BACTERIOLOGICAL QUALITY AND SAFETY OF RAW BEEF FROM SELECTED OUTLETS IN WINDHOEK (NAMIBIA)

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ABSTRACT

Inappropriate slaughtering and retail operations can compromise food safety specifically in densely populated areas like informal markets. The bacteriological quality and prevalence of *Salmonella* spp. and *Escherichia coli* O157:H7 on beef samples from the three different outlets (supermarket, butchery and open market) were assessed to determine their safety for human consumption. A total of 138 of beef samples were collected at random from three different outlets. The bacteriological quality of the beef samples were performed using the total plate count and total coliform count method, while standard culture methods were used for *Salmonella* spp. and *E. coli* O157:H7 isolation and detection. Based on results, the mean total plate count of beef from the open markets, butchery and supermarkets were 3.83 Log CFU/g, 3.90 Log CFU/g and 4.31 Log CFU/g, respectively. The mean TPCs on beef samples from supermarket A, B, C, D, E and F were 4.72, 4.87, 4.38, 4.90, 3.48 and 2.61 Log CFU/g, respectively. The mean TPCs on beef samples from open market X, Y and Z were 4.33, 4.28 and 2.95 Log CFU/g, respectively. The mean coliform count of beef samples from the open markets, butchery and supermarkets were 2.08 Log CFU/g, 1.71 Log CFU/g and 1.31 Log CFU/g, respectively. The mean CCs from supermarket A, B, C, D, E and F were 1.49, 2.80, 0.33, 0.00, 2.74 and 0.93 Log CFU/g, respectively. The mean CCs on beef samples from open market X, Y and Z were 2.59, 2.80 and 0.89 Log CFU/g, respectively. Based on the results, only 25.0 % of beef samples tested for total plate count and 26.4 % of samples tested for total coliform count exceeded the acceptable limit. The overall prevalence of *Salmonella* spp. and *E. coli* O157:H7 on beef samples were 68 (49.3 %) and 8 (5.80 %) respectively. The prevalence of *Salmonella* spp. on beef samples from the open

market 31 (67.4 %), followed by butchery 24 (52.2 %) and lowest prevalence from supermarkets was 13 (28.3 %). The highest prevalence of *E. coli* O157:H7 on beef samples from open markets was 5 (10.9 %), followed by butchery 2 (4.35 %) and supermarkets 1 (2.17 %). The prevalence of *Salmonella* spp. on beef samples from supermarket A, B, C, D, E and F were 33.0 %, 22.0 %, 0.00 %, 57.0 %, 50.0 % and 20.0 %, respectively. The prevalence of *Salmonella* spp. on beef from open market X, Y and Z were 75.0 %, 63.0 % and 50.0 %, respectively. The prevalence of *E. coli* O157:H7 on beef samples from supermarket A, B, C, D, E and F were 0.00 %, 0.00 %, 0.00 %, 0.00 %, 0.00 % and 20.0 %, respectively. The prevalence of *E. coli* O157:H7 on beef samples were 13.0 %, 0.00 % and 21.0 % from open market X, Y and Z, respectively. A significant correlation ($P<0.01$) was found between TPC, TCC, *Salmonella* spp. and *E. coli* O157:H7. Beef collected from the outlets had low microbial counts and hence fit for human consumption. However, there was presence of *Salmonella* spp. and *E. coli* O157:H7 in beef samples from the outlets that can pose serious threat to the consumer and hence, testing may be necessary to avoid foodborne disease outbreaks.

Keywords: Microbial, quality, prevalence, TPC, CC, Salmonella spp., *E. coli O157:H7, fresh beef, Windhoek, Namibia*

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ABBREVIATIONS

HACCP	-	Hazard Analysis Critical Control Point
FAO	-	Food and Agricultural Organization
CDC	-	Centers for Disease Control and Prevention
WHO	-	World Health Organization
ISO	-	International Organization for Standardization
STEC	-	Shiga Toxin *Escherichia coli*
BPW	-	Buffered Peptone Water
PCA	-	Plate Count Agar
EMB	-	Eosin Methylene Blue
TPC	-	Total Plate Counts
CC	-	Coliform Counts
FDA	-	Food and Drug Administration
USDA	-	United States Department of Agriculture
DNA	-	Deoxyribonucleic Acid
EU	-	European Union
CVL	-	Central Veterinary Laboratory
IFA	-	Immune-Fluorescent Antibody
XLD	-	Xylose Lysine Desoxycholate
BSA	-	Bismuth Sulfite Agar
MKTTn	-	Muller-Kauffmann Tetrathionate novobiocin
RVS	-	Rappaport-Vassiliadis with Soya
H_2S	-	Hydrogen Sulfite

CIDRAP	-	Centre for Infectious Disease Research and Policy
HUS	-	Hemolytic Uremic Syndrome
PCR	-	Polymerase Chain Reactions
ELISA	-	Enzyme-Linked Immunosorbent Assay
CFU	-	Colony Forming Unit
NSF	-	Non-Sorbitol Fermenting
SMAC	-	Sorbitol MacConkey Agar
LAB	-	Lactic Acid Bacteria
GMP	-	Good Manufacturing Practices
MAWF	-	Ministry of Agriculture, Water and Forestry
IMS	-	Immuno-magnetic Separation
ANOVA	-	Analysis of Variance
ICMSF	-	International Commission of Microbiological Specifications for Foods
MHSS	-	Ministry of Health and Social Service
FSIS	-	Food Safety and Inspection Service
CCPs	-	Critical Control Points
MMWR	-	Morbidity and Mortality Weekly Results
CFU	-	Colony Forming Unity
API	-	Analytical Profile Index
GHP	-	Good Hygiene Practices

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DECLARATION

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Johannes Nkandi

CHAPTER 1: INTRODUCTION

1.1 Introduction

Most developing countries are faced with higher prevalence of food poisoning outbreaks. Food-borne diseases remains the most significant food safety hazards worldwide associated with beef (Maripandi & Al-Salamah, 2010). Generally, beef is one of the most consumed foods in the Republic of Namibia particularly the city of Windhoek. Beef is highly perishable food and its high in nutritional value such as proteins, minerals, vitamins and fat (Prescott, Harley & Klein, 2002). The composition of beef is ideal for growth of a wide range of spoilage bacteria and pathogenic bacteria (Mayr *et al.,* 2003).

