THE MICROBIOLOGICAL SAFETY STATUS OF BEEF PRODUCED IN THE KUMASI METROPOLIS

By

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DECLARATION

I hereby declare that this submission is my own work piece towards MPhil. in Meat Science. To the best of my knowledge, it contains neither materials already published by others nor materials which have been accepted for the award of any other degree of the University, except where due acknowledgement has been made in the text.

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ABSTRACT

Meat is an important part of the diet for good growth. Almost all the essential amino acids could be found in it. Problems associated with meat eating cannot be overlooked. Ingestion of contaminated meat is one of the risks in meat eating and many food poison cases were found to have resulted from contaminated meat dishes. Looking at the way beef is produced and distributed and displayed for sales in the Kumasi Metropolis, there could be a possible chance of contamination. The study investigated the kinds of microbes and their levels found on thirty (30) different carcasses, considering the sticking knives, the inner surface of the carcasses after evisceration, the surface of the carcasses after skin removal and the last knives used in trimming the carcasses in the Kumasi Abattoir Company Limited slaughter plant. Samples taken were swabs from the sites mentioned above. Twenty (20) butchers were selected from Atonsu and Mayaanka markets where their benches, knives and meat surfaces were swabbed. The microbial quality of four hundred and eighty (480) swab samples were aseptically collected and analyzed using standard microbiological techniques. Pieces of beef samples collected from the slaughter plant had mean pH range of 6.41 and 8.40 with standard deviations of ±0.03 and ±0.05 respectively. Beef pieces collected from the markets also had mean pH range of 7.21 to 8.68 with standard deviations of ±0.04 and ±0.24. It was observed that the slaughter plant did not practise many hygienic slaughter practices that would produce beef of acceptable levels of microbial loads. The sticking knives recorded mean log values of 13.41 to 15.23 CFU/cm^2 for TPC, 8.05 to 9.41 MPN/cm^2 for TCC, 5.21 to 6.38 MPN/cm^2 for FCC, 2.61 to 4.33 MPN/cm^2 for ECC and 3.93 to 5.27 CFU/cm^2 for SC. The inner surface of the carcasses after evisceration recorded mean log values 13.25 to 14.56 CFU/cm^2 for TPC, 8.26 to 9.74 MPN/cm^2 for TCC, 4.73 to 6.18 MPN/cm^2 for FCC, 2.59 to 4.20 MPN/cm^2 for ECC and 3.49 to 4.78 CFU/cm^2 for

SC. The surface of the carcasses after skin removal recorded mean log values of 13.60 to 14.66 CFU/cm^2 for TPC, 7.81 and 9.14 MPN/cm^2 for TCC, 4.46 from 6.11 MPN/cm^2 for FCC, 2.81 to 4.85 MPN/cm^2 for ECC and 3.62 to 5.59 CFU/cm^2 for SC. The last knives used in trimming carcasses recorded mean log values of 13.66 and 14.78 CFU/cm^2 for TPC, 7.81 to 9.39 MPN/cm^2 for TCC, 4.76 to 6.14 MPN/cm^2 for FCC, 2.76 to 4.20 MPN/cm^2 for ECC and 3.71 to 5.27 CFU/cm^2 for SC. It was observed that the butchers did not care about microbial safety of the beef they sold. High levels of microbial loads were recorded from the samples collected from their benches, knives and the meat surfaces in the markets. The benches recorded mean log values of 13.66 to 15.18 CFU/cm^2 for TPC, 7.92 to 9.11 MPN/cm^2 for TCC, 5.70 to 7.01 MPN/cm^2 FCC, 3.48 and 5.17 MPN/cm^2 for ECC and 4.47 to 5.99 CFU/cm^2 for SC. The knives recorded mean log values 13.46 to 14.55 CFU/cm^2 for TPC, 8.67 and 9.42 MPN/cm^2 for TCC, 6.42 and 7.33 MPN/cm^2 for FCC, 3.26 and 5.14 MPN/cm^2 for ECC and 5.28 and 6.03 CFU/cm^2 for SC. The meat surfaces also recorded mean log values 13.46 to 14.55 CFU/cm^2 for TPC, 7.85 to 9.14 MPN/cm^2 for TCC, 4.27 and 7.08 MPN/cm^2 for FCC, 3.53 to 4.92 MPN/cm^2 for ECC and 4.57 and 5.97 CFU/cm^2 for SC. Other microbes like *Pseudomonas spp.*, *Enterobacteriaceae, Staphylococcus spp.* and *Bacillus spp.* were also found on the samples taken from both the slaughter plant and the markets at different levels. The beef produced at K.A.C.L. slaughter plant and the samples from the Atonsu and Mayaanka markets had very bad microbiological status. K.A.C.L management and the butchers were more concerned with their business than the health of the public.

DEDICATION

To my parents, Mr. Yaw Acheampong and Mrs. Felicia Acheampong, my siblings, Rita Acheampong, Clement Acheampong, Atta Agyeman Duah and Ammah Amoah Acheampong not forgetting my grandmother Madam Mary Nyarko and Yaw Boateng Ampadu for their love, encouragement and support through this level of my education.

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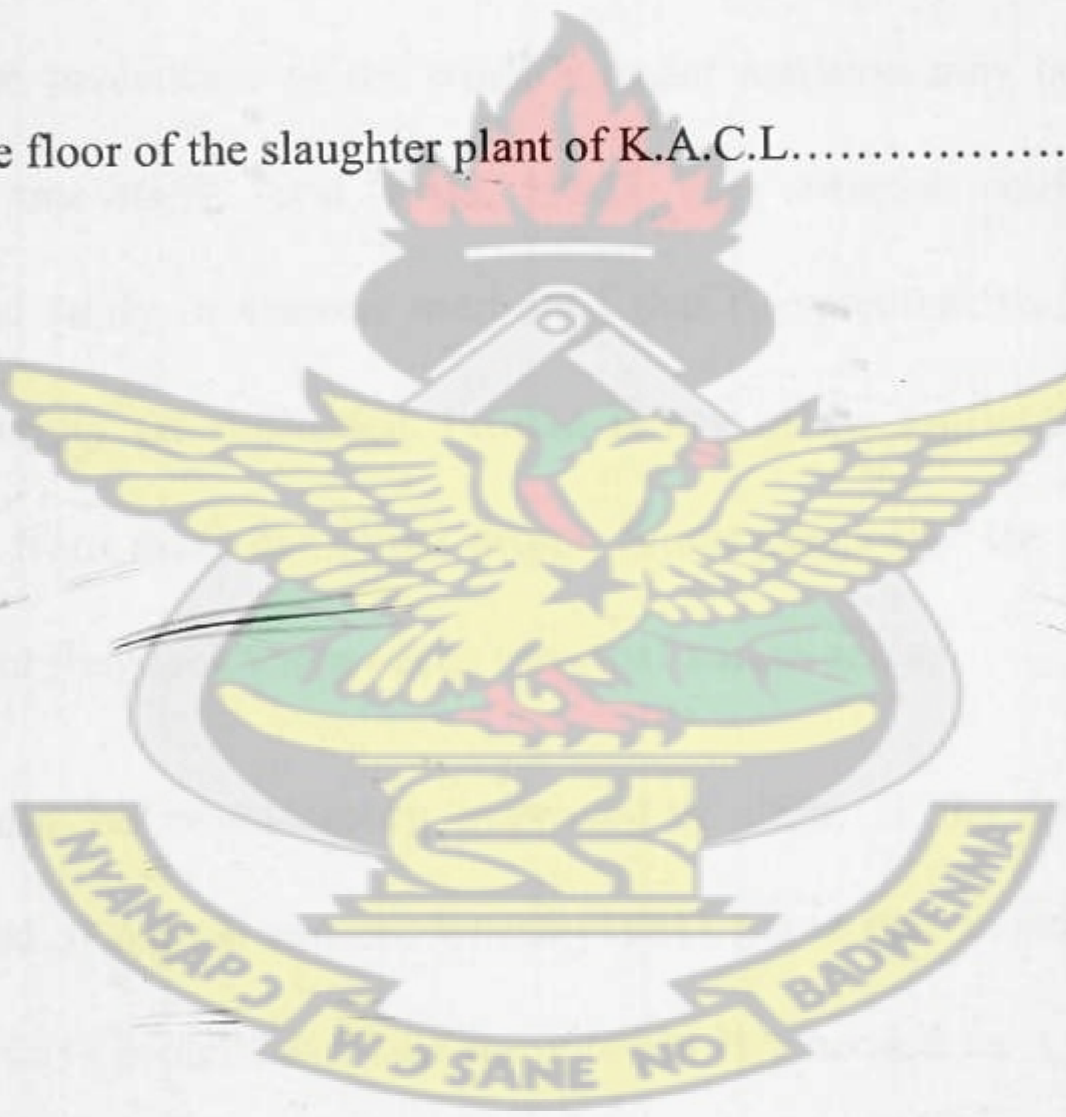


CHAPTER ONE

1.0 INTRODUCTION

Living things source nutrients needed for growth, maintenance and productive activities from the food they eat. The food for such purpose should be balanced in the right proportion for growth, development and maintenance as well as production. There should be different kinds of food stuff that can satisfy the various nutrients required. A large percentage of the world's under nutrition may be due to the high consumption of one staple food (FAO, 1992). This situation could be remedied if there is a careful study in various food stuff that complement each other when the nutrients they supply are discussed. Protein is one of the nutrients needed and this could be gotten from plant or animal origin. Some plants like the legumes are very rich in protein but they lack some of the essential amino acids.

Many diets could be considerably improved if there is the inclusion of small quantities of food of animal source. Jensen (1981) found that as little as 25g of meat will give about 45% protein and half the vitamin B_{12} needed by a child daily. Meat refers to the edible parts of mammals but this definition has been broadened to include all animals as well as fish, poultry, shellfish and some uncommon species like frog and alligators (Nakai and Modler, 2000). Hammer (1987) suggested that aside the skeletal muscles and associated fats, organs like lungs, brain, skin, liver, kidneys, bone marrow, blood and some other internal organs could be referred to as meat. Meat is a kind of food that complements many diets especially those food stuff from plant sources.

Meat and meat products have high concentrations of good quality protein containing the almost all essential amino acids which are absent in plant protein. There is the supply of most of the easily absorbed minerals and vitamins from meat. Components of food which are found in higher concentrations than a few milligrams or micrograms per 100g of a particular kind of food and that they often give energy are called macronutrients. Fortunately these nutrients make up about 98% of the edible portion of meat with water included (Simonsen *et al.,* 1988). The components of a typical meat are about 20% protein, 70% water, 5% lipid and 5% other nutrients like carbohydrates, salts, vitamins and a host of others (Nakai and Modler, 2000). There is high biological value protein in meat with vitamins like vitamin $B_{12,}$ niacin, vitamin $B_{6,}$ vitamin D and minerals like zinc, phosphorus and iron are in abundance in meat. Long chain omega-3 polyunsaturated fats, riboflavin, selenium and pantothenic acid could be well sourced from meat.

With all these and other benefits derived from meat, the risks associated with the eating of meat cannot be overlooked. The issue of food security is complicated where protein sources like meat and its products, fish and fishery products are commodities associated with high risk with respect to contamination by pathogens and their toxins and other possible adulterants (Yousuf *et al.*, 2008). One of the risks involved in eating meat is the possible ingestion of contaminated meat. As much as meat has a high concentration of nutrients needed by humans for healthy living, microorganisms also find meat as a good source of food for their growth and survival. Their presence in meat may render the meat unwholesome for humans.

Proper slaughterhouse design and Good Manufacturing Practices (GMP) are very important to ensure meat safety. The safety of our food is an issue of public health interest all over the world.

It becomes more important if food is produced, handled and processed in an environment likely to be highly contaminated (Anonymous, 1996). Though there are advances in modern technology, food borne illnesses have become important concern in both developed and developing countries. A number of fresh foods especially those from animal source are highly susceptible to microbial invasion and for that matter food poisoning.

The raw meats in retail shops have been found to be potential means for spreading food-borne diseases and therefore there is a need for the practice of Hazard Analysis Critical Control Point (HACCP) and the education of consumers on food safety (Zhao *et al.*, 2001).

Raw meats could be easily contaminated with bacteria which might be very harmful to humans (Burgess *et al.*, 2005; Tutenel *et al.*, 2003). Bacterial food contamination is widespread and occurs when our environments are untidy and the foods are not hygienically produced and maintained. Soyiri *et al.* (2008) found that many slaughterhouses in Ghana have very poor facilities for meat production and do not have HACCP systems available and this can result in heavy loads of microbes. This heavy microbial load makes the meat unwholesome for human consumption or lead to incidence of food poisoning.

Sources of microbes in meat could be inherent micro-flora found in the tissues of animals, the air, surroundings, or contamination due to unhygienic slaughtering conditions, poor handling practices and bad processing conditions.

Hobbs and Roberts (1993) reported that the major bacterial pathogens are *Salmonella spp, Staphylococcus aureus, Clostridium botulinum, Clostridium perfringens, Bacillus cereus* and *Escherichia coli.* These microbes cause microbiological and biochemical changes in meat and result in the production of toxic substances leading to the events of sicknesses like typhoid fever, cholera or other fatal diseases and even death in the

extreme cases (Soyiri *et al.*, 2008). Yeast and moulds also cause meat spoilage and for that matter a threat to meat eating habit (Anonymous, 1996).

According to Hobbs and Roberts (1993), more than 74% of incidences of food poisoning are as result of meat dishes. Food safety in general is evaluated in terms of acceptable levels of risk. Given the scope and magnitude of meat production and supply in our Ghanaian setting, it is very difficult to ensure that all the meat is kept free from potential sources of contamination.

Meat and for that matter beef safety is enhanced by systematically concentrating upon lowering chances for pathogenic bacteria contamination at every point from animal production, meat production and processing to distribution, preparation and even consumption. Different microbes are introduced onto meat as contaminants at different stages of beef production and processing when proper sanitary measures are lacking (Sumner *et al.*, 2003; Ebel *et al.*, 2004). Some of the microbes are inherent in the healthy animals on low counts but due to poor handling of animals before and during slaughter, they increase in number. Others are gotten onto the meat by contaminated equipment used or from the hands and clothes of the personnel handling the meat especially where personal hygiene is poor.

Meat quality is compromised when contamination cannot be controlled. The type and level of contamination are studied in order to maintain and improve the hygienic status and quality of meat produced by a slaughterhouse (Inthavong *et al.*, 2006).

Post-mortem meat inspection has been designed to ensure the safety and wholesomeness of meat in the slaughterhouse.

Another factor which is very important with regards to microbial contamination and proliferation is temperature. The ambient temperature within which production and storage of meat are done contribute to how safe the meat would be.

There are certain temperature ranges within which microbes thrive very well and increase in their numbers with all other conditions being available.

This means that if the temperature under which meat is produced and sold is controlled then most of the microbes could also be controlled. Other factors like pH, water activity, nutrient availability, initial microbial load would also affect the contamination of meat and meat products.

The mode of transporting the meat to the retail centres could also be a possible means of introducing and increasing the numbers of already existing pathogenic microbes.

The system of beef production in Kumasi is such that animals are brought to the abattoir for slaughter services at a fee. The owners of the animals or the butchers would like to see what happens to their animals throughout the slaughter process.

The whole plant becomes so congested with many people and there are no sanitary measures in place. There is cause for panic as Soyiri *et al.* (2008) reported that there are no HACCP principles in almost all the slaughter houses in Ghana. Retail cuts with large surfaces exposed to environment with good oxygen penetration, nutrients and water could lead to high levels of microbial load (Forest *et al.*, 1985). Ayres (1995) concluded that the way retail cuts are displayed provides a good platform for microbial activities which ends in meat spoilage and poisoning.

Though certain standards require absence of certain microbes in food, this is not practicable in raw meat (Bell *et al.*, 1997). The presence of bacteria at certain levels is inevitable in Kumasi with regards to production processes. Regular microbial load assessment is important in order to enhance meat quality assurance (Yousuf *et al.*, 2008).

Many stakeholders and experts advocate the development of a scientific and risk based food safety scheme in which hazards and their remedies would be available using data on sources, distribution and reduction of the hazards (Batz *et al.*, 2005).

The need for microbial levels assessment of beef in Kumasi is important to ensure that the quality of beef consumed can be guaranteed as microbiologically safe.

This has become important looking at the way of production and the manner of display of beef during sales.

1.1. OBJECTIVES:

1.2. General Objectives:

The study seeks to assess the general microbial loads of beef consumed by the public in the Kumasi metropolis, implications of uncontrolled temperatures during production, sales and even after sales in the event of leftovers. The effect of poor sanitation and the absence of certain practices that improve safety in both the slaughter house and the meat shops would also be taken into consideration.

1.3 The specific objectives were to:

I. Identify potential pathogenic microbes on the beef.

II. Establish the microbiological safety of beef in the Kumasi Metropolis.

CHAPTER TWO

2.0. LITERATURE REVIEW

2.1.0. CATTLE SLAUGHTER PROCESS

2.1.1. PRE–SLAUGHTER PRACTICES

Most of the cattle in Ghana are raised by the extensive system on grasslands where they are left to find food in an open area. Their basic feed is grass and other forages they find during grazing. Large populations of these livestock are found in the Northern part of Ghana. Veterinary extension services are provided to assist farmers in animal production. The animals being sent to the animal market or abattoir should be certified by a veterinarian as healthy and free from certain zoonotic diseases like tuberculosis through inspection which is done immediately before slaughter.

Cattle are also imported from neighbouring countries like Burkina Faso to Ghana for the purpose of meat production. These animals are quarantined and examined for any malfunctions and zoonotic diseases before they are released for sale on the market.

The animals are transported in long articulated trucks where about twenty cattle are put in a truck. Since the abattoir is not able to buy the animals and slaughter for butchers to buy the carcass, the animals are sent to the 'kraal'. The kraal is the place provided by the abattoir where the animals are kept for prospective buyers to buy before they are sent to the slaughter house. The distance between the kraal and the slaughter plant is a few meters so the animals are driven to the plant on foot. The ages of the cattle are not normally considered in our setting since they are not the fast growing ones. It is usually difficult to find the particular farm where an animal is coming from since records on animals from birth till they are ready for slaughter are mostly not available.

The animals are not sold on the basis of their calculated weight but on the physical appearance and with experience one can easily differentiate between a good animal and a bad one though there could be errors sometimes.

Cattle go through the process of slaughter before they are converted into meat. There are several stages in this process. These stages have many effects on the quality of the meat in terms of tenderness and microbiological safety. There are variations in the slaughter techniques employed by different countries and these depend on the religion of the workers in the abattoir and the safety regulation enforced in that country (Karama, 2005). There are several accepted methods of slaughtering cattle and making it ready for consumption as meat. The ultimate result of each method is to convert the live animals into meat and other edible products. Interaction of animals for slaughter and the process involved in turning animals into edible products influence the microbiological status of the meat and its products at the end (Fernandes, n.d.).

Regulation on Animal Welfare demands that all animals are stunned before slaughtered by the required method and instrument except under certain exempted situations (Fernandes, n.d.). Most of the advanced countries and some developing countries require by law that animals must be made unconscious before they are slaughtered (FAO, 2001). The effect of stunning is to eliminate pain, discomfort and stress from the procedure of slaughter.

The Jewish (Kosher) and Islamic (Halal) methods of slaughter are examples of the exceptional cases when the Animal Welfare is compromised in respect to stunning (FAO, 2001). There are three main methods of stunning animals before slaughter. These include the percussion, electrical and gas stunning methods. The percussion method causes physical damage to brain by the use of captive bolt or the gun.

Electrical stunning is also done by the use of electric current to make the animal unconscious for a few seconds. The gas stunning on the other hand is done by exposing the animals to a certain concentration of a gas like CO_2 which renders the animals unconscious.

2.1.2. CATTLE SLAUGHTER PRACTICES

Sticking is done immediately after stunning to prevent the animal from regaining consciousness. In cattle, the carotid artery is severed to effect bleeding. The effectiveness of bleeding depends on the kind of stunning method used. Certain stunning methods leave the carcasses with blood splashes on the muscles. Animals are hanged so that bleeding is enhanced to prevent retention of blood and its associated effects (Hedrick *et al.*, 1994).

When proper bleeding of the animals is done, 60% of the total blood is drained off and this is very good for long shelf life of the meat (Swatland, 2000). The efficiency of bleeding could be said to be very important requirement of slaughter operations in order to get meat and meat products of high quality (Warriss, 1977).

The high pH (7.35-7.45) (Kolb, 1984) of blood with its high protein content enhances quick putrefaction (Mucciolo, 1985). Therefore the shelf life of poorly bled carcasses is short. Bleeding is followed by dressing of the carcass which involves the removal of head, feet, hide, excess fat, viscera and offals. This is done to separate the edible parts of the carcass from the inedible portions.

This stage should be carried out without any delay since this could lead to contamination of the carcass (Lawrie, 1984).

Primal and whole cuts are then prepared from carcasses after chilling them. Chilling is done to reduce the microbial activities and also to improve other biochemical aspect of meat to achieve a good product (Anonymous, 1997).

2.2.0. THE BUTCHERS' SHOPS

The demand for beef has recently risen following the noise about avian and swine flu diseases in the whole country (Soyiri *et al.*, 2008). Almost all Ghanaians depend on butchers in our markets for the supply of beef (King *et al.*, 2000).

Activities at the butchers' shops are as important as what goes on in the slaughterhouse when safe and quality beef should be guaranteed. The quality of the meat could be compromised when certain important measures are not taken most especially issues concerning safety (Forest *et al.*, 1985).

Any wooden bench or table with a few kilograms of beef could be regarded as a butcher's shop without much consideration of the environment and even whether the butcher is trained or not. Bacteria growth and proliferation are easy where the conditions under which beef is stored for sale are untidy and the beef itself is unhygienically kept (Soyiri *et al.*, 2008). Beef is sold in this part of the world in open populated traditional markets. Beef is retailed in the market at the butchers' shops on tables exposed to the environment from the time the meat is displayed till everything gets finished at end of the day.

Meat exposed to an environment where flies are available have *coliforms* on it since flies are their known vectors. Unlike in the developed countries where meat is sold under controlled environment, just the opposite happens in the less developed world.

Adequate refrigeration and proper temperature control is very important in meat retail shop for safe meat to be guaranteed, though meat could get contaminated even before it gets out of the slaughterhouse (Anonymous, 1997). Retail cuts of fresh meat are sold with good surface area exposure to the environment with more readily available water, nutrient and oxygen for bacterial growth (Forest *et al.*, 1985). Growth of microbes on food surfaces is mainly influenced by water activity, temperature and pH (Ross, 1999).

Ayres (1995) further reported that the way retail cuts are displayed presents conducive conditions for microbial growth and proliferation which eventually result in spoiled meat. The ideal way to display retail cuts for sale is to package the cuts and keep under low temperature (Gill, 1998).

Though stainless steel is usually the common contact surface because of its smoothness, durability and low oxidative properties (Lauzon, 1998), beef is usually kept on wooden benches for display during sales in Ghana.

With certain levels of microbes already on the meat (Jamilah *et al.*, 2008) the microbial numbers increase as the day goes by with increase in temperature, availability of oxygen in the surrounding air and moisture from water which is occasionally sprinkled on the meat (Ross, 1999).

The porous nature of the wood surface presents an easy way of entrapment of bacteria (Lauzon, 1998). Some of these bacteria have the ability to adhere to hard surfaces like the wooden bench.

The number of Total Viable Count and *Enterobacteriaceae* found on a work surface which makes that surface acceptable or unacceptable are found in **Table 1.**

Bacteria multiply and produce extracellular polymeric material forming what is called *biofilm* (Lauzon, 1998). Other bacteria may be entrapped in such *biofilm* and they

could be protected from active compounds in detergents during cleaning (Lauzon, 1998).

The sanitation in the markets where butchers' shops are usually located in Ghana is very poor (Soyiri *et al.*, 2008). The personal hygiene of the butchers is an important thing since it also influences the kinds of microbes and their numbers on the beef (Nouichi and Hamdi, 2009). The usual thing is that butchers are more concerned about their business than good hygienic practices that would affect the beef they sell (Soyiri *et al.*, 2008).

In Europe, it is a legal requirement for all meat counter and butcher shops to have in place food safety management scheme which is HACCP principles based (Anonymous, 2000).

A different thing happens in Ghana as anyone who has money can go into the business without any form of training or even any certification. Due to the limited capacity of the Ghana Food and Drugs Board, it is very difficult to implement food safety systems to improve food safety in the country in general (Soyiri *et al.*, 2008).

Table 1. Mean values for the number of colonies on work surfaces

	Acceptable range	Unacceptable
Total viable counts (TVC)	0-10/cm^2	> 10/cm^2
Enterobacteriaceae	0-1/cm^2	> 1/cm^2

Source: (Anonymous, 2007).

2.3.0. CONTAMINATION OF BEEF

In the presence of unwanted substances in food which could be physical, chemical or microorganism, this food could be regarded as contaminated. The contaminant may be harmful or not depending on what effect it would have on the meat and the consumer. Meat could be contaminated with a whole lot of foreign matter when precautions are not taken seriously. Carcass contamination level depends on the cleanliness of the animal before slaughter, the types and number of microbes introduced during meat production and processing, the temperature as well as the time of storage, transportation and distribution conditions (Nortje *et al.,* 1990). Contamination could occur horizontally when an initially treated carcass gets contaminated again, thus cross contamination could also be a major problem in a slaughter plant. Pathogenic microbes could easily spread from equipment, material and staff at work to non contaminated carcasses.

Operations during cattle slaughter like bleeding, dressing and evisceration, storage and distribution make microbial contamination of the sterile muscles by microbes on the skin, in the digestive tract and in the environment possible and easy (Bacon *et al.,*2000). A good portion of beef carcass contamination starts with dirt, dust and faecal matter found on the hide and usually occurs during hide removal (Elder *et al.,* 2000).

Evisceration and carcass dressing are both critical stages where muscles get microbial contamination (Cutter *et al.,* 2000) and measures could be implemented to correct the problem (Bacon *et al.*, 2000).

Carcasses and cuts could get contaminated by direct deposition of the microbes or by an indirect contact by equipment, workers, installations and even the air around (Borch and Arinder, 2002).

It has been found that in the slaughterhouse workers and the tools and equipment they use could easily spread contaminants into the internal organs of beef carcasses (Abdalla *et al.*, 2010).

Salmonella spp and *E. coli* were isolated from the hands of workers in the meat industry (Dickson and Anderson, 1992). Gill (1998) in a further study found that certain practices like storage and display of meat in the retail shops also make contamination of beef possible.

Contamination is highly possible during the periods of processing, transport and distribution due to equipment failure, accidents or negligence (IM/NRC, 1998) and even the absence of approved equipments for loading and unloading of meat during transportation and distribution. Unacceptable high levels of microbes were found during analysis of beef and goat meat from Accra (Mensah *et al.*, 2001).

2.3.1. PHYSICAL CONTAMINATION

The physical contaminants of meat are foreign objects which may be present without any major changes to the chemical and biochemical composition of the meat. These foreign matters may cause injury and or sickness to consumers during and after consumption (Folks, 2001). Wagstrom (2004) reported that physical contaminants could enter meat during the pre-harvest activities due to items like broken needles. Hair, stones, machinery pieces, splinters from pallets, knife blades, bolts and nuts, plastic, wood and many more are the contaminants likely to be found on meat during production and processing of meat and other meat products (Wagstrom, 2004).

A research revealed that sources of foreign materials in food such as meat include ingested metals and plastics from animals, veterinary instruments, tips of saws used in carcass splitting and others (Wallin and Haycock, 1998). The pieces of wood from the cutting board and bench at the retail shops are also important (Lauzon, 1998).

2.3.2. CHEMICAL CONTAMINATION

Chemical contamination of meat may occur as a result of residues of agrochemicals or pharmaceuticals in animal production or due to toxins. Excess addition of even approved chemical ingredients to food may compromise the safety of the food (FDA, 2004).Toxic compounds that may be dangerous when ingested by humans have numerous effects on them. Most of the contaminants which find their way into the food chain may be pesticides, heavy metals and other chemicals from the livestock production (Anonymous, 2009a) and also during the production of meat.

High levels of pesticides residues may be found in meat resulting from high concentration of the residue in the body tissues of animals due to pests and vector control through dipping or even grazing on feeds with high levels accumulated residues of these chemicals (Darko and Acquaah, 2007). Heavy metals may leak into food when food equipment and utensils corrode (Tybor, 1990).

This may happen when corroded surfaces come in contact with highly acidic foods. There are certain levels of metal left on beef during preparation at the stage of singeing (Santhi *et al*., 2008). The kind of material used in the singeing process whether wood or scrap tyre would influence the kind of heavy metals left on the meat and also the levels of such metals. The water used in cleaning the singed carcass also has effect on presence and levels of heavy metals on the meat (Obiri-Danso *et al*., 2008). Failure to rinse equipments properly during cleaning and sanitizing would result in contamination when the equipment is used in food preparation.

Tybor (1990) reported that with proper personnel training on cleaning and sanitizing, the problem could be controlled. Chemical residue associated issues will continue to be of concern and some of them may become major issues needing attention from time to time.

Monitoring pragrammes are important in checking the levels of chemical residues in the food chain which may pose environmental and health hazard (Jayashree and Vasudevan, 2007).

2.3.3. BIOLOGICAL CONTAMINATION

This kind of contamination is usually associated with living organisms which might be microscopic or visible to the eye. The visible ones could be identified and easily controlled or eliminated if possible. The visible ones are called parasites which are either single or multi-celled organisms. They live within or upon the host organism causing harm at the end.

Parasites are larger than viruses and bacteria with sizes usually greater than 10 micrometers (μm). They only cause infection not intoxication as foodborne organisms. Microorganisms like viruses are unable to multiply in foods but only survive in the environment and later transported via food to human. Normally, parasites go through structural changes in their life cycle periods. Only the form of their structure which is transmissible via food is a cyst which is inert and resistant to the external conditions of the host similar to bacteria though less resistant to heat.

Entamoeba histolytica, Toxoplasma gondii and *Giardia lambla* include single-celled parasites which caused foodborne disease outbreaks in the United States (USDA-ERS.2001). Immunocompromised people are more affected by these kinds of organisms (USDA-ERS.2001). The multi-celled types are usually found in food as

their eggs, larvae or other forms. When ingested into the body, they hatch or become active resulting in development of new parasites.

Trichinella spiralis is reported to cause foodborne disease (trichinosis) in USA (USDA-ERS, 2001).

Tapeworm species are also important parasites found in meat like *Taenia saginata* in beef and *Taenia solium* in pork but infection from them are rare.

The microscopic organisms are numerous and they are in the groups of moulds, yeasts and bacteria. They get onto the meat through various means. They are found as the living organism itself or toxins they produce during metabolism (ICMSF, 1996). They cause various foodborne illnesses which happen to humans who ingest levels of these microbes that are enough to cause that. The microbes are difficult to control especially when sanitation and hygienic practices are poor in places where the meat is prepared (Lawrie, 1991).

2.4.0. SOURCES OF CONTAMINATION

There are mechanisms in the body which control microbial numbers through defensive actions of the white blood cells and antibodies. These mechanisms are lost during exanguination.

