbiological quality of RTE meals in accordance with the microbiological limits suggested by the Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche; a reference Public Institution on food hygiene and veterinary public health, in every 25 g of meat, limit of total mesophilic aerobes (TMA) is $\leq$ 4.0 Log cfu/g (Osimani *et al*., 2015).

Coliforms are counts generally adopted as an efficient parameter for estimating the overall hygiene of foods and their presence in heat-treated meat can most likely be

ascribed to cross contamination (Osimani *et al*., 2015). According to the Italian guidelines for the microbiological quality in every 25 g of RTE meat should be ≤ 3 Log cfu/g (Osimani *et al*., 2015).

Handling, processing and storage are some of the factors affecting the microbial status of RTE foods (Akbar and Anal, 2014; Roy *et al*., 2011). The bacterial load may vary from place to place due to many factors which include environmental condition favorable for bacterial proliferation and mainly issue of hygienic measurements to avoid post-contamination of food (Amare *et al*., 2019).

A study was conducted by Tavakoli and Riazipour (2008) to evaluate the extent of bacterial contaminations of foodstuffs and the study revealed that bacteria and coliform counts (mean ± SD) in grilled ground meat were $1.14 \times 10^5 \pm 1.51 \times 10^2$ (cfu/g) and $1.98 \times 10^2 \pm 0.94 \times 10$ cfu/g, respectively (Tables 2.2 and 2.3).

Table 2.2: Mean total bacterial contamination (cfu/g) in four different foodstuffs

Centre	Grilled ground meat	Chicken	Fish	Grilled chicken
A	5.5×10^1	0	0	0
B	* 3.02×10^2	*2.07×10^2	0	*2.17×10^2
C	* 2.64×10^2	*1.06×10^2	0	1×10^2
D	*2.51×10^2	0	0	*1.58×10^2
E	* 1.16×10^2	0	0	*1.58×10^2
F	* 2.01×10^2	5.8×10^1	0	0
Mean	1.98×10^2	6.1×10^2	0	1.05×10^2

*higher than standards contamination load (Tavakoli and Riazipour, 2008)

Table 2.3: Mean total coliform contamination (cfu/g) in four different foods

Centre	Grilled ground meat	Chicken	Fish	Grilled chicken
A	1.26×10^3	1.67×10^3	2.29×10^3	2.91×10^3
B	$*2.91 \times 10^5$	4.64×10^4	5.91×10^4	8.22×10^4
C	6.59×10^4	6.01×10^4	4.26×10^4	6.39×10^4
D	$*2.2 \times 10^5$	4.19×10^4	5.2×10^4	6.43×10^4
E	2.67×10^2	3.46×10^2	5.67×10^2	2.25×10^3
F	9.67×10^4	2.09×10^4	$*1.79 \times 10^5$	$*1.58 \times 10^5$
Total mean	1.14×10^5	2.85×10^4	5.59×10^4	6.23×10^4

*Higher than standards contamination load (Tavakoli and Riazipour, 2008)

The study also revealed that unhygienic practices such as unclean hands of vendors, dirty aprons, unclean cutting board and grilled meat coming into contact with raw meat were the causes of the higher coliform counts (Tavakoli and Riazipour, 2008). Another study on the total mean aerobic bacterial count by Amare *et al*. (2019) with four different food items vended in Gondar, Ethiopia was 6.64×10^4 cfu/g with loads varying from 1×10^4–1.86×10^5 cfu/g.

Food Standards Australia New Zealand (2016) report indicates that aerobic plate count of foods that have received heat treatment should have bacteria levels between $<10^3$ log cfu/cm^2 and10^4 log cfu/cm^2. It was revealed by Adzitey *et al*. (2019), that grilled beef samples met the criteria of the Food Standards Australia New Zealand and therefore, relatively safe for consumption (Table 2.4).

Table 2.4: Total aerobic bacteria count in raw and grilled beef collected from Nyankpala

Sample	Log cfu/cm2
Raw beef (T1)	3.59[a]
Grilled beef (0h, T2)	2.94[b]
Grilled beef (1h 30min, T3)	2.83[b]
SEM	0.42
P value	0.00

Key: SEM = Standard Error of Means. The superscripts, a & b signifies difference at P<0.05 and vice versa (Adzitey *et al*., 2019).

Adio *et al*. (2014) likewise reported a total viable count (TVC) of 2.8 x 10^6 to 5.465 x 10^6 cfu/g and a total coliform count of 0.2 x 10^5 to 6.35 x 10^5cfu/g from ready to eat (RTE) barbecue meat (suya) sold on the streets of Lagos State, Nigeria (Table 5).

Table 2.5: Total aerobic and coliform microbial population in RTE barbecue meat (suya)

Sample name	Total viable count on nutrient agar (NA)cfu/g	Coliform count on MacConkey agar cfu/g
Ebute Metta 1	2.8×10^6	0.02×10^5
Mushin 2	0.06×10^6	3.75×10^5
Oshodi 3	5.05×10^6	3.4×10^5
Ikorodu 4	4.05×10^6	2.6×10^5
Shomolu 5	7.7×10^6	4.75×10^5
Ketu 6	8.9×10^6	6.1×10^5
Ojota 7	0.06×10^6	4.35×10^5
Surulere 8	7.65×10^6	4.1×10^5
Ikeja 9	5.465×10^6	6.15×10^5
Island 10	9.4×10^6	6.35×10^5

Source: Adio *et al*. (2014).

Adio *et al*. (2014) study revealed that hygienic condition of the meat was below acceptable standard for human consumption. It was also noted that aseptic processing and handling techniques were not adequately employed by the grilled RTE meat (suya) venders to reduce microbial loads of the meat. With regards to coliform contaminations, center A was found to be in the standard range (10^2 cfu/g) while other centres had higher contamination loads. Grilled chicken samples taken from centers B, E and D and chicken samples taken from centre B and C also had higher contamination than the standard range (Tavakoli and Riazipour, 2008).

Salek (2000) conducted an assessment on 100 RTE samples taken from meat foods offered in clinical centres of Shahid Beheshti University of Medical Sciences. Mean total bacterial count detected were 2.04×10^5, 2.16×10^2, 2.45×10^4 and 2.25×10^4 cfu/g in samples of grilled ground meat, grilled chicken, chicken and hamburger, respectively.

Likewise, Agbodaze *et al*. (2005) carried out a study to determine the microbial load (mainly Salmonella, Shigellae, *E. coli*, and Staphylococcus) in 'khebab' (grilled RTE meat) samples bought from Osu, Nima and Accra Central. The study revealed khebab from Osu had a total plate count (TPC) of 5.02, Accra Central samples had TPC of 4.08 and those from Nima had TPC of 4.80 log10 cfu/g of khebab. Samples from Accra central recorded the highest mean coliform count (5.12) whilst samples bought from Osu and Nima recorded 4.41 and 3.70 $\log_{10}$ cfu/g, respectively. Results on the extent of contamination of khebab bought from vending sites in the Accra Metropolis are presented in Table 2.6.

Table 2.6: Microbial load of khebab samples from three selling centres in the Accra metropolis

Location	Sample no.	Total count (log10 cfu/g)	Coliforms (log10 cfu/g)	Faecal coliform (log10 cfu/g)
Osu	001	4.20	0.00	4.28
	002	5.65	5.41	5.28
	003	6.25	4.36	4.84
	004	5.55	4.32	4.41
	005	5.85	4.00	4.30
	006	3.36	5.38	6.46
	007	4.89	4.72	0.00
	008	4.77	5.30	0.00
	009	4.97	5.34	5.04
	010	4.74	5.36	5.15
Accra	011	5.84	4.97	4.46
Central	012	5.38	4.46	4.28
	013	4.61	3.74	5.15
	014	4.23	5.11	4.30
	015	5.61	6.32	5.41
	016	5.28	5.40	5.41
	017	5.22	5.60	0.00
	018	4.93	5.38	5.23
	019	3.90	5.11	5.26
	020	5.65	5.11	5.26
Nima	021	4.91	3.86	3.65
	022	5.87	0.00	3.80
	023	5.95	0.00	0.00
	024	3.95	0.00	4.36
	025	4.39	5.30	4.89
	026	4.48	5.38	5.04
	027	5.23	7.00	4.23
	028	3.54	4.71	3.98
	029	4.74	3.69	3.72
	030	4.97	7.01	3.95

Source: Agbodaze *et al*. (2005)

It was noted that Khebab samples bought from Osu had the highest total plate count (TPC) which ranged between 3.36 – 6.25 log10cfu/g. TPC for Central Accra and Nima had ranges of 3.90 –5.84 and 3.54 – 5.95 log10cfu/g respectively.

Olayinka *et al*. (2008) determined the microbial quality of suya and found aerobic mesophiles and coliform counts to range from 0.07 to 2.22 x 10^5 colony forming units (cfu) per gram of Suya (Table 2.7). It was also revealed that exposure to higher temperature for a longer time during roasting could help reduce the numbers of these groups of microorganisms which constitute food safety risk in Suya and related foods (Olayinka *et al*., 2008). The foods with coliforms contamination in general mostly results from cross contamination through unhygienic processing equipment, poor hygiene practices, poor personal hygiene and generally from unhygienic handling of foods (Jay *et al*., 2005).

Table 2.7: Microbial counts (10^5 cfu per g / ml) of Suya samples from selected locations

Location	Aerobic mesophiles	Coliforms	Salmonellae
I	1.40^{bc}	0.20^{bc}	0.07^{ab}
II	0.07^{a}	0.12^{a}	ND
III	0.21^{a}	0.13^{a}	0.03^{ab}
IV	1.11^{b}	0.24^{c}	0.17^{b}
V	2.22^{d}	0.21^{bc}	0.13^{ab}
VI	1.73^{c}	0.16^{ab}	0.10^{ab}

Source: Olayinka *et al*. (2008). Note: Values with different superscripts are significant at P≤ 0.05

Ampaw (2018), studied prevalence of *Listeria monocytogenes* in 'Khebab', a street-vended spicy grilled meat in the Accra Metropolis and recorded total aerobic and coliforms counts (Table 2.8). Table 2.8: Mean microbial count of grilled meat ('Khebab')

Table 2.8: Mean microbial count of grilled meat ('Khebab')

Location	TVC CFU/g	TCC CFU/g
Adentan		
Banana Inn	6.596	6.394
Bubuashie	6.378	4.984
Cantoment	5.497	4.699
Dansoman	5.565	6.033
Dome	6.786	
Dzorwulu	6.924	5.607
James Town	6.555	5.31
Korle-bu	5.154	5.363
Kwashieman	6.58	5.013
Labone	6.953	5.708
Latebiorkorshie	5.093	5.539
Legon	6.885	
Mamobi	6.58	5.48
Madina	7.267	5.296
Nima	6.2	5.489
North Kaneshie	6.922	
Roman Ridge	6.927	4.097
Sowutuom	4.732	4.826
Tabora	6.23	

KEY: TVC = Total Viable Count TCC = Total Coliform Count (Ampaw, 2018).

As shown in Table 2.8, Madina vending area recorded the highest load of TVC (7.267 log10 cfu/g) whilst Sowutuom vending area had the least TVC of 4.732 log10 cfu/g. In the case of the total coliform count (TCC), the highest contamination was found in the Banana Inn vending area (6.394 log10 cfu/g) whilst the lowest count of 0.00 log10 cfu/g was found in the Dome, Legon, North Kaneshie, and Tabora vending areas. These microbial counts were thought to have implicated the sanitation of all these selected areas (Ampaw, 2018). This implies that, the sanitary conditions were unsatisfactory due to occurrence of coliforms in general and even in grilled 'Khebabs' (Ampaw, 2018).

2.16 Salmonella Spp.

According to Ryan and Ray (2004) *Salmonella* are generally dispersed in nature and are responsible for food poisoning, typhoid fever and paratyphoid fever. CDC (2014) stated that infants, the elderly, and those with weakened immune systems are severely affected by Salmonella infections. Animals could harbour the bacteria which making products obtained from them often implicated as vehicles for Salmonella transmission. Hence, foods from animal originm are vehicles for salmonellosis (Institute of Food Technologists (IFT), 2004).

2.17 Classification and Nomenclature of *Salmonella Spp*.

The genus *Salmonella* was named after Dr. Daniel Salmon, a veterinary bacteriologist at the United States Department of Agriculture (USDA) (Gast, 2003; Salyers and Whit, 2002). The genus Salmonella comprises of two species,

Salmonella bongori and *Salmonella enterica* (WHO 2003; Solari *et al.,* 2003). Within *Salmonella enterica* there are six subspecies (figure 2); *Salmonella enterica* subspecies *enterica* (I), *Salmonella enterica* subspecies *salamae* (I), *Salmonella enterica* subspecies *arizonae* (IIa), *Salmonella enterica* subspecies *diarizonae* (IIb), *Salmonella enterica* subspecies *houtenae* (IV) and *Salmonella enterica* subspecies *indica* (VI) (WHO 2003). These subspecies can be further classified into approximately 50 serogroups based on their lipopolysaccharide (LPS) O antigen component (Sabbagh *et al*., 2010). Langridge *et al*. (2008) summarizes the classification of the genus Salmonella as in the figure (Figure 2).

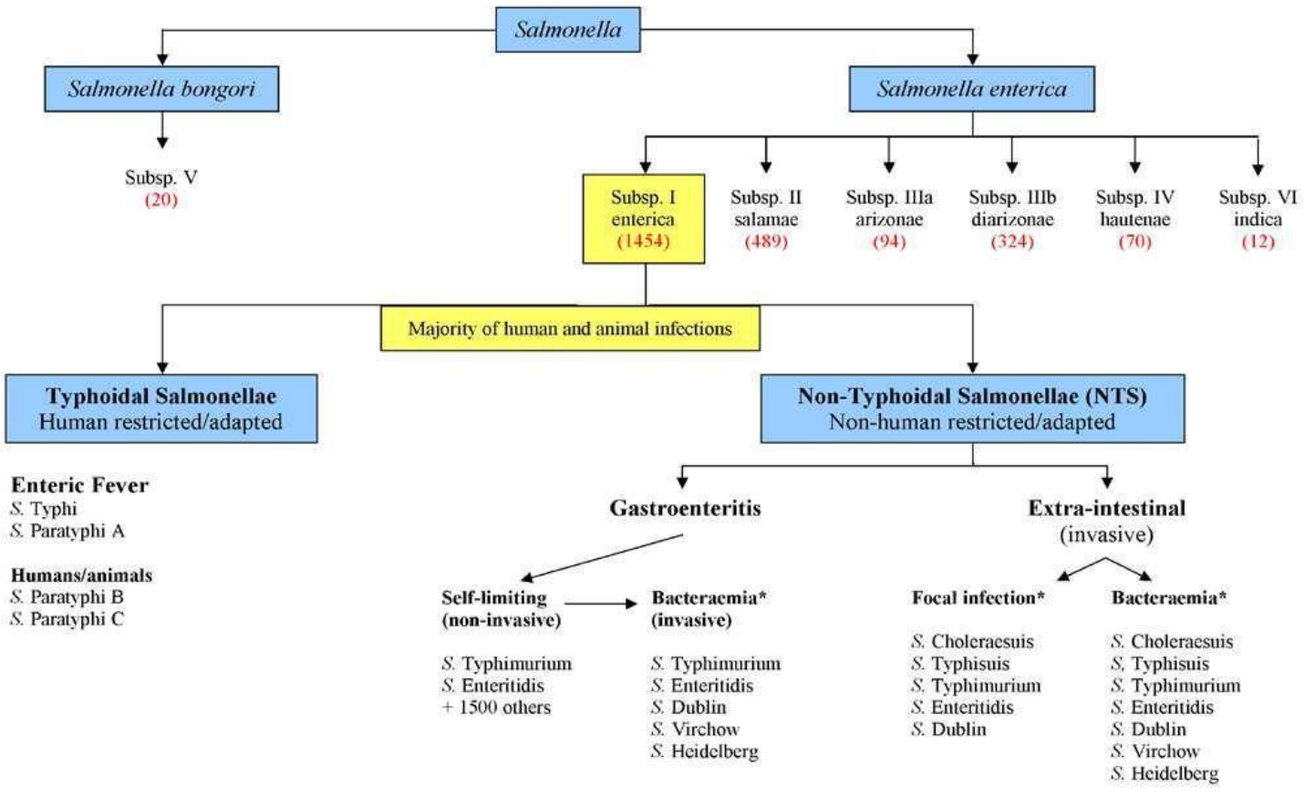

Figure 2: Classification of the genus Salmonella (Langridge *et al.*, 2008)

Note: Numbers in brackets indicate the total number of serotypes included in each subspecies. *Common serotypes are listed but other serotypes may cause bacteraemia or focal infection; subsp = subspecies.

Another system of classifying various Salmonella is by using the antibody interaction with surface antigens by Kauffman and White (Porwollik, 2011; Achtman *et al*., 2012). The Kaufman-White classification system, classifies *Salmonella enterica* into six subspecies with each subspecies further grouped into serovars. The serovar, is a type of classifying *Salmonella* to subspecies on the basis of the type of antigens that are located on the organism (Porwollik, 2011; Achtman *et al*., 2012). Over 2500 potential pathogens of *Salmonella* serovars have been reported (Bell and Kyriakides, 2002; Crum-Cianflone, 2008; Saroj *et al.*, 2009). Card (2009) offers a general overview of the number of Salmonella serovars in the table (Table 2.9).