The muscle tissues of healthy living animals maybe become infected with a wide range of microorganisms during slaughter, processing and handling (Pal, 2012). During slaughter process, microorganisms that reside in the gastro-intestinal tract of cattle often spills and spread on the meat surface under faulty and poor processing conditions (Oosterom, 1991; Biswas *et al*., 2009). During hide removal, the hide can also contaminate the meat once come into contact with the meat surface (Edeza, Quiroz & Felix, 2012). Additionally, contamination can occur due to poor handling when convert the beef carcasses to retail cuts. Cutting and mincing beef carcasses cause contamination through subsequent meat handling (Adzitey *et al*., 2011). Microbial contamination of beef surface can also occur via contact with unclean cutting knives, chopping boards, saw and grinders and by contact with meat handlers or by vehicle during transporting beef carcasses (Rombouts & Nout, 1994; Adzitey *et al*., 2011). According to Smith and Lechman (2003) beef products can also be

contaminated with the roundworm *Trichinella* larvae by meat grinders and other equipment used for processing pork. This roundworm has been frequently involved in serious human disease outbreaks (Oivenen *et al.*, 2002). The ambient temperature and the duration of storage time for meat are crucial factors in the growth of spoilage and pathogenic bacteria (Delmore, 2009).

The initial load of contaminating microorganisms in excess influences the shelf life of beef become unwholesome and unfit for human consumption. However, beef under hot and humid climatic conditions tends to deteriorate more rapidly and become a major vehicle for gastrointestinal infections problems (Akinro, Ologunagba & Yahaya, 2009). The high levels of microbial load causes biochemical and microbiological changes in beef causing increased incidences of illnesses and other fatal human diseases (Soyiri, Agbogli & Dongde, 2008). Spoilage bacteria that can cause discoloration, bad odors and slime on beef surfaces includes *lactic acid bacteria*, *Pseudomonas* spp., *Acinetobacter* spp., and *Moraxella* spp.(Kraft, 1992).

Food-borne infections normally results from consumption of contaminated beef with pathogenic bacteria (Pal, 2012). The food poisoning microorganisms such as *Salmonella* spp., *E. coli*, *Listeria monocytogenes* and *Campylobacter jejuni* can cause food borne infection and intoxication (Kraft, 1992). The global surveillance data indicated that there was increment in the incidences of food-borne infections associated with the consumption of contaminated beef (Mukhopadhyay, Pillai, Pal & Ajay, 2009; Minami *et al.*, 2010), demonstrating the importance of controlling food pathogens in food processes. However, the children, elderly and immune-suppressed

individuals are most vulnerable to food-borne infections (Kiiyukia, 2003; Adak *et al*., 2005).

Microbial contamination can be reduced in beef production chain through the establishment of food safety systems in food processing industries. Good Manufacturing practices (GMP) is one of the effective tool of hygiene and sanitations during food processing (Food Agriculture Organization [FAO]/ World Health Organization [WHO], 2013). According to Silliker (1980) the practices can help to reduce the level of both spoilage and pathogenic microorganisms effectively. Another important food safety tool is the Hazard Analysis Critical Control Point (HACCP) plan which has been applied to identify and control food-borne pathogens (Zweifel, Baltzer & Stephan, 2005). Monitoring the presence of microorganisms in beef is an important step of HACCP plan to prevent food-borne pathogens. The microbiological data are used to identify the sources of bacteriological contamination of food products (Brown *et al*., 2000). Microbiological examination of food samples can be used to draw reliable conclusions regarding the food hygiene (Zweifel, Blatzer & Stephan, 2005).

However, there is relatively few surveys and lack of information on the microbiological status of beef offered for retail sale in Windhoek city. Several food borne outbreaks were attributed to meat particularly beef (Thomas, Lallo & Badric, 2006). The aim of the study was to assess microbial quality of beef from supermarkets, butchery and open markets in Windhoek, the capital city of Namibia.

1.2 Statement of the problem

Raw beef sold in Namibian outlets especially the open markets is often displayed in the open air at ambient temperature with lot of houseflies, dusts and bad human handling. Therefore, it was necessary to test for TPC, CC and pathogenic bacteria such as *Salmonella* spp. and *E. coli* O157:H7 to assess microbial quality and safety of beef sold at the outlets.

Figure 1. Meat for sale in open markets.

Furthermore, people in Namibia have faced many kinds of health issues due to hygiene problems. Some of these issues become so serious, especially for small children and elderly people. According to Theresia (2015) at least of 300 children under age of 5 and more than 270 adults from Okuryangava, Babylon and Okahandja Park have visited clinics suffering from diarrhea and vomiting. However, it is still not clear about the causes and effects of this type of illness in Namibia. *Salmonella* spp. are major public health concern because they cause salmonellosis and gastroenteritis (CDC, 2007). *E. coli* O157:H7 is a serious public health concern and is linked to high mortality (Bielaszewska *et al*., 2007).

1.3 Overall objective

The main objective of this study was carried out to assess the bacteriological quality and investigate *Salmonella* spp. and *E. coli* O157:H7 on beef samples generated from three different outlets.

1.4 Specific objectives

In order to achieve the main aim of the study, the following specific objectives were set:

1. To determine Total Plate Counts (TPC), Coliform Counts (CC) and investigate the prevalence of *Salmonella* spp., *E. coli* O157:H7 on beef samples collected from supermarkets, butcheries and open markets in Windhoek.
2. To compare total plate counts, total coliform counts and the prevalence of *Salmonella* spp., *E. coli* O157:H7 on beef samples collected from supermarkets, butcheries and open markets in Windhoek.
3. To determine and compare the total plate counts, coliform counts and the prevalence of *Salmonella* spp., *E. coli* O157:H7 on beef samples collected from six supermarkets (A, B, C, D, E and F) and three open markets (X, Y and Z).
4. To compare the relationship between TPC, CC, *Salmonella* and *E. coli* O157:H7

1.5 Significance of the study

The total plate count and total coliform count are keys indicators in the field of hygiene management. They indicate the number of microorganisms present in the samples which should not exceed the guide value. The study will help provide

information on the microbial load of the beef in order to control and prevent foodborne diseases.