This makes invasion by microorganisms into the muscles of meat animals much easier where the surroundings of production is already full of these microbes. This invasion becomes easy especially in a filthy environment. Pathogenic bacteria generally found on slaughtered animals are either part of the indigenous microflora of animals or those that result from the breeding of these bacteria on equipment which lead to cross contamination at the end (WHO, 1990).

Amezquita *et al.* (n.d.) found two main sources of pathogenic microorganisms in meat and meat products as the living animals and the environment in which meat and its

products are processed. Live animals may be asymptomatic carriers of pathogenic bacteria or even highly contaminated which may be the source of any later contamination of meat (Letellier *et al.*, 1999).

The cleanliness of an animal is determined by the climate and geographical location, holding conditions and the transportation method during its sale (Sofos *et al.*, n.d.). Animals raised in feedlots may have the surfaces of their carcass occupied more by microbes of intestinal origin (Sofos, 1994). On the other hand, carcasses of animals finished on pasture may have more of the microbes with their origin in soil (Sofos *et al.*, 1999).

These kinds of animals may frequently carry different quantities of manure, bedding and soil on their skins as they enter the abattoir (Karama, 2005).

Adhered mud, bedding and manure on the skin of animals make a large contribution to microbial contamination during skin removal.

Karama (2005) reported that the skins of animals ready for slaughter may carry as many as log 9 bacteria load of faecal or soil origin per cm^2 of the skin. Microorganisms usually found on the skin include *Staphylococci, Micrococci, Pseudomonads, Yeast* and *Moulds*. Food-borne pathogens likely found in mud and faeces are *E.coli, Clostridium perfringens* and *Salmonella spp* (Van Donkersgoed *et al.*, 1997).

Normally the muscles are sterile in live healthy animals whereas lymph nodes, some organs and importantly exposed surfaces to the environment like exterior hide, fleece, the bucal cavity as well as the gastrointestinal passage which is highly contaminated (Gill, 1998). The hides and fleece of the animals being processed for beef could also contribute to the microbial loads during beef harvest (Anonymous, 1997).

Collins and Wall (2004) reported that contaminated hide of cattle could be the immediate source of most microbes found on carcasses during and after slaughter.

The viscera and skin have been found to be a good source of human pathogens and spoilage microbes (Gracey and Collins, 1992).

The general contamination sources of carcasses could be the slaughtered animals themselves, the slaughter plant environment and the staff at work (Bell and Hathaway, 1996). Instruments like knives, hooks, rails used in the entire production process could be vehicles of contamination if adequate and proper cleaning and sanitizing are not done (Clayton, 2002). The stick knife, if not sterile can be a source of microbial contaminants during exsanguinations and these microbes could swim into the blood stream and eventually deposited on the carcass (Jay, 2000). Microbes which originate from the skin are among those that end up on the carcass via the stick knife (Jay, 2000). He went further and reported that others too may be found on the freshly dehaired carcasses or even onto freshly cut surfaces.

The air around the place of slaughter and sale could be a source of contaminants though there is little documentation. Airborne microbes seem to be contributors of carcass contamination. Jay (2000) reported that certain microflora on skin becomes airborne which could contaminate even dressed carcasses. A study by Rahkio and Korkeala (1997) revealed that there is a correlation between carcass microbial contamination and air microbiological contamination. They believed the airborne microbes originate from lairages and the skins of animals. The microbes found in air and dust that can persist are mostly the gram-positive ones and a number of moulds and some yeast as well (Jay, 2000). He went further to say that the microorganisms in the air are those that regularly reseeded to the environment. Coughs and sneezes from workers in slaughter plant and butcher's shop could contribute to meat contamination especially when they have infections and also practise poor personal hygiene (Anonymous, 1997).

The hygienic practices of the slaughter plant's staff and the butchers in their shops are paramount to the general sanitation of the plant or meat shop (Higgins, 2002).

He went further to say that if the sanitation is poor then contamination of food and for this case meat is highly likely to occur.

Jay (2000) reported that the microbial community on the hands and outer clothes of slaughterhouse staff generally reflect the habits and the kind of environment around them and microbes in question and they may originate from soils, water, dust and other environmental source. He went further to say that other important sources of the microbes are nasal cavities, mouth and on the skin that may enter the meat when personal hygiene is poorly practised.

This kind of situation is highly possible in settings when the slaughter staff does not cover their nose, mouths, heads and do not wear the proper clothes. Dirty workers' hands, clothes and equipment could be intermediate sources of beef contaminants (Gill, 1998). Furthermore, certain bacteria like *S.aureus* is very likely to be isolated from workers' hands and other likely sources include cuts and scratches especially when the fellow is infected and practices poor personal hygiene (Anonymous, 1997).

Sanitation of food contact surfaces is crucial in the process of food since those surfaces could spread microbial contaminants in products (Lauzon, 1998). Butchers keep the bulk of the beef they sell on wooden benches during sales. With the beef surface exposed to the environment, dust and other contaminants easily find their way on them. The wood being porous and absorbent allows organic material and bacteria as well to be entrapped and cause contamination later (Lauzon, 1998) when fresh meat is put on it.

Cattle were starved before slaughter during transportation to the slaughter house and this makes them vulnerable to microbial invasion (Callaway *et al.*, n.d.).

Volatile Fatty Acids (VFA) and other organic acids are responsible for checking the microbial numbers in the rumen and intestinal tract, (Russell and Diez-Gonzalez 1998).

But during fasting the concentration of VFA and the other organic acids reduce which allows the rumen and intestinal microbial population to rise (Van Immerseel *et al.*,2006) hence transportation where there is usually starvation makes shedding of microbes more easy.

The population of *E.coli, Enterobacter* and total anaerobic bacteria increased in the entire intestinal tract in a research work where the animals were starved for some time (Gregory *et al.*, 2000) and the numbers of *Salmonella* and *E.coli* in the rumen also rose up (Grau *et al.*, 1969). A further study revealed that fasting prior to slaughter can apparently cause *E.coli* negative animals to become positive (Kudva *et al.*, 1995).

Poor method of evisceration is one of the ways microbes are introduced onto meat. Punctures into the intestinal contents with the usual heavy microbial load may leave carcasses contaminated (Jay, 2000). He went further to say that it is important to avoid punctures as the rumen of a ruminant like cattle contains approximately 10^{10} bacteria per gram. The lymph nodes also contain large numbers of microbes especially bacteria. These lymph nodes are embedded in fat in red meats so if care is not taken and the nodes are cut through, the heavy microbial load finds its way on the carcasses (Jay, 2000) and even contaminate other carcasses when good sanitation is not practised.

2.4.1. ORGANISMS INVOVLED IN MEAT CONTAMINATION

Meat could be contaminated with many kinds of organisms. These could be found in various places on the earth. Some of them could be useful in many ways. During the preparation of some special meat products like pepperoni, the fermentation effect of bacteria is employed. Yeast could be very helpful in the bakery industry. On the other hand, some of these organisms which are pathogenic could pose as threats to other living organisms like humans.

Depending on the group an organism may belong, it would have a particular shape and size. Some of them are visible to the human eyes whiles others may not. There are various viruses, bacteria and other infectious agents that could be found on meat.

2.4.2. YEASTS

Yeasts are unicellular organisms generally having larger cells than bacteria. Yeasts size may range from 1-5 μm in width and 5-30 μm or even more in length. Though usually oval, some of them are elongated or spherical in shape. Yeasts lack flagella and any other means of locomotion. They form smooth and glistening colonies like bacteria when cultured on an agar medium.

Yeasts reproduce by budding which is projection of a cell developing independent of the mother cell. They are very useful in the food industry as fermentation organisms as in the preparation of wine, bread, beer, cakes and other fermented foods. Yeasts are more tolerant to osmotic pressure of the medium in which they find themselves than moulds and bacteria. Yeasts contamination and unwanted growth on food result in spoilage which reduces the shelf-life of the food. They may contaminate meat through improper sanitation of equipment or through the air (Rochon and Belliveau, 2006).

Yeasts growth is enhanced at conditions like low water activity, low pH, high salt or sugar content which inhibit bacteria growth (Rochon and Belliveau, 2006).

2.4.3. MOULDS

They are filamentous fungi that are seen in a form of a tangled mass that spreads fast when growing and the mass can cover several inches of an area within 2-3 days. Mycelium refers to the total mass or even any large portion of the growth. The mycelium compresses filaments or branches known as hyphae. Moulds are found on food by their whisker-like or fuzzy appearance.

The types of moulds important in food multiply by ascospores, zygospores or conidia. The ascospores of certain genera are identified by their extreme ability to withstand heat (Jay, 2000). Moulds are tolerant to osmotic pressure and can grow at a_w value of 0.6. They usually spoil cereals grains rather than bacteria. Low pH and water activity with high salt and sugar contents of a substrate improves the growth of moulds (Rochon and Belliveau, 2006).

2.4.4. BACTERIA

Bacteria are a group microorganisms that could be found almost anywhere in the world. The structure of bacterial cell is described in **Figure 1**. They are one-celled living microbes that have cell walls. Cells of bacteria differ in shapes and sizes from about 0.5–5.0 micrometers (μm) in length. Most bacteria belong to one of three groups depending on their shape. They could take the shapes of rod (rod shaped bacteria are known as bacilli), sphere (cocci is the name given to bacteria which are spherical in shape) and spiral (spiral shaped bacteria are called spirilla).

Others with more complex shapes belong to groups different from the groups mentioned above. They could also be grouped into aerobic (those that require oxygen to survive) and anaerobic ones (those that do not need oxygen and even die when kept in an oxygenated environment).

Bacterial Cell Structure

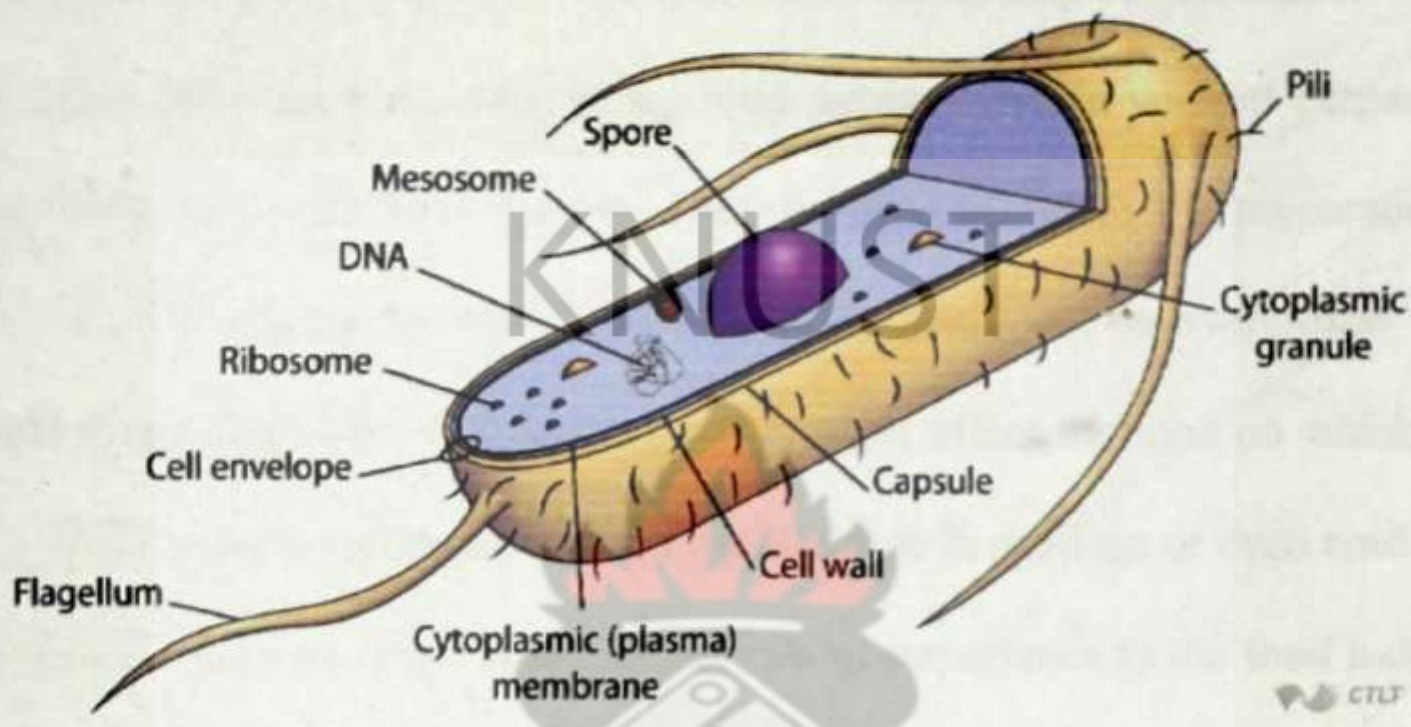


Figure 1. Source: Jay, (2000).

Bacteria are also grouped into Gram positive (cell wall able to bind with agent used in staining) or Gram negative (cell wall unable to bind with staining agent) based on Gram Staining Method. Certain bacteria are Autotrophic and they obtain the carbon they need from carbon-dioxide, they are also known as autotrophs. Some autotrophs use sun light directly to produce sugar from carbon. Heterotrophic bacteria on the other hand depend on carbon and sugar from the environment in which they are found, they are also known as heterotrophs. This group is usually found either as commensals or parasites where some of them could be pathogenic to their hosts.

Bacterial cells increase when a particular cell divides into two; each divided portion grows into full size and divides into two again. Specific bacteria have certain peculiar conditions for growth and reproduction.

Though bacteria share certain traits with viruses, the former have the ability to multiply in or on food.

Large numbers are easily reached under optimum conditions. There are various types of these bacteria which are helpful to humans whiles others may be pathogenic.

Different kinds of bacteria are used in the food industry like in yoghurt preparation and other foods that need fermentation. They are also useful in the preparation of vaccines. The bad effects bacteria cause to humans could not be overlooked when bacteria are being discussed. The activities of bacteria affect the food on which they grow. For instance their metabolic activity would result in spoilage or even render the food poisonous. There are many types of bacteria of importance to the food industry. They cause spoilage and also food poison when proper care is not taken. Examples of pathogenic bacteria which are found on meat are *Salmonella, Staphylococcus aureus, Clostridium botulinum, Clostridium perfringens, Bacillus cereus* and *Escherichia coli* (Hobbs and Roberts, 1993).

2.4.5. GROWTH OF BACTERIA

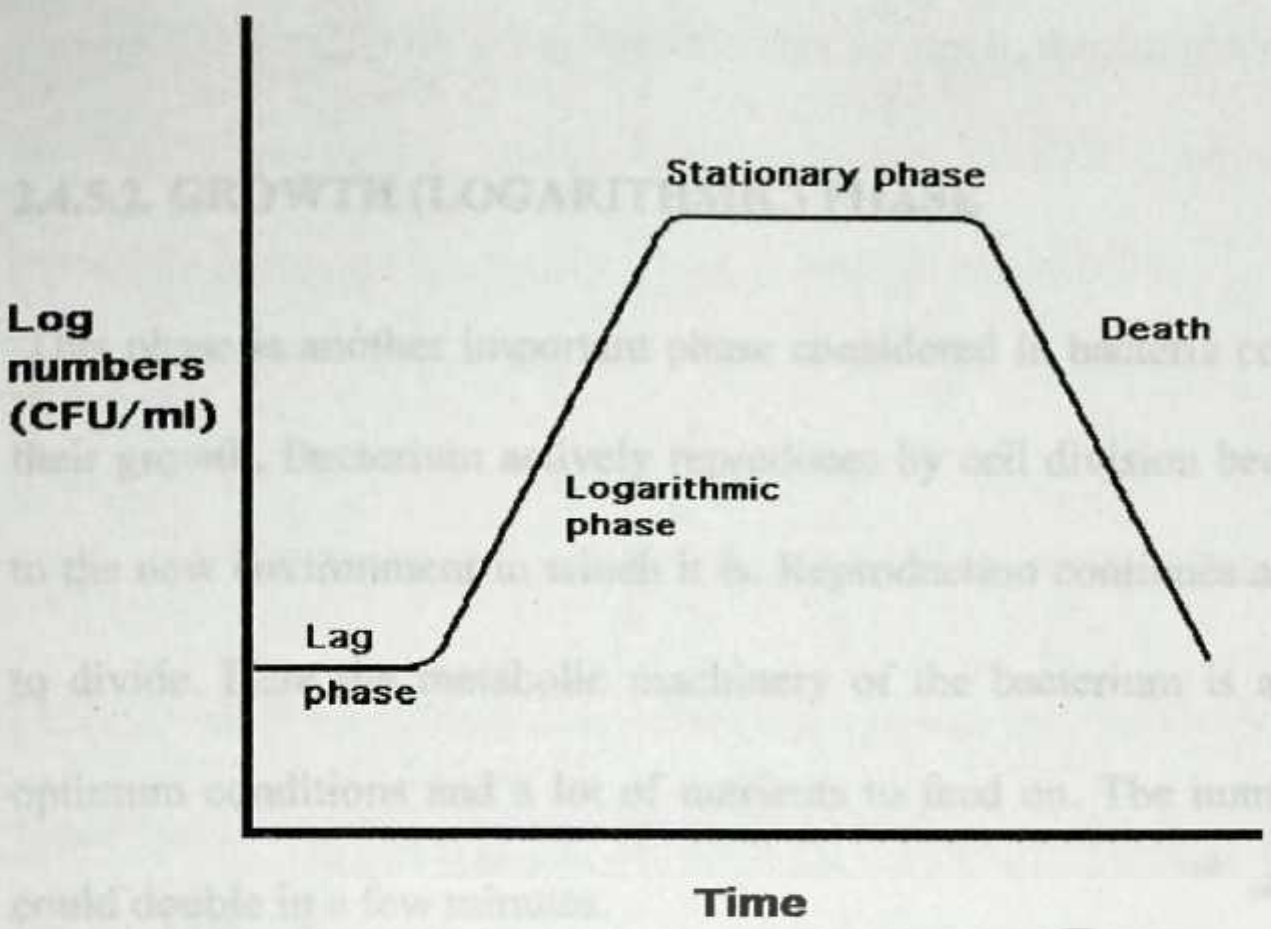
Bacteria growth in food is a very complex process. This is influenced by genetic, biochemical and environmental factors. The increase in cellular composition which may lead to increase in microbial size, population number or even both is known as bacterial growth. Bacteria, like any other living organisms require nutrients with other optimum conditions to grow very well (Anonymous, 1997). Meat provides the needed nutrients for the growth of bacteria and they include proteins, phospholipids, fatty

acids and carbohydrate (especially glycogen) and other soluble non-protein nutrients (Anonymous, 2009b).

Bacteria are the most important microbes that affect food and in this situation beef. Just like normal microbes, they grow as they consume the nutrients and produce waste products.

Bacterial growth rate depends on nutrient availability and the toxic bacterial end-products concentration in or on the medium of growth (Anonymous, 1997). At the time of high nutrient availability bacterial cells begin to grow and reproduce fast and easily. On the other hand, the buildup of toxic bacterial waste makes the cells stop the fast and easy growth and reproduction, bacteria begin to die off.; hence the growth of bacteria is self-limiting. Bacterial growth cycle has four distinct phases which are represented on the growth curve of bacterial cells. A graphical presentation of the growth curve is represented in **Figure 2**. The first part is the lag phase where a bacterium cell lands on a substrate (beef) and here growth is very slow. The growth or rapid phase is the next and it is characterized by exponential increase in the number of bacteria.

The third phase is called the stationary phase with reduced or constant rate of growth. The death phase is the last to complete the growth cycle where almost all the cells might have died due to certain unfavorable conditions.



Hypothetical bacterial growth curve.

Figure 2. Bacterial Growth Curve. Source: Anonymous, (1997).

2.4.5.1. LAG PHASE

This is the first phase of bacterial growth when they are introduced onto a suitable substrate. The lag phase is a period of bacterial growth where their cells get adjusted physiologically to the environment in which they are found. Bacterial growth is restricted during this period, for instance when a bacterium is transferred from a work surface onto the meat. Here the bacteria replicate their genes and in the event of spores, the spores are differentiated into the vegetative cells. Duration of the lag phase is dependent on the temperature, the initial microbial numbers (large numbers shortens the phase) and the physiological history of the organisms for instance where they were actively growing before they were transferred. The factors mentioned above could be manipulated to extend the lag phase thereby increasing shelf life and decreasing the rate at which spoilage would ensue. Rapid chilling of meat prolongs the lag phase (Anonymous, 1997).

2.4.5.2. GROWTH (LOGARITHMIC) PHASE

This phase is another important phase considered in bacteria control with regards to their growth. Bacterium actively reproduces by cell division because it adjusted well to the new environment in which it is. Reproduction continues as the new cells begin to divide. Here the metabolic machinery of the bacterium is actively running with optimum conditions and a lot of nutrients to feed on. The number of bacterial cells could double in a few minutes.

2.4.5.3. STATIONARY PHASE

This phase only occurs when bacterial numbers reach a figure of over one hundred million per gram(10^8/g) which is more than ten times the number to cause spoilage (Husband, n.d.) which may also end in food poisoning. When bacterial cells have been multiplied for some time, there is accumulation of toxins and other bacterial metabolism end-products. This situation results in the death of some of the bacteria. Growth of bacteria still occurs bringing about no net increase in bacteria numbers.

2.4.5.4. DEATH PHASE

The eventual outcome of the stationary growth is the death of more cells than those produced. At this time, the rate of dying cells exceeds the growth rate causing a net reduction in the number of living bacterial cells. By the time the growth curve reaches this point, spoilage might have ensued and the likelihood of food poisoning when not properly cooked or processed before human consumption.

Though the number of living bacteria may be small, they might have produced spores or other toxins during metabolism which may also be dangerous to human health especially to the immunocompromised (Jamilah *et al.*, 2008)

2.5.0. FACTORS THAT AFFECT BACTERIAL GROWTH

There are various factors that affect the growth and survival of bacteria on meat. These factors could be grouped broadly as intrinsic or extrinsic factors (Rombout and Wout, 1994). Both groups have their ways of influence on the growth of microbes. Factors that are the physical and chemical characteristic of the meat itself that affect the growth of microbes are referred to as intrinsic factors.

These factors include moisture content, pH, water activity, oxidation-reduction potential, availability of nutrients, physical structure and the possible presence of some natural antimicrobial agents (Prescott *et al.*, 2002). The extrinsic factors usually refer to the effects of the environment around the meat. Forest *et al.* (1985) found that temperature, moisture or water activity and availability of oxygen are the important factors that affect microbial growth on meat and meat products.

2.5.1. WATER ACTIVITY (a_w)

The ratio of water vapour pressure of food substrate to the vapour pressure of pure water when they have the same temperature is called water activity [$a_w = p/p_o$, where p = vapour pressure of the solution and p_o = vapour pressure of the solvent (normally water)] (Jay, 2000). This is a measure of the availability of water for biological activity and this relates to water in the free form present in the food.

The total moisture content in meat present is in the free and bound forms. The bound water is important for hydration of hydrophilic molecules and also for dissolving solutes but never available for biological use. The bound water does not contribute to water activity. Free water on the other hand makes biological activities in the meat possible. This free water is important in the growth of microbes. It is necessary for transportation of nutrients and waste materials. Enzymatic reactions, cellular material synthesis and other biochemical reactions are made possible by the free water in the meat.

Pham (2001) on the other hand defined water activity as the availability of water for deteriorative changes or microbial growth in a particular food. Water activity affects microbes' behaviour through the growth, sporulation, production and stability of toxins, survival during processing and at store and the easy of recovery on media. Each microbe has specific maximum, optimum and minimum water activity level for growth. Microbial growth is reduced below a minimum water activity level and microbial cells are viable for a while.

A further reduction of the water activity level makes microbial cells lose their viability completely and this loss is very fast at the beginning but gradually slows down. Fresh foods like meat, fruits and vegetables have their a_w values (0.97 - 0.99) are very close to optimum for many microbes to grow, (FDA, 2001b).

Most kinds of meat have high levels of water content in reference to a_w which are normally found approximately 0.99 which is conducive for most microbes to survive (Rao *et al.*, 2009). The response of an indicator organism runs through a particular taxonomic class. The Gram negative microbes are normally more sensitive to reduced or low a_w but on the other hand Gram positive ones are more tolerant (FDA, 2001b).

Reddy (n.d.) gave the minimum values of a_w which allow certain microbes grow and cause spoilage in food in **Table 2**.

The a_w could be manipulated in foods by a number of means, including addition of solutes such as salt or sugar, physical removal of water through drying or baking, or binding of water to various macromolecular components in the food.

Table 2: Lowest water activity (a_w) values for most of the different groups of microorganisms spoiling food

Group of Microorganism	Minimal (a_w) value
Bacteria	0.91
Yeasts	0.88
Moulds	0.80
Halophilic bacteria	0.75
Xerophilic fungi	0.65
Osmophilic yeasts	0.60

Source: Reddy, (n.d).

2.5.2. pH

The measure of acidity and alkalinity is called pH (pH = –log10 [H+]). pH is measured on the scale of 0-14 where values close to 0 signifies high acid content and those close to 14 being alkaline but 7 is the neutral pH point. The pH of food is one of the factors that affect the physicochemical properties of that meat (Wajda1 *et al.*, 2004).Soon after slaughter; the glycogen in the animals becomes depleted. The easy way of bacteria invasion and shortened shell-life of meat are also influenced by the pH. The normal meat pH is ranged from 5.4-5.8 (Anonymous, 2003). Meat becomes very susceptible to microbial invasion even under the best production and processing conditions when the ultimate pH is high (Hedrick *et al.*, 1994).

Microorganisms found on meat whether spoilage or pathogenic ones have a certain pH range to survive in. Most microbes can grow over the range of muscle pH values of 5.4-7 (Anonymous, 2009a). The ultimate pH of meat is important for its resistance to spoilage since many bacteria grow optimally at about pH of 7 and not grow well below pH of 4 or above 9 (Walker and Betts, 2000).

This results from the depletion of reserved glycogen due to crude methods of restraining animals before slaughter which leads to stress (Anonymous, 2003).

In the combination of other growth factors, pH becomes a very important parameter which determines the kind of microflora found on a substrate like meat. For instance, in a situation of the absence of oxygen, the composition of the microflora on red lean meat would depend on the muscle pH (Anonymous, 2009b).

Meat pH is an important determinant of microbial growth and the higher the pH of beef, the higher the spoilage potential and therefore the shorter the shelf life and other associated problems (Newton and Gill, 1981).

Certain minimum pH reduce the growth rate of some bacteria normally found associated to meat. *E.coli* growth is inhibited at pH of 5.0 that for *Salmonella spp* is at 4.6 (Soyiri *et al.*, 2008). It is important to find ways of reaching such minimum pH points or even getting close to such pH values during the production and processing of beef.

2.5.3. TEMPERATURE

One of the critical environmental factors that affect bacterial growth is temperature. Temperature is an essential parameter in the measure of food safety, quality and wholesomeness. It affects the shelf-life of the food and whether that food would cause food poisoning too. Microorganisms and even inherent enzymes found in foods are temperature dependent for efficiency.

The grouping of bacteria is done roughly on the basis of suitable temperature for growth. They are classified as psychrotrophs (grow below 20^0C), mesophiles (grow within the temperatures of 20^0C and 45^0C) and thermophiles (grow above of 45^0C). The psychrotrophs and the mesophiles are the types of bacteria important to the meat industry. The *pseudomonas spp* are spoilage microbes whereas the food poisoning ones like *Salmonella* and *E. coli* are psychrotrophs and mesophiles respectively (Anonymous, 1997).

2.5.4. GASEOUS ENVIRONMENT

The gaseous composition of the atmosphere surrounding food has a profound influence on the growth of many microorganisms on that food. Bacteria which contaminate meat are usually found confined to the surface of the meat where there is readily available oxygen (Anonymous, 2009a). Oxygen concentration around the meat influences the growth of bacteria as they are grouped into strict aerobes, obligate anaerobes, facultative anaerobes and micro-aerophilic (Reddy, n.d.). Like the *Pseudomonas*, they can only grow in the presence of oxygen. *Clostridium* on the other hand grows only under anaerobic conditions.

Moulds and most yeast are aerobic and so are usually found on the surfaces of food for enough oxygen. The way to control the spoilage aerobes brought in the idea of vacuum packaging. It is very obvious that in a market where meat is sold in the open, growth of aerobic bacteria is easy and the shelf life of the product is always suspected to be short.

2.6.0. BEEF BACTERIAL CONTAMINATION

2.6.1. INDICATOR ORGANISMS

Organisms grouped under taxonomic, ecological or physiological class whose absence or presence gives indirect evidence about a specific feature in the past history of the sample in question is termed indicator organisms (Harrigan and McCance, 1976). Buchanan (2000) also said that indicator organisms are a group of microbes that show that a particular food has been exposed to situations that pose a rise in risk and that a pathogen contamination may have occurred. He further said that the food might have been held under favourable conditions for pathogenic organisms' growth. Indicator organisms have been used as indicator for microbiological safety, hygiene during slaughter and processing and the extent of shelf life of meat. In this study the indicator organisms were Total Plate Count (TPC), Total *coliforms* (TC), Faecal *coliforms* (FC) and others like *Enterobacteriaceae, Staphylococcus spp., Bacillus spp* and *Pseudomonas spp.*

2.6.2. TOTAL PLATE COUNT (TPC)

This is used to quantify the microorganism populations in product samples (Maturin and Peeler, 1998). Plate count determination is important for all products because of its use as indicator utility, condition and the length of storage of products before stabilizing processes like freezing (ICMSF, 1978).

Total Plate Count can also be used to check Good Commercial Practice (GCP) and based on such findings can aid in controlling in-plant sanitation (ICMSF, 1986). The TPC increases significantly over time under uncontrolled temperature of a product.

Homogenates or aliquots are diluted 10-folds dilutions, spread on agar medium and incubated at 37^0C.The Total Plate Count values of carcasses not chilled in excess of 10^7 are either entirely contaminated or have had exposure of conditions that permit microbial growth to an extent that spoilage can be detected easily (ICMSF, 1978). Nouichi and Hamdi (2009) found log 4.48CFU/cm^2 as the total plate count on the surfaces of bovine carcasses in a slaughterhouse in Algeria. A study by El-Hadef *et al.* (2005) revealed a mean log TPC of 5.34CFU/cm^2 in a slaughterhouse.

Mean log counts of 2.42 CFU/cm^2, 1.82 CFU/cm^2 and 1.3 CFU/cm^2 were recorded as TPC of a study in Australia (Phillips *et al.*, 2006).

A research done at five slaughterhouses in Switzerland obtained superficial TPC of bovine carcasses ranging from 2.1 to 3.1 Log CFU/cm^2 (Zweifel and Stephan, 2003).

By the end of a slaughter process, the surfaces of bovine carcasses are likely to have TPC of 10^3-10^5 CFU/cm^2 (ICMSF, 1980). Other research done in the USA recorded TPC values ranging from 2.68 - 7 Log CFU/cm^2, (Cook *et al.*, 1997). Hudson *et al.* (1996) reported mean TPC values between 1.98 Log CFU/cm^2 and 4.14 Log CFU/cm^2. Although setting categories for acceptance or rejection of carcasses on the basis of TPC, some countries have set scales for that purpose (Hudson *et al.*, 1996).

Recommended levels of total plate count made by Agricultural and Resource Management Council of Australia and New Zealand (ARMCANZ) brought the following descriptions : Excellent, Good, Acceptable and Marginal for listed levels of total plate count in **Table 3** (Anonymous, 2003).