Table 2.9: Species, subspecies and serovars of Salmonella genus

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

Source: Card (2009)

The serotype *Salmonella enterica* subspecie *enterica* (subspecies I), mostly cause human infections and also infects warm-blooded animals (Christenson, 2013).

According to Molbak *et al.* (2006), *Salmonellae* subspecies *enterica* is the most important zoonotic serotype and is found in the first subspecies, ssp. *enterica*.

2.18 Morphology and Biochemical Characteristics of Salmonella Enterica

Salmonella is a gram-negative, non-spore forming rod and facultative anaerobe that can ferment glucose and belong to the family *Enterobacteriaceae* (Grimont *et al.*, 2000). Most strains are motile with peritrichous flagella and can reduce nitrate to nitrite (Grimont *et al.*, 2000). The organism is mesophilic with optimum growth temperature in the range of 32 – 37°C but capable of growth within a wide temperature range of 6 – 46ºC (Olsen *et al.*, 2000). Salmonella is ubiquitous in the environment originating from the gastrointestinal tracts of domesticated and wild animals and can be present without causing apparent illness (Olsen *et al.*, 2000). Most infections result from the ingestion of foods of animal origin contaminated with Salmonella species such as beef, chicken and pork (Olsen *et al.*, 2000).

The ideal pH for multiplication of Salmonella is 7, but can survive in pH values between 4 and 9 (Gast, 1997). They grow in culture medium for enterobacteria and on blood agar. Colonies are 2 to 4mm in diameter, with smooth and round edges (Gast, 1997). Colonies may remain viable for a long time when stored in peptone broth (Gast, 1997).

Biochemically, Salmonella strains have the ability to catabolize nutrients, and catabolize D-glucose and other carbohydrates, except lactose and sucrose, with production of acid and gas (Quinn *et al.*, 202). They are catalase positive and oxidase

negative, they do not ferment malonate, they do not hydrolyze urea and do not produce indole, and they can use citrate as a sole source of carbon, and may produce hydrogen sulphide (Quinn *et al*., 202). The bacterium itself is surrounded by a mucus layer, which contributes to its resistance to phagocyte digestion, and has a fringe of fimbria located around its outer surface that are used in cell adhesion (Hirsh *et al*., 2004; Quin *et al*., 2002).

2.19 Prevalence of Salmonella in Ready to Eat Meat

According to the Food and Agriculture Organization (FAO) (1997), ready to eat food is any food (including beverages) usually consumed raw, or any food that is handled, processed, mixed, cooked, or prepared in any other way, which is consumed without any further handling.

Ready to eat (RTE) meats are also meats or meat products that are in edible form without additional preparation to achieve food safety (Bilatu, 2012). *Salmonella* spp. and *Campylobacter spp*. are recognized as two of the most important foodborne pathogens that can cause severe infections in humans and economic losses worldwide (Antunes *et al*., 2016; Khan *et al*., 2018). Their presence is monitored in different steps of the food chain, and especially in finished raw and ready-to-eat (RTE) products, as a safety criterion for the consumer, representing a very important tool for implementing efficient food safety systems (Antunes *et al*., 2016; Khan *et al*., 2018). Mamber *et al*. (2018) indicated that Salmonella can occur in ready-to-eat (RTE) meats and poultry products. Out of all (181) ready-to-eat poultry meat samples tested by Akba and Anal (2015), only one (0.55 %) was found contaminated

with *Salmonella*. However, Tavakoli and Riazipour (2008) evaluated grilled chicken and grilled ground meat and found out that the prevalence of Salmonella was 0% (0/54) in each. Also, Tareq *et al.* (2014) evaluated the presence of Salmonella, *L. monocytogenes*, and *E. coli* O157:H7 in Mediterranean RTE chicken and beef products sold in Jordanian restaurants (Table 10).

Salmonella, *Listeria monocytogenes*, and *Escherichia coli* O157:H7 in ready-to-eat (RTE) meat products are considered major concern for food control Authorities worldwide (Tareq *et al*., 2014). Salmonella, *L. monocytogenes*, and *E. coli* O157:H7 have been isolated from various types of RTE meat products in the Mediterranean region (Harakeh *et al*., 2005; Cabedo *et al*., 2008). Handling, processing and storage are some of the factors affecting the microbial status of RTE foods (Akbar and Anal, 2014; Roy *et al*., 2011).

These foodborne pathogens can cause severe illnesses or death to humans, especially high-risk individuals. Major Foodborne pathogens cause an estimated 9.4 million cases of foodborne illness, 55,961 hospitalizations, and 1,351 deaths each year in the United States (Osaili *et al*., 2013). Fifty percent of the deaths result from consumption of foods contaminated with Salmonella, *L. monocytogenes*, or *E. coli* O157:H7 (Rahn *et al*., 1992).

Table 2.10: Prevalence of *Salmonella spp* in ready-to-eat chicken and beef products in Jordan

		No. of samples positive for:	
Product	No. of samples	Salmonella	*Listeria monocytogenes*
Chicken			
Shawirma	301	3	12
Roasted	157	1	1
Burger	20	0	0
Total	478	4	13
Beef			
Shawirma	42	0	1
Pastry	163	0	5
Kubba	115	1	0
Kebab	92	0	2
In pita bread	90	0	0
Burger	48	0	0
Total	550	1	8
Grand total	1,028	5	21

Source: Tareq *et al.* (2014)

Tavakoli and Riazipour (2008) assessed the prevalence of various bacteria in RTE meats and found out that there was no Salmonella contamination in grilled ground meat and

grilled chicken as in the table (Table 2.11).

Table 2.11: Comparison of the prevalence of different bacteria in meats

Food type	Sample size	*E. coli* contamination (%)	*Staphylococcus aureus* contamination (%)	Salmonella contamination (%)	*Listeria monocytogenes*(%)
Grilled ground meat	54	38.9	55.6	0	0
Chicken	54	5.6	0	0	0
Fish	54	0	0	0	0
Grilled chicken	54	5.5	0	0	0
Mean		12.5	13.8	0	0

Souce: Tavakoli and Riazipour (2008)

2.20 Prevention of Salmonella in RTE Meats

Non-typhoidal *S. enterica* infections are a major public health problem world-wide. Reduction of these diseases presents a serious and challenging problem. This pathogen has several animal reservoirs (Hoelzer *et al*., 2011). The incidence of non-typhoidal salmonellosis continues to rise along with rates of emergence of antibiotic resistant strains and increased centralization of food production. Therefore, it is important to monitor every step of food production, from handling of raw products to preparation of finished foods (Hoelzer *et al*., 2011).

The prudent use of antimicrobial agents in both humans and animals is necessary to minimize the further emergence of antibiotic resistant strains (Getenet, 2008). Salmonella contamination from food handlers has been shown to make a significant contribution to human foodborne illness in several developing countries (Catherine *et al.*, 2001).

The World Health Organization (WHO) (2006) indicated that safe food handling and proper cooking will help keep you and your family safe from foodborne bacteria. WHO (2006) has since recommended four food safety steps: clean; wash hands and surfaces often, separate; separate raw meats, cooked or grilled foods and poultry from other foods, cook; cook all poultry to an internal temperature of 73.9 °C, and chill; refrigerate promptly.

Beshatu (2014) reported that in many urban centers, eating and drinking in public establishments, such as hotels, restaurants, and snack bars is a common practice in many countries. These establishments prepare, handle, and serve large quantities of food and drink to large groups of people within a short period of time implying a possible risk of infections if sanitary and hygienic norms are not strictly followed (Beshatu, 2014). The world health status review indicates that the health problem of developing nations is mainly linked to inadequate sanitation (Kumie *et al*., 2002).

Better education of food industry workers in basic food safety and restaurant inspection procedures may prevent cross-contamination (Kumie *et al*., 2002). Food handling errors can lead to outbreaks. Improvements in farm animal hygiene, slaughter plant practices, and packing operations may help prevent salmonellosis caused by contaminated foods (Doyle *et al*., 2009). In the future, irradiation may greatly reduce contamination of raw meat (CDC, 2008). Strategies for reducing foodborne illness require a comprehensive farm-to-table approach (Catherine *et al.*, 2001). Hoelzer *et al.* (2011) and Buncic (2006), reported that in order to control Salmonella infection, an individual should cook foods thoroughly, prevent cross-

contamination of heat-treated foods, avoid consumption of undercooked or raw eggs, store heat-treated foods at less than 4°C or greater than 60°C to prevent growth. Furthermore, reduce carriage of livestock by vaccinating or dosing with antibiotics or probiotics, exclude infected or carrier-status individuals from handling food, control rodents and insects and dispose of sewage in a sanitary manner (Hoelzer *et al*., 2011).

Different processing methods for meats exist. For instance, hard smoking involves using much salt and smoking at a low temperature until little moisture is left in it. The salt preserves the meat by inhibiting the growth of the micro-organisms. Hot smoking also requires a temperature of at least 65.56ºC so that the food can be cooked and flavoured with smoke at the same time. Cooking is much longer than grilled meats, in lower temperatures. Cold smoking on the other hand, requires temperature less than 37.78ºC (Essuman, 1982). In this case, the meat is not cooked at all, but rather the meat is flavoured and sealed with the smoke barrier so that bacteria cannot cause it to spoil. Salt in meat reduces the moisture content in the meat through osmotic effect. Growth of most microorganisms is inhibited when water activity is lowered due to residual salt (Essuman, 1982).

2.21 Antibiotic Resistance of Salmonella Isolates

In recent years, strains of Salmonella resistant to antimicrobial drugs have spread worldwide with isolates resistant to quinolones being reported with increasing frequency in several African countries (Eng *et al*., 2015). In Ghana, earlier reports have indicated the presence of Salmonella strains showing multi-resistance to

antimicrobials routinely used as therapeutic agents (Andoh *et al*., 2017).. The development of resistance to fluoroquinolones and extended-spectrum β-lactamses (ESBL) which may have been used either for prophylaxis, therapeutic treatment of humans or as growth promoters in livestock feed (Andoh *et al*., 2017).

The major mechanism for this resistance is through the production of specific enzymes to hydrolyze the associated extended-spectrum cephalosporins. Molecular analysis indicates that the resistance genes, mostly blaCMY-2 and blaCTX-M-3, are plasmid- borne and can be transmitted among bacterial organisms of the same or different species, resulting in wide-spread resistance and this has increased to as high as 70% in many areas of the world (Su and Chiu, 2007). However, the resistance rate differs among serotypes. *Salmonella* Enteritidis is generally more susceptible to antimicrobial agents, while *S*. Typhimurium exhibits a much higher resistance (Su and Chiu, 2007). One of the major reasons for the higher antimicrobial resistance observed in *S*. Typhimurium may be the emergence of a distinct multidrug-resistant strain of definitive phage type 104 (DT104) in the United States and Europe in the early 1990s (CDC, 2009). This unique strain is characterized by its associated resistance to five (5) antimicrobial agents, ampicillin (A), chloramphenicol (C), streptomycin (S), sulfonamide (Su), and tetracycline (T) (Su and Chiu, 2007). As of the mid-1990s, *S*. Typhimurium DT104 had become one of the most prevalent strains among human isolates of Salmonella, second only to *S*. Enteritidis strain PT4 in the United Kingdom (CDC, 2009).

In the United States, a recent report from the National Antimicrobial Resistance Monitoring System (NARMS) indicated that the risk of bloodstream infections in patients infected with antimicrobial resistant non-typhoid Salmonella, particularly *S.* Typhimurium, is 2-fold greater than in those infected with pan-susceptible strains (Rabatsky-Her *et al.*, 2004). Information from FoodNet also indicates a more than 4-fold risk of hospitalization in patients with resistant Salmonella infections (Rabatsky-Her *et al.*, 2004). Similarly, a Danish study reported that infection with quinolone-resistant *S.* Typhimurium was associated with a 3-fold higher risk of invasive illness or death within 90 days of infection, compared with that observed for infection with pan-susceptible strains (Voetsch *et al.*, 2004).

Fluoroquinolones are one of the alternatives in the treatment of invasive infections caused by Salmonella (Su and Chiu, 2007). However, resistance to fluoroquinolones has been frequently reported in many countries (Su and Chiu, 2007). A particularly worrisome situation in Taiwan is that fluoroquinolone resistance was found in clinical isolates of *S. Choleraesuis* in 2000 and this resistance has increased rapidly, to approximately 70% in 2003 (Su and Chiu, 2007).

In Akwatia in the Eastern Region of Ghana, Puopelle (2014) isolated *Salmonella* spp. from humans and reported a resistance of 76.30% to trimethoprim/sulfamethoxaxole. All the *Salmonella* species (100.00%) were susceptible to ciprofloxacin and norfloxacin Puopelle (2014).Four Salmonella isolates from humans in the Tamale Metropolis were analyzed by Saba *et al.* (2013)

and the results showed that all *Salmonella* spp. were all susceptible to amoxicillin-clavulanate, ciprofloxacin, gentamicin, tetracycline and chloramphenicol.

2.22.0 Antibiotic Sensitivity Test

Determination of bacterial resistance to antimicrobials is an important part of the management of infections in patients (American Society for Microbiology (ASM) (ASM, 2016). Antimicrobial susceptibility test (AST) is crucial in assisting the clinician in selecting the most suitable agent for treating that disease (Atlas, 1995). The methods used in AST include broth dilution, agar dilution tests, disk diffusion, the automated AST systems, mechanized specific tests, genotypic methods and the E – test (Atlas, 1995). Many guidelines including the National Committee for Clinical Laboratory Standards (NCCLS) are available for antimicrobial susceptibility testing and subsequent interpretative criteria (NCCLS 2006; Craig, 1993).

2.22.1 Disk Diffusion Method

The disk diffusion method, which is also known as the Kirby - Bauer method (Bauer *et al*., 1966) has been standardized and is a viable alternative to broth dilution methods for laboratories without the resources to utilize the newer automated methods for broth micro-dilution testing (Hudzicki, 2009). The Kirby-Bauer method involves the use of 6-mm filter paper disks, tablets or strips that have been impregnated with antibiotic with a known concentration of an antimicrobial compound to determine whether a particular bacterium is susceptible or resistant to the antibiotic. After inoculating the organism on a solid culture media (Mueller-

Hinton (MH) agar), the disk is placed aseptically onto the media plate. The antibiotic in the disk diffuse into the culture medium in decreasing amount further away from the disk. The rate of diffusion through the agar is not as rapid as the rate of extraction of the antimicrobial out of the disk, therefore the concentration of antimicrobial is highest closest to the disk and a logarithmic reduction in concentration occurs as the distance from the disk increases (Jorgensen and Turnidge, 2007). The rate of diffusion of the antimicrobial through the agar is dependent on the diffusion and solubility properties of the drug in MH agar (Bauer *et al*., 1966) and the molecular weight of the antimicrobial compound. Larger molecules will diffuse at a slower rate than lower molecular weight compounds. These factors, in combination, result in each antimicrobial having a unique breakpoint zone size indicating susceptibility to that antimicrobial compound (Bauer *et al*., 1966). If the bacterium is inhibited by the concentration of the antibiotic, there will be no growth in the immediate area around the disk. This area is called the zone of inhibition and establishes the organism as either sensitive or resistant to the antibiotics used (Finegold *et al*., 1978). The disc diffusion method is commonly used in most Laboratories because it is convenient, efficient and less expensive (Atlas, 1995).

2.22.2 Broth Dilution

According to Atlas (1995), the broth dilution method is often referred to as the "gold standard". In this broth dilution method, standardized microbial inoculum is tested against different specific concentrations of an antimicrobial agent in a standardized liquid medium (Atlas, 1995). This method can be carried out in two ways; either by

macro-dilution or micro-dilution. Macro-dilution is done in tubes with at least 2ml of broth, whereas micro-dilution is done in small micro titration plates containing broth volume of 0.05 to 0.1ml (Balows *et al*., 1991; Craig, 1993). According to Balows *et al*. (1991), the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation is referred as the minimum inhibitory concentration (MIC).

2.23.0 Classes of Antibiotics

2.23.1 Beta-Lactams

Beta-lactams are a wide range of antibiotics, the first of which to (Solensky, 2012). All beta-lactam antibiotics contain a beta-lactam ring; they include penicillins, such as amoxicillin, and cephalosporins. They work by interfering with the synthesis of peptidoglycan, an important component of the bacterial cell wall, and are mostly used against gram-positive bacteria (Solensky, 2012). Bacteria can, however, develop resistance to beta-lactams via several routes, including the production of enzymes that break down the beta-lactam ring. Penicillins are the most commonly prescribed antibiotics, with amoxicillin being the most common in the class Solensky (2012).

2.23.2 Sulfonamides

Prontosil, a sulfonamide, was the first commercially available antibiotic, developed in 1932. A significant number of sulfonamide antibiotics were subsequently developed (Finch et al., 2010).. Sulfonamides are broad-spectrum antibiotics capable of acting on both Gram-positive and Gram-negative bacteria (Finch *et al*.,

2010). Unlike the beta-lactams, they do not act by directly killing the bacteria, but by inhibiting bacterial synthesis of the B vitamin folate, thus preventing growth and reproduction of the bacteria. In the present day, sulfonamides are rarely used, partially due to the development of bacterial resistance, but also due to concern about unwanted effects such as hepatotoxicity (Finch *et al*., 2010).

