KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY,

KUMASI, GHANA

Prevalence of *Listeria monocytogenes* in 'Khebab', a Street-Vended Spicy Grilled Meat

By

Barnabas Abane Ampaw (BSc Laboratory Technology)

A Thesis Submitted to the Department of Food Science and Technology,

Faculty of Bioscience,

College of Science,

In partial Fulfillment of the Requirements for the award of Master of Science Degree in

FOOD QUALITY MANAGEMENT

JUNE, 2018

DECLARATION

This research report contains no material, previously published by another person, nor submitted to any other university or institution for the award of degree except where due acknowledgement has been made in text. References from the work of others have been clearly acknowledged.

Barnabas Abane Ampaw ……. ……

Student (PG4368015) signature date

Certified by:

Dr. F.C. Mills-Robertson …….. ……...

Supervisor signature date

Certified by:

F. Wireko-Manu …… ……..

Head of Department signature date

DEDICATION

This dissertation is dedicated to my mother Madam Veronica Leticia Abane, for her abundant prayers, patience and moral support. I am very grateful for her encouragements and advices.

ACKNOWLEDGEMENTS

A special appreciation goes to Dr. F.C Mills-Robertson of the Department of Biochemistry and Biotechnology, Kwame Nkrumah University of Science and Technology who has urged me on through several academic discussions. Thank you for the many hours we spent discussing relevant things that needed to be done as far as this research is concerned even on mobile phones at night.

Mr. Cornelius Takyi Arthur (Head of Animal Health and Food Safety Division, Council for Scientific and Industrial Research), I say thank you for your assistance at the Microbiology laboratory, in practical, theoretical support and encouragement throughout this research.

Thank you Mrs. Frances Nathan Mensah, laboratory technician of the Microbiology Department (Animal Research Institute) for your assistance in the microbiological analysis. I also thank Mr Enock Arthur, for his assistance in media purchasing, sample collection and laboratory analysis. Also to Mrs. Rachel Marfo, for her assistance through editing, Malik Ganyo, Eugene Owusu-Osafo, Richard Owusu Boateng and Alex Asiedu, I say thank you for your encouragement and moral support. Mrs. Margaret Mante Abossey, thank you for your encouragement and support.

I also wish to thank the lecturers of the MSc. Food Quality Management programme, especially, Dr. F.C. Mills-Robertson.

I acknowledge all my colleagues at the Food Quality Management programme, Accra campus for their lasting support through the whole study. Finally, I thank the Management of Council for Scientific and Industrial Research/Animal Research Institute for giving me the opportunity to undertake my research at their Microbiology Laboratory.

ABSTRACT

Meat and poultry products are reported to be the most frequently implicated vehicles of transmission of *Listeria monocytogenes*, a Gram-positive bacterium. *Listeria monocytogenes* is a foodborne pathogen causing diseases from severe blood and/or central nervous system infections to mild gastroenteritis. This study investigated the prevalence of *Listeria monocytogenes* in 'Khebab', a Street-Vended Spicy Grilled Meat in the Accra Metropolis. A survey was carried out using a structured questionnaire that had both observational and responsive questions for both the processor and the consumer. Microbiological laboratory analysis was employed using pour plate method, streak method and biochemical tests to isolate and identify contaminants in the 'khebab' samples. A total of 120 samples comprising of 60 skewed raw meats and 60 grilled skewed meats were analyzed. Results showed that, out of the 400 respondents who were Consumers, 196 (49%) were females while 204 (51%) were males. About half (49.75%) of the Respondents were in the age group ranging from 20-39 years. Those that were less than 20 years were 21.5% while only 12.5% were in the age range of fifty years and above ((≥50. About 361 (90.25%) of the respondents indicated that they consume 'Khebabs" while only 39 (9.75%) indicated that they do not consume "khebabs". A total of 20 processors who were all males and between the ages of 30-49 were recruited for this study. Out of the total number (20) 70% were in the age range of 30-39 while 30% were between 40 and 49 years. Six (6) had had Senior High School (SHS) education, seven (7) with Junior High School education (JSS), three (3) with primary school education while four (4) were illiterates. Majority (55%) of the processors bought their raw meat from the open market while 45% bought their raw meat from slaughter houses.

The calculated Cohen's Kappa co-efficient (*k*) was 0.02 implying a fair agreement in terms of *Listeria monocytogenes* contamination. Out of a total of 120 samples of meat consisting of 60 raw meats and 60 grilled meats ('khebabs') tested, 92 samples (76.67%) were positive for *Listeria monocytogenes* with 45 samples (48.91%) being raw meat and 47 samples (51.09%) being grilled meat. James Town vending area recorded the highest count of 5.046 ± 0.977 $\log_{10}$cfu/g whilst the least count of 2.239 ± 0.337log10 cfu/g was recorded in the Labone vending area for the raw meat. After the meat had been grilled, Banana Inn vending area recorded microbial count of 5.929 ± 1.064 $\log_{10}$cfu/g whilst the least count of 2.739 ± 0.370 $\log_{10}$cfu/g was recorded in the Tabora vending area. Madina had the highest load of TVC (7.267 $\log_{10}$ cfu/g) whilst Sowutuom vending area had the least TVC of 4.732 $\log_{10}$ cfu/g. Total coliform count (TCC) was the highest in the Banana Inn vending area (6.394 $\log_{10}$ cfu/g) whilst the lowest count of 0.00 $\log_{10}$ cfu/g was in the Dome, Legon, North Kaneshie, and Tabora vending areas. For the *E. coli* (EC) contamination, the highest level of 7.009 $\log_{10}$cfu/g was found in the Dansoman vending area whilst the lowest level of 0.00 $\log_{10}$ cfu/g was found in the Dome, Sowutuom, and Tabora vending areas. Thus, there should be education on food safety issues in order to protect the immuno-compromised individuals such as diabetics, AIDS patients, those with renal failure, organ transplant patients, cancer patients and pregnant women who are prone to *Listeria monocytogenes* and other pathogens. *Listeria monocytogenes* limit for all ready-to-eat food should be less than 100 colony-forming units per 25g or 25ml portion of the food according to WHO microbiological guidelines for ready-to-eat food. The microbial loads are therefore not below the WHO threshold value.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER ONE

1.0. INTRODUCTION

1.1. BACKGROUND INFORMATION

Foods that are prepared and/or sold by vendors in streets and other public places for immediate consumption or later time consumption without processing or preparation further are called street vended foods. Many study reports have indicated the proliferation of street-vended foods probably due to business being generally profitable and requiring low capitalization (Cho *et al*., 2011; WHO-INFOSAN, 2010). Foods implicated in the transmission of foodborne diseases are street vended foods (Chomvarin *et al*., 1993; Gillespie *et al*., 2000; Fang *et al*., 2003; Mahale *et al*., 2008). 'Khebab', a spicy grilled animal protein, is a street vended ready-to-eat meat easily accessible to most Ghanaians. The meat is from either a goat, cow, sheep among others. 'Khebab' is, however, a type of potentially hazardous food (PHF) that may provide suitable medium for the rapid growth of microorganisms. Such a food can affect the health of consumers when they are contaminated by microbial pathogens or their toxins and may even result in death (Agbodaze *et al*., 2005). Food pathogens levels do change during processing, storage and meal preparation thereby making it difficult to determine the number of microorganisms or the concentration of the toxins from the microorganisms at the time of ingestion by the consumer (Devlieghere *et al*., 2004). Some of the pathogens associated with meat are *Escherichia coli*, *Salmonella* spp., *Clostridium* spp, and *Listeria monocytogenes* among many others. According to Roberts (1994), infective dose of *Listeria monocytogenes* seems to be more than 100 viable cells, depending on the strain of the pathogen and the host's susceptibility. Listeriosis is observed when *L. monocytogenes* is detected in blood, cerebrospinal

fluid, the placenta or foetus, and other such normally sterile areas in the body (Ramaswamy *et al*., 2007).

Quantitative microbial risk assessment (QMRA), shows the microbial risk involved with the consumption of specific food that are contaminated and forecasts the level of illness that a pathogen can cause in a given affected population (Forsythe, 2002). Controlling the risk may, thus, aid the safety of 'khebabs' patronized by consumers. The safety of the meat can also be possible when the risk analysis is employed so that, the microbial hazards with respect to the contaminated meat can be controlled by the processors. Microbiologically safe foods could be provided to consumers when risk analysis is employed to control microbial hazards in foods by both regulatory authorities and food processors (Duffy *et al*., 2006).

1.2. PROBLEM STATEMENT

The transmission of many foodborne diseases implicates street foods (Chomvarin *et al*., 1993; Gillespie *et al*., 2003; Mahale *et al*., 2008). Poultry products as well as meat mostly and frequently serve as a conduit for transmission of *Listeria monocytogenes* that causes human listeriosis. Ready-to-eat foods usually implicated in outbreaks of invasive listeriosis include smoked meat such as 'khebabs', smoked fish, and multi-ingredient prepared foods such as sushi, chicken and salad among many others. *Listeria monocytogenes* is able to grow or survive at refrigeration temperatures as well as relatively low pH, high salt and low water activity conditions in so many foods (Luchansky *et al*., 2017). According to Appiah and Dogbe (2010), some of the foods that *Listeria monocytogenes* usually affect are meat and fish among others. This study evaluated the contamination by *Listeria monocytogenes* of processed ready to eat meat (khebabs) in some selected areas in Accra Metropolis.

1.3. JUSTIFICATION

Ready-to-eat foods usually implicated in outbreaks of invasive listeriosis include smoked meat such as 'khebabs', smoked fish, and multi-ingredient prepared foods such as sushi, chicken and salad among many others (Chomvarin *et al*., 1993; Gillespie *et al*., 200; Fang *et al*., 2008). It is hoped that information from this study may reveal consumer knowledge on the quality of 'khebabs' consumed in selected areas in the Accra Metropolis. Data may reveal the demographic characteristics and the insight of hygienic practices by processors of 'khebabs' in the areas selected. It is also expected that the level of contamination of 'khebabs' sold in the various areas in the Accra Metropolis would be revealed. This may help in the after-study interventions and education by regulators and researchers as well as policy makers on the best practices for safer foods advocated by the World Health Organization (http://www.who.int/foodsafety/publications/5keysmanual/en/).

1.4. STUDY OBJECTIVES

1.4.1. Main Objective

The study is to evaluate the prevalence of *Listeria monocytogenes* in 'Khebabs', a Streets-Vended Spicy Grilled Meat.

1.4.2. Specific objectives

1. To assess the knowledge of consumers on the quality of 'Khebabs' processed in the selected areas in the Accra Metropolis
2. To assess the demographic characteristics and general knowledge of processors of 'Khebabs' in the selected Accra Metropolis
3. To determine the load of *Listeria monocytogenes* in raw skewed meat and grilled skewed meat in twenty selected areas in the Accra Metropolis

CHAPTER TWO

2.0. LITERATURE REVIEW

2.1. REVIEW OF MICROBIOLOGICAL FOOD SAFETY

Food serves as a source of energy and nutrients required by human for survival, however, it also serves as a conduit for transportation and growth of pathogens. These pathogens are responsible for foodborne illness in humans and animals. Correctly associated foodborne illness by people with flu-like gastrointestinal symptoms such as diarrhea and vomiting are just a few of the symptoms caused by foodborne pathogens. Economic losses and death which still affect all countries worldwide are associated with foodborne diseases (Thurston, 2006).

According to Iyer *et al*. (2013), in the developing countries foodborne pathogens are known to be the most cause of illness and death, 1.8 million people approximately are annually killed. Deeper tissues invasions by some pathogens or production of toxins which are absorbed consequently causes systemic symptoms, such as kidney failure, fever, anaemia, headache and death. The Center for Disease Control and Prevention (CDC), forecasted 76 million cases of foodborne illness each year, with 325,000 hospitalizations resulting in 5,000 deaths. Some foods are dangerous for some people due to their likelihood of containing a particular pathogen. For instance, *Listeria monocytogenes* infecting a pregnant woman affects the developing foetus also and may kill it even though *Listeria monocytogenes* doesn't often cause illness in the general population.

Food safety which describes preparation, storage and handling of food in ways that prevent foodborne illness must keep on being one of the highest needs of the sustenance of the food industry worldwide. Microbiological safety of food can be

attained if both the manipulators (WHO, 2002) and the foods are continually monitored (Gilling *et al*., 2001).

2.2. 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 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 intrinsic 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 *Escherichia coli* 0157:H7, *Listeria monocytogens, Camphylobacter jejuni, Clostridium perfringens, Salmonella* spp., and *Staphylococcus aureus* (Clarence *et al*., 2009).

The use of contaminated food ingredients and equipment in domestic kitchen may also serve as major sources of high number of foodborne pathogens (Medeiros *et al*., 2001; Beumer and Kusumaningrum, 2003; Redmond and Grifith, 2003)

2.3. THE MAGNITUDE OF FOODBORNE ILLNESSES

Broad degree of foodborne illness incidents are caused by foods that are improperly prepared or mishandled at homes, eatery or markets. High cases of foodborne illness

are reported worldwide with their corresponding death. Table 2.1 shows the percentage death of the corresponding foodborne illness cases.

Table 2.1: Percentage death to Foodborne Illnesses

Pathogens	Cases	Illness	Death	% Death
Listeria monocytogens	**2,493**	**2,298**	**499**	**21.71**
E. coli non- O157-STEC	31,229	921	26	2.82
E. coli- O157:H7	62,458	1,843	52	2.82
Mackerelella non-typhoidal	1,341,873	15,608	553	3.54
Campylobacter spp.	1,963,141	10,539	99	0.95

(Source: CDC, 2009)

2.4. READY-TO-EAT PROCESSED MEAT ('KHEBAB')

Meat in Ghana is mostly contaminated with foodborne pathogens, potential causes of many foodborne infections (WHO, 1997; Adzitey *et al*., 2010a; Adzitey *et al*., 2011b). The most frequently implicated vehicles of transmission of Listeria monocytogenes are meat and poultry (Jay *et al*., 2005). 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* have all been implicated in meat contamination with high levels of microbes like *Salmonella, Staphylococcus aureus, Listeria monocytogenes, Escherichia coli* and others of which are often implicated in outbreak of food borne disease (Bean *et al*., 1990). According to Dyckman and Lansburg (2002), meat that is undercooked (not well grilled) may affect the health of consumers therefore food safety hazard considered most important is attributed to undercooked poultry and meat. 'Khebab' is a very popular street vended food and may therefore affect the health of many consumers if not well grilled. The surroundings where these livestock are kept as well as methods involved in processing after slaughtering also can cause contamination by microorganisms (Adeyemo, 2002). Livestock farming in Ghana

contributes a lot towards the development of the country through meat production and other products like the skin for bags , belts among others (Ansah *et al*., 2006). The live production of animals such as cattle, goats, sheep and their meat production depicts the meat consumption per year in Ghana. Goats, cattle, sheep and poultry are commonly reared in Ghana (Teye and Salifu, 2006) and the demand for meat and its products being high, as shown in Tables 2.2 and 2.3.