The presence of *Salmonella* and *E. coli* O157:H7 on beef has been a major challenge for public health because of its potential to cause illnesses. Knowledge of how these pathogens disseminates through retail points is important in understanding how beef retailing procedures contribute to contamination and subsequent human infections. Therefore, this research provides information on the presence of *Salmonella* and *E. coli* O157:H7 on beef samples which can be used to estimate the potential threat at retail points for proper control and management

Lack of good hygiene practices and HACCP plans in the retail processing procedures increases food-borne pathogens and microbial contamination. The study may help to raise awareness among food handlers on the adverse effect of poor hygiene in order to improve and strengthen hygienic retail practices to avoid microbial contamination. The findings of the study may be useful to the relevant authorities to help initiate and enforce regulations regarding food safety.

1.6 Limitation of the study

To carry out the research project, there were number of different constraints in the research process that were dealt with. Firstly, it was possible that some of the local retailers may refuse to allow researchers to sample beef from their shops thereby reducing the variety of sampling sites from the Windhoek area. Another concern is that lack of equipment and reagents will limit the smooth completion of the study.

The sample size was small due to logistical challenges and hence the samples collected might not be representative of all outlets.

CHAPTER 2: LITERATURE REVIEW

2.1 Meat composition and nutritional values

Meat is the most perishable of all important foods since it contains sufficient nutrient needed to support the growth of microorganisms (Magnus, 1981; Mayr *et al.*, 2003). The chief constituents of meat are water, protein and fat. Other components include iron, phosphorus, zinc and B vitamins (USDA/ARS/NDL, 2012). The major primary unit of meat is called carcass and it represents the ideal meat after head, hide, intestine, blood. The edible parts of a carcass include lean flesh and edible glands or organs such as heart, liver, kidney, tongue and brain. Despite the nutritional benefits, meat has been identified as potential vehicle for transmitting food-borne diseases due to its high protein content, low in carbohydrates, approximately neutral pH and high water activity. These compositions create favorable conditions for the growth and survival of bacteria (Bhandare, Sherikar, Paturkar, Waskar & Zende, 2007; Yousuf *et al.*, 2008)). Most meat have high water content corresponding to the water activity approximately 0.99 which is suitable for microbial growth (Rao, Thulasi & Ruban, 2009). Conditions of the storage including factors such as temperature and humidity which are critical in the growth of spoilage and pathogenic microorganisms on beef and hence effective refrigeration or freezing are prior methods (Delmore, 2009). Age and sex of the animal has a major influence on the quality of meat that is produced from animals.

2.2 Sources of microbial contamination in meat

The meat tissues of healthy animals are regarded as sterile but the presence of microorganisms on meat often comes from contamination during and after slaughter. Microbial contamination can come from the skin or intestinal tract of animal during slaughter (Okonko *et al*., 2010). The hairs, skin or hide of animals are naturally contaminated by a variety of microorganisms and hence microbial contamination of carcasses normally occur during skinning, evisceration, processing at abattoirs and retail outlets (Featherstone, 2003; Okonko *et al*., 2010; Adzitey *et al*., 2011).

The soil adhering to the skin and faeces are the main source of microbial contamination of meat (Featherstone, 2003). Soil has comparable numbers (10^7) of bacteria per gram of soil whereas faeces are more contaminated and have microbial counts of 10^9 and coliforms counts of 10^8 per gram of faeces (Featherstone, 2003; Unc & Goss, 2004). The instruments used during dressing and evisceration such as knives, saws and cleavers can be potential sources of contamination (CDC, 2007; Fernandes, 2009).

The vehicles used to transport meat from abattoirs to retail outlets may be sources of contamination due to lack of regular cleanliness. The meat carcasses are transported to retail outlets in dirty vehicles which grossly contaminate the meat (Raji, 2006; Adzitey *et al*., 2011). However, the meat contamination also comes from dusts, insects and flies since the meat is usually not well covered during transportation (Galland, 1997).

The abattoirs and retail outlets environments are major source of microbial contamination on meat. The meat contamination often results from poor handling, contaminated water, contaminated knives and contaminated tables to display meat intended for sale (Okonko *et al*., 2010; Osama & Gehan, 2011). Ozlem (2005) and Adetunde *et al*. (2011) reported high levels of fecal contamination in the slaughterhouse and hence microbial contamination could begin at the slaughterhouses. Agbodaze *et al.* (2005) reported that the occurrence of *Enterobacteriaceae* in beef originated from fecal contamination due to unsanitary practices and inappropriate storage conditions in the abattoirs. According to Adzitey *et al*. (2011), the other possible sources of contaminations includes chopping boards, containers, meat handlers, vehicle for transporting carcasses and the meat selling environment.

Refrigerator or freezers are essential storage facilities used to prevent spoilage of meat and keep meat safe for long period of time. Clarence *et al*. (2009) reported that beef carcasses that remains on the stalls at ambient room temperature for too long favor fast growth and multiplication of microorganisms. Therefore, meat must be kept in refrigerated conditions during retailing in order to have minimal bacterial contamination.

Food handlers can expose meat to microorganisms during meat handling due to lack of knowledge of personal hygiene and sanitation (Clarence *et al*., 2009). According to Kondaiah, Anjaneyulu and Mandal (2011), the microorganisms that are harboured in the hands, hairs, nose and mouth can be transferred onto food during preparation, packaging, processing and service. Okonko *et al*. (2010) reported that food can be

infected with microorganisms as a result of coughing and sneezing from those who handle and process these foods.