2.23.3 Aminoglycosides

Aminoglycosides inhibit the synthesis of proteins in bacteria, eventually leading to cell death (Guilfoile and Alcamo, 2007). They are only effective against certain Gram-negative bacteria, as well as some Gram-positive bacteria, but are not absorbed during digestion, so must be injected (Source). In the treatment of tuberculosis, streptomycin was the first drug found to be effective; however, due to issues with toxicity of aminoglycosides, their present-day use is limited (Guilfoile and Alcamo, 2007).

2.23.4 Tetracyclines

Tetracyclines are broad-spectrum antibiotics, active against both Gram-positive and Gram-negative bacteria (Lara *et al*., 2010). Like the sulfonamides, they inhibit protein synthesis, inhibiting growth and reproduction of bacteria. Their use is decreasing due to increasing instances of bacterial resistance; however, they still find use in treatment of acne, urinary tract, and respiratory tract infections, as well as chlamydia infections. They must be taken in isolation, often two hours before or after eating, as they can easily bind with food, reducing their absorption Lara *et al*. (2010).

2.23.5 Chloramphenicol

Chloramphenicol is a broad-spectrum antibiotic which acts by inhibiting protein synthesis, and thus growth and reproduction of bacteria (Lara *et al.*, 2010). Due to the possibility of serious toxic effects, in developed countries it is generally only used in cases where infections are deemed to be life-threatening, although it is also occasionally used in the treatment of eye infections (Lara *et al.*, 2010).Despite this, it is a much more common antibiotic in developing countries due to its low cost and availability, and is recommended by the World Health Organization as an effective first line treatment for meningitis in those countries with a low income (Lara *et al.*, 2010).

2.23.6 Macrolides

Macrolides are mainly effective against Gram-positive bacteria; however, they act in a bacteriostatic manner, preventing growth and reproduction by inhibiting protein synthesis (Kalan and Wright, 2011).Their effectiveness is marginally broader than that of penicillins, and they have been shown to be effective against several species of bacteria that penicillins are not. Whilst some bacterial species have developed resistance to macrolides, they are still the second most commonly prescribed antibiotics in the National Health Service (NHS) of the United Kingdom (UK), with erythromycin being the most commonly prescribed in this class (Lara *et al.*, 2010).

2.23.7 Glycopeptides

Glycopeptides include the drug vancomycin; commonly used as a 'drug of last resort', when other antibiotics have failed. Whilst this used to be the last line of defense against infections, particularly Methicillin-resistant S. aureus (MRSA), the more recent development of newer antibiotics in other classes has provided other options (Kalan and Wright, 2011).Nonetheless, there remain strict guidelines on the circumstances in which vancomycin can be used to treat infections, in order to delay the development of resistance (Source). The bacteria against which glycopeptides are active are otherwise somewhat limited, and in most cases, they inhibit growth and reproduction rather than killing bacteria directly (Kalan and Wright, 2011).

2.23.8 Oxazolidinones

Oxazolidinones are active against Gram-positive bacteria, and act by inhibiting protein synthesis, and hence growth and reproduction (Zaffiri *et al*., 2013). Linezolid, approved for use in 2000, was the first marketed antibiotic in the class, although the compound cycloserine has been used as a second line tuberculosis treatment since 1956 (Zaffiri *et al*., 2013). They further stated that resistance to linezolid seems to be developing relatively slowly since its introduction.

2.24.9 Ansamycins

Villain-Guillot *et al*., (2007) reported that this class of antibiotics is effective against Gram-positive bacteria, as well as some Gram-negative bacteria and that they inhibit the production of RNA; which has important biological roles inside the cells of the

bacteria, and as such leads to the death of the bacterial cells. A subclass of these antibiotics, rifamycins, is used to treat tuberculosis and leprosy. Uncommonly, ansamycins can also demonstrate anti-viral activity (Villain-Guillot *et al*., 2007).

2.24.10 Quinolones

Quinolones are bactericidal compounds that interfere with the replication and transcription of DNA in bacteria cells (Villain-Guillot *et al*., 2007). They are broad-spectrum antibiotics, and are widely used for urinary tract infections, as well as other hospital-acquired infections where resistance to older classes of antibiotics is suspected (Villain-Guillot *et al*., 2007). Additionally, their use for veterinary purposes is widespread; a use that has been criticized in some quarters for hastening the development of resistance. Resistance to quinolones can be particularly rapid in its development; in the US, they were the most commonly prescribed antibiotics in 2002, and their prescription for unrecommended conditions or viral infections is also thought to be a significant contributor to the development of resistance (Sharma *et al*., 2009).

2.24.11 Streptogramins

These are a group of cyclic peptide antibiotics that inhibit the synthesis of bacteria proteins (Aronson 2015). Mast and Wohlleben (2014) reported that streptogramins are unusual in that they are usually administered as a combination of two antibiotic drugs from the different groups within the class: streptogramin A and streptogramin B. On their own, these compounds only show growth-inhibiting activity, but

combined they have a synergistic effect and are capable of directly killing bacteria cells, by inhibiting the synthesis of proteins. They are often used to treat resistant infections, although resistance to the streptogramins themselves has also developed (Mast and Wohlleben, 2014)

2.24.12 Lipopeptides

Discovered in 1987, lipopeptides are the most recent class of antibiotics, and are bactericidal against Gram-positive bacteria (Thorne and Alder, 2002). Daptomycin is the most commonly used member of this class; it has a unique mechanism of action, disrupting several aspects of cell membrane function in bacteria. This unique mechanism of action also seems to be advantageous in that, currently, incidences of resistance to the drug seem to be rare – though they have been reported. It is given via injection, and commonly used to treat infections in the skin and tissue (Thorne and Alder, 2002).

2.25 Antibiotic Resistance of Salmonella Isolated from Ready to Eat Meat

In Thailand, out of all 181 RTE poultry meat samples tested by Akbar and Anal (2015), only one (0.55 %) was found to be contaminated with Salmonella. The isolate showed resistance to ampicillin, chloramphenicol, tetracycline and nalidixic acid but susceptible to cefotaxime, norfloxacin, erythromycin and sulfamethoxazole-trimethoprim.

In Nigeria, a study conducted by Abdullahi *et al.*, (2012), showed that, high proportions of *Salmonella Typhi* and Salmonella Paratyphi A were resistant to

ampicillin, chloramphenicol and cotrimoxazole. All the isolates were however susceptible to ciprofloxacin and ofloxacin.

In Abidjan, Cote d"Ivoire, a study done by Boni-Cissé *et al*., (2012), showed that resistant to amoxicillin and amoxicillin-clavulanic acid by Salmonella isolates were 74.2% and 58.1% respectively. Resistant to ciprofloxacin was also found to be 14%.

In Ghana, multi drug resistant was observed in 52% (30/58) of *Salmonella Typhi* isolates in a study conducted by Mills-Robertson *et al*., (2002). Resistance to antimicrobial agents is a great challenge to clinicians in the management of infections (Mølbak, 2005).

Antibiotic susceptibility test by Adzitey *et al*. (2015) on 45 Salmonella species isolated from beef and its related samples showed an overall resistance, intermediate and susceptibility of 35.50% (144/405), 7.90% (32/405) and 56.54% (229/405), respectively. All Salmonella species (100%) examined were resistant to vancomycin but susceptible to ciprofloxacin. A large percentage of the Salmonella species were also resistant to erythromycin (75.56%) and susceptible to gentamicin (86.67%), ceftriaxone (73.33%), suphamethoxazole/trimethoprim (68.89%), chloramphenicol (62.22%), tetracycline (57.78%) and amoxycillin/clavulanic acid (57.78%). Intermediate resistances were observed for all the antibiotics except vaconmycin and ciprofloxacin.

2.26 Salmonella Detection Methods

Several methods have been developed for the detection, identification and molecular characterization of Salmonella species (Sen *et al*., 2007).

Generally, detection methods are based on physiological and biochemical markers of the organism (Aktar *et al*., 2016). Cultural methods are based on nutrient acquisition, biochemical characteristics, and metabolic products unique to *Salmonella* spp. More rapid immunological and molecular screening methods of detection have been devised to detect cell surface markers and nucleic acids, respectively (Odumeru and León-Velarde, 2012).

Some of the various culture-based methods and rapid methods currently available for the detection of Salmonella in foods and food ingredients can take from 4 to 7 days in order to isolate and confirm the presence of Salmonella from the sample (Eriksson and Aspan, 2007).

Conventional culture methods used for the isolation of Salmonella include non-selective pre-enrichment followed by selective enrichment and plating on selective and differential agars (Eriksson and Aspan, 2007). Suspected colonies are then confirmed biochemically and serologically. According to Sandel *et al.* (2003) and Gracias and Mckillip (2004), enrichment of samples are done purposely to recover partially injured cells of bacteria due to heat, cold, acids or osmotic shock in a non-selective pre-enrichment media for example Buffered Peptone Water (BPW). Another purpose is to multiply the number of target cells (microorganism) as they are generally not uniformly distributed in the foods, occurs in small amount and

might be in a mixed microbial population. Selenite Cysteine broth, Rappaport Vasiliadis Soy broth, Tetrathionate broth or Muller-Kauffmann Tetrathionate-Novobiocin broth are some examples of primary enrichment media and Xylose lysine Deoxycholate (XLD) agar, Bismuth Sulphite agar, Brilliant Green agar are some examples of selective media (Sandel *et al*., 2003; Gracias and Mckillip, 2004). A number of alternative methods for the detection of Salmonella in foods have been developed including, immune-assays, nucleic acid hybridization and polymerase chain reaction (PCR) techniques (Li *et al*., 2000).

2.27 Media for Isolating Bacteria and Their Classification

According to Andrew (2006), microbial culture is used to determine the type of microbes and their abundance in the samples being tested. The report further added that culture media contains nutrients and other physical growth parameters needed for microbial growth. They in addition indicated that, individual microorganisms can thrive on different culture media. Media may be grouped into solid or liquid, synthetic or non-synthetic (Andrew, 2006) and based on the use grouped as basic, enrichment, non-selective and selective media (Garrard, 2013).

2.28.0 Sources of Microbial Contamination of Grilled RTE Meat

Salmonella spp. contaminates variety of foods. Production of food that practically has no organism is impossible (Montville and Matthews, 2005). They have been associated with cheese, raw (unpasteurized) milk, deli meats, salad, fish and smoked fish, ice cream and hot dogs (Montville and Matthews, 2005). Salmonella have

mostly been implicated in salmonellosis outbreaks in ready to eat foods (Sagoo *et al.*, 2003; Mauro *et al*., 2008). The reason is that, these ready-to-eat foods are eaten without any further treatment or processing (Ampaw, 2018). Poultry products as well as meat products have been identified as the most common implicated modes of transmission (Jay *et al*., 2005).

2.28.1 Poor Hygiene Practices

Poor hygienic practices during slaughtering and marketing process of meats is a major contributing factor to various pathogens being isolated from beef, chevon and mutton sold in various markets places (Sulley, 2006; Soyiri *et al*., 2008; Adzitey *et al*., 2011).

The use of contaminated food ingredients and equipment serve as major sources of greater number of food borne pathogens (Medeiros *et al*., 2001; Beumer and Kusumaningrum, 2003; Redmond and Grifith, 2003). The unclean water, bowls, knives, apparels and other items used by vendors contribute immensely in contaminating the meat Ampaw, 2018). The sale of grilled meat at unhygienic areas; closeness to open gutters make it possible for pathogens to contaminate the meat (Ampaw, 2018).

2.28.2 Cross-Contamination

A contaminated food is a food that contains microorganisms such as bacteria, fungus, parasites, viruses, or toxins produced by microorganisms (United Nations Food and Agriculture Organization (FAO) and the Pan American Health

Organization (PAHO), 2017). The contamination is caused by the transmission of a hazard present in a food to another food that is safe, via surfaces or utensils that have contact with both, without the requisite cleaning and disinfection (FAO and PAHO, 2017). In addition to faecal contamination, cross-contamination of foods by Salmonella during food preparation can be an important source of foodborne illness (Odumeru and León-Velarde, 2012). The most frequent cases of cross-contamination occur when the handler (vender) allows a raw food to come into contact with a food ready to be consumed, by using the same cutting boards or kitchen utensils (FAO and PAHO, 2017). Another example of this type of contamination is when meat is grilled and the same cutting board is used for both raw and cooked meat (FAO and PAHO, 2017). Salmonella spp. is highly pervasive, usually associated with the soil, dust, silage, water, waste from slaughter houses, effluent from sewage and many others (Jay *et al*., 2005). The meat can therefore be contaminated straight from the slaughter house or during transportation to processing sites (Jay *et al*., 2005). Formation of biofilms at retail or vendor points can result in cross contamination of the RTE meat (Mauro *et al*., 2008; Smigic *et al*., 2016).

2.28.3 Grilled RTE Meat Processing Equipment, Processing Methods and Vendors

Handling, processing and storage are some of the factors affecting the microbial status of RTE foods (Akbar and Anal, 2014; Roy *et al*. 2011). Unclean food processing equipment have biofilms formed on them (Mauro *et al*., 2008**)**. A number of studies have implicated food handlers as Salmonella carriers and thus serve as a

potential source of infection of enteric fever. Feglo *et al*. (2004) reported that, 2.3% of food vendors were Salmonella carriers in Kumasi, Ghana. Mensah *et al*. (1997) also reported a prevalence of 3.2% involving 176 food vendors in Accra Ghana. Unclean hands of processors and vendors and their apparels tend to habour pathogens thereby aiding the formation of biofilms (Smigic *et al*., 2016). Also, approximately $1x10^3$-$1x10^4$ viable microorganisms are shed per minute by human and food handlers without any symptoms of the related illness and are estimated to shed around 10^9 pathogens per gram of faces (Forsythe, 2000). The report further stated that 10^7 counts of pathogenic microbes are present in the fingernails of food handlers. Irrespective of the temperatures, pH and salt concentration at these areas the pathogen finds themselves, as far as the processors or venders are concern, they are able to grow. This enables pathogens to colonize and adapt to various environment (Jay *et al*., 2005; Mauro *et al*., 2008); major sources of greater number of foods borne pathogens (Medeiros *et al*., 2008). Ready-to-eat is produced from fresh meat through modifications that employ one or more procedures, such as the addition of seasoning, and heat treatment among others. These processes are known to prolong the shelf life of the RTE meat and these processes have a lot of importance since they prevent contamination by bacteria (Stobart, 2017). Spices such as ginger, black pepper and others have antioxidant properties which help preserve meat (Stobart, 2017). The red pepper, garlic, ginger, onion, black peppers are the most common spices used in preserving and flavoring meat (Stobart, 2017) in Ghana. Thus, RTE meats are mostly processed in order to prolong shelf life, enable incorporation of non-meat components into it thereby increasing the volume and

improving other desirable qualities such as colour, texture, and flavor (Stobart, 2017).

2.29 Regulations of *Salmonella Spp*. In Food

A zero-tolerance for the *salmonella spp*. has been declared by United Kingdom and United States of America (Gallagher *et al*., 2003). Tolerance levels for the pathogen have been set by most countries in the European Union (EU) (Gallagher *et al*., 2003). The tolerance levels set by these countries are due to the counts of the organism in the food which tells whether the food is acceptable or unacceptable (Montville and Matthews, 2005). This simply implies that, the zero-tolerance offers no additional protection for consumers (Montville and Matthews, 2005). In the Italian guidelines for the microbiological quality RTE meals in accordance with the microbiological limits suggested by the Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche; a reference Public Institution on food hygiene and veterinary public health, in every 25g of meat, limits of *Salmonella spp*. and coliforms should be ≤ 3 Log cfu/g and that of total mesophilic aerobes (TMA), ≤ 4.0 Log cfu/g. Mamber *et al*. (2018), reported that the Safe Food for Canadians Regulations (SFCR) indicated the lethality of *Salmonella spp*. for meat products containing no poultry as 6.5 $\log_{10}$, meat products containing poultry meat other than turkey as 7.0 $\log_{10}$ and products containing turkey meat only and no other poultry meat 7.0 as $\log_{10}$. It has been estimated that consumption of *Salmonella enterica* with food at the rate of 10^3 cfu/g to 10^9 cfu/g can be an infectious dose for human being depending on their immunity (Parry, 2006). About 10^5 *Salmonella* cells per gram of food can initiate infection

(Jay *et al*., 2003). Burrows and Renner (1999) also reported that 10^4 *Salmonella* per litre of water is required to initiate an infection. According to Wannissorn (2001), the *Salmonella* inoculum needed for infection to occur depends on the type of strain and the physiological wellbeing of the host. For example those producing little gastric acids such as the aged and people who frequently use antacids could reduce the infective dose to 10^3 cells. However, for people who have been vaccinated against *Salmonella* infection, the infective dose can increase to 10^9 cells (Raffatellu *et al*.., 2006). According to the Australian Standard (2002) and the European Union (2002), the levels of bacteria count and the levels of acceptability are present in Table 2.12.

Table 2.12: Standards for bacteria counts

	Total Viable Count	
Category	AUS[a] (log cfu/g)	EU[b] (log cfu/g)
Excellent	< 3.0	< 2.8
Good	3.0-4.0	-
Acceptable	-5.0	-
Marginal	> 5.0	> 4.3

Note ; [a] Australian Standard (AUS) (2002), [b] European Union (EU) 2002).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Location of Study

The research was carried out in Bolgatanga, the Upper East Regional capital and laboratory tests were conducted in the Spanish laboratory of the University for Development Studies, Nyankpala campus. Bolgatanga is located in the Upper East Region, approximately, between latitudes 10°30' and 10°50' North and longitudes 0°30' and 1°00' West (Ghana Statistical Service (GSS), 2014). It is also the capital of Bolgatanga Municipality. It is bordered to the north by the Bongo District, south and east by the Talensi and Nabdam Districts, and to the west by the Kassena / Nankana Municipality. It covers a total land area of 729 square kilometers (Ghana Statistical Service (GSS), 2014). The map (Fig. 3) shows Bolgatanga, where the samples were collected for the study.

