UNIVERSITY OF EDUCATION, WINNEBA

COLLEGE OF TECHNOLOGY EDUCATION, KUMASI

MICROBEAL CONTENT OF MEANT AND POULTRY PRODUCT SOLD AT KUMASI CENTRAL MARKET

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A Dissertation in the DEPARTMENT OF TECHNOLOGY EDUCATION, Faculty of VOCATIONAL AND TECHNICAL EDUCATION, submitted to the Graduate Studies, University of Education Winneba, in partial fulfilment of the requirement for the award of Master of Technology in (Catering and Hospitality Education) degree

AUGUST, 2016

DECLARATION

CANDIDATE'S DECLARATION

I, **SALAM NAFISATU** hereby declare that this dissertation is the result of my own original work and that no part of it has been presented for another degree in this University or elsewhere.

SIGNATURE:……

DATE:……..……

SUPERVISOR'S DECLARATION

I hereby declare that the preparation and presentation of this dissertation were supervised in accordance with the guidelines on supervision of dissertation laid down by the University of Education, Winneba

SUPPERVISOR'S NAME: **DR. GILBERT OWIAH SAMPSON**

SIGNATURE:……

DATE:……..……

ACKNOWLEDGEMENT

First of all, I would like to express my heart feeling thanks to almighty Allah without His assistance nothing would be have happened.

I would like to express my gratitude to my major advisor and supervisor for Dr. Gilbert Sampson for his resourceful comments and suggestion throughout the research period and throughout the research period and at the time of write-up process of this thesis. My heart-felt thanks is also forwarded to my co-advisor Yaw Aviamu of Faculty of Agriculture Library, K.N.U.S.T, for his valuable comments at the time of the research as well as during thesis write-up process.

My special thank goes to Chris Asare of Clinical laboratory, K.N.U.S.T for his help and guidance during the lab work and the analysis of the results and also during editing of the whole draft of the paper. Above all, I am grateful to my friends and colleagues who have given me encouragement.

DEDICATION

I dedicate this work to my husband; Mr. Malik Boigu of K.N.U.S.T, Faculty of Agric who encouraged me all the way and made sure that I give it all it takes to finish that which I have stated.

I also dedicate this work to my children; Sarah (Mama) and Ridwan (Mo) who have been affected in every way possible by the quest. Thank you. My love for you can never be quantified and to my parents and siblings, I say thank you for your support.

May Allah bless you all.

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ABSTRACT

Meat is an integral aspect of human diet from ancient times and has seen variety and improvement over the years through the advancement in technology and industrialization. In developing countries such as Ghana, meat quality particularly raw meat from abattoirs and markets has been a matter of health concern due to the poor hygienic practices persisting at these sites. This study set out to investigate the microbial quality of the meat sold on the Central market of the Kumasi Metropolis in the Ashanti region of Ghana. The sampling comprised four meat; chevon, beef, chicken and offals which were taken from abattoirs and sale points mainly streets within and around the Central market and analyzed at the microbiology Laboratory of the Department of Biochemistry, Kwame Nkrumah University of Science and Technology, KNUST. The analysis comprised the total aerobic count, total Coliform count, *Escherichia coli*, *Staphylococcus aureus* and *Salmonella* using the ISO protocols. The results obtained from the study indicated the meat to available on the markets to the generally not safe and of poor microbial quality. The total aerobic count indicated the presence of some aerobic microorganisms in appreciable loads; Chicken recording the least aerobic contamination of averagely 3.57×10^5 cfu/g with cow and goat meat recording 2.2×10^6 cfu/g and 2.1×10^7 cfu/g, respectively. Offals recorded a mean load of 9.49×10^6 cfu/g. All the meat samples recorded the presence of Coliforms in levels exceeding the threshold ranging from 5.0×10^2 cfu/g to 1.67×10^6 cfu/g. Cow recorded the least Coliform load of 5.1×10^3cfu/g whereas chicken recorded 1.94×10^4 cfu/g with goat and offals recording 2.6×10^5 cfu/g and 3.8×10^5 cfu/g respectively. Though undesirable, the outcome of this study indicated the presence of *Escherichia coli* in all the samples. Cow recorded the highest *E.coli*

contamination with a mean level of 2.38×10^5cfu/g with offals, goat and chicken recording levels of 1.97×10^5 cfu/g, 7.34×10^4 cfu/g and 1.47×10^4 cfu/g respectively. The various microorganisms were detected in the meat samples taken from the Central market of the Kumasi metropolis; *Escherichia coli*, *Enterococci* spp., *Staphylococcus* spp and some Coliforms and aerobic microorganisms that could not be generically identified.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of Study

One excellent source of protein in our diet is meat (Ayhan, 2000). It has long been known for its high nutrients composition hence consumed by many people worldwide (Kim & Dave, 2009, Erol, 2007). The protein profile of meat has been described as superior to the presence of all the essential amino acids and vitamins required by the body (Bryan & Doyle, 2004). The protein and vitamins especially vitamin A and B12 in meat is not available in plant sources (Wabeck, 2002). The increased demand for meat is as a result of higher disposable income and the human desire for a greater variety in their diets (Erkmen, 2010).

In Ghana for instance about 60,000 people are believed to sell an estimated $100 million worth of food annually and these individuals rely on butchers for their supply of beef (Sackey & Zdolec, 2001). In general, the consumers judge meat quality from its appearance, texture, juiciness, water holding capacity, firmness, tenderness, odor and flavor. According to Cross *et al.* (1986), those meat features are among the most important and perceptible that influences the initial and final quality judgment by consumers. Furthermore, the quality of poultry meat gathers quantifiable properties of meat such as water holding capacity, shear force, drip loss, cooking loss, pH, shelf life, collagen content, protein solubility, cohesiveness, and fat binding capacity, which are indispensable for processors involved in the manufacture of value-added meat products (Allen *et al*., 1998).

Adu-Gyamfi, Nketsiah-Tabiri & Boating, (2009), asserted that this has resulted into a drastic fall in the consumption of chicken products in recent times as against a sharp increase in demand for beef consumption. Meat market makes an important contribution to the well-being of people but this is not without its health hazards (Farkas, 2006).

There is considerably high food related infections such as diarrhea, typhoid fever and cholera recorded in hospitals and clinics worldwide. In the past, people have expressed worry about the role of meat and meat products in food poisoning but available records show that more than 74% of cases of food poisoning worldwide are due to meat dishes (Kozacinski & Zdolec, 2006). Meat is highly prone to microbial contamination due to its rich source of nutrients which provide a suitable environment for growth of microbes (Steinkraus, 1994). The microbial growth can lead to meat spoilage and food borne infections in human resulting in economic losses (Adu-Gyamfi, et al., 2009). Contamination of meat and poultry with foodborne pathogens remains an important health issues, because it can lead to illness if there are malprachers in handling, cooking, or post-cooking storage of the products. In Ghana, foodborne illness causes human suffering and loss of productivity and adds significantly to cost of food production and health care (Bircan & Barringer, 2002).

The widespread distribution of raw meat and meat products which are potential vehicle for transmitting food borne diseases makes the consequences of meat contamination more serious. Therefore, there is the need for increased implementation of Hazard Analysis of Critical Control Point (HACCP) and consumer food safety education efforts. HACCP

refers to any actions and activities that can be undertaken to prevent or eliminate food safety hazard or reduce it to an acceptable level by identifying potential risk areas and putting appropriate measures to avoid contamination (ICMSF, 1988). Numerically, the most important biological hazard known to cause these illnesses are *Salmonella* and *Compylobacterspp* (WHO, 2009). Different microbes get introduced at each stage of meat processing after slaughtering, and these tend to contaminate the meat (Andrews &Bäumler, 2005).

Raw beef sold at retail outlets is subjected to a long chain of slaughtering and transportation where each step poses a potential risk of microbial contamination (Teye and Okutu, 2009). Majority of markets where they sell raw beef and kitchen particularly in the Kumasi Central market have no HACCP systems in place and the handling, processing and sale of meat (beef) is done under unhygienic conditions. The state of health of animals prior to slaughtering can also contribute to the microbial quality of meat from such animals. These conditions coupled with the high ambient temperature, high humidity, lack of portable water and poor handling practices expose meat to microbial contamination and rapid deterioration. There is no available literature on the level of contamination of fresh beef and kitchen sold in the Kumasi central market despite generally poor sanitation in the market, and poorly designed slaughtering, processing and transport facilities for handling raw meat.

Modern poultry processing requires high rate of throughput to meet consumer demand, as poultry meat can easily be contaminated with microorganisms, due to many factors, as nutrients, high water activity and neutral pH (Teye & Okumu, 2009). However, healthy

broilers entering slaughter processing might be highly contaminated by microorganisms, including food borne pathogens such as *Salmonella* species, *Campylobacter* species and other bacteria and these pathogens tend to disseminate in the processing plant (Mead *et al.,* 1994). They can be found on the surfaces of feet, feathers, skin and also in the intestines. During processing, a high proportion of these organisms will be removed, but further contamination can occur at any stage of the processing operation (Teye&Okumu, 2009). The procedure for converting a live, healthy bird into a safe and wholesome poultry product provides many opportunities for micro-organisms to colonize on the surface of the carcasses. During the various processing operations, opportunities exist for the contamination of the carcass from the environment, the process in the plant itself, contamination via knives, equipment, the hands of workers and also by cross-contamination from carcass to carcass. Some processing operations increase contaminating micro-organisms or encourage their multiplication (Teye&Okumu, 2009).

As a result, the microbial population changes from mainly Gram-positive rods and micrococci on the outside of the live chicken to Gram-negative micro-organisms on the finished product (Conner, Davis & Zhang, 2001). Efforts should be made to prevent the build-up of contamination peaks during processing. Rinsing of the carcasses, especially during defeathering and evisceration is therefore of great importance (Small &Buncic 2009). Spoilage bacteria grow mainly on the skin surfaces, in the feather follicles and on cut muscle surfaces under the skin.

1.2 Statement of the Problem

In spite of the increased consumer demand on food safety standards for beef in central market, there are still poor hygiene and sanitary practices along the food production chain which contribute to unacceptable level of microbial load in meat. This poses a health risk to consumers. Although several studies have been conducted to assess the degree of meat losses due to contamination of carcasses and offal, detection of zoonotic conditions through post mortem inspection and occurrence of Thermophilic *Compylobacter*spp in meat. In other parts of the world, there is dearth of knowledge on the microbial profile of meat along the production chain from the abattoir to Kumasi central market.

Extremely perishable meat provides favourable growth condition for various microorganisms. Meat is also very much susceptible to spoilage due to chemical and enzymatic activities. The breakdown of fat, proteinand carbohydrates of meat results in the development of off-odours, off-flavour and slim formation which make the meat objectionable for human consumption (Dave & Ghaly, 2011). This can result to loss of revenue by shop owners due to spoilage of meat. It is therefore, necessary to control meat spoilage in order to prevent the causes of sickness and cholera out breaks in human settlement.

1.3. Objective of the Study

The study was carried out to determine the microbial profile and associated risk factors in beef and poultry production chain from abattoir to retail meat outlets in Kumasi central market.

1.4 Specific Objectives of the Study

This study was therefore undertaken to specifically:

1. Determine the microbial load of poultry and meat in Kumasi Central Market
2. Characterize the type of microbes found on poultry and meat sold in Kumasi Central Market
3. To identify risk factors that contribute to microbial contamination of beef and chicken in Kumasi Central market

1.6. Significance of the Study

Slaughterhouses in Ghana are behind achieving full implementation of HACCP systems and in an environment soaked with filth and insanitary conditions, microbial contamination is inevitable. In order to improve on hygienic conditions in slaughterhouses and enhance food safety, it is important to assess current hygienic practices of butchers and the microbial load of the meat (beef) they sell to the public. The study was important because Kumasi central market appeared to be a fast growing market in terms of population and economic activities due to the operations of Metropolis which has brought its attendant influx of people and their negative impacts on the environment. The result of the study would be beneficial in sense that it would help propose recommendations that when implemented could help reduce the potential risk of food borne intoxications in the metropolis.

1.7. Organization of the Study

The study is divided into five chapters. Chapter one deals with the introduction, which gives a background of the study. It also highlights on the objectives, research questions, hypotheses significance of the study and organization of the study. Chapter two covers the review of relevant literature to the study. The methodology used to undertake the project is also described in chapter three. Detail results and discussions of all the study components are presented in chapter four. The conclusions and recommendations from the results have been presented in chapter five.