Table 2.2: Selected animal species live production in Ghana from 2006 to 2010

Animal/ Year	2006	2007	2008	2009	2010	Average
Cattle	1,359,000	1,373,000	1,392,000	1,438,000	1,454,000	1,403,200
Goats	3,997,000	4,196,000	4,405,000	4,625,000	4,855,000	4,415,600
Sheep	3,314,000	3,420,000	3,529,000	3,642,000	3,759,000	3,532,800
Pigs	477,000	491,000	506,000	521,000	536,000	506,200
Chickens	34,030	37,038	39,816	43,320	47,752	40,391.2

Source: FAOSTA (2012)

Table 2.3: Selected animal species Meat production (tons) in Ghana from 2006 to 2010

Animal/Year	2006	2007	2008	2009	2010	Average
Beef	23,865	23,419	25,350	25,538	25,775	24,789
Chevon	11,170	13,083	13,663	14,273	14,273	13,292
Mutton	10,370	10,773	15,881	16,389	16,914	14,065
Pork	15,456	16,506	16,968	17,506	17,506	16,788
Chicken	31,493	41,730	44,460	47,970	51,675	43,466
Game meat	61,667	57,864	64,951	69,276	74,100	65,572
Meat total	191,021	198,093	220,243	232,516	244,742	217,323

Source: FAOSTA (2012)

2.4.1. Nutritional Information of Meat

Ready source of nutrients for microorganisms are from meat tissue. Meat contains 0.2% glucose and 0.4% amino acids, both of which are first nutrients to be metabolized by microflora (Dainty and Mackey, 1992). Meat and meat products are of so much importance since they are the actual source of all the B-complex vitamins that include thiamin, riboflavin, biotin, vitamins B6 and B12. They are also best sources of some minerals such as iron, copper, zinc and manganese. Meat plays a very vital role in preventing zinc deficiency and particularly iron deficiency (Dainty and Mackey, 1992).

2.4.1.1. Raw Meat and Grilled Meat Processing

According to Dyckman and Lansburg (2002), undercooked meat may affect health of consumers therefore the most vital food safety hazard involves undercooked meat and poultry. Some of the common pathogens associated with meat contaminations are *Salmonella* spp., *Escherichia coli, Clostridium perfringens, Listeria monocytogenes* and many others.

2.4.1.2. Sources of Contamination of Raw Meat and Grilled Meat

Contamination of fresh meat may be due to improper handling and improper hygiene thereby affecting health of consumers (Koussemon et al., 2008). Cross contamination of the food product after the heat treatment is possible and may subsequently lead to the growth of pathogen (Buncic *et al*., 1990; Zaika *et al*., 1989). Possible source of contamination are through slaughtering of unhealthy animals, butchers using dirty water to wash meat, flies contamination as processing is done in an unhygienic environments, transport of meat through rickety vehicles, and using equipment such as knife that are contaminated. The perishable nature of meat is due to the sufficient

nutrient it contains that support microbial growth (Huda *et al*., 2010). The highest significant food safety hazard involves foods from animals (Kivi *et al*., 2007; Maripandi and Al-Salamah, 2010).

2.5. LISTERIOSIS

Listeria monocytogenes causes listeriosis. *Listeria monocytogenes* is a facultative, intracellular, non-spore forming, Gram-positive and with a tumbling end-over-end motility at room temperature (Brooks *et al*, 1998). *Listeria* is a foodborne pathogen characterized into seven species namely*: L. murrayi, L. innocua, L. ivanovii, L. seeligeri, L. welshimeri, L. grayi, L. monocytogenes* (Collins *et al*., 1991; Jones, 1992). Among them, only *L. ivanovii* and *Listeria monocytogenes* are pathogenic (Robinson *et al.,* 2000). The difference between *Listeria monocytogenes* and non-pathogenic strains is the ability of *Listeria monocytogenes* to lyse red blood cells. *Listeria monocytogenes is* distributed widely through many potential routes in order to infect humans. The pathogen has the ability to cross the intestinal, placental and blood-brain barriers in human which leads to gastroenteritis, maternofetal infection and meningoencephalitis respectively. Extreme infections though rare is common with healthy adults and young children through the ingestion of highly contaminated food (Swaminathan and Gerner-Smidt, 2007). Individuals such as diabetics, AIDS patients, those with renal failure, organ transplant patients, cancer patients who are considered immune-compromised are at risk of listeriosis as well as elderly adults. Pregnant women, foetus and also newborns are also at high risk (Vazquez-Boland *et al*. 2001). Some of the common fatal symptoms seen in pregnant women are pre-term labour, amnionitis, spontaneous abortion, stillbirth as well as early onset sepsis (Vazquez-Boland *et al*., 2001).

2.5.1. Occurrence of Listeria monocytogenes

Asymptomatic faecal carriers of the pathogen involve patients with gastro enteritis, pregnant women, workers of slaughter house, workers at the laboratory handling *Listeria* patients undergoing haemodialysis of food handlers, as well as some healthy people.

Some of the symptoms commonly seen in older adults and those with weakened immune systems involves fever, muscle aches, nausea and diarrhea. When infection spreads to the nervous system, other symptoms may include stiff neck, headache confusion and convulsions. In pregnant women, it leads to miscarriages, stillbirth, premature deliveries and infection of the newborn.

Listeria monocytogenes is accepted to have its development helped at a water movement (aw) $\geq$ 0.92 utilizing sodium chloride as the solute. The pathogen best develops at (aw) $\geq$0.97 (Jay *et al*., 2005). The base water action for the development of most strains is 0.93, some develop at (aw) 0.90 as some tend to make due for long stretches at (aw) as low as 0.83 (Montville and Matthews, 2005). In one of the examinations, a Scott A strain of Listeria monocytogenes in a fluid entire egg was warmed at 60 ^{o}C for 3.5 minutes. The D-value was 2.1 minutes (Atlas, 1997). A similar strain in a fluid entire egg to which NaCl of 10% was included and warmed at 63^{o}C for 3.5 minutes gave a D-value of 13.7 minutes (Bartlett and Hawke, 1995). It was watched that, regardless of a higher temperature (63^{o}C) was utilized for fluid entire egg with 10% salt. The D-value was around four times that of the fluid entire egg without salt. This was because of the 10% salt which brought down the water movement of the item from 0.98 to 0.915 (Linton *et al*., 1990). This infers, for salty nourishment frameworks when the temperature is brought down, the capacity of the pathogen to get by at high salt focus expands (Ryser and Marth, 1988). The security

of cured items, for example, salted fish or meat is along these lines ensnared. The pathogen can develop at a pH levels in the vicinity of 4.4 and 9.4 in research facility media.

2.5.2. Meat Contaminated by *Listeria monocytogenes*

Occurrence of *Listeria monocytogenes* within slaughter houses and meat processing facilities has been associated with environmental colonization, because of its ability to adapt and survive even on 'clean' equipment and rooms (Lunden *et al*., 2000). *Listeria monocytogenes* enters through infected animals and raw meat or intermediate products processed by suppliers (Boerlin and Piffaretti 1991; Gill and Jones 1995; Fenlon *et al*., 1996; Nesbakken *et al.*, 1996; Sammarco *et al*., 1997).

2.5.3. Detection of Listeria monocytogenes

Generally, detection of *Listeria monocytogenes* in food include culturing on selective media, description of colonial characteristics on solid selective media, morphology, biochemical and confirmatory tests (Prentice and Neaves, 1992). The pathogen is a poor contender and would along these lines not develop well when other organisms are present.

2.5.4. Factors Influencing *Listeria monocytogenes* in Meat Initially.

Ready source of nutrients for microorganisms is provided by the meat tissue. Meat contamination by microbes can occur from many sources and at different stages of processing or slaughtering. The initial contamination by *Listeria monocytogenes*, right from the live animal, slaughter house, storage and processing is based on water activity, pH, temperature and preservatives in the food which supports the growth of this pathogen (Gill, 1979; Borch *et al*., 1996).

2.5.5. Contributing Factors of Contamination from Processors and Vendors

Listeria monocytogenes 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; Swaminathan and Gerner-Smidt 2007). Mostly implicated in listeriosis outbreaks are ready to eat foods (Mauro *et al*., 2008).The reason is that, these ready-to-eat foods are eaten without any further treatment or processing. Poultry products as well as meat products have been identified as the most common implicated modes of transmission (Jay *et al*., 2005).

2.5.5.1. Poor Hygiene Practices

The use of contaminated food ingredients and equipment in domestic kitchen serves 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 others used by the processors and vendors aid in contaminating the meat. The sales of grilled meat at unhygienic areas, such as, closeness to open gutters, also make it possible for the pathogen to contaminate the meat.

2.5.5.2. Cross-Contamination

Listeria monocytogenes is highly ubiquitous, usually associated with the soil, dust, silage, water, waste from slaughter houses, effluent from sewage and many others (Prescott *et al*., 1995; Garbutt, 1997; Black, 1999; Jay *et al*., 2005). The meat can therefore be contaminated straight from the slaughter house or during transportation to processing sites (Glass and Doyle, 1990).

2.5.5.3. Sterile Processing Equipment, Processors and Vendors

Unclean hands of processors and vendors and their apparels tend to harbour this pathogen thereby aiding the formation of biofilms. Unclean food processing equipment have biofilms formed on them (Mauro *et al.*, 2008). 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 the pathogen to colonize and adapt to various environment (Jay *et al*., 2005; Mauro *et al*., 2008). The pathogen exhibits a very unique tumbling motility too at a temperature between 20-25^{o}C but not at 35^{o}C (Prentice and Neaves, 1992).

2.5.5.4. 'Khebab' (Meat) Processing Methods

'Khebab' 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 meat or 'khebab and these processes have a lot of importance since they prevent contamination by bacteria since bacteria are of health concern in meat (Table: 2.4). Salted and smoked meat is processed for flavour and consequently preserved due to the presence of salt, smoke and heat treatment. Seasoning such as ginger, black pepper and others have antioxidant properties. Also, red pepper and ginger have bacteriostatic properties which help preserve the meat. The red pepper, garlic, ginger, onion, black peppers are the most common spices used in preserving and flavoring meat in Ghana. The spices are usually ground for uniform dispersion on meat products. Thus, 'khebabs' 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 flavour.

Table 2.4: Sources of Bacteria of Health Concern in Meat

Organism	Principal source
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 & Wood, 1992)

The number of cases and mortality rate of food-borne illness caused by these pathogens are high; for example, *Salmonella* 31% of food related deaths, *Listeria* (28%), *Campylobacter* (5%), and *Escherichia coli* O157:H7 3% (Mead *et al*., 1999).

Hard smoking of meat is the common method employed and it involves much salty and smoking at a low temperature until little moisture is left in it. The salt preserves the meat as it inhibits the growth of the micro-organisms. Hot smoking requires a temperature of at least 65.56^0C 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 37.78 ^{0}C. 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).

2.5.5.5. Regulations of *Listeria monocytogenes* in Food

A zero-tolerance for the pathogen has been declared by United Kingdom and United States of America; however, set 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. The zero-tolerance policy faces two challenges, that is, listeriosis incidence in the USA is same as in the EU which allows < 100 CFU/g (Montville and Matthews, 2005). This simply implies that, the zero-tolerance offers no additional protection for consumers. The International Commission on Microbiological Specifications for foods (ICMSF, 1996) concludes that, if the count of *Listeria monocytogenes* does not exceed 100 CFU/g of food at the point of consumption, the food is acceptable. France believe it is possible to expect zero counts of *Listeria monocytogenes* in raw foods, particularly, given the inevitable presence of the pathogen in food processing environment (Tompkin, 2002).

CHAPTER THREE

3.0. MATERIALS AND METHODS

3.1. STUDY DESIGN

This study employed a mixed-method approach. The first phase of the study comprised of a descriptive survey design to obtain baseline data using structured questionnaires from the consumers and processors of 'khebabs'. Observation method was also used to ascertain the actual practices that might contribute to the contamination of the 'khebabs'. The second phase comprised of a cross-sectional design to obtain data on the microbial contamination of 'khebabs' using laboratory analysis.

The survey was carried out to yield information about the consumption patterns (type, mass and frequency consumed) of 'khebab' and the hygienic practices by 'khebab' processors in twenty selected areas in the Accra Metropolis. The laboratory analysis involved the isolation and identification of *Listeria monocytogenes* on 'khebabs' processed in selected areas in the Accra Metropolis.

3.2. STUDY SITES

Samples were collected randomly from twenty selected geographical areas of the Accra Metropolis (Figure 3.1). The population density as well as patronage of 'Khebabs' were reasons for the selection of sites. The longitudes and latitudes of the specific sampling points of the selected areas were recorded as follows:

Adentan (5.743260 and -0.151818), Banana Inn (5.542305 and -0.254963), Bubiashie (5.573681,-0.231882), Cantonment (5.572526 and -0.186610), Dansoman (5.531570 and -0.285453), Dome (5.63846052 and -0.24210993), Dzorwulu (5.607384 and -0.206485), James Town (5.540029 and -0.205956), Korle-Bu (5.537406, -0.227398), Kwashieman (5.588847 and -0.261483), Labone (5.563013 and -0.170127), Latebiorkorshie (5.548149 and -0.240324), Legon (5.640173 and -0.167042), Maamobi

(5.597001 and -0.192969), Nima (5.581542 and -0.198869), North Kaneshie (5.598811 and -0.232324), Roman Ridge (5.602401 and -0.195165), Tabora (5.619192 and -0.261937), Sowutuom (5.631570 and -0.285453), and Madina (5.678669 and -0.168909).

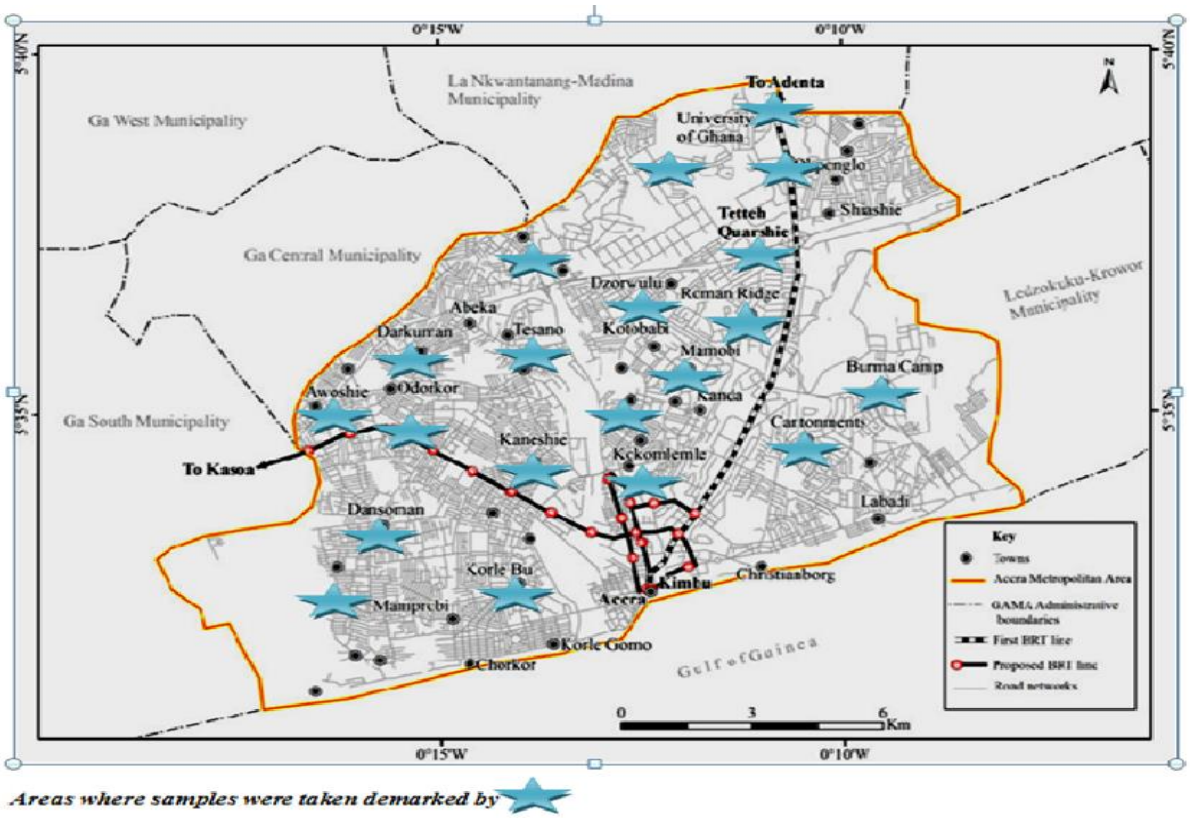

Figure 3.1: Map showing selected sample sites/areas in the Accra Metropolis

3.3. SURVEY AND SAMPLING SITES

A total of 400 consumers consisting of 20 each from the 20 selected areas were interviewed with questionnaires (Appendix A). Twenty (20) 'khebab' processors for the 20 selected areas were also interviewed with questionnaires (Appendix B) regarding their processing methods and practices.