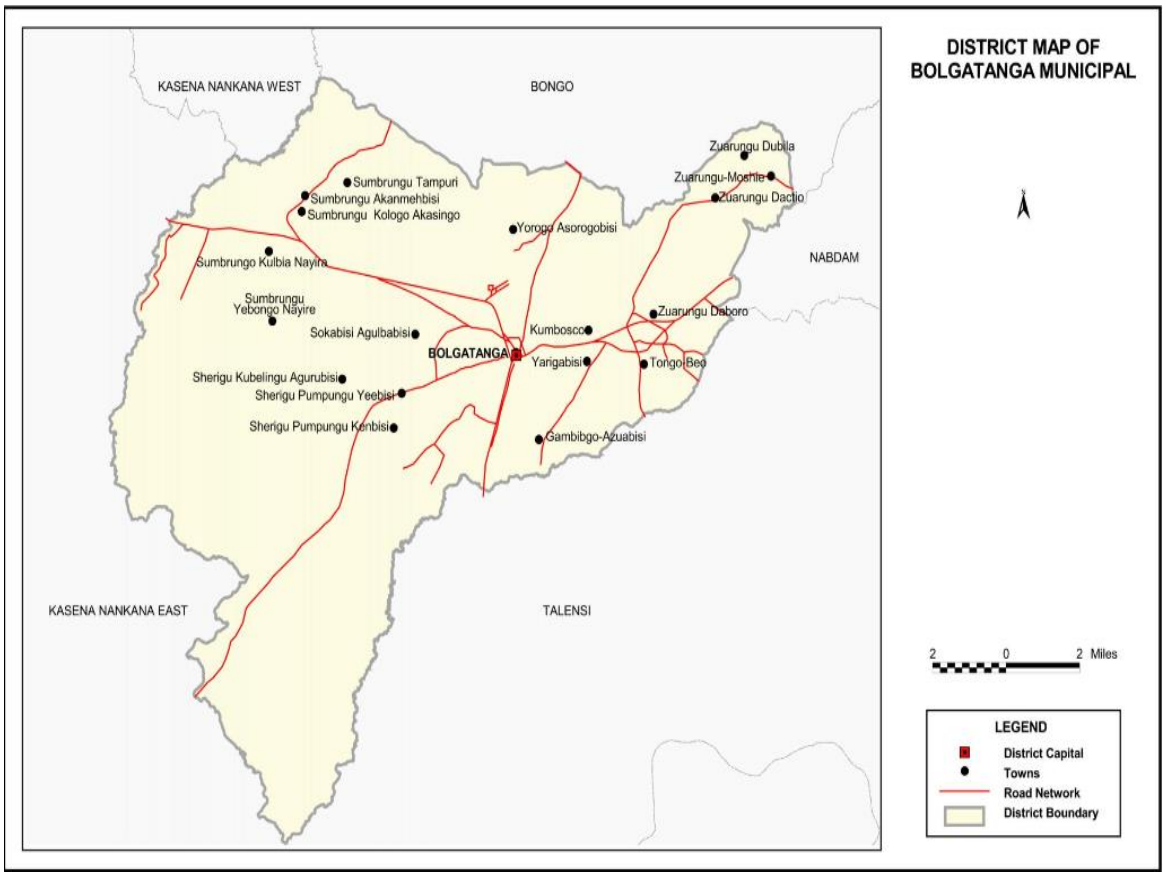

Figure 3: Map of Bolgatanga, capital of the Upper East region

3.2 Study Design

The study employed a mixed-method approach to obtain the data required. The first part of the study entailed a descriptive survey, intended to obtain baseline data using semi-structured questionnaires from the grilled RTE meat vendors and consumers. Observation method was also used to establish the actual practices that perhaps contribute to the contamination of the grilled RTE meats. The next part made was a cross-sectional study to get data on the microbial contamination of grilled TRE meats using laboratory investigation techniques. The survey was conducted to gather information about the consumption patterns including the type, mass and frequency

of consumption of grilled RTE meats and the hygiene practices by venders in all three hundred RTE meat vender shops in Bolgatanga. The laboratory analysis involved the isolation and identification of *Salmonella enterica* on grilled RTE meats in all the 300 randomly selected vendor shops in Bolgatanga.

3.3.0 COLLECTION OF SAMPLES

3.3.1 Survey and Sampling

Three hundred (300) grilled RTE meat venders were selected at random and interviewed with questionnaires (Appendix IA) regarding their knowledge, attitudes and practices on meat safety. Also, a total of 382 consumers were randomly sampled and interviewed on meat safety using questionnaires as attached in Appendix 'IB'.

3.3.2. Consumers' Survey

The number of consumers was determined by queuing in the population of Bolgatanga, (66,685) (GSS, 2014) into the sample Size Calculator Anonymous (2020) available at https://www.calculator.net/sample-size-calculator. html?type =1&cl=95& ci=5&pp=50&ps=66685&x=39&y=22

Using a confidence level 95%, the result was 382 consumers hence this number of consumers was randomly selected and interviewed.

3.3.3 Vendors' Survey

Three hundred (300) grilled RTE meat vendors were selected at random in the capital and information on their methods of handling the RTE meats, knowledge,

attitudes as well as practices were obtained with the aid of semi-structured questionnaires (Appendix IA). The vendors shops were also physically observed during the interview.

3.4.0 Swab Sample Collection from RTE Meat Vendors Shops

Prior to swab sample collection, vendors were visited as familiarization tour and to explain the rational and significance of the study to them. The vendors were engaged in the study after they had given their approval and they were assured of confidentiality. Grilled RTE meat swabs totaling 300 were collected from the vendor shops between the period of January to December, 2019. They were made up of chevon (n=50), mutton (n=50), pork (n=50), beef (n=50), guinea fowl (n=50) and chicken (n=50).

For each swab, a meat area of 10 cm^2 was swabbed. Isopropyl Alcohol (70%) was used to sanitize hands after each swab and vendor shop respectively to prevent cross contamination. Swabbed samples were stored in ice-filled ice chest and transported immediately to the University for Development Studies (UDS) Spanish Laboratory, Nyankpala Campus for microbial analysis.

3.4.1 Laboratory Analyses

The laboratory investigations encompassed bacterial load (TPC), isolation, identification and antibiotic susceptibility tests for *Salmonella enterica* isolated from grilled RTE meats. Confirmation of all presumptive *Salmonella enterica* was

conducted using standard tests for bacteriological analysis as in the Bacteriological Analytical Manual (BAM) (BAM, 2007).

3.4.2 Total Aerobic Plate Count

For the total bacterial counts, RTE meat swabs were added to 10 ml of 0.1 % buffered peptone water and homogenized for 2 minutes. Subsequently, decimal serial dilutions from 10^{-1} to 10^{-4} were made in 10 ml 0.1 % buffered peptone water using 1 ml aliquot. Plating was done on plate count agar and incubated aerobically at 37°C for 24 hours before colonies were counted and reported as colony forming unit per cm^2 (cfu/cm^2). Number of colony forming unit per cm^2 was calculated using the formula described by (BAM, 2007) as follows:

$$N = \sum C / [(1 * n_1) + (0.1 * n_2)] * (d)$$

Where:

N= Number of colonies per cm^2

$\sum C$ = Sum of all colonies on all plates counted

n_1 = Number of plates in first dilution counted

n_2= Number of plates in second dilution counted

d = Dilution from which the first counts were obtained.

3.4.3.0 Isolation and Identification of Salmonella Enterica

Isolation and identification of Salmonella enterica was carried out using a modified method according to the Food and Drug Administration-Bacteriological Analytical Manual (FDA-BAM)-USA (Wallace and Hammack, 2007).

3.4.3.1 Pre-Enrichment

Swabs from section 3.4 were pre-enriched in 10ml buffered peptone water (BPW) and incubated aerobically at 37°C for 24 hours. This helped in recovery of the organism, sensitivity and specificity for detection of *Salmonella* (Myint *et al*., 2006).

3.4.3.2 Selective Enrichment

After pre-enrichment, 0.1ml of pre- enriched aliquots was transferred into 10ml Rappaport-Vassiliadis (RV) and Selenite Cystine (SC) broths. Samples in RV broths were incubated aerobically at 42°C for 24 hours while samples in SC broths were incubated at 37°C for 24hours.

3.4.3.3 Isolation

After enrichment, a loopful of aliquots from RV and SC broths were streaked on Xylose Lysine Deoxycholate (XLD) and Brilliant green agar. On the Xylose Lysine deoxycholate (XLD) agar, pink colonies with or without black centers and those with large, glossy black centers were isolated as presumptive. On Brilliant Green Agar (BGA), typical *Salmonella* colonies appear as pinkish-white or red colonies surrounded by a red halo in the medium

3.4.3.4.0 Confirmation of Salmonella Enterica

3.4.3.4.1 Gram Stain

A moderate smear of cell concentration was air- dried; heat-fixed for one (1) minute with crystal violet staining reagent. The slide was then gently washed in an indirect stream of tap water for two (2) seconds. Gram's iodine (mordant) was used to flood the slide and waited for one (1) minute. It was then again washed in a gentle and indirect stream of tap water for two (2) seconds. The decolorizing agent was then added drop by drop to the slide until the decolorizing was running clear from the slide. The Counterstain, Safarin was at this point used to flood the slide and waited between thirty (30) seconds to one (1) minute. The slide was again washed in a gentle and an indirect stream of tap water until no colour appears in the effluent and blot-dried with absorbent paper. The result of the staining procedure was observed under oil immersion using a Bright field microscope. Pink-stained or red-stained rods bacteria were recorded as gram-negative bacteria.

3.4.3.4.2 Triple Sugar Iron (TSI)

This was used primarily to differentiate members of the Enterobacteriaceae family from other gram-negative rods on the basis of their sugar fermentation patterns. This test employs Triple Sugar Iron Agar which is designed to differentiate organisms based on the differences in carbohydrate fermentation patterns and hydrogen sulphide production. An agar slant of the special medium has multiple sugars comprising a pH-sensitive dye (phenol red), 1% lactose, 1% sucrose, 0.1% glucose as well as sodium thiosulphate and ferrous sulphate used for carrying out the test.

The carbohydrate fermentation is indicated by the production of gas and a change in the colour of the pH indicator from red to yellow. Using a straight inoculation needle, the top of a well-isolated colony was touched and the TSI inoculated by stabbing through the center of the medium to the bottom of the bottle and then streaked on the surface of the agar slant. With the cap left loosely, the bottle was incubated at 32^0C for 24hours. Reaction of the medium was examined, recorded, and interpreted. Akline/ acid (red slant/ yellow butt) reaction was interpreted as an indication of dextrose fermentation only. Acid/acid (yellow slant/ yellow butt) reaction was also noted as fermentation of dextrose, lactose and /or sucrose. An alkaline/alkaline (red slant, red butt) reaction showed the absence of carbohydrate fermentation results. Blackening of the reaction medium indicated that hydrogen gas was present. Also, bubbles or cracks in the agar showed that there was production of gas (CO_2 and H_2 formation).

3.4.3.4.3 Latex Agglutination

A suspected colony was mixed in a test circle with one drop of the test latex. Using a sterilised loop, a second colony was mixed continuously for 10 to 15 seconds in a second test circle with a drop of control latex. In a circular motion, the card was gently rocked for not more than two (2) minutes and observed with the aid of a magnifying glass for agglutination. The latex kits were returned to the refrigerator and subsequently, the results were read and interpreted as follows: Positive if agglutination of the test latex occurs within two (2) minutes, no agglutination of the control latex occurs within the two (2) minutes. On the other hand, negative readings

were recorded if no agglutination of either the test latex or the control latex occurs within two (2) minutes. Agglutination occurring after the two (2) minutes and agglutination of both the test latex and the control latex were ignored and results regarded as un-interpretable. However, cultures of such colonies were re-streaked and checked using biochemical and serological procedures.

3.4.3.4.4 Lysine Iron Agar (LIA)

Lysine iron agar (LIA) slants tests organisms for their ability to deaminate lysine or decarboxylate lysine (Sagar Aryal, 2019). Lysine iron agar or LIA is a differential media used to distinguish bacteria that are able to decarboxylate lysine and/or produce hydrogen sulfide from those that cannot. The LIA is made up of enzymatic digest of gelatin (5 g), yeast extract (3 g), dextrose (1 g), L-lysine (10 g), ferric ammonium citrate (0.5 g), sodium thiosulfate (0.04 g), bromocresol purple (0.02 g and agar (13.5 g) per 1000 mL and at a pH of 6.7. The medium is first turbed, sterilized and slanted in order that a short slant and a deep butt are formed. A straight inoculating needle was used to inoculate LIA by stabbing twice through the center of the medium to the bottom of the tube and then streaked on the slant. The tube was capped tightly and incubated at 35^0C – 37^0C in ambient air for 24 hours. The tube was examined at 24hours and 48hours for growth and colour changes in the tube butt, slant, and for blackening at the apex of slant.

3.5 Antimicrobial Susceptibility of Salmonella Enterica Isolates

The disk diffusion method of Bauer *et al*., (1966) was used to determine the antibiotic resistance of *salmonella enterica* isolates against the following antibiotics; ciprofloxacin (Cip) 5µg, amoxicillin (AMC) 20µg, azithromycin 15µg, telcoplanin (TEC) 30µg, gentamicin (Cn) 10µg, tetracycline (Te) 30µg, chloramphenicol (C) 30 µg, ceftriaxone (Cro) 30µg, suphamethoxazole/ trimethoprim (Sxt) 23.75µg. The disks were purchased from Oxoid Limited, Basingstoke, UK. Pure cultures of salmonella were grown overnight in Tryptic Soy Broth (TSB) (Oxoid Limited, Basingstoke, UK) at 37^0C and the concentration adjusted using sterile TSB until a 0.5 McFarland turbidity was attained. One hundred microliters (100µl) of the culture was then spread plated unto Mueller Hinton agar (Oxoid, Basingstoke, UK) using a sterile cotton swab. Four antimicrobial disks were placed on the surface of the agar plate at a distance to avoid overlapping of inhibition zones. The plates were incubated at 37^0C for 24 hours and the inhibition zones were measured and interpreted using European Committee on Antimicrobial Susceptibility Testing (EUCAST) (2019).

3.6 Statistical Analysis

The data obtained was analyzed using binary logistic of IBM Statistical Package for the Social Sciences (SPSS) Version 20. Test for statistical difference was done using Wald's chi-square at 5% significance level.

CHAPTER FOUR

4.0 RESULTS

4.1. Socio-Demographic Characteristics of Vendors

The socio-demographic characteristics of the grilled RTE meat vendors are shown in Table 4.1. The result of this study revealed that 293 (97.7%) of the vendors were males (Table 4.1). It further indicates that majority of the vendors had ages ranging from 21–40 years (77.3%), followed by 41-60 years (22%), and only 0.7% were below 21 years old. Also, apart from the 89.5% of them who were Ghanaians, 2.4%, 5.4% and 2.7% were Burkinabes, Malians and Nigerians respectively. The majority, 73.7% had basic education as their highest educational level with 24.7% having no education at all. Majority of the meat vendors were Muslims (63.0%) and had work experience of six years and above (43.3%). Also, many meat vendors interviewed (95.0%) sell their respective grilled RTE meat product types based on consumer preference but few (3.0%) do it for religious reasons and mainly sell grilled meat as full-time business (93.7%).

Table 4.1: Socio-demographic characteristics of respondents

Variable	Frequency	Percentage (%)
Gender		
Male	293	97.7
Female	7	2.3
Age		
Below 21 years	2	0.7
21-40 years	231	77.3
41-60 years	66	22.1
Nationality		
Ghanaian	265	89.5
Burkinabe	7	2.4
Malian	16	5.4
Niger	8	2.7
Marital status		
Married	274	91.3
Single	25	8.3
Divorced	1	0.3
Religion		
Christianity	92	30.7
Islamic	189	63.0
Traditional	15	5.0
Others	4	1.3
Educational background		
None	74	24.7
Basic	221	73.7
Secondary	5	1.7
Tertiary	0	0
Years in business		
Less a year	0	0
1-5 years	131	43.7
6-10 years	130	43.3
Above 10 years	39	13.0
Type of grilled RTE meat sold		
Pork	50	16.7
Mutton	49	16.3
Guinea fowl	51	17.0

Chevon	49	16.3
Chicken	50	16.7
Beef	51	17.0
Reason for product preference		
Cheaper	6	2.0
Consumer preference	285	95.0
Religion	9	3.0
Occupational status		
Full time	281	93.7
Part time	19	6.3
Alternative occupation if part-time		
Farming	22	7.3
Teaching	0	0.0
Number of shops		
One	290	96.7
Two	10	3.3
Total		

4.2 Knowledge of Meat Safety and Contamination by Vendors

The study revealed 98.3% of the 300 respondents have heard about meat safety (Table 4.2). Also, the result showed that all the meat vendors knew that meat can be contaminated with germs such as bacteria as a result of poor handling. The study further identified that a good number of the meat vendors (85.5%) have received some form of training on meat safety and 96% of them were aware that contaminated meat can cause foodborne disease/illness. A greater proportion of the vendors (99.0%) were aware that eating and drinking while selling meat increases the risk of meat contamination and all the respondents were aware that regular washing of hands and using sterilized gloves reduces the risk of meat contamination. In addition, as high as 98.7% indicated that they know that there is the need to take leave from work when infected with any skin disease. Many vendors (94.7%) know that it is necessary to refrigerate leftover meat indicating that it is very effective (97.0%).