CHAPTER TWO

2.0 REVIEW OF RELATED LITERATURE

2.1 Introduction

In this chapter, the researcher review literature relevant to the study. That is the researcher look at the relevant literature under the following headings:

- Indicator Organisms on Meat
- Common Microbial Present in Meat and Meat Products
- Bacterial pathogens associated with food poisoning
- Staphylococcus aureus
- *Salmonella* spp.
- *Escherichia coli* serotypes
- Meat as Food
- Beef as food
- Meat Consumption and Related Health Issues
- Meat Quality
- Microorganisms Found in Meat
- Meat Bacteria of Health Concern
- Poultry Meat
- Contamination during handling and processing

2.2. Indicator Organisms on Meat

The safety of raw meat products can be estimated based on indicator organism including TVC, TCC and TFC counts of mesophilic (Barros *et al*., 2007). Their presence indicates the possibility of finding pathogenic bacteria. TVC gives a quantitative idea about the presence of microorganisms such as bacteria, yeast and mould in samples. The coliform bacteria group consists of several genera of bacteria within the family *Enterobacteriaceae*. Total coliforms are a group of bacteria that are widespread in nature. All members of the total coliforms group can occur in human faeces, but some can also be present in animal manure, soil, sub-merged wood and in other places outside the human body. The usefulness of total coliforms as an indicator of faecal contamination depends on the extent to which the bacteria species found are faecal and human in origin. Faecal coliforms are good indicator of contamination from human or other animal waste products and they indicate greater risk of exposure to pathogenic organisms than total coliforms (Moore and Griffith, 2002). Control measures that reduce the number of bacterial load will reduce the risk of pathogenic bacteria on meat.

2.3. Common Microbial Present in Meat and Meat Products

Microorganisms of relevance with regard to meat hygiene include helminths, moulds, bacteria and viruses. Within these groups, bacteria play the most important role. Parasites are of insignificant value in meat which has passed meat inspection, or where efficient internal parasite control programmes or measure are in place. The most frequently identified bacterial pathogen associated with consumption of beef products are *Salmonella* spp, *Compylobacter*spp, *Staphylococcus aureus, Escherichia coli, Listeria monocytogenes, Clostridium perfringens, Yersinia enterocolitica*, *Bacillus cereus and*

Vibrio parahaemolyticus (Biswas e*t al.,* 2011). *Compylobacter*spp, *Salmonella* spp and *Escherichia coli* are often present in fresh meat and poultry (Zhao *et al.,* 2001). Ali *et al.* (2010) reported the food borne pathogens isolated from meat samples in retail meat shops. They included *Escherichia coli* O157:H7, *Listeria* spp, *Salmonella enteritidis*and *Shigella*species while in meat handling equipments in retail shops were *Staphylococcus* and *Shigella*spp. Soyiri*et al.* (2008) isolated *Staphylococcus aureus, Bacillus cereus, Clostridium perfringens*and *Escherichia coli* in beef samples from butchers. Moreover, the faecal coliforms such as *Escherichia coli* are generally considered as indisputable indicators of faecal contamination from warm blooded animals (Yousuf *et al*., 2008).

2.4 Bacterial Pathogens Associated with Food Poisoning

2.4.1 Staphylococcus Aureus

Staphylococcus aureus*is* a normal flora in human and animals, their presence in foods are indications of excessive human handling (Clarence *et al.,* 2009). Staphylococcus aureusis a Gram positive coccus, resistant to heat, drying and radiation. Its strains can be pathogenic and relatively non pathogenic. They produce disease when the bacteria contaminate food. They produce some enzymes which are implicated in staphylococcal invasiveness and many extracellular substances some of which are heat stable enterotoxins that render the foods dangerous even though it appears normal. Once the bacteria have produced toxin, the food can be extensively and properly cooked, killing the bacteria without destroying the toxin. Many of their toxins are gene–based that is carried on plasmids. The intensity of the signs and symptoms may vary with the amount of contaminated food ingested and susceptibility of the individuals to the toxin. Some

signs and symptoms of staphylococcal food poisoning include: Nausea, vomiting, abdominal cramp, prostration and diarrhea.

Since *Staphylococcus aureus* can colonize on various sites of food animals asymptomatically, such as pig or cow, these animals may serve as reservoir and/or a transmission vehicle of spreading *S. aureus* and Multidrug Resistant *Staphylococcus aureus* (MRSA). Food products derived from the animals may be contaminated with *S. aureus* or MRSA during slaughtering and processing. MRSA has been isolated from meat or dairy products in several countries including Netherlands, Italy, Australia, Japan and United States (Buxton & Fraser, 2007).

2.4.2 *Salmonella* spp.

Salmonella species such as *Salmonella typhi*is a bacterium that causes typhoid fever (enteric fever), an acute, life-threatening febrile illness (APHA, 1984). The disease is a cause for concern and a major public health problem in developing countries (Asia, Africa); especially in Kenya due to poor sanitary conditions and lack of or inadequate potable water. It is mainly transmitted through food, drink, or water, contaminated with urine or faeces of infected people or a chronic carrier. Since 1987, *Salmonella enteritidis* has been one of the most frequently isolated salmonellae associated with food borne outbreaks, which have been linked to consumption of chickens, eggs, and foods that contain eggs and it presents an interesting challenge from an epidemiologic perspective (Zhang and Conner, 2001). Infections with nontyphoidal *Salmonella* have increased during the last 3–4 decades, and although a decrease has been reported over the last

decade, *Salmonella* infections continue to be a major public health concern in many countries. These salmonellae are zoonotic, and the infections are generally food borne (Forbes and Weissfield, 2002).

The main reservoir of zoonotic *Salmonella* is food animals, and the main sources of infections in industrialized countries are animal-derived products, notably fresh meat products and eggs (Beach, Murano and Acuff, 2002). Rapid spread of a limited number of successful *Salmonella* clones in different sectors of food animal production (swine, broiler chickens, and particularly layer hens) has been suggested as the most important cause of this increase. Salmonellosis may occur in small, contained outbreaks in the general population or in the large outbreaks in hospitals, restaurants, or institutions for the children or the elderly (APHA, 1984).

2.4.3 Escherichia coli serotypes

Certain types of *Escherichia coli* can cause food borne illness (APHA,1984). *Escherichia coli* O157: H7 outbreaks due to plants and animal produce have become increasingly common (ASNS, 2003). While half of produce associated outbreaks were due to kitchen-level cross-contamination, which calls for further prevention efforts targeting food preparers, the other half were due to produce already contaminated with *Escherichia coli* O157: H7 before purchase (Gauri, 2006). *Escherichia coli,* which are normal flora of the human and animal intestine, have been identified as a leading cause of food borne illness all over the world. *Escherichia coli* and *Escherichia coli* 0157: H7 strain has previously been isolated from meat samples (Hussein, 2007). However, diarrhea caused by

enterotoxigenic *Escherichia coli* (ETEC) is highly prevalent in young children in developing countries as well as in travelers. It spreads through contaminated water and food (Fayad and Naji,2009). The potentially high mortality associated with *Escherichia coli* and *Escherichia coli* 0157: H7 strain infection, therefore make its presence in any food material worrisome and of serious public health sconcern as most of the outbreaks recorded has been traced to consumption of beef contaminated with the *Escherichia coli* 0157:H7 strain (Gauri, 2006).

In spite of the wide knowledge of the organism and its interaction, there seem to be no report on the prevalence of the organism in Africa and particularly Kenya. An *E. coli*out break infection in the United States of America in 1997 resulted in the recall of 11 million kilograms of ground beef (APHA, (2002). Most incidents of food-borne diseases are due to the *E. coli* bacteria (Soyiri, Agbogli, & Dongdem, 2008).

2.5. Meat as Food

Meat is flesh of animal that is eaten as food (Lawrie and Ledward, 2006). Most often meat refers to skeletal muscle and associated fat and other tissues, but it may also describe other edible tissues such as offals (i.e. meat other than meat flesh, including brain, heart, kidney, liver, pancreas, spleen, thymus, tongue and tripe) (Lawrie and Ledward, 2006). Conversely, meat is sometimes used in a more restrictive sense to refer to the flesh of mammalian species (pigs, cattle, lambs, etc.) raised and prepared for human consumption, to the exclusion of fish and other seafood. Humans have hunted and killed animals for meat since prehistoric times. The advent of civilization allowed the

domestication of animals such as chickens, sheep, pigs and cattle, and eventually their use in meat production on an industrial scale (Robert *et al.,* 2000). Meat is produced by killing an animal and cutting flesh out of it. These procedures are called slaughter and butchery respectively. There is ongoing research into producing meat in -vitro that is, outside of animals (McArdle, 2000).

Meat is composed mainly of water and protein, and is usually eaten together with other food. Though it can be eaten raw, it is normally eaten after it has been cooked and seasoned or processed in a variety of ways. Unprocessed meat will spoil within hours or days. Spoilage is caused by the practically unavoidable infection and subsequent decomposition of meat by bacteria and fungi, which are borne by the animal itself, by the people handling the meat, and by their implements (Tutenel *et al.,* 2003). Meat can be broadly classified as "red" or "white" depending on the concentration of myoglobin in muscle fibre. When myoglobin is exposed to oxygen, reddish oxymyoglobin develops, making myoglobin-rich meat appear red. The redness of meat depends on species, animal age, and fibre type. Red meat contains more narrow muscle fibres that tend to operate over long periods without rest, while white meat contains more broad fibres that tend to work in short fast bursts. The meat of adult mammals such as cows, sheep, goats and horses is generally considered red, while chicken and turkey breast meat is generally considered white (Lawrie and Ledward, 2006). The nutritional composition of red meats changes depending on breed, feeding, season and meat cut. However lean red meat shows consistency in high protein content, essential vitamins and minerals, relatively low fat content and moderate in cholesterol (Williams, 2007). Meat is a complete protein food

with all the essential amino acids needed for the human body. It is digested slowly, largely because of the presence of fats. Meat consumption varies worldwide, depending on cultural or religion preferences, as well as economic conditions. Vegetarians choose not to eat meat because of ethical, economic, environmental, and religious or health concerns that are associated with meat production and consumption (Sofos, 2008).

2.6. Beef as food

Beef is the meat from bovines, especially cattle (*Bosprimigenius*). Beef can be obtained from cows (adult female cattle), bulls (adult male cattle), heifers (young sexually matured but unmated female cattle) or steers (castrated male cattle). Beef muscle meat can be cut into steaks, roasts or short ribs or can be processed into corned beef and trimmings, minced or used in sausages. The tail, testicles, tongue and the internal organs such as liver, stomach, pancreas brain, heart, and intestines are other parts that are eaten. Beef harvested from steers have more muscle and less fat than that of heifers. Often older cattle with tougher meat are the ones used for beef when they have past their reproductive prime (Raloff, 2003). Twenty-five percent (25%) of meat produced worldwide is beef and it is the third most widely consumed meat in the world after pork and poultry at 38% and 30% respectively (Raloff, 2003). The United States, Brazil, and China are the world's three largest consumers of beef (USDA, 2009). The world's largest exporters of beef are Brazil, India, Australia and the United States in that order (USDA, 2009).

2.7. Meat Consumption and Related Health Issues

The intake of meat varies widely throughout the world (Speedy, 2003). Available records indicate that overall meat consumption is on the rise in the developed nations of the world and that the U.S. remains the highest consumer of total meat (FAO, 2003). Carrie *et al*. (2011) reported that red meat still represents the largest proportion of meat consumed in the U.S. despite a shift toward increased poultry consumption. They further indicated that only a quarter of the meat consumed in U.S. is processed. On per capita basis, the U.S. is the leading meat consumer in the world with 124kg/capita/year higher than the global average of 38kg/capita/year. Africa and South Asia are the least consumers of meat. Their consumption is between 3 and 5 kg/capita/year (Speedy, 2003). The consumption of meat in Ghana is 9.2 kg/capita/year and this is supplemented by a relatively higher intake of fish (26.2 kg/capita/year) (FAO, 2003). On daily basis in the U.S. and other developed countries, meat takes a significant proportion of the normal diet contributing more than 15% energy, 40% protein, and 20% fat (FAO, 2003). The demand for meat in developing countries continues to grow as the production and consumption of meat increases with available income (Speedy, 2003). There appears to be an emerging trend in dietary requirements where meat has taken the place of cereals and other foods of plant origin though meat selection and consumption vary by education, race, age, and gender (Krebs-Smith, 1998).