A scale for logarithmic mean of Total Aerobic Count (CFU/cm^2) as seen in **Table 4**. Results of high aerobic counts on meat usually implies poorer quality and reduction in shelf life (Eisel, 1997).

Table 3. Description and levels of TPC suggested by Agricultural and Resource Management Council of Australia and New Zealand (ARMCANZ).

Category	TPC/cm^2 or /g
Excellent	<1,000 (10^3)
Good	1,000-10,000 (10^3-10^4)
Acceptable	10,000-100,000 (10^4-10^5)
Marginal (Action required)	100,000-1,000,000 (10^5-10^6)

Modified from Anonymous, (2003).

Table 4. A scale for acceptance or rejection of carcasses on the basis of Aerobic Plate Count.

Description	Log CFU/cm^2 of aerobic plate count
Excellent	<2.0
Good	2.0-2.9
Fair	3.0-3.4
Bad	>4.5

Source: Karama, (2005).

2.6.3. Total *Coliforms*

Coliforms are a collection of relatively harmless bacteria that belong to the family *Enterobacteraceae.* They live in large numbers in soils, plants and intestines of warm and cold blooded animals. *Coliforms* aid in digestion of food. Total *coliforms* are Gram-negative aerobic and facultative anaerobic, rods, non-spore forming microbes. They are able to produce acid from glucose and other carbohydrates.

Coliforms are a group of indicator organisms with the capability of fermenting lactose where there is the production of acid and gas within a period of 24-48 hours at a temperature of 35^0C (APHA, 1998). By elevated temperature tests, *coliforms* can be grouped into faecal and non-faecal *coliforms.* These *coliforms* get discharged in relatively high levels ($2x10^9$ *coliforms* /day/capita) via human and animal faeces though not all of them are of faecal source,(Bitton, 2005). A study by El-Hadef *et al.* (2005) recorded total *coliforms* levels of log 2 CFU/cm^2. A lower level of log 1.7 CFU/$cm^2$was found in a research by Ware *et al.* (2001).

2.6.4. Faecal *Coliforms*

Faecal *coliforms* are group of *coliforms* that have their growth restricted to the gastrointestinal tract of humans and warm-blooded animals. They are tolerant to high temperatures and are capable fermenting lactose at 44.5^0C (Bitton, 2005). Microflora of faecal origin like faecal *coliforms* are by far the predominant pathogens found on final dressed carcasses (WHO, 1990). These types of *coliforms* are found in the faeces of the humans and the above described animals. This group includes members of three genera *Escherichia, Klebsiella* and *Enterobacter*. A range of log 4.38-4.77MPN/cm^2 was reported by Arenas de Moreno *et al.* (n. d.).

2.6.5. *Escherichia coli (E.coli)*

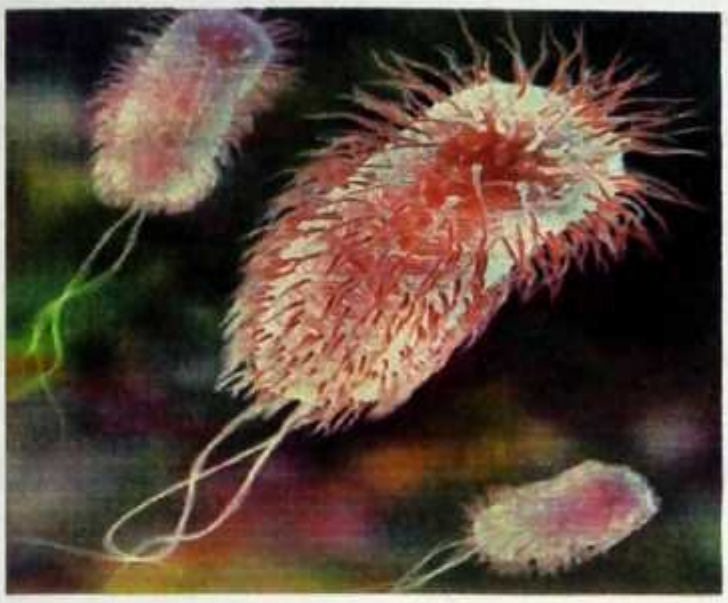


Figure 3. Source: Anonymous, (n.d.)

E.coli is a facultative anaerobe which is usually in the mammalian intestinal tract (Drasar and Barrow, 1985). Its life style is that of fecal-oral and it can constitute 1% of the total gastrointestinal microbes of mammals (Winfield and Groisman, 2003). Cattle have shown up to 30% asymptomatic carriers of *E.coli O157:H7* in a herd (Callaway *et al.*, 2006; Reinstein *et al.*, 2007).

Cattle ability to shed E.coli is widespread but very irregular (Meyer-Broseta *et al.*, 2001). Cattle themselves and the environment they are found are very important sources of pathogenic *E.coli* (Elder *et al.*, 2000).

The shedding of *E.coli* is influenced by the season of the year (Sargeant *et al.*, 2007). There are high levels during the wet season and relatively low when the weather is dry (Naumova *et al.*, 2007). The manure of cattle is capable of harboring certain strains of *E.coli* at environmental temperatures of >49^{0}C (Wang *et al.*, 1996).

There is the possibility of the faeces sticking on the skin of the animals which eventually contaminate the meat at slaughter when care is not taken. *E.coli* on meat does not necessarily indicate the presence of pathogens but simply means there is the risk of pathogens of faecal origin like *Salmonella spp, E.coli 0157:H7 and Campylobacter spp* (Caliciouglu *et al.*, 1999).

Furthermore, the presence of *E.coli* in meat gives a general indication of either direct or indirect fecal contamination of the meat (Clarence *et al.*, 2009). *E.coli's* presence may be assigned to contamination of environmental origin and the resultant growth in or on the meat (Herrera, 2001). It is reported that the prevalence rate of *E.coli* is 85.65% in a research with fresh meat samples from an abattoir and a traditional open market in Nigeria where samples showed 100% *E.coli* prevalence (Enabulele and Uriah, 2009). Stannard (1997) reported that the presence of *E.coli* in a slaughterhouse is an indication of either a good or bad hygienic practices

While some of the strains are commensal, others can be pathogenic to humans and they are normally found in foods of animal origin (Drasar and Barrow, 1985). The presence of *E.coli* as a pathogen does not directly cause food poisoning. Temperatures down to 10^0C give conducive growth conditions to the organism but its growth rate is reduced at temperatures below 10^0C (Anonymous, 1997).

E.coli is able to produce heat- stable toxins at temperatures above 18^0C which can poison meat even where there has been adequate heat application to destroy the organism itself (Anonymous, 1997).

When there is early and sufficient chilling of meat, the growth of the organism is retarded and the toxin is not formed at all (Anonymous, 1997).

Certain strains of *E.coli* are able to cause human hemorrhagic colitis and the popularly known strain is *E.coli O157:H7* (Scotland *et al.*, 1990).

The strain of *E.coli* capable of causing human illnesses and also able to colonize cattle differ geographically and also on temporal basis (Cookson et al., 2007; Wang et al., 2000). The Agricultural and Resource Management Council of Australia and New Zealand (ARMCANZ) has suggested certain levels of *E.coli* in fresh meat as seen in **Table 5**.

Table 5. Description and levels of *E.coli* suggested by Agricultural and Resource Management Council of Australia and New Zealand (ARMCANZ)

Category	*E.coli*/cm^2 or /g
Excellent	Not detected
Good	1-10
Acceptable	10-100
Marginal (Action required)	100-1,000 (10^2-10^3)

Source: Modified from Anonymous, (2003).

2.6.6. *Salmonella spp*

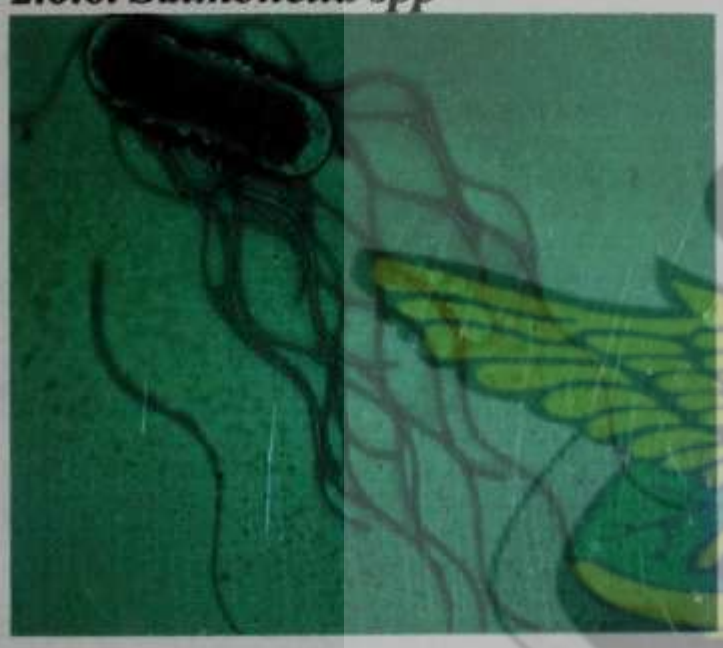


Figure 4. Source: Unnevehr, (2003)

Salmonella spp belong to the family of the *Enterobactericea*. There are many serotypes of this organism.

There are over 2,000 kinds of *Salmonellae* identified to be pathogenic to humans (D'Aoust, 1997). The common one of interest is *S. typhimurium*.

Many microbes cannot withstand the osmotic stresses during certain processing and preservation methods. However, *Salmonellae* are more tolerant to these conditions (Clayton, 2002).

The ability of survival to varying range of environmental conditions, *Salmonella spp* grow within a wide variety of meat products. The prevalence of *Salmonella spp* on beef has been reported as 0.31% of 1171 test samples (Anonymous, 2003).

Reported survival ability of *Salmonellae* in meat products is in temperature range of 5.2°C-45°C, water activity range of 0.945-0.999 and pH range of 4.5-9.5 (Jay, 1992). They can survive with or without a host organism (Berends *et al.*, 1997). Animal faeces, raw meats, factory surfaces as well as kitchen surfaces could be the environmental sources of the organism (FDA, 2001b).

The gastrointestinal tracts and lymph of many animals could be sources of *Salmonella spp*. Other potential sources of *Salmonella spp* are transport vehicles, holding pens, knocking boxes, workers and the equipment (Beach *et al.*, 2002).

Animals asymptomatically carry these microbes and hence make their detection very difficult, (Anderson *et al.*, 2001). Presence of *Salmonellae spp* on raw meat is a reflection of their presence in the live animal than a result of poor hygiene (ICMSF, 1978).

Many microorganisms cannot endure the osmotic stresses that are encountered with high salinity and low water activity; therefore cured products are rarely carriers of pathogenic bacteria.

However, *Salmonellae* are some of the more tolerant pathogens to these conditions (Clayton, 2002). The salmonellosis problem is the result of a continuous faecal-oral cycle, as this genus of organisms is able to survive with and without a host organism (Myint, 2004).

In many cases, the carrier animals are asymptomatic and are therefore difficult to detect (Anderson *et al.*, 2001). The carriers can then shed the pathogens in their faeces, providing the potential for spread of the organisms.

Also, the ability of these bacteria to adapt to a varying range of environmental conditions, allows them to survive within a wide variety of pork products.

Documented survival of *Salmonella spp* in meat products has been observed among temperatures 5.2°C-45°C, water activities 0.95-0.99 and pH values 4.5-9.5 (Jay, 1992).

2.6.7. OTHER BACTERIAL FLORA

Depending on the kind of conditions prevailing, bacteria like *Pseudomonas spp, Bacillus spp, Staphylococcus spp* and others could also be found on beef. Many research works have found different levels of these microbes on the surface and in muscles of beef. Buys (2000) found *Enterobacteriacea, Staphylococcus spp, Bacillus spp* and *Pseudomonas spp* after characterizing the microbial flora on beef and poultry carcasses. In another development, the above named microbes are the usual ones found on meat (ICMSF, 1980). These microbes could either be Gram-negative or positive. According to Gill (1983), the predominance of a particular bacteria group depends on their resistance to pH and temperature.

In a similar study in an abattoir in Sudan, the samples analysed recorded 14.76% and 10.54% for *Staphylococcus spp* and *Bacillus spp* (Abdalla *et al.*, 2009).

2.7.0. MEAT SPOILAGE

Spoiled meat could result from damages or injuries from poor handling of animals before slaughter. The injuries or damages could be caused by insects, physical injury during production, packaging and even storage.

Enzymatic degradation of meat, and also the activities of microorganisms could lead to spoilage (Jackson and Mcgowan, 2001).

Meat spoilage by microbes is due to rise in their number, utilization of nutrients in the meat, their enzymatic activities which leads to bad flavours because of the breakdown of certain nutrients in the meat (Reddy, n.d.). Meat spoilage is evident when microbial numbers are as low as one million per cm^2 (Anonymous, 2009a).

Spoilage bacteria found on dressed carcasses are usually due to cross-contamination from environmental origin e.g. contact surfaces like tables and other tools other than from human source (WHO, 1990).

Aerobic bacteria are predominant on meat in an oxygenated condition (Anonymous, 2009a) which are usually *Pseudomonas*, spoilage bacteria. The nutrients in meat are the same ones microbes used for growth and thus survival making meat spoilage very easy when conditions are favourable. Meat begins to spoil soon after animals are slaughtered as a result of physical, chemical and biological processes during the period of meat production (Hedrick *et al*., 1994). When the aerobes on the meat use nitrogen-containing compounds like amino acids, the end products of the microbial activity would include stinky amines like ammonia, putrescines and cadaverine and also sulphur containing compounds (Anonymous, 2009b).

All these resultant compounds together cause the 'off' odours and flavours generally known as putrid (Anonymous, 2009a). Jackson and Mcgowan (2001) found that meat could be subjected to changes by inherent enzymes.

By microbial activities, fats in meat could get oxidized chemically and these microbes could grow on meat causing organoleptic, textural and visual changes after they release metabolic wastes (Jackson and Mcgowan, 2001).

2.8.0. FOOD-BORNE DISEASES AND REPORTS

The outbreak of food- borne diseases is an important health problem in the world with its resulting economic problems. Several meat-borne pathogenic microbes have been discovered within the last two decades in the United States (Amezquita *et al.*, n.d.). They added that these pathogenic microbes cause various disease outbreaks, deaths and the associated economic losses. Jay (2000) said that these food borne diseases come third after cardiovascular and respiratory diseases in USA. Adak *et al.* (2005) also reported that it is a major cause of sickness and death on the globe.

The mean occurrences of food- borne diseases in the developed and developing countries are 38.3 and 915.8 in hundred thousand population respectively (Tavakoli and Riazipour, 2008).

Adams and Moss (2002) found that the rate of the food-borne diseases incidence has increased from 19 cases in hundred thousand population in 1985 to 62 cases in hundred thousand population in Australia and in Spain from 30 cases in 1983 to 116 cases within the same population in 2001.

Tavakoli and Riazipour (2008) reported that many developing countries like Ghana do not have accurate data on the incidence of food borne diseases.

The incidence of food born diseases seems to be higher in the developing countries than that of the developed world due to poor and unhygienic conditions of production, processing, distribution and even points of sales conditions and also low health educational levels in these countries (Tavakoli and Riazipour, 2008).

The prevalence and incidence of food borne diseases in the developing countries are higher when compared to that of the developed countries (Tokassian *et al.*, 2004).

Food related illness were considerably high with cholera (2,216), typhoid fever (65,333) and diarrhoea (331,998) (Soyiri *et al.*, 2008). The true picture may not be seen because there are no epidemiological systems in place (Soyiri *et al.*, 2008).

An estimate of 60 people die and 73, 000 fall sick because of *E.coli O157:H7* every year (Mead *et al.*, 1999) and reports indicate an estimated amount of $1billion spent each year (USDA-ERS, 2001).

Pathogens isolated from meat may cause self-limiting enteric illnesses or systemic and even fatal diseases to the immuno-compromised, the young and even the elderly (Marshall and Bal'a, 2001).

Approximately 69% of the reported cases of bacterial food borne illnesses are caused by gram negative bacteria (Clarence *et al.*, 2009).

Most of these gram negative bacteria are of faecal origin which usually contaminates the meat through poor handling during production and processing (Turtura, 1991).

Many of the bacterial infections are caused by toxins produced by the bacteria. Karama (2005) found that the toxins may express their pathogenic effects on target cell in a direct manner. Others could interact with the cells of the immune system which would end in the release of immunological mediators resulting in pathophysiological effects (Karama, 2005).

Eley (1994) described two main kinds of those toxins as endotoxin (a part of the outer membrane of Gram-negative bacteria) and the exotoxins that complicated both by the Gram-positive and negative organisms.

Currently research has revealed that there is a continuous development and adaptation of resistant pathogenic microbes to antibiotics and gradually to the traditional food preservation methods, like heat application, solar drying, low water activity, low pH, and chemical additives (IFT, 2006).

The existence of certain strains of pathogenic microbes is evident where they have enhanced their ability to survive in their hosts, low infective doses and improved virulence after exposure to common environmental stresses for some time (Samelis and Sofos, 2003).With such developments in the microbes, they pose a threat to human health and the situation has its associated problems if they are left unchecked.

Salmonella spp is one of the most common causes of bacterial gastroenteritis in humans in the world (Nouichi and Hamdi, 2009). Red meat and poultry are the major sources of the organism. The continuous faecal-oral cycle make salmonellosis a problem (Berends *et al*., 1997).

Even though the real occurrence of salmonellosis is difficult to assess, it is believed that there is an increase in the incidence of the disease in most parts of the world (Forshell and Wierup, 2006). Nausea, vomiting and diarrhoea with or without fever are the most common clinical evidence of the disease. The true incidence of salmonellosis is difficult to evaluate because of lack of epidemiological system in Ghana, which is particularly true in developing countries.

However, the number of outbreaks particularly in humans has increased considerably in most parts of the world (Forshell and Wierup, 2006).

The most common clinical presentation is gastroenteritis with nausea, vomiting, and diarrhoea with or without fever. The severity of sickness with developing haemolytic uremic syndrome, high rate of mortality especially in immune-compromised persons is appreciably higher in *E.coli* than *Salmonella spp* even though illnesses reported due to *E.coli* are fewer than *Salmonella spp* (Mead *et al*.,1999).

2.9.0. CONTROL OF CONTAMINATION IN BEEF PRODUCTION

All conditions and measures crucial to safety assurance and suitability of food through the entire food production chain are referred to as food hygiene (FAO/WHO, 1999). Food safety objectives are very important in the quest for safe and suitable food (Anonymous, 2004).

Good sanitation measures, hygienic practices, handling and processing of meat, applications of decontamination measures, storage conditions, manner of distribution and retail display all influence the type and level of meat contamination.

Research has revealed that proper sanitations measures in slaughter plants can effectively reduce microbial contamination of carcasses (Arthur *et al.*, 2007).

Every food production or processing system must have a quality assurance mechanism in place to ensure safety.

Quality assurance implies in principle that potential microbiological risks found and the necessary control measures are implemented throughout the meat production chain (WHO, 1990). By a systematic assessment of hazards or risks, developing control measures and focusing on measures to prevent them, meat safety could be achieved (Anonymous, 2004). Varieties of microorganisms have to be considered during the process of risks or hazards identification.

Hazards identified in a meat production or processing chain may differ from one country to the other, the health status of animals, slaughter procedures, the kinds of meat products and the system of distribution as well as storage (WHO, 1990).

Considering the system of meat production, distribution and storage during sales principles applied in Ghana, strict implementation of international production standards cannot be applied here.

But there is the need to put into practice a certain level of good hygiene in meat production and sales (Soyiri *et al.*, 2008). Throughout the whole production process, the potential effects of production activities on meat safety and suitability should be crucial at all times (Codex, 1997).

Meat production, processing, distribution, storage and its sales activities require tailor-made hygiene and safety measures which suite the local and national situation (FAO/WHO., 2003). In the case of developing a meat hygiene programme in Ghana, it would not be appropriate to set standards that would require for instance refrigeration of meat during sales looking at the way meat is sold in Ghana.

There are three basic measures in the development of practical Meat Hygiene Programme. These are Good Hygienic Practices (GHP), the Hazard Analysis and Critical Control Point (HACCP) system and Risk assessment (Codex, 1997).

Good hygienic practices provide the baseline for food control measures. In general, GHP consists of practices regarding conditions and mechanisms necessary for assurance of safety and suitability of food. GHP guidelines describe the quantitative specification of acceptable levels of contaminants in food (Codex, 1997). Good sanitation and personal hygiene in the meat production, processing and sales conditions are very important especially in a developing country like Ghana.

The environmental sanitation is very important since the immediate surroundings of meat production; processing or sales outlet could be a major source of contamination (Codex, 1997).

The level of personal hygiene practised by workers in meat production, processing and sales facilities is also important when safety and suitability of the product is to be assured.Frequent cleaning of equipment and tools during working hours and thorough cleaning after work is also important.

The Hazard Analysis Critical Control Points (HACCP) system is the widely recommended system for assurance of food safety and suitability for consumption.

A HACCP system is a prepared document in accordance with the HACCP principles to ensure control of hazards significant to food safety in line with the food production chain (Anonymous, 2004).

Many research works have revealed the importance of HACCP in maintaining and ensuring safe food. Wagude (1999) found significant decreases in levels of pathogenic microbes in a research done in a slaughterhouse after the implementation of HACCP system in South Africa. She found 14% of the baseline samples as *E. coli* positive but after the HACCP implementation, no sample was positive to the *E. coli* test that was run.

CHAPTER THREE

3.0. MATERIALS AND METHODS

3.1.0. RESEARCH SITE

The main research area was the Kumasi metropolis. It is the capital city of the Ashanti Region. Kumasi is the second most densely populated city in Ghana. There were two specific research sites where samples were taken for this study, the slaughter plant and the butchers' shops. These were chosen because they are the sources of beef in Kumasi. The Kumasi Abattoir Company Limited (K.A.C.L.) slaughter plant was chosen because that is where the animals are converted into beef. Two different markets, Atonsu and Maayanka markets which source their beef from K.A.C.L. were selected as where samples from the butchers would be taken.

3.1.1. THE SLAUGHTER PLANT

The study considered the cattle slaughter plant of Kumasi Abattoir Company Limited (K.A.C.L.). The plant is located at Kaase-Ahinsa area. This is an Industrial area where the environment is highly suspected to be contaminated with industrial waste. The market for the sale of livestock is also just around the plant. There is also a market for food stuff very close to the slaughter plant. The Kumasi Abattoir Company Limited (K.A.C.L) is the main source of beef for many meat markets in the Kumasi metropolis and even certain parts of Obuasi.

Cattle are slaughtered in a section different from sheep and goats as well as pigs. The research concentrated on the section for cattle only. That section has two main lines of slaughter, lines A and B. This section has a capacity of about 200-300 cattle/per day. There are twenty workers in each line. Prior to the start of the work, enquiries were made about how operations are carried out in the Abattoir.

The production manager and the foreman of the plant were given explanation into the work for cooperation. The production manager spoke to the workers in the plant for them to cooperate where they needed to.

3.1.2. THE BUTCHERS' SHOPS

Butchers slaughtered their cattle at K.A.C. L. slaughter plant at a certain fee. Only the slaughter services are provided by the plant. Transportation of the carcasses to the market is the problem of the butchers which makes them resort to ways that would not increase the production cost. The Maayanka market just near the Abattoir and the Atonsu markets were the places where samples were taken to represent the butchers' shops. These two markets were selected because the butchers source their beef from the abattoir. Ten butchers were randomly selected for the work in each market. It was very difficult to explain and convince them on what the work was about. Most of them did not allow samples to be taken from their meat.

3.2.0. PREPARATION OF COTTON SWABS

Already manufactured pyrogen-free cotton-tipped swabs of about 1inch in thickness (Sea Pearl, Cel Mart International Inc, USA) were used. Since the dry swabbing method was going to be used, the swabs were kept in their packs aseptically till they were ready to be used.

3.3.0. PREPARATION OF BROTHS

3.3.1. BUFFER PEPTONE WATER

The formulation was done with peptone mix 10.0g, sodium chloride 5.0g, disodium hydrogen phosphate 3.5g, potassium dihydrogen phosphate 1.5g (Park Scientific Limited, Northampton, UK). The product is in the powder form. 20g of the powder was weighed and added to 1litre of distilled water. The solution was mixed thoroughly and 10ml distributed into each screw cap glass containers. Sterilization was done by autoclaving at 121^0C for 15 minutes. The final pH was 7.2 ± 0.2.

3.3.2. MacConkey BROTH (OXIOD)

This is a powdery substance with the composition of 20g of peptone, lactose 10g, sodium chloride 5g, neutral red 0.075g and bile salt 5g (Oxoid Limited, England). 40g of the manufactured powder was added to 1litre of distilled water and allowed to dissolve and settle for about 10minutes. 5ml of the solution was put into each test tube and fitted with Durham's tubes. The test tubes containing the solution were sterilized by autoclaving at 121^0C for 15 minutes. The final pH of 7.4 ± 0.2 at a temperature of 25^0C was reached.

3.3.3. TRYTOPHAN BROTH (Scharlau)

The product in the powdered form has a composition of 10g of meat peptone, L-tryptophan 1.0g, 5g of sodium chloride (Scharlau Chemie S.A, Barcelona, Spain). 16g of the powder was added to 1litre of deionised water. 9ml of the solution was distributed into each test tube and then sterilized by autoclaving at 121^0C for 15 minutes. The final pH reached was 7.0 ± 0.2.

3.3.4. CM0699 SELENITE CYSTINE BROTH BASE (OXIOD)

5.0g of tryptone, 4.0g/m of lactose, sodium L-cystine 0.01g/m and 10g/m of disodium phosphate 4g of sodium bi-selenite as manufactured into powder (Oxoid Limited, England). 19g of the powder was suspended in 1litre of distilled water. The suspension was warmed for complete dissolution. 9ml of the solution was dispensed each into screw cap containers of depth of at least 60mm. Sterilization was done in a boiling water bath for 15 minutes. The broth was not autoclaved before use.

3.4.0 PREPARATION OF AGARS

3.4.1. PLATE COUNT AGAR (SP)-[MO221]

It is a powdery substance of the formulation of tryptone 5.0g/L, yeast extract 2.5g/L, glucose 1.0g/L and agar-agar 12.0g/L (Park Scientific Limited, Northampton, UK). 20.5g of the powder was weighed into 1litre of deionised water. Sterilization was done by autoclaving at 121^0C for 15 minutes. Cool to 47^0C before use.

3.4.2. *SALMONELLA-SHIGELLA* AGAR (M0240)

The composition of the agar is in g/l., 5.0 beef extract, balance peptone 5.0, sodium citrate 5.0, sodium thiosulphate 8.5, agar-agar 13.5, lactose 5.0, 8.5 bile salts, No. 3 ferric citrate 1.0, brilliant green 0.00033 and 0.025 of neutral red (Park Scientific Limited, Northampton, UK). The product is in the powder form. 60g of the powder was added to 1litre of distilled water. The powder was allowed to soak for about 10 minutes.

The solution was swirled to mix then allowed to boil. The solution was allowed to cool to 47^0C. The agar was shaken to mix well and then poured into petri dishes. The surfaces of the poured agar were allowed to dry before inoculation. No autoclaving was done. A final pH of 7.0 ± 0.2 was reached.

3.4.3 NUTRIENT AGAR

This medium has a formulation of 1g/l of meat extract, 2g/l of yeast extract, peptone 5g/l, 5g/l of sodium chloride and 15g/l of agar (BIOTEC Laboratories Limited, United Kingdom). This product is in the powder form. 28g of the powder was suspended in 1litre of water. The solution was brought to boil for complete dissolution. Sterilization was done by autoclaving at 121^0C for 15 minutes.

3.5.0. REAGENTS

The only reagent used in the analysis of the microbes was the Kovac's indole reagent.

3.5.1. KOVAC'S INDOLE REAGENT

The ingredients per liter of the reagent are 5g of paradimethyl-aminobenzaldehyde, 75 ml of amylalcohol and 25 ml of hydrohydrochloric acid as prepared (Merck KGaA, Germany).

3.6.0. SAMPLE COLLECTION

3.6.1. SLAUGHTER PLANT

The slaughter plant has two lines, lines A and B for beef production. Each line has a capacity of slaughtering about 200 cattle per day. Fifteen carcasses were considered as sources of samples from each line. Dry swab method was used with sterile pyrogen-free cotton swabs within an area of $100cm^2$ on the carcasses and $52.5cm^2$ on the knives. The swabs taken were put into screw cap glass containers of 10ml of Buffer Peptone Water (pH 7.0±2.0) after the swabbing was done.

Swabs were taken from the knives used for the neck sticking, the surface of the flesh under the skin after dehiding, inside of the carcass after evisceration, knives used for final trimming. The general sanitation in the plant was evaluated by simple physical observation of the number of times the floor was washed, workers washed their hands, knives and other equipment used in the slaughter process were also washed.

Swabs taken were immediately put into the containers containing the peptone water, kept on ice at a temperature of about 2^0C and sent to the laboratory for the microbial analysis immediately. About 50g of meat from each carcass swabbed was also collected to check for pH.

3.6.2. BUTCHERS' SHOPS

Twenty randomly selected meat shops were chosen for the work. The butchers were informed of what was going to be done. Most of them were reluctant to comply initially since they thought it was an exercise to send them out of business. The general sanitation of their surroundings was evaluated by the presence of washing basin and towel, whether they have covered their heads, noses and mouths.

Swabs were taken from their benches, knives and the surfaces of the meat. The dry swab method was employed by swabbing within an area 100cm^2 on the benches and meat surface and 52.5cm^2 on the knives with sterile pyrogen-free cotton swabs. The swabs taken were immediately put into the peptone containing container and kept on ice at about 2^{0}C. The samples collected were then sent to the laboratory for microbial analysis to commence. About 50g of meat from each butcher was also collected to check the pH.

3.7.0. pH MEASUREMENT OF BEEF

The pH of the meat samples were measured by the Mettler Toledo MP-220 pH meter. The instrument was calibrated by standard buffer solution before use. The pH of the meat samples were determined by inserting the pH meter into the meat directly and the readings taken.

This exercise was done three times for each sample and the mean and standard deviation of the three readings were recorded. After each sample measurement, the rod was washed with distilled water before taking measurement of the next sample.

3.8.0. MICROBIOLOGICAL ANALYSIS

Contents of the swabs taken from both the slaughter plant and the butchers' shops were brought to laboratory on ice within 1$^1/_2$ hours. The samples were vortexed vigorously for about a minute into solution by stirring each swab into a bottle containing 10ml of Buffer Peptone Water (BPW). The various assays were used to identify the various microbes.

3.8.1. TOTAL PLATE COUNT (TPC)

1ml of each homogenate was used to make a 10-fold serial dilution up to 10^{-15}. The diluents 10^{-13}, 10^{-14}, 10^{-15} were put on petri dishes. About 10ml of the Plate Count Agar (PCA) was poured into the petri dishes at a temperature of 47^0C and mixed thoroughly with the 1ml of the diluents and swirled in clockwise and anticlockwise directions. The petri dishes were inverted and incubated at 37^0C for 24 hours under aerobic conditions. One of the three petri dishes was selected and the visible colonies were counted with Stuart Scientific illuminated colony counter. Colonies of distinct appearance were selected for sub-culturing.