Table 4.2: Knowledge of meat safety and contamination

Parameters	Response	
	Yes, n(%)	No, n(%)
Vendors' response about hearing of meat safety	295(98.3)	5(1.7)
Knowledge on meat contamination	300(100)	0(0)
Knowledge on meat-borne diseases	289(96.3)	6(2.0)
Received training on meat safety	254(85.5)	43(14.5)
Aware that eating and drinking while selling meat increases risk of meat contamination	297(99.0)	3(1.0)
Aware that regular washing of hands reduces the risk of meat contamination	300(100)	0(0.0)
Aware that using sterilized gloves reduces risk of meat contamination	298(99.3)	2(0.7)
Know that there is the need to take leave from work when infected with skin disease	296(98.7)	4(1.3)
Know that it is necessary to refrigerate leftover meat	284 (94.7)	16 (5.3)

n=Number of respondents

4.3 Meat Vendors' Responses to Hygienic Practices

Practices of meat safety among grilled RTE meat vendors sampled from Bolgatanga are presented in Table 4.3. Majority 182 (60.7%) of the meat vendors sourced meat from the abattoir citing safety and quality as their main reasons (60.7%). However, 37% (111/300) vendorsobtained their meat through backyard slaughtering with the excuse that such meat were readily available (36.7%). Most, 48.0% (144/300) of the meat vendors sold their grilled RTE meat on tables with a net covering the meat and 40.3% (121/300) sold in glass sieves. Majority 91.7% (275/300) of the vendors indicated that they wash their chopping tables at the beginning and at the end of work each day, whilst a few (6%) wash them at the beginning of work. 99.3% (298/300) of vendors indicated that they disinfect their meat shops at least once a week 42.7% (128/300), with majority of them 57.0% (171) disinfecting their shops

twice a week with mostly isopropyl alcohol 94.7% (284) and iodine 4.0 % (12). Majority, 99.7% (299) of the vendors said that they always wash their hands before touching the grilled RTE meat and equipment 100.0% (300) for selling RTE meat. Out of these grilled RTE meat vendors who wash their equipment, 99.3% (298) wash with detergent and water, rinse with only water 76.0% (228) and warm water 24.0% (72). All the meat vendors' responses to sterilization of their knives and equipment were yes which they do daily 53.8% (161), weekly 42.1% (126) and twice a week 3.3% (10). Comparatively, more RTE meat vendors 98.0% (294) wore apron during work and this number, 91.3% (272) wash them daily. However, only a few of the vendors 9 (3.0%) always wear clean gloves during work with the majority 68.0% (204) who sometimes wear clean gloves, 21.7% (65) who rarely wear clean gloves and as much as 7.3% (22) admitted never using clean gloves during work. The results also revealed that majority of the vendors 98.3% (295) do not smoke during work and just a small fraction 1.7% (5/300) do smoke but at home. Physical observation of the neatness level of vendors also showed that a greater number 65.3% (196) of them were very clean, 33.0% (99) scoring clean appearance with only 1.3% (4) and 0.3% (1) of them appearing dirty and very dirty, respectively. In addition, majority 94.7% (284) of the vendors stored leftover grilled RTE meat in refrigerators with 5.3% (16) of them smoking it.

Table 4.3: Vendors' responses to hygienic practices

Variables	Frequency	Percentage (%)
Source of meat for grilling		
Backyard slaughter	111	37.0
Abattoir	182	60.7
Imported carcass	7	2.3
Others	0	0
Reason for choice of source		
Safe and quality	182	60.7
Readily available	110	36.7
Cheap	8	2.7
Others	0	0
What do you sell meat on/in?		
An open table	7	2.3
Table with a net covering the meat	144	48.0
Glass sieve	121	40.3
Others	28	9.3
Frequency of washing cutting tables		
At the beginning of work	20	6.7
At the end of work	5	1.7
At the beginning and at the end of work	275	91.7
Once a week	0	0
Others	0	0
Disinfection of shop		
Yes	298	99.3
No	2	0.7
How often vendors disinfect shop		
Once a week	128	42.7
Twice a week	171	57.0
Once a month	0	0
Others	1	0.3
Type of disinfectant used		
Isopropyl alcohol	284	94.7
Iodine	12	4.0
Hydrogen peroxide	4	1.3

Frequency of washing hands before touching meat

Always	299	99.7
Sometimes	1	0.3
Rarely	0	0
Never	0	0

Do you wash your equipment for selling RTE meat

Yes	300	100.0
No	0	0

Materials used for washing equipment

Only water	1	0.3
Detergent and water	298	99.3
Others	1	0.3

What do you use to rinse equipment after washing

Water	228	76.0
Warm water	72	24.0

Sterilization of cutting tools and other equipment

Yes	283	94.3
No	17	5.7

Frequency of sterilization of cutting tools and equipment

Daily	161	53.8
Twice a week	10	3.3
Weekly	126	42.1
Others	2	0.7

Wear apron during work

Yes	294	98.0
No	5	1.7

How often vendors wash apron

Everyday	272	91.3
Twice a week	18	6.0
Once a week	8	2.7

Frequency of wearing clean gloves by vendors during work

Always	9	3.0
Sometimes	204	68.0
Rarely	65	21.7
Never	22	7.3
Smoking by vendors		
Yes	5	1.7
No	295	98.3
Neatness of vendors on their appearance		
Very dirty	1	0.3
Dirty	4	1.3
Clean	99	33.0
Very clean	196	65.3
Handling of leftover meat		
Refrigeration	284	94.7
Salting	0	0
Smoking	16	5.3
Frying	0	0

4.4. Willingness of Meat Vendors to Adopt Meat Safety Practices

The eagerness of the grilled RTE meat vendors towards meat safety is shown in Table 4.4. The meat sellers were all willing to sell meat in an enclosure place. They were also willing to always clean work area before start of work, wash knives and other equipment, and wash their hands before selling RTE meat. Almost all (99.7%) the vendors were also ready to avoid touching RTE meat with wounded hands and rubbing hands on their face, hair, etc while selling RTE meat. Furthermore, 74.7% of

the vendors disagreed to wearing rings (including wedding rings and watches) while selling and they were willing to separate raw meat from grilled RTE meat (74.7%) and use separate equipment to handle raw and grilled RTE meat (74.0%). Also, all (100.0%) the vendors were very enthused to cover their mouth and nose when coughing or sneezing and also willing to disinfect their shops regularly. Majority (99.7%) of the vendors indicated their readiness to always wear clean apron and use it as a towel to clean their hands (89%). In addition, 86.7% of vendors prefer to wear clean gloves and 92.7% were willing to avoid using the same towel to clean many places in the meat shop. All the vendors said they did smoke while selling RTE meat, but were willing to be trained on meat safety, and adhere to food safety rules. Majority (99.7%) of the vendors were also willing to refrigerate leftover meat.

Table 4.4: Readiness of meat vendors to adopt meat safety practices

Parameter	Level of readiness		
	Agree, no.(%)	Uncertain, no.(%)	Disagree, no.(%)
Always ready sell meat in an enclosure	100.0	0	0
Willing to clean work area before start of work	100.0	0	0
Will wash tables knives and equipment before start of work	100.0	0	0
Wash hands before selling RTE meat	100.0	0	0
Will not touch RTE meat with wounded hand	99.7	0.3	0
Will avoid rubbing hands on face, hair, et c while selling	99.7	0.3	0
Will wear jewelry including wedding ring and watch while handling RTE meat	6.3	19.0	74.7
Will not necessarily separate raw and RTE meat	7.0	18.3	74.7
Will use separate equipment to handle raw and RTE meat	74.0	22.3	3.7
Will cover mouth and nose when coughing or sneezing	100.0	0	0
Will disinfect shop regularly	100.0	0	0
Like to use clean apron	99.7	0.3	0
Will not refreeze defrosted RTE meat	76.7	22.7	0.7
Will use clean apron as towel to clean hands	89.0	5.7	5.3
Will use the same towel to clean many places in the meat shop	4.3	3.0	92.7
Like to use clean gloves	86.7	12.0	1.3
Not smoke while selling	100.0	0	0
Like to be trained on meat safety	100.0	0	0
Will refrigerate leftover RTE meat	99.7	0.3	0
Will adhere to food safety rules	100.0	0	0

n=Number of respondents

4.5. Socio-Demographic Characteristics of RTE Meat Consumers

The socio-demographic characteristics of the grilled RTE meat consumers are shown in Table 4.5. The result of this study showed that 71.7% (274) of the respondents were males whiles the minority 28.3% (108) were females. It further indicates that majority of the respondents (65.4%) had ages between 21 and 40 years, 22.8% were aged between 41-60 with only 9.2% and 2.6% below 21 and above 60 years respectively. The study also revealed that 73.8% of the respondents were single and 24.1% of them were married. In addition, 66.5%, 20.7% and 11.3% of the respondents were Christians, Muslims and traditionalists respectively. The research further showed that majority (54.2%) of the respondents obtained basic education and close to 13% had no education at all. Also, apart from 34.3% of them who were Frafra by tribe, Builsas (11.8%), Kassenas (9.4%), Manprusis (4.7%) and Others (39.8%) made up for the tribe distribution of the rest of the respondents. Moreover, the study showed that a greater proportion (69.6%) of the consumers preferred grilled RTE guinea fowl to chevon (11%), pork (8.4%), mutton (5.8%) and beef (5. %) citing good taste (89.3%) for their choice. However, few others (7.9%) suggested it was healthy as the reason for their preference and majority (57.3%) often consume it once a week and once a month (29.8%) mainly when they go out with friends in the evening (86.9%).

Table 4.5: Socio-demographic characteristics of RTE meat consumers

Variable	Frequency	Percentage (%)
Gender		
Male	274	71.7
Female	108	28.3
Age		
Below 21 years	35	9.2
21-40 years	250	65.4
41-60 years	87	22.8
Above 60 years	10	2.6
Marital status		
Married	92	24.1
Single	282	73.8
Divorced	7	1.8
In a relationship	1	.3
Religion		
Christianity	254	66.5
Islamic	79	20.7
Traditional	43	11.3
Others	1	.3
Educational background		
None	48	12.6
Basic	207	54.2
Secondary	86	22.5
Tertiary	32	8.4
Others	9	2.4
Tribe		
Frafra	131	34.3
Builsa	45	11.8
Kasena	36	9.4
Manprusi	18	4.7
Others	152	39.8
Type of RTE meat preferred		
Pork	32	8.4

Mutton	22	5.8
Guinea fowl	266	69.6
Chevon	43	11.3
Beef	19	5.0
Reason for your preference		
Readily available	3	.8
Has good taste	341	89.3
It is healthy	30	7.9
It is cheap	6	1.6
It is safe	2	.5
How often do you consume		
Daily	20	5.2
Once a month	114	29.8
2-3 times a week	29	7.6
Once a week	219	57.3
What prompts you consume it		
My mouth sweet me	22	5.8
When i go out with friends in the evening	332	86.9
For home consumption	27	7.1
Others	1	0.3

4.6 Knowledge of Consumers on Meat Safety and Contamination

As in Table 4.6, it was revealed that most (91.8%) of the 382 RTE meat consumers interviewed have heard about meat safety mostly from veterinary officers (80.7%). Also, the result showed that 88.9% of them knew that meat can be contaminated with pathogens such as bacteria through poor handling. However, the study indicated that a good number of the consumers (76.6%) did not know that eating, drinking and smoking by RTE vendors increases their risk of contamination with only. Also, 94.0% of the respondents were aware that regular hand washing and the use of sterilized gloves by vendors reduces the risk of contamination.

Table 4.6: Knowledge of consumers on meat safety and contamination

Parameters	Response	
	Yes,n(%)	No,n(%)
Have you ever heard of meat safety	358 (91.8)	21 (5.5)
Do you know that meat can be contaminated by poor handling	327 (88.9)	26 (7.1)
Do you know that eating, drinking and smoking by vendors while RTE meat increases the risk of contamination	70 (19.0)	282 (76.6)
Do you know that regular washing of hands by vendors reduces the risk of contamination	359 (94.0)	23 (6.0)

4.7. Consumers' Responses to Hygienic Practices

From the study conducted, many of the consumers (94.7%) have the opinion that RTE leftover meat should be refrigerated as shown in the Table 4.7. On the other hand, others (27.5%) thought the meat should be smoked for preservation. A little above half of the respondents (54.5%) bought their grilled RTE meat by the roadside, while 19.9% of them bought from drinking bars mostly displayed on tables with wire mesh covering (44.5%) and in glass sieves (38.5%). However, 15.2% bought their grilled RTE meat normally displayed on open tables. Also, 69.6% of the respondents do not wash their hands before touching or eating their RTE meat and the few (24.8%) who did wash, washed with only water (73.3%).

Table 4.7: Consumers' responses to hygienic practices

Variables	Frequency	Percentage (%)
How should leftover grilled RTE meat be stored		
Referigeration	256	67.0
Salting	8	2.1
Smoking	105	27.5
Frying	13	3.4
Where do you buy your grilled RTE meat		
Market	63	16.5
Road side	208	54.5
Restaurant	35	9.2
Drinking bar	76	19.9
How is the RTE meat that you buy normally displayed		
On open table	58	15.2
Table with wire mesh covering	170	44.5
Glass sieve	147	38.5
Others	7	1.8
Do you wash your hands before touching or eating RTE meat?		
Yes	93	24.8
No	261	69.6
What do you use to wash if yes?		
Only water	220	73.3
Soap and water	80	26.7
Where do you eat your RTE meat		
On the street	47	12.3
At home	85	22.3
In a drinking bar	222	58.1
On the vendors table	28	7.3

4.8 Readiness of RTE Meat Consumers to Adopt Meat Safety Practices

Meat safety practices among grilled RTE meat consumers sampled from the Upper East regional capital, Bolgatanga are presented in Table 4.8. Most (94.5%) of the RTE meat consumers were very willing to buy RTE meat in an enclosure and ready to avoid buying from a vendor who was coughing or sneezing (95.0%) and rubbing the hands on the face, nose or hair (96.6%). Also, 89.8% want raw and grilled RTE meats to be separated with separate equipment for handling each. Also they want RTE meat vendors to disinfect their shops regularly (96.3%), want vendors to wear apron, gloves and mouth mask while selling RTE meat (90.8) but did not want to see RTE meat vendors' wearing jewelry including wedding rings and watches while handling RTE meat (46.3%).

Table 4.8: Readiness of RTE meat consumers to adopt meat safety practices

Parameter	Level of readiness		
	Agree, n(%)	Uncertain, n(%)	Disagree, n(%)
Will always buy grilled RTE meat in an enclosure	361 (94.5)	7 (1.8)	7 (1.8)
Will not buy RTE meat from a vendor who is coughing and sneezing	363 (95.0)	7 (1.8)	12 (3.1)
Will not like vendors to rub their hands on face, nose, or hair	369 (96.6)	0	13 (3.4)
Will want raw and grilled RTE meat to be separated with separate equipment for handling each	343 (89.8)	25 (6.5)	14 (3.7)
Will want RTE meat vendors to disinfect their shops regularly	368 (96.3)	14 (3.7)	0
Will want vendors to wear apron, gloves and mouth mask while selling RTE meat	347 (90.8)	7 (1.8)	28 (7.3)
Will not want vendors to wear jewelry including wedding ring and watch while handling RTE meat	92 (24.1)	113 (29.6)	177 (46.3)

4.9 Aerobic Bacteria Count of Grilled RTE Meat Samples

Microbial quality of grilled RTE meats from Bolgatanga revealed the mean total plate count (TPC) of beef, chicken, chevon, guinea fowl, mutton and pork were 3.368 log_{10} cfu/cm^2, is 2.526 log_{10}cfu/cm^2, 4.852 log_{10}cfu/cm^2, 4.057 log_{10} cfu/cm^2, 4.171log_{10} cfu/cm^2 and 4.02log_{10}cfu/cm^2, respectively. It was also noted that chevon had the highest count of 4.852 log_{10} cfu/cm^2, whilst the least count of 2.526 log_{10} cfu/cm^2 was recorded for chicken. There were significant differences ($P<0.001$) among bacterial count of the various meat types as shown in Table 4.9. However, there were no significant differences ($P>0.05$) among grilled guinea fowl meat, mutton and pork.