Meat in the diet provides an important source of protein and micronutrients such as iron, zinc, and vitamins (Stipanuk, 1999). However, high intake of meat, fats and sugars in diets coupling with sedentary lifestyle have been implicated in the high rate of obesity and diet-related chronic diseases in the world (Mente *et al.*, 2009). There is direct

correlation between high meat consumption and high rates of chronic diseases including cardiovascular disease (CVD) and cancer. Cardiovascular diseases (diseases of the heart) are the current leading causes of morbidity and mortality in the U.S. and other westernized countries (WHO, 2009). According to a report by Cross *et al.* (2007), health risks associated with meat consumption may vary depending on the animal the meat is derived from as well as rearing, processing, and preparation methods. Meat cooking and processing techniques such as smoking, curing, salting or addition of chemical preservatives lead to the formation of carcinogenic compounds, such as *N*-nitroso compounds (NOCs), heterocyclic amines (HCAs) and polycyclic aromatic hydrocarbons (PAHs) (Cross *et al*., 2007). The fat content in red meat and dietary cholesterol has been closely linked to chronic diseases (Lichtenstein *et al.,* 2006). A large body of evidence suggests that vegetarians may be at lower risk for CVD, hypertension, diabetes mellitus, obesity, and cancer (Fraser, 2009). In that case, meat should be eaten in moderation and without too much attendant fat so that it can make a valuable contribution to body development and function (Callow, 2009).

2.8. Meat Quality

The term “meat quality” is used to describe a range of attributes of meat. Many factors determine the quality in meat. It includes requirements of food safety and animal welfare. It also includes the sensory appeal of meat such as palatability (visual appearance, smell, firmness, juiciness, tenderness, and flavor) and perceived healthiness, especially in relation to the amount and type of fat and other fatty components (Aberle *et al.,* 2001). Quality of meat describes how attractive the meat is to consumers. Meat must look good

to consumers before satisfying their palate when they decide to buy it. The expectations of the consumer in terms of aroma, tenderness, juiciness, flavor, colour, wholesomeness and nutrition must be met once the meat is bought, cooked, and served (FAO, 2012). Flavour is interwoven with aroma to bring out the sensation the consumer has during eating. Flavour and aroma are perceptions and depend on the ability to smell through the nose and on the sensations of salty, sweet, sour and bitter on the tongue. Meat flavor is affected by type of species, diet, cooking method and method of preservation (e.g. smoked or cured) (FAO, 2012). The source of flavor in meat is the fat. The different flavors among different kind of meat (beef, pork, chicken, turkey, mutton and chevron) come from fatty components. Fat acts as one of precursors of flavor by combining with amino acids from proteins and other components when heated. The aroma and juiciness of meat products can be improved using spices and cooking method (Dinh, T. N. T, 2006).

The tenderness depends on textural characteristics, composition of meat, breeds, sex and many other factors. Tenderness of meat is also based on ease of chewing, which is contributed by the fibrous nature of muscle (Gerrard and Grant, 2003). The appearance of meat is the visual meat quality which is based on colour, marbling and water holding capacity. Marbling is small streaks of fat that are found within the muscle and can be seen in the meat cut. Marbling has a beneficial effect on juiciness and flavour of meat. Colour of meat should be normal and uniform when cut through. Another aspect of meat quality is smell. This will differ slightly based on species and breeds. Meat product should have a normal smell without any rancid or strange smelling odour (FAO, 2012).

2.9 Microorganisms Found in Meat

Microorganisms are minute living creatures found everywhere in nature and in human environments, including our meat supply. They are too small to be seen with the naked eye unless microscope. Microorganisms include bacteria, yeasts, molds and viruses. Some microorganisms are useful for the production of specialty meat products, while others are pathogenic which means they have the ability to cause meat spoilage leading to food borne illness (Abaidoo and Obiri-Danso, 2008). Therefore meat should be stored in the coldest part of refrigerator or be stored frozen to prevent contamination by microorganisms. Good hygienic practices are extremely important to prevent microbial contamination in meat and other foods in addition to proper handling, cooking and cooling practices (Doyle, 2007).

2.10 Meat Bacteria of Health Concern

The presence of pathogens in our environment is life threatening and poses serious potential health hazards due to their wide range of diversity and complexity. The ability of some of them to survive and or proliferate under refrigeration and in reduced oxygen concentration and for some pathogens, their low numbers do not debar them from causing diseases (IFT, 2004). The way and manner in which farm animals are reared (husbandry practices), slaughtered, processed and transported to the market influence greatly the microbiological condition of carcass meat. When meat is not properly handled, processed and preserved can support growth of a wide range of microorganisms due to its high nutrients content. Contact between hide and carcass allows a multitude of microorganisms to be introduced into the carcass. These contaminating microorganisms

are derived from the animal's pre-slaughter environment and may be of faecal, soil, water or feed origin (Bell, 1997). Certainly, high numbers of microorganisms exist in meat animals intestinal tracts and some of these may find their way to the carcass surfaces during slaughter (Bell, 1997). Table1 illustrates the primary source of these carcass microbes from animal's pre-slaughter environment. Raw meat have been found to contain high numbers of micro-organisms like *salmonella, Clostridium perfringens, staphylococcus aureus, Yersinia enterocolitica, Listeria monocytogenes Campylobacter jejuni and Eschericia coli.* Some of these are pathogenic and are often incriminated in outbreak of food borne disease (Bean *et al.,* 1990). In order to cause a disease, pathogens must successfully invade some parts of the body and either produce more of themselves or produce a toxin which interferes with normal body process (Abaidoo & Obiri-Danso, 2008).

Table 1: Sources of bacteria of health concern in meat

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

Source: Church &Wood (1992)

Growth of bacteria on meat is dependent on the storage temperature, pH, moisture content, oxygen availability and the general handling of the carcass. Low storage temperatures results in a significant decrease in the rate of microbial growth as well as a

reduction in the diversity of the microbial flora. The fairly high moisture content of meat also supports the growth of wide variety of bacteria. The pH of meat which ranges between 5.3 and 6.5 is ideal for microbial proliferation. Several factors such as feeding and handling practices at the time of slaughter affect the pH of meat (NACMCF, 1993). Food borne pathogens contaminate carcasses and causing a major public health problem. Microbial contamination decreases the shelf-life of food and promotes food borne illness. Outbreaks of food-borne diseases have led to considerable illness and even death. It is reported that every year from 24 to 81 million cases of food-borne illness are recorded in USA, out of which 50% are associated with meat and poultry (Unneveher, 2000). Out of ten (10) pathogens tracked by FoodNet (a reporting system used by public health agencies in United States that captures food-borne illness in over 13% of the population), *Salmonella*, *Campylobacter,* and *Shigella*are responsible for most cases of food-borne illness. The estimated number of cases and mortality rate of food-borne illness caused by these pathogens are high with *Salmonella* causing 31% of food related deaths, followed by *Listeria* (28%), *Campylobacter* (5%), and *Escherichia coli* O157:H7 3% (Mead *et al.,* 1999). It is estimated that 13.8 million cases of foodborne illness are due to known agents. Out of these cases roughly 30% are due to bacteria. Bacteria are the causative agents of 60% of foodborne illness requiring hospitalization (Table 2). It is generally accepted in the scientific community that the true incidence of foodborne disease is under reported and that the international impact of foodborne illness is difficult to estimate (Mead *et al.,* 1999). Nevertheless, about 2.1 million children in developing countries die of diarrheal- related illnesses annually. It is suspected that food or water is the vehicle for many of these illnesses (WHO, 2009). Because food is biological in nature and is capable

of supplying consumers with nutrients, it is equally capable of supporting the growth of contaminating microorganisms (IFT, 2004).

Table 2: Foodborne disease in the United States, including estimated annual prevalence

Bacteria	Potential Food Contamination	Number of Illness
Clostridium perfringens	Meat, meat products and gravies.	248,520
Salmonella spp.	Raw meats, poultry, eggs, milk and dairy products, fish, shrimp, yeast, coconut, sauces, salad dressings (i.e., homemade items containing unpasteurized eggs and no or insufficient acidification for destroying pathogens.	1,341,873
Staphylococcus aureus	Meat and meat products, poultry, egg products, salads (chicken, potato, macaroni), cream-filled bakery products, milk and dairy products.	185,060
Shigella spp.	Salads (potato, tuna, chicken, macaroni raw vegetables, bakery products (e.g. in stools, tenesmus cream-filled pastries), sandwich fillings, milk and dairy products, poultry.	89,648
Campylobacter spp.	Raw chicken, beef, pork, shellfish and raw milk	1,963,141

Source: IFT (2004)

2.11 Poultry Meat

Poultry meat products provide animal protein of high biological value for consumers at all ages, where they contain all the essential amino acids required for human growth, higher proportion of unsaturated fatty acids and less in cholesterol value. Moreover, Poultry meat products are highly desirable, palatable, digestible and nutritious for all ages. Further processing of poultry meat involves conversion of raw poultry carcasses into value added products e.g. reconstructed products, cold cuts or breaded products. Advantages of further processing of poultry meat are improving juiciness and flavor, shelf life and water holding capacity (Sahoo, et al. 1996). Unfortunately, such products offer ideal medium as microbial growth for they are highly nutritious, have a favorable pH, and are normally lightly salted or not salted at all (Edel, 2004). Aerobic plate counts in food samples may be useful to indicate quality, shelf life and post heat processing contamination (Gast, 2003) as well as, total bacterial count is considered as an index of quality, which gives an idea about the hygienic measures during processing and helps in assessing the keeping quality of the product (Aberle *et al*., 2001). Food handlers are the primary source of *S.aureus* contamination in the processing plant. Most staphylococcal intoxications involving poultry products are related to recontamination of cooked product by food handlers, followed by improper holding temperature (NACMCF, 1997).

Enterococci recognized as important nosocomial pathogens causing endocarditis, bacteremia, and central nervous system infections as well as neonatal, respiratory tract, urinary tract and other infections (Franz et al., 2011). *Escherichia coli* are an important organism in the food microbiology; besides being involved in food-borne gastroenteritis,

it is considered as a good indicator of possible fecal contamination (Foster, 2002). Their presence in poultry cuts and its products indicates a lack of proper sanitation.

Throughout the world, the production and consumption of chicken has increased. The annual production of chicken meat in Mauritius is 47000 tons (Mead, 2007). The consumption of chicken for the year 2012 was 35.7 Kg per capita (Frazierand Westhoff (2008) compared to 28.06 Kg per capita in the year 2000 (Mead, 2007). Chicken consumption has considerably increased since it represents a major component of the human diet and chicken is an important low cost source of animal protein (Mead, 2007).

Meat is a highly perishable product. If it is not stored, processed, packaged and distributed correctly; it will spoil quickly and become hazardous due to microbial growth (Kabour, 2011). The level of microorganism present in meat products can be reduced only when they are further processed (Rahman, 2008). If spoilage microorganisms such as *Brochothrixthermosphacta* and *Pseudomonas* spp. are present and grow to a high number, the meat will spoil and will be unfit for consumption (Wagner, 2004). Pathogens, such as *Salmonella* spp., *Escherichia coli* and *Staphylococcus aureus* can also grow and cause illness either by multiplication in the human body (food infection), producing toxins (food intoxication) or multiplying and releasing toxins in the body (food toxico-infection). The presence of pathogens in the food supply is considered to be undesirable and they are the major cause of gastrointestinal disease throughout the world (Pichharidt, 2004).

Unhygienic practices prevailing in poultry slaughterhouses and retail outlets can lead to unsafe and low quality chicken product (Naito & Takahara (2006). Pichharidt (2004) noted that poor hygienic practices among poultry meat handlers have often been reported. And emphasized that about 253 kg of poultry were seized and destroyed by the Public Health Officers (PHOs) of the Ministry of Health and Quality of Life of Mauritius.

2.12 Contamination during Handling and Processing

Bacteriological quality of meat products is strongly influenced by the prevailing hygiene condition during their production and handling (Yang and Slavik, (2008). The carcass of a healthy animal slaughtered for meat and held in a refrigerated room is likely to have only minimal surface bacteriological contamination while the inner tissues are sterile. After chilling, further processing of beef carcasses can result in product contamination. When carcasses and cuts are subsequently handled through the food distribution channels where they are reduced to retail cuts, they are subjected to an increasing number of micro-organisms from the cut surfaces (Uyttendaele & Debevere, 2009).

Contamination subsequently occurs by the introduction of micro-organisms on the meat surfaces in operations performed during cutting, processing, storage, and distribution of meat (Turantas, 2001). However, if the meat is kept clean by preventing contamination through dirty hands, clothing, equipment and facilities and the meat is kept cold and covered, there will be little or no contamination by micro-organisms whether bacteria, yeasts, moulds, viruses or protozoa (Swartz, 2002). Fresh meat cutfrom the chilled carcasses has its surface contaminated with micro-organisms characteristic of the environment and the implements used to cut the meat (Uyttendaele & Debevere, 2009).