3.3.1. Consumer Survey

The number of consumers denoted by n was determined using the formula from Moore and McCabe (1993):

$$\mathbf{n = (Z\alpha_{/2} / 2m)\ 2}$$

n = number of consumers
α= significance
$\mathbf{Z_{\alpha}}$ = critical value

Table 3.1: Confidence intervals and the respective significance and critical values

Confidence (1–α) g 100%	Significance (α)	Critical Value (Z_α)
90%	0.1	1.645
95%	*0.05*	*1.96*
98%	0.02	2.326
99%	0.01	2.576

A confidence interval of 95% was chosen so that the margin of error would be 5%. It implied that,

$Z_{0.025}$ = 1.95, where m (error of margin) = 5%, α= 0.05 and Confidence interval = 95%

$$n= (Z_{\alpha/2}/2m)^{\ 2}$$

$$n = [(1.96/0.1)^2]$$

$$n = 19.6^2$$

$$19.6\approx20$$

$$n = 20^2 =400$$

The total number (n=400) was obtained since 19.6≈20. This implied that 400 respondents for all the twenty (20) selected areas was required.

3.3.2. Processor Survey

Twenty (20) processors were selected based on areas where much of the 'khebabs' were produced and consumed and their processing methods and general handling of the meat

as well as practices. They were therefore interviewed with structured questionnaires (Appendix B) to obtain information on their practices. The meat processing sites were the interviewing points in order to observe the methods and practices.

3.4. LABORATORY ANALYSES

The laboratory examinations needed to do with isolation, identification and *Listeria monocytogenes* enumeration and furthermore the general microbial checks/counts (total coliform count, total plate count and *Escherichia coli* count) on the raw meat and the processed meat ('khebab'). Affirmation of all presumptive *Listeria monocytogenes* isolates was carried out utilizing Standard tests (Board et al., 1992). Aseptic methods were utilized for sampling and microbiological investigations.

In all, 120 samples were taken for the lab investigations involving sixty (60) raw meat and sixty (60) grilled ready- to-eat 'khebab' (Table 3.2). Samples were put into sterile sample bags appropriately labeled with ice packs put on them until bacteriological examination.

3.4.1. Sample Collection from the Processing Sites

Selected sites were visited prior to starting the study in order to explain to the vendors the basis and significance of study. The vendors were engaged in the study after they had given their approval and they were assured of confidentiality. Raw meat and grilled meat totaling one hundred and twenty (120) were collected from the selected areas. Hands were sanitized with 70% alcohol in the process to prevent contamination. The vendors helped in sample selection and collection as well as putting samples into sample bags to avoid cross contaminations.

3.4.2. Enumeration of Microbes

The total viable counts, total coliform counts, and *Escherichia coli* counts were determined for all the 120 samples using the protocol from the Center for Food Safety and Applied Nutrition (USA) while the procedure recommended by the International Organization for Standardization procedure (ISO17604:2015) was used for the enumeration, isolation, and identification of *Listeria monocytogenes*. Ten grams (10 g) of each sample were weighed aseptically into sterile plastic stomacher bags and macerated. A 1:10 dilution was prepared by adding 90 mL of 0.1% sterile peptone water (Merck, Darmstadt-Germany). The 1:10 suspension was mixed thoroughly through ten- fold serial dilutions carried out by aseptically transferring 1ml of the 1:10 suspension into a 9 mL of the diluent, and serially diluted in the same buffer solution, starting from 1:10 through 10^{-6}.

3.4.2.1. Total viable count (TVC)

Total viable counts were carried on Standard Plate Count Agar (Merck, Darmstadt-Germany). About 1 mL of diluent was added to 9 mL molten Standard Plate Count Agar (Merck, Darmstadt-Germany) kept at 45-50^{0}C in a water bath (Grant, OLS 200). Using pour plate technique, mixing was well done by rotation and then poured into a 9cm sterile Petri dish, allowed to set and incubated at 37^{0}C for 18-24hrs.

3.4.2.2. Total coliform count (TCC)

Membrane Lactose Glucuronide Agar (Oxoid, CM1031, USA) was used for the total coliform count. Applying the pour plate count technique, 1 mL of diluent was added to 9 cm Petri dish. Then, 9 mL molten Membrane Lactose Glucuronide Agar (Oxoid, CM1031, USA) kept at 45-50^{0}C in water bath (Grant, OLS 200) was added to the sample in the Petri dish. Mixing well by swirling was done and allowed to set before

incubation at 37^0C for 18-24hrs. Plates that showed cream to yellowish colonies were selected and counted using a colony counter (Stuart Scientific, UK).

3.4.2.3. *E. coli* count

Membrane Lactose Glucuronide Agar (Oxoid, CM1031, USA) was used for the *E. coli* count. Applying the pour plate count technique, 1 mL of the diluent was added into 9cm Petri dish. Then, 9 mL molten Membrane Lactose Glucuronide Agar (Oxoid, CM1031) kept at 45-50^0C in water bath was added to the sample in the Petri dish. Mixing was well done by swirling, allowing to set and incubated at 37^0C for 18-24hrs. Plates that showed greenish colonies were selected and counted using colony counter (Stuart Scientific, UK).

3.4.2.4. *Listeria* count

One milliliter (1 mL) of diluent was added to a 9 mL molten chromogenic *Listeria* Selective Agar (Oxoid, CM1084) which was supplemented (Oxoid, SR0226E and SR0244E) and kept at 45-50^0C in water bath. Using pour plate technique (ISO 11290), mixing was well done by rotation and poured into a 9 cm sterile Petri dish, allowed to set and incubated aerobically at 30^0C for 96 hrs. Plates showing greenish colonies when viewed at 45^o were selected and counted using a colony counter (Stuart Scientific, UK). Colonies counted were multiplied by the dilution factor and the results expressed as $x*10^y$cfu/g, where x is colony counted, y is the dilution factor and cfu/g as unit.

3.4.3. Isolation and Identification of Microbes

Ten milliliter portions of 1:10 suspension were centrifuged at 10,000 rpm for 30 minutes in a refrigerated centrifuge (4°C). After decanting the supernatant, a loop-full of the pellet was aseptically streaked onto Blood agar (Merck, Darmstadt-Germany) and

MacConkey agar (Merck, Darmstadt-Germany) using the plate–out technique (Heritage *et al*, 1996). Cultures were incubated aerobically at 37^0C for 18-24hrs in bacteriological incubator (Wagtech, USA). Impure cultures on primary media were purified by sub-culturing onto selected secondary media such as Membrane Lactose Glucuronide Agar, Xylose Lysine Deoxycholate, Brilliant Green Agar, Mannitol Salt Agar, and Blood Agar.

After overnight incubation (96 hours for *Listeria* isolates), colonial morphology of organisms based on their physiological characteristics were observed for size, shape, outline, colour and change in medium. Standard microbiological techniques, including Gram staining, cellular morphology, and biochemical tests such as Motility Indole Urea (MIU) (Lioflichems.r.l. Bacteriology Products, 610236, Italy), Catalase, Triple Sugar Iron (TSI) (Oxoid, CM 0277, Hamsphire – England), Indole Methyl Red Vorges-Proskeur Citrate (IMViC) test, carbohydrates Oxidation/Fermentation (O/F) test (to detect gas and or acid production) among others were used to isolate and identify the organisms.

3.4.3.1. Isolation and Identification of *Salmonella*

One (1) mL of the diluent was added to 9 mL of Selenite Cystine broth (Oxoid, CM 0699). This was mixed thoroughly and incubated at 37^0C overnight. Using plate-out technique, sub-cultures were made from the broth aseptically onto Xylose Lysine Deoxycholate (XLD) medium (Oxoid, CM0469). Cultures were incubated at 37^0C for 24-48 hrs and examined for the physiological characteristics of colonies on the media (colonial morphology). Colonies showing slight to heavy blackening due to hydrogen sulphide production were isolated and identified as *Salmonella* species. Organism was confirmed using tests based on Latex Agglutination (Oxoid, FT0201), Motility Indole

Urea (MIU) (Lioflichems.r.l. Bacteriology Products, 610236, Italy) and Triple Sugar Iron (TSI) agar (Oxoid, CM 0277, Hampshire – England) tests.

3.4.3.2. Isolation, Identification of *Escherichia coli*

All lactose fermenting colonies on MacConkey agar were selected and aseptically sub-cultured onto Membrane Lactose Glucuronide Agar (Oxoid, CM1031) to isolate and identify *Escherichia coli*. Cultures were incubated at 45^0C for 24-48 hours in a bacteriological incubator. After incubation, green colonies observed at an angle of 45^0 to a light source, were isolated and identified to be *Escherichia coli*. The organism was confirmed using cell morphology and Indole Methyl Red Vorges-Proskeur Citrate (IMViC) test which are some of the confirmatory tests for *E. coli*.

3.4.3.3. Isolation, Identification of *S. aureus*

Using sterile inoculating loop, all colonies showing colonial and cellular morphology of *S. aureus* were plated from the Blood Agar (Lioflichems.r.l. Bacteriology Products, 610005, Italy) onto Mannitol Salt Agar (Oxoid, CM 0085, Hamsphire-England). Cultures were incubated at 37^0C overnight. Colonies showing golden yellow to cream pigments with pinkish background were selected and based on the biochemical tests such as catalase and coagulase, the organisms were identified as *S. aureus.*

3.4.3.4. Presumptive and confirmatory identification for *Listeria*

Purification of *Listeria monocytogenes* was done as the streaking on a nutrient agar was done before incubating plates for 24 hours at 37ºC (Board *et al*., 1992). Testing for the purified colonies from the nutrient agar plates for their Gram reaction was done as described by Black (1999). Pure colonies obtained through Gram reaction were used for subsequent confirmatory identification tests.

3.4.3.4.1. Catalase test

The catalase test procedure described by Elmer W. Koneman (2006, medical), was used**.** A catalase test performed separates the *Listeria monocytogenes* from other *Listeria* species. The enzyme catalase present in the organism mediates the breakdown of hydrogen peroxide (H_2O_2) into oxygen and water. The oxygen thereby produces a rapid effervescence of bubbles indicating a positive test. This occurred with most of the colonies that were picked with a sterile loop onto a slide after placing few drops of 3% hydrogen peroxide using a capillary pipette.

3.4.3.4.2. H_2S Production in TSI (Triple Sugar Iron)

Hydrogen sulphide (H_2S) production test was performed in order to detect the hydrogen sulphide (H_2S) gas produced by an organism. The sterile inoculating wire was used to touch the top of the isolated colony and then inoculated by stabbing through center of the medium to the bottom of the tube then streaking the surface of the agar slant. It was then incubated in the tube for 24 hours at 35^oC. The production of H_2S when sulphur–containing amino acids are decomposed indicating a positive test.

3.4.3.4.3. β-haemolysis test

β-haemolysis which is associated with complete lysis of red cells surrounding the colony is caused by two hemolysins O and S where the O is inactive in the presence of oxygen and S being oxygen-stable cytotoxin. The presumptive *Listeria monocytogenes* isolates were picked with a sterile loop and streaked on the blood agar. It was then incubated for 24 hours at 37^oC. Clear bands around the lines of streak noticed indicated a positive β–haemolysis (Board *et al.*, 1992).

3.4.3.4.4. Indole Test

An indole test was performed to determine the ability of the organism to split tryptophan molecule into indole which is one of the metabolic degradation product of the amino acid tryptophan. Tryptone Soya Broth (Oxoid, CM129) was inoculated with the picked colonies and incubated for 24 hours at 37^0C. The bright red colour at the interface of the broth and reagent within seconds after adding reagent indicated the presence of indole positive test.

3.4.3.4.5. Motility Test

The motility test was done to find out if the organisms present were motile or non-motile. The picked colonies using the inoculating needle were stabbed into motility media. Growth away from the stab line indicated a positive test.

3.4.3.4.6. Urease Test

This was performed in order to differentiate organisms based on their ability to hydrolyze urea with the enzyme urease. The surface of a urea agar slant was streaked with a portion of picked colonies that was done using a sterilized loop. Cap of the test tube was closed and the content in test tube was incubated 37^0C for 48 hours. The colour change of phenol red from light orange to pink within 24 hours indicated urea being hydrolyzed with the enzyme urease

CHAPTER FOUR

4.0. RESULTS

4.1. DEMOGRAPHIC CHARACTERISTICS OF CONSUMERS

A shown in Figure 4.1, a total of 400 respondents comprising of 196 (49%) females and 204 (51%) males were recruited for the study. Table 4.1, depicts that majority (27.75%) of the respondents were in the age group ranging from 30-39 years and resided in Adentan, Cantoments, Dansoman, Dzorwulu, Labone, Legon, Roman Ridge, and Sowutuom vending areas. The age group range of 20-29 years made up 22% of the respondents and located in the Bubiashie, Dome, and Kwashieman vending areas while those that were less than 20 years were 21.5% and located in the Banana Inn, James Town, Korle-bu, Latebiorkorshie, Madina, Maamobi, Nima, and Tabora. Only 12.5% were in the age range of fifty and above ((≥50) and located in the North Kaneshie vending area. About 361 (90.25%) of the Respondents indicated that they consume 'Khebabs" while only 39 (9.75%) indicated that they do not consume "khebabs". Kwashieman and Maamobi recorded the highest number of consumers (100%) of which majority of them were females (80%). North Kaneshie recorded the least consumers (70%) compared with the other locations with 45% of the respondents being female consumers (Figure 4.2).