3.8.2. SUB-CULTURE OF COLONIES

Prepared Nutrient Agar was poured into petri dishes and allowed to solidify. Colonies on counted petri dishes with distinct features like colour or certain morphological appearance were sub-cultured to make identification much easier.

These colonies were taken with a loop which was sterilized by flaming with naked flame. The colonies picked by the sterile loop were carefully streaked onto the agar in the petri dishes. The dishes were labeled according to the label of the petri dish from which a particular colony was picked. The dishes were incubated at 37^0C for 24 hours under aerobic conditions.

The sub-cultured colonies grew all over the plates. Pure cultures were transferred onto Nutrient Agar in test tubes which were tilted before solidifying. These test tubes were left in the fridge for 24 hours before transfer was done.

The growths on the petri dishes were then streaked onto the agar in the test tubes and incubated at 37^0C for 24 hours. The growths in the test tubes were kept in a fridge for bacterial identification later.

3.8.3. IDENTIFICATION OF BACTERIA

3.8.3.1. PREPARATION OF SLIDES

The identification was done by Gram's staining. Grease-free slides were used for the staining of colonies selected from sub-cultures in test tubes. Since the smears were taken from agar culture, a drop of water was put on the slide and then a little of the culture was taken by a sterile loop. The smear was then spread by the loop on the slide and fixed onto it by passing the slide on Bunsen flame 2 or 3 times.

3.8.3.2. THE GRAM STAIN

The bacterial smear was stained with a few drops of 0.5% crystal violet for 2 minutes. The crystal violet was then washed with water and drained off. Few drops of dilute iodine were put on the slides for about 2 minutes and then washed off with water. The crystal violet and iodine formed a purple /black complex inside the bacterial cell. A careful drip of absolute alcohol onto the smear was done and then allowed to run off. This activity was done three times and then washed off with water. The alcohol dissolved the lipid layer around the Gram negative cells and made the crystal violet/iodine complex to wash out. A counter stain was done with 1% safranin for 2 minutes and then washed with water and drained off the slides. The slides were then ready for observation under a microscope.

3.8.3.3. MICROSCOPY

The slides were examined under microscope without cover slips. The stained smears were found with a low power objective X10 or X20. A small drop of immersion oil was directly put onto the smears. The smears were examined with an oil immersion (X100) lens. The Gram-positive and Gram-negative bacteria were identified according to the dichotomous keys based on morphological and biochemical features, (Dainty *et al.*, 1979).

3.9.0. INDENTIFICATION OF INDICATOR ORGANISMS

3.9.1. TOTAL *Coliforms* COUNT (TCC)

The method used was the Most Probable Number (MPN), (AOAC, 1995) to find the Total *coliforms* of each swab that was taken. The three tubes-MPN method was employed. The test tubes were filled with 5ml of MacConkey broth with Durham's tubes and sterilized before use. The test tubes were labeled in accordance with the number of diluents and each tube containing MacConkey broth had corresponding diluents bearing the same number.

A serial dilution with factor ten was done from 1 -10 and 1ml of each diluents was inoculated into each test tube containing the 5ml sterile MacConkey broth according to the label found on it. The test tubes on test tube racks were then incubated at 37^0C for 24 hours under aerobic conditions.

After the incubation period, the test tubes were observed to find changes in the colour of the broth from purple to yellow signifying either the presence or absence of the organisms and their numbers when present. The MPN table was used to find the number of the organisms present in every sample.

3.9.2. FAECAL *Coliforms* COUNT (FCC)

The MPN method, (AOAC, 1995) was used in determining faecal *coliforms*. The three tubes-MPN method was what made enumeration of the faecal microbes. 1ml of the diluents was inoculated into test tubes containing 5ml of sterile MacConkey broth with Durham's tubes bearing the same number. Serial dilutions from 1-10 were done by inoculating 1ml of the diluents into the 5ml MacConkey broth in test tubes with Durham tubes in them.

The test tubes on racks were put into the 44^0C incubator for 24 hours under aerobic conditions. After the 24 hours, the test tubes were observed to find the change in colour of the MacConkey broth from purple to yellow. Test tubes which showed the expected change were from 1-7 though not all the three tubes for each factor changed from purple to yellow. The MPN table was used to determine the number of organisms in each sample.

3.10.0. ISOLATION OF SPECIFIC PATHOGENS

3.10.1. *E.coli*

The test tubes containing MacConkey broth incubated at 44^0C were observed for change in colour from purple to yellowish colour for the presence of faecal *coliforms*. The tubes showing the change in colour of the broth were considered as positive to the test done.

E.coli was then tested for by inoculating contents of the positive tubes into sterile test tubes containing tryptophan broth. The test tubes containing tryptophan broth were labeled according to the number on each test tubes containing MacConkey broth. 1ml

of changed MacConkey broth was put into a corresponding tube containing 5ml tryptophan broth. The tubes were incubated at 44^0C for 24 hour before observation. The tryptophan broth tubes became turbid after the incubation period.

3.10.1.1. INODLE TEST

Confirmatory test for the presence of *E.coli* was done by the indole test. This was done by putting into the tubes 0.3-.0.5ml of KOVAC'S reagent. The presence of red colour ring at the meniscus of the tryptophan broth in the test tubes indicated the sample was positive for *E.coli*. Not all three test tubes for each dilution factor showed positive. The MPN table was used to find the approximate numbers present in each sample.

3.10.2. *Salmonella spp*

3.10.2.1. PRE-ENRICHMENT OF ORGANISMS

The swabs taken kept in the Buffer Peptone Water (BPW) found in screw cap glass containers were incubated under aerobic conditions at 37^0C for 24 hours. Observation made after the incubation period revealed the BPW turned from light golden colour to a turbid culture.

3.10.2.2. ENRICHMENT OF ORGANISMS

1ml of the pre-enriched BPW culture of each sample was inoculated into 9ml of prepared of Selenite Cystine Broth Base (CM0699-OXIOD). The enriched cultures were subsequently incubated at 44^0C under aerobic conditions for a period of 24 hours. The light golden colour of the Selenite was changed into a turbid culture after

the incubation period. A serial dilution was done with each enriched culture from the factor of 1-10. 1ml of each diluent was transferred onto a petri dish where *Salmonella-Shigella* agar (SS agar) was poured on the inoculum at a temperature of 47^0C. The agar and the inoculum were mixed thoroughly and then left for the surface to dry. The plates were then inverted and incubated at 44^0C for 24 hours.

Observations made after the incubation period revealed red colonies had either black inner centres or not. The red colonies with black centres were considered to be the *Salmonella spp*. The colonies were then counted with the electronic Stuart Scientific illuminated colony counter.

3.11.0. STATISTICAL ANALYSIS OF DATA

Three swabs were taken for each particular site. The mean values for swabs were calculated to represent the site swabbed. For statistical analysis purposes, the counts of microbes were converted into log values of colony forming units per cm^2 (CFU/cm^2) and Most Probable Number per cm^2 (MPN/cm^2). Data in tables were arranged per microbes identified at a specific point in the study.

There were five different microbes identified at four different places on the carcasses in the abattoir and three different places in the butchers' shops. There were thirty different carcasses swabbed from the abattoir and twenty from the meat shops. There were four hundred and eighty (480) samples taken from the abattoir and one hundred eighty (180) samples were also taken from the two selected markets.

Mean log CFU/cm^2 or log MPN/cm^2 and standard deviation (SD) values were also computed for each type of microbe at every point of sample collection by Microsoft Excel 2007 (2003-2007 Microsoft Corporation).

Analysis of variance (ANOVA) was determined for data using Genstat recovery 3. Comparison among the means was done by Statistix version 9.0 ($p = 0.05$). Pie charts were drawn using Microsoft Excel 2007.

CHAPTER FOUR

4.0. RESULTS AND DISCUSSIONS

4.1.0. RESULTS

The study was conducted to evaluate the microbiological status of beef produced at the Kumasi Abattoir and sold also at selected butchers' shops in two selected markets (Maayanka and Atonsu markets) of the Kumasi Metropolis. Samples collection for the study was done for a period of three months from the beginning of April to the end of June of 2010.Though laboratory analysis continued right after each sampling day, final completion of lab analysis took a little longer. These results present the counts for Total Plate Count (TPC) and *Salmonella spp* Count (SC) in log CFU/cm^2, Total *coliforms* Count (TCC), Faecal *coliforms* Count (FCC) and *E.coli* Count (ECC) in log MPN/ cm^2. Other bacterial flora like *Enterobacteriacea, Bacillus spp*, *Staphylococcus spp* and *Pseudomonas spp* were also identified on samples taken and represented in percentage.

4.1.1. OBSERVATIONS IN THE PLANT

Production started as early as 6am and ended around mid-day. Each production step had different set of workers performing a particular function. The recommended pre-slaughter practices like stunning do not go on at the abattoir. Animals slaughtered at the abattoir do not belong to the company but rather the butchers. Each butcher and his apprentices entered the plant with the idea of protecting their own. At every stage of production there were many people around but only few were staff of the abattoir. There were no inspections by the veterinary meat inspectors in the plant at any point of production unless the inspection table where the liver, kidney and heart were

checked for any abnormalities in their size, colour, shape or even the presence of any unusual feature.

Blood drained from the animals on the floor was not washed away until several animals were slaughtered. Knives, rails, hooks and other equipment used in the production process were not washed after they have been used on one carcass.

There was so much congestion in the plant without any footbath at the entrance. There were no sinks or washing basins around for regular hand-washing. The staff did not cover their heads, noses and mouths. Some of them even wore jewellery and there was so much noise inside the plant. If anybody around had an air borne disease, this could be transmitted easily.

The traditional meat inspection went on by the veterinary meat inspectors on duty where they cut through the meat at points like the shank to check if there were any presence of organisms whiles the other organs like the liver and kidney were also inspected on the inspection table. Cuts were made through these organs to check if there were any abnormalities. Though this inspection aims at ensuring safety, it did not consider the issue of microbial contamination during the slaughter process (Hudson *et al*., 1996).

The following is a description of how cattle are slaughtered at the Kumasi abattoir where part of this study was conducted.

Driving of animals to the slaughter plant from the kraal

↓

Restraining animal for sticking

↓

Sticking

↓

Bleeding

↓

Animal hoisted on rail by the hind leg and bleeding continues

↓

Head and legs cutting

↓

Skin removal or Flaying

↓

Evisceration

↓

Veterinary Inspection

↓

Carcass trimming and splitting

↓

Moved to loading bay

↓

Ready for transportation to retail shops

Figure 5: Cattle slaughter process in K.A.C.L. flow –diagram.

4.1.2. OBERVATION MADE AT THE BUTCHERS' SHOPS

Beef from the abattoir arrived at the shops either in Taxi boots or at the back of Pick-up tracks covered with polythene sheets. There were no temperature control considerations during the transportation of the meat. A butcher's shop consisted of a table or beneh under a shed with different sizes of knives and a weighing scale. Those in the big markets have permanent shed with different sizes of table depending on the quantity of beef the individual sells. The butchers said the surfaces of their tables are washed thoroughly at the end of each working day.

The sharp knives they used in cutting portions of their stock for their customers left many crevices on the surfaces of their table tops. The crevices could hold blood and bits of beef portions in them which could contaminate the next day's stock (Lauzon, 1998). Beef was displayed in the open without any temperature control system. The meat was then at the mercy of the weather, flies and other environmental pollutants. Water was occasionally sprinkled on the surface of the meat to keep the surface a bit wet. The butchers had devised a way of driving away flies by using polythene bags torn at one side but still held together at the bottom and held to a stick. Unfortunately, there were refuse dumps close to the markets and even the immediate surroundings were not tidy. Trimmings made during sales were left till the end of the day. The attire the butchers themselves wore could also be a source of contamination since they were dirty with blood stains. One group of the butchers visited had a freezer for leftover meat but it was in an insanitary condition. No sanitary inspector was ever met during the period of sample collection.

4.1.2. pH OF CARCASSES AT THE SLAUGHTERHOUSE

The pH of the thirty carcasses sampled in the study gave a range of pH measure between a mean 6.41 and 8.40 with standard deviations of ±0.03 and ±0.05 respectively. **Table 6** gives the pH values measured for each carcass.

4.1.3. pH OF BEEF AT THE BUTCHER' SHOPS

Twenty butchers' shops from two selected markets (Maayanka and Atonsu markets) in Kumasi were visited and samples collected. Beef found on their tables were sampled and the pH of the meat checked. The results of the pH reading revealed a range from 7.21 to 8.68 with standard deviations of ±0.04 and ±0.24 respectively. **Table 7** gives the mean pH values with their standard deviations of each beef sampled from the butchers' tables.

Table 6. Mean pH values and their standard deviations for 30 beef carcasses sampled in the slaughterhouse of the Kumasi Abattoir

Carcass number	Mean pH reading	Standard Deviation(±)
1	8.37	0.15
2	8.21	0.17
3	8.15	0.13
4	7.76	0.08
5	8.15	0.06
6	6.65	0.05
7	7.56	0.26
8	7.07	0.08
9	6.70	0.26
10	6.89	0.17
11	7.33	0.04
12	6.87	0.04
13	7.53	0.05
14	6.91	0.09
15	8.40**	0.05
16	8.30	0.05
17	8.36	0.08
18	7.53	0.06
19	6.92	0.07
20	8.05	0.05
21	6.57	0.07
22	8.30	0.02
23	7.10	0.05
24	6.50	0.06
25	8.31	0.05
26	7.29	0.05
27	8.31	0.03
28	6.41*	0.03
29	7.62	0.03
30	7.21	0.04

*** and ** represent the minimum and maximum range respectively.**

Table 7. Mean pH values of beef sold in some twenty selected meat shops in the Atonsu and Maayanka markets in Kumasi

Carcass number	pH reading	Standard Deviation(±)
1	7.50	0.10
2	7.21*	0.04
3	7.44	0.10
4	7.55	0.05
5	7.28	0.27
6	8.08	0.05
7	7.40	0.08
8	8.35	0.34
9	8.68**	0.24
10	8.09	0.36
11	8.15	0.28
12	7.64	0.90
13	8.59	0.08
14	7.55	0.05
15	7.45	0.07
16	8.34	1.00
17	8.29	0.27
18	7.79	0.32
19	8.17	0.20
20	7.91	0.18

*** and ** represent the minimum and maximum range respectively.**

4.2.0. BACTERIAL COUNTS AT THE SLAUGHTERHOUSE

4.2.1.1. TOTAL PLATE COUNT (TPC)

The log means for the TPC for sticking knives used on thirty carcasses in the Kumasi Abattoir slaughter plant ranged from 13.41 to 15.23 CFU/cm^2 with standard deviations of ±0.59 and ±0.57 respectively. A range of 13.25 to 14.56 CFU/cm^2 was recorded for the log means of TPC of the inner surface of the thirty carcasses with standard deviations of ±1.99 and ±0.56 respectively.

The log means recorded for the surface of the carcasses after skin removal had a range of 13.60 to 14.66 CFU/cm^2 with ±0.59 and ±1.01 as their respective standard deviations for thirty carcasses. The TPC found on the last knives used for trimming of the thirty carcasses fell within the range of 13.66 and 14.78 CFU/cm^2 with their respective standard deviations as ±0.60 and ±1.17. There were no significant differences among the levels of TPC enumerated at different sampling sites at the slaughterhouse in this study as shown in **Table 8**.

4.2.1.2. TOTAL *COLIFORM* COUNT (TCC)

The log means for TCC found on sticking knives for sticking thirty cattle in the Kumasi Abattoir slaughter plant ranged from 8.05 to 9.41 MPN/cm^2. Their standard deviations were ±0.51 and ±0.61 respectively. A range of 8.26 to 9.74 MPN/cm^2 for TCC was found at the inner surface of thirty carcasses at the slaughter plant with ±0.99 and ±1.54 as the respective standard deviations for the range given above. Log mean values of TCC recorded for thirty carcasses after skin removal fell within a range of 7.81 and 9.14 MPN/cm^2 with standard deviations of ±0.54 and ±0.60 respectively. The log means of TCC found on the last knives used for trimming of the thirty carcasses before loading recorded a range of 7.81 to 9.39 MPN/cm^2 with respective standard deviations of ±0.65 and ±0.03.

There were no significant differences ($p<0.05$) observed among the levels counted and this can be seen in **Table 9.**

Table 8. Mean values of Total Plate Count (TPC) expressed as log CFU/cm^2 with their standard deviations (SD) for 30 beef carcasses at two different parts of the carcasses and two different knives used on the carcasses during beef production in the Kumasi Abattoir slaughter plant.

Carcass number	Sticking knife (±SD)	Inner surface after evisceration (±SD)	Surface of carcass after skin removal (±SD)	Last knife used for trimming (±SD)
1	13.86 (0.58)	14.29 (1.01)	13.96 (0.59)	14.42 (0.99)
2	14.52 (1.01)	14.32 (1.02)	14.66(1.01)**	14.47 (1.01)
3	13.90 (0.58)	13.97 (0.58)	14.29 (1.01)	14.14 (0.56)
4	14.20 (0.58)	14.56(0.57)**	14.29 (1.00)	14.78(1.17)**
5	15.23 (0.57)**	13.94 (0.59)	14.31 (1.00)	14.20 (1.14)
6	13.87 (0.57)	14.19 (0.99)	14.30 (1.00)	14.51 (0.99)
7	14.24 (0.58)	14.25 (1.00)	14.32 (1.01)	14.84 (0.58)
8	14.20 (1.16)	14.22 (0.99)	14.65 (0.56)	14.46 (0.99)
9	14.51 (1.00)	13.55 (0.58)	14.30 (1.00)	13.83 (0.59)
10	14.58 (0.01)	13.99 (1.16)	13.65 (0.56)	13.90 (0.59)
11	14.34 (0.98)	14.10 (1.01)	13.97 (0.58)	14.23 (0.57)
12	14.58 (1.01)	13.93 (0.58)	13.96 (0.59)	14.53 (1.01)
13	14.53 (0.99)	13.57 (0.59)	13.96 (0.57)	14.47 (1.00)
14	13.91 (0.58)	13.62 (0.58)	13.63 (0.58)	13.66 (0.60)*
15	14.56 (1.00)	13.83 (1.15)	13.93 (0.59)	13.79 (0.57)
16	13.83 (0.58)	13.89 (0.57)	13.62 (0.58)	13.78 (0.59)
17	14.88 (0.59)	13.84 (0.58)	13.56 (0.58)	14.44 (0.99)
18	14.54 (0.99)	13.95 (0.58)	13.63 (0.58)	13.84 (0.58)
19	13.92 (0.56)	13.60 (0.56)	13.64 (0.58)	13.86 (0.56)
20	13.90 (0.57)	13.96 (0.59)	13.65 (0.56)	14.17 (0.58)
21	13.80 (0.58)	13.25 (1.99)*	14.28 (0.99)	13.77 (0.58)
22	13.82 (0.58)	13.57 (0.56)	13.61 (0.58)	13.75 (0.59)
23	13.78 (0.59)	13.63 (0.57)	13.65 (0.58)	13.82 (0.57)
24	13.87 (0.58)	13.89 (0.58)	13.62 (0.58)	13.79 (0.57)
25	13.95 (0.57)	13.64 (0.58)	13.67 (0.58)	13.81 (0.59)
26	13.89 (0.58)	13.95 (0.57)	13.98 (0.59)	14.26 (0.59)
27	13.88 (0.58)	13.63 (0.58)	13.99 (0.58)	13.81 (0.58)
28	13.94 (0.57)	14.33 (1.00)	14.02 (0.58)	13.87 (0.58)
29	13.41 (0.59)*	13.61 (0.58)	13.64 (0.57)	13.79 (0.58)
30	13.86 (0.59)	14.22 (1.01)	13.60 (0.59)*	13.78 (0.58)

*** and ** represent the minimum and maximum range respectively.**

Table 9. Mean values of total *coliform* count (TCC) expressed as log MPN/cm^2 with their standard deviations (SD) for 30 beef carcasses at two different parts of the carcasses and two different knives used on the carcasses during beef production in the Kumasi Abattoir slaughter plant.

Carcass number	Sticking knife (±SD)	Inner surface after evisceration (±SD)	Surface of carcass after skin removal (±SD)	Last knife used for trimming (±SD)
1	8.34 (0.65)	8.26 (0.99)*	7.81 (0.54)*	8.27 (1.20)
2	8.32 (1.22)	8.63 (0.60)	8.56 (1.03)	8.56 (0.94)
3	8.59 (1.22)	9.04 (2.12)	8.33 (0.98)	8.54 (1.19)
4	8.27 (0.57)	8.71 (1.12)	8.15 (1.11)	8.51 (1.02)
5	8.42(1.22)	8.98(1.51)	8.39 (1.01)	8.43 (0.97)
6	8.93 (1.07)	9.10 (1.52)	7.93 (1.17)	8.40 (0.95)
7	8.81 (0.96)	8.33 (1.71)	8.06 (1.20)	7.82 (0.52)
8	8.06 (0.59)	9.00 (2.06)	8.91 (1.17)	8.55 (1.02)
9	8.64 (0.95)	9.02 (2.10)	9.05 (0.64)	8.22 (1.09)
10	8.61 (1.05)	8.80 (1.10)	7.97 (0.65)	8.42 (0.94)
11	8.26 (1.20)	9.04 (1.47)	8.26 (1.16)	8.56 (0.95)
12	8.63 (1.04)	9.06 (0.54)	8.28 (1.11)	8.10 (1.15)
13	8.37 (1.19)	9.00 (0.54)	8.92 (1.12)	8.17 (1.21)
14	9.00 (0.56)	9.74 (1.54)**	8.15 (1.14)	8.08 (1.22)
15	8.23 (0.58)	9.37 (1.98)	8.82 (1.09)	9.39 (0.03)**
16	8.35 (1.12)	8.41 (1.65)	8.88 (0.64)	7.81 (0.65)*
17	8.63 (0.98)	9.15 (1.52)	8.96 (1.13)	8.21 (1.17)
18	9.03 (1.12)	8.48 (1.69)	9.14 (0.60)**	8.15 (1.14)
19	8.50 (1.05	9.26 (2.06)	8.72 (0.96)	8.27 (1.11)
20	8.99 (1.16)	8.47 (0.94)	8.59 (0.98)	8.54 (1.04)
21	8.67 (1.02)	9.47 (0.06)	8.48 (1.01)	8.16 (1.21)
22	9.02 (1.18)	9.45 (0.97)	8.67 (1.17)	8.56 (0.97)
23	8.61 (0.97)	9.11 (1.55)	8.50 (0.95)	8.60 (1.04)
24	8.31 (1.17)	9.48 (1.99)	8.26 (1.20)	8.86 (0.60)
25	8.36 (1.12)	8.92 (1.48)	7.87 (0.60)	8.82 (1.21)
26	9.03 (1.20)	9.11(2.04)	8.32 (1.21)	8.51 (0.99)
27	8.05 (0.51)*	9.63 (0.50)	7.97 (0.66)	8.40 (1.05)
28	8.68 (1.04)	8.88 (1.15)	9.00 (1.10)	8.57 (1.10)
29	8.35 (0.60)	8.98 (2.12)	9.12 (0.59)	8.21 (0.55)
30	9.41(0.61)**	9.04 (2.03)	8.54 (1.04)	8.21 (1.08)

*** and ** represent the minimum and maximum range respectively.**

4.2.1.3. FAECAL *COLIFORMS* COUNT (FCC)

Log mean values of Faecal *Coliform* Count (FCC) found on sticking knives for sticking thirty cattle at the Kumasi Abattoir slaughter plant recorded a range of 5.21 to 6.38 MPN/cm^2 with standard deviations of ±1.21 and ±0.55 respectively. FCC found at the inner surface of the carcasses after evisceration also ranged from 4.73 to 6.18 MPN/cm^2 with standard deviations of ±0.65 and ±0.56 respectively. The range obtained for FCC on the surface of the carcass after evisceration was from 4.46 from 6.11 MPN/cm^2 with standard deviations of ±0.09 and ±0.59 respectively. On the last knives used for trimming carcasses, FCC counts ranged from 4.76 to 6.14 MPN/cm^2 with their standard deviations as ±0.52 and ±0.59 respectively. The counts made were not significantly different ($p<0.05$) from one another as seen in **Table 10**.

4.2.1.4. *E.COLI* COUNT (ECC)

The log means recorded for *E.coli* count (ECC) found on sticking knives for the thirty carcasses sampled ranged from 2.61 to 4.33 MPN/cm^2 with their respective standard deviations as ±0.05 and ±0.52. ECC found at the inner surface of carcasses after evisceration had a range from 2.59 to 4.20 MPN/cm^2 having standard deviations ±0.59 and ±0.56 respectively. The surface of the carcasses after skin removal recorded ECC counts from 2.81 to 4.85 MPN/cm^2 with standard deviations of ±0.53 and ±0.06 respectively. A range of 2.76 to 4.20 MPN/cm^2 with standard deviations of ±0.58 and ±0.13 respectively was found on the last knives used for trimming the carcasses. The counts recorded were not significantly different ($p<0.05$) from one another as seen in **Table 11**.

Table 10. Mean Faecal *Coliform* Count (FCC) values expressed as log MPN/cm^2 with their standard deviations (SD) for 30 beef carcasses at two different parts of the carcasses and two different knives used on the carcasses during beef production in the Kumasi Abattoir slaughter plant.

Carcass number	Sticking knife (±SD)	Inner surface after evisceration (±SD)	Surface of carcass after skin removal (±SD)	Last knife used for trimming (±SD)
1	6.21 (0.61)	4.73 (0.65)*	4.98 (0.57)	5.92 (0.44)
2	6.57 (0.56)	6.14 (0.64)	5.80 (0.92)	5.08 (0.08)
3	6.38 (0.55)**	6.18 (0.56)**	4.93 (0.57)	5.10 (0.56)
4	5.36 (0.58)	5.64 (1.08)	5.95 (1.14)	5.49 (0.97)
5	5.51 (0.95)	6.15 (0.62)	5.51 (0.90)	5.75 (1.09)
6	5.63 (0.98)	5.35 (0.98)	4.82 (0.64)	5.27 (0.53)
7	5.60 (1.04)	5.06 (0.59)	5.43 (0.99)	5.37 (0.97)
8	5.21 (1.21)*	5.63 (1.08)	5.36 (0.88)	6.00 (0.52)
9	5.95 (0.57)	5.94 (0.57)	5.39 (1.02)	4.84 (0.64)
10	5.27 (1.17)	5.45 (0.96)	5.14 (1.14)	5.24 (1.14)
11	5.61 (0.97)	5.38 (0.97)	4.46 (0.09)*	6.14 (0.59)**
12	5.48 (0.89)	5.01 (1.14)	6.11 (0.59)**	5.84 (1.09)
13	5.84 (1.09)	5.41 (0.97)	5.08 (1.13)	5.80 (1.08)
14	5.36 (0.71)	4.74 (0.55)	4.76 (0.58)	5.83 (1.19)
15	5.89 (1.12)	5.49 (1.19)	4.82 (0.55)	5.77 (1.19)
16	5.58 (1.01)	4.74 (0.58)	5.41 (0.97)	4.81 (0.56)
17	6.37 (0.05)	6.01 (0.57)	4.81 (0.57)	5.74 (1.19)
18	5.25 (1.14)	5.35 (0.97)	5.05 (1.13)	5.72 (1.07)
19	5.82 (1.07)	4.76 (0.54)	4.81 (0.55)	4.76 (0.52)*
20	5.83 (1.19)	5.39 (0.97)	5.14 (1.12)	5.48 (0.92)
21	5.62 (1.04)	5.44 (0.99)	4.81 (0.63)	5.58 (0.99)
22	5.54 (0.89)	4.82 (0.64)	5.20 (1.08)	5.44 (0.89)
23	5.63 (0.99)	4.82 (0.56)	5.56 (0.98)	5.77 (1.14)
24	5.64 (0.95)	5.73 (1.10)	5.47 (0.96)	5.10 (1.09)
25	5.61 (0.96)	5.36 (0.92)	4.85 (0.53)	5.10(1.06)
26	5.54 (0.87)	5.66 (1.11)	5.47 (0.99)	5.71 (1.09)
27	5.60 (0.96)	5.33 (0.90)	4.81 (0.62)	5.15 (1.01)
28	6.28 (0.54)	5.18 (0.64)	4.88 (0.66)	4.78 (0.54)
29	5.64 (0.95)	4.81 (0.56)	5.25 (0.55)	5.20 (1.09)
30	5.58 (0.98)	5.39 (0.98)	5.15 (0.53)	5.46 (0.92)

*** and ** represent the minimum and maximum range respectively.**

Table 11.Mean values of *E. coli* count (ECC) expressed as log MPN/cm^2 with their standard deviations (SD) for 30 beef carcasses at two different parts of the carcasses and two different knives used on the carcasses during beef production in the Kumasi Abattoir slaughter plant.

Carcass number	Sticking knife (±SD)	Inner surface after evisceration (±SD)	Surface of carcass after skin removal (±SD)	Last knife used for trimming (±SD)
1	3.27 (1.02)	3.09 (0.75)	4.15 (0.65)	3.25 (0.46)
2	3.76 (1.18)	3.28 (0.06)	4.26 (0.55)	4.20 (0.13)**
3	3.86 (0.55)	2.97 (0.58)	4.16 (0.38)	3.43 (0.95)
4	3.82 (1.07)	3.60 (0.45)	4.39 (0.07)	3.30 (0.96)
5	3.37 (0.84)	3.22 (0.05)	3.96 (0.43)	3.34 (0.89)
6	3.55 (0.94)	3.27 (0.87)	3.16 (1.10)	3.69 (0.61)
7	3.30 (0.94)	3.19 (0.92)	2.81 (0.53)*	2.76 (0.58)*
8	3.97 (0.57)	2.59 (0.59)*	3.64 (0.93)	2.87 (1.03)
9	3.39 (0.98)	3.29 (1.04)	3.00 (0.57)	3.60 (0.39)
10	3.43 (0.95)	3.68 (0.61)	3.69 (0.97)	3.36 (1.02)
11	3.50 (0.96)	3.20 (0.99)	3.63 (1.04)	2.77 (0.62)
12	3.23 (0.95)	2.82 (1.12)	3.58 (0.89)	3.54 (1.06)
13	3.40 (0.96)	2.93 (1.57)	3.29 (1.11)	3.62 (1.04)
14	3.64(1.11)	3.59 (1.12)	4.01 (1.10)	3.17 (1.11)
15	2.86 (0.55)	3.71 (1.09)	3.71 (1.02)	3.92 (0.46)
16	3.25 (0.56)	3.70 (1.10)	3.79 (0.97)	3.04 (1.20)
17	3.57 (1.01)	2.85 (0.53)	3.74 (0.89)	3.30 (0.98)
18	3.75 (0.94)	3.37 (0.95)	4.16 (1.09)	3.10 (1.09)
19	4.14(1.42)	3.08 (1.17)	4.11 (0.66)	3.54 (1.01)
20	3.82 (1.39)	3.10 (1.14)	3.71 (0.99)	3.16 (1.10)
21	3.54 (0.99)	3.06 (1.13)	3.63 (1.08)	3.50 (0.95)
22	4.02 (2.33)	2.94 (0.46)	4.19 (0.63)	2.98 (0.50)
23	2.83 (0.63)	3.45 (0.99)	4.85 (0.06)**	3.97 (0.53)
24	2.61 (0.05)*	4.04 (0.53)	3.54 (0.58)	2.78 (0.68)
25	2.88 (0.52)	3.77 (0.60)	4.54 (0.55)	3.87 (0.61)
26	4.17 (0.60)	3.98 (0.67)	3.81 (0.94)	3.05 (0.51)
27	3.60 (0.04)	4.20 (0.56)**	4.28 (0.63)	2.89 (0.69)
28	2.94 (0.54)	3.45 (0.99)	4.67 (0.58)	3.37 (0.97)
29	4.17 (0.59)	3.44 (1.02)	4.50 (0.56)	3.45 (0.88)
30	4.33 (0.52)**	3.85 (0.59)	4.48 (0.65)	2.98 (0.62)

*** and ** represent the minimum and maximum range respectively.**

4.2.1.5. *SALMONELLA SPP* COUNT (SC)

Log means of *Salmonella spp* count (SC) found on sticking knives used on the thirty carcasses sampled at the abattoir ranged from 3.93 to 5.27 CFU/cm^2 with ±0.62 and ±0.54 as their respective standard deviations. The inner surfaces of the carcasses after evisceration had a range from 3.49 to 4.78 CFU/cm^2 with their standard deviations as ±0.62 and ±0.59 respectively. SC values recorded at the surfaces of the carcasses after skin removal were from 3.62 to 5.59 CFU/cm^2 with standard deviations of ±0.05 and ±2.01 respectively. A range from 3.71 to 5.27 CFU/cm^2 with their standard deviations as ±0.06 and ±0.64 respectively was recorded for SC on the last knives used for final trimming carcasses. The values recorded were not significantly different ($p<0.05$) from one another. **Table 12** shows the value of *Salmonella spp* count recorded.