Employees are the largest contamination source and employees who do not follow sanitary practices, contaminate food that they touch with spoilage and pathogenic micro-organisms. Employees come in contact with these micro-organisms through work and other parts of the environment while their hands, hair, nose and mouth, harbor micro-organisms that can be transferred to food during processing, packaging, preparation and service by touching, breathing, coughing or sneezing (Rheinheimer, 2002). Therefore, in the prevention of meat contamination, personal hygiene plays an important role as there are as many as 200 different species of micro-organisms on a healthy human body (Swartz, 2002).

Carcass contamination not removed by trimming or washing at slaughter is spread to newly exposed surfaces, which in turn can potentially decrease the shelf life of retail cuts and ground beef in retail meat display cases (Pichharidt, 2004; Mead, 2000). The process of chopping and grinding enables bacteria present on the meat surface, to be distributed throughout the product (Goktan, 2000; Ergeldi, 2010). The ultimate shelf life of ground beef depends on the bacterial level of the trimmings, sanitary conditions during processing, time and temperature of processing and storage (Goktan, 2000; Ergeldi, 2010). Ground meat is especially good growth medium because of the extensive surface area provided by the grinding and because these organisms are distributed throughout the product, whereas on the uncut meat the bacteria would be present almost entirely on the outer surfaces (Goktan, 2000; Ergeldi, 2010). Freshly minced meat constitutes one of the most challenging of meat products for quality assurance and public health protection (Banwart, 2009). If retail mince samples show microbiological counts well in excess of

106 per gram it is an indication of poor quality and a potential hazard, which can markedly increase if the mince is held in ambient temperature and for these reasons, the storage of unfrozen minced meat is prohibited in many countries (Goktan, 2000). The storage life of ground beef that contains 1 million bacteria per gram is approximately 28 hours at 15.5 °C. At a normal refrigerated storage temperature of approximately -1 to 3 °C, the storage life exceeds 8 days (Goktan, 2000).

Shelf life is therefore obviously influenced by the initial load of contaminating micro-organisms and there is evidence that poorly cleaned mincing equipment can contribute to a lot of contamination (Dominguez & Zumalacarregui, 2002). Minced meat, unless maintained under refrigerated conditions, rapidly deteriorates. Strict sanitary fabrication practices of beef carcasses can (a) reduce total bacterial counts of beefsteaks, (b) reduce the percentage of typical Gram-negative spoilage bacteria of steaks, and (c) reduce off-odour development of refrigerated vacuum-packaged steaks (Goktan, 2000).

CHAPTER THREE

3.0 METHODOLOGY

3.1. Introduction

This chapter describes the research methodology applied in this study. The discussions in this chapter include; research design, study area, observation and checklist, samples collection, chemical reagents, meat sample preparation, poultry sample preparation, microbiological analysis and statistical analysis.

3.2. The Study Area

This study was carried out in Kumasi Central Market in Kumasi Metropolis, the capital town of the Ashanti Region of Ghana. The Kumasi Central Market (*also known as* Kejetia Market) is an open-air market in the city of Kumasi the capital of Ashanti. Kumasi Central Market is in the Forest region of Ashanti on the Ashanti land Peninsula.

3.3 Samples Collection

Samples of fresh beef and chicken were taken from eight (8) vendors from in the Kumasi central markets. Freshly cut beefsteaks from the fore or hind limb areas were sampled. Eight samples each weighing 100 g were aseptically collected in sterile polythene pouches, sealed and transported on ice to the KNUST Microbiological Laboratory for microbiological analysis within some few hours of collection. This exercise was repeated weekly for three weeks in October 2016. A total of twenty-four (24) fresh samples were used.

3.4 Chemical Reagents

The agars used were products of OXOID Laboratories, Basingstoke Hampshire, England. They included Plate Count Agar used for the isolation of total viable count; Brilliant E.coli agar of *Escherichia coli*; *Brilliant green agar and yassiliads broth* for the isolation of *Salmonella;* Mannitol Salt Agar for isolation of *staphylococcus*.

3.4.1 Preparation of Plate Count Agar

Plate Count Agar (Nutrient agar) was prepared by suspending 23.5 grams in 1000 ml (1 liter) distilled water and heated to boil to dissolve completely. It was sterilized at 121°C for 15 minutes in sealed bottle. The sterilized agar was left to cool at 50°C before pouring into sterile Petri plates.

3.4.2 Preparation *Escherichia.coli*

The presence of *E.coli* in the samples will be confirmed and enumerated on the Brilliance E. coli/Coliform Selective medium. The agar will be prepared according to the directive of the manufacturer (28.1 g in a litre of distilled water and bring to boil no further sterilization is needed). The agar will be brought to cool at 50ºC and poured into sterile agar plates after which sterility check will be conducted for 24 hours. The inoculation will be done by adding a volume of 1 ml of the dilution in triplicate fashion unto the sterile agar plates and spreading uniformly (spread plate). The inoculated plates will be incubated for 24 hours and observation made for E. coli detected by violet colonies and Coliforms characterized by purple colonies. The detected colonies will then be enumerated and recorded.

3.4.3 Preparation of Mannitol Salt Agar

Agar powder (111 g) was suspended in 1 liter of distilled water and brought to boil to dissolve completely. It was sterilized by autoclaving at 121°C for 15 minutes.

3.5 Meat Sample Preparation

Ten grams (10 g) of beef and chicken meat sample were weighed and aseptically taken into a sterile jar containing 90 ml sterile normal diluents. It was homogenized with a pulsifier for 15 seconds and a 1 ml aliquot of homogenate was transferred to a test tube containing 9 ml sterile distilled water to make 10-1 dilution and shaken well with vortex mixer. Serial dilutions up to 10-4 were prepared for the microbiological analysis.

3.6 Microbiological Analysis

The procedures described below were used to test for presence of microorganisms in beef. Colonies on selected plates were counted using a colony counter. The morphological characteristics of colony such as colour, shape and size were examined to facilitate grouping and identification.

3.6.1 Diluent preparation and serial dilution

The diluent used in this study was buffered peptone water from Biolab which was prepared according to the manufacturer's instruction on label. The stock dilution was prepared by dissolving 10 g of sample in 90ml of sterile diluent and shaking for 30 seconds. The subsequent dilutions were prepared by adding 1ml aliquot of the stock solution in 9ml of sterile diluent in succession.

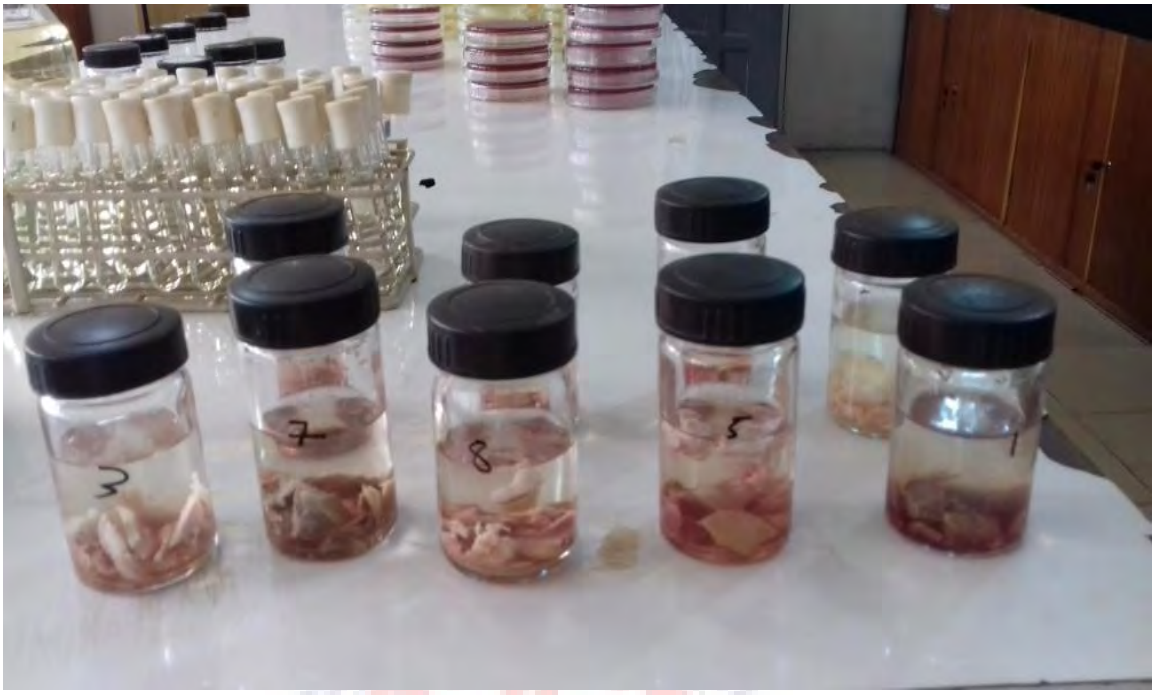

Plate 1: Serial dilution of meat samples for microbial assay

3.6.1 Total Viable Count (TVC)

Total Viable Counts were isolated and enumerated by spread plate method and grown on Plate Count Agar (PCA). Serial dilutions of up to 10-4 were prepared by diluting 10 g of the sample into 90 ml of sterilized distilled water. One milliliter (1ml) aliquots from each of the dilutions were inoculated into Petri dishes with already prepared PCA. The plates were then inverted and incubated at 35 ⁰ C for 24 hours. After incubation all white spot or spread were counted and recorded as total viable count using the colony counter.

3.6.3 Enumeration of Staphylococcus Species

Staphylococcus species were isolated and enumerated by spread plate method and grown on Salt Mannitol Agar (SMA). Serial dilutions of 10-1 to 10-4 were prepared by diluting 10 g of sample into 90 ml of sterilized distilled water. One milliliter aliquots from each of the dilution were inoculated into Petri dishes with already prepared SMA. The inoculum was evenly spread with a sterile bent rod and allowed to dry for 15 minutes at room temperature. The plates were inverted and incubated at 35 ºC for 24 hours. After incubation yellow colonies were counted and recorded as *Staphylococcus* counts using the colony counter.

3.6.4 Enumeration of *Escherichia coli*

The presence of *E.coli* in the samples will be confirmed and enumerated on the BrillianceE.coli/Coliform Selective medium. The agar will be prepared according to the directive of the manufacturer (28.1 g in a litre of distilled water and bring to boil. No further sterilization is needed). The agar will be brought to cool at 50ºC and poured into sterile agar plates after which sterility check will be conducted for 24 hours. The inoculation will be done by adding a volume of 1 ml of the dilution in triplicate fashion unto the sterile agar plates and spreading uniformly (spread plate). The inoculated plates will be incubated for 24 hours and observation made for E.coli detected by violet colonies and Coliforms characterized by purple colonies. The detected colonies will then be enumerated and recorded

3.6.5 Enumeration of *Salmonella/Shigella*

The detection of Salmonella spp. will be carried out in line with the ISO protocol for food microbiology. The media to be used are Brilliant Green agar (BGA) and Xylose Lysine Deoxycholate agar (XLD). Both agars will be prepared according to the manufacturer's instructions (52 g in a litre for BGA and 56.68 g in a litre of distilled water for XLD. Bring to the boil and bring to cool at 50^{o}C. No further sterilization is needed). The agar plates will be prepared and checked for sterility overnight. The test is a qualitative one and progresses through three phases; pre-enrichment, enrichment and inoculation unto agar plates.

The pre-enrichment will be done by inoculating 1 ml of the stock dilution in 9 ml of 10% bacteriological peptone. This will be incubated for 24 hours at 37^{o}C. The next phase will involve pipetting 100 µL of the peptone with sample into 9 ml Rappaport Vassiliads Broth (RVB) followed by another 24 hours incubation period. Phase three will involve adding 10 µL of the RVB unto the BGA and XLD agar plates and the inoculated plates will be incubated for another 24 hours. Observation will then be made for the presence of red colonies with black centres.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSIONS

4.1 Microbial Quality Assessment

The microbial assay conducted on the meat from the various sites showed varying trends across sites and meat type. The general phenomenon observed across all samples however was high microbial contamination which exceeded the safe limits.

4.1 Total Aerobic Count (TAC)

The total aerobic count is needful in establishing the degree of contamination of a sample. The results obtained did indicate the presence of some aerobic microorganisms on the meat sold on the local market at varying amounts across sellers and meat type. Chicken recorded the least aerobic contamination of averagely 3.57×10^5 cfu/g with beef and chevon meat recording 2.2×10^6 cfu/g and 2.1×10^7 cfu/g, respectively. These recorded counts exceed the acceptable or safe limit for readily consumable foods but meat is mostly processed by heat treatments such as roasting, frying, smoking and boiling prior to consumption (Jiménez-Colmenero, *et al*., 2001). It is thus expected that the mode of processing should reduce the population of these aerobic contaminants to acceptable numbers (Dickens, *et al*., 1994) but this might not be the case due to a couple of factors such as not cooking the food for longer period for it to kill the microorganism.