Table.4.1: Demographic Characteristics of the Consumers

	Age (%)				
Location	**<20**	**20-29**	**30-39**	**40-49**	**≥50**
Adentan	3(15)	4(20)	**7(35)**	4(20)	2(10)
Banana Inn	**7(35)**	4(20)	5(25)	2(10)	2(10)
Bubiashie	4(20)	**5(25)**	2(10)	4(20)	5(25)
Cantoment	0(00)	2(10)	**9(45)**	7(35)	2(10)
Dansoman	3(15)	4(20)	**8(40)**	3(15)	2(10)
Dome	4(20)	**6(30)**	4(20)	3(15)	3(15)
Dzorwulu	2(10)	2(10)	**9(45)**	4(20)	3(15)
James Town	**10(50)**	5(25)	3(15)	1(5)	1(5)
Korle-bu	**7(35)**	5(25)	2(10)	5(25)	1(5)
Kwashieman	4(20)	**9(45)**	3(15)	2(10)	2(10)
Labone	1(5)	4(20)	**8(40)**	4(20)	3(15)
Latebiorkorshie	**9(45)**	7(35)	3(15)	1(5)	0(00)
Legon	0(00)	2(10)	**9(45)**	6(30)	3(15)
Madina	**6(30)**	5(25)	4(20)	2(10)	3(15)
Mamobi	5(25)	**7(35)**	3(15)	4(20)	1(5)
Nima	**6(30)**	4(20)	3(15)	2(10)	5(25)
North Kaneshie	4(20)	4(20)	5(25)	1(5)	**6(30)**
Roman Ridge	0(00)	2(10)	**8(40)**	6(30)	4(20)
Sowutuom	4(20)	3(15)	**10(50)**	1(5)	2(10)
Tabora	**7(35)**	4(20)	6(30)	3(15)	0(00)

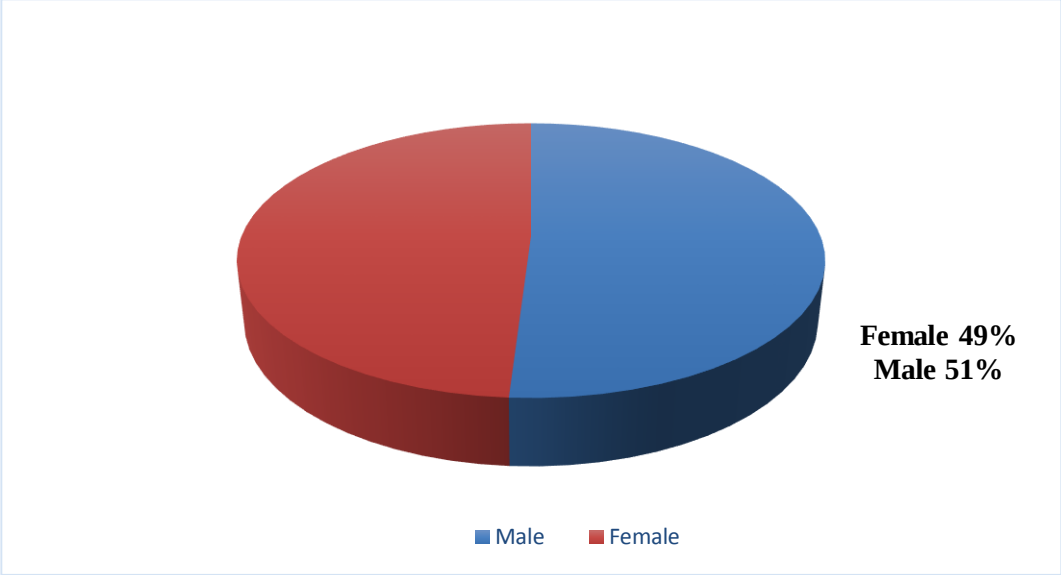

Figure 4.1: Gender of consumers

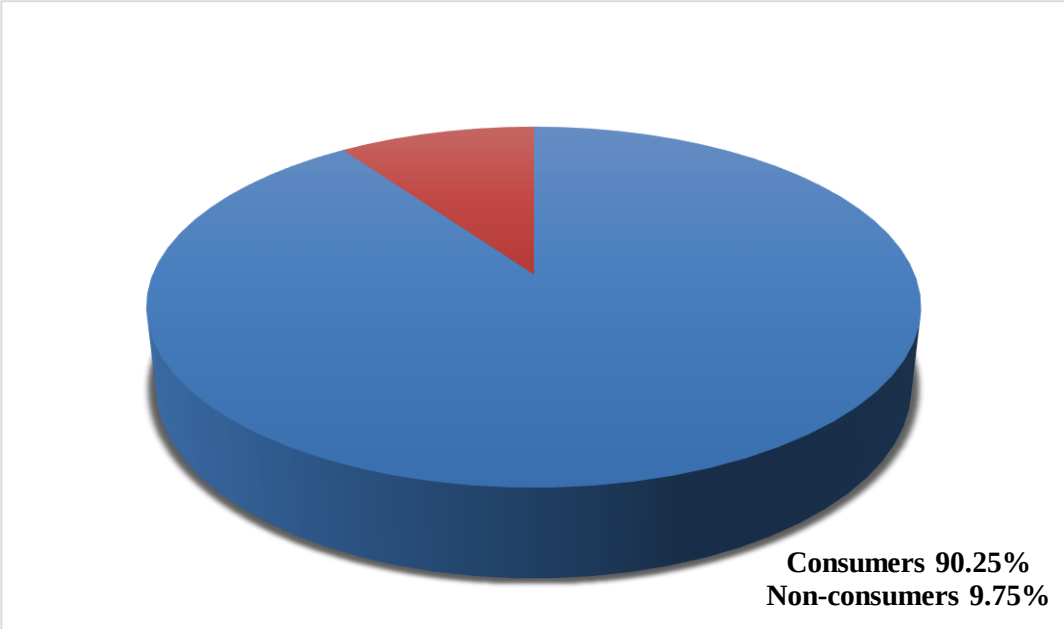

Figure 4.2: Consumers and non-consumers

4.2. DEMOGRAPHIC CHARACTERISTICS OF THE PROCESSORS

A total of 20 processors were all males and between the ages of 30-49 were recruited for this study. Out of the total number 70% were in the age range of 30-39 while 30% were between 40 and 49 years (Table 4.2). Six (6) had had Senior High School (SHS)

education, seven (7) with Junior High School education (JSS), three (3) with primary school education while four (4) were illiterates. Majority (55%) of the Processors bought their raw meat from the open market while 45% bought their raw meat from slaughter houses (Figure 4.3). Eight (40%) of the processors sold their 'khebabs' in a close glass cage while twelve (60) sold their 'khebabs' in the open (Figure 4.4).

Table.4.2: Demographic Characteristics of the Processors

		Age		
Location	**Gender**	**20-29**	**30-39**	**40-49**
Adentan	M		√	
Banana Inn	M		√	
Bubiashie	M		√	
Cantoment	M			√
Dansoman	M		√	
Dome	M		√	
Dzorwulu	M		√	
James Town	M			√
Korle-bu	M			√
Kwashieman	M		√	
Labone	M		√	
Latebiorkorshie	M		√	
Legon	M		√	
Madina	M		√	
Mamobi	M			√
Nima	M			√
North Kaneshie	M			√
Roman Ridge	M		√	
Sowutuom	M		√	
Tabora	M		√	

M = male √= selected range

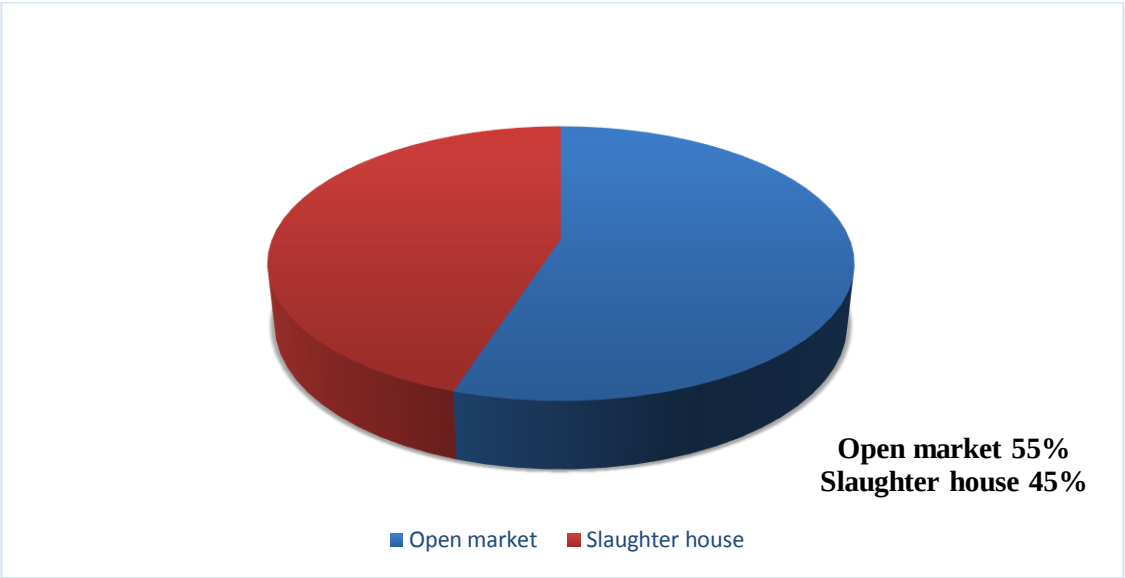

Figure 4.3: Source of raw meat

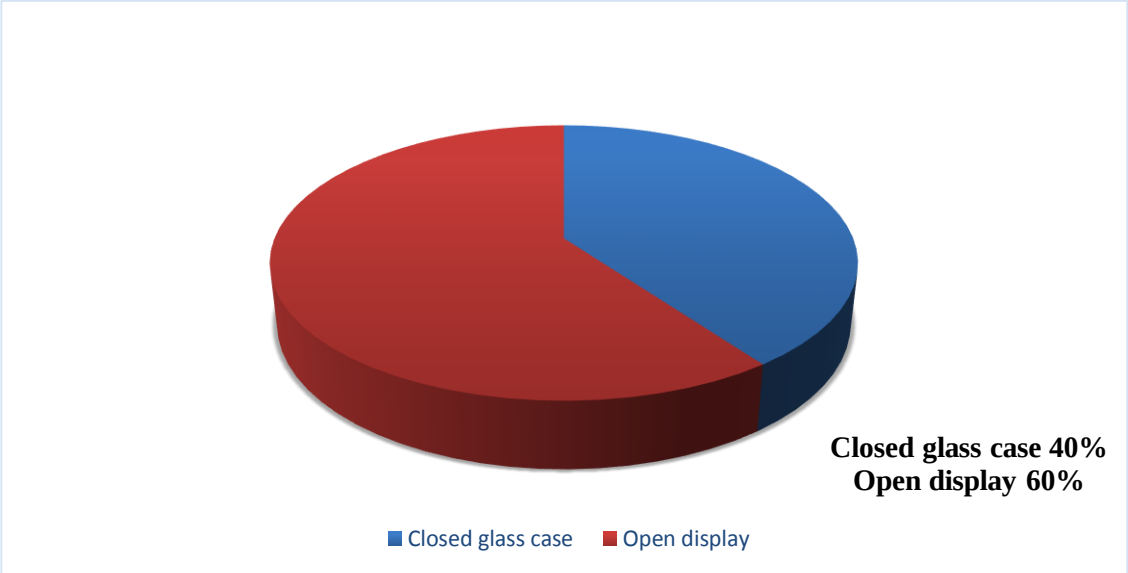

Figure 4.4: Mode of storage of meat

4.3. PREVALENCE OF LISTERIA MONOCYTOGENES IN THE 'KHEBABS'

As shown in Figure 4.5, from a total of 120 samples of meat consisting of 60 raw meats and 60 grilled meats ('khebabs') tested, 92 samples (76.67%) were positive for *Listeria monocytogenes*. Out of these 92 samples being positive for *Listeria monocytogenes*, 45 samples (48.91%) were raw meat and 47 samples (51.09%) were grilled meat. Samples that were negative for *Listeria monocytogenes* were 28.

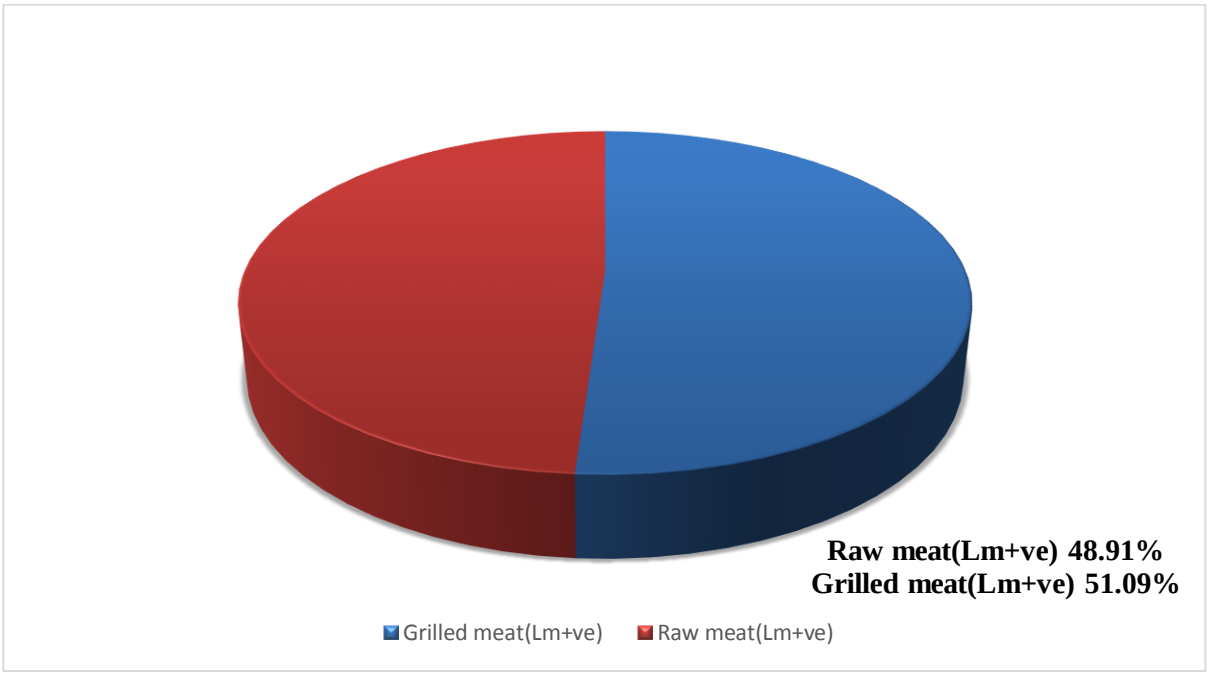

Figure 4.5: Prevalence of *Listeria monocytogenes* in the 'Khebabs'

Key: (Lm +ve) = *Listeria monocytogenes* detected

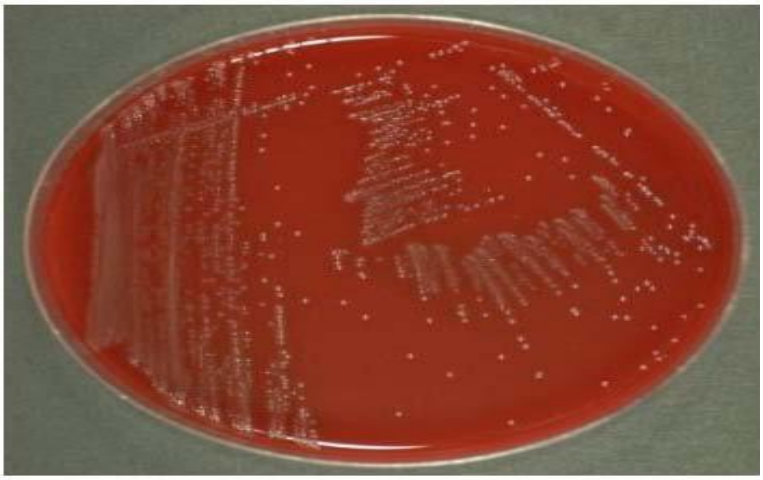

Plate 4.1: A representative blood agar plate showing β -haemolysis of *L. monocytogenes*

4.4. Listeria Counts in both the raw and grilled Meat ('Khebab')

As shown in Table 4.3, Madina vending area recorded the highest count of 6.190 log_{10}cfu/g whilst the least count of 0 log10 cfu/g was recorded at both Cantoment and Roman Ridge vending area for the grilled meat. Dome recorded the highest listeria count of 6.30 log_{10}cfu/g whilst the least listeria count of 0 log_{10}cfu/g was recorded at both Labone and North Kaneshie vending areas.