2.3 The effects of microbial contamination on meat

Beef can become spoiled by the actions of spoilage microorganisms which appear as discoloration and slime formation on the meat (Walter, 1975; Jackson *et al*., 2001). The fresh and spoiled beef cuts are presented in **Figure 2.** The changes are noticeable when the bacterial count ranges from 10^7 CFU/cm^2 to 10^8 CFU/cm^2 and hence meat is regarded as spoiled and unfit for human consumption (Ayres, 1960). The biochemical and microbiological changes in the meat often lead to production of noxious substances which results into increased incidences of illnesses and other fatal human diseases (Walter, 1975). As a result, consumers can acquire various bacterial infections due to contamination which results from poor handling practices and lack of knowledge on sources of microbial contamination of food animals and meat (Adeyemo, Adeyemi & Awosanya, 2009). The author further stated that the other challenges of microbial contamination on meat includes food poisoning, spoilage of the meat and rejection of carcasses which results into reduction of income to farmers as well as meat sellers.

Figure 2. The fresh and spoiled beef cuts

2.4 Microorganisms present in meat

The most important microorganisms with regard to meat hygiene include molds, helminths, parasite, bacteria and viruses. Among these groups, bacteria are the most important microorganisms. The most common pathogenic bacteria that has been associated with meat spoilage include *Salmonella* spp., *Escherichia coli, Compylobacter* spp., *Staphylococcus aureus, Listeria monocytogenes, Yersinia enterocolitica, Clostridium perfringens, Bacillus cereus and Vibrio parahaemolyticus* (Biswas *et al.,* 2011).

Salmonella spp., *Compylobacter* spp and *Escherichia coli* were common pathogens often present in fresh meat and poultry (Zhao *et al.*, 2001). However, *Escherichia coli* O157:H7, *Salmonella enteritidis, Listeria* spp., and *Shigella* species are commonly recovered from fresh meat and poultry. *Staphylococcus aureus, Bacillus cereus, Clostridium perfringens* and *Escherichia coli* were isolated in beef samples from butchers (Soyiri *et al.,* 2008).

2.5 Microbial quality control and indicator microorganisms

Food safety system is the most efficient way to reduce microbial contamination in foods. Good sanitation and hygienic practices in food processing plants can reduce the level of both spoilage and pathogenic micro-organisms in foods (Silliker, 1980). The HACCP plan in food processing plants can also reduce the risk of microbial contamination throughout food production chain (Molla, Alemayehu & Salah, 2003; Zweifel & Stephan, 2005). The United States Department of Agriculture (USDA) and Food and Drug Administration (FDA) reported the five main principles of HACCP

plan required to prevent food safety problems in food industry (Khandke & Mayes, 1998).

Total plate count is one of the indicators used to estimate the microbiological quality of raw meat products (Yousef & Carlstrom, 2003; Mukhopadhyay *et al*., 2009). The TPC indicates the chances of finding pathogenic bacteria in food samples. Total plate count estimates the presence of microorganisms such as bacteria, yeast and mold in food (Hatakka, 2000). Total plate counts can also provide an estimate of overall bacterial populations but the higher level of total plate count relates to poor quality foods and reduced shelf-life (Ray, 2004). The results could reflect the level of hygiene for food handling and storage. A high count of microorganisms exceeding 7.00 Log CFU/g of TPC is an indication for meat spoilage and potential health hazards. The total plate count exceeding 5.00 Log CFU/g for raw meat is unacceptable and meat hygiene must be urgently improved (ICMSF, 1986; FAO, 2007).

Coliforms are gram-negative, rod-shaped facultative anaerobic bacteria which are identified by production of gas from glucose and other sugars as well as fermentation of lactose to acid and gas within 48 h at 35ºC (Hitchins, Feng, Watkins, Rippey & Chandler, 1998). The growth of these organisms is very slow in foods stored at 5 °C and only in some occasions growth has been observed between 3 and 6°C. The coliform group of organisms consists of species from the genera *Escherichia, Klebsiella, Enterobacter,* and *Citrobacter*, and includes *E. coli*. For this reason, coliforms are used as indicator microorganisms to serve as a measure of fecal contamination (Greenberg & Hunt, 1985).

Although, some coliforms are found in the intestinal tract of man, most are found throughout the environment and have little sanitary significance (Greenberg & Hunt, 1985). The total coliform count is a good indicator of fecal contamination of human or other animals and they indicate greater risk of exposure to pathogenic organisms. Higher coliform counts generally correlates with higher levels of food-borne pathogens of faecal origin (Jay, 2000). The *Enterobacteriaceae* that exceeds 3.00 Log CFU/g when found on fresh meat are not acceptable and meat hygiene along meat handling chain must be urgently improved (ICMSF, 1986; FAO, 2007).

2.6 *Salmonella*

2.6.1 Introduction

It is speculated that *E. coli* O157:H7 and *Salmonella* diverged from a common ancestor and hence *Salmonella* diverged from the genus *Escherichia* about 120-160 years ago (Lawrence, 1999; Wray & Wray, 2000). On the basis of the degree of sequence divergence, it can be estimated that a common ancestor of the genus existed about 25 to 40 million years ago (Baumler, Tsolis, Ficht & Garry, 1998). *Salmonella* was first discovered by the bacteriologist D. E. Salmon from porcine intestine in 1884 (Lin & Cheng, 2007).

Salmonellosis is an important public health problem causing real morbidity and essential economic impact worldwide (Workman, 1999). According to Crump, Luby and Mintz (2004), *Salmonella* causes approximately 1.3 billion human infections annually. It causes nearly 1.4 million human illnesses each year in the US alone

resulting in 116,000 hospitalizations and 600 deaths (Lin, 2005; Majowicz *et al*., 2010; Hale *et al*., 2012). According to Olsen *et al*. (2001), there has been an alarming rate of *Salmonella* isolations among infants and old persons in US.

Salmonellosis had imposed huge economic burden on individuals and society globally (Bishwa, Frederick & Martin, 2004; Jordan *et al*., 2006). Approximately US$ 2.7 billion was linked to Salmonellosis in US alone (USDA, 2011). According to Korsak *et al*. (2006), the cost linked to food-borne salmonellosis in Europe ranged between € 560 million and € 2.8 billion during 1999. In comparison to the developing world, the global estimate of typhoid fever alone was about 21.7 million cases and 200,000 deaths annually (Crump *et al*., 2004).