Microbial growth are influenced by mass, time, temperature and surface area (Gillooly, *et al*., 2001), thus if these conditions are not optimized the rate and efficiency of the growth is affected. Though the heat treatments used in the processing of meat have the potential

of making them safe, it is not a matter of absolute certainty as these factors of temperature, time and surface area vary from time to time and place to place as well as some of the measures have the potential of forming spores during processing and return to their vegetative where conditions are favourable.

If the processing of the meat is not effective then a high initial microbial contamination may result in a cooked/processed meat of bad microbial quality thus posing health threats to consumers. This makes the quality of the raw or unprocessed meat of essence and interest.

A careful look at the results in Table 4.1 shows chevon meat recorded the highest contamination and this is evident from the pictorial presentation of the mean results in Figure 4.1

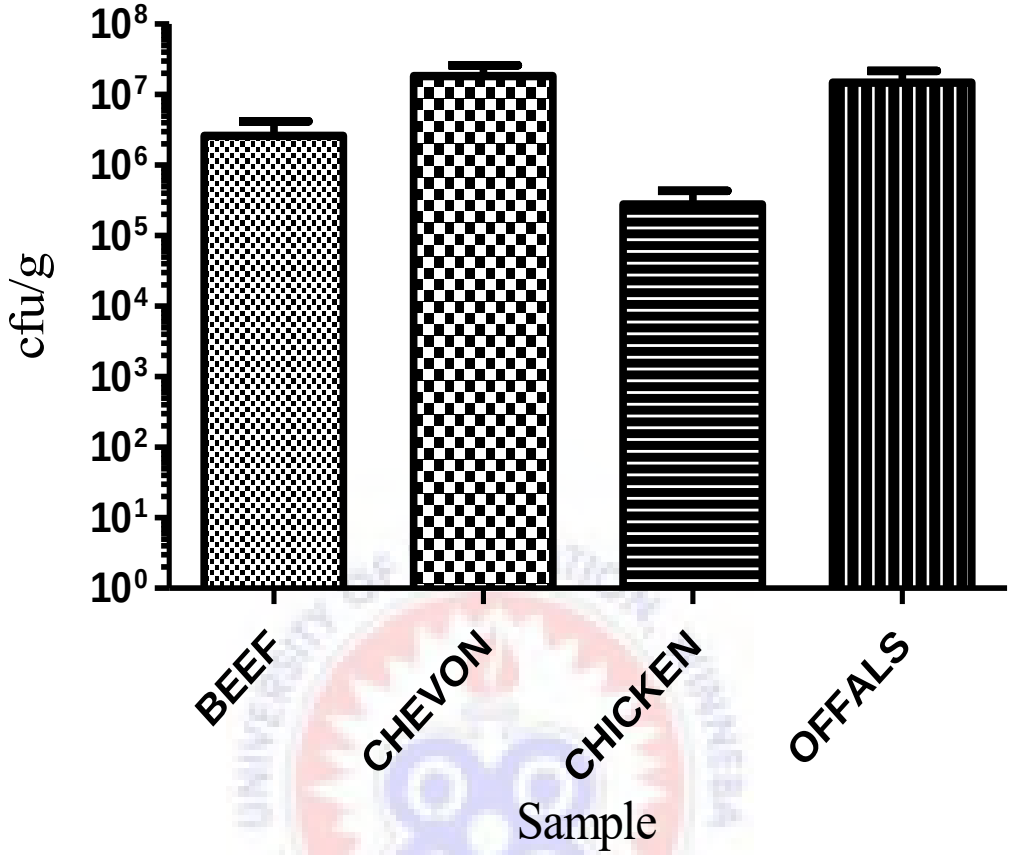

Figure 4.1: Mean aerobic count of raw meat sold at the Kumasi central market within Kumasi Metro.

Table 4.1: Total aerobic count of raw meat within Kumasi Metropolis

Sample	Vendor	Load (cfu/g)
BEEF	SHD 1	$5.2\times10^5 \pm 4.16$
	SHD 2	$1.46\times10^5 \pm 5.03$
	SHD 3	$4.7\times10^5 \pm 3.61$
	STR 1	$8.2\times10^6 \pm 7.51$
	STR 2	$3.8\times10^6 \pm 3.51$
CHEVON	SHD 1	$1.37\times10^7 \pm 4.73$
	SHD 2	$4.7\times10^7 \pm 3.46$
	SHD 3	$1.79\times10^7 \pm 7.02$
	STR 1	$5.4\times10^6 \pm 7.02$
	STR 2	$6.3\times10^6 \pm 5.02$
CHICKEN	CLDSTR 1	$8.8\times10^5 \pm 3.51$
	CLDSTR 2	$4.38\times10^4 \pm 12.12$
	CLDSTR 3	$5.23\times10^4 \pm 4.23$
	STR 1	$2.73\times10^5 \pm 311$
	STR 2	$1.46\times10^5 \pm 6.11$
OFFALS	SHD 1	$4.1\times10^6 \pm 4.51$
	SHD 2	$3.46\times10^7 \pm 5.11$
	SHD 3	$2.89\times10^6 \pm 11.02$
	STR 1	$8.32\times10^5 \pm 4.12$
	STR 2	$5.02\times10^6 \pm 6.11$

SHD- shed STR-street CLD-cold store

The statistical analysis using the Bartlett's corrected test at a Confidence Intervals (CI) of 95% showed significant difference in the levels of contamination of the different meat recording a P value of <0.0001 and this is obvious from Figure 4.1 with the order being chicken, beef, offal and chevon in increasing order of contamination levels.

The high level of contamination can be attributed to the flaws in the production line from the abattoir through transportation to the point of sale. The typical practice at the abattoirs involves handling the meat with bare hands without proper aseptic techniques, direct contact of the meat with the floor which is not aseptically treated before and after close of work, direct exposure of the meat to the air and immediate environment throughout the processing, use of unsterilized equipments in the processing and transportation of the processed meat under uncontrolled and unhygienic conditions to the point of sale as observed at the time of sampling.

The sale locations, be it in the streets or the abattoir sheds had similar persisting conditions as was observed at the abattoirs. The meat is left at the mercy of flies and dust in the open environment and is handled without proper aseptic measures. These appalling practices persisting at the sites accounts greatly for the contamination levels observed in the study. There was significant difference between the outcomes of the street and abattoir shed samples at 95% Confidence Interval (CI) using a Two-way ANOVA at P value of 0.05 (fig. 4.2). This clearly depicts that the contamination levels of the meat is independent of the sale point which could be due to either pre-contamination from the source and transportation of the meat (abattoir) or the persisting similar environmental conditions and bad hygiene practices at the sale points.

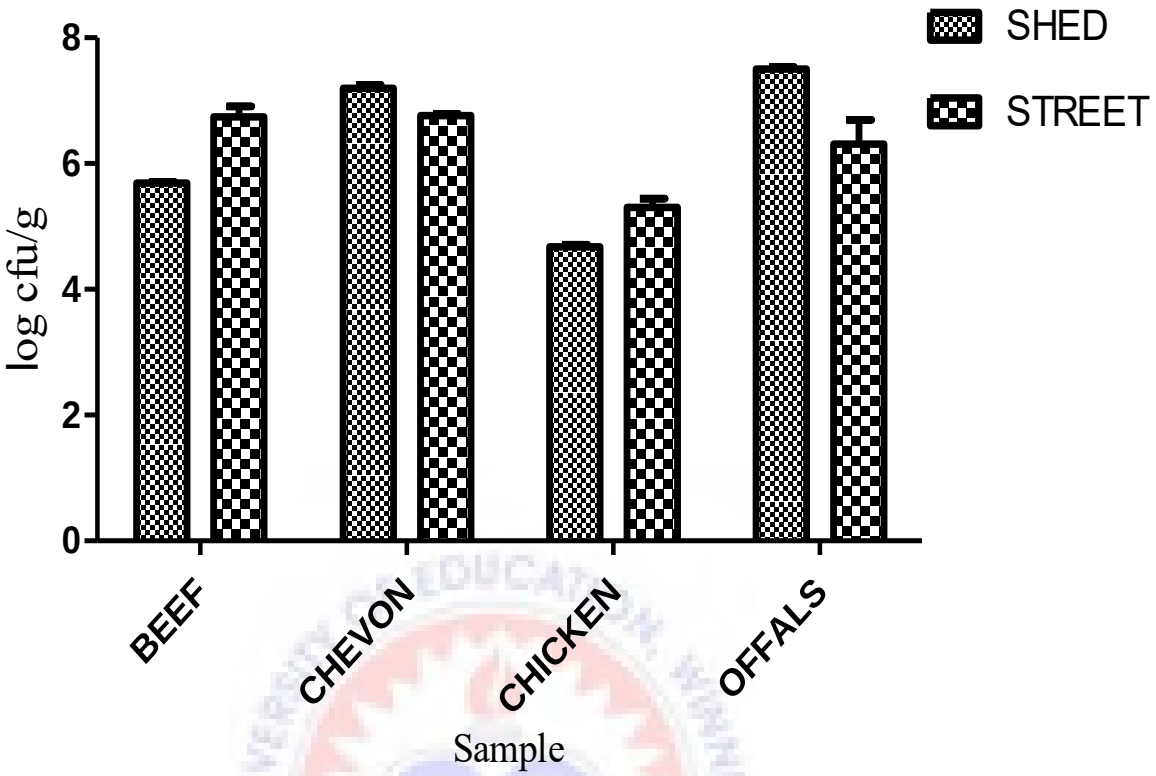

Figure 4.2: Comparative study of the contamination levels of Street and Shed meat

This point out to the danger one is exposed to in consuming improperly cooked meat irrespective of the point of sale be it from the street or the abattoir. This calls for consumer discretion in meat processing for food particularly for commercial purposes as these are done in large quantities thus the high tendency of misprocessing in the favor of saving time and energy.

4.2 Total Coliform Count

Coliforms are a class of organisms which are of prime importance in the area of food safety as they have been implicated in a couple of food poisoning and contamination cases recorded across the globe. This makes Coliforms pathogenic and of concern to health and safety of consumers of meat. The family of Coliforms consists of organisms

such as *Salmonella* spp. particularly the *S.typhi* which is the causative organism of typhoid which is one of the food borne illnesses very difficult to treat (Thiruvengadam *et al*., 1973). Other Coliforms are *Klebsiella pneumoniae* which causes pneumonia, *Enterococci* spp., and the infamous *Escherichia coli* responsible for diarrhea experienced from food contamination.

These causes most countries to have a sharp eye for Coliforms in foods with the standards ranging from 0 to 10 cfu/g as the tolerable limits but in Ghana the Coliform must be zero(0). The results obtained upon the assessment of meat sold on the Kumasi central market showed extreme levels of Coliform contamination relative to the standards. This is clearly an indication of the poor hygiene and sanitary conditions of the immediate environment of the meat.

The results generally showed coliform counts ranging from 5.0×10^2 cfu/g to 1.67×10^6 cfu/g which exceed the safe and acceptable limit, with chevon recording the highest whilst beef meat recorded the least count. The argument of further processing of meat (boiling, smoking and frying) before consumption thus making preprocessing contamination less significant could be raised in this instance, yet the chance of misprocessing cannot be overlooked. Any failure to get rid of these coliforms from the meat prior to consumption exposes the consumer to health risks as this class of organisms are deemed pathogenic.

Fig. 4.3 shows the coliform count of meat sold at the Kumasi central market.