Table 4.3: Mean Microbial Count on both the Raw and Grilled Meat ('Khebab')

Location	TVC	EC	TCC	LC(raw meat)	LC(grilled meat)
			Log_{10}cfu/g		
Adentan				5.6	5.88
Banana Inn	6.596	6.556	6.394	5.83	4.3
Bubuashie	6.378	5.491	4.984	6.11	5.18
Cantoment	5.497	6.483	4.699	4	---
Dansoman	5.565	7.009	6.033	5.36	5.26
Dome	6.786	---	---	6.3	5.59
Dzorwulu	6.924	6.825	5.607	5.93	4
James Town	6.555	6.812	5.31	5.93	4.48
Korle-bu	5.154	6.827	5.363	6.04	5
Kwashieman	6.58	6.634	5.013	6.18	5.2
Labone	6.953	6.176	5.708	---	4.85
Latebiorkorshie	5.093	6.164	5.539	5.53	5.32
Legon	6.885	5.602	---	5.74	5.53
Mamobi	6.58	6.51	5.48	0	5.89
Madina	7.267	4.748	5.296	5.46	5.34
Nima	6.2	6.792	5.489	---	4
North Kaneshie	6.922	6.792	---	4	---
Roman Ridge	6.927	5.491	4.097	4.3	4.48
Sowutuom	4.732	---	4.826	4.6	4.3
Tabora	6.23	---	---	5.7	6.19

KEY: **TVC** = Total Viable Count, **EC** = *E. coli* Count, **TCC** = Total Coliform Count, **LC** = Listeria Count

4.5. DISTRIBUTION OF *LISTERIA MONOCYTOGENES* AT THE SELECTED VENDING AREAS

As shown in Figure 4.6, Banana Inn vending area recorded the highest pathogen contamination level in the grilled meat whilst Cantoment, Dome, Laterbiorkorshie, North Kaneshie and Tabora vending area recorded the least pathogen contamination level. The grilled meat ('khebab') also had Banana Inn vending area recording the highest pathogen contamination while Cantonment, Dome, Laterbiorkorshie, North Kaneshie, and Tabora vending areas recorded the least pathogen contamination.

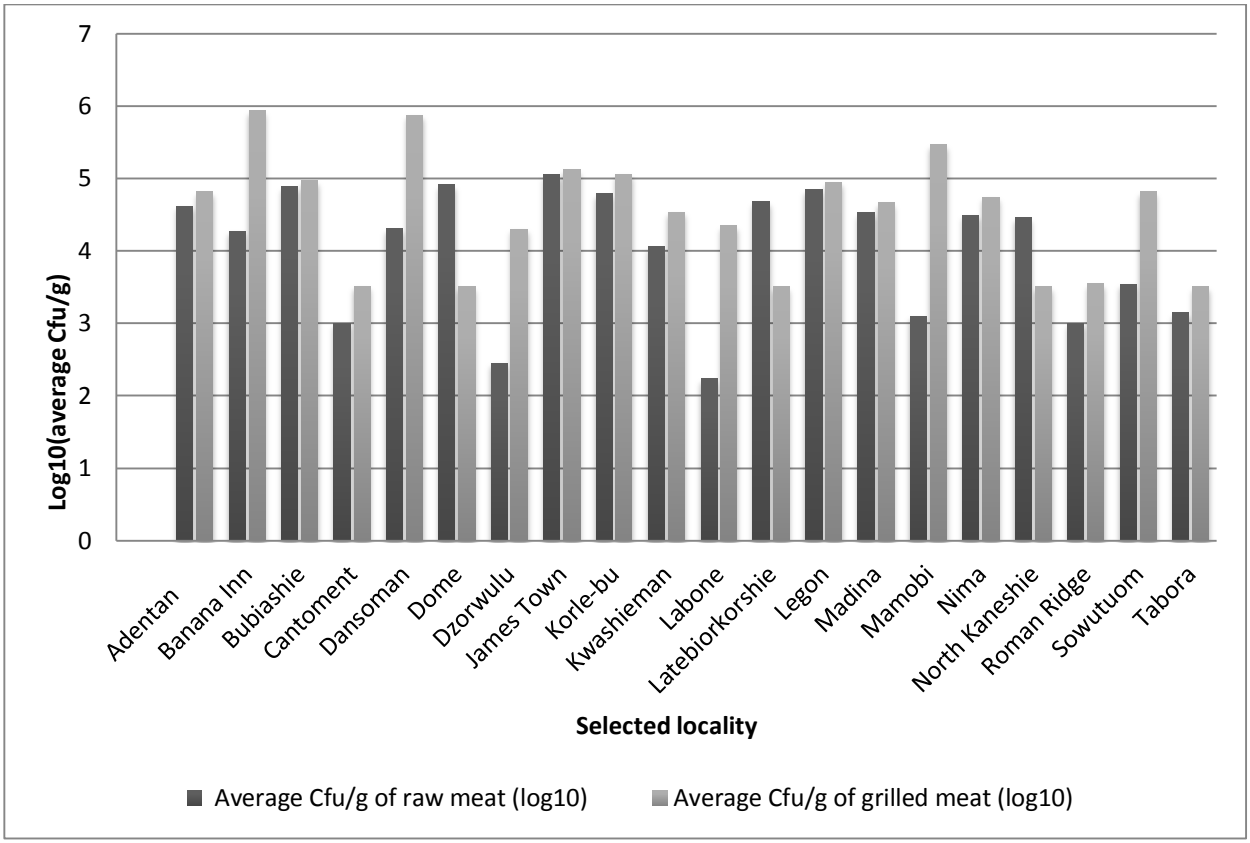

Figure 4.6: Load of pathogens on both raw and grilled meat at the selected localities

4.6. Microbial Counts of TVC, EC and TCC on the Grilled Meat ('Khebab')

As shown in Table 4.3, Madina had the highest load of TVC (7.267 $\log_{10}$ cfu/g) whilst Sowutuom vending area had the least TVC of 4.732 $\log_{10}$ cfu/g. In the case of the total coliform count (TCC), the highest contamination was found in the Banana Inn vending area (6.394 $\log_{10}$ cfu/g) whilst the lowest count of 0.00 $\log_{10}$ cfu/g was found in the Dome, Legon, North Kaneshie, and Tabora vending areas. For the *E. coli* (EC) contamination, the highest level of 7.009 $\log_{10}$cfu/g was found in the Dansoman vending area whilst the lowest level of 0.00 $\log_{10}$ cfu/g was found in the Dome, Sowutuom, and Tabora vending areas.

CHAPTER FIVE

5.0 DISCUSSION

This study sought to assess prevalence of *Listeria monocytogenes* in raw and grilled meat ("khebab") in selected vending areas in the Greater Accra Region of Ghana. Survey of the "khebab" processors revealed that about 80% had had some form of formal education and therefore could be inferred that majority of them have an insight now with respect to safe grilled "khebabs". The survey also revealed that "khebab" processing is mainly a man's job as all the 20 processors were males with majority of them in the age range from 20 to 39 years. Processors all being males could be due to the nature of the job.

'Khebab' is generally prepared using meat, usually from cattle, goat, sheep, chicken, guinea fowl, and others with the meat cut into reasonable sizes, seasoned and grilled. Most of the processors indicated that they purchased their meat from the open market and/or slaughterhouses and transported to the site of processing. Thus, the contamination of 'khebabs' by *Listeria monocytogenes*, in this study, could be attributed foremost to the source of purchase and contact surfaces during transportation of the meat from the open market or slaughter houses.

The occurrence of *Listeria monocytogenes* within slaughter houses and meat processing facilities is associated with environmental colonization, because of its ability to adapt and survive even on 'clean' equipment and rooms (Lunden *et al.*, 2000). The improper handling of the meat during and after transportation could also be the reason for the presence of the pathogen in most of the meat samples. Improper handling and improper hygiene may lead to the contamination of fresh meats and eventually affect health of consumers (Koussemon *et al.*, 2008). This could be through unclean hands, bowls, knives, clothes and others of processors or vendors.

The presence of the pathogen on the meat samples could also emanate from the animals from which the meat was obtained. *Listeria monocytogenes* are known to enter individuals through infected animals and raw meat or intermediate products processed by suppliers (Boerlin and Piffaretti 1991; Gill and Jones 1995; Fenlon *et al*., 1996; Nesbakken *et al*., 1996; Sammarco *et al*., 1997).

It was observed that most of the processing sites were close to open gutters and also in dusty areas with the dust serving as vehicles for the contaminants. This could be a contributing factor to the pathogen contaminating the meat samples since *Listeria monocytogenes* is usually detected in the soil, dust, water, decay vegetation among others (Garbutt, 1997; Jay *et al*., 2005).

The microbial counts performed were Total Viable Count, *E. coli* Count, Total Coliform Count and *Listeria* Count. The isolates identified among the *E. coli* and Coliforms were *Salmonella* spp., *Staphylococcus* spp., *Bacillus* spp., and others. The mean counts for the raw meat in $\log_{10}$ for the various selected areas were less than their respective mean counts for the grilled meat of the selected areas apart from Dome, Laterbiorkorshie and North Kaneshie. These areas had their mean counts for the raw meat more than that of the grilled meat. The microbial count reduction after grilling implies that, either the effect was from the spices before grilling or from the heat from the grill. The mean microbial counts for *Listeria* for both the raw and grilled meat exceeded the infective dose for *Listeria monocytogenes* in terms of viable cells. The least count recorded was for Tabora which was $\log_{10}(3.15 \pm 1.63)$ for raw meat and $\log_{10}(2.739 \pm 0.370)$ for grilled meat which is greater than $\log_{10}(2)$. *Listeria monocytogenes* limit for all ready-to-eat food should be less than 100 colony-forming units per 25g or 25ml portion of the food according to WHO microbiological guidelines for ready-to-eat food. The microbial loads are therefore not below the

WHO threshold value.

The calculated Cohen's Kappa co-efficient (*k*) was 0.02, indicating a fair agreement in terms of *Listeria monocytogenes* contaminations as far as the raw meat contamination and the grilled 'Khebab' contaminations are concerned. The *E. coli* counts and coliform counts could be attributed to cross contamination through unhygienic processing equipment, poor hygiene practices, poor personal hygiene and others. The foods with coliforms contamination in general and *E. coli*, in particular, mostly results from unhygienic handling of foods (Hobbs and Roberts, 1987; Jay *et al*., 2005).

The hazard in this study, *Listeria monocytogenes*, is responsible for the foodborne disease listeriosis. The immuno-compromised individuals such as diabetics, AIDS patients, those with renal failure, organ transplant patients, cancer patients and also elderly adults are generally at risk from listeriosis. Pregnant women, foetus and also newborns are also at high risk (Vazquez-Boland *et al*. 2001). The pathogen was detected in 92 samples (76.67%) out a total of 120 samples of meat consisting of 60 raw meat and 60 grilled meat ('Khebabs'). Also, out of the 120 samples of which 92 samples are positive for the pathogen, 37.5% was for raw meat and 39.17% was for grilled meat. Samples that were negative for *Listeria monocytogenes* contamination out of the same 120 samples recorded 23.33% where 12.5% was for raw meat and 10.83% was for grilled meat.

Listeria monocytogenes occurring in raw meat even from the slaughter house to the processing point suggests that the meat products could be a vehicle for the transmission of the pathogen to consumers. The level of microbial counts implicates the sanitation of all these selected areas. This implies that, the sanitary conditions were unsatisfactory due to occurrence of coliforms in general and *Escherichia coli* even in the grilled 'Khebabs'.

This should therefore be a food safety issue in order to protect the immuno-compromised individuals such as diabetics, AIDS patients, those with renal failure, organ transplant patients, cancer patients and pregnant women who are prone to this pathogen.

CHAPTER SIX

6.0. CONCLUSIONS AND RECOMMENDATIONS

6.1. CONCLUSIONS

Most of the "khebab" processors had some form of formal education and had some insight with respect to safety of grilled "khebabs". *Listeria monocytogenes* was detected in majority of the 'Khebab' samples of the 20 selected areas of Accra metropolis. *Listeria monocytogenes* occurred more in the grilled meat than the raw meat probably due to cross contamination during processing which could be either from the processor himself through his unclean hands or clothes, processing equipment and poor sanitation of processing site. All the processors were males with ages ranging from30 to 39 years and 40 to 49 years. *Listeria monocytogenes* was detected in the grilled skewed meat more than the raw skewed meat samples collected in the 20 selected areas in Accra Metropolis.

6.2. RECOMMENDATIONS

1. Further studies on the occurrence (prevalence and concentration) of the pathogen in sausages, guinea fowls, chicken that are also grilled together with the meat as 'khebabs' should be conducted.
2. Investigations of the role of other pathogenic *Listeria species (Listeria evanovii)* in Ghana should be undertaken.

REFERENCES

Ababio P. F. and Adi D. D., (2012). Evaluating Food Hygiene Awareness and Practices of Food Handlers in the Kumasi Metropolis; *Internet Journal of Food Safety*, **14**: 35-43.

Adam, M.R. & Moss, M.O. (2008). Food Microbiology, 3rd Ed. RSC Publishing, Cambridge. **229:** 224 – 231.

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

Adzitey F, Teye GA, Ayim AG and Adday S (2010). Microbial quality of chevon and mutton sold in Tamale Metropolis of Northern Ghana. *Journal of Applied Sciences and Environmental Management***14**: 53-55.

Adzitey F, Teye GA, Kutah WN and Adday S (2011). Microbial quality of beef sold on selected markets in the Tamale Metropolis in the Northern Region of Ghana. *Livestock Research for Rural Development* **23**(1): 2011.