2.6.2 Taxonomy of *Salmonella* spp.

The genus *Salmonella* is divided into two species; *S. enterica* and *S. bongori* (Grimont & Weill, 2007). The *S. enterica* is further divided into six subspecies; II, *S. enterica* subsp. *Salamae,* I, *S. enterica* subsp. *enterica,* IIIa, *S. enterica* subsp. *Arizonae,* VI, *S. enterica* subsp. *Indica,* IIIb, *S. enterica* subsp. *Diarizonae,* IV, *S. enterica* subsp. *Houtenae* (Brenner, Villar, Angulo, Tauxe & Swaminathan, 2000). Species and subspecies of the *Salmonella* genus are shown in **Table 1** below. With regard to food safety *S. enterica* subsp. *enterica* is of major concern because the strains within these serogroups are known to cause 99 % of *Salmonella* infections in humans (Bell & Kyriakides, 2002). The *Salmonella enterica* is comprised of the subspecies *S.* Typhimurium, *S.* Enteriditis and *S.* Typhi (Bopp, Brenner, Fields, Wells & Strockbine, 2003).

Table 1. Species and subspecies in the *Salmonella* genus.

Salmonella subspecies	Species	Name of serovars
S.enterica	Enterica	1478
	salamae	498
	arizonae	94
	diarizonae	327
	houteane	71
	indica	12
S. bongori	-	21
Total		2501

Names are retained only for subspecies *enterica* serovars and they are no longer italicized as per newer convention. The first letter is a capital letter "*S*" followed by the serovar names of subspecies *enterica* e.g. Typhimurium or Montevideo. At the first citation of the serotype the genus name is given followed by the word "serotype" (Brenner *et al*., 2000). The antigenic formulae are used to name *Salmonella* serotypes as shown in **Table 2**. The designation includes: (i) subspecies designation (subspecies I trough VI), (ii) O (somatic) antigens followed by a colon, (iii) H (flagellar) antigens (phase 1) followed by a colon, and (iv) H antigens (phase 2, if present) i.e. *Salmonella* serotype IV 45: g, z51:- (Brenner *et al*., 2000). Currently, the nomenclature system used at the Centers for Disease Control and Prevention (CDC) for the genus *Salmonella* is based on recommendations from the World Health Organization (WHO) Collaborating Centre for Reference and Research on *Salmonella* at the Pasteur Institute, Paris, France (Popoff & Le Minor, 1997).

Table 2. Example of antigenic formula of selected *Salmonella enterica* subsp. *enterica* serotypes according to Kaufmann-White scheme.

Serotype	Serogroup	Somatic antigens(O)	Flagella (H) antigens	
			Phase 1	Phase 2
S. Paratyphi A	A	1, 2, 12	A	(1, 5)
S. Typhimurium	B	1, 4, (5), 12	I	1, 2
S. Agona		4,12	f, g, s	-
S. Derby	B	1, 4, (5), 12	f, g	(1, 2)
S. Typhi	B	9, 12, (Vi)	C	1, 2
S. Enteriditis	D	1, 9, 12	g, m	1,7
S. Infantis	D	6, 7, 14	R	1, 5
S. Virchow		6, 7	R	1, 2
S. Gallinarum		1, 9, 12	-	-
S. Dublin		1, 9, 12 [Vi]	R	1, 2

2.6.3 Characteristics of *Salmonella*

Salmonella are gram-negative rods that belong to the Family *Enterobacteriaceae* (Yan *et al*., 2003; Garry, Ouattarra, Williams & Pesta 2009). The authors further reported that *Salmonella* live in the intestinal tract of infected animals and humans. These are small microscopic facultative anaerobic pathogens which are usually motile by peritrichous flagella except *S.* Pullorum and *S.* Gallinarum that are non-motile because they lack flagella (Krieg & Holt, 1984; Holt, Krieg, Sneath, Staley & William, 2002). The size of the rods range from 0.7-1.5 μm - 2.2-5.0 μm and the colonies are 2-4 mm in diameter (Holt *et al*., 2002).

Salmonella is able to produce hydrogen sulfide, except *Salmonella* Choleraesuis and most *Salmonella* Paratyphi A strains (ISO 6579:2002). *Salmonella* are catalase positive, oxidase negative, but methyl red and Simmons's citrate positive and indole

and voges-proskauer negative (Holt *et al*., 2002). *Salmonella* is able to produce acid and gas from glucose, reduce nitrates to nitrites, and produce hydrogen sulfide and decarboxylate to cadaverine and ornithine to putrescine lysine and usually use citrate as the sole carbon source (Minor, 1984). *Salmonella* catabolizes glucose and lysine, although it fails to metabolize lactose, sucrose and urea and they are chemo-organotrophic organisms, containing both respiratory and fermentative type of metabolism (John *et al*., 1994).

Salmonella is difficult to control because of its ability to survive extreme environmental conditions. According to D'aoust (1991), the optimal growth temperature for *Salmonella* is 37 °C and the temperature range for growth is 7 °C to 45 °C, pH for growth range between 4 to 9 and water activities for growth is above 0.94. *Salmonella* are heat labile and they are inactivated at ordinary cooking temperatures (> 70 °C) (D'aoust, 1991). *Salmonella* is able to tolerate up to 20 % salt concentration and survive under freezing conditions (from -23 °C to -18 °C) for as long as seven years (Bell & Kyriakides, 2002).

2.6.4 Serological identification of *Salmonella*

Salmonella nomenclature evolves from a concept of serotyping one species based on the serologic identification of O (somatic) and H (flagellar) proposed by Kauffmann using a series of independent agglutination tests (Jay, 2000; Grimont & Weill, 2007). The Centre for Infectious Disease Research and Policy (CIDRAP) classified members of the *Salmonella* species into more than 2501 serotypes (Popoff & Le Minor, 1997).

The new serotypes are listed in the annual updates of the Kauffmann-White scheme (Brenner *et al.*, 2000).