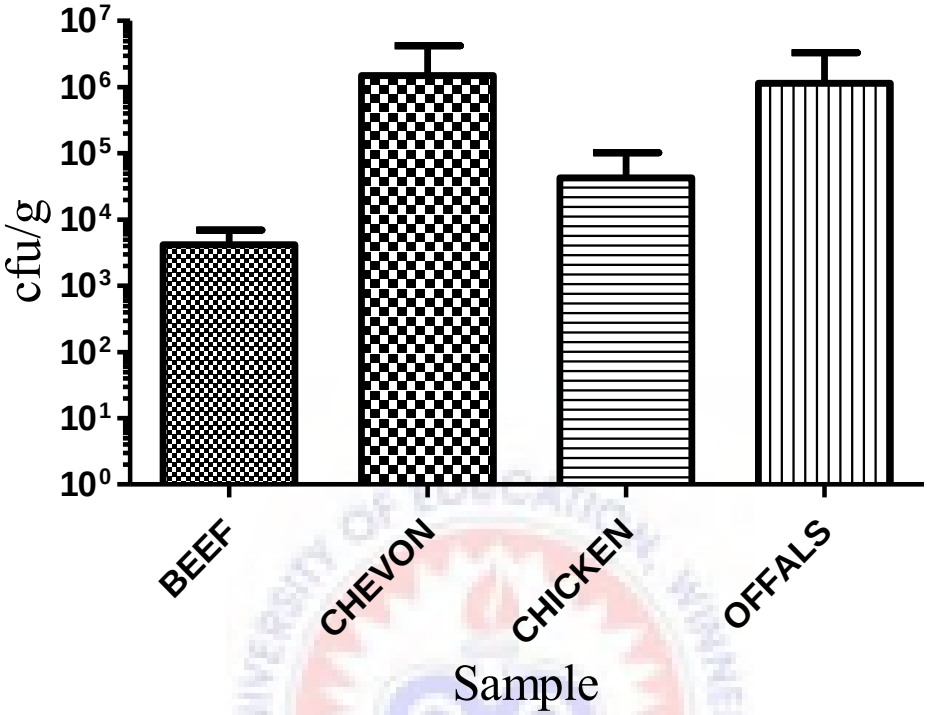

Figure 4.3: Coliform count of meat from Kumasi central market within Kumasi Metro

Statistical analysis using the Bartletts corrected test at CI of 95% showed significant difference ($P<0.05$) between the coliform counts of the different meat with similar pattern as the total aerobic count. However, there was no significance difference between the chevon meat and the offals meat for as the coliform count was generated. Unlike the total aerobic count where chicken recorded the least contamination, beef rather recorded the least contamination of 5.1×10^3cfu/g whereas chicken recorded 1.94×10^4 cfu/g. This could be due to the high water retention capacity of chicken meat as opposed to beef meat (Adu-Gyamfi, Nketsiah-Tabiri & Boating, (2009). Coliforms thrive in conditions of high

moisture and protein which are obtained in Chicken meat. Another factor could be the duration of storage as the chicken was obtained from the cold store and could have been stored for longer periods as opposed to the beef meat from the abattoirs which do not stay that long on the shelf thus relatively little time for coliform proliferation.

In trying to establish the link between the environment and the contamination levels, the statistical probing using a Two-way ANOVA showed no significant difference between the street and abattoir sold meat at a P value of 0.1692. This again rules out the notion of the abattoir meat being more preferred than the ones on the streets.

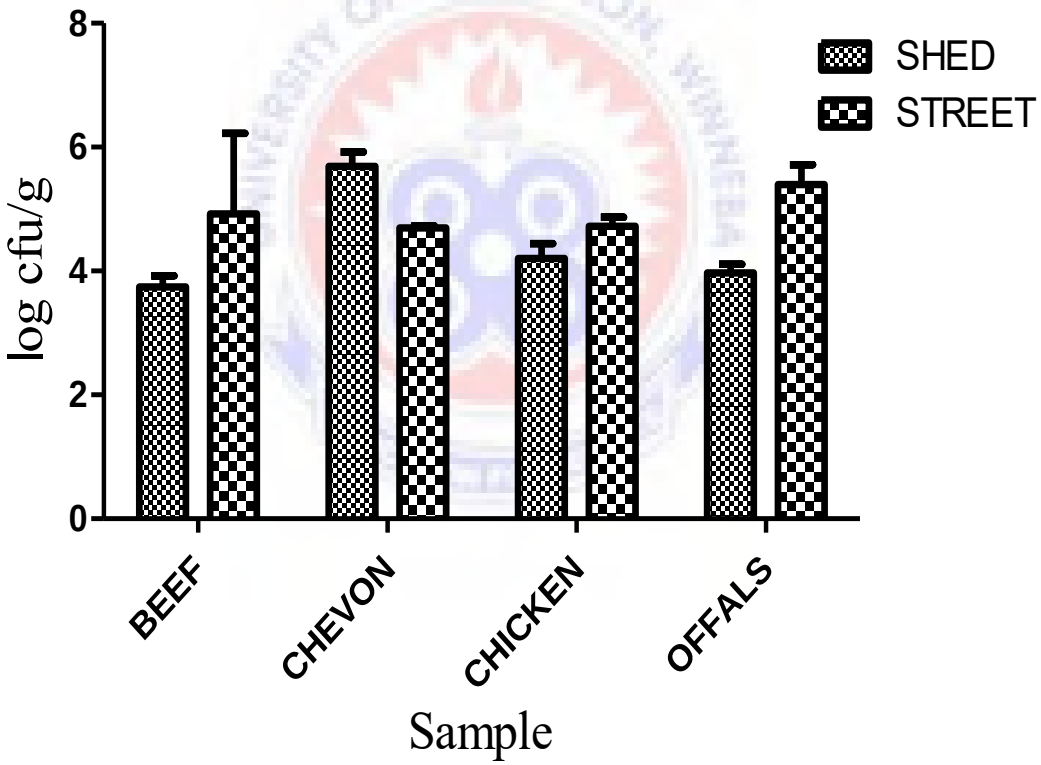

Figure 4.4: Comparative study of Coliform contamination of street and shed meat

4.2.1 Escherichia coli

One typical Coliform that is of significance in food safety is *Escherichia coli* and is mostly used as the indicator for faecal contamination and human induced contamination. The detection of *E.coli* strains particularly the pathogenic strains in food is deemed a high alert factor thus the acceptable or tolerable limit of *E.coli* being set at 0cfu/g by the ISO and AOAC. The *E.coli* assay of the meat revealed an alarming phenomenon of high contamination levels in all the meat. This raises health alerts as the organism in question is deemed pathogenic.

The results obtained showed beef meat to have recorded the highest *E.coli* contamination with a mean level of 2.38×10^5cfu/g which exceeds the tolerable limit of 0 by Ghana standard Authority thus revealing the poor microbial quality of the meat. The next sample in the order of contamination is the offals with a prevailing level of 1.97×10^5 cfu/g followed by chevon meat with a level of 7.34×10^4 cfu/g, these all once stand far above the tolerable limit thus are deemed highly unsafe for human consumption ruling out the effect of further processing prior to consumption. Chicken meat, which is ranked the 'safest' with the least contamination of 1.47×10^4 cfu/g still stands far above the safe limit.

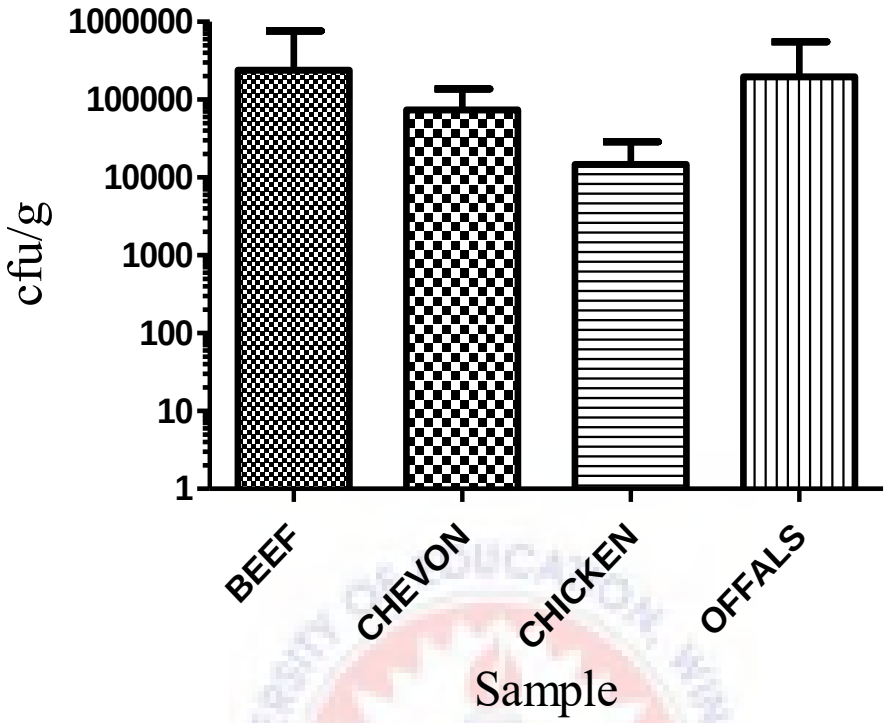

Figure 4.5 *Escherichia coli* contamination of meat from the local market within Kumasi Metro.

The occurrence of high Coliforms and *E.coli*presence in such meat products is linked to two major classes of intrinsic and extrinsic factors; the former being the contamination sourced from the intestinal faecal matter of these organisms due to improper treatment and processing (Grauke *et al*., 2002).

The digestive tract particularly the sections of the small and large intestines are known to house appreciable numbers of these coliform and *E.coli* which are to aid in digestion thus a mutualistic relationship between the host and organism (Hooper & Gordon,

2001).However when the population of these organisms exceed the threshold then symptoms such as diarrhea persist (Gorbach *et al*., 1971). Due to this phenomenon it could occur that the faecal matter from the offals could contaminate the meat thus resulting in such high Coliform and *E.coli* presence.

The extrinsic factors could be attributed to the human interface and factors such as poor sanitary practices of improper hand washing which could transmit these organisms from the contaminated human to meat as the meat is handled with the bare hands. The improper handling of the meat from the point of kill to sale can also contribute to the high contamination recorded.

Table 4.2: Coliforms and *Escherichia coli* from meat from the Kumasi central market within Kumasi metro

Sample	Vendor	TTC	*E.coli*
BEEF	SHD 1	$4.20\times10^{3}\pm16.0$	$2.20\times10^{3}\pm3.05$
	SHD 2	$3.70\times10^{3}\pm2.52$	$1.40\times10^{4}\pm9.14$
	SHD 3	$8.80\times10^{3}\pm16.2$	$5.10\times10^{3}\pm7.09$
	STR 1	$4.20\times10^{3}\pm4.51$	$4.51\times10^{6}\pm4.51$
	STR 2	$1.67\times10^{6}\pm3.51$	$2.30\times10^{3}\pm2.08$
CHEVON	SHD 1	$5.40\times10^{4}\pm5.03$	$9.40\times10^{4}\pm8.19$
	SHD 2	$2.90\times10^{5}\pm3.52$	$1.80\times10^{3}\pm3.01$
	SHD 3	$8.31\times10^{5}\pm8.62$	$1.75\times10^{5}\pm6.65$
	STR 1	$4.70\times10^{4}\pm7.02$	$3.70\times10^{4}\pm4.04$
	STR 2	$5.3\times10^{4}\pm4.11$	$4.43\times10^{4}\pm2.08$
CHICKEN	CLDSTR 1	$2.78\times10^{4}\pm7.09$	$8.90\times10^{3}\pm7.51$
	CLDSTR 2	$4.91\times10^{2}\pm5.12$	$1.40\times10^{4}\pm9.14$
	CLDSTR 3	$5.01\times10^{3}\pm4.23$	$4.20\times10^{3}\pm3.12$
	STR 1	$3.72\times10^{4}\pm13.01$	$3.90\times10^{4}\pm4.73$
	STR 2	$9.50\times10^{3}\pm8.02$	$7.42\times10^{3}\pm2.41$
OFFALS	SHD 1	$5.29\times10^{5}\pm3.51$	$6.96\times10^{4}\pm4.73$
	SHD 2	$1.17\times10^{5}\pm5.58$	$6.40\times10^{4}\pm9.14$
	SHD 3	$6.80\times10^{3}\pm4.51$	$3.20\times10^{3}\pm3.00$
	STR 1	$7.82\times10^{5}\pm6.12$	$2.73\times10^{5}\pm5.11$
	STR 2	$4.82\times10^{5}\pm4.31$	$8.30\times10^{4}\pm3.14$

SHD- shed STR-street CLD-cold store

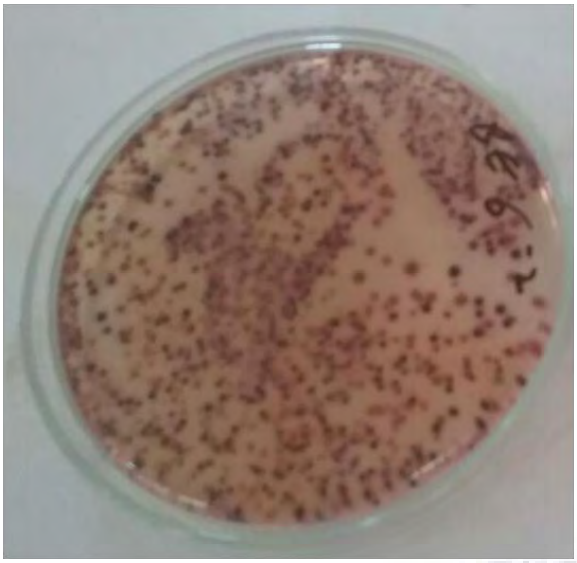

Plate 2: *E.coli* from meat on *BE.coli*

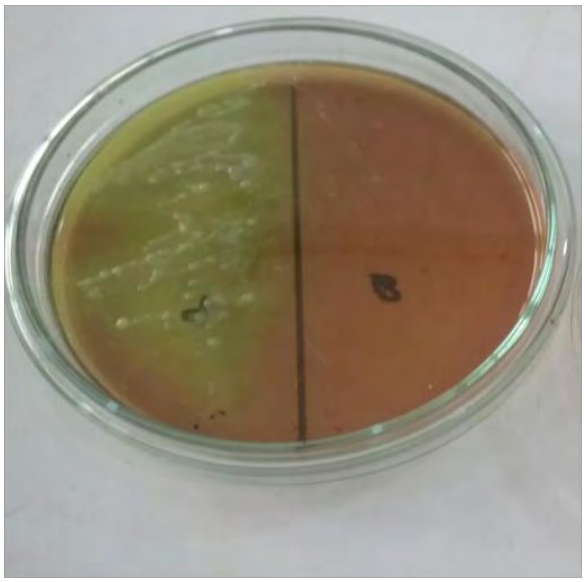

Plate 3: Negative *Salmonell*a test on BGA

Plate 4: Coliforms from meat on VRBLA

Statistical analysis using the Bartletts test for equal variances at a CI of 95% showed significant difference in the levels of E.coli contamination across the different meat samples at a P value of <0.0001. This goes to show the different meat per their different treatments and peculiar practices during processing results in varying levels of contamination.