Agbodaze, D., P.N. Nmai, F. Robertson, D. Yeboah-Manu, K. Owusu-Darko and K. Addo, 2005. *Microbiological quality of khebab consumed in the Accra metropolis*. Ghana. Med. J., **39**: 46-49.

Allan JT, Yan Z, Genzlinger LL, Kornacki. JL (2004) Temperature and biological soil effects on the survival of selected foodborne pathogens on a mortar surface. *J Food Prot* **67**(12**)**: 2661–2665

Ansah, T., Dzoagbe, G. S. K., Djang-Fordjour, K. T., Agbolosu, A. A., & Wesseh, A. (2006). The role of churches in the sustainability of livestock production in the Northern Region. The Savanna Farmer. A magazine on Sustainable Agriculture. The Association of Church Development Project (ACDEP) of Ghana. 35.

Ansah, T., Dzoagbe, G. S. K., Teye, G. A., Adday, S., & Danquah, J. K. (2009). Microbial quality of table eggs sold on selected markets in the Tamale municipality in the Northern Region of Ghana. Livestock Research for Rural Development. **21** (8):2009.

Appiah, J. (2010). Risk assessment of consuming milk and milk products contaminated with *Listeria monocytogenes* from informal markets in Ashiaman, Accra. MPhil Thesis.

Argenti, O. 2000. Food for the cities: food sully and distribution policies to reduce urban food insecurity. "Food into cities" Collection, DT/43-00E. FAO, Rome. Arnold, London. 217

Atlas, R.M. (1997). Principles of Microbiology, 2nd Edition. Wm. C. Brown Publishers, Boston.

Atlas, R.M. (1997). Principles of Microbiology, 2nd Edition. Wm. C. Brown Publishers, Boston.

Bartlett, R.M., & Hawke, A.E. (1995). Heat resistance of Listeria monocytogenes Scott A & HAL 957El in various liquid egg products. *J. Food Protect*. **58**: 1211-1214.

Basti, A.A., Misaghi, A., Salehi, T.Z., & Kamkar, A. (2006). Bacterial pathogens in fresh smoked and salted Iranian fish. *Food Control***17:** 183 – 188.

Bell, C., Kyriakides, A., 1998.Listeria. A Practical Aroach to the Organism and its Control in Foods. Blackie Academic &Professional, London

Bemrah, N., Sanaa, M., Cassin, M.H., Griffiths, M.W. & Cerf, O. (1998). Quantitative risk assessment of human listeriosis from consumption of soft cheese made from raw milk. *Prev. Vet. Med*. **37:** 129-145.

Beumer, R. 1997. Listeria monocytogenes detection and behaviour in food and in the environment. Thesis work. University of Wageningen. ISBN 90-5485-6327

Beumer, RR & Kusumaningrum, H. 2003. Kitchen hygiene in daily life. International Bio-deterioration & Biodegradation **51**(4):299-302.

Bille J., Catimel B., Bannerman E., Jacquet C. Yersin M. N., Caniaux I. Monget D. &Rocourt J., 1992. API Listeria, a new and Promising One –Day System To Identify Listeria Isolates. *Journal of Alied and Enviromental Microbiology* **58**:1857- 1860.

Bille, J. (1990). Epidemiology of listeriosis in Europe, with special reference to the Swiss outbreak. . In: Miller, Smith & Somkuti. Pp. 71-74

Black, J.G. (1999). Microbiology: Principles and Explorations. 4th Ed. Prentice Hall, New Jersey. 386, 600.

Board, R.G., Jones, D., & Skinner, F.A. (1992). Identification Methods in Alied and Environmental Microbiology. Blackwell Scientific Publications, Oxford. 193, 197.

Boerlin, P. & Piffaretti, J.C. (1991) Typing of human, animal, food, and environmental isolates of Listeria monocytogenes by multilocus enzyme electrophoresis. Alied and Environmental Microbiology 57, 1624–1629.

Borch, E., T. Nesbakken, and H. Christensen. 1996. *Hazard identification in swine slaughter with respect to foodborne bacteria*. Int. J. Food Microbiol. **30**:9-25.

Bradshaw, J.G., Peeler, J.T., & Corwin, JJ. (1985). Thermal resistance of Listeria monocytogenes in milk. *J. Food Protect.* **48** :743-755.

Brock, T.D. & Madigan, M.T. (1991). Biology of Microorganisms. 6th Ed. Prentice Hall, New Jersey.p p 553

Brooks, G.F., Butel, J.S.and Morse S. A., 1998. Jawetz, Melnick & Adlbergs Medical Microbiology. 21st ed. Stamford, CT: Appleton & Lange.

Broome, C.V., Gellin, B. & Schwartz, B. (1990). Epidemiology of listeriosis in the United States. CRC Press, New York. . 61–65.

Bryan F. L. (1982). Diseases transmitted by foods (A classification and summary); second edition, U.S. Department of Health and Human Services (HHS), Centers for Disease Control (CDC), Atlanta.

Buchanan, R.L., Damert, W.G., Whiting, R.C., & van Schothorst, M. (1997). Use of epidemiologic and food survey data to estimate a purposefully conservative dose-response relationship for Listeria monocytogenes levels and incidence of listeriosis. *J. Food Protect.***8**, 918 – 922.

Buncic, S., Paunovic, L., &Radisic, D. 1990. The fate of Listeria monocytogenes in fermented sausages and in vacuum-packaged frankfurters. *J. Food Prot*. **54**:413-417.

Bunning, V.K., Crawford, R.G., & Tierney, J.T. (1990). Thermotolerance of Listeria monocytogenes and Salmonella typhimurium after sublethal heat shock. *Al. Environ. Microbiol*.**56**:3216-3219.

Cassin, M.H., Paoli, G.M., McColl, R.S., and Lammerding, A.M.(1996). A comment on hazard assessment of Listeria monocytogenes in the processing of bovine milk. *J. Food Protect.***59**, 341-343.

Chambers PG, Grandin T, Heinz G, Srisuvan T (2001). Guidelines for human handling, transport and slaughter of livestock. Food and Agriculture Organisation (FAO) Publishers, Thailand.

Chant, S. (1999). Informal sector activity in the third world city. In Pacione, M. (ed). Alied Geography: Principles and Practice, London, Routledge Company.

Cho, S.-Y., Lim, C.-J., Lee, K.-H., Kim, K.-S., Ha, S.-D., 2011. Assessment of microbial contamination levels of street-vended foods in Korea. *Journal of Food Safety***31**, 41-47.

Chomvarin, C, Kotimanusvaniji, D Rhompruk (1993). Study on the correlation between the enterotoxin producing *Staphylococcus aureus* isolated from prepared food and cooks. Srinagarind Hos. Med. j. (6)231-242.

Clarence S. Y., Obinna C.N. & Shalom N.C. (2009) 'Assessment of bacteriological quality of ready-to-eat food (Meat pie) in Bennin City metropolis, Nigeria', *African journals of Microbiology research, vol.3*, **6**, 390-395.

Codex Alimentarius Commission, CAC. (2003). Principles and guidelines for the conduct of microbiological risk assessment (CAC/GL-30, 1999). 53 – 62. FAO.

Colburn, K. G., Kaysner, C. A., Abeyta, C. &Wekeel, M. (1990). Listeria species in a California Coast Estuarine Environment. *Alied and Environmental Microbiology*. **56**(7):2007-2011.

Collins, D. M., Wallbanks, S., Lane, J. D., Shah, J., Nietupski, R., Smida, J., Dorsch, M. and

Dainty, R. H., & B. M. Mackey, 1992. The relationship between the phenotypic properties of bacteria from chill-stored meatand spoilage processes. *J. Al. Bact.***73**:103S–114S

Department of Nutrition and Food Science, University of Ghana. Unpublished.

Devlieghere, F.; Francois, K.; Vereecken, K. M.; Geeraerd, A. H.; Van Impe, J. F. and Debevere, J. (2004), Effect of chemicals on the microbial evolution in foods. *J. Food Prot.*, **67**, 1977-1990.

Dobge, E. (2010). Risk of Listeria monocytogenes ingestion in consuming coleslaw from food vendors in the Accra metropolis. M.Phil Thesis. Department of Nutrition and Food Science, University of Ghana. Unpublished.

Dobge, E. (2010). *Risk of Listeria monocytogenes ingestion in consuming coleslaw from food vendors in the Accra metropolis*. M.Phil Thesis. Department of Nutrition and Food Science, University of Ghana. Unpublished.

Duffy, L.L.,Vanderlinde, P.B., &Grau, F.H. 1994.Growth of Listeria monocytogenes on vacuum-packed cooked meats; effects of low pH, aw, nitrite and ascorbate. *International Journal of Food Microbiology***23**: 377-390.

Dyckman, L.J. & J.E. Lansburgh, 2002. Meat and Poultry: Better USDA Oversight and Enforcement of Safety Rules Needed to Reduce Risk of Food-Borne Illness. In: Food Safety is anyone Watching, Smyth, V.L. (Ed.). Nova Science Publishers Inc., New York, USA.,

Elmer W. Koneman. Koneman's Color Atlas and Textbook of Diagnostic Microbiology. Lippincott Williams & Wilkins, 2006 -Medical-1565 pages

Embarek, P.K.B. 1994. Presence, detection and growth of Listeria monocytogenes in seafood: a review. *International Journal of Food Microbiology* **23**: 17-34.

Essuman, K.M. (1982). *The Effects of hot smoke-curing on the protein and organoleptic quality of West African herrings* (Sardinella eba) *processed in improved traditional ovens*. MSc. Thesis, Department of Nutrition and Food Science, University of Ghana

Fang, T, Que-Kim, W, Chia-Wei, L, Min-Ju,H, Tzu-Hui, W (2003). Microbiological quality of 18^{o}C ready-to-eat foods products sold in Tawian. *International Journal of food micro-biology*. **80**: 241-250

FAO/WHO.(2004). Risk assessment for Listeria monocytogenes in ready-to-eat foods, Interpretative Summary. Microbiological Risk Assessment Series 4 and 5. Rome.

Farber, J.M. & Peterkin, P.I. (1991). Listeria monocytogenes, a food-borne pathogen. Microbiological Reviews **55**, 476-511.

Farber, J.M., & B.E. Brown. (1990). Effect of prior heat shock on heat resistance of Listeria monocytogenes in meat. Al. Environ. Microbiol. 56: 1584-1587.

Farber, J.M., Ross, W.H., Harwig, J. (1996). Health risk assessment for Listeria monocytogenes in Canada. *Int. J Food Microbiol*. **30**, 145-156.

Farber, J.M., Wang, S.L., &Cai, Y.(1998). Changes in populations of Listeria monocytogenes inoculated on packaged fresh-cut vegetables. *J. Food Protect*. **61**: 192-195.

Fedio, W.M. & Jackson, H. (1989). Effect of tempering on the heat resistance of Listeria monocytogenes. *Lett. Al. Microbiol*.**9**:157-160.

Fehlduson, F. (1999). Foodborne bacterial pathogens. Marcel Dekker, New York.

Fellows, P. and Hilmi, M., (2011), "Selling street and snack foods", FAO, Rome.

Fenlon, D.R., Wilson, J. &Donachie, W. (1996). The incidence and level of Listeria monocytogenes contamination of food sources at primary production and initial processing. *Journal of Alied Bacteriology***81**, 641–650.

Food & Agriculture Organization (FAO). (2000). The state of world fisheries and aquaculture. FAO Fisheries Department, Rome.

Food Standard Australia New Zealand (2009), Guidelines for the microbiological examination of ready-to-eat foods,

Forsythe S. J. (2002). The microbiological risk assessment of food.Blackwell Publishing, Oxford, United Kingdom, Pp. 216.

Gallagher, D. L., Ebel, E.D., &Kause, J.R. (2003). Food Safety and Inspection Service (FSIS) risk assessment for Listeria monocytogenes in Deli Meats. United States Department of Agriculture.

Garbutt, J. (1997). Essentials of Food Microbiology. Arnold, London. 162 – 164.

Ghosh M, Wahi S, Kumar M, Ganguli A (2007). Prevalence of enterotoxigenic

Gilbert, R.J. (1992). Provisional microbiological guidelines for some ready-to-eat foods sampled at point of sale: Notes for PHLS Food Examiners. Public Health Serv. Lab. Q. 9:98-99.

Gill, C.O. & Jones, T. (1995) The presence of Aeromonas, Listeria and Yersinia in carcass processing equipment at two pig slaughtering plants. Food Microbiology 12, 135–141.

Gill, C.O. and T. Jones, 2000. Microbiological sampling of carcasses by excision or swabbing. *J. Food Prot.*, **63**: 167-173.

Gillespie, I, Little, C, Mitchell, R (2000). Microbiological examination of cold ready to eat sliced meats from catering establishments in United Kingdom. *J. Appl. Microbiol*,**88**: 467-474.

Gilling S., Taylor E. A., Kane K. and Taylor J. Z. (2001). Successful hazard analysis critical control point implementation in the United Kingdom: understanding the barriers through the use of a behavioral adherence model; *J. Food Protect*., **64**: 710-715.

Gilling, S.; Taylor, E. A.; Kane, K.; Taylor, J. Z. (2001), Successful hazard analysis critical control point implementation in the United Kingdom: understanding the barriers through the use of a behavioral adherence model. *J. Food Protect*, **64**, 710-715.

Glass, K.A., & Doyle, M.P. (1990). Fate of Listeria monocytogenes in processed meat products during refrigerated storage. Al. Environ. Microbiol. 55:1565–1569.

Guizani, N. &Mothershaw, A. (2007). Fermentation as a method for food preservation. In Rahman M.S. (ed). Handbook of food preservation.2nd ed. CRC Press, Boca Raton. 215

Hass, A. &Kreft, J. (1988). Listeria – biotechnological aspect of a pathogenic microorganism. *Inter. Industrial Biotech*. **8**:17 – 32.

Heinitz, M.L., Ruble, R.D., Wagner, D.E., &Tatini, S.R. (2000). Incidence of Salmonella in fish and seafood. *J. Food Protect*. **63**:579–592.

Hobbs, B.C. & Roberts, D. (1987). Food Poisoning and Food Hygiene. 5th Ed. Edward Arnold, London. p 217

Huda, N., Y.H. Shen, Y.L. Huey, R. Ahmad and A. Mardiah, 2010. *Evaluation of physico-chemical properties of Malaysian commercial beef meatballs*. Am. J. *Food Technol*., **5**: 13-21.

Ihekoronye, A.I. &Ngoddy, P.O.(1985). Integrated food science and technology for the tropics. Macmillan Publishers, New York. 339.

Ikeh, M.A.C., Obi, S.K.C., Ezeasor, D.N., Ezeonu, I.M., &Moneke, A.N. (2010). Incidence and pathogenecity profile of Listeria sp. isolated from food and environmental samples in Nsukka, Nigeria. African Journal of Biotechnology Vol 9(30). 4776-4782.

International Commission on Microbiological Specification of Foods (ICMSF). (1996). Microorganisms in Foods. 5th ed., Aspen Publishers, Inc., Gaithersburg.