Salmonella species has specific O-antigen groups whereby different serovars are distinguished within O-groups using the combination of O- and H-antigens that are present. Each serotype has a specific antigenic formula. The O-antigens are represented by Arabic numbers (CDC, 2011). The H antigens of phase 1 are designated with small letters, and those of phase 2 are designated by Arabic numerals (Jay, 2000).

According to Todar (2005), the somatic (O) antigen is a carbohydrate antigen which is heat stable and alcohol resistant. The author further explains somatic antigen as a polymer of O sub-units and each of the O sub-unit is consisting of four to six sugars. The variation of the sugar components of the O sub-unit leads to variation in O antigen (CDC, 2011).

Flagellar (H) antigen is a protein called flagellin that is present in the flagella (Jay, Loessner & Golden, 2005). Most H antigens are diphasic strains and some H antigens are monophasic (*S.* Enteritidis, *S.* Risen) (Sifin, 2004). Most of *Salmonella* species have two types of flagellar antigens known as phase 1 and phase 2. Few *Salmonella* species do not have flagella antigens when they express as non-motile.

2.6.5 *Salmonella*: pathogenesis and epidemiology

Salmonella are capable of producing serious food-borne infections that present gastroenteritis. *Salmonella* are potentially pathogenic to both humans and animals

(CDC, 2007). Humans are infected with *Salmonella* through the digestive system when they consume contaminated food (Collins & Thato, 2011). Foods of animal origin have been a major vehicle for transmission of *Salmonella* to humans (Vaeteewootacharn *et al*., 2005).

Despite hygiene improvements, food processing and education of food handlers, *Salmonella* is still dormant as the significant public health problem worldwide (CDC 2007). According to Foley, Lynne and Nayak (2008), approximately 95 % cases of salmonellosis are associated with the consumption of contaminated food products. The animal population such as poultry, livestock's, pets and reptiles are asymptomatic carriers of *Salmonella* (CDC Vitalsigns, 2011; Hoelzer, Moreno & Wiedmann, 2011). *Salmonella* are shed in the feces of warm and cold-blooded animals which can be transferred to humans through direct contact (CDC, 2007; USDA/ Food Safety and Inspection Service [FSIS], 2012).

The common disease syndromes are mild to moderate gastroenteritis i.e. diarrhea, abdominal cramps, and fever, chills, headache, nausea and vomiting (Bell, 2002). The author further reported that the period of incubation range from 8 to 28 days. *S.* Typhi and *S*. Paratyphi are responsible for causing enteric fever (Guthrie, 1991). The antibiotics of choice for treatment of enteric fever are ampicillin, chloramphenicol or trimethoprim-sulfamethoxazole (Monteville & Matthews, 2008). The symptoms are usually self-limiting (usually last for 4-7 days) and patients recover without seeking medical attention (Josefsen, 2004).

Salmonella has been able to survive in the stomach and multiply to reach the intestines and interacts with non-phagocytic cells such as the epithelial cells of the intestinal mucosa to cause infection (Bell, 2002; Hensel, 2004). The infection then spread from the intestine to the blood stream and cause systemic syndromes such as gastroenteritis, septicemia, bacteremia and typhoid fever (Bell, 2002). According to Alphons, Asten and Dijk (2005), *Salmonella* contains virulence factors such as virulence-plasmids, toxins, fimbriae and flagella to help it establish an infection. Typically immunocompromised infants and elderly persons are at higher risk for severe illness. The presence of *Salmonella* in more than 25 g of raw meat is considered unsafe for human consumption.

Some *Salmonella* serotypes such as *S.* Typhi, *S.* Paratyphi, *S.* Choleraesuis and *S.* Dublin cause invasive salmonellosis in human (Chiu, Lin & Ou, 1999). Invasive salmonellosis is fatal and the antimicrobial treatment is essential in this circumstance (Su & Chiu, 2007). The elderly, infants and individuals with impaired immune systems are more likely to suffer (CDC, 2007).

Some of the *Salmonella* serovars are host preference and hence they cause diseases in a limited number of host species (Uzzau *et al*., 2000). An example of host-restricted serovars include *S.* Paratyphi and *S.* Typhi which cause typhoid and paratyphoid fever in humans, *S.* Gallinarum that are host-adapted in poultry, *S.* Abortusequi in horses, *S.* Abortusovis in sheep and *S.* Typhisuis in swine (WHO, 2005). *S.* Enteriditis and *S.* Typhimurium infect both man and animals causing gastrointestinal infections and enteric fever (Velge, Cloeckeart & Barrow, 2005). According to Velge *et al*. (2005),

the serotypes produce typhoid-like infections in mice and in humans and asymptomatic in chicken intestines.

2.6.6 Distribution of *Salmonella* serovars

The incidence of salmonellosis has increased rapidly worldwide over the years. The *S.* Enteriditis and *S.* Typhimurium are most common serotypes that have been recovered from human, animal, foods, feeds and environment sources globally during 2000 - 2005 (WHO, 2006). According to Foley *et al*. (2008), *S.* Typhimurium, *S.* Newport, *S.* Enteriditis, *S.* Heidelberg and *S.* Javiana are common *Salmonella* serovars isolated in the United States.

S. Typhimurium and *S.* Enteriditis are more prevalent in the developing world causing most disease outbreaks in sub-Sahara countries (Morpeth, Ramadhani & Crump, 2009). A study conducted on children and adults in Malawi reported the higher isolation rate of *S.* Typhimurium (75 %) and *S.* Enteriditis (21 %) during the period of 1998 to 2004 (Gordon *et al*., 2008). *S.* Hiduddify and *S.* Kedougou in poultry were reported in Nigeria and Thailand (Holly, Morrine, Timothy & David, 2014).

2.6.7 The virulence genes of *Salmonella*

Most of the *Salmonella* virulence factors are clustered on chromosome referred to as *Salmonella* pathogenicity islands (Shea, Hensel, Gleeson & Holden, 1996). *Salmonella* invade the cells through intestinal epithelium to reach deeper tissues without causing major damage to the intestinal mucosa in the early step of infection (Galan & Curtiss III, 1991).