Table 12. Mean *Salmonella spp* count (SC) values expressed as log CFU/cm^2 with standard deviations (SD) for 30 beef carcasses at two different parts of the carcasses and two different knives used on the carcasses during beef production in the Kumasi Abattoir slaughter plant.

Carcass number	Sticking knife (±SD)	Inner surface after evisceration (±SD)	Surface of carcass after skin removal (±SD)	Last knife used for trimming (±SD)
1	5.01 (0.51)	4.81 (0.49)	3.65 (0.05)	4.06 (0.54)
2	4.44 (0.08)	3.49 (0.62)*	3.70 (0.05)	4.73 (0.03)
3	4.48 (0.06)	4.12 (0.97)	3.62 (0.05)*	3.71 (0.06)*
4	4.77 (1.14)	4.78 (0.59)**	3.78(0.53)	3.78(0.04)
5	5.01 (0.45)	4.20 (0.05)	4.26 (0.57)	4.39 (0.55)
6	4.17 (0.89)	4.24 (0.96)	4.48 (0.98)	4.63 (0.96)
7	4.52 (0.96)	3.69 (0.61)	3.79 (0.55)	4.59 (1.01)
8	4.22 (0.58)	4.43 (0.98)	4.59 (1.02)	4.61 (0.93)
9	4.29(1.20)	4.43 (0.97)	4.50 (0.96)	4.89 (0.54)
10	4.58 (1.08)	4.50 (0.98)	4.59 (1.01)	4.69 (0.98)
11	3.93 (0.62)*	4.49 (1.01)	4.58 (1.01)	4.63 (0.95)
12	4.63 (0.97)	4.41 (0.95)	4.95 (0.58)	4.54 (0.95)
13	5.27 (0.54)**	3.78 (0.60)	3.90 (0.59)	5.27 (0.64)**
14	4.64 (0.98)	4.09 (1.14)	4.56 (0.97)	4.57 (0.98)
15	4.58(1.07)	4.08 (0.54)	4.52 (1.03)	4.80 (1.03)
16	4.53 (0.95)	4.43 (0.98)	3.91 (0.60)	4.27 (0.61)
17	5.22 (0.58)	4.23 (1.04)	4.10 (0.55)	4.11 (0.60)
18	4.53 (1.03)	4.45 (1.01)	4.49 (1.01)	4.58 (1.01)
19	4.52 (0.94)	3.77 (0.60)	4.61 (0.99)	4.66 (0.96)
20	4.55 (1.03)	3.78 (0.58)	5.59 (2.01)**	4.60 (0.93)
21	4.50 (0.96)	4.37 (1.03)	4.49 (1.03)	3.85 (0.58)
22	4.56 (0.97)	4.08 (1.14)	4.52 (1.03)	4.54 (1.08)
23	4.61 (0.98)	4.54 (0.98)	4.61 (1.03)	4.55 (1.04)
24	4.50 (0.93)	4.05 (0.58)	4.54 (1.01)	4.29 (1.12)
25	4.46 (0.97)	4.43 (0.98)	4.54 (0.97)	3.60 (0.60)
26	4.55 (0.98)	4.06 (1.16)	4.51 (0.99)	4.86 (1.09)
27	4.53 (0.95)	3.75 (0.54)	4.63 (0.97)	3.93 (0.60)
28	4.62 (0.96)	4.45 (1.02)	4.35 (0.56)	4.64 (1.01)
29	4.50 (1.07)	3.74 (0.55)	3.88 (0.58)	4.17 (0.56)
30	4.52 (1.03)	4.72 (1.13)	3.82 (0.57)	4.22 (0.54)

*** and ** represent the minimum and maximum range respectively.**

4.2.2.0. BACTERIAL COUNTS AT THE BUTCHERS' SHOPS

4.2.2.1. TOTAL PLATE COUNT (TPC)

TPC log mean values for beef displayed on butcher's benches at the butchers' shops gave a range from 13.31 to 14.72 CFU/cm^2 with standard deviations of 0.58 and 0.55 respectively. The knives used by the butchers to cut meat during sales also had a TPC range from log 13.66 to 15.18 CFU/cm^2 with their respective standard deviations as 0.57 and 0.58. TPC values recorded from samples collected on meat surfaces at the butchers' shops ranged from log 13.46 to 14.55 CFU/cm^2 with standard deviations of 0.56 and 0.57 respectively. The values recorded were not significantly different ($p<0.05$). **Table 13** shows the TPC values recorded at twenty butchers' shops.

4.2.2.2. TOTAL *COLIFORMS* COUNT (TCC)

Benches at the butchers' shops had TCC values ranging from 7.92 to 9.11 MPN/cm^2 with standard deviations of ±0.61 and ±0.58 respectively. TCC values recorded from samples taken from knives at the butchers' shops ranged from 8.67 and 9.42 MPN/cm^2 with standard deviations of ±0.99 and ±0.52 respectively. Meat surfaces at the butchers' shops had TCC values ranging from 7.85 to 9.14 MPN/cm^2 with standard deviations of ±0.61 and ±0.52. There were no significant differences ($p<0.05$) among the values recorded. All the values are found in **Table 14**.

Table 13.Mean values of Total Plate Count (TPC) expressed as log CFU/cm^2 with their standard deviations (SD) for 20 butchers' shops on the surfaces of their benches, knives and the meat at the Atonsu and Maayanka markets in Kumasi.

Shop number	Benches (±SD)	Knives (±SD)	Meat surface (±SD)
1	14.12 (1.01)	13.66 (0.57)*	14.26 (1.01)
2	13.74 (1.15)	14.28 (1.01)	14.20 (1.15)
3	14.43 (0.59)	14.60 (1.15)	14.17 (0.99)
4	14.33 (0.57)	14.88 (0.58)	13.46 (0.56)*
5	13.31 (0.58)*	14.88 (0.56)	14.20 (1.01)
6	14.21 (1.02)	14.54 (1.00)	13.55 (0.59)
7	14.72 (0.55)**	14.58 (1.00)	14.21 (0.99)
8	14.06 (0.99)	14.51 (1.00)	14.20 (1.01)
9	14.10 (1.00)	14.53 (1.01)	14.20 (1.00)
10	14.14 (0.99)	14.57 (0.99)	14.21 (0.99)
11	14.06 (1.00)	14.59 (0.99)	14.22 (1.01)
12	14.10 (0.99)	14.53 (1.00)	14.20 (1.00)
13	14.15 (0.99)	14.53 (1.01)	14.24(1.01)
14	14.09 (0.99)	15.18(0.58)**	14.23 (1.01)
15	13.46 (0.58)	14.21 (0.57)	14.21 (0.98)
16	14.16 (1.02)	14.53 (1.01)	14.23 (0.99)
17	14.09 (1.00)	14.53 (0.98)	14.55(0.57)**
18	13.81 (1.14)	14.20 (0.58)	14.55 (0.57)
19	14.13 (1.02)	14.50 (1.05)	13.89 (0.58)
20	14.19 (1.01)	14.54 (0.99)	14.19 (1.01)

*** and ** represent the minimum and maximum range respectively.**

Table 14. Mean values of Total *Coliform* Count (TCC) form count expressed as log MPN/cm^2 with their standard deviations (SD) for 20 butchers' shops on the surfaces of their benches, knives and the meat at the Atonsu and Maayanka markets in Kumasi.

Shop number	Benches (±SD)	Knives (±SD)	Meat surface (±SD)
1	8.77 (1.13)	8.72 (1.02)	8.77 (0.88)
2	8.30 (1.08)	8.67 (0.99)*	8.49 (0.94)
3	8.62 (0.97)	8.69 (1.01)	8.59 (0.98)
4	8.49 (0.91)	9.41 (0.62)	8.28 (0.60)
5	7.92 (0.61)*	9.09 (0.56)	8.59 (1.02)
6	8.69 (0.60)	8.54 (1.18)	8.26 (1.18)
7	8.42 (0.99)	9.15 (0.59)	8.87 (0.59)
8	8.48 (1.04)	8.79 (1.04)	8.51 (1.04)
9	8.54 (1.04)	8.81 (1.07)	8.53 (1.07)
10	8.46 (1.06)	8.79 (1.01)	8.51 (1.01)
11	8.81 (1.17)	8.75 (1.05)	8.48 (1.05)
12	8.39 (1.01)	9.14 (0.59)	8.86 (0.59)
13	8.41 (1.01)	9.42 (0.52)**	9.14 (0.52)**
14	9.11 (0.58)**	8.70 (1.01)	8.52 (0.95)
15	8.47 (0.96)	9.15 (0.66)	7.85 (0.61)*
16	8.48 (1.04)	8.81 (1.03)	8.43 (1.01)
17	8.48 (0.97)	9.10 (0.56)	8.48 (0.97)
18	8.48 (1.01)	8.74 (1.06)	8.42 (0.98)
19	8.52 (0.97)	9.11 (1.19)	8.55 (0.92)
20	9.10 (0.56)	9.08 (1.20)	8.86 (1.10)

*** and ** represent the minimum and maximum range respectively.**

4.2.2.3. FAECAL *COLIFORMS* COUNT (FCC)

The log means of FCC found on benches were recorded within a range of 5.70 to 7.01 MPN/cm^2 and standard deviations of ±0.62 and ±2.14 respectively. Log means recorded for FCC found on knives fell within 6.42 and 7.33 MPN/cm^2 and their standard deviations of ±0.55 and ±0.61 respectively. A range of 4.27 and 7.08 MPN/cm^2 with standard deviations of ±0.63 and ±0.56 respectively.

The log means recorded were not significantly different ($p<0.05$) from one another. **Table 15** gives the values of the count recorded.

4.2.2.4. *E.COLI* COUNT (ECC)

Log means of ECC identified on benches fell within 3.48 and 5.17 MPN/cm^2 having standard deviations of ±0.60 and ±0.10 respectively. The knives used by the butchers also recorded values within the range of log 3.26 and 5.14 MPN/cm^2 having standard deviations of ±0.54 and ±0.63 respectively. A range of log 3.53 to 4.92 MPN/cm^2 with standard deviations of ±0.59 and ±0.54 respectively. Values recorded were not significantly different ($p<0.05$) from one another. These values are seen in **Table 16**.

Table 15. Mean faecal *coliforms* count expressed as log MPN/cm^2 with their standard deviations (SD) for 20 butchers' shops on the surfaces of their benches, knives and the meat at the Atonsu and Maayanka markets in Kumasi.

Shop number	Benches (±SD)	Knives (±SD)	Meat surface (±SD)
1	6.26 (1.01)	6.43 (1.05)	6.88 (0.59)
2	6.35 (1.03)	6.42 (0.97)*	6.92 (0.47)
3	6.11 (0.56)	6.54 (1.05)	6.76 (0.54)
4	6.93 (0.63)	6.83 (0.52)	7.08 (0.56)**
5	6.37 (1.03)	7.20(0.54)	6.97 (0.67)
6	6.15 (0.53)	6.61 (1.04)	6.76 (0.53)
7	6.37 (0.97)	6.61 (0.98)	6.35 (1.03)
8	6.05 (0.60)	7.33 (0.55)**	6.37 (0.95)
9	6.72 (0.57)	6.60 (0.99)	4.27 (0.63)*
10	6.30 (0.99)	6.54 (1.04)	6.66 (0.54)
11	6.33 (0.99)	6.60 (0.96)	5.97 (0.60)
12	6.30 (0.98)	6.55 (1.06)	5.74 (0.59)
13	6.33 (0.96)	6.55 (0.96)	6.94 (0.57)
14	6.37 (0.95)	6.61 (0.98)	7.06 (0.54)
15	6.35 (0.97)	6.65 (1.03)	6.42 (1.03)
16	5.70 (0.62)*	6.68 (1.01)	6.30 (1.02)
17	6.36 (1.03)	7.30 (0.61)	6.30 (1.01)
18	6.35 (0.98)	7.28 (0.59)	6.97 (0.60)
19	6.36 (0.97)	6.57 (0.97)	6.39 (1.04)
20	7.01 (2.14)**	6.91 (0.68)	6.35 (1.08)

*** and ** represent the minimum and maximum range respectively.**

Table 16. Mean *E coli* counts expressed as log MPN/cm^2 with their standard deviations (SD) for 20 butchers' shops on the surfaces of their benches, knives and the meat at the Atonsu and Maayanka markets in Kumasi.

Shop number	Benches (±SD)	Knives (±SD)	Meat surface (±SD)
1	3.86 (1.09)	4.16 (0.76)	4.21 (0.93)
2	3.48 (0.60)*	4.96 (0.50)	4.77 (0.43)
3	4.73 (0.46)	4.35 (0.88)	4.18 (0.90)
4	4.13 (0.41)	4.86 (0.66)	4.43 (0.53)
5	3.86 (0.48)	4.23 (0.90)	4.67 (0.44)
6	4.29 (1.10)	4.49 (0.93)	3.89 (1.14)
7	4.16 (0.97)	3.26 (0.54)*	4.92 (0.54)**
8	4.28 (0.94)	4.53 (0.91)	4.20 (0.96)
9	5.17 (0.10)**	4.99 (0.49)	4.21 (1.07)
10	4.20 (1.04)	4.49 (0.92)	4.12 (1.02)
11	3.52 (0.66)	4.42 (0.94)	3.78 (1.10)
12	4.08 (0.92)	4.02 (0.61)	3.53 (0.59)*
13	4.20 (0.99)	4.38 (0.95)	4.12 (0.95)
14	4.15 (0.99)	5.14 (0.63)**	4.58 (0.61)
15	4.18 (1.05)	4.53 (1.05)	4.35 (0.96)
16	4.58 (0.49)	4.16 (0.59)	4.04 (1.08)
17	4.16 (1.00)	4.93 (1.19)	4.15 (1.01)
18	3.54 (0.64)	3.83 (0.52)	4.77 (0.54)
19	3.80 (0.63)	4.06 (1.10)	4.17 (1.04)
20	4.15 (1.00)	4.36 (1.02)	4.07 (1.09)

*** and ** represent the minimum and maximum range respectively.**

4.2.2.5. *SALMONELLA SPP* COUNT (SC)

The log means of SC found on benches had a range from 4.47 to 5.99 CFU/cm^2 with standard deviations of ±0.66 and ±0.57 respectively. The SC means found on knives fell with log 5.28 and 6.03 CFU/cm^2 with standard deviations of ±1.08 and ±0.64 respectively. The log means of SC identified on meat surfaces had a range of 4.57 and 5.97 CFU/cm^2 with their respective standard deviations as ±0.50 and ±0.56.

The counts recorded were not significantly different ($p<0.05$) from each one another as seen in **Table 17.**

Table 17. Mean *Salmonella spp counts* (SC) expressed as log MPN/cm^2 with their standard deviations (SD) for 20 butchers' shops on the surfaces of their benches, knives and the meat at the Atonsu and Maayanka markets in Kumasi.

Shop number	Benches (±SD)	Knives (±SD)	Meat surface (±SD)
1	5.23 (0.17)	5.90 (0.56)	5.41 (0.98)
2	5.73 (0.57)	5.71 (0.49)	5.97 (0.56)**
3	5.01 (0.67)	6.28 (0.15)	5.73 (1.08)
4	4.77 (0.63)	5.91 (0.52)	5.74 (0.68)
5	5.56 (0.39)	5.59 (0.94)	5.67 (0.51)
6	4.69 (0.49)	5.60 (0.80)	4.57 (0.50)*
7	5.31(0.95)	5.58 (1.02)	5.21 (0.80)
8	5.20 (0.89)	5.32 (1.17)	5.72 (1.16)
9	5.39 (1.08)	5.28 (1.08)*	5.14 (0.91)
10	5.25 (1.05)	5.52 (0.97)	5.26 (1.03)
11	4.47 (0.66)*	5.60 (0.96)	5.24 (1.06)
12	5.67 (1.09)	5.26 (1.19)	5.30 (1.07)
13	5.37 (1.09)	5.59 (0.99)	5.42 (1.03)
14	5.70 (1.13)	5.62 (0.93)	5.06 (1.11)
15	5.99 (0.57)**	6.03 (0.64)**	5.46 (0.97)
16	5.35 (0.91)	5.63 (1.10)	5.34 (0.96)
17	5.75 (1.13)	6.01 (1.17)	5.73 (1.15)
18	6.10 (0.51)	5.72 (0.96)	5.45 (1.02)
19	5.45 (0.97)	5.70 (1.01)	5.38 (0.94)
20	5.00 (1.11)	5.64 (0.94)	5.39 (0.97)

*** and ** represent the minimum and maximum range respectively.**

4.2.2.6. BACTERIAL IDENTIFICATION

4.2.2.6.1. BACTERIAL ISOLATES FROM THE ABATTOIR

One hundred isolates picked from the Total Plate Counts were characterised. Proportions of isolates identified per sampling site were 25 isolates for each sampling site as shown in **Table 18.** The bacteria flora identified were *Enterobacteriaceae, Staphylococcus spp., Bacillus spp* and *Pseudomonas spp.*

The predominant flora was *Enterobacteriaceae* 38%, followed by *Staphylococcus spp.* 23%, *Bacillus spp.*21% and *Pseudomonas spp.*18% as shown in **Figure 6**.

Out of the 25 isolates recovered on sticking knives 32% (8/25) were seen as *Enterobacteriaceae,* 24% (6/25) as *Staphylococcus spp.*, 28% (7/25) as *Bacillus spp.* and 16% (4/25) as *Pseudomonas spp.*

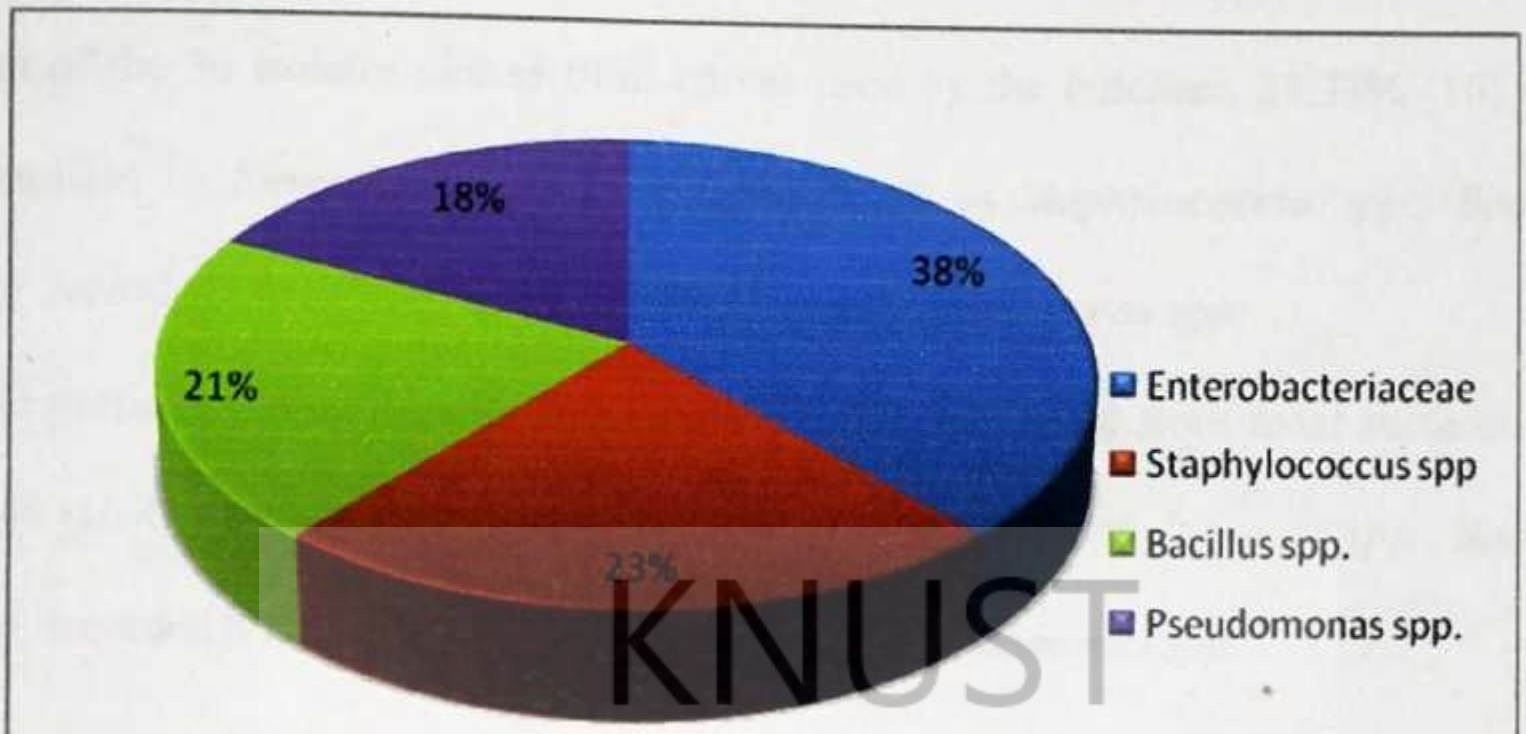
The 25 isolates recovered at inner surface of carcasses after evisceration were divided into 36% (9/25) as *Enterobacteriaceae,* 28% (7/25) as *Staphylococcus spp.*, 16% (4/25) as *Bacillus spp.* and 20% (5/25) as *Pseudomonas spp.*

The proportional distribution for the 25 isolates recovered on surface of carcass after skin removal was 36% (9/25) for *Enterobacteriaceae,* 16% (4/25) for *Staphylococcus spp.*, 28% (7/25) for Bacillus *spp.* and 20% (5/25) for *Pseudomonas spp.* 48% (12/25) of the isolates recovered on last knife used for trimming were *Enterobacteriaceae,* 24% (6/25) as *Staphylococcus spp.*, 12% (3/25) as *Bacillus spp.* and 16% (4/25) as *Pseudomonas spp.*

Table 18. Distribution of bacterial isolates identified at four sampling sites on 30 carcasses at the Kumasi Abattoir slaughterhouse.

Bacterial isolates	Sticking knives	Inner surface of carcass after evisceration	Surface of carcass after skin removal	Last knives used for trimming of carcass	Percentage
Enterobacteriaceae	32% (8)	36% (9)	36% (9)	48% (12)	38%
Staphylococcus spp	24% (6)	28% (7)	16% (4)	24% (6)	23%
Bacillus spp.	28% (7)	16% (4)	28% (7)	12% (3)	21%
Pseudomonas spp.	16% (4)	20% (5)	20% (5)	16% (4)	18%
Total number of isolates identified per sampling site	25	25	25	25	100

Figure 6: Distribution of bacterial isolates identified on beef carcasses in the Kumasi Abattoir slaughter plant.



4.2.2.6.2. BACTERIAL ISOLATES FROM THE BUTCHERS' SHOPS

One hundred bacterial isolates picked from the total plate counts were identified and characterised. The proportion of isolates used were 30 for benches, 30 for knives and 40 for meat surfaces as shown in **Table 19**.

The bacteria flora identified were *Enterobacteriaceae, Staphylococcus spp., Bacillus spp* and *Pseudomonas spp.* The predominant flora was *Enterobacteriaceae* 33%, followed by *Staphylococcus spp.* 24%, *Bacillus spp.*19% and *Pseudomonas spp.*24% as shown in **Figure 7**.

Of the 30 isolates recovered on benches, 43.33% (13/30) were *Enterobacteriaceae, Staphylococcus spp.* recorded 26.67% (8/30), 13.33% (4/30) were identified as *Bacillus spp* and 16.67% (5/30) were identified as *Pseudomonas spp.*

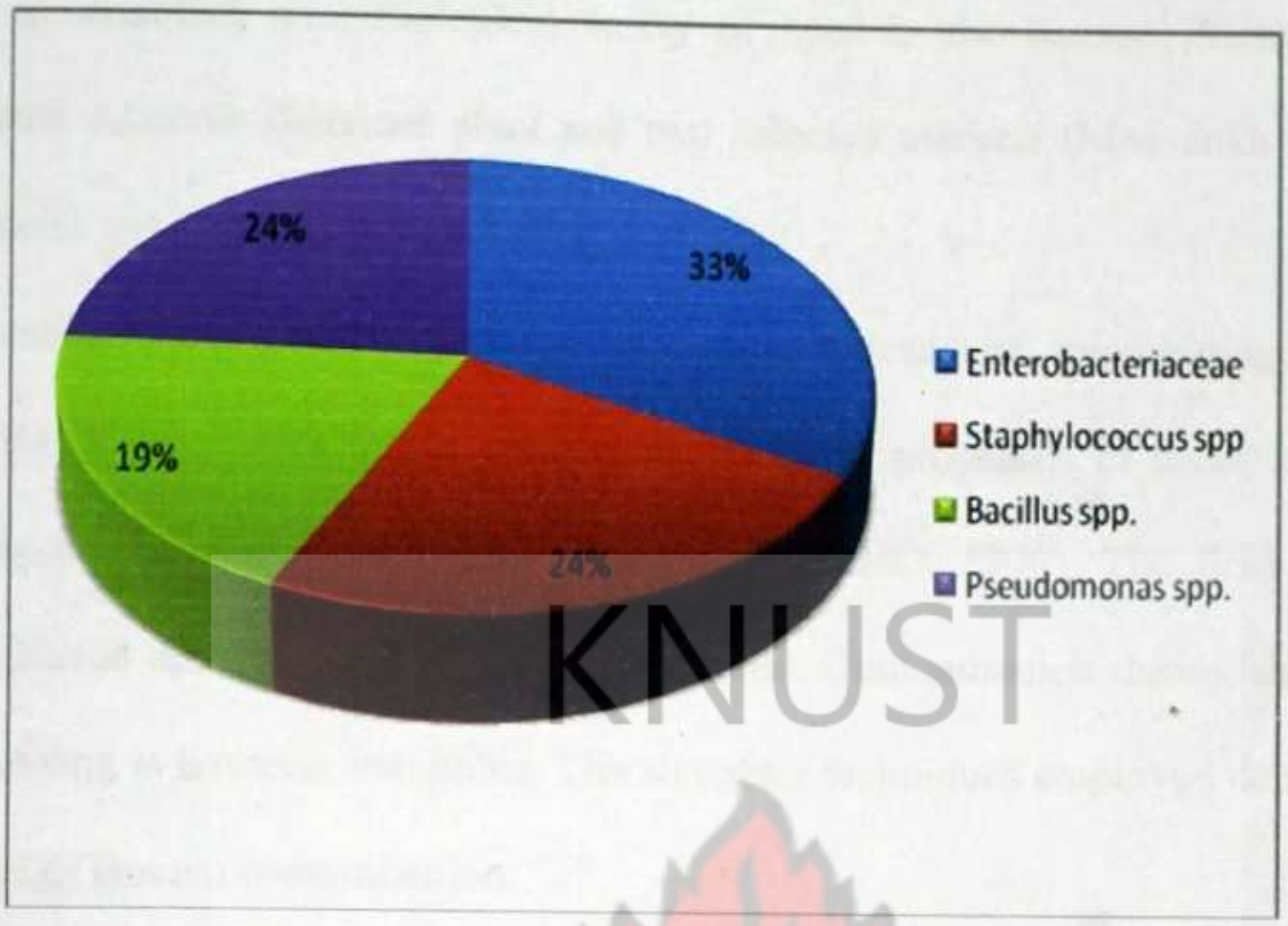
Out of the 30 isolates picked from knives used by the butchers, 33.33% (10) were identified as *Enterobacteriaceae,* 23.33% (7/30) as *Staphylococcus spp., Bacillus spp.* recorded 30% (9/30) and 13.33% (4/30) as *Pseudomonas spp.*

The proportional distribution for the 40 isolates recovered from meat surfaces was 25% (10/40) as *Enterobacteriaceae,* 22.5% (9/40) for *Staphylococcus spp., Bacillus spp.* recorded 15% (6/40) and 37.5% (15/40) as *Pseudomonas spp.*

Table 19. Distribution of bacterial isolates identified at three sampling sites at 20 different butchers' shops at two different markets in Kumasi.

Bacterial isolates	Benches	Knives	Meat surfaces	Percentage
Enterobacteriaceae	43.33% (13)	33.33% (10)	25% (10)	33%
Staphylococcus spp	26.67% (8)	23.33% (7)	22.5% (9)	24%
Bacillus spp.	13.33% (4)	30% (9)	15% (6)	19%
Pseudomonas spp.	16.67% (5)	13.33% (4)	37.5% (15)	24%
Total number of isolates identified per sampling site	30	30	40	100

Figure 7: Distribution of bacterial isolates identified at butchers' shops at the Atonsu and Mayaanka markets in Kumasi.



4.3.0. DISCUSSION

The objectives of this study were to identify potential pathogenic microbes on beef and to establish microbiological safety of beef in the Kumasi Metropolis. The Kumasi Abattoir slaughter plant and two selected markets (Maayanka and Atonsu markets) were used as research sites.

Generally, meat quality depends predominantly on its microbiological quality (WHO, 1990). Aside nutritional and organoleptic properties of meat, hygiene and safety have become crucial to meat quality (WHO, 1990). The flesh of healthy slaughtered animals can be assumed as sterile. Contamination during slaughter and processing is however inevitable. The slaughter techniques employed determines the extent of carcass contamination.

In this study, the microbiological status of beef carcasses were assessed in order to determine how the carcasses compare in terms of bacterial counts on the surfaces of sticking knives, inner surface of carcass after evisceration, the surface of the carcass after skin removal and the last knives used for trimming in the slaughter plant. The microbiological status of beef sold in the butchers' shops were also assessed in order to find how beef sold by different butchers compare in terms of bacterial counts on the benches used for sales, knives used during sales and the surfaces of the beef being sold. All the results found in this work were higher than results given by other authors elsewhere.