Iyer, A., Kumosani, T., Yaghmoor, S., Barbour, E., Azhar, E.andHarakeh, S. 2013. Escherichia coli and Salmonella spp. in meat in Jeddah, Saudi Arabia. *J. Infect. Dev. Ctries.***7**(11):812-818.

Jay, J.M. (2003). Modern Food Microbiology, 6th Ed. Aspen Publishers, Inc., Gaithursburg. 485 – 505.

Jay, J.M., Loessner, M.J. & Golden, D.A. (2005). Modern food microbiology. 7th ed. Springer Science+Business Media, Inc. 591.

Jay, J.M., Loessner, M.J. and Golden, D.A. (2005) Modern Food Microbiology. New York: Springer.

Jemmi, T. &Keusch, A. (1994). Occurrence of Listeria monocytogenes in freshwater fish farms and fish-smoking plants. Food Microbiology, Volume 11, Issue 4, 309-316.

Kalliopi, R., Valentina, A., Rosalinda U., Paola, D., & Luca C. (2008). Detection, quantification and vitality of Listeria monocytogenes in food as determined by quantitative PCR. *International Journal of Food Microbiology***121** (2008): 88 – 105.

Kamat, A.A & Nair, P.M. (1994). Incidence of Listeria species in Indian sea foods and meat. *J. Food Saf*. **14**: 117-1130.

Karunasagar, I., Segar, K. & Goebel, W. (1992). Incidence of Listeria species in tropical sea foods. In: Listeria 1992. Abstract no. 155. Eleventh International Symposium on Problems of Listeriosis (ISOPOL XI). May 11th-14th. Copenhagen, Denmark.

Kathariou, S. (2002). Listeria monocytogenes virulence and pathogenicity, a food safety perspective. *J. Food Protect,***65:** 1811-1829.

Khairuzzaman, M., Chowdhury, F. M., Zaman, S., Mamun, A.A. and Bari, L. (2014) "Food Safety Challenges towards Safe, Healthy, and Nutritious Street Foods in Bangladesh", *International Journal of Food Science,* vol. 2014, 483519.

KhairuzzamanMd., F. M. Chowdhury,S.Zaman, A. A. Mamun, Md. L.Bari, 2014.*Food safety challenges towards safe, healthy, and nutritious street foods in Bangladesh*. International Journal of Food Science, Volume 2014,pp.**1**-9.

Kivi, M., A. Hofhuis, D.W. Notermans, W.J. Wannet and M.E. Heck et al., 2007. A beef-associated outbreak of Salmonella Typhimurium DT104 in the Netherlands with implications for national and international policy. *Epidemiol. Infect.*,**135**: 890-899.

Kornacki JL (2000) .The nuts and bolts of food safety. *Food Testing Anal***40**:18–22

Koussemon, M., R. Koffi-Nevry, K. Tano, M. Traore& A. Kamenan, 2008. Assessing the microbiological quality and conditions of sales of Cyprinuscarpio, Arius sp. and Cybiumtritor: Three fish species mostly consumed in Cote d'Ivoire. *J. Fish. Int*., **3**: 1-6.

Kwiatek, K. (2000). Occurrence of Listeria monocytogenes in selected foods of animal origin. Bull. *Vet. Inst. Pulawy***48**, 269-272.

Lado B, Yousef AE (2007) Characteristics of *Listeriamonocytogenes* important to food processors. Ch 6 In: Ryser ET, Marth EH (eds) Listeria, listeriosis and food safety. 3rd ed, CRC Press Taylor & Francis Group, Boca Raton, 157–213

Lammerding, A.M. (1998). An overview of microbial food safety risk assessment. *J. Food Prot.***60**:1420.

Lammerding, A.M., & J.M. Farber. (1994). The status of Listeria monocytogenes in the Canadian food industry. *Dairy Food Environ. Sanit*. **14**:146-150.

Lammerding, A.M., Fazil, A.M. & Paoli, G.M. (2001). Microbial Food Safety Risk Assessment. In Downs, F.P. and Ito, K. (eds). Compendium of methods for the microbiological examination of foods. American Public Health Association, Washington DC. 267 – 277.

Leimeister-Wächter M.,& Chakraborty T. 1989. Detection of listeriolysin, the thiol-dependent hemolysin in Listeria monocytogenes, Listeria ivanovii, and Listeria seeligeri. *Infection and Immunity.***57**: 2350-2357.

Lindqvist, R. &Westoo, A. (2000). Quantitative risk assessment for Listeria monocytogenes in smoked or gravad salmon and rainbow trout in Sweden. *International Journal of Food Microbiology***58**, 181 – 196.

Linton, R.H., Pierson, M.D., & Bishop, R. (1990). Increase in heat resistance of Listeria monocytogenes Scott A by sublethal heat shock. *J. Food Protect.***53**:924-927.

Lober, B. (2007). Listeriosis. In Goldfine, H. & Shen, H. Listeria monocytogenes: pathogenesis and host response. Springer Science+Business Media, LLC. p 13

Lunden, J.M., Miettinen, M.K., Autio, T.J. &Korkeala, H.J. (2000) Persistent Listeria monocytogenes strains show enhanced adherence to food contact surface after short contact times. *Journal of Food Protection***63**, 1204±1207.

Mackey, B.M., & N. Bratchell. (1989). The heat resistance of Listeria monocytogenes. Lett. *Al. Microbiol.***9**:89-94.

Mackey, B.M., Pritchet, C., & Norris. P. (1990). Heat resistance of Listeria: Strain differences and effects of meat type and curing salts. *Lett. Al. Microbiol*. **10**:251-255.

Mahale, D.P, Khade, R.G, Vaidya (2008). *Microbiological analysis of street vended fruit juices from Mumbai city*, India. Internet Journal of Food safety. **10**: 31-34.

Mahmood, M.S., Ahmed, A.N. & Hussain, I. (2003). Prevalence of Listeria monocytogenes in poultry meat, poultry meat products and other related inanimates at Faisalabad. *Pakistan Journal of Nutrition***2** (6): 346-349.

Manani T. A., Collison E. K., Mpuchane S. 2006. Microflora of minimally processed frozen vegetables sold in Botswana. *Journal of Food Protection* **69**:2581 – 2586.

Maripandi, A. & A.A. Al-Salamah, 2010. Multiple-antibiotic resistance and plasmid profiles of Salmonella enteritidis isolated from retail chicken meats. Am. *J. Food Technol.*, **5**: 260-268.

Maripandi, A. and A.A. Al-Salamah, 2010. *Multiple-antibiotic resistance and plasmid profiles of Salmonella enteritidis isolated from retail chicken meats*. Am. J. Food Technol., **5**: 260-268.

Mauro, C., Pierluigi, D. C., Domenico, M., Zanardi, E., Festino, A.R., Ghidini, S., Vergara, A., and Ianieri, A. (2008). Sources and tracking of Listeria monocytogenes in a cold-smoked processing plant. Ann. Fac. Medic. Vet. di Parma Vol. XXVIII 97-104

Mckellar, R. C. (1993), Effect of preservatives and growth factors on secretion of listeriolysin O by Listeria monocytogenes. *J. Food Protect*, **56**, 380-384.

McKellar, R.C., Moir, R. &Kalab, M. (1994) Factors influencing the survival and growth of Listeria monocytogenes on the surface of Canadian retail wieners. *J Food Protect*, **57**, 387–392.

Mead, P.S., Slutsker, L., Dietz, V., McCaig, L.F., Bresee, J.S., Shapiro, C., Griffin, P.M., and Tauxe, R.V. 1999.Food-related illness and death in the United States. Emerg. Infect. Dis. **5**: 607-625

Medeiros L., Hillers V. N., Kendall P. A. and Mason A. (2001).*Evaluation of food safety education for consumers;* J. of Nutrition Educ., **33**: 27-34.

Mensah, P., Yeboah-Manu, D., Owusu-Darko, K., &Ablordey, A. (2002). Street foods in Accra, Ghana: how safe are they? Bulletin of the World Health Organization 2002; 80:546- 554.

Microbiological Examination of Foods. 3rd Ed. Washington D.C.: American Public Health Association.

Miller, R.K., 2002. Factors affecting the quality of raw meat, In: Meat processing Improving quality. Joseph, K., K. John & D. Ledward (Eds.), CRC Press, FL, USA, : 26-63. ISBN: 978-1-59124-484-4

Montville, T.J. & Matthews, K.R. (2005). Food Microbiology: An Introduction. ASM Press, Washington D.C. 159 – 171.

Muinde, O.K. and Kuria. E. (2005) *Hygienic and Sanitary Practices of Vendors of Street Foods in Nairobi, Kenya*. African Journal of Food Agriculture and Nutritional Development, **5**, 1-14.

Munhande, C. &Makaye, P. (2008). The contribution of informal food trading to urban food security in Zimbabwe: the case of Gweru 200-2007. Journal of Sustainable Development in Africa, Vol. 10 No. 2.

Murray, E. G. D., Webb, R. E. & Swann, M. B. R. (1926). A disease of rabbits characterized by a large mononuclear leucocytosis caused by a hithero undescribed bacillus. Bacterium monocytogenes (n. sp). *J. Path. Bacteriol*.**29**:40 – 439.

Nesbakken, T., Kaerud, G. &Caugant, D.A. (1996) Pathways of Listeria monocytogenes contamination in the meat processing industry. *International Journal of Food Microbiology* **31**, 161–171.

Okutani, A., Okada, Y., Yamamoto, S., &Igimi, S. Nationwide survey of human Listeria monocytogenes infection in Japan. Epidemiol. Infect. (2004), 132, 769 – 772.

Prentice, G.A. &Neaves, P. (1992). The Identification of Listeria Species. In Board, R.G., Jones, D. & Skinner, F.A. (eds). Identification Methods in Alied and Environmental Microbiology. Blackwell Scientific Publications, New York. 283-296.

Prescott, L.N., Harley, J.P. & Klein, D.A. (1995). Microbiology. 3rd ed. Wm. C. Brown Publishers, Dubuque. 850.

Promoting local innovation in Northern Ghana. The Association of Church Development Projects (ACDEP). 7(2), pp. 35-37.

Rahman, M.S. &Perera, C.O. (2007). Drying and food preservation. In Rahman M.S. (ed). Handbook of food preservation.2nd ed. CRC Press, Boca Raton. p414

Rahman, M.S. &Perera, C.O. (2007). Drying and food preservation. In Rahman M.S. (ed). Handbook of food preservation.2nd ed. CRC Press, Boca Raton. 414

Rahman, S.F., 1999a. Post-harvest handling of foods of animal origin. In: Handbook of food preservation. Rahman. S.F. (ed). Marcel Dekker, NY,: 47-54. ISBN: 0-8247-0209-3.

Ramaswamy, V., V. M. Cresence, J. S. Rejitha, M. U. Lekshmi, K. S. Dharsana, S. P. Prasad, and H. M. Vijila. 2007. "Listeria*: Review of Epidemiology and Pathogenesis*." Journal of Microbiology, Immunology, and Infection **40**: 4–13.

Redmond E. C. and Griffith C. J. (2003). Consumer food handling in the home: a review of food safety studies; J. Food Prot., **66**: 130–161.

Reij MW, Den Aantrekker ED (2004) Recontamination as a source of pathogens in processed foods. *Int J Food Microbiol***91**:1–11

Robinson, R. K., Batt, C. A. & Patel, P. D. (editors) (2000). *Encyclopedia of Food Microbiology. San Diego, CA: Academic Press Staphylococcus aureus and Shigella spp. in some raw street vended Indian foods*. Int J Environ Health Res. **17**(2):151–157.

Rodriquez-Lazaro, D.,Hernandez, M., Scortti, M., Esteve, T., Vazquez-Boland, J.A., &Pla, M. 2004. Quantitative Detection of Listeria monocytogenes and Listeria innocua by Real-Time PCR: Assessment of hly, iap and lin02483 Targets and AmpliFluor Technology. *Journal of Alied and Enviromental Microbiology***70**: 1366-1377.

Ryser, E.T. & Marth, E.H. (1988). Survival of Listeria monocytogenes in cold-pack cheese food during refrigerated storage. *J. Food Protect*. **51**:615-621.

Sammarco, M.L., Ripabelli, G., Ruberto, A., Iannitto, G., & Grasso, G.M. (1997) Prevalence of salmonellae, listeriae and yersiniae in the slaughterhouse environment

and on work surfaces, equipment, and workers. *Journal of Food Protection***60**, 367–371.

Schlech W.F. (1999). Pathogenesis and immunology of Listeria monocytogenes. PatholBiol (Paris) 44: 775–782

Schmidhuber, J. &Tubiello, F.N. (2007). Global food security under climate change. National Academy of Sciences, USA. 104, 50:1970319708

Seeliger, H. P. R. & Jones, D. (1986). Genus Listeria pirie, 1940,. In: Bergey's Manual of Systematic Bacteriology, Vol. 2 (Sheath, Mair, Sharpe & Holt (ed.), Williams & Wilkins, Baltimore MD. 338.

Seeliger, H. P. R. & Jones, D. (1986).Listeria. In Bergey's Manual of Systematic Bacteriology, vol. 2, . 1235–1245. Edited by P. H. A. Sneath,N. S. Nair, N. E. Sharpe & J. G. Holt. Baltimore: Williams & Wilkins.

Seeliger, H. P., &Hohne, K. 1979. Serotyping of Listeria monocytogenes and related species. Methods Microbiology. 13:31-49.

Shelef, L.A. (1989). Survival of Listeria monocytogenes in ground beef or liver during storage at 4oC and 25oC. *J. Food Protect.***52**:379–383.

Slutsker L, Schuchat A (1999). Listeriosis in humans. In: Ryser ET, Marth EH (Eds.), *Listeria*, Listeriosis and Food Safety, 2nd Edition. Marcel Dekker, New York, 75-96.

Stackebrandt, E. (1991). Unrooted tree or network showing the phylogenetic inter – relationship of listeria and other G+C – content gram – positive taxa. *Int. J. System. Bacteriol*. **41**:240 – 246.

Sutherland, P.S. and Porritt, R.J. (1997) Listeria monocytogenes. In: Food-borne microorganisms of public health importance, 5th Edition. AIFST (NSW Branch), Sydney. 333-378.

Swaminathan, B. (2001). In Doyle, M. P., Beuchat, L.R., & Montville, T.J. (eds). Food Microbiology: Fundamentals and Frontiers, 2nd Ed. ASM Press, Washington D.C. 383-409.

Teye, G. A. and Salifu, S. (2006). The contribution of the various ruminant species to meat production in the Tamale Metropolis. The Savanna Farmer **7**, 12, *2006.*

The United Kingdom Health Protection Agency (HPA). (2009). Detection and enumeration of Listeria monocytogenes and other Listeria species. Issue No. 3.1. Standards Unit, Department for Evaluations, Standards and Training. Reference No. F19i3.1

The United States Centre for Disease Control and Prevention (CDC). (2009). Emerging pathogens and their estimated annual outbreaks of foodborne illnesses in the USA.

The United States Centre for Disease Control and Prevention (CDC). (2011). Outbreak of listeriosis Linked to Cantaloupe.

Thurston C. (2006). The food safety enhancement act; how a laboratory information management system (LIMS) can help you be prepared; *IFST*, **24** (3).