Table 4.9: Aerobic bacteria count of the samples

RTE meat type	Bacteria load (log_{10} cfu/cm^2)
Beef	3.368^{ab}
Chicken	2.526^{a}
Chevon	4.852^{c}
Guinea fowl	4.057^{bc}
Mutton	4.171^{bc}
Pork	4.02^{bc}
s.e.d.	0.294
P-value	<.001

s.e.d: standard error of difference, P-value: probability value

4.10 Prevalence of Salmonella Enterica In the Ready-To-Eat (RTE) Meats

The Table 4.10 shows the prevalence of *Salmonella enterica* in the grilled RTE meat samples obtained from Bolgatanga. Out of a total of 300 samples of meat consisting of 50swabs each from grilled RTE beef, chicken, chevon, guinea fowl, mutton, pork tested, only 2% (6/300) were positive for *Salmonella enterica* and 98% (249/300) grilled RTE meat samples that were negative for *Salmonella enterica*. Guinea fowl recorded a prevalence of 2/50 whilst beef had no *Salmonella enterica.*

Table 4.10: Prevalence of *Salmonella enterica* in the ready-to-eat (RTE) meats

Meat type	Total Sample tested	Number positive	Prevalence
Mutton	50	1	2
Chevon	50	1	2
Pork	50	1	2
Guinea Fowl	50	2	4
Chicken	50	1	2
Beef	50	0	0
TOTAL	300	6	2

4.11 Pairwise Comparisons

From the table (Table 4.11), there were no significant differences (P>0.05) among the prevalence of *Salmonella enterica* in all the grilled RTE meat types obtained from Bolgatanga.

Table 4.11: Pairwise comparison of prevalence of *Salmonella enterica* in RTE meats

Meat type	Meat type	Mean Difference	Standard Error	Degrees of freedom	Significance
Beef	Chevon	0.02	0.02	1	0.312
	Chicken	0.02	0.02	1	0.312
	Guinea Fowl	0.04	0.028	1	0.149
	Mutton	0.02	0.02	1	0.312
	Pork	0.02	0.02	1	0.312
Chevon	Beef	-0.02	0.02	1	0.312
	Chicken	0	0.028	1	1.000
	Guinea Fowl	0.02	0.034	1	0.557
	Mutton	0	0.028	1	1.000
	Pork	0	0.028	1	1.000
Chicken	Beef	-0.02	0.02	1	0.312
	Chevon	0	0.028	1	1.000
	Guinea Fowl	0.02	0.034	1	0.557
	Mutton	0	0.028	1	1.000
	Pork	0	0.028	1	1.000
Guinea fowl	Beef	-0.04	0.028	1	0.149
	Chevon	-0.02	0.034	1	0.557
	Chicken	-0.02	0.034	1	0.557
	Mutton	-0.02	0.034	1	0.557
	Pork	-0.02	0.034	1	0.557
Mutton	Beef	-0.02	0.02	1	0.312
	Chevon	0	0.028	1	1.000
	Chicken	0	0.028	1	1.000
	Guinea Fowl	0.02	0.034	1	0.557
	Pork	0	0.028	1	1.000
Pork	Beef	-0.02	0.02	1	0.312
	Chevon	0	0.028	1	1.000
	Chicken	0	0.028	1	1.000
	Guinea Fowl	0.02	0.034	1	0.557
	Mutton	0	0.028	1	1.000

4.12 Antibiotic Susceptibility of *Salmonella Enterica* Isolates from The Samples

The six (6) *Salmonella enterica* isolates tested against nine (9) commonly used antibiotics revealed 44.44% of the isolates were susceptible, 20.37% were intermediately resistant and 35.19% were resistant to the various antibiotics (Table 4.12). Telcoplanin had highest resistance (100%) followed by azithromycin (83.33%). However, the isolates were 100% susceptible to sulfamethoxazole/trimethoprim with chloramphenicol and gentamycin recording 83.33% susceptibility each for the *Salmonella enterica* isolates.

Out of the six (6) *Salmonella enterica* isolated, two (2) were resistant to two (2) and four (4) antibiotics (33.33%), respectively and one (1) was resistant to three (3) and five (5) antibiotics (16.67%), respectively.

Table 4.12: Antibiotic Susceptibility patterns of the *Salmonella enterica* isolates

Antimicrobial	Resistant (%)	Intermediate resistant (%)	Susceptible (%)
Amoxycillin/Clavulanic acid 30ug (AMC)	66.67	0.00	33.33
Azithromycin 15ug (AZM)	83.33	0.00	16.67
Ceftriaxone 30ug (CRO)	0.00	50.00	50.00
Chloramphenicol 30ug (C)	0.00	16.67	83.33
Ciprofloxacin 5ug (CIP)	0.00	16.67	83.33
Gentamycin 10ug (CN)	16.67	50.00	33.33
Telcoplanin 30ug (TEC)	100.00	0.00	0.00
Tetracycline 30ug (TE)	50.00	50.00	0.00
Sulfamethoxazole/Trimethoprim (SXT)	0.00	0.00	100.00
Total	35.19	20.37	44.44

4.13 Antibiotic Resistance Profile and Multiple Antibiotic Resistance Index of *Salmonella Enterica* Isolates

All the *Salmonella enterica* isolates exhibited different antibiotic resistant patterns. *Salmonella enterica* isolated from grilled RTE mutton had the highest resistance profile (Amc-Azm-Tec-Te-C) with resistance to five (5) different antibiotics whereas isolates from grilled RTE pork and guinea fowl have the lowest resistance profile (Azm-Tec) and (Amc-Tec) respectively, being resistance to two (2) antibiotics each (Table 4.15).

Table 4.13 shows the antibiotic resistance profile and multiple antibiotic resistance (MAR) index of individual *Salmonella enterica* isolated from different RTE meats. The *Salmonella enterica* isolates exhibited six (6) different antibiotic resistant patterns with MAR index ranging from 0.22 to 0.56. The majority of the isolates were resistant to two (2) and four (4) antibiotics respectively. Also, two isolates were resistant to three (3) and five (5) antibiotics (2 isolates; MAR index 0.33 and 0.56) respectively. The two isolates that were resistant to four antibiotics exhibited AzmTecCnTe (1 isolate) and AmcAzmTecTe (1 isolate) antibiotic resistant patterns respectively. Also the two isolates that were resistant to two antibiotics exhibited the resistant patterns AzmTec (1 isolate) and AmcTec (1 isolate), respectively. Lastly, the one isolate each that was resistant to three and five antibiotics had resistant patterns AmcAzmTec and AmcAzmTecTeC, respectively.

Table 4.13: Antibiotic resistance profile and multiple antibiotic resistance indexes of individual *Salmonella enterica* isolates

Codes	Meat type	Number of Antibiotics	Antibiotic resistant profile	MAR index
S7	Mutton	5	AmcAzmTecTeC	0.56
P3	Pork	2	AzmTec	0.22
Go44	Chevon	4	AzmTecCnTe	0.44
G44	Guineafowl	2	AmcTec	0.22
G49	Guineafowl	3	AmcAzmTec	0.33
C16	Chicken	4	AmcAzmTecTe	0.44

CHAPTER FIVE

5.0 DISCUSSION

Salmonella infection is a worldwide public health problem, often associated with insanitary food processing and preparations, unsafe water supplies and inadequate sanitary conditions (Addo *et al*., 2007). Salmonellosis is among the prevalent foodborne infections affecting Ghanaians, but data on this is limited. Nonetheless, Typhoid fever, caused by *Salmonella typhi* has been ranked eleventh (11th) among the top twenty causes of outpatient morbidity in Ghana between 2002 and 2013 with 339,877 total cases in 2013 (Ghana Health Service (GHS), 2015).

5.1 The Knowledge, Attitude and Practices of RTE Meat Vendors on Microbiological Meat Safety

5.1.1 Socio-Demographic Characteristics of Grilled RTE Meat Vendors

The result of this study revealed that the majority of the grilled RTE meat vendors were males (Table 4.1). It further indicates that majority of the respondents had ages ranging from 21-40 (77.3%), similar to earlier research by Ampaw (2018) who indicated that 70% of RTE meat processors were in the age range of 30-39. Apart from the majority who were Ghanaians, few were Burkinabes, Malians and Nigerians. Also, 73.7% had basic education which implies that they would have an idea about safety of grilled meat. High illiteracy among vendors would have had negative impact on knowledge of meat safety practices. This result is consistent with earlier findings by Ampaw (2008) and FAO (2016) which revealed that close to 80% of RTE meat ('khebab') processors and street food venders (SFV) in Accra

metropolis had at least formal basic education. Majority of the meat vendors were Muslims, (63.0%) this might be the reason for the higher frequency of hand washing among the meat vendors since most of them wash their hands several times a day prior to entering the mosque for prayers as also observed by Adzitey *et al*. (2018). Many of the vendors had work experience of six years and above (43.3%). With this experience it is expected that they were conversant with matters relating to handling and selling of meat. Also, many meat vendors interviewed sell their respective grilled RTE meat product types based on consumer preference but a few did it for religious reasons on a full time.

5.1.2 Knowledge of RTE Meat Vendors on Meat Safety and Contamination

The study revealed that majority of the meat vendors had heard about meat safety. Also, the result showed that all the meat vendors knew that meat can be contaminated with germs such as bacteria as a result of poor handling. The study further identified that a good number of the meat vendors (85.5%) have received some form of training on meat safety and were aware that contaminated meat can cause foodborne disease/illness (96.0%). A greater proportion of the vendors (99.0%) were aware that eating and drinking while selling meat increases risk of meat contamination and all the respondents were aware that regular washing of hands and using sterilized gloves reduces the risk of meat contamination. In addition, 98.7% indicated that they know that there is the need to take leave from work when infected with skin disease. Many vendors (94.7%) know that it is necessary to refrigerate leftover meat because of its effectiveness in preserving it. Refrigerated

meat keeps longer and contributes to less microbial contamination. The result of this study is consistent with work by Adzitey *et al*. (2018) that revealed that meat sellers were aware of the necessary precautions needed to reduce the risk of contamination in meat. Furthermore, majority (94.3%) of the vendors sterilize their cutting equipment daily (53.8%) and weekly (42.1%); and this helps to reduce the spread of pathogens such *Salmonella spp*., *Escherichia coli Staphylococcus aureus* among others.

5.1.3 Vendors' Responses of Hygienic Practices

Practices of meat safety among grilled RTE meat vendors from Bolgatanga are presented in Table 15. A good number of the vendors (60.7%) sourced their meat from the abattoir for grilling because that was thought to be safe and quality (60.7%), similar to findings by Sulleyman *et al*. (2018). They reported that meat sellers in Accra metropolis mainly obtained their meat from the abattoir. However, as high as 37% of the vendors carry out backyard slaughter, against the instructions of the local veterinary and health authorities. This may be due to the absence of slaughter houses or abattoirs for different categories of animals in the region. The incidence of this practice may pose a health risk to consumers since meat produced from this is not inspected by veterinary officers. Majority of the vendors sold their grilled RTE meat on tables with net covering the meat (48.0%) and glass sieves (40.3%) which prevent flies, dust and other contaminants. These findings are contrary to results from a study by Sulleyman *et al*. (2018) in Accra, Ghana, that meats were sold on open tables which cannot provide any protection from contamination. Also, 91.7% of the

vendors wash their cutting tables and other equipment at the beginning and at the end of work with detergent and water (99.3%), disinfect their shops (57.0%) with isopropyl alcohol (94.7%). These results were partially similar to earlier studies by Adzitey *et al*. (2020) which indicated that meat sellers in Tamale metropolis wash and scrap their tables and equipment three or times a day, but failed to disinfect their shops and sterilize their knives and other equipment, respectively. The majority (99.7%) of the meat vendors always washed their hands before touching the grilled RTE meat since their hands can spread pathogens as a result of cross contamination (Bas *et al*., 2006). Contrary, Amare *et al*. (2019) found that 50% of the RTE food vendors did not hand wash their hands frequently ith soap and water while selling food. Almost all the vendors (98.0%) wore aprons during this study, contrary to a previous report by Adzitey *et. al* (2018). However, 68.0% did not wear gloves regularly during work, similar to earlier works by Adzitey *et al*. (2018) and Sulleyman *et al*. (2019) with a minority (1.7%) who indicated that they smoke and even at home. This is contrary to the findings of Sulleyman *et al*. (2018). The majority (65.3%) of the meat vendors interviewed appeared very clean. Being "clean" means that less than a quarter of the vendors' clothes were not stained by fresh meat particles/ blood splashes, while being "dirty" means that half of the vendors' clothes were stained with either fresh or old meat particles/blood splashes (Adzitey *et al*., 2020). A high proportion of the vendors (94.7%) stored leftover meat in refrigerators and 5.3% by smoking, which is consistent with work by Adzitey *et al*. (2018) which showed that close to 84% of butchers store their leftover meats in a refrigerator.

5.1.4 Readiness of RTE Meat Vendors to Adopt Meat Safety Practices

All of the grilled RTE meat vendors were ready to sell meat in an enclosure, clean work area before start of work, wash tables knives and equipment before start of work, wash hands before selling RTE meat and ever willing to avoid either touching RTE meat with wounded hand (99.7%) nor rubbing hands on face, hair, et c while selling (99.7%). Furthermore, vendors, (99.7%) were very eager to wear clean clothing when selling meat, willing to use clean gloves (86.7%). However, they were very unwilling to wear jewelry including wedding ring and watch while handling RTE meat (74.7%). In addition, they were all ever ready to avoid smoking while selling, but will like to be trained on meat safety, refrigerate leftover RTE meat and adhere to food safety rules. The willingness of the RTE meat vendors shows their preparedness to accept and to adopt practices required to ensure meat safety.

5.2 Knowledge, Attitude and Practices of Rte Meat Consumers on Microbiological Meat Safety

5.2.1 Socio-Demographic Characteristics of Rte Meat Consumers

The socio-demographic characteristics of the grilled RTE meat consumers are shown in Table 17. The result of this study showed that 274 (71.7%) of the respondents were males whiles the minority 108 (28.3%) were females. This may be as a result of the fact that normally more men go out with friends than women since majority of the respondents said they are usually prompted to consume RTE when they go out with friends (86.9%) and mostly eat their RTE meat in the drinking bars (58.1%). It further indicates that, majority of the respondents (65.4%) had ages between 21-40 years, 22.8% were aged between 41-60 years, with only 9.2% and 2.6% below 21 and above 60 years, respectively. This may be attributed to the fact that most consummers were at a youthful age and single (73.8%) due to youthful exuberance. The study also revealed that 73.8% of the respondents were single with just 24.1% of them being married. In addition, 66.5%, 20.7% and 11.3% of the respondents were Christians, Muslims and traditionalists, respectively. This might be because Christians dominate in Bolga and perhaps go out more or used to outings. The research further showed that majority (54.2%) of the respondents obtained basic education and close to 13% had no education at all. This might have accounted for their high knowledge on meat safety (93.7%) and hygiene practices. Also, apart from 34.3% of them who were Frafra by tribe, Builsas (11.8%), Kassenas (9.4%), Manprusis (4.7%) and Others (39.8%) make up for the tribe distribution of the rest of the respondents. This may be due to fact that many people from different works of

life and tribes are in the capital town. Moreover, the study showed that a greater proportion (69.6%) of the consumers preferred grilled RTE guinea fowl to chevon (11%), pork (8.4%), mutton (5.8%) and beef (5.0%) citing good taste (89.3%) for their choice. However, few others (7.9%) suggested it was healthy as the reason for their preference and majority (57.3%) often consume it once a week and once a month (29.8%) mainly when they go out with friends in the evening (86.9%). This may be attributed to high cost of grilled guinea fowl (GHC 40 on the average).

5.2.2 Knowledge of Consumers on Meat Safety and Contamination

As in Table 18, it was revealed that most (91.8%) of the 382 RTE meat consumers interviewed have heard about meat safety mostly from veterinary officers (80.7%). This may partly be attributed to the fact that many (54.2%) of the consumers had at least basic education. Also, the results showed that 88.9% of them knew that meat can be contaminated with pathogens such as bacteria through poor handling. However, the study indicated that a good number of the consumers (76.6%) did not know that eating, drinking and smoking by vendors while selling RTE meat increases the risk of contamination with only a few (19.0%) who were aware of the risk. This may mean that stakeholders are not adequately carrying out sensitization programs on meat safety. Also, 94.0% of the respondents were aware that regular hand washing and the use of sterilized gloves by vendors reduces the risk of contamination.

5.2.3 Consumers' Responses to Hygienic Practices

From the study conducted, many consumers (94.7%) have the opinion that RTE leftover meat should be refrigerated. On the other hand, others (27.5%) think it should be smoked. This might be because many consumers preferred guinea fowl (69.6%). More than half of the respondents (54.5%) bought their grilled RTE meat by the roadside with 19.9% of them buying from drinking bars mostly displayed on tables with wire mesh covering (44.5%) and in glass sieves (38.5%). However, 15.2% buy their grilled RTE meat normally displayed on open tables. The reason for this may be that buying from the roadside, on open table and on table with net is cheaper than buying from the restaurants and in glass sieves. Also, 69.6% of the respondents do not wash their hands before touching or eating their RTE meat, this may be because many eat RTE meat with tooth-picks and the few (24.8%) who do, wash with only water (73.3%) and preferably eat it in the drinking bar (58.1%), this may me be due to the fact that majority (73.8%) are single and ate it at home. These, 22.3% may be the married consumers who will want to consume it at home with their families. In addition, the study revealed that out of the 69.6% who wash their hands before touching or eating their RTE meat, 26.7% of them wash with only water. This may be due to inadequate hand washing materials at drinking bars and habits of infrequent hand washing since many (58.1%) of the respondents take their meat there.

5.2.4 Readiness of RTE Meat Consumers to Adopt Meat Safety Practices

Meat safety practices among grilled RTE meat consumers sampled from the Upper East regional capital, Bolgatanga are presented in Table 4.8. Most (94.5%) of the consumers were very willing to buy RTE meat in an enclosure and ready to avoid buying from a vendor who was coughing or sneezing (95.0%) and rubbing the hands on the face, nose or hair (96.6%). This may be attributed to their knowledge of meat safety and that meat can be contaminated with germs (91.8%). Also, 89.8% will want raw and grilled RTE meats to be separated with separate equipment for handling each, will want RTE meat vendors to disinfect their shops regularly (96.3%), will want vendors to wear apron, gloves and mouth mask while selling RTE meat (90.8%), but are unwilling to see RTE meat vendors' wearing jewelry including wedding rings and watches while handling meat (46.3%).