4.3.1.0. OBSERVATIONS IN THE SLAUGHTER PLANT

Production started at 6am on each morning of sampling day. This time was very good since it was usually the period within the day when the temperature is low. Low temperature during meat production is very important in controlling bacteria growth and proliferation in an event of contamination (Husband, n.d.).

Animals were moved from the kraal to the slaughter plant by walking them in ropes. This practice is inappropriate for animal welfare reasons. Cortesi (1994) reported that in the developed world, there is a rise in demand for humane slaughter of animals which reduces unnecessary pains to the animals being slaughtered. It is important that slaughter is done without unnecessary pains but with an effective bleeding method (Gracey and Collins, 1992).

Though stunning is one of the processes required in a humane slaughter process, it was not practised in the plant of K.A.C. during this study. Gil & Durão (1985) found that if stunning is done adequately, animals are left unconscious which must last until bleeding to avoid suffering and improve thorough bleeding. It is not clear whether stunning is not done for lack of stunning instruments.

The rope on one of the hind legs was twisted until the animal fell on the floor. The horns of the animal were ceased and used to turn the neck till an appropriate area of the neck is exposed for sticking. Sticking of animals is done on the floor of the plant with sharp knives and bleeding starts here before the animal was held up on a rail. There may be introduction of bacteria from the unsterile knives used from one animal to the other (Jay, 2000) or even from the floor where the sticking was done (Gill, 1998).

This method of sticking was employed because there are no modern mechanisms in place to do the sticking as done in the developed countries where the blood is collected in a chamber.

The workers pushed the carcasses after they have been held up on the rail. They have to push the carcasses from one stage of the production process to the next. Though some of the workers wore gloves, they did not wash their hands regularly and sterilization was not done at all.

This means they touched the carcasses with their unclean hands which was a possible means of contamination. Gill (1998) confirmed this observation that dirty hands of workers, clothes and equipment could be an intermediate source of beef contaminants. This means there is also the possibility of cross-contamination of carcasses in the plant. Not only do they not wash their hands regularly, their heads, noses and mouth are left uncovered.

Jay (2000) reported that a slaughter plant with this kind of situation could have problems of contamination control especially when the workers do no practise good personal hygiene.

The skin removal process was done by ripping which involves some touching of the carcasses. The dirty and unwashed skins carry many microbial contaminants (Anonymous, 1997) which were touched by the workers at the flaying point. There was a chance of contamination and cross contamination. The skins were usually stained with blood and faeces which confirm what Collins and Wall (2004) reported that contaminated skins could be immediate source of most microbes found on beef and this can be seen in **Figure 8**. The immediate surroundings of the point of skin removal leave a lot of questions to be answered about a possible contamination of the carcasses.

Whiles others in some other parts of the world are using automated skin removal mechanisms, the abattoir at the time of this study used the manual method of skin removal without any method of washing or decontamination measures.

Figure 8. A photograph of the process of skin removal of cattle at K.A.C.L. slaughter plant.

Evisceration was done with bear hands which were not washed from one carcass to the other. The speed at which the process is carried out suggests a possible punch into the intestinal contents. When there are punches into the intestinal contents, carcasses may be left contaminated with heavy microbial loads (Jay, 2000). The immediate surroundings of the place of the evisceration confirmed that there could be possible contamination either physically or microbiologically.

Cross contamination in this situation was highly possible as the workers dirty and blood stained aprons could also touch the carcasses as the evisceration process went on.

Cross contamination is inevitable in a production plant where there are multiple contacts with contaminated tools and workers' hands without regular cleaning and sterilization. The clothes of the workers as seen in **Figure 9** were not in the condition for use in a food production facility.

Figure 9. A photograph of the process of evisceration of cattle at K.A.C.L. slaughter plant.

Veterinary inspection of carcasses did not involve the microbiological status of these carcasses. Post-mortem meat inspection has been designed to ensure the safety and wholesomeness of meat (Gill, 2000).

However, what goes on at K.A.C.L. slaughter plant does not meet the required standard. This confirms the report made by Hudson *et al.* (1996) that the inspections do not adequately consider microbial contamination of meat during slaughter.

The carcasses were pushed to the splitting stage of production with bare hands. There was no washing of the saw used on one carcass to the next. Excess fats on the carcasses were trimmed and the spinal cord removed at this stage in the same manner.

The hooks used in hanging the carcasses were rusty and were not sterilised after use on one carcass to another. Hooks and rails could serve as sources of contamination when adequate cleaning and sanitizing are not done (Clayton, 2000).

The noise and congestion in the plant could also contribute to meat contamination. Sneezes and coughs from such a congested slaughter plant could also influence the levels of microbial load especially when those people have infections (Anonymous, 1997). This congestion is believed to have effect on the microbes in the air circulating in the plant and this is confirmed in a report by Rahkio and Korkeala (1997).

The floor of the plant was left untidy during sampling periods till the work for the day ended when thorough washing of the plant was done. This is seen in **Figure 10** as trimmed-off pieces of carcasses were left on the floor. The water hose used for washing of knives and hands when the workers felt like was also left on the floor. The state of the structures found in this picture leaves one to wonder if the plant adheres to strict hygienic practices during slaughter. Again re-contamination and cross contamination in a slaughter plant of this condition is inevitable.

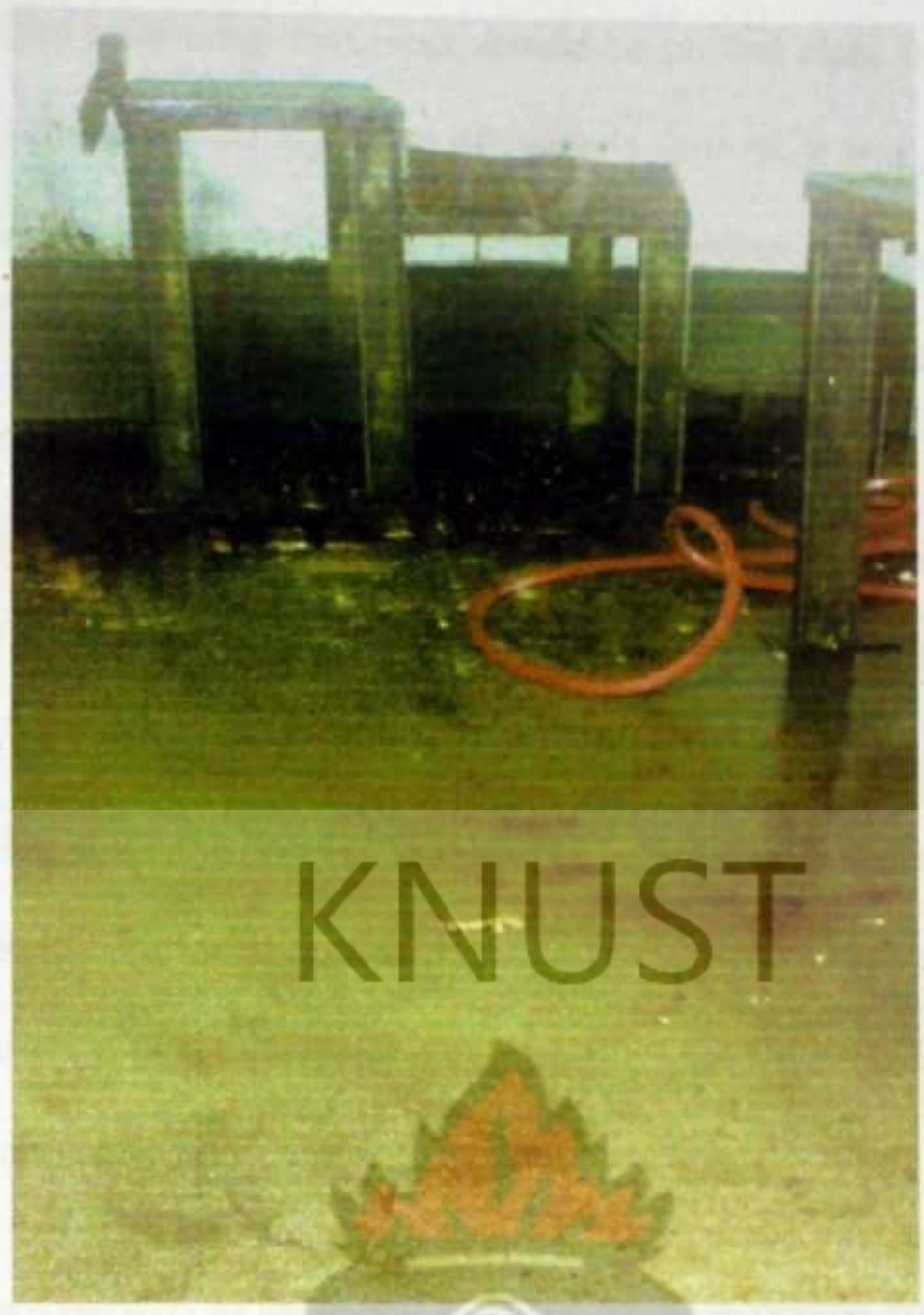


Figure 10. A photograph of the floor of a section of the slaughter plant of K.A.C.L.

4.3.1.1. OBSERVATIONS AT THE BUTCHERS' SHOPS

By-laws regarding transportation of fresh beef and other hygienic practices with respect to meat handling were not enforced. Beef was transported at convenience of the butchers. Most of the butchers were not aware of existing by-laws because they had little or no education.

Though beef transportation was done in early parts of the day around 9am, the likelihood of finding beef of short shelf life on the market is very high since there would not be any introduction of cold condition to the meat during the time of sales. Beef was transported to the markets without any temperature control which enhanced microbial growth proliferation.

Anonymous (1997) indicated that meat should be chilled right from the slaughter house in order to prolong the lag phase of bacteria growth. It was observed that the meat markets as research sites were located near refuse damps. These kinds of conditions are favourable for flies which could carry and deposit pathogenic microbes on the beef.

Unfortunately the butchers did not seem to care about the safety of their products. Beef displayed on wooden benches could be contaminated especially benches with crevices which may harbour microbes (Lauzon, 1998).

The butchers displayed the whole bulk of beef they had for their daily sales on benches, exposed to the open environment without any fly netting or temperature control mechanism in place. This manner of display has a direct effect on the quality of the meat in terms of its microbiological safety.

4.3.1.2. pH RECORDED FOR BEEF IN THE SLAUGHTER HOUSE AND THE MARKETS

The pH values recorded for the thirty carcasses in the slaughter plant were relatively higher (**Table 6**) compared to the normal range of 5.4-5.8 (Anonymous, 2003). This could be attributed to the poor pre-slaughter conditions the animals were exposed to. The depletion of reserved glycogen due to pre- slaughter stress (Anonymous, 2003) makes the ultimate pH high and would favour microbial growth and proliferation.

The value of the ultimate pH of meat is crucial to its resistance to microbial invasion and spoilage (Walker and Betts, 2000).

This means that the carcasses sampled were highly susceptible to be invaded by microbes which may reduce the shelf life and consequently affect the microbiological safety of meat from these carcasses.

It was observed that beef from the twenty butchers' benches in two different markets also recorded pH values not significantly different ($p<0.05$) from the values recorded in the slaughter plant (**Table 7**). These values were higher compared to those recorded by Soyiri *et al.* (2008). The values were not expected to be lower than those found in the slaughter plant since they were pieces from carcasses from the slaughterhouse. Such high pH values favour growth of many meat microbes which leave the meat unsafe for consumption and also cause fast spoilage (Newton and Gill, 1981).

4.3.2.0. BACTERIAL COUNTS IN THE SLAUGHTERHOUSE

4.3.2.1. TOTAL PLATE COUNT (TPC)

Levels of TPC values obtained in a study were generally accepted as the basis for microbial contamination of carcasses and a helpful indicator of the general hygiene (Zweifel and Stephan, 2003) and prevailing sanitary conditions of the slaughterhouse.

The TPC values (log 13.41 to 15.23 CFU/cm^2 for sticking knives, log 13.25 to 14.56 CFU/cm^2 for inner surface of the carcass after evisceration, log 13.60 to 14.66 CFU/cm^2 for surface of the carcass after skin removal, log 13.66 and 14.78 CFU/cm^2 for the last knives used trimming the carcasses) recorded in the slaughter plant were on the higher side compared to results reported by Nouichi and Hamdi (2009) and EL-Hadef *et al.* (2005) who reported a TPC value of log 5.34CFU/cm^2.

The values recorded in this study were far above the requirement of the Ghana Standards Board (<1.0x10^{4}CFU/g). The International Commission on Microbiological Specification (ICMS, 1980) requires TPC of 10^3-10^5CFU/cm^2.

Furthermore, the TPC values recorded in this work exceeded the ICMS threshold value of 10^7CFU/cm^2 expected of fresh and not chilled meat. The ICMS would thus classify these carcasses as entirely contaminated or those which had exposure to conditions favourable for microbial growth and could easily get spoiled (ICMS, 1978).

According to Eisel (1997) high TPC values in a plant indicates poor quality and reduced shelf life of carcasses as a result of poor and unhygienic practices during slaughter. The results showed in this work would have been classified under the "marginal" category in the scale given by ARMCANZ (Anonymous, 2003). By the scale of acceptance and rejection used in the UK, the carcasses sampled in the plant would be put under "bad" since the values were above log 4.5CFU/cm^2.

The absence of significant differences ($P<0.05$) among the values is an indication of generalised contamination resulting from little cleaning during the production process.

4.3.2.2. TOTAL COLIFORMS COUNT (TCC)

The TCC values (log 8.05 to 9.41 MPN/cm^2 for sticking knives, log 8.26 to 9.74 MPN/cm^2 for inner surface after evisceration, log 7.81 and 9.14 MPN/cm^2 for surface of the carcass after skin removal and log 7.81 to 9.39 MPN/cm^2 for last knives for trimming carcasses) recorded in this work were very high compared to that found by El-Hadef *et al.* (2005) and Ware *et al.* (2001) in slaughterhouses. These higher values are expected to have come as a result of contaminated carcasses with dirt.

Though samples were taken from different locations, they all seem to fall within the same range.

There is the need to check the problem since the higher TCC found on a carcass, the greater the possibility of finding high levels of faecal and non-faecal *coliforms* on that sample.

Contamination from one stage of the harvest process is carried to the next since there were no washing of hands, equipment and tools and even the carcasses. Difference in values could have been obtained if the slaughter plant practised some good degree of hygiene and Good Manufacturing Practices (GMP).

4.3.2.3. FAECAL *COLIFORMS* COUNT (FCC)

FCC values are useful indicators of faecal contamination of a sample being worked on and the higher the values obtained, the greater the expectation of finding other microbes of faecal origin (Zweifel and Stephan, 2003). High values of FCC were recorded in this study (log 5.21 to 6.38 MPN/cm^2 for sticking knives, log 4.73 to 6.18 MPN/cm^2 for inner surface of carcasses after evisceration, log 4.46 from 6.11 MPN/cm^2 for surface of carcasses after skin removal and log 4.76 to 6.14 MPN/cm^2 for last knives used trimming carcasses) compared to log 4.38 - 4.77MPN/cm^2 found by Arenas de Moreno *et al*. (n.d.).

It was expected that parts of carcasses in close association with the hides and viscera would have high values because these sites have been found to be good sources of spoilage and pathogenic microbes (Gracey and Collins, 1992). The insignificant differences observed among the levels recorded in this study are evidence of a possible cross contamination. There is correlation between the cleanliness status of animal and the level of faecal contamination of its carcass during harvest (Herrera, 2001). Almost all the cattle ready for slaughter were soiled with faeces and dirt. No washing of these animals were observed.

Research has demonstrated that regular washing of hands by slaughter plant personnel during harvest could help reduce cross contamination. Carcass washing with clean, uncontaminated water could also help decrease superficial microbial contamination (Dickson and Anderson, 1992).

4.3.2.4. *E. COLI* COUNT (ECC)

All the samples in this research showed positive reaction to the *E.coli* test as against 26% out of 1, 296 found by Arenas de Moreno *et al.*, (n.d.). 85.65% was found to be positive in similar work done in an abattoir in Nigeria (Enabulele and Uriah, 2009) compared to 100% positive *E coli* in this work. Though the Ghana Standards Board requires no detection of *E.coli* on fresh beef, this study obtained various levels of *E.coli* contamination (log 2.61 to 4.33 MPN/cm^2 for sticking knives, log 2.59 to 4.20 MPN/cm^2 for inner surface of carcasses after evisceration, log 2.81 to 4.85 MPN/cm^2 for surface of the carcasses after skin removal and log 2.76 to 4.20 MPN/cm^2 for last knives used trimming of carcasses). The presence of *E.coli* is a useful indicator of hygienic slaughter practices (Stannard, 1997). By the criteria set by ARMCANZ, the carcasses in this study would be put under "marginal" category (Anonymous, 2003). There is either direct or indirect faecal contamination of carcasses (Clarence *et al.*, 2009) which could have an environmental origin (Herrera, 2001).

E.coli contamination in a slaughterhouse could also be as result of cross contamination from one step of the production process to the next because of lack of Good Hygienic Practices (GHP). These hygienic practices include regular washing of hands, equipment and tools and also the floor of the plant itself.

Though carcasses pass the approval of the veterinary inspection, this inspection leaves the microbial status of the carcasses unchecked (Gill, 2000).

The detection of *E.coli* on meat means a possible find of other pathogens of faecal origin (Caliciouglu *et al.*, 1999).

It is important to prevent *E.coli* contamination and its possible subsequent growth on meat because it is able to produce heat stable toxins above 18^0C (Anonymous, 1997). Sufficient chilling of carcasses after dressing could retard the growth of *E.coli*, (Anonymous, 1997) but this was not observed in this study since carcasses were sent to the loading bay to be transported to the markets. Reduction of faecal contamination in a slaughter plant is crucial to obtaining low levels of *E.coli* counts on carcasses and knives used in a plant.

4.3.2.5. *SALMONELLA* SPP COUNT (SC)

Salmonella spp were detected on all the samples taken from the slaughterhouse though it has been reported that the prevalence rate is 0.31% of 1171 test samples in Australia (Anonymous, 2002). The prevalence rates of *Salmonella spp* were 1.0% for steer-heifers and 2.7% for cow-bull carcasses in the United States of America (Sofos *et al.*, 1999) compared to 100% obtained in this study at various levels (log 3.93 to 5.27 CFU/cm^2 for sticking knives, log 3.49 to 4.78 CFU/cm^2 for inner surface of carcasses after evisceration, log 3.62 to 5.59 CFU/cm^2 for surface of the carcasses after skin removal and log 3.71 to 5.27 CFU/cm^2 for last knives used in trimming of carcasses).

Salmonella spp are found in the gastrointestinal tracts and the lymph nodes of many animals which could contaminate the carcasses during harvest when care is not taken. This high prevalence rate is an indication of lack of hygiene during evisceration processes (Karama, 2005). This means that the samples from sticking knives should not have recorded levels of *Salmonella spp*.

There is a possibility of the organisms originating from other sources including transport vehicles, holding pens, equipment and tools as well as workers in the plant (Beach *et al.*, 2002). Cross contamination is also suspected to be another cause of the detection of the organism at all sampling sites.

ICMSF (1978) reported that carcass contamination by *Salmonella spp* is a reflection of their presence in the live animal than as a result of poor hygiene during production. There is therefore a high possibility that the animals slaughtered at the Kumasi abattoir during this study were asymptomatic carriers of *Salmonella spp* (Anderson *et al.*, 2001).

Contamination control of the organism at the slaughterhouse is very important in controlling contamination at the butchers' shops and the subsequent cause of infections.

4.3.3.0. BACTERIAL COUNTS AT THE BUTCHERS' SHOPS

4.3.3.1. TOTAL PLATE COUNT (TPC)

The TPC values (log 13.66 to 15.18 CFU/cm^2 for benches, log 13.46 to 14.55 CFU/cm^2 knives and log 13.46 to 14.55 CFU/cm^2 for the meat surface) recorded on the surfaces sampled in the shops were similar to those found in the slaughterhouse. It could be said that carcasses were contaminated from the slaughterhouse (Anonymous, 1997).

Though results recorded were expected to be higher than those found in the slaughterhouse, the opposite happened. This could be explained that the bacteria had reached the stationary phase of their growth process.

These levels recorded in this study were unacceptably high like the results obtained by Mensah *et al.* (2001) from beef and goat microbial analysis from Accra.

The knives and the meat surfaces recorded high values in this work and this could be attributed to the heavy loads on the carcasses (Jamilah *et al.*, 2008).

The values recorded for the benches in this study on the other hand could be as a result of entrapment of bacteria (Lauzon, 1998) which could also cause cross contamination. The levels found in this study (log 13.66 to 15.18 CFU/cm^2 for benches and log 13.46 to 14.55 CFU/cm^2 knives) were higher compared to limits required of working surfaces (Anonymous, 2007). This could mean that the crevices on the surfaces of the benches do not get proper cleaning.

The high level of TPC especially on the meat surface suggests a poor keeping quality, shortened shelf life and possibly presence of pathogenic microbes (Eisel, 1997). It was expected that the values recorded in this work at the butchers' shops would be higher than the level found in the slaughterhouse since bacteria proliferate with time and other growth factors (Ross, 1999) but result found in this research was not different could. It may be due to the fact that the surfaces sampled were dry as a result of exposure to the environment with high temperature during the time of sampling. There seem to be a non-significant reduction in TPC values found in the market compared to that of the slaughterhouse.

The bacteria flora might have reached the death phase of their growth cycle where they might have produced spores and toxins which might be dangerous to the health of the immunocompromised (Jamilah *et al.*, 2008). Meat spoilage might have ensued as spoilage is evident by microbial numbers as low as one million per cm^2 (Anonymous, 2002) though organoleptic and textural tests were not done on the beef sampled.

4.3.3.2. TOTAL *COLIFORMS* COUNT (TCC)

Total *coliforms* count (log 7.92 to 9.11 MPN/cm^2 for benches, log 8.67 and 9.42 MPN/cm^2 for knives and log 7.85 to 9.14 MPN/cm^2 for meat surface) found on samples at the market were high and similar to that found in the slaughterhouse. This high level suggests that there was a heavy contamination during slaughter, transportation or even during offloading and also the manner of display.

The values obtained in this study were not significantly different from the values got in the slaughterhouse because there were no decontamination measures in place by time of the study. High levels of total *coliforms* could mean possible presence of other pathogenic *coliforms* like *Salmonella spp*. Their reduction is non-significant compared to the values found in the slaughterhouse possibly as a result of the effect of dry knives, meat and bench surfaces where samples were taken.

The butchers did not have measures in place to reduce the levels of contamination, which they were even not aware of. Butchers in Ghana are more concerned about their business not how hygienic their premises should be (Soyiri *et al.*, 2008).

4.3.3.3. FAECAL *COLIFORMS* COUNT (FCC)

FCC values are useful indicators of faecal contamination of a sample being worked on and the higher the values obtained, the greater the expectation of finding other microbes of faecal origin (Zweifel and Stephan, 2003). High values of FCC were recorded in this study (log 5.21 to 6.38 MPN/cm^2 for sticking knives, log 4.73 to 6.18 MPN/cm^2 for inner surface of carcasses after evisceration, log 4.46 from 6.11 MPN/cm^2 for surface of carcasses after skin removal and log 4.76 to 6.14 MPN/cm^2 for last knives used trimming carcasses) compared to log 4.38 - 4.77MPN/cm^2 found by Arenas de Moreno *et al*. (n.d.).

It was expected that parts of carcasses in close association with the hides and viscera would have high values because these sites have been found to be good sources of spoilage and pathogenic microbes (Gracey and Collins, 1992). The insignificant differences observed among the levels recorded in this study are evidence of a possible cross contamination. There is correlation between the cleanliness status of animal and the level of faecal contamination of its carcass during harvest (Herrera, 2001). Almost all the cattle ready for slaughter were soiled with faeces and dirt. No washing of these animals were observed.

Research has demonstrated that regular washing of hands by slaughter plant personnel during harvest could help reduce cross contamination. Carcass washing with clean, uncontaminated water could also help decrease superficial microbial contamination (Dickson and Anderson, 1992).

4.3.3.4. *E.COLI* COUNT (ECC)

The prevalence of *E.coli* was 100% in the samples taken from the markets and were similar to what was reported by Enabulele and Uriah (2009) in a traditional open market in Nigeria. The levels recorded in this study were higher (log 3.48 and 5.17 MPN/cm^2 for benches, log 3.26 and 5.14 MPN/cm^2 for knives and log 3.53 to 4.92 MPN/cm^2 for meat surface) than those values found at the slaughterhouse (log 2.61 to 4.33 MPN/cm^2 for sticking knives, log 2.59 to 4.20 MPN/cm^2 for inner surface of carcasses after evisceration, log 2.81 to 4.85 MPN/cm^2 for surface of the carcasses after skin removal and log 2.76 to 4.20 MPN/cm^2 for last knives used trimming of carcasses). This increase in the levels is significant ($p>0.05$) and could be attributed to contamination during transportation of the carcasses, offloading or even during display for sales.

The possibility of cross contamination from the benches on which the carcasses are displayed for sale cannot be overlooked since the top of the benches had crevices. Poor sanitary conditions in the markets could also be a contributing factor.

Though the surfaces had dried up due to high temperatures of the weather, the microbes might have been in the growth phase when sampling was done. This is a very serious situation since the microbes might form spores or leave behind toxins (Jamilah *et al.*, 2008) which would make the meat unwholesome for consumption. Though this high prevalence does not directly mean the presence of the pathogenic strain like *E.coli O157:H7*, the possibility of other pathogens of faecal origin are highly suspected. By the standards given by ARMCANZ, the beef found in these two markets are under the "marginal" category (Anonymous, 2003).

Contamination control in general in the shops should be considered very crucial to safe meat on the bench for sale.

4.3.3.5. *SALMONELLA* SPP COUNT (SC)

Salmonella spp invasion on meat is an indication of faecal contamination. A 100% prevalence rate obtained in this study is high compared to 0.31% of 1171 test samples (Anonymous, 2002). This high prevalence is worrying since pathogenic strains of the organism could possibly be present.

The SC values (log 4.47 to 5.99 CFU/cm^2 for benches, log 5.28 and 6.03 CFU/cm^2 for knives and log 4.57 and 5.97 CFU/cm^2 for meat surface) recorded in this work for the markets were significantly higher ($p>0.05$) than values found at the slaughterhouse (log 3.93 to 5.27 CFU/cm^2 for sticking knives, log 3.49 to 4.78 CFU/cm^2 for inner surface of carcasses after evisceration, log 3.62 to 5.59 CFU/cm^2 for surface of the carcasses after skin removal and log 3.71 to 5.27 CFU/cm^2 for last knives used in trimming carcasses).

Salmonella spp are able to tolerate conditions unfavourable to microbial growth (Clayton, 2002) making them grow even at high environmental temperatures and reduced water activity.

A possible cross contamination could not be ruled out in that the offals were not separated from the carcasses during transportation and the intestinal organs are usually contaminated with faecal matter. Another possible source is from the surfaces of the benches since the benches also recorded higher values though non-significant from the knives and the meat surfaces in the butcher's shops.

4.3.3.6. BACTERIAL ISOLATES FROM THE ABATTOIR

The indicator organisms isolated from samples analysed in this study showed various levels with *Enterobacteriaceae* as the predominant flora followed by *Staphylococcus spp., Bacillus spp.,* and *Pseudomonas spp.* along sampling sites in the slaughterhouse (**Figure 6**). Most of them identified were similar to what are normally found on meat (ICMSF, 1980).

Karama (2005) gave a similar report of *Enterobacteriaceae* predominance in an abattoir. The high level of *Enterobacteriaceae* (38%) isolated suggested poor hygienic practices in the plant with direct contamination from intestinal tract during meat harvest (Karama, 2005). This could have occurred at any point of sampling since sterilization was not done along the slaughter process.

The level of *Staphylococcus spp* (23%) recorded in this study was higher than what (14.76%) was reported by Abdalla *et al.* (2009).

This means the samples analysed did not pass the zero test set by the Ghana Standards Authority (Soyiri *et al.*, 2008). The level of *Bacillus spp.* (21%) found in this study is higher than that (10.54%) reported by Abdalla *et al.* (2009).

The low level of *Pseudomonas spp.* (18%) could be due to the fact that samples taken were not chilled since *Pseudomonas spp* are psychrophiles (Gill, 1983). Though this level is low, the *Pseudomonas spp.* could have come from water especially from taps and hose pipes (Gill, 1987) used during the splitting of the carcasses in the abattoir.

4.3.3.7. BACTERIAL ISOLATES FROM THE BUTCHERS' SHOPS

The organisms isolated are usually found associated with fresh meat (ICMSF, 1980). The predominant group isolated at the markets were *Enterobacteriaceae* (33%) followed by *Staphylococcus spp.* (24%), *Pseudomonas spp.* (24%) and *Bacillus spp.* (19%) respectively (**Figure 7**). The predominance of *Enterobacteriaceae* at sampling sites especially on meat could be due to contamination from the slaughterhouse because of poor sanitation during harvesting. This raises a serious concern because beef is sold in the open environment and there is high possibility of exposure to microbes contaminated air.

Beef sold by the butchers could easily get bad since *Enterobacteriaceae* play a major role in aerobic meat spoilage (Karama, 2005). Most strains of *Enterobacteriaceae* are capable of releasing H_2S which with decarboxylated amino-acids are responsible for off-odours. This indicates that *Enterobacteriaceae* have high spoilage potential under favourable conditions (Gill, 1986).

Pseudomonas spp. (24%) recorded an increase in percentage over that found in the slaughterhouse (18%). This could be as a result of the poor quality of the water (Lihelac and Colin, 1979) which is used at the shops by butchers to keep the surfaces of beef moist.

Pseudomonas spp. is usually associated with meat spoilage (Anonymous, 2009a) and the higher their levels found on meat, the lower the shelf life of the meat.

The other bacterial isolates are also important since they cause illnesses in humans after ingestion through meat. *Staphylococcus spp* and *Bacillus spp.* presence on benches, knives and beef surfaces in the markets are also important since they are organisms of public health concern.

CHAPTER FIVE

5.0. CONCLUSION AND RECOMMENDATIONS

5.1. 0. CONCLUSION

This work provides a baseline data on microbiological status of bovine carcasses produced at the Kumasi Abattoir slaughter plant and that sold at Atonsu and Maayanka meat markets in the Kumasi metropolis.

E.coli and *Salmonella spp* were found on beef at both the abattoir and meat shops. The other microbial contamination indicators like the Total Plate Count, Total *Coliforms* Count, Faecal *Coliforms* Count were also high and gave an indication of poor sanitation. There is the need for awareness creation and education on this issue since these organisms are pathogenic and could cause diseases when ingested in high levels.

There is an indication of contamination and cross contamination especially from faecal source in the slaughter plant. The manner in which animals are transported to the abattoir is also most likely to contribute to the poor state of their skins during slaughter. The trucks that carry the animals to the kraal are loaded with more than the expected number which does not allow them space to move freely. Most of the animals ready for slaughter had dirty skins and no washing was done before slaughter.

There were no decontamination interventions, hot or cold water spray wash, pasteurization, chemical spray wash or even production of meat under controlled temperature during the slaughter process and this accounted for similar results recorded for samples taken from the markets.