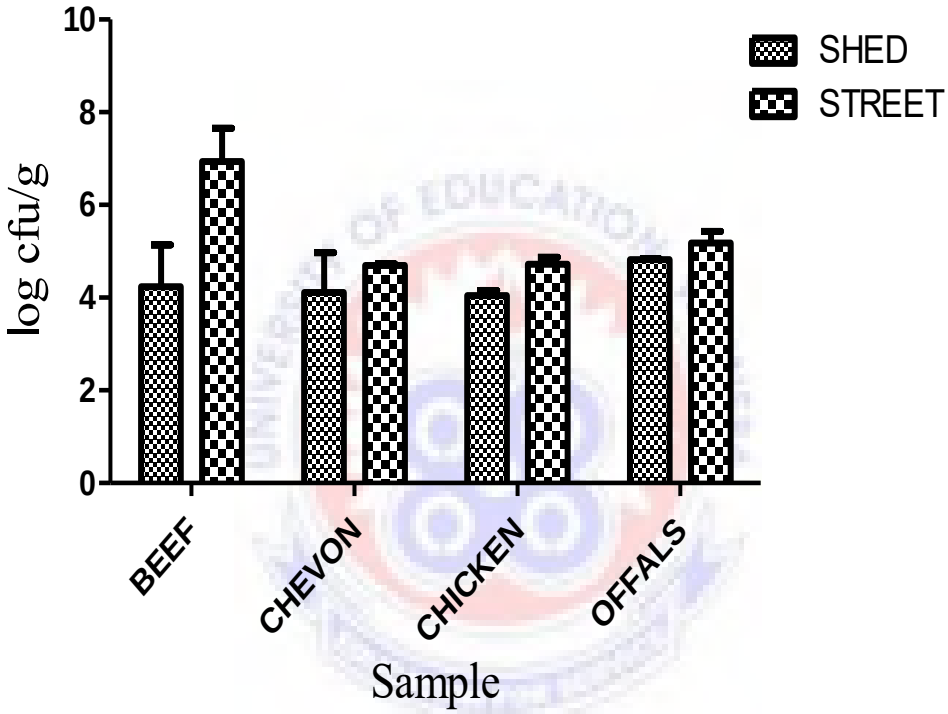

Figure 4.6: Comparative study of *Escherichia coli* contamination of street and shed meat

4.3 Staphylococcus aureus

Staphylococcus aureus has been implicated as the causative organism of some clinical boils coupled with other symptoms such as high temperature, headaches and uneasy feelings (Liu *et al*., 2011).

The qualitative assay for the presence of *S. aureus* indicated high contamination as all the samples tested positive to the presence of the organism, representing a 100% occurrence. This raises a health alarm as clearly confirms the poor hygiene practices and sanitary state of the environment within which the meat is processed and sold. *S. aureus* is an indicator of environmental and human induced contamination as the organism is mostly found to inhabit living hosts such as man (Liu *et al*., 2011).

The common practice among the meat dealers is handling the meat with the bare hands which exposes the meat to direct contact with the organisms if the person is infected. The use of gloves would help reduce the case of *S. aureus* contamination.

Table 4.3: Qualitative assay of *Staphylococcus aureus* in meat

Sample	Vendor	Presence/Absence	Strength	Inference
BEEF	SHD 1	✓	++	Staphylococcus aureus is not to be detected in consumable products with an acceptable limit of 0. This makes the meat samples not wholesome and acceptable.
	SHD 2	✓	++	
	SHD 3	✓	+	
	STR 1	✓	+++	
	STR 2	✓	++	
CHEVON	SHD 1	✓	++	
	SHD 2	✓	+	
	SHD 3	✓	++	
	STR 1	✓	++	
	STR 2	✓	+++	
CHICKEN	CLDSTR 1	✓	+	
	CLDSTR 2	✓	++	
	CLDSTR 3	✓	+	
	STR 1	✓	++	
	STR 2	✓	++	
OFFALS	SHD 1	✓	+	
	SHD 2	✓	++	
	SHD 3	✓	+++	
	STR 1	✓	+++	
	STR 2	✓	++	

SHD- shed STR-street CLD-cold store

The detection of S.aureus in the samples renders them unsafe for consumption according to the ISO regulations. This is due to the enormous health risks the consumer is exposed to upon consumption of S.aureus infested food (Liu *et al*., 2011).

The detection of *S.aureus* in the samples correlates with the observations made at the sites at the time of sampling. There persisted quite appalling health and sanitary practices at most sites visited with all the vendors handling the meat with bare hands thus posing the direct transfer or interaction of the meat with infected persons.

The weakness of the claim of this study with regards to the *S.aureus* menace was the inability to conduct confirmatory tests on all the isolates either biochemically or by molecular techniques to validate the findings as to the isolates being of the *aureus* species and not other *Staphylococcus* species. This however does not disclaim the findings of this work as the test protocol used is an internationally accepted tool in *S.aureus* detection (ISO, 2005).

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The meat available on the local market in the Kumasi metropolis particularly the Central market and its environs can be broadly classified as not safe for consumption especially in the absence of proper and thorough post-purchase processing on the part of the consumer before consumption.

The total aerobic count indicated the presence of some aerobic microorganisms in appreciable loads; Chicken recording the least aerobic contamination of averagely 3.57×10^5 cfu/g with beef and chevon meat recording 2.2×10^6 cfu/g and 2.1×10^7 cfu/g, respectively. Offals recorded a mean load of 9.49×10^6 cfu/g.

All the meat samples recorded the presence of Coliforms in levels exceeding the threshold ranging from 5.0×10^2 cfu/g to 1.67×10^6 cfu/g. Beef recorded the least Coliform load of 5.1×10^3cfu/g whereas chicken recorded 1.94×10^4 cfu/g with chevon and offals recording 2.6×10^5 cfu/g and 3.8×10^5 cfu/g respectively.

Though undesirable, the outcome of this study indicated the presence of *Escherichia coli* in all the samples. Beef recorded the highest *E.coli* contamination with a mean level of 2.38×10^5cfu/g with offals, chevon and chicken recording levels of 1.97×10^5 cfu/g, 7.34×10^4 cfu/g and 1.47×10^4 cfu/g respectively.

The various microorganisms were detected in the meat samples taken from the Central market of the Kumasi metropolis; *Escherichia coli*, *Enterococci* spp., *Staphylococcus* spp and some Coliforms and aerobic microorganisms that could not be generically identified

The quality of the meat is not hinged on the quantitative measures of microbial populations but also the qualitative dimension as well. The unwholesome condition of the meat is premised on the detection of pathogenic or disease causing microorganisms in the meat rendering them unsafe for consumption. The study identified some lactose fermenting Coliforms to be present in the meat samples which are a class of pathogenic microorganisms. Further analysis showed specifically *Escherichia coli* and *Enterococci* spp suspected to be *Enterococcus feacalis* were present in the meat samples analyzed at relatively alarming levels as well. The study however indicated no *Salmonella typhi* and *Shighella* spp. were present in the meat sample which is a good indicator as these are classified as highly pathogenic in food microbiology.

The biochemical profiling indicated some *Staphylococcus* spp. to be present as well in the meat samples but failure to identify the specific species is attributed to unavailability of primers for the genetic assay and the lack of the required biochemical tool to identify the species.

Gram stain, catalse, citrate and TSI profile of some isolated aerobic organisms indicated the presence of *Bacillus* spp., *Staphylococcus* spp. *Escherichia coli* and *Enterococci* spp. were present in the meat samples.

Table 5.1: Biochemical profile of isolated species

Isolate	Gram R.	Shape	Argmt.	Catalase	Citrate		TSI		Gas
					Slunt	Butt	Slunt	Butt	
1	Negative	Rods	Chains	+	G	G	Y	Y	-
2	Positive	Rods	Singular	+	G	G	Y	Y	-
3	Negative	Rods	Chains	+	G	G	Y	Y	-

- Denotes negative Y Denotes Yellow Gram R. Denotes Gram reaction

+ Denotes positive Argmt Denotes arrangement

G Denotes green

The field observational studies coupled with the findings of the laboratory analysis clearly spells out some risk factors associated with the contamination of meat sold on the markets from the abattoir to the point of sale (production line). The first identified factor is the handling and processing of meat at the abattoirs. The prevailing mode of slaughter and processing at the abattoirs is highly unhygienic (direct contact of the meat with soil and bare floor, use of unsterilized instruments in operation, direct handling of meat without hand washing, use of unsterilized surfaces).

Another factor is the mode of transportation of the meat from abattoir to the point of sale. The current system of using “kia” and “aboboyaa” vehicles (vehicles with open carriages) exposes the meat to the polluted atmosphere and allows for contact with flies and other flying creatures which introduce more contamination. These vehicles are not properly cleaned and sanitized prior to conveying the meat thus there also exist the risk of contamination from contact with contaminated surfaces of the vehicles.

The third factor is the prevailing conditions at the point of sale which from observation were highly inappropriate and not ideal for safety and health. The meat is exposed directly to the environment and left at the mercy of flies which is a major source of contamination. The handlers are culprits of the same error of direct handling without proper hand washing and sanitation.

5.2 Recommendation

In view of the findings of this study it is recommended for further studies to be carried out to elucidate the pathogenicity of the isolated strains of organisms; *Escherichia coli*, *Enterococci* spp., *Staphylococcus* spp. by either molecular techniques or detailed biochemical profiling to establish the risk associated with consuming foods infested with such organisms.

Work should also be done in the area of estimating the direct impact of the various malpractices at the abattoirs and sale points on the microbial quality (risk factors) to enable the development of proper systems to control such occurrences and thereby improve the microbial quality of the meat available on the markets.