Tompkin, R.B. (2002). Control of Listeria monocytogenes in the food-processing environment. *J. Food Protect*. ***65***:709–725.

United States Centre for Disease Control and Prevention (CDC). (2009). Annual outbreaks of food-borne illnesses.

Vazquez-Boland, J. A., M. Kuhn, P. Berche, T. Chakraborty, G. Dominguez-Bernal, W. Goebel, B. Gonzalez-Zorn, J. Wehland, and J. Kreft. 2001 Listeria pathogenesis and molecular virulence determinants Clin. Microbiol. Rev. **14** 584–640

Wanyenya, I.; Muyanja, C.; Nasinyama, G. W. (2004), Kitchen practices used in handling broiler chickens and survival of *Campylobacter* spp. on cutting surfaces in Kampala, Uganda. *J. Food Protect.*,**67**, 1957-1960.

Warriss, P. D. (2001). Meat hygiene, spoilage, and preservation: Meat Science, an introductory text, school of veterinary science, University of Bristol; CABI International, UK. Pp.182-192.

WHO-INFOSAN., 2010. Safety of street-vended food. INFOSAN Information Note No. 3/2010, June 30, 2010.http://www.who.int/foodsafety/fs_management/No_03_StreetFood_Jun10_en.pdf.

Zaika, L.L., Palumbo, S.A., Smith, J.L., Del Corral, F., Bhaduri, S., Jones, C.O., & Kim, A.H. 1989. Destruction of Listeria monocytogenes during frankfurter processing. *J. Food Prot.***53**:18-2

APPENDICES

APPENDIX A: CONSUMER QUESTIONNAIRE

DEPARTMENT OF FOOD SCIENCE, KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

PREVALENCE OF *LISTERIA MONOCYTOGENES* IN READY TO EAT MEAT IN ACCRA

Dear respondent, this questionnaire is to help gather some information on the consumption of processed grilled meat (Khebabs) in Ghana, as part of an Msc Food Quality Management Thesis on the topic above. The information you provide remains confidential and will be used for academic purposes only. Thank you.

Date: _____

Location: _____

Please thick (√) or circle the responses that you desire. Where appropriate, write out your own responses in the spaces provided.

BACKGROUND INFORMATION

Sex:
Male=M
Female=F

Age:
1=Less than 20 years
2=20-29 years
3=30-39 years
5=40-49 years
5=50-59 years and above

Highest level of education
1=None

2=Primary
3=Middle School/JHS
4=Secondary
5=Tertiary
6=Other, specify……

PATTERNS FOR MEAT CONSUMPTION
Do you consume grilled meat?
1=Yes
2=No

If yes to 4, which of the following processed meat do you consume?
1=Grilled meat
2=Fried meat
3=Boiled meat
4=Smoke meat
5=Others, specify……

Which spice of processed meat do you consume?
1=Goat
2=Sheep
3=Cow
4=Other, specify……..

How often do you consume the meat product?

Skewed grilled meat with lots of species
1=Daily
2=Once a month
3=2-3 times a week
4= Once a week
5= Specify……..

Which of the meat do you consume?
1=Goat
2=Sheep
3=Cow
4=Chicken
Specify……..

How many do u consume at an instance?
1=1 stick
2=2 sticks
3=3 sticks
4=more than 3 sticks

CONSUMPTION BY HIGH RISK GROUPS
I. CHILDREN

Do you have children (Above 3 years) in your home?

1=Yes
2=No

How often do they consume grilled meat?
1=Daily
2=Once a month
3=2-3 times a week
4= Once a week
5= Specify……..

Which of the meat do they consume?
1=Goat
2=Sheep
3=Cow
4=Chicken
Specify……..

III. PREGNANT WOMEN

MEAT HANDLING PRACTICES

Where do you buy your raw meat?
1=Informal market
2=Slaughter houses
3=House to house vendors
4= Others, specify……

How much processed meat do you often purchase?
1=enough quality for one meal
2= enough quality for three days
3= enough quality for one week
4= enough quality for one month

How do you store raw meat?
1=In refrigerator
2=Steaming
3=Boil
4=Others, specify……
In what form(s) do you consume processed meat?
1=Grilled with species
2=Grilled with pepper
3= Others, specify……

APPENDIX B: PROCESSOR QUESTIONNAIRE

DEPARTMENT OF FOOD SCIENCE, KWAME NKRUMAH UNIVERSITY

OF SCIENCE AND TECHNOLOGY

RISK ASSESSMENT FOR *LISTERIA MONOCYTOGENES* IN READY TO EAT MEAT IN ACCRA

Dear respondent, this questionnaire is to help gather some information on the consumption of processed grilled meat (Khebabs) in Ghana, as part of anMsc. Food Quality Management Thesis on the topic above. The information you provide remains confidential and will be used for academic purposes only. Thank you.

Date: _____
Location: _____

Processor Code: _____

Please thick or circle the responses that you desire. Where appropriate, write out your own responses in the spaces provided.

BACKGROUD INFORMATION

Sex:
1=Male=M
2=Female=F

Age:
1=Less than 20 years
2=20-29 years
3=30-39 years
5=40-49 years
5=50-59 years and above

Highest level of education
1=None
2=Primary
3=Middle School/JHS
4=Secondary
5=Tertiary
6=Other, specify……

How long have you been in the processing business?
1= 1-5 years
2= 6-10 years

3=11-15 years
4= 16-20 years
5= More than 20 years

What kind meat products do you process? Tick as many as apply to you.
1=Smoke meat
2=Boiled meat
3=Grilled meat
4=All the above
5=Other, specify……

B: RAW MEAT ACQUISITION
What kind of meat do you process?
1=Goat meat
2= Cow meat
3= Chicken meat
4= Guinea fowl meat
5=Other, specify……..

Where do you get your raw meat?
1=Vendors
2=Slaughter houses
3=Cold store
4=Open market
5=Other, specify…….

What species of meat do you process?
1=Goat
2=Sheep
3=Cow
4=Specify…….

Do you inspect fresh/raw meat before purchasing?
1= Yes
2= No

If yes to Q27, what do you look out for?
1= Color of meat
2= Texture of meat
3= Odor of meat
4= Specify…….

D. RAW MEAT TRANSPORTATION

How long does it take to transport raw meat to the processing site?
1=Less than 30 minutes
2=30mins – 1 hour
3=More than 1 hour, less than 10 hours
4=More than 10 hours, less than 24 hours
5=More than 24 hours

How do you transport the raw meat to the processing site?
1= By foot
2= Public transport
3= Private transport
4= Refrigerated truck/van
5= Other, specify…….

What containers do you use to carry the raw meat during transportation?
1=Bowls
2=Sacks
3=Ice chest
4=Other, specify……

E. PROCESSING OF MEAT
Do you wash your hands before starting processing?
1= Yes
2= No

What do you use to wash your hands?
1=Only water
2=Water and soap
3= Specify……

How long do you keep the meat before processing?
1=Less than 30 minutes
2=30mins – 1 hour
3=More than 1 hour, less than 1 week
4=More than 1 day, less than 1 week

How do you keep raw meat before starting processing?
1=At room temperature
2=In a fridge
3=In a freezer
4=Other, specify……

Describe how you process your meat.

How do you know when raw meat is adequately processed?

How much meat do you process at a time/what constitute a batch?
1=15 kilos
2=20 kilos
3=20-30 kilos
4=> 30 kilos
What do you do to keep raw meat from spoiling when processing is delayed?

F. HANDLING AND STORAGE OF GRILLID MEAT

Where do you store processed meat?
1= Regular room
2= Boiling
3=In refrigerator
4=In deep freezer, freezer compartment of refrigerators
5=Other, specify……

How is the processed meat stored?
1= In sacks
2= In bowls
3= In solid boxes (not perforated)
4= On shelves
5= Other, specify……

For how long after processing do you store meat before selling?
1=Less than 1 day
2=1-3 days
3=More than 3 days, less 1 week
4=1 week – 1 month
5=More than a month

G. TRANSPORTATION OF GRILLED MEAT

Do you process meat before you transport to where you grill them?
Approximately how long does it take to transport processed meat from the storage/processing site to where you grill them?
1=Less than 30 minutes
2=30mins – 1 hour
3=1-3 days
4=4-12 hours
5=More than 12 month

How do you transport processed meat to where you grill them?
1= By foot
2= Public transport
3= Private transport
4= Refrigerated truck/van
5= Other, specify……

Where exactly do you grill and sell the meat?
1=Drinking bars
2=Market places
3=On the street in traffic
4=At churches
5=Specify……

THANK YOU

APPENDIX C: RAW DATA OF RESULTS FOR THE VARIOUS ISOLATES

Area	Count	Raw meat	*Grilled meat*	*Area*	Count	Raw meat	*Grilled meat*
Adenta	**TVC** (x10^5cfu/g)	172	246	***Dome***	**TVC** (x10^5cfu/g)	12	121
	TCC (x10^4cfu/g)	166	0		**TCC** (x10^4cfu/g)	186	42
	E. coli (x10^5cfu/g)	274	0		***E. coli*** (x10^5cfu/g)	102	39
	LC (x10^4cfu/g)	40	76		**LC** (x10^4cfu/g)	200	39
Banana Inn	**TVC** (x10^5cfu/g)	204	102	***Dzorwulu***	**TVC** (x10^5cfu/g)	2	234
	TCC (x10^4cfu/g)	20	15		**TCC** (x10^4cfu/g)	0	0
	E. coli (x10^5cfu/g)	108	19		***E. coli*** (x10^5cfu/g)	0	125
	LC (x10^4cfu/g)	68	2		**LC** (x10^4cfu/g)	3	1
Bubiashie	**TVC** (x10^5cfu/g)	3	160	***Jamestown***	**TVC** (x10^5cfu/g)	1	106
	TCC (x10^4cfu/g)	240	13		**TCC** (x10^4cfu/g)	200	36
	E. coli (x10^5cfu/g)	180	8		***E. coli*** (x10^5cfu/g)	190	168
	LC (x10^4cfu/g)	129	15		**LC** (x10^4cfu/g)	86	3
Cantoment	**TVC** (x10^5cfu/g)	220	2	***Korlebu***	**TVC** (x10^5cfu/g)	210	2
	TCC (x10^4cfu/g)	210	10		**TCC** (x10^4cfu/g)	240	3
	E. coli (x10^5cfu/g)	42	82		***E. coli*** (x10^5cfu/g)	124	1
	LC (x10^4cfu/g)	1	0		**LC** (x10^4cfu/g)	110	10
Dansoman	**TVC** (x10^5cfu/g)	2	2	***Kwashieman***	**TVC** (x10^5cfu/g)	246	80
	TCC (x10^4cfu/g)	239	30		**TCC** (x10^4cfu/g)	200	20
	E. coli (x10^5cfu/g)	12	1		***E. coli*** (x10^5cfu/g)	200	81
	LC (x10^4cfu/g)	23	18		**LC** (x10^4cfu/g)	150	16

Area	Count	Raw meat	*Grilled meat*
Labone	**TVC** (x10^5cfu/g)	2	242
	TCC (x10^4cfu/g)	0	51
	E. coli (x10^5cfu/g)	0	21
	LC (x10^4cfu/g)	0	7
Laterbiorkshie	**TVC** (x10^5cfu/g)	104	1
	TCC (x10^4cfu/g)	0	68
	E. coli (x10^5cfu/g)	9	2
	LC (x10^4cfu/g)	34	21
Legon	**TVC** (x10^5cfu/g)	2	152
	TCC (x10^4cfu/g)	476	0
	E. coli (x10^5cfu/g)	1	4
	LC (x10^4cfu/g)	55	34
Mamobi	**TVC** (x10^5cfu/g)	3	72
	TCC (x10^4cfu/g)	0	22
	E. coli (x10^5cfu/g)	0	53
	LC (x10^4cfu/g)	0	78
Nima	**TVC** (x10^5cfu/g)	94	40
	TCC (x10^4cfu/g)	136	0
	E. coli (x10^5cfu/g)	156	0
	LC (x10^4cfu/g)	29	22

Area	Count	Raw meat	*Grilled meat*
North Kaneshie	**TVC** (x10^5cfu/g)	254	242
	TCC (x10^4cfu/g)	210	7
	E. coli (x10^5cfu/g)	62	185
	LC (x10^4cfu/g)	0	1
Roman Ridge	**TVC** (x10^5cfu/g)	163	247
	TCC (x10^4cfu/g)	10	0
	E. coli (x10^5cfu/g)	8	0
	LC (x10^4cfu/g)	1	0
Tabora	**TVC** (x10^5cfu/g)	1	17
	TCC (x10^4cfu/g)	1	0
	E. coli (x10^5cfu/g)	3	0
	LC (x10^4cfu/g)	2	3
Sowutuom	**TVC** (x10^5cfu/g)	2	2
	TCC (x10^4cfu/g)	129	8
	E. coli (x10^5cfu/g)	31	4
	LC (x10^4cfu/g)	4	2
Madina	**TVC** (x10^5cfu/g)	205	185
	TCC (x10^4cfu/g)	182	6
	E. coli (x10^5cfu/g)	438	0
	LC (x10^4cfu/g)	50	156

APPENDIX D: BIOCHEMICAL ANALYSIS

Purification of *Listeria monocytogenes* was done by streaking on nutrient agar before incubating plates at 37ºC for 24 hours (Board *et al*., 1992). The pure colonies obtained through the Gram reaction were used for the subsequent confirmatory identification tests. *Listeria monocytogenes* was subjected to test such as Catalase test, Indole, Urease test, β- Haemolytic test, and others (Plate 4.1).

Plate 4.2: Biochemical tests of meat samples showing different reactions

Table 4.3: Biochemical characterization of the *L. monocytogenes* in the grilled samples

Isolate	Catalase reaction	TSI	H_2S	MIU	Urea	Cit.	β-H	Presumptive organism	Confirmed organism
Jt	+	+	+	+	-	-	+	***Lm***	***Lm***
Tb	+	+	+	+	-	+	+	***E. coli, Lm***	-
Lb	+	-	+	-	-	+	+	***Enterobacter* spp.**	-
Nm	+	+	-	+	+	-	+	***Salmonella* spp., *Lm***	***Lm***
Mb	+	+	-	+	-	-	+	***Salmonella* spp.**	***Lm***
Bsh	+	-	-	+	-	-	-	***E. coli***	-
NK	+	-	-	-	-	-	+	***Enterobacter* spp, *Listeria* spp.**	-
Lt	+	+	+	+	+	+	-	***Proteus mirabilis, Pseudomonas***	-
Dm	+	+	-	+	+	+	+	***Enterobacter aerogenes***	***Lm***
Kw	+	+	-	+	-	-	+	***Morganellamorganii***	-

Key:
Jt(Jamestown), **Tb**(Tabora), **Lb**(Labone), **Nm**(Nima), **Mb** (Mamobi), **Bsh**(Bubiashie), **NK**(NorthKaneshie), **Lt**(Latebiorkorshie), **Dm**(Dome), **Kw**(Kwashieman), **TSI** (Triple Sugar Iron test), **MIU** (Motility Indole Urease test), **β – H** (Beta Haemolysis), **Lm** (*Listeria monocytogenes*)