Poor or lack of supervision by managers of the plant to check whether production staff adhere to safety standards could also be another contributing factor.

The plant lacks modern meat production equipment to control contamination and improve safety of meat. The equipment and tools in place at the time of this study were old and even certain parts had to be improvised in order to make production possible.

Also the bacterial loads found on sticking knives were not different from those found at the inner surface after evisceration, surface of carcass after skin removal and the last knives used for trimming. This implies that cross contamination at this level is also highly possible.

Poor methods of evisceration and skin removal also contributed to the high levels of microbes in this study. Congestion of people and their behaviour in the plant could also be a contributing factor to the high levels of microbes found. Some of these people touch the carcasses, sneeze, talk and laugh without any consideration of contamination in case they carried some contaminants. Some of them entered the plant with their dirty clothes which were blood stained and this could have been a source of microbes.

Though some of the production staff had some level of education, frequent training of the people on the importance of hygienic production conditions for meat were lacking.

Most of them did not have much knowledge on meat production especially on the issue of why producing safe meat is crucial to sustaining their jobs and also improving on it.

Though the Ghana Standards Authority (GSA) has a limit for TPC and other indicators of contamination in meat, there were no mechanisms in place to check that in the slaughter plant.

The Food and Drugs Board (FDB) were said to have never visited the plant to check what goes into meat production in terms of safety at the time the study was done. Though the veterinary inspectors attached to the plant inspect carcasses, this inspection is geared towards eliminating carcasses with lesions. The role of FDB in the production of safe meat for the public has been overlooked. FDB supervisory role goes beyond just the safety of the finished products but also the conditions under which the raw materials are kept and then processed into the product. The FDB does not play a part in even how meat gets to the butchers' benches for consumers to purchase for use. There are by-laws at the Kumasi Metropolitan Assembly to check this but the FDB should also educate and train butchers on the consequences of not adhering to such by-laws.

The location of the plant could have also contributed to high levels of contaminants on the meat in this study since the surrounding air could have carried some of the microbes. The nearness of the plant to a refuse damp needs attention since most of the microbes isolated are of faecal origin.

Chemical contamination could also be another problem since the plant is in an industrial area. Smoke and other waste from those industries could also have bad effect on the safety of the meat produced by the abattoir though this study did not consider those other contaminants. There were no safety systems like HACCP in place.

With all these lapses in ensuring safe beef, there were no laid down conditions for carcass rejection not even on the basis of poor transportation method.

Beef harvest was done according to standards which are less expensive since butchers were not ready to pay any extra cost. Beef sold at the Atonsu and Maayanka markets where samples were taken had poor microbiological status.

The butchers did not care so much about microbial safety. They were not aware of the implications of poor hygiene on the quality of the meat, so hygienic practices were not their concern.

The manner of beef transport to their shops also provides another opportunity for not only microbes but also other contaminants onto the meat. The butchers were more concerned about making profit than selling safe meat to consumers. Issue of leftover meat for sale leaves a lot to be desired because even the safety of fresh beef on the benches of butchers cannot be guaranteed looking at the production and handling conditions before reaching the consumers.

Safe food is important in the lives of sound and strong humans so safety of food like beef should not be compromised.

In conclusion, the microbiological safety status of beef found in Kumasi based on this study is very bad and worrying hence the need for improvement.

The Ghana Standards Authority requires between 10^3 to 10^5 CFU/g or cm^2 of Total Plate Count for fresh beef and no detection of *E.coli* and other pathogenic microbes on the meat but findings from this study did not meet that standard.

The implications of having such contaminated meat on the market are many since there seem to be no checks on what really happens from transportation of the animals till the meat gets to butchers for sale. The consequences of such unfortunate conditions would also mean that the public is at risk of falling ill when such unsafe meat is consumed.

5.2.0. RECOMMENDATION

It could be of interest to K.A.C.L. to implement efficient quality assurance systems in the slaughter plant. The staff in the plant should be provided with personal protective equipment which would ensure safety of the meat they produce. This should include education and training of workers in the area of safety especially concerning contamination and its effects on the meat.

The abattoir should begin to check the levels of microbes in the plant during slaughtering to help find ways of reducing microbial contamination.

Management of the plant must find a Standard Sanitary Operating Procedures (SSOP) which would fit into their style of operation and involve certain procedures like chilling after dressing of carcasses. The need for decontamination measures like washing of animals ready for slaughter, hands of workers and equipment could help reduce the microbial loads. Improved and better evisceration method that would not punch the intestinal contents is needed. This could reduce the levels contamination toward improving safety of beef.

Congestion of people in the plant could also be a contributing factor to the high levels of microbes found. If management of the company could restrict movements in the plant or better still provide footbaths at the entrance of the plant, it could help in ensuring safety.

Though HACCP is the best recommended safety standard in food production, its implementation in the abattoir seem very difficult but certain aspects of the system such regular washing of both equipment hands of workers and sterilization could be adopted.

Butchers need education on the need for good personal hygiene as well as around their shops. The by-laws regarding the manner in which they operate should be explained to them as something that would not take them out of business but would

rather improve their work. These by-laws must be enforced by the appropriate institution for compliance. The Metropolitan Assembly should educate the butchers on the proper methods of transporting the beef to their shops with safety consideration which will also be affordable to them.

The GSA and FDB should be interested in educating the abattoir staff and enforcing the right standards of meat production and handling in the abattoir. The butchers who sell the meat should not be left out.

There should be a general education of the public on the consequences on consuming unsafe meat. This would help the public to be selective and watch out for bad meat on the market. The government should also equip the GSA and FDB with the necessary logistics to enable them ensure safety of the meat sold to the public.

A further study to identify ways of reducing contamination in both the slaughter house and the meat shops is very necessary. Other types of meat should also be subject to this kind of study.

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APPENDICES

APPENDIX A
RAW VALUES FROM THE SLAUGHTERHOUSE
A1: VALUES FOR TOTAL PLATE COUNT ON STICKING KNIVES

CARCASS NUMBER	REP 1	REP 2	REP 3
1	1.75E+15	1.80E+16	1.78E+15
2	1.68E+15	1.84E+16	1.72E+17
3	1.80E+15	1.76E+15	1.82E+16
4	1.76E+15	1.80E+16	1.78E+16
5	1.90E+17	1.95E+16	1.88E+17
6	1.84E+15	1.80E+15	1.76E+16
7	1.98E+15	2.02E+16	1.95E+16
8	1.84E+17	1.78E+15	1.80E+15
9	1.69E+15	1.72E+16	1.70E+17
10	2.00E+16	1.97E+16	2.08E+16
11	1.10E+17	1.20E+15	1.16E+16
12	1.98E+16	2.02E+17	1.96E+15
13	1.73E+17	1.80E+15	1.78E+16
14	2.05E+15	1.97E+16	1.89E+15
15	1.94E+16	1.87E+17	1.91E+15
16	1.77E+15	1.80E+16	1.83E+15
17	1.89E+17	1.86E+16	1.79E+16
18	1.82E+16	1.79E+17	1.85E+15
19	2.06E+15	1.96E+16	2.11E+15
20	1.92E+15	1.88E+16	1.98E+15
21	1.54E+16	1.48E+15	1.60E+15
22	1.55E+15	1.60E+16	1.65E+15
23	1.76E+15	1.80E+15	1.88E+16
24	1.80E+16	1.78E+15	1.84E+15
25	1.89E+16	1.92E+15	1.94E+15
26	1.76E+15	1.80E+15	1.83E+16
27	1.85E+15	1.90E+16	1.89E+15
28	2.19E+15	2.15E+16	2.07E+15
29	1.68E+15	1.73E+16	1.66E+15
30	1.75E+15	1.80E+15	1.83E+16

A2: VALUES FOR TOTAL PLATE COUNT ON THE INNERSURFACE OF THE CARCASSES AFTER EVISCERATION

CARCASS NUMBER	REP 1	REP 2	REP 3
1	1.98E+17	2.00E+16	1.89E+15
2	2.08E+16	2.16E+17	1.97E+15
3	1.98E+16	2.07E+16	2.00E+15
4	1.70E+16	1.68E+17	1.72E+16
5	1.88E+16	1.79E+15	1.90E+16
6	1.60E+16	1.54E+15	1.48E+17
7	1.76E+15	1.74E+17	1.80E+16
8	1.68E+16	1.70E+15	1.65E+17
9	1.58E+15	1.64E+16	1.68E+15
10	2.15E+17	2.20E+15	2.09E+15
11	1.25E+16	1.30E+17	1.27E+15
12	1.76E+16	1.84E+15	1.90E+16
13	1.68E+15	1.70E+15	1.75E+16
14	1.96E+16	2.00E+15	1.86E+15
15	1.45E+17	1.50E+15	1.47E+15
16	1.68E+16	1.71E+15	1.63E+16
17	1.52E+16	1.48E+15	1.45E+16
18	1.89E+16	1.91E+15	1.93E+16
19	1.74E+16	1.69E+15	2.11E+15
20	2.00E+16	1.96E+16	1.87E+15
21	1.78E+13	1.83E+15	1.69E+17
22	1.75E+15	1.68E+16	1.80E+15
23	2.03E+15	1.96E+16	1.94E+15
24	1.68E+16	1.72E+16	1.65E+15
25	1.98E+15	2.01E+16	2.06E+15
26	1.88E+16	1.94E+16	1.98E+15
27	1.96E+15	1.98E+15	2.00E+16
28	2.24E+16	2.08E+17	2.11E+15
29	1.93E+16	1.89E+15	1.95E+15
30	1.68E+16	1.70E+17	1.60E+15

A3: VALUES FOR TOTAL PLATE COUNT ON THE SURFACE OF THE CARCASSES AFTER SKIN REMOVAL

CARCASS NUMBER	REP 1	REP 2	REP 3
1	2.08E+16	1.90E+16	1.87E+15
2	2.20E+16	2.00E+17	2.10E+16
3	2.00E+16	1.88E+15	1.96E+17
4	1.98E+17	1.96E+16	1.94E+15
5	2.10E+17	2.07E+15	1.98E+16

6	2.00E+16	1.98E+15	1.96E+17
7	2.15E+17	2.09E+15	2.10E+16
8	2.09E+16	1.99E+17	2.11E+16
9	2.00E+15	1.98E+17	2.05E+16
10	2.10E+15	2.00E+16	2.17E+15
11	2.00E+15	1.97E+16	2.02E+16
12	2.03E+16	1.97E+16	1.89E+15
13	1.95E+16	2.00E+15	1.97E+16
14	2.05E+15	1.97E+16	1.89E+15
15	1.90E+16	1.86E+16	1.78E+15
16	1.95E+16	1.89E+15	1.98E+15
17	1.70E+15	1.67E+16	1.63E+15
18	2.00E+15	1.97E+16	1.95E+15
19	2.10E+15	2.05E+16	1.97E+15
20	2.10E+15	1.98E+16	2.08E+15
21	1.94E+15	1.90E+16	1.87E+17
22	1.87E+16	1.90E+15	1.84E+15
23	2.15E+15	2.09E+16	2.00E+15
24	1.98E+16	1.96E+15	1.93E+15
25	2.15E+15	2.23E+16	2.18E+15
26	2.00E+15	2.09E+16	2.12E+16
27	2.10E+16	2.13E+16	2.07E+15
28	2.30E+16	2.26E+15	2.19E+16
29	2.00E+15	1.99E+16	2.04E+15
30	1.86E+15	1.90E+16	1.79E+15

A4: VALUES FOR TOTAL PLATE COUNT ON LAST KNIVES USED FOR TRIMMING CARCASSES

CARCASS NUMBER	REP 1	REP 2	REP 3
1	1.40E+17	1.36E+16	1.42E+15
2	1.52E+16	1.60E+17	1.54E+15
3	1.52E+16	1.64E+15	1.57E+16
4	1.50E+17	1.46E+17	1.40E+15
5	1.74E+17	1.82E+15	1.80E+15
6	1.70E+16	1.68E+17	1.72E+15
7	1.68E+16	1.70E+17	1.66E+16
8	1.50E+16	1.48E+17	1.54E+15
9	1.59E+15	1.70E+16	1.68E+15
10	1.98E+16	1.89E+15	1.94E+15
11	1.88E+16	1.90E+16	1.92E+15
12	1.79E+17	1.82E+16	1.69E+15
13	1.58E+15	1.49E+16	1.60E+17
14	1.16E+15	1.00E+15	1.20E+16

15	1.50E+15	1.48E+16	1.52E+15
16	1.42E+15	1.50E+16	1.46E+15
17	1.44E+16	1.39E+17	1.48E+15
18	1.65E+15	1.70E+16	1.69E+15
19	1.80E+15	1.69E+16	1.76E+15
20	1.66E+15	1.72E+16	1.59E+16
21	1.42E+16	1.39E+15	1.47E+15
22	1.32E+15	1.41E+16	1.39E+15
23	1.62E+15	1.59E+16	1.64E+15
24	1.52E+15	1.49E+16	1.54E+15
25	1.65E+16	1.58E+15	1.54E+15
26	2.00E+15	2.09E+16	2.12E+16
27	1.57E+15	1.55E+15	1.60E+16
28	1.76E+15	1.80E+16	1.83E+15
29	1.46E+15	1.50E+16	1.54E+15
30	1.43E+15	1.49E+16	1.50E+15

A5: VALUES FOR TOTAL COLIFORM COUNT ON STICKING KNIVES

NUMBER	REP 1	REP 2	REP 3
1	6.40E+10	5.30E+09	4.40E+09
2	3.40E+11	2.10E+09	1.90E+09
3	5.30E+11	4.20E+09	3.90E+09
4	4.40E+10	5.30E+09	3.90E+09
5	3.60E+11	2.80E+09	2.70E+09
6	4.30E+10	5.30E+11	3.90E+09
7	3.50E+09	3.90E+10	2.90E+11
8	2.80E+09	2.70E+09	2.90E+10
9	2.70E+10	2.40E+09	1.90E+11
10	2.40E+11	1.90E+09	2.10E+10
11	2.30E+11	1.90E+09	2.00E+09
12	2.60E+10	2.30E+11	1.90E+09
13	2.90E+11	2.70E+09	2.40E+09
14	2.70E+10	2.40E+11	2.30E+10
15	2.40E+10	2.10E+09	1.90E+10
16	2.30E+11	2.60E+09	2.70E+09
17	2.40E+10	2.10E+11	2.30E+09
18	2.60E+11	2.40E+11	2.80E+09
19	2.00E+11	1.60E+09	1.40E+10
20	2.80E+11	2.40E+09	2.10E+11
21	2.40E+09	2.30E+10	2.60E+11
22	2.70E+11	2.40E+09	2.60E+11
23	2.00E+11	2.30E+09	2.40E+10
24	2.60E+09	2.40E+11	2.00E+09
25	2.90E+09	2.40E+11	2.60E+09
26	2.60E+11	2.90E+11	2.30E+09

27	3.40E+09	2.70E+09	2.30E+10
28	2.80E+11	2.40E+10	2.30E+09
29	2.40E+09	2.90E+10	2.40E+10
30	2.80E+11	2.70E+10	3.40E+11

A6: VALUES FOR TOTAL COLIFORM COUNT ON THE INNER SURFACE OF THE CARCASSES AFTER EVISCERATION

NUMBER	REP 1	REP 2	REP 3
1	2.00E+09	1.60E+10	1.90E+09
2	1.90E+10	2.00E+10	2.10E+09
3	2.10E+09	2.40E+10	2.60E+11
4	2.40E+11	2.60E+09	2.10E+09
5	1.90E+11	2.10E+09	2.00E+10
6	2.80E+09	2.60E+11	2.70E+10
7	2.10E+09	2.30E+09	2.00E+10
8	2.40E+09	2.00E+10	2.10E+11
9	2.30E+09	2.10E+10	2.40E+11
10	2.80E+11	3.40E+09	2.60E+09
11	2.70E+09	2.40E+11	2.00E+10
12	2.70E+10	2.40E+11	2.30E+09
13	2.40E+10	2.10E+11	2.00E+09
14	2.80E+11	2.40E+10	2.60E+11
15	2.70E+11	2.30E+09	2.10E+11
16	3.50E+09	2.40E+09	2.10E+10
17	3.50E+11	2.90E+09	2.70E+10
18	3.60E+09	2.90E+09	2.70E+10
19	3.90E+09	4.20E+10	3.60E+11
20	3.50E+09	2.90E+10	2.60E+09
21	3.50E+11	2.80E+11	2.70E+09
22	3.40E+11	2.80E+10	2.40E+10
23	2.80E+11	2.60E+09	2.90E+10
24	2.90E+09	3.50E+11	2.70E+11
25	4.30E+09	3.90E+10	3.50E+10
26	3.60E+09	2.30E+10	2.60E+11
27	2.40E+11	2.00E+11	1.60E+10
28	4.40E+11	3.60E+09	2.80E+09
29	1.90E+09	2.00E+10	2.30E+11
30	2.80E+10	2.40E+09	2.00E+11

A7: VALUES FOR TOTAL COLIFORM COUNT ON THE SURFACE OF THE CARCASSES AFTER SKIN REMOVAL

NUMBER	REP 1	REP 2	REP 3
1	2.70E+10	3.40E+09	2.90E+09
2	3.90E+11	3.50E+10	3.40E+09
3	1.90E+11	2.10E+09	2.40E+10
4	3.50E+09	2.70E+11	2.90E+09
5	2.30E+09	2.60E+10	2.40E+11
6	1.60E+09	1.90E+11	2.00E+09
7	2.80E+11	2.90E+09	1.90E+09
8	4.30E+11	3.60E+09	3.40E+11
9	2.90E+11	2.10E+10	2.40E+11
10	5.30E+10	4.40E+09	3.50E+09
11	4.20E+09	3.90E+11	3.60E+09
12	4.30E+09	3.60E+11	4.40E+09
13	4.30E+09	3.90E+11	3.50E+11
14	3.50E+09	2.90E+11	2.70E+09
15	3.60E+09	2.90E+11	2.70E+11
16	3.60E+10	4.20E+11	2.90E+10
17	4.40E+09	3.90E+11	4.30E+11
18	2.80E+10	3.50E+11	2.70E+11
19	4.30E+11	5.30E+09	6.40E+10
20	4.20E+09	3.60E+10	3.90E+11
21	2.90E+11	3.40E+10	2.70E+09
22	2.40E+11	2.10E+09	2.00E+11
23	3.50E+10	2.70E+11	3.40E+09
24	4.40E+11	3.90E+09	3.60E+09
25	2.90E+09	3.60E+10	3.90E+09
26	5.30E+11	4.40E+09	3.90E+09
27	5.30E+10	4.20E+09	3.60E+09
28	4.20E+11	5.30E+09	4.40E+11
29	3.50E+11	2.80E+10	2.30E+11
30	3.50E+11	2.90E+09	4.20E+10

A8: VALUES FOR TOTAL COLIFORM COUNT ON THE LAST KNIVES USED FOR TRIMMING CARCASSES

CARCASS NUMBER	REP 1	REP 2	REP 3
1	2.40E+11	2.10E+09	1.90E+09
2	2.10E+09	1.60E+11	2.00E+10
3	3.90E+09	4.30E+11	3.60E+09
4	1.50E+09	2.00E+10	1.60E+11
5	1.40E+11	1.60E+09	1.30E+10
6	1.30E+10	1.50E+09	1.20E+11

7	1.60E+09	1.40E+10	1.90E+09
8	1.90E+09	1.60E+10	2.10E+11
9	1.60E+11	2.00E+09	2.10E+09
10	1.40E+10	1.20E+11	1.60E+09
11	2.00E+09	1.60E+11	2.10E+10
12	1.60E+09	1.40E+11	1.30E+09
13	1.60E+09	1.90E+11	1.50E+09
14	1.60E+11	1.30E+09	1.20E+09
15	1.30E+11	1.20E+11	1.40E+11
16	1.90E+10	1.50E+09	1.40E+09
17	2.00E+09	1.90E+11	1.60E+09
18	1.50E+11	1.90E+09	1.40E+09
19	2.10E+09	1.90E+11	2.40E+09
20	1.90E+11	1.60E+09	2.00E+10
21	1.60E+09	1.90E+11	1.40E+09
22	2.30E+10	1.90E+09	1.60E+11
23	1.90E+09	2.10E+10	2.30E+11
24	1.90E+11	2.00E+10	1.50E+10
25	1.90E+11	1.60E+11	1.40E+09
26	2.10E+09	1.90E+11	1.20E+10
27	1.50E+11	1.30E+10	1.20E+09
28	2.00E+10	2.40E+11	1.50E+09
29	1.50E+10	2.00E+09	2.10E+10
30	2.10E+09	1.90E+09	1.50E+11

A9: VALUES FOR FAECAL COLIFORM COUNT ON STICKING KNIVES

NUMBER	REP 1	REP 2	REP 3
1	4.30E+08	3.90E+07	3.60E+07
2	4.40E+07	4.20E+08	3.90E+08
3	2.90E+07	2.60E+08	2.70E+08
4	2.60E+06	2.40E+07	2.80E+07
5	2.00E+06	1.50E+07	1.60E+08
6	2.40E+07	2.30E+06	2.10E+08
7	1.90E+06	2.10E+07	2.30E+08
8	2.10E+08	1.90E+06	1.50E+06
9	2.10E+08	2.40E+07	2.00E+07
10	1.60E+06	2.00E+06	1.90E+08
11	2.40E+06	2.00E+07	2.10E+08
12	1.10E+08	1.90E+06	2.00E+07
13	2.00E+06	1.60E+08	1.50E+08
14	2.10E+08	2.00E+06	1.90E+07
15	2.10E+06	2.00E+08	1.60E+08
16	2.00E+06	2.10E+08	1.90E+07
17	1.40E+08	1.20E+08	1.10E+08

18	2.00E+06	1.90E+08	2.10E+06
19	1.60E+08	2.00E+06	1.30E+08
20	1.90E+08	1.60E+08	1.50E+06
21	2.00E+06	2.40E+08	2.30E+07
22	2.70E+06	1.60E+08	1.40E+07
23	2.40E+06	2.10E+07	2.30E+08
24	2.60E+07	2.40E+06	1.90E+08
25	2.00E+08	2.10E+07	2.40E+06
26	2.40E+06	2.00E+07	1.30E+08
27	2.30E+06	2.10E+07	1.90E+08
28	2.40E+07	2.00E+08	2.10E+08
29	2.60E+07	1.90E+08	2.40E+06
30	2.00E+07	2.10E+06	1.90E+08

A10: VALUES FOR FAECAL COLIFORM COUNT ON THE INNER SURFACE OF THE CARCASSES AFTER EVISCERATION

NUMBER	REP 1	REP 2	REP 3
1	3.60E+06	2.80E+07	1.50E+06
2	7.50E+08	6.40E+07	5.30E+07
3	3.50E+08	2.90E+08	3.40E+07
4	5.30E+08	4.40E+07	3.60E+06
5	3.60E+08	2.90E+08	2.70E+07
6	2.40E+07	2.30E+06	2.10E+08
7	2.40E+06	2.70E+07	2.40E+07
8	2.40E+06	1.60E+08	2.00E+08
9	2.10E+08	1.90E+07	1.60E+08
10	2.80E+08	3.40E+06	2.40E+07
11	2.80E+06	2.40E+08	2.10E+07
12	2.60E+06	2.10E+08	1.90E+06
13	2.70E+06	2.40E+08	2.60E+07
14	2.70E+06	2.60E+06	2.40E+07
15	1.30E+06	1.60E+08	1.40E+08
16	2.40E+06	2.60E+07	2.70E+06
17	2.40E+08	2.00E+08	2.30E+07
18	2.40E+06	2.30E+07	2.10E+08
19	2.90E+06	2.70E+06	2.40E+07
20	2.70E+06	2.40E+08	2.30E+07
21	2.70E+06	2.60E+08	2.90E+07
22	3.60E+07	2.80E+06	2.90E+06
23	2.80E+06	3.50E+06	2.90E+07
24	2.00E+08	2.90E+06	2.70E+08
25	1.90E+08	2.40E+07	2.70E+06
26	2.40E+06	1.90E+08	2.10E+08

27	2.40E+07	1.60E+08	2.60E+06
28	2.80E+06	3.50E+07	3.60E+07
29	2.80E+06	2.90E+07	3.40E+06
30	2.90E+06	2.60E+08	1.90E+07

A11: VALUES FOR FAECAL COLIFORM COUNT ON THE SURFACE OF THE CARCASSES AFTER SKIN REMOVAL

NUMBER	REP 1	REP 2	REP 3
1	3.90E+06	4.30E+07	5.30E+06
2	5.30E+08	7.50E+06	6.40E+07
3	4.40E+06	3.90E+07	3.60E+06
4	4.30E+06	3.90E+08	4.20E+08
5	4.40E+06	2.80E+07	2.70E+08
6	3.60E+07	2.90E+06	2.70E+06
7	2.80E+06	2.60E+07	2.70E+08
8	2.80E+06	2.70E+07	1.60E+08
9	2.60E+08	2.40E+07	2.40E+06
10	3.60E+06	2.90E+08	2.60E+06
11	3.60E+06	2.80E+06	2.40E+06
12	2.90E+08	2.70E+07	2.80E+08
13	2.60E+06	2.40E+08	2.80E+06
14	2.90E+06	2.40E+06	2.70E+07
15	3.50E+06	2.90E+06	2.80E+07
16	2.80E+06	2.60E+07	2.40E+08
17	3.50E+06	2.90E+07	2.70E+06
18	2.60E+06	2.40E+06	2.30E+08
19	3.40E+06	2.90E+06	2.80E+07
20	3.40E+06	2.90E+06	2.70E+08
21	2.90E+06	2.70E+06	3.40E+07
22	3.50E+06	4.20E+06	2.80E+08
23	4.30E+06	3.90E+08	2.80E+07
24	2.80E+08	3.40E+06	2.70E+07
25	3.50E+06	3.40E+06	2.90E+07
26	2.80E+06	2.60E+08	3.50E+07
27	2.80E+06	3.40E+07	2.90E+06
28	2.40E+06	4.30E+07	4.20E+06
29	4.20E+06	3.90E+07	3.50E+07
30	2.80E+07	3.40E+06	2.90E+07

A12: VALUES FOR FAECAL COLIFORM COUNT ON THE LAST KNIVES USED FOR TRIMMING OF CARCASSES

NUMBER	REP 1	REP 2	REP 3
1	1.40E+08	2.30E+07	2.60E+07
2	5.30E+06	7.50E+06	6.40E+06
3	2.90E+07	3.50E+06	2.80E+06
4	1.90E+07	1.40E+08	1.60E+06
5	1.60E+06	1.20E+08	1.30E+08
6	2.40E+06	2.00E+07	1.90E+07
7	1.40E+06	1.20E+08	1.10E+07
8	1.20E+08	9.00E+07	1.30E+07
9	1.60E+06	2.00E+07	1.50E+06
10	1.90E+06	1.90E+08	2.10E+06
11	1.90E+08	1.50E+07	1.30E+08
12	1.60E+08	2.00E+06	1.50E+08
13	1.90E+06	1.60E+08	1.20E+08
14	1.50E+06	1.90E+08	1.60E+08
15	1.30E+06	1.60E+08	1.40E+08
16	1.90E+06	1.50E+07	1.40E+06
17	1.50E+08	1.30E+08	1.20E+06
18	1.60E+06	1.10E+08	1.20E+08
19	1.60E+06	1.40E+06	1.20E+07
20	1.90E+06	1.60E+07	1.30E+08
21	2.00E+06	2.10E+07	1.90E+08
22	1.20E+08	1.30E+07	2.00E+06
23	1.20E+08	1.50E+06	1.60E+08
24	1.90E+06	1.30E+06	1.20E+08
25	2.00E+06	1.30E+06	1.10E+08
26	1.20E+08	1.10E+08	1.50E+06
27	2.00E+06	1.90E+06	1.10E+08
28	2.00E+06	1.30E+07	1.20E+06
29	1.50E+08	1.90E+06	2.00E+06
30	1.90E+06	1.30E+08	1.40E+07

A13: VALUES FOR E. COLI COUNT ON STICKING KNIVES

NUMBER	REP 1	REP 2	REP 3
1	2.80E+04	1.50E+06	2.30E+04
2	1.60E+06	1.30E+06	1.30E+04
3	9.00E+05	9.00E+04	7.00E+05
4	1.50E+06	2.00E+04	1.40E+06
5	9.00E+05	1.90E+04	1.10E+05
6	1.90E+05	2.10E+04	1.60E+06
7	1.10E+05	9.00E+05	1.20E+04
8	1.10E+05	9.00E+05	1.20E+06

9	1.40E+04	1.30E+06	1.20E+05
10	1.50E+04	1.60E+05	1.20E+06
11	1.50E+05	1.60E+06	1.90E+04
12	7.00E+05	1.10E+05	9.00E+03
13	1.60E+04	1.30E+06	1.10E+05
14	9.00E+05	1.20E+04	1.10E+06
15	2.30E+04	1.60E+05	1.50E+04
16	2.00E+05	2.10E+04	1.90E+05
17	1.90E+05	1.90E+04	2.00E+06
18	2.10E+04	1.60E+06	1.90E+05
19	2.00E+04	1.60E+06	1.90E+05
20	1.90E+05	2.00E+04	1.60E+06
21	1.90E+06	1.60E+05	2.00E+04
22	2.30E+04	2.60E+04	2.70E+08
23	1.50E+04	1.90E+05	1.60E+04
24	1.90E+04	2.10E+04	2.40E+04
25	2.10E+04	1.60E+05	1.90E+04
26	1.60E+05	1.50E+06	2.00E+06
27	2.30E+05	2.10E+05	1.90E+05
28	2.40E+04	2.10E+04	1.90E+05
29	1.90E+06	1.60E+05	1.50E+06
30	2.80E+05	2.40E+06	2.10E+06

A14: VALUES FOR E. COLI COUNT ON THE INNER SURFACE OF THE CARCASSES AFTER EVISCERATION

NUMBER	REP 1	REP 2	REP 3
1	9.00E+05	3.90E+04	5.30E+04
2	2.10E+05	1.60E+05	2.00E+05
3	2.10E+05	1.90E+05	2.00E+04
4	2.40E+05	1.30E+06	2.00E+05
5	1.60E+05	1.90E+05	1.50E+05
6	2.00E+05	2.40E+04	1.30E+06
7	2.00E+04	1.40E+06	1.30E+05
8	2.00E+04	1.90E+05	1.60E+04
9	2.40E+05	1.90E+06	1.60E+04
10	2.00E+05	2.40E+06	2.30E+05
11	1.90E+05	1.40E+06	1.50E+04
12	1.30E+06	1.40E+04	1.60E+04
13	1.90E+06	2.00E+05	1.60E+03
14	1.90E+06	2.00E+04	1.60E+06
15	2.80E+04	2.40E+06	2.00E+06
16	2.70E+04	2.40E+06	1.90E+06
17	3.40E+04	3.50E+04	2.90E+05

18	2.40E+04	1.90E+06	2.90E+05
19	2.40E+04	2.60E+04	2.70E+06
20	2.60E+06	2.70E+04	2.90E+04
21	2.30E+06	2.40E+04	2.70E+04
22	5.30E+04	4.20E+04	2.90E+05
23	2.80E+06	2.70E+05	2.90E+04
24	1.90E+06	2.60E+06	2.70E+05
25	2.90E+06	2.80E+05	2.60E+05
26	2.40E+06	2.30E+06	1.60E+05
27	3.90E+06	2.90E+06	3.60E+05
28	2.70E+06	2.90E+05	2.80E+04
29	2.90E+06	2.80E+05	2.60E+04
30	3.60E+05	3.40E+06	2.90E+05