Salmonella have an invasion gene (*invA*) which allows the bacterium to invade the cells (Galan & Curtiss III, 1991). It contains virulence plasmid which is essential for it to survive and grow in the host cells (Galan & Curtiss III, 1991). The plasmids of various *Salmonella* serotypes differ in size that ranges between 50 kb to 285 kb and it contains highly conserved 8 kb region with the *spv* locus that encodes the *spvR* regulatory gene and 4 structural *spvABCD* genes (Guerra, Soto, Helmuth & Mendoza, 2002). The author further reported that the *spv* operon fully expresses the virulence of *Salmonella*.

2.6.8 *Salmonella* control

Surveillance and inspection programs play a critical role in the reduction of food-borne illnesses (Friis & Sellers, 2004). According to Mølbak, Olsen and Wegner (2006), the developed countries have systems in place to report the occurrence of outbreaks, although the surveillance reports are subjected to some limitations: 1) physicians do not always request a stool culture of suspected cases; 2) people do not always look for medical aid when they get infected; 3) not all positive cases are reported and shared in the database, and 4) differences in health-care seeking behaviors among age groups is variable. These factors affect the accurate determination of the magnitude of illness caused by *Salmonella* (Mølbak *et al*., 2006). Food safety programs involve the investigation of *Salmonella* cases, laboratory analysis of food, regular inspection of foods and their production chain, appropriate legislation for the prevention of *Salmonella* and educating those who work with foods as well as consumers to effectively reduce *Salmonella* in foods (Todd, 1997).

The prevention of *Salmonella* in foods remains a challenge during food production chain. The HACCP plans and other safety measures demonstrate successful effective reductions in the occurrence and levels of pathogenic bacteria at different processing steps along the production lines to keep critical points under control (Bell & Kyriakides, 2002). The combination of temperature and pressure on sprays and chemical treatments such as dioxide, acidified sodium chlorite, ozone, organic acids, trisodium phosphate and cetylpyridinium chloride are used to decontaminate meat carcasses and hence reduce *Salmonella* contamination (Bell & Kyriakides, 2002).

2.6.9 *Salmonella* detection

Conventional culture method is the most reliable and accurate technique for food-borne pathogen detection (Rodriguez & Hernandez, 2006). The process of *Salmonella* detection begin with pre-enrichment in buffered peptone water or lactose broth, followed by selective enrichment in Rasspaport - Vasilliadis (RV) broth, Selenite Cysteine Broth (SC), Tetrathionate broth (TT) and then plate the cultured media on Brillant green agar, Bismuth sulfite agar or XLD (Mølbak *et al.*, 2006). The author further reported that the culture method is time-consuming, labor-intensive and took up to 8 days to get final test results. The culture method suffer from poor specificity due to difficulties in recovering sub-lethally injured cells, identification of typical colonies and high degree of false-positive results (Naravaneni & Jamil, 2005).

Enzyme-Linked Immuno-sorbent Assay (ELISA), immune-fluorescent antibody (IFA) techniques and radioimmunoassay are the immunological methods used to detect *Salmonella* based on the specific binding of antibodies to antigens (Gooding &

Choudary, 1997). According to Gooding and Choudary (1997), the immunological methods are used to prepare the samples for further identification of *Salmonella* using immunomagnetic separation technique. The immunological method took only 10-28 h to obtain the results, which is faster than conventional methods (Gooding & Choudary, 1997).

Other various methods which include immunofluorescence assay, the latex agglutination and the enzyme immunoassay were developed for the detection of *Salmonella* in foods (Mølbak *et al*., 2006). According to Mølbak *et al*. (2006), the enzyme immunoassay is rapid and sensitive with relative stable reagents and thus minimum equipment are required. The main limitation of immunoassay technique is that it gives a high percentage of false positive reactions due to non-specific nature of the polyclonal antibodies which was resolved by the use of Monoclonal Antibody which is specific to *Salmonella* (Fung, 2002).

Polymerase Chain Reaction (PCR) is the most widely used nucleic acid based technique to replicate target Deoxyribonucleic Acid (DNA) sequence. PCR amplification produces millions of copies of a targeted DNA in few hours (Persing, 1993; Nguyen, Khan & Lu, 1994). PCR technique has been used to amplify genes specific to *Salmonella* and also to detect genes involved in the virulence of *Salmonella* (Bej, Mahbubani, Boyce & Atlas, 1994). One of the drawbacks of PCR detection methods of DNA are the sensitive nature of the method and the reagents which make it prone to inhibition (Wilson, 1997).

2.7 *Escherichia coli* O157:H7

2.7.1 Introduction

E. coli was first discovered from feces of human neonates by a German pediatrician, Theodore Escherich in 1884 (Bray & Beavan, 1948; Khan & Steiner, 2002). *E. coli* live in digestive tracts of human and animals and is considered to be part of the normal flora of the intestine (Todar, 2005). *E. coli* are large and diverse group of bacteria that are generally harmless. *E. coli* are indicator organisms for fecal contamination and breaches in hygiene (Eblen, 2008).

E. coli O157:H7 was first isolated from a Californian woman with grossly bloody diarrhea in 1975 (Riley *et al*., 1983). *E. coli* O157:H7 was also firstly isolated from cattle in Argentina in 1977 (Fernandez, 2008). Since then, many pathogenic *E. coli* strains have been discovered. Hundreds of *E. coli* serotypes were identified based on somatic polysaccharide chain (O), flagellar (H) and capsular (K) surface antigen profiles using modified Kauffman scheme (Edwards & Ewing, 1972).

The increasing numbers of cases of *E. coli* O157:H7 outbreaks have been reported worldwide (Effler *et al*., 2001; Tarr, Gordon & Chandler, 2005). The most recent *E. coli* O157:H7 food-borne outbreak occurs when the ill people have consumed ground beef purchased from Fairbank farms causing numerous outbreaks in United States (Josefa, Phyllis, Collen, Patricia & David, 2015). *E. coli* O157:H7 strains were responsible for approximately 73,480 illnesses annually in the United States alone resulting in more than 2,000 hospitalizations and 60 deaths each year (Mead *et al*., 1999). The annual costs of *E. coli* O157:H7 infections were $405 million from 1996

to 2004, including $370 million premature deaths, $30 million medical care and $5 million lost in production (Frenzen, Drake & Angulo, 2005).