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APPENDIX

Sample No. Blank								
		Dilution						
Media	**Replicate**	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
PCA	1	1	0	0	0	0	0	
	2	0	0	0	0	0	0	
	3	0	0	0	0	0	0	
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
VRBLA	1	0	0	0	0	0	0	
	2	0	0	0	0	0	0	
	3	0	0	0	0	0	0	
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
B E.coli	1	0	0	0	0	0	0	
	2	0	0	0	0	0	0	
	3	0	0	0	0	0	0	
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
PBA	1	0	0	0	0	0	0	
	2	0	0	0	0	0	0	
	3	0	0	0	0	0	0	
XLD	1	Not detected						
	2	Not detected						
	3	Not detected						
BGA	1	Not detected						
	2	Not detected						
	3	Not detected						
Sample No.sample 1								
		Dilution						
Media	**Replicate**	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
PCA	1	TNTC	TNTC	337	34	6	1	
	2	TNTC	TNTC	321	41	9	0	
	3	TNTC	TNTC	329	38	4	0	
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
VRBLA	1	57	6	0	0	0	0	
	2	27	4	0	0	0	0	
	3	41	3	0	0	0	0	
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
B E.coli	1	21	8	0	0	0	0	
	2	25	5	0	0	0	0	
	3	22	7	0	0	0	0	
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
PBA	1	Presence of growth						
	2	Presence of growth						
	3	negative						

XLD	1	Not detected						
	2	Not detected						
	3	Not detected						
BGA	1	Not detected						
	2	Not detected						
	3	Not detected						
Sample No.sample 2								
		Dilution						
Media	**Replicate**	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
PCA	1	TNTC	360	49	6	0	0	
	2	TNTC	342	57	9	0	0	
	3	TNTC	351	51	5	0	0	
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
VRBLA	1	40	8	0	0	0	0	
	2	35	4	0	0	0	0	
	3	37	12	0	0	0	0	
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
B E.coli	1	25	3	0	0	0	0	
	2	19	1	0	0	0	0	
	3	21	5	0	0	0	0	
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
PBA	1	Presence of growth						
	2	Presence of growth						
	3	Presence of growth						
XLD	1	Not detected						
	2	Not detected						
	3	Not detected						
BGA	1	Not detected						
	2	Not detected						
	3	Not detected						
Sample No.sample 3								
		Dilution						
Media	**Replicate**	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
PCA	1	TNTC	316	50	16	1	0	
	2	TNTC	328	43	12	0	0	
	3	TNTC	319	48	14	5	0	

		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
VRBLA	1	95	5	0	0	0	0	
	2	73	9	0	0	0	0	
	3	81	6	0	0	0	0	
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
B E.coli	1	57	1	0	0	0	0	
	2	43	2	0	0	0	0	
	3	52	5	0	0	0	0	
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
PBA	1	Presence of growth						
	2	Presence of growth						
	3	Presence of growth						
XLD	1	Not detected						
	2	Not detected						
	3	Not detected						
BGA	1	Not detected						
	2	Not detected						
	3	Not detected						
Sample No.sample 4								
		Dilution						
Media	**Replicate**	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
PCA	1	TNTC	140	20	0	0	0	
	2	TNTC	151	28	0	0	0	
	3	TNTC	146	25	2	0	0	
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
VRBLA	1	4	0	0	0	0	0	
	2	6	0	0	0	0	0	
	3	3	0	0	0	0	0	
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
B E.coli	1	0	0	0	0	0	0	
	2	0	0	0	0	0	0	
	3	0	0	0	0	0	0	
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
PBA	1	Presence of growth						
	2	Presence of growth						
	3	Presence of growth						
XLD	1	Not detected						

	2	Not detected						
	3	Not detected						
BGA	1	Not detected						
	2	Not detected						
	3	Not detected						
Sample No.1								
		Dilution						
Media	**Replicate**	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
PCA	1	203	67	10	0	0	0	
	2	227	63	5	0	0	0	
	3	218	69	7	0	0	0	
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
VRBLA	1	131	57	2	0	0	0	
	2	157	69	1	0	0	0	
	3	142	53	0	0	0	0	
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
B E.coli	1	109	12	1	0	0	0	
	2	96	19	0	0	0	0	
	3	111	13	0	0	0	0	
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
BPA	1	Presence of growth						
	2	Presence of growth						
	3	Presence of growth						
XLD	1	Not detected						
	2	Not detected						
	3	Not detected						
BGA	1	Not detected						
	2	Not detected						
	3	Not detected						
Sample No.2								
		Dilution						
Media	**Replicate**	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
PCA	1	TNTC	145	21	2	0	0	
	2	TNTC	153	17	0	0	0	
	3	TNTC	141	24	1	0	0	
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
VRBLA	1	87	19	0	0	0	0	

	2	103	14	3	0	0	0	
	3	96	21	1	0	0	0	
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
B E.coli	1	12	1	0	0	0	0	
	2	17	0	0	0	0	0	
	3	16	3	0	0	0	0	
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
BPA	1	Presence of growth						
	2	Presence of growth						
	3	Presence of growth						
XLD	1	Not detected						
	2	Not detected						
	3	Not detected						
BGA	1	Not detected						
	2	Not detected						
	3	Not detected						
Sample No.3								
		Dilution						
Media	**Replicate**	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
PCA	1	TNTC	118	11	3	0	0	
	2	TNTC	124	11	0	0	0	
	3	TNTC	121	15	1	0	0	
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
VRBLA	1	37	4	0	0	0	0	
	2	46	8	0	0	0	0	
	3	42	5	0	0	0	0	
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
B E.coli	1	13	4	0	0	0	0	
	2	17	6	0	0	0	0	
	3	20	10	0	0	0	0	
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
BPA	1	Presence of growth						
	2	Presence of growth						
	3	Presence of growth						
XLD	1	Not detected						
	2	Not detected						
	3	Not detected						

BGA	1	Not detected						
	2	Not detected						
	3	Not detected						
Sample No.4								
		Dilution						
Media	**Replicate**	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
PCA	1	TNTC	TNTC	180	35	5	0	
	2	TNTC	TNTC	202	42	1	0	
	3	TNTC	TNTC	192	39	3	0	
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
VRBLA	1	63	12	0	0	0	0	
	2	68	15	1	0	0	0	
	3	72	10	2	0	0	0	
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
B E.coli	1	32	2	0	0	0	0	
	2	29	5	0	0	0	0	
	3	35	3	0	0	0	0	
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
BPA	1	Presence of growth						
	2	Presence of growth						
	3	Presence of growth						
XLD	1	Not detected						
	2	Not detected						
	3	Not detected						
BGA	1	Not detected						
	2	Not detected						
	3	Not detected						
Sample No.5								
		Dilution						
Media	**Replicate**	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
PCA	1	TNTC	210	84	4	0	0	
	2	TNTC	141	87	12	0	0	
	3	TNTC	180	78	8	0	0	
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
VRBLA	1	1	0	0	0	0	0	
	2	0	0	0	0	0	0	
	3	0	0	0	0	0	0	
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	

B E.coli	1	0	0	0	0	0	0	
	2	0	0	0	0	0	0	
	3	0	0	0	0	0	0	
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
BPA	1	Presence of growth						
	2	Presence of growth						
	3	Presence of growth						
XLD	1	Not detected						
	2	Not detected						
	3	Not detected						
BGA	1	Not detected						
	2	Not detected						
	3	Not detected						
Sample No.6								
		Dilution						
Media	**Replicate**	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
PCA	1	TNTC	TNTC	TNTC	74	12	2	
	2	TNTC	TNTC	TNTC	82	21	0	
	3	TNTC	TNTC	TNTC	89	18	0	
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
VRBLA	1	TNTC	TNTC	165	72	12	2	
	2	TNTC	TNTC	172	64	20	0	
	3	TNTC	TNTC	169	68	17	1	
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
B E.coli	1	TNTC	TNTC	118	27	2	0	
	2	TNTC	TNTC	123	34	0	0	
	3	TNTC	TNTC	114	31	1	0	
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
BPA	1	Presence of growth						
	2	Presence of growth						
	3	Presence of growth						
XLD	1	Not detected						
	2	Not detected						
	3	Not detected						
BGA	1	Not detected						
	2	Not detected						
	3	Not detected						

Sample No. 1								
		Dilution						
Media	**Replicate**	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
PCA	1	TNTC	TNTC	296	46	6	-	
	2	TNTC	TNTC	308	37	1	-	
	3	TNTC	TNTC	301	41	3	-	
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
VRBLA	1	TNTC	224	83	12	-	-	
	2	TNTC	231	79	9	1	-	
	3	TNTC	228	88	16	-	-	
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
B E.coli	1	TNTC	68	11	-	-	-	
	2	TNTC	75	15	2	-	-	
	3	TNTC	66	18	1	-	-	
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
PBA	1							
	2	Present						
	3							
XLD	1							
	2	None Detected						
	3							
BGA	1							
	2	None Detected						
	3							

Sample No. 2								
		Dilution						
Media	**Replicate**	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
PCA	1	TNTC	TNTC	85	10	2	-	
	2	TNTC	TNTC	92	7	-	-	
	3	TNTC	TNTC	88	12	1	-	
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
VRBLA	1	201	33	6	-	-	-	
	2	187	39	3	-	-	-	
	3	192	37	7	-	-	-	
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
B E.coli	1	82	13	-	-	-	-	
	2	97	17	4	-	-	-	
	3	89	21	2	-	-	-	
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
PBA	1							
	2	Present						
	3							
XLD	1							
	2	None Detected						
	3							
BGA	1							
	2	None Detected						
	3							

Sample No. 3								
		Dilution						
Media	**Replicate**	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
PCA	1	TNTC	TNTC	TNTC	172	32	12	
	2	TNTC	TNTC	TNTC	186	27	5	
	3	TNTC	TNTC	TNTC	178	36	9	
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
VRBLA	1	182	49	8	-	-	-	
	2	197	53	5	1	-	-	
	3	185	59	7	-	-	-	
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
B E.coli	1	TNTC	85	11				
	2	TNTC	101	6	2			
	3	TNTC	96	13				
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
PBA	1							
	2			Present				
	3							
XLD	1							
	2	None Detected						
	3							
BGA	1							
	2	None Detected						
	3							

Sample No. 4								
		Dilution						
Media	**Replicate**	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
PCA	1	TNTC	TNTC	TNTC	-	-	-	
	2	TNTC	TNTC	TNTC	-	-	-	
	3	TNTC	TNTC	TNTC	-	-	-	
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
VRBLA	1	TNTC	212	48	4	-	-	
	2	TNTC	219	41	-	-	-	
	3	TNTC	215	56	3	-	-	
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
B E.coli	1	TNTC	184	27	2	-	-	
	2	TNTC	197	34	-	-	-	
	3	TNTC	173	29	1	-	-	
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
PBA	1							
	2				Present			
	3							
XLD	1							
	2	None Detected						
	3							
BGA	1							
	2	None Detected						
	3							

Sample No. 5								
		Dilution						
Media	**Replicate**	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
PCA	1	TNTC	TNTC	TNTC	TNTC	284	72	
	2	TNTC	TNTC	TNTC	TNTC	296	89	
	3	TNTC	TNTC	TNTC	TNTC	286	83	
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
VRBLA	1	TNTC	TNTC	TNTC	189	56	8	
	2	TNTC	TNTC	TNTC	197	67	12	
	3	TNTC	TNTC	TNTC	181	71	7	
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
B E.coli	1	TNTC	TNTC	296	39	1	-	
	2	TNTC	TNTC	274	47	2	-	
	3	TNTC	TNTC	289	41	-	-	
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
PBA	1							
	2				Present			
	3							
XLD	1							
	2	None Detected						
	3							
BGA	1							
	2	None Detected						
	3							

Sample No. 6								
		Dilution						
Media	**Replicate**	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
PCA	1	TNTC	TNTC	192	47	9	-	
	2	TNTC	TNTC	207	53	2	-	
	3	TNTC	TNTC	221	61	7	-	
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
VRBLA	1	TNTC	39	4	-	-	-	
	2	TNTC	53	3	-	-	-	
	3	TNTC	47	6	-	-	-	
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
B E.coli	1	TNTC	33	2	-	-	-	
	2	TNTC	36	-	-	-	-	
	3	TNTC	41	3	-	-	-	
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
PBA	1							
	2			Present				
	3							
XLD	1							
	2	None Detected						
	3							
BGA	1							
	2	None Detected						
	3							

Sample No. 7								
		Dilution						
Media	**Replicate**	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
PCA	1	TNTC	TNTC	TNTC	292	43	-	
	2	TNTC	TNTC	TNTC	284	49	-	
	3	TNTC	TNTC	TNTC	297	45	-	
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
VRBLA	1							
	2							
	3							
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
B E.coli	1							
	2							
	3							
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
PBA	1							
	2			Present				
	3							
XLD	1							
	2	None Detected						
	3							
BGA	1							
	2	None Detected						
	3							

Sample No. 8								
		Dilution						
Media	**Replicate**	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
PCA	1	TNTC	TNTC	TNTC	132	23	1	
	2	TNTC	TNTC	TNTC	139	26	-	
	3	TNTC	TNTC	TNTC	141	27		
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
VRBLA	1	TNTC	TNTC	72	18	2	-	
	2	TNTC	TNTC	83	23	-	-	
	3	TNTC	TNTC	89	21	2	-	
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
B E.coli	1	TNTC	182	23	2	-	-	
	2	TNTC	169	29	4	1	-	
	3	TNTC	173	34	5	-	-	
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
PBA	1							
	2		Present					
	3							
XLD	1	None Detected						
	2							
	3							
BGA	1							
	2	None Detected						
	3							

Sample No. 9								
		Dilution						
Media	**Replicate**	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
PCA	1	TNTC	TNTC	TNTC	142	52	2	
	2	TNTC	TNTC	TNTC	147	59	7	
	3	TNTC	TNTC	TNTC	138	54	3	
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
VRBLA	1	TNTC	112	8	-	-	-	
	2	TNTC	116	12	-	-	-	
	3	TNTC	123	9	1	-	-	
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
B E.coli	1	TNTC	53	6	-	-	-	
	2	TNTC	71	11	-	-	-	
	3	TNTC	67	9	-	-	-	
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
PBA	1							
	2			Present				
	3							
XLD	1							
	2	None Detected						
	3							
BGA	1							
	2	None Detected						
	3							

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