5.3 Microbiological Safety of Ready to Eat Meats

5.3.1 Aerobic Bacteria Count of Grilled RTE Meat Samples

Muscle tissues of healthy animals are essentially free of microorganisms and can be contaminated with both pathogenic and non-pathogenic microorganisms at the time of slaughter and post-slaughter conditions, when these are done poorly or under any faulty processing condition (Prescott *et al.*, 2002; Warriss, 2000; Adzitey *et al.*, 2014). Possible source of contamination of grilled RTE meat may be due to improper handling and improper hygiene thereby affecting health of consumers (Koussemon *et al.*, 2008). Cross contamination of the food (meat) products after the heat treatment is possible and may subsequently lead to the growth of pathogens (Buncic *et al.*, 1990).

Microbial quality of grilled RTE meats from Bolgatanga revealed the following mean total plate count (TPC) for beef, chicken, chevon, guinea fowl meat, mutton and pork to be 3.368 $\log_{10}$ cfu/cm^2, is 2.526 $\log_{10}$ cfu/cm^2, 4.852 $\log_{10}$ cfu/cm^2, 4.057 $\log_{10}$ cfu/cm^2, 4.171 $\log_{10}$ cfu/cm^2 and 4.02 $\log_{10}$ cfu/cm^2, respectively. Chevon recorded the highest count of 4.852 $\log_{10}$ cfu/cm^2 whilst the least count of 2.526 $\log_{10}$ cfu/cm^2 was recorded for chicken. There were significant differences ($P<0.001$) among bacterial count of the various meat types as shown in Table 4.9. However, there were no significant differences ($P>0.05$) among grilled guinea fowl meat, mutton and pork. The Ghana Standard Board (GSA) (2019) recommends that bacterial contamination for grilled meat should be < 5log10 cfu/g. This implies that all the grilled RTE meats met the acceptable limit set by the GSA. Adzitey *et al.* (2015), reported that meat sample with microbial load above 10^6 cfu/cm^2 is said to be

unsatisfactory; hence all RTE meat types tested were satisfactory and met the Ghana Standard Board's recommendation. Warriss (2000) reported that when the microbial load is above 10^7 CFU g^{-1} spoilage of meat in eminent. In this research, none of the RTE meats were spoiled since their loads were all less than 10^7 CFU g^{-1}. The results of this study are in line with Adzitey *et al*. (2020) who revealed that microbial load of ready-to-eat (RTE) meats obtained from Bolgatanga ranged from 4.02 to 4.85 log cfu/cm^2.

Compared the study, Ampaw (2018) reported a highest and lowest total viable count (TVC) of 7.267 $\log_{10}$ cfu/g and 4.732 $\log_{10}$ cfu/g, respectively in 'Khebabs' vended on some streets in the Accra Metropolis. This may be due to the good hygienic practices being observed by the vendors contrary to suggestions by Ampaw (2018). Agbodaze *et al*. (2005) and Edema *et al*. (2008) said that 'Khebab' and Suya (grilled meat product) were prepared and sold under largely unhygienic and un-safe conditions, but in accordance with findings by Ansari-Lari *et al*. (2010) that showed a high level of knowledge and general sanitary measures among food handlers. Annan-Prah *et al*. (2011) analyzed 'Khebab' samples sold on the streets of Cape Coast and found bacterial contamination levels of 5×10^4cfu/g, slightly higher than the result of this study. This may be attributed to probably higher level of heat applied during the grilling process in this study.

During sampling and data collection in this research, it was physically observed that the neatness level of vendors was 65.3%. Also, 48.0% of the meat vendors sold their grilled RTE meat on tables with a net covering the meat and 40.3% of the vendors

sold in glass sieves; at variance with findings from Ampaw (2008) where 'Khebab' was found to be displayed openly on tables close to open, dirty gutters. Also, majority 275(91.7%) of the vendors indicated that they wash their chopping tables at the beginning and at the end of work each day in contrast to findings from Agbodaze *et al*. (2005) and Adzitey *et al*. (2015) in a previous study. These may be some of the reasons for the low levels of bacterial load detected in this study. This study however gives an indication that possibly, pathogenic bacteria species such as *Staphylococcus* spp., *Salmonella* spp., *Escherichia coli*, *Streptococcus* spp., *Proteus* spp. and *Bacillus* spp. among others may be present in various RTE meats sold in the Upper East Regional capital. Also, Adio *et al*. (2014) reported a total viable count (TVC) on ranging from 2.8 x 10^6 to 5.465 x 10^6 cfu/g and a total coliform ranging from 0.2 x 10^5 to 6.35 x 10^5cfu/g from ready to eat (RTE) barbecue meat (suya) sold on the streets of Lagos State, Nigeria. Their findings were attributed to inadequate aseptic processing and handling techniques employed by the grilled RTE meat (suya) vendors to reduce microbial loads of the meat, contrary to findings in this study.

Agbodaze *et al*. (2005) carried out a study to determine the microbial load in 'Khebab' (grilled RTE meat) samples bought from Osu, Nima and Accra Central. It was found out that Osu recorded mean total plates count (TPC) of 5.02 $\log_{10}$ cfu/g, Accra Central samples had TPC of 4.08 $\log_{10}$ cfu/g and those from Nima had TPC of 4.80 $\log_{10}$ cfu/g of 'Khebab'. Samples from Accra Central recorded the highest mean coliform count (5.12 $\log_{10}$ cfu/g) whilst samples bought from Osu and Nima recorded 4.41 and 3.70 $\log_{10}$ cfu/g, respectively. This result was largely attributed to poor hygiene and sanitary measures by the 'Khebab' (RTE meat) vendors contrary to

findings of this study. Tavakoli and Riazipour (2008) reported a mean of total bacterial and coliform counts in grilled ground meat samples were 1.14×10^5 cfu/g and 1.98×10^2 cfu/g, respectively lower than findings in this research.

El-Hassan *et al*. (2018) reported that a processed meat product (Tsire) in Kano State, Nigeria contains bacterial load ranging from $1.4x10^4$cfu/ml to $2.95x10^5$cfu/ml, lower than the results of this study. This may be due stricter adherence to safety and hygiene practices than what was observed in this study.

5.3.2. Prevalence of Salmonella Enterica In the Ready-To-Eat (RTE) Meats

According to Akbar and Anal (2013), *Salmonella enterica* is a pathogen known for its ability to cause enteric fever worldwide. The table (Table 22) shows the prevalence of *Salmonella enterica* in the grilled RTE meat samples obtained from Bolgatanga. As indicated in the table, from a total of three hundred (300) samples of meat consisting of fifty (50) RTE meat swabs each of grilled RTE beef, chicken, chevon, guinea fowl, mutton and pork tested, six (6/300) samples representing two percent (2%) were positive for *Salmonella enterica*. On the other hand, grilled RTE meat samples that were negative for *Salmonella enterica* were 98%. Guinea fowl recorded the highest prevalence (2/50) whilst beef recorded no (0/50) prevalence of *Salmonella enterica.* Out of a total of 1,028 RET meat samples evaluated by Tareq *et al*. (2014) for the presence of Salmonella in Mediterranean RTE chicken and beef products sold in Jordanian restaurants, 5 were positive for Salmonella representing 0.5%. Terentjeva *et al*. (2017) also reported a 0% (0/364) for Salmonella in RTE meats. These results show a lower prevalence (0.5%) compared to the findings in this

study and this may be due to the fact that in this study, majority (54.5%) of the RTE meat were sold by the road side (exposed to possible contamination) instead of restaurants in the case of Tareq *et al*. (2014). A study by Akbar and Anal (2014) on 181 RTE poultry meat samples also revealed that the prevalence of Salmonella was 0.55%. The results of this study are also similar to findings of Cabedo *et al*. (2008) who reported 1.5% Salmonella prevalence in RTE frozen chicken croquettes, and from Spain and Khaitsa *et al*., (2007) who reported 1.1% Salmonella contamination in RTE turkey meat products from USA, but contrary to Angkititrakul *et al*. (2005) who reported 75% Salmonella prevalence in retail chicken meat samples in Khon Kaen Provence of Thailand, EL Hassan *et al*. (2018) who recorded 21.7% in processed meat product (Tsire) and Chomvarin *et al*. (2006) who reported that 4.3% of the samples were positive for Salmonella. This may be attributed to good sanitary practices adopted by the grilled RTE meat vendors in Bolgatanga and the processes of preparing the ready to eat meats with the application of heat contributes to the low prevalence of *Salmonella enterica* observed in this study. Monitoring and control of Salmonella in RTE meat products are an important task, as the results of the present and previous studies confirm the presence of different Salmonella species from RTE food products all over the world.

5.4 Antibiotic Susceptibility of Salmonella Enterica

5.4.1 Antibiotic Susceptibility of Salmonella Enterica Isolates from The Samples

According to the European Food Safety Authority (EFSA) (2004), there are many factors that contribute to the increasing antibiotic resistance around the world. They include overprescribing (to treat minor infections), misuse (to treat viral infections),

self-medication (availability of antibiotics without a prescription), improper treatment regimens (overly long use), fake or substandard antibiotics (containing too little or no active antimicrobial agent), poor infection and prevention control practices in healthcare settings, overuse of antibiotics in veterinary practices and factory farming and consuming meat products from animals that were given feed-added antibiotics.

The antibiotic susceptibility patterns of the *Salmonella enterica* isolates is shown in Table 4.12. From a total of six (6) *Salmonella enterica* isolates tested against nine (9) commonly used antibiotics, 44.44% of the isolates were susceptible, 20.37% were intermediate resistant and 35.19% were resistant to the various antibiotics used. Telcoplanin had highest resistance (100%) followed by Azithromycin (83.33%). However, the isolates were 100% susceptible to Sulfamethoxazole/Trimethoprim contrary to findings of Terentjeva *et al*. (2017) who reported 40% resistance to sulfamethoxazole, with chloramphenicol and Gentamycin recording 83.33% susceptibility each for the *Salmonella enterica* isolates. Terentjeva *et al*. (2017) reported that 62% (13/21) of Salmonella isolates from meat and meat products were resistant to at least one antimicrobial agent. Also, 25% (5/20) were resistant to nalidixic acid, ciprofloxacin, ampicillin and 20% (4/20) to tetracycline. All isolates were susceptible to ceftazidime, cefotaxime, meropenem, azithromycin and tigecycline. *Salmonella* Typhimurium exhibited antimicrobial resistance more often (87.5%) than other serovars. The results of this study (35.19% resistance, 20.37% intermediate and 44.44% susceptible) are almost in line with findings from Adzitey *et al*. (2019) study which reported an overall resistance of 35.50% (144/405), 7.90%

(32/405) intermediate resistant and 56.54% (229/405) antimicrobial susceptibility for 45 Salmonella species isolated from RTE meat samples. All Salmonella species (100%) examined were resistant to vancomycin but susceptible to ciprofloxacin. A large percentage of the Salmonella species were also resistant to erythromycin (75.56%) and susceptible to gentamicin (86.67%), ceftriaxone (73.33%), suphamethoxazole/trimethoprim (68.89%), chloramphenicol (62.22%), tetracycline (57.78%) and amoxycillin/clavulanic acid (57.78%). Intermediate resistances were observed for all the antibiotics except vaconmycin and ciprofloxacin. Intermediate resistance refers to those Salmonella species that were not clearly resistant or susceptible. It has been suggested in clinical diagnoses that patients with intermediate results can be given a higher dosage of antibiotics (Lorian, 2005). Organisms that exhibit intermediate resistance also have the tendency to easily become resistant (Adzitey *et al*., 2012).

5.4.2 Antibiotic Resistance Profile and Multiple Antibiotic Resistance Index of Salmonella Enterica Isolates

According to the World Health Organization (WHO), antibiotic resistance has reached “dangerously high” levels on a global scale (Anonymous, 2019). The increasing prevalence of antimicrobial1 resistance (AMR) threatens the success and continuation of clinical medicine (Lodato and Kaplan, 2013). This threat decreases the ability to successfully treat numerous infectious diseases, at the same time increases health risks for immune-compromised patients (Lodato and Kaplan, 2013). The increased public health threats caused the World Health Organization (WHO) to

declare AMR as one of the three greatest threats to human health (WHO, 2012). Table 5 shows the antibiotic resistance profile and multiple antibiotic resistance index of individual *Salmonella enterica* isolates. Out of the six (6) *Salmonella enterica* isolated, two (2) were resistant to two (2) and four (4) antibiotics (33.33%), respectively and one (1) was resistant to three (3) and five (5) antibiotics (16.67%), respectively.

From the table, *Salmonella enterica* isolated from grilled RTE mutton had the highest resistance profile (AmcAzmTecTeC) with resistance to five (5) different antibiotics whereas isolates from grilled RTE pork and guinea fowl have the lowest resistance profile (AzmTec) and (AmcTec) respectively, being resistance to two (2) antibiotics each.

Table 25 shows the antibiotic resistance profile and multiple antibiotic resistance (MAR) index of individual *Salmonella enterica* isolated from different RTE meats. The *Salmonella enterica* isolates exhibited six (6) different antibiotic resistant patterns with MAR index ranging from 0.22 to 0.56. The majority of the isolates were resistant to two (2) and four (4) antibiotics (4 isolates; MAR index of 0.22 and 0.44), respectively. Also, two isolates were resistant to three (3) and five (5) antibiotics (2 isolates; MAR index 0.33 and 0.56), respectively. All the *Salmonella enterica* isolates exhibited different antibiotic resistant patterns. The two isolates that were resistant to four antibiotics exhibited AzmTecCnTe (1 isolate) and AmcAzmTecTe (1 isolate) antibiotic resistant patterns, respectively. Also the two isolates that were resistant to two antibiotics exhibited the resistant patterns AzmTec

(1 isolate) and AmcTec (1 isolate), respectively. Lastly, the one isolate each that was resistant to three and five antibiotics had resistant patterns AmcAzmTec and AmcAzmTecTeC, respectively. The results of this are in line with earlier findings by Adoh *et al*. (2017) which revealed that fifty-eight (42.3%) of all *Salmonella enterica* isolated from humans in Ghana showed multiple antimicrobial resistance (MAR).

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

1. Ready-to-eat meats in the Bolgatanga Municipality were contaminated with various microbes ranging from 2.526 $\log_{10}$ cfu/cm^2 to 4.852 $\log_{10}$ cfu/cm^2, but were within acceptable limit.
2. *Salmonella enterica* were found in some of the ready-to-eat (RTE) meat (2%) samples.
3. All the *Salmonella enterica* isolates exhibited multidrug resistance.
4. Good personal and environmental hygiene were responsible for the reduced cross contamination of the RTE meats.

6.2 Recommendations

1. Further research should investigate the presence of resistance genes and genetic characterization of the *Salmonella enterica* isolates.
2. Consumers of meat should try their best to know the sources (health status) of meat they buy.
3. Consumers of RTE meats in Bolgatanga should take the necessary precautions prior to consumption.

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APPENDICES

APPENDIX.1A: Questionnaire for ready-to-eat (RTE) meat vendors

University for Development Studies; Faculty of Agriculture

Animal Science Department

This study seeks to evaluate the knowledge, attitude and practices of grilled ready-to-eat (RTE) meat consumers on microbiological safety of RTE meats sold on the streets of Bolgatanga, the Upper East Regional capital.

Please, information given will be treated with high level of confidentiality and used only for academic purposes.

Tick where appropriate or state accordingly [√].

PART I: PERSONAL PROFILE

1. Gender: Male [ ] Female [ ]
2. Age (years): a. below 21 [ ] b. 21- 40 [ ] c. 41 - 60 [ ] d. above 60 [ ]
3. Nationality: a. Ghanaian b. Burkinabe c. Malian d.Niger e. Others (specify)…
4. Marital status: a. Married [ ] b. Single [ ] c. Divorced [ ] d. Others [ ]
5. Religion: a. Christianity [ ] b. Islamic [ ] c. Traditional [ ] d. Others [ ]
6. Educational background: a. None [ ] b.Basic [ ] c. secondary [ ] d.Tertiary [ ] e. Others (specify)…….

PART II: OCCUPATIONAL PROFILE

6. How long have you been selling RTE meat?

a. Less than 1 year [ ] b. 1 -5 years [ ] c. 6-10 years [ ] d. above 10 years [ ]

7. Which of the following do you sell?

a. Pork [ ] b. Mutton [ ] c. Guinea fowl [ ] d. Chevon [ ] e. Chicken f. Beef g. Others [ ]

8. Why do you prefer selling the selected meat type?

a. Cheaper [ ] b. Consumer preference [ ] c. Religion [ ] d. Others (specify)….

9. Do you sell meat as a full or part time activity?

a. Fulltime [ ] b. Part-time [ ]

If part time, what work do you do?

10. How many meat shops do you have?

a. One [ ] b. Two [ ] c. More than three [ ]

PART III: KNOWLEDGE OF MEAT VENDORS ON MEAT SAFETY

11. Have you ever heard of meat safety?

a.Yes [ ] b. No [ ] If yes, where……..