2.7.2 The species *Escherichia coli* and *Escherichia coli* O157:H7

Escherichia coli (*E. coli*) are considered harmless in human's intestine until 1935 when one strain was identified to cause diarrhea outbreak on infants. *Escherichia coli* are gram-negative and rod-shaped bacteria that usually move with the help of flagella. They are classified as members of the Family *Enterobacteriaceae* (Ewing, 1986). According to Ewing (1986), *E. coli* are facultative anaerobic bacteria and hence they are able to grow in the presence and absence of oxygen. *E. coli* are also able to respond to environment signals such as temperature, chemicals and pH. Identification of *E. coli* is determined by using Indole, Methy Red, Voges-Proskauer and Citrate tests. *E. coli* is negative for Voges-Proskauer test and Citrate test while it is positive for Indole test and Methy Red test (Todar, 2005).

Pathogenic strains are classified on the basis of their virulence properties, mechanisms of pathogenicity, clinical symptoms, and the presence of distinct O and H antigens (Doyle *et al.*, 1997). The grouping include; Enterotoxigenic *E. coli* (ETEC), Enteroinvasive *E. coli* (EIEC), Enterohaemorrhagic *E.coli* (EHEC) Enteropathogenic *E. coli* (EPEC) and Enteroaggregative *E. coli* (EAggEC) and of these groupings, EHEC has a potential to cause the most severe illness (Todar, 2005).

E. coli O157:H7 has been known as Verotoxin producing *E. coli* (VTEC) because it produces toxins which are toxic to Vero (Africa green monkey kidney) (Effler *et al.*,

2002). *E. coli* which produces Stx toxins are referred to as enterohaemorrhagic *E. coli*, verotoxin producing *E coli* (VTEC) and Shiga-Toxigenic *E. coli* (STEC) (Donnenberg & Whittam, 2001; Effler *et al*. (2002); Montville & Matthews, 2005).

2.7.3 Characteristics of *Escherichia coli* O157:H7

E. coli O157:H7 differs from other *E. coli* strains because it possesses the outer membrane with a lipopolysaccharide component which is different from the cytoplasmic membrane (Beneduce, Spano & Massa, 2003). *E. coli* O157:H7 is not able to ferment sorbitol and test negative for glucuronidase (Beneduce *et al*., 2003). *E. coli* O157:H7 has two types of antigen. The O157 antigen of *E. coli* O157:H7 is defined by the carbohydrate composition and structure within the lipopolysaccharide. The H7 antigen is determined by the unique polypeptide composition of the flagella (Beneduce *et al*., 2003).

E. coli O157:H7 growth and survival can be influenced by environmental factors. Okrend, Rose and Lattuada (1990) reported that *E. coli* O157:H7 does not grow at a temperature of 44.5 ˚C but other *E. coli* strains grow at temperature of 46 °C. *E. coli* O157:H7 is able to survive a temperature of 5 °C for 35 days (Weagant, Bryant & Bark, 1994). It also survive on mangoes and papayas stored at -20 °C for at least 180 days (Stfreshn & Danyluk, 2010). *E. coli* O157:H7 was found more tolerant to acid when it is in stationary growth phase or starved during its Log-phase of growth (Arnold & Kaspar, 1995). The high concentrations of salt (≥ 8.5%) showed an inhibitory effect on the growth of *E. coli* O157:H7 (Jay, 2000).

2.7.4 Sources of *Escherichia coli* O157:H7

The *E. coli* O157:H7 infection occurs from consumption of contaminated food, unpasteurized milk, disinfected water, contact with cattle and also contact with the infected person (Willshaw, Cheasty, Smith, O'Brien & Adak, 2001). Furthermore, cross-contamination during food preparation in kitchen can also lead to infection. Cattle are the main carriers of *E. coli* O157:H7, as they carry it in their intestinal tract, hides and faeces (Bettelheim, 1996; Elder *et al*., 2000; Pennington, 2010; Rahimi, Kazemeini & Salajegheh, 2012). The skin of animal and intestinal tract is major source of *E. coli* O157:H7 contamination (Chapman, Siddons, gerdan & Harkin, 1997; McEvoy *et al*., 2003). Drinking water, feed and the environment are also main sources of STEC infection in cattle (Rahimi *et al*., 2012).

The food products of bovine origin such as ground beef and raw milk have been frequently associated the largest outbreaks of *E. coli* O157 (Meng, Zhao & Doyle, 1998; Hajian, Rahimi & Mommtaz, 2011). *E. coli* O157:H7 outbreaks were also linked to ground beef hamburgers, ready-to-eat cold meats, milk, yoghurt, butter, ice creams, apple juices, grapes, spinach, lettuce, unpasteurized fruit juices and water (Morbidity and Mortality Weekly Results [MMWR], 2008).

2.7.5 Symptoms of *Escherichia coli* O157:H7 infection

E. coli O157:H7 infections are mild to life-threatening in susceptible individuals who show a range of symptoms such as hemolytic-uremic syndrome, haemolytic colitis and thrombotic thrombocytopenia purpura (HUS) (Bielaszewska *et al*., 2007). Other symptoms include vomiting, nausea and fever (Josefa *et al*., 2015). The normal

incubation period is around 3 to 9 days. Hemorrhagic colitis is characterized by diarrhea with visible blood, abdominal tenderness and abdominal cramps (Josefa *et al.*, 2015).

E. coli O157:H7 is responsible for the many cases of hemolytic uremic syndrome in North America. The illness may be complicated by hemolytic uremic syndrome whereby red blood cells are destroyed and the kidneys fail (Bielaszewska *et al*., 2007). The children of <5 years of age and the elderly are highly susceptible to HUS. Severe colitis often results in intestinal necrosis and the development of colonic structures (Rahimi *et