A15: VALUES FOR E. COLI COUNT ON THE SURFACE OF THE CARCASSES AFTER SKIN REMOVAL

NUMBER	REP 1	REP 2	REP 3
1	1.60E+06	1.30E+05	1.90E+06
2	1.30E+06	2.80E+06	2.40E+05
3	1.10E+06	2.80E+05	1.40E+06
4	1.50E+06	1.30E+06	1.10E+06
5	2.80E+05	1.50E+06	2.70E+05
6	1.60E+04	1.90E+04	1.40E+06
7	1.90E+04	1.50E+04	1.40E+05
8	2.60E+04	2.40E+05	1.90E+06
9	2.60E+04	2.40E+05	2.40E+04
10	2.70E+04	2.60E+05	2.40E+06
11	2.00E+04	2.40E+06	2.30E+05
12	2.60E+04	1.60E+06	1.90E+05
13	2.40E+04	2.00E+06	2.30E+04
14	2.90E+04	2.40E+06	2.30E+06
15	2.80E+06	2.70E+05	2.60E+04
16	3.40E+04	3.50E+05	2.90E+06
17	2.30E+06	2.60E+05	3.90E+04
18	4.20E+04	3.90E+06	2.70E+06
19	3.90E+06	2.90E+05	2.80E+05
20	2.90E+04	2.40E+05	2.80E+06
21	4.40E+04	3.90E+06	6.40E+04
22	4.40E+06	3.50E+05	3.60E+05
23	4.40E+06	3.50E+06	3.40E+06
24	3.60E+05	4.20E+05	3.90E+04
25	3.90E+06	4.20E+05	3.60E+06
26	3.50E+05	2.90E+06	3.90E+04
27	5.30E+06	4.40E+05	4.20E+05

28	6.40E+06	5.30E+05	4.40E+06
29	2.80E+06	3.90E+05	4.30E+06
30	3.90E+06	3.60E+06	2.80E+05

A16: VALUES FOR E. COLI COUNT ON THE LAST KNIVES USED FOR TRIMMING OF CARCASSES

NUMBER	REP 1	REP 2	REP 3
1	2.80E+04	1.50E+05	2.00E+05
2	9.00E+05	6.00E+05	1.10E+06
3	1.50E+04	1.60E+05	1.20E+06
4	9.00E+05	1.20E+05	1.10E+04
5	9.00E+05	1.50E+04	1.10E+05
6	1.10E+05	1.30E+06	1.20E+05
7	1.40E+05	1.60E+04	1.20E+04
8	9.00E+03	6.00E+05	1.10E+04
9	6.00E+05	1.20E+05	1.30E+05
10	1.10E+04	1.30E+05	1.20E+06
11	1.50E+04	1.60E+05	1.20E+04
12	1.10E+04	9.00E+05	6.00E+05
13	1.10E+06	1.40E+04	7.00E+05
14	1.60E+04	1.50E+06	2.00E+04
15	9.00E+05	7.00E+05	1.30E+05
16	1.20E+04	1.40E+06	1.10E+04
17	1.10E+06	1.20E+04	9.00E+04
18	1.50E+04	1.60E+04	1.20E+06
19	2.00E+06	1.90E+04	1.60E+05
20	1.40E+06	1.60E+04	1.90E+04
21	1.90E+04	1.50E+06	1.60E+05
22	2.70E+04	2.40E+04	1.90E+05
23	9.00E+05	1.20E+05	1.10E+06
24	1.50E+04	1.90E+05	1.10E+04
25	2.00E+06	1.90E+05	1.60E+05
26	1.20E+05	1.10E+05	1.50E+04
27	2.60E+04	2.40E+05	1.10E+04
28	1.40E+04	1.10E+05	1.20E+06
29	1.90E+04	1.60E+05	1.10E+06
30	2.00E+04	2.40E+04	2.60E+05

A17: VALUES FOR SALMONELLA COUNT ON STICKING KNIVES USED

NUMBER	REP 1	REP 2	REP 3
1	1.40E+06	1.20E+07	9.00E+06
2	1.20E+06	1.50E+06	1.70E+06
3	1.30E+06	1.70E+06	1.40E+06
4	1.50E+05	1.30E+07	1.50E+07
5	1.30E+07	1.70E+06	7.00E+06
6	1.00E+05	8.00E+05	6.00E+06
7	1.40E+07	1.70E+05	2.20E+06
8	1.70E+06	1.90E+05	2.10E+06
9	2.50E+07	1.90E+05	2.30E+05
10	2.20E+06	1.60E+05	2.30E+07
11	1.80E+05	2.30E+06	2.10E+05
12	2.30E+06	2.40E+05	2.10E+07
13	2.10E+07	2.30E+06	1.90E+07
14	2.50E+06	2.30E+05	2.10E+07
15	2.10E+06	1.70E+05	2.30E+07
16	1.90E+05	1.50E+07	2.00E+06
17	1.90E+06	2.20E+07	1.60E+07
18	1.70E+07	2.20E+06	1.50E+05
19	1.50E+07	1.80E+06	2.00E+05
20	1.80E+07	2.30E+06	1.60E+05
21	1.90E+06	1.70E+05	1.40E+07
22	2.00E+05	2.10E+06	1.70E+07
23	2.20E+07	1.90E+06	2.40E+05
24	1.70E+06	1.90E+05	1.40E+07
25	1.50E+05	1.80E+06	1.30E+07
26	1.90E+05	1.70E+07	2.00E+06
27	2.00E+05	1.80E+06	1.60E+07
28	2.40E+05	2.20E+06	2.00E+07
29	1.70E+06	1.40E+05	1.90E+07
30	1.50E+05	1.70E+07	2.10E+06

A18: VALUES FOR SALMONELLA COUNT ON THE INNER SURFACE OF THE CARCASSES

NUMBER	REP 1	REP 2	REP 3
1	1.00E+07	1.50E+07	1.80E+06
2	1.20E+05	1.60E+06	1.50E+05
3	1.20E+06	1.50E+05	1.30E+07
4	1.10E+07	1.30E+06	1.50E+07
5	1.40E+06	1.60E+06	1.80E+06
6	1.40E+07	1.70E+05	2.20E+06
7	2.10E+05	2.50E+06	2.30E+05

8	2.50E+06	2.90E+05	2.60E+07
9	2.90E+05	2.70E+06	2.50E+07
10	3.20E+05	2.90E+07	3.40E+06
11	2.90E+06	3.10E+05	3.30E+07
12	3.00E+06	2.70E+05	2.10E+07
13	2.90E+05	3.00E+06	2.60E+05
14	2.80E+05	2.60E+05	2.50E+07
15	2.26E+06	2.80E+06	2.90E+05
16	2.50E+07	2.80E+06	2.70E+05
17	2.00E+06	1.70E+07	1.40E+05
18	2.80E+07	3.00E+06	2.60E+05
19	2.60E+05	2.90E+06	2.70E+05
20	2.60E+05	2.80E+06	3.00E+05
21	2.20E+05	2.50E+07	2.40E+06
22	2.50E+07	2.70E+05	2.60E+05
23	3.50E+05	3.20E+07	3.70E+06
24	2.40E+05	2.20E+06	2.60E+06
25	2.80E+05	2.50E+07	2.70E+06
26	2.50E+07	2.20E+05	2.70E+05
27	2.60E+05	2.90E+05	2.40E+06
28	2.80E+05	2.70E+06	3.00E+07
29	2.60E+05	2.40E+06	2.70E+05
30	2.60E+05	2.40E+07	2.30E+07

A19: VALUES FOR SALMONELLA COUNT ON THE SURFACE OF THE CARCASSES AFTER SKIN REMOVAL

NUMBER	REP 1	REP 2	REP 3
1	5.00E+05	4.00E+05	4.50E+05
2	3.90E+05	4.50E+05	4.80E+05
3	3.70E+05	4.20E+05	4.60E+05
4	2.90E+05	3.10E+05	2.50E+06
5	3.60E+06	4.00E+05	4.20E+06
6	3.00E+07	2.70E+06	3.30E+05
7	3.20E+05	2.80E+05	2.70E+06
8	3.90E+06	4.10E+07	3.70E+05
9	3.50E+05	3.20E+06	2.90E+07
10	4.20E+06	3.70E+05	3.90E+07
11	4.00E+06	3.80E+07	3.60E+05
12	4.10E+07	3.90E+06	4.30E+06
13	3.50E+05	3.90E+05	3.70E+06
14	4.00E+05	3.50E+07	3.40E+06
15	3.30E+06	3.50E+07	3.10E+05
16	3.50E+05	3.80E+05	4.00E+06
17	2.50E+06	2.80E+06	2.90E+05

18	3.10E+05	2.90E+06	3.30E+07
19	4.30E+06	3.90E+07	4.00E+05
20	4.10E+07	3.70E+05	3.90E+09
21	2.90E+05	3.00E+06	3.30E+07
22	3.10E+05	3.60E+07	3.30E+06
23	4.40E+07	4.00E+06	3.80E+05
24	3.50E+07	3.20E+05	3.70E+06
25	3.40E+06	3.80E+05	3.30E+07
26	3.50E+05	2.90E+06	3.40E+07
27	3.80E+07	4.30E+05	4.60E+06
28	4.80E+06	5.00E+05	4.60E+06
29	3.20E+05	3.50E+06	3.80E+05
30	2.90E+05	3.00E+06	3.30E+05

A20: VALUES FOR SALMONELLA COUNT ON THE LAST KNIVES USED FOR TRIMMING OF CARCASSES

NUMBER	REP 1	REP 2	REP 3
1	2.50E+06	2.70E+05	3.20E+05
2	3.00E+06	2.80E+06	2.60E+06
3	3.10E+05	2.70E+05	2.40E+05
4	2.90E+05	3.20E+05	3.50E+05
5	3.00E+05	2.80E+06	2.50E+06
6	2.00E+07	2.40E+05	2.30E+06
7	1.90E+06	2.10E+05	2.20E+07
8	1.80E+07	2.20E+06	2.50E+05
9	1.70E+07	2.10E+06	1.90E+06
10	2.50E+07	2.80E+05	2.40E+06
11	2.50E+05	2.20E+06	2.00E+07
12	1.90E+06	2.00E+05	1.60E+07
13	1.80E+06	2.20E+07	2.40E+07
14	1.80E+07	2.00E+05	2.10E+06
15	3.30E+06	3.50E+07	3.10E+05
16	2.30E+06	1.90E+05	2.10E+06
17	1.30E+06	1.70E+06	1.40E+05
18	1.70E+06	2.10E+05	2.20E+07
19	2.60E+05	2.40E+06	2.20E+07
20	2.60E+05	1.80E+06	1.90E+07
21	2.00E+05	1.70E+06	1.50E+05
22	1.50E+05	1.80E+06	2.20E+07
23	2.00E+07	1.70E+05	1.90E+06
24	2.20E+05	2.50E+05	2.00E+07
25	1.30E+05	1.70E+05	1.60E+06
26	1.80E+07	1.50E+07	2.10E+05
27	1.90E+05	2.20E+06	2.10E+05

28	2.20E+05	2.40E+06	2.30E+07
29	1.30E+06	1.80E+05	2.00E+06
30	2.10E+05	1.70E+06	1.90E+06

APPENDIX B

RAW VALUES FROM THE ATONSU AND MAAYANKA MARKETS

B1: VALUES FOR TOTAL PLATE COUNT ON BENCHES

NUMBER	REP 1	REP 2	REP 3
1	1.30E+15	1.34E+16	1.29E+17
2	1.20E+16	1.18E+15	1.16E+16
3	1.25E+17	1.28E+16	1.23E+16
4	1.00E+17	9.70E+15	1.02E+16
5	1.00E+15	9.70E+15	8.90E+14
6	1.56E+15	1.62E+16	1.70E+17
7	1.09E+17	1.12E+17	1.21E+16
8	1.15E+15	1.20E+16	1.09E+17
9	1.23E+16	1.28E+15	1.30E+17
10	1.36E+16	1.42E+15	1.33E+17
11	1.07E+16	1.19E+17	1.22E+15
12	1.23E+16	1.27E+17	1.35E+15
13	1.42E+16	1.39E+17	1.45E+15
14	1.23E+16	1.18E+17	1.25E+15
15	1.34E+16	1.29E+15	1.40E+15
16	1.38E+15	1.45E+16	1.50E+17
17	1.28E+17	1.10E+16	1.30E+15
18	1.36E+17	1.40E+15	1.29E+16
19	1.36E+16	1.40E+17	1.29E+15
20	1.30E+16	1.34E+17	1.27E+15

B2: VALUES FOR TOTAL PLATE COUNT ON KNIVES USED IN THE MEAT SHOPS

NUMBER	REP 1	REP 2	REP 3
1	1.08E+15	1.10E+16	1.15E+15
2	1.00E+15	9.80E+14	1.04E+16
3	9.80E+14	1.00E+16	9.60E+15
4	1.88E+17	1.90E+16	1.79E+16
5	1.74E+17	1.86E+16	1.92E+16
6	1.82E+15	1.78E+16	1.84E+17
7	1.79E+15	1.86E+16	1.83E+17
8	1.80E+17	1.77E+15	1.83E+16

9	1.74E+15	1.79E+16	1.84E+17
10	1.77E+15	1.85E+16	1.72E+17
11	1.70E+16	1.75E+15	1.69E+17
12	1.81E+17	1.79E+15	1.77E+16
13	1.84E+17	1.76E+16	1.74E+15
14	1.73E+17	1.69E+16	1.71E+15
15	1.82E+16	1.79E+16	1.86E+15
16	1.81E+17	1.75E+15	1.72E+16
17	1.83E+15	1.78E+16	1.69E+17
18	1.78E+16	1.80E+15	1.85E+16
19	1.84E+17	1.78E+16	1.73E+15
20	1.85E+16	1.79E+17	1.82E+15

B3: VALUES FOR TOTAL PLATE COUNT ON MEAT SURFACES AT THE MEAT SHOPS

1.76E+15	1.80E+16	1.84E+17
1.50E+15	1.48E+16	1.46E+15
1.00E+15	1.28E+16	1.39E+17
1.50E+15	1.46E+17	1.44E+16
1.40E+15	1.32E+15	1.28E+16
1.62E+16	1.59E+17	1.55E+15
1.68E+16	1.57E+15	1.64E+15
1.60E+16	1.64E+15	1.59E+17
1.58E+15	1.63E+17	1.54E+16
1.55E+16	1.62E+17	1.59E+15
1.59E+16	1.63E+17	1.69E+15
1.63E+17	1.59E+15	1.70E+16
1.63E+16	1.58E+17	1.60E+15
1.59E+15	1.64E+16	1.66E+17
1.64E+15	1.69E+16	1.73E+17
1.65E+16	1.56E+17	1.68E+15
1.64E+17	1.69E+15	1.73E+16
1.63E+16	1.58E+17	1.68E+16
1.59E+16	1.63E+15	1.65E+16
1.49E+16	1.54E+15	1.60E+17

B4: VALUES FOR TOTAL COLIFORM COUNT ON BENCHES

NUMBER	REP 1	REP 2	REP 3
1	2.40E+08	2.90E+09	2.90E+10
2	4.30E+11	5.30E+10	3.60E+09
3	4.40E+10	3.90E+11	4.20E+09
4	3.90E+10	2.60E+11	2.90E+09
5	2.60E+11	2.40E+10	2.10E+10
6	2.40E+09	2.30E+07	2.10E+08
7	2.90E+09	2.30E+10	2.70E+11

8	3.40E+11	2.90E+09	2.70E+10
9	4.20E+10	3.40E+11	2.90E+09
10	3.40E+11	2.70E+10	2.60E+09
11	2.80E+11	2.90E+09	3.40E+11
12	2.40E+11	2.60E+10	2.30E+09
13	2.40E+10	2.70E+11	2.60E+09
14	2.90E+11	2.70E+11	2.80E+10
15	1.34E+16	1.29E+15	1.40E+15
16	1.38E+15	1.45E+16	1.50E+17
17	1.28E+17	1.10E+16	1.30E+15
18	1.36E+17	1.40E+15	1.29E+16
19	1.36E+16	1.40E+17	1.29E+15
20	1.30E+16	1.34E+17	1.27E+15

B5: VALUES FOR TOTAL COLIFORM COUNT ON KNIVES

NUMBER	REP 1	REP 2	REP 3	REP1 CFU
1	2.90E+10	2.60E+10	2.70E+09	5.52E+08
2	2.70E+11	2.40E+10	2.30E+09	5.14E+09
3	2.80E+10	2.40E+11	2.60E+09	5.33E+08
4	3.40E+11	2.90E+09	2.70E+10	6.48E+09
5	3.50E+11	2.80E+09	2.70E+10	6.67E+09
6	3.90E+09	4.20E+11	3.60E+09	7.43E+07
7	3.90E+10	3.50E+11	2.90E+10	7.43E+08
8	3.50E+10	3.40E+11	2.80E+09	6.67E+08
9	3.90E+11	3.60E+10	2.80E+09	7.43E+09
10	3.50E+11	2.90E+10	3.40E+09	6.67E+09
11	3.40E+11	2.90E+10	2.70E+09	6.48E+09
12	3.90E+10	3.40E+11	2.80E+10	7.43E+08
13	3.40E+10	2.80E+11	2.70E+11	6.48E+08
14	2.80E+11	2.40E+10	2.70E+09	5.33E+09
15	4.20E+11	3.40E+10	2.80E+10	8.00E+09
16	3.90E+11	3.40E+09	2.90E+10	7.43E+09
17	2.90E+11	3.60E+10	2.80E+10	5.52E+09
18	3.40E+11	2.80E+10	2.60E+09	6.48E+09
19	3.90E+11	2.90E+09	2.70E+11	7.43E+09
20	3.40E+11	2.80E+11	2.60E+09	6.48E+09

B6: VALUES FOR TOTAL COLIFORM COUNT MEAT SURFACES AT THE MEAT SHOPS

NUMBER	REP 1	REP 2	REP 3
1	7.50E+09	6.40E+09	4.30E+11
2	2.00E+11	1.90E+11	2.10E+11
3	3.50E+11	2.90E+09	4.20E+10
4	4.30E+11	3.00E+08	4.20E+11
5	4.30E+09	3.90E+11	3.50E+10

6	3.90E+09	4.20E+11	3.60E+09
7	3.90E+10	3.50E+11	2.90E+10
8	3.50E+10	3.40E+11	2.80E+09
9	3.90E+11	3.60E+10	2.80E+09
10	3.50E+11	2.90E+10	3.40E+09
11	3.40E+11	2.90E+10	2.70E+09
12	3.90E+10	3.40E+11	2.80E+10
13	3.40E+10	2.80E+11	2.70E+11
14	3.60E+09	2.90E+11	3.40E+10
15	3.60E+10	2.90E+09	3.40E+09
16	2.80E+11	2.60E+10	2.70E+09
17	2.90E+11	3.40E+09	2.70E+10
18	2.90E+10	2.60E+09	2.40E+11
19	4.20E+09	3.60E+10	2.90E+11
20	3.60E+11	3.90E+09	2.80E+11

B6: VALUES FOR FAECAL COLIFORM COUNT ON BENCHES

NUMBER	REP 1	REP 2	REP 3
1	9.00E+08	7.00E+08	6.00E+08
2	1.50E+07	1.30E+09	1.40E+08
3	1.10E+09	1.60E+07	9.00E+07
4	9.00E+08	7.00E+07	4.00E+08
5	2.60E+07	2.40E+07	2.10E+09
6	2.90E+08	3.50E+07	2.80E+08
7	2.10E+08	2.30E+09	2.70E+07
8	2.30E+07	2.60E+08	2.40E+08
9	2.40E+09	2.30E+08	2.60E+08
10	1.90E+09	2.10E+08	2.00E+07
11	2.30E+08	2.00E+09	2.10E+07
12	2.00E+08	2.10E+07	1.90E+09
13	2.10E+08	2.40E+07	2.00E+09
14	2.60E+07	2.30E+08	2.10E+09
15	2.40E+08	2.30E+07	2.00E+09
16	2.30E+07	2.60E+08	2.10E+07
17	2.30E+09	2.60E+08	2.00E+07
18	2.30E+07	2.40E+08	2.10E+09
19	2.40E+08	2.10E+09	2.40E+07
20	2.00E+08	2.60E+11	2.10E+07

B7: VALUES FOR FAECAL COLIFORM COUNT ON KNIVES

NUMBER	REP 1	REP 2	REP 3
1	1.20E+09	9.00E+08	4.00E+07
2	7.00E+08	9.00E+06	4.00E+08
3	1.10E+08	9.00E+08	3.00E+08

4	2.00E+07	1.40E+09	1.60E+08
5	2.00E+09	1.90E+08	1.50E+08
6	2.40E+09	2.10E+08	2.00E+07
7	2.00E+08	2.30E+07	2.10E+09
8	2.60E+08	2.40E+09	2.30E+09
9	2.40E+08	1.90E+09	2.00E+07
10	1.90E+09	2.00E+08	1.60E+07
11	1.90E+08	2.00E+09	2.40E+07
12	2.10E+09	1.90E+08	1.60E+07
13	1.90E+07	2.10E+08	1.60E+09
14	2.10E+09	2.00E+08	2.30E+07
15	2.60E+08	2.40E+09	2.10E+07
16	2.80E+08	2.40E+09	2.30E+07
17	2.10E+08	2.40E+09	2.30E+09
18	2.40E+09	2.10E+08	2.00E+09
19	2.40E+08	1.90E+07	1.60E+09
20	2.60E+09	1.90E+08	1.60E+08

B8: VALUES FOR FAECAL COLIFORM COUNT ON MEAT SURFACES

NUMBER	REP 1	REP 2	REP 3
1	6.40E+09	4.40E+08	3.50E+07
2	1.90E+07	6.00E+08	7.00E+08
3	1.90E+08	1.40E+09	1.10E+07
4	2.70E+09	2.60E+09	2.40E+07
5	2.70E+07	1.90E+09	1.60E+08
6	2.80E+08	2.40E+09	2.90E+08
7	2.40E+09	2.30E+08	2.10E+07
8	2.40E+08	2.10E+09	2.60E+07
9	2.10E+09	1.50E+08	2.40E+09
10	2.40E+08	1.90E+09	2.10E+08
11	2.00E+08	2.10E+08	1.90E+07
12	2.60E+08	2.30E+07	2.70E+07
13	2.10E+09	1.90E+08	1.60E+09
14	2.70E+08	2.40E+09	2.30E+09
15	2.70E+09	2.40E+07	2.90E+08
16	2.10E+09	1.90E+07	2.00E+08
17	2.10E+08	2.00E+09	1.90E+07
18	2.10E+09	2.00E+09	1.90E+08
19	2.90E+09	2.40E+07	2.10E+08
20	2.80E+09	2.10E+08	1.90E+07

B9: VALUES FOR E.COLI COUNT ON BENCHES

NUMBER	REP 1	REP 2	REP 3
1	1.90E+07	1.60E+05	2.00E+07
2	2.30E+06	2.40E+05	2.10E+05
3	2.70E+05	2.60E+06	2.90E+07

4	2.00E+07	1.90E+05	1.60E+06
5	1.90E+07	1.50E+07	1.20E+05
6	1.50E+05	2.00E+06	2.40E+07
7	1.50E+06	1.30E+07	1.50E+05
8	2.10E+05	2.00E+06	1.60E+07
9	1.40E+07	1.90E+07	1.20E+07
10	1.30E+06	1.60E+05	1.90E+07
11	1.90E+06	1.50E+05	1.30E+05
12	9.00E+06	1.50E+06	1.30E+05
13	1.50E+05	1.90E+06	1.40E+07
14	1.60E+05	1.50E+07	1.20E+06
15	1.90E+06	1.50E+07	1.20E+05
16	1.90E+06	2.00E+06	1.40E+07
17	1.60E+05	1.60E+07	1.20E+06
18	1.60E+05	1.90E+06	1.40E+05
19	1.60E+06	1.30E+06	1.20E+05
20	1.60E+05	1.60E+07	1.10E+06

B10: VALUES FOR E.COLI COUNT ON KNIVES

NUMBER	REP 1	REP 2	REP 3
1	1.50E+07	1.60E+05	1.20E+06
2	1.40E+07	1.60E+05	1.20E+07
3	2.00E+05	1.90E+05	1.60E+06
4	1.30E+07	6.00E+06	7.00E+05
5	9.00E+06	1.10E+06	7.00E+06
6	1.90E+05	1.60E+06	1.40E+07
7	1.90E+05	2.10E+07	1.60E+06
8	1.40E+07	1.90E+06	2.10E+05
9	1.40E+06	1.10E+07	9.00E+06
10	1.50E+06	1.40E+07	2.00E+05
11	1.60E+05	1.40E+06	1.20E+07
12	1.20E+06	1.30E+06	1.10E+05
13	1.50E+05	1.20E+07	1.10E+06
14	1.30E+07	1.10E+07	1.40E+06
15	2.00E+06	1.90E+07	1.50E+05
16	2.00E+06	1.60E+05	1.40E+06
17	1.90E+05	2.50E+07	1.90E+07
18	1.90E+05	1.50E+05	1.30E+06
19	1.50E+05	1.30E+05	1.10E+07
20	1.30E+06	1.20E+07	1.10E+05

B11: VALUES FOR E.COLI COUNT ON MEAT SURFACES

NUMBER	REP 1	REP 2	REP 3
1	1.90E+06	1.60E+05	1.40E+06
2	2.40E+06	1.50E+07	1.60E+07
3	1.30E+06	1.90E+05	1.10E+06
4	1.40E+05	1.10E+05	1.30E+06

5	1.50E+06	1.10E+05	6.00E+06
6	1.50E+05	1.90E+05	1.60E+07
7	2.00E+06	1.50E+07	1.90E+07
8	1.60E+05	1.90E+06	1.30E+07
9	1.60E+06	1.90E+07	1.40E+05
10	1.30E+07	1.50E+06	1.20E+05
11	1.50E+05	1.30E+05	1.10E+07
12	1.90E+05	1.60E+06	1.30E+05
13	1.30E+06	1.50E+05	1.20E+07
14	2.10E+06	1.90E+07	1.40E+06
15	2.40E+05	2.00E+07	2.30E+06
16	1.10E+06	9.00E+04	1.30E+07
17	1.50E+06	1.40E+07	1.30E+05
18	1.40E+06	1.10E+07	1.30E+07
19	1.90E+06	1.40E+07	1.20E+05
20	1.60E+07	9.00E+05	1.10E+05

B9: VALUES FOR SALMONELLA ON BENCHES

NUMBER	REP 1	REP 2	REP 3
1	1.10E+06	9.00E+06	1.30E+08
2	1.40E+08	1.20E+07	9.00E+07
3	9.00E+07	1.50E+07	1.20E+08
4	1.50E+06	1.20E+07	1.10E+08
5	1.00E+08	1.80E+06	2.70E+07
6	1.80E+07	2.20E+06	3.00E+08
7	2.00E+07	1.80E+08	2.30E+06
8	1.20E+08	1.70E+07	2.00E+06
9	1.20E+08	9.00E+06	1.40E+06
10	2.00E+08	1.80E+07	1.60E+06
11	1.10E+06	1.70E+07	1.40E+06
12	2.20E+08	2.60E+06	1.80E+08
13	3.00E+07	2.50E+08	1.70E+06
14	2.40E+08	2.50E+06	2.10E+08
15	1.70E+08	2.20E+07	2.50E+08
16	2.50E+07	1.70E+08	2.60E+06
17	2.40E+08	2.60E+08	2.80E+06
18	33E+	2.80E+08	2.20E+08
19	3.00E+07	2.50E+08	2.90E+06
20	2.60E+06	1.90E+08	2.00E+06

B10: VALUES FOR SALMONELLA ON KNIVES

NUMBER	REP 1	REP 2	REP 3
1	6.00E+07	1.00E+08	1.20E+06
2	1.00E+06	1.30E+07	7.00E+07
3	7.00E+07	1.00E+07	6.00E+05
4	7.00E+07	1.20E+06	1.10E+07
5	8.00E+07	1.20E+07	1.00E+08
6	1.20E+08	2.50E+07	3.00E+06

7	2.00E+06	2.20E+08	1.80E+07
8	3.00E+06	2.40E+08	1.80E+06
9	1.80E+08	2.50E+06	2.30E+06
10	2.00E+06	1.70E+08	1.50E+07
11	1.90E+08	2.10E+07	2.30E+06
12	2.20E+08	2.60E+06	1.50E+06
13	2.20E+08	1.70E+07	2.30E+06
14	1.80E+08	2.30E+07	2.50E+06
15	3.10E+08	2.50E+07	2.30E+07
16	2.70E+08	2.40E+07	1.70E+06
17	3.00E+08	2.40E+06	2.20E+08
18	2.80E+07	2.50E+08	3.00E+06
19	2.50E+08	3.00E+07	2.40E+06
20	1.80E+08	2.40E+06	2.80E+07

B11: VALUES FOR SALMONELLA ON MEAT SURFACE

NUMBER	REP 1	REP 2	REP 3
1	2.50E+08	2.80E+06	2.40E+07
2	2.00E+06	1.90E+07	2.10E+06
3	1.70E+06	2.20E+06	2.00E+07
4	1.50E+08	1.20E+07	9.00E+05
5	1.00E+06	1.80E+07	2.70E+06
6	1.70E+06	1.40E+07	2.20E+06
7	1.00E+08	1.70E+07	2.50E+06
8	2.70E+08	2.30E+08	2.40E+06
9	1.10E+08	1.40E+07	1.70E+06
10	2.10E+08	1.60E+07	1.80E+06
11	2.00E+08	1.70E+07	1.50E+06
12	2.30E+08	1.70E+06	2.10E+07
13	3.00E+08	2.60E+06	2.30E+07
14	2.40E+06	2.80E+06	2.20E+08
15	2.90E+06	2.50E+08	3.30E+07
16	1.80E+07	2.20E+08	2.70E+06
17	2.30E+08	2.50E+06	2.70E+08
18	2.60E+06	2.80E+08	3.10E+07
19	2.80E+06	2.10E+08	2.40E+07
20	3.00E+07	2.40E+06	2.10E+08

APPENDIX C

CALCULATIONS

C1: CONVERSION OF RAW VALUES INTO VALUES OF CFU/cm^2

$$\text{COUNT CFU/cm}^2 = \frac{\text{recorded count x volume of diluent x dilution factor}}{\text{surface area swabbed}}$$

C2: C1: CONVERSION OF RAW VALUES INTO VALUES OF MPN/cm^2

$$\text{MPN/cm}^2 = \frac{\text{recorded MPN x volume of diluent x dilution factor}}{\text{surface area swabbed}}$$

C3: CONVERSION OF CFU/cm^2 OR MPN/cm^2 TO LOG_{10} VALUES BY EXCEL FORMULA OR CALCULATOR

LOG_{10} CFU/cm^2 = LOG_{10}(CFU/cm^2 VALUE)

LOG_{10} MPN/cm^2 = LOG_{10}(MPN/cm^{2}VALUE)