12. Do you know meat can be contaminated with bacteria/germs by poor handling?

a. Yes [ ] b. No [ ]

13. Do you also know that contaminated meat can cause meat borne diseases?

a. Yes [ ] b. No [ ]

If yes, give some examples of meat borne diseases you know…….

14. Have you undergone any training course regarding safe meat handling?

a. Yes [ ] b. No [ ] If yes, where (specify): ……..

15. Are you affiliated to or a member of any meat sellers association?

a. Yes [ ] b. No [ ] If yes, please state the association: ……

16. Are you aware that eating and drinking while selling meat increases the risk of meat contamination?

a. Yes [ ] b. No [ ]

17. Do you know that washing hands before and at regular intervals during the working day, especially after visiting the lavatory (toilet) reduces the risk of meat contamination?

a. Yes [ ] b. No [ ]

18. Are you aware that using sterilized gloves during work reduces the risk of meat contamination?

a. Yes [ ] b. No [ ]

19. Do you know it is necessary to take leave from work when infected by any skin disease?

a. Yes [ ] b. No [ ]

20. How do you preserve leftover RTE meat?

a. Refrigeration [ ] b. Salting [ ] c. Smoking d. Frying e. Others (specify)...

21. Why do you choose this method ? a. Easily available b. Cheap c. Very effective

PART IV: MEAT SAFETY PRACTICES AMONG MEAT VENDORS

22. Where do you get your meat from?

a. Backyard slaughter [ ] b. Abattoir [ ] c. Imported carcass [ ] d. Others (specify):…….

23. Briefly explain why you choose such sources?

a. Safe and quality b. Readily available c. Cheap d. Others (specify):……

24. Do you sell meat on/in?

a. An open table [ ] b. Table with a net covering the meat [ ] c. Glass sieve d. Others (specify):……..…

25. How often do you wash your chopping/cutting tables?

a. At the beginning of work [ ] b. At the end of work [ ] c. a and b

c. Once a week [ ] d. Others (specify):……

26. Do you disinfect your meat shop?

a. Yes [ ] b. No [ ]

27. How often do you disinfect the shop?

a. Once a week [ ] b. Twice a week [ ] c. Once a month [ ] d. Others (specify)……

28. What disinfectant do you use?

a. Isopropyl alcohol, **[ ]** b**.** iodine **[ ]** c. hydrogen peroxide d. Pine-Sol e. Others

29. Do you wash your equipment used for selling meat?

a. Yes [ ] b. No [ ]

30. What do you use to rinse your equipment after washing?

a. Water [ ] b. Warm water [ ] c. Alcohol d. Others (specify)……..

31. How often do you wash your hands before touching the RTE meat?

a. Always [ ] b. Sometimes [ ] c. Rarely [ ] d. Never [ ]

32. What do you use to wash your equipment?

a. Only water [ ] b. Detergent and water [ ] c. Others (specify) ……

33. Do you ever sterilize your knives and other equipment?

a. Yes [ ] b. No [ ] If yes, how often …….

34. Do you wear an apron during work?

a. Yes [ ] b. No [ ]

35. How often do you wash it, if yes?

a. Every day b. Twice a week c. Once a week d. Others (specify)……

36. Do you wear gloves during work?

a. Always [ ] b) Sometimes [ ] c) Rarely [ ] d) Never [ ]

37. How often do you wash the gloves?

a. Every day b. Twice a week c. Once a week d. Others (specify)……

38. Do you smoke?

a. Yes [ ] b. No [ ] If yes, where do usually do it?……

39. On a scale of 1 to 4, score meat venders on neatness of clothes.

1= very dirty [ ] 2= dirty [ ] 3= clean [ ] 4= very clean [ ]

(Interviewer should observe and provide an appropriate answer).

Table 1: Key for the assessment

Score	Description
Score of 1 or very dirty	Fresh and old meat particles/blood splashes found all over the front of the sellers' clothes.
Score of 2 or dirty	Half of the clothes covered with either fresh or old meat particles/blood splashes.
Score of 3 or clean	Less than quarter of the clothes is covered with meat, only fresh meat particles/blood splashes.
Score of 4 or very clean	When meat particles/blood splashes were not found on the sellers' clothes.

PART V: ATTITUDE OF RTE MEAT VENDORS TOWARDS MEAT SAFETY

On a scale of 1 to 3, state your readiness towards ensuring meat safety.

Use: 1= Agree

2= Uncertain

3= Disagree

40. Sell RTE meat in a neat enclosure.

41. Work area must be cleaned before start of work.

42. Wash my tables, knives and other equipment before start of work.

43. Hands should be washed before selling RTE meat.

44. Selling RTE meat with dirty hands should be avoided.

45. RTE meat should not be touched with wounded hand.

46. We should not rub our hands-on face, hair, etc. while selling.

47. Jewelry (including wedding ring) and a watch can be worn while handling RTE meat.

48. Raw meat and RTE meat should not necessarily be separated.

49. Separate equipment must be used to handle raw meat and RTE meat.

50. We must cover our mouth and nose when coughing or sneezing.

51. Disinfecting my meat shop regularly.

52. Wear clean apron when selling.

53. Defrosted RTE meat should not be refrozen.

54. Clean apron can be used as a towel to clean hand.

55. The same towel can be used to clean many places in the meat shop.

56. Like to use clean gloves.

57. We should not smoke while selling RTE meat.

58. Be trained on meat safety issues.

59. Always refrigerate leftover RTE meat.

60. Adhere to food safety rules and regulations.

Appendix IB: Questionnaire for grilled ready-to-eat (RTE) meat consumers

University for Development Studies; Faculty of Agriculture

Department of Animal Science

This study seeks to evaluate the knowledge, attitude and practices of grilled ready-to-eat (RTE) meat consumers on microbiological safety of RTE meats sold on the streets of Bolgatanga, the Upper East Regional capital.

Please, information given will be treated with high level of confidentiality and used for academic purposes only.

Tick where appropriate or state accordingly [√].

PART I: PERSONAL PROFILE

1. Gender: Male [ ] Female [ ]
2. Age (years): a. below 21 [ ] b. 21- 40 [ ] c. 41 - 60 [ ] d. above 60 [ ]
3. Marital status: a. Married [ ] b. Single [ ] c. Divorced [ ] d. In a relationship [ ] e. Others [ ]
4. Religion: a. Christianity [ ] b. Islamic [ ] c. Traditional [ ] d. Others [ ]
5. Educational background: a. None [ ] b.Basic [ ] c. secondary [ ] d.Tertiary [ ] e. Others (specify)…….

6. Tribe: a. Frafra [ ] b. Bulisa [ ] c Kasena d. Manprusi e. Others (specify)……

PART II: PATTERN OF MEAT CONSUMPTION

7. Do you consume grilled meat? Yes [ ] No [ ]

8. If yes, which of the following grilled RTE meat do you prefer? a. Pork [ ] b. Mutton [ ] c. Guinea fowl [ ] d. Chevon [ ] e. Beef [ ]

9. What is your reason for your preference in 8 above? a. readily available [ ] b. has good taste [ ] c. it is healthy [ ] d. it is cheap [ ] e. It is safe [ ] f. (specify)……

10. How often do you consume grilled RTE meat products? a) Daily [ ] b. Once a month [ ] c. 2-3 times a week [ ] d. Once a week [ ] e. Others (specify)……..

11. What prompts you to consume RTE Meat? a. my mouth sweet me [ ] b. when I go out with friend(s) in the evening[ ] c. for home consumption d. Others (specify)……

PART III: KNOWLEDGE OF MEAT CONSUMERS ON MEAT SAFETY

12. Have you ever heard of meat safety?

a. No [ ] b. Yes [ ]

13. If yes, by who or what means? a. Health officer [ ] b. Media [ ] c. Veterinary officer [ ] d. Teacher [ ] e. Others (specify)……

14. Do you know meat can be contaminated with bacteria/germs by poor handling during grilling and can cause meat-borne diseases? a.Yes[ ] b. No [ ]

If yes give examples of some of the meat-borne diseases…….

15. Are you aware that eating, drinking and smoking by vendors while selling grilled RTE meat to you increases the risk of its contamination?

a. Yes [ ] b. No [ ]

16. Do you know that when the vendor washes hands before and at regular intervals during the working day, especially after visiting the lavatory (toilet) reduces the risk of meat contamination?

a. Yes [ ] b. No [ ]

17. In your opinion how should left-over grilled RTE meat be preserved?

a. Refrigeration [ ] b. Salting [ ] c. Smoking d. Frying e. Others (specify)..

Why did you choose this method?.....

PART IV: MEAT SAFETY PRACTICES AMONG RTE MEAT CONSUMERS

18. Where do you buy grilled RTE meat from?

a. Market [ ] b. Road side [ ] c. Restaurant [ ] d. Drinking bar [ ]

e.Others (specify):……

19. How is the grilled RTE meat (in 18 above) normally displayed?

a. On an open table [ ] b. Table with a net covering the meat [ ] c. Glass sieve d. Others (specify):……..…

20. Do you wash your hands before touching or eating RTE meat ?

a. Yes [ ] b. No [ ]

21. If yes what do you use a. only water, b. soap and water, c. only warm water d. soap and warm water e.Others (specify):……

22. Where do you eat your RTE meat? a. On the street [ ] b. At home [ ]

c. In a drinking bar [ ] d. On the venders table [ ]

PART V: ATTITUDE OF RTE MEAT CONSUMERS TOWARDS MEAT SAFETY

On a scale of 1 to 3, state your readiness to ensure that you consume meat that is microbiologically safe.

Use: 1= Agree

2= Uncertain

3= Disagree

23. Buy grilled RTE meat displayed in a neat enclosure where the work area and the equipment are cleaned before and the end of work respectively.

24. I will not buy RTE meat from a vendor who is coughing and sneezing

25. Vendors should not rub their hands on face, nose, hair, etc. while selling.

RTE meat

26. Raw meat and grilled RTE meat should be separated with separate equipment for handling them.

27. Vendors must disinfect their meat shops regularly.

28. Vendors wear apron, gloves and mouth mask while selling meat

29. Jewelry (including wedding ring) and a watch can be worn while handling RTE meat.

Appendix II Analysis of Salmonella Using SPSS Version 18 **GET**

Generalized Linear Models

Case Processing Summary		
	N	Percent
Included	300	100.0%
Excluded	0	0.0%
Total	300	100.0%

Categorical Variable Information				
			N	Percent
Dependent Variable	Salmonella spp.	0	294	98.0%
		1	6	2.0%
		Total	300	100.0%
Factor	Meat type	Beef	50	16.7%
		Chevon	50	16.7%
		Chicken	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)	.000	1	.997
Meattype	.657	5	.985

Dependent Variable: Salmonella spp.

Model: (Intercept), Meattype

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test
			Lower	Upper	Wald Chi-Square
(Intercept)	3.892	1.0102	1.912	5.872	14.843
[Meattype=Beef]	18.674	11237.6229	-22006.662	22044.010	.000
[Meattype=Chevon]	-2.551E-015	1.4286	-2.800	2.800	.000
[Meattype=Chicken]	-2.252E-015	1.4286	-2.800	2.800	.000

[Meattype=Guinea Fowl meat]	-.714	1.2415	-3.147	1.719	.331
[Meattype=Mutton]	-2.824E-015	1.4286	-2.800	2.800	.000
[Meattype=Pork]	0[a]	.	.	.	.
(Scale)	1[b]				

Estimated Marginal Means: Meat type

Estimates				
Meat type	Mean	Std. Error	95% Wald Confidence Interval	
			Lower	Upper
Beef	1.00	.000	.00	1.00
Chevon	.98	.020	.87	1.00
Chicken	.98	.020	.87	1.00
Guinea Fowl meat	.96	.028	.85	.99
Mutton	.98	.020	.87	1.00
Pork	.98	.020	.87	1.00

Pairwise Comparisons

(I) Meat type	(J) Meat type	Mean Difference (I-J)	Std. Error	df	Sig.
Beef	Chevon	.02	.020	1	.312
	Chicken	.02	.020	1	.312
	Guinea Fowl meat	.04	.028	1	.149
	Mutton	.02	.020	1	.312
	Pork	.02	.020	1	.312
Chevon	Beef	-.02	.020	1	.312
	Chicken	.00	.028	1	1.000
	Guinea Fowl meat	.02	.034	1	.557
	Mutton	.00	.028	1	1.000
	Pork	.00	.028	1	1.000
Chicken	Beef	-.02	.020	1	.312
	Chevon	.00	.028	1	1.000
	Guinea Fowl meat	.02	.034	1	.557

	Mutton	.00	.028	1	1.000
	Pork	.00	.028	1	1.000
Guinea Fowl meat	Beef	-.04	.028	1	.149
	Chevon	-.02	.034	1	.557
	Chicken	-.02	.034	1	.557
	Mutton	-.02	.034	1	.557
	Pork	-.02	.034	1	.557
Mutton	Beef	-.02	.020	1	.312
	Chevon	.00	.028	1	1.000
	Chicken	.00	.028	1	1.000
	Guinea Fowl meat	.02	.034	1	.557
	Pork	.00	.028	1	1.000
Pork	Beef	-.02	.020	1	.312
	Chevon	.00	.028	1	1.000
	Chicken	.00	.028	1	1.000
	Guinea Fowl meat	.02	.034	1	.557

	Mutton	.00	.028	1	1.000

Pairwise Comparisons

(I) Meat type	(J) Meat type	95% Wald Confidence Interval for Difference	
		Lower	Upper
Beef	Chevon	-.02	.06
	Chicken	-.02	.06
	Guinea Fowl meat	-.01	.09
	Mutton	-.02	.06
	Pork	-.02	.06
Chevon	Beef	-.06	.02
	Chicken	-.05	.05
	Guinea Fowl meat	-.05	.09
	Mutton	-.05	.05
	Pork	-.05	.05
Chicken	Beef	-.06	.02

	Chevon	-.05	.05
	Guinea Fowl meat	-.05	.09
	Mutton	-.05	.05
	Pork	-.05	.05
Guinea Fowl meat	Beef	-.09	.01
	Chevon	-.09	.05
	Chicken	-.09	.05
	Mutton	-.09	.05
	Pork	-.09	.05
Mutton	Beef	-.06	.02
	Chevon	-.05	.05
	Chicken	-.05	.05
	Guinea Fowl meat	-.05	.09
	Pork	-.05	.05
Pork	Beef	-.06	.02
	Chevon	-.05	.05

	Chicken	-.05	.05
	Guinea Fowl meat	-.05	.09
	Mutton	-.05	.05

Pairwise comparisons of estimated marginal means based on the original scale of dependent variable Salmonella spp.

Overall Test Results

Wald Chi-Square	Df	Sig.
6.165	5	.290

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

(I) Meat type	(J) Meat type	Std. Error	df	Sig.	
Beef	Chevon	.020	1	.312	No
	Chicken	.020	1	.312	No
	Guinea Fowl meat	.028	1	.149	No
	Mutton	.020	1	.312	No
	Pork	.020	1	.312	No
Chevon	Beef	.020	1	.312	No
	Chicken	.028	1	1.000	No
	Guinea Fowl meat	.034	1	.557	No
	Mutton	.028	1	1.000	No
	Pork	.028	1	1.000	No
Chicken	Beef	.020	1	.312	No
	Chevon	.028	1	1.000	No
	Guinea Fowl meat	.034	1	.557	No
	Mutton	.028	1	1.000	No

	Pork	.028	1	1.000	No
Guinea Fowl meat	Beef	.028	1	.149	No
	Chevon	.034	1	.557	No
	Chicken	.034	1	.557	No
	Mutton	.034	1	.557	No
	Pork	.034	1	.557	No
Mutton	Beef	.020	1	.312	No
	Chevon	.028	1	1.000	No
	Chicken	.028	1	1.000	No
	Guinea Fowl meat	.034	1	.557	No
	Pork	.028	1	1.000	No
Pork	Beef	.020	1	.312	No
	Chevon	.028	1	1.000	No
	Chicken	.028	1	1.000	No
	Guinea Fowl meat	.034	1	.557	No
	Mutton	.028	1	1.000	No

Appendix IV: Analysis of Total Aerobic Count Using GenStat Version 12.1

GenStat Release 12.1 (PC/Windows Vista) 11 February 2020 23:59:39

Registered to: The NULL Corporation

GenStat Twelfth Edition
GenStat Procedure Library Release PL20.1

Data imported from Excel file: C:\Users\EKLI\Desktop\ADUAH MARTIN\DATA ENTERIES.xlsx
on: 12-Feb-2020 0:01:06
taken from sheet ""Sheet1"", cells A2:B37

Identifier	Values	Missing	Levels
Sources	36	0	6

Identifier	Minimum	Mean	Maximum	Values	Missing
log_Cfu_cm2	2.130	3.834	5.727	36	0

Analysis of variance

Variate: log_Cfu_cm2

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sources	5	22.4916	4.4983	16.64	<.001
Residual	30	8.1106	0.2704		
Total	35	30.6022			

Message: the following units have large residuals

units 1	-1.033	s.e.	0.475
units 17	-1.183	s.e.	0.475
units 18	-1.183	s.e.	0.475

Tables of means

Variate: log_Cfu_cm2

Grand mean 3.834

Sources	Beef	Chevon	Chicken	Guinea fowl meat
	3.368	5.135	2.526	3.909

Sources	Mutton	Pork
	4.183	3.885

Standard errors of differences of means

Table	Sources
rep.	6
d.f.	30
s.e.d.	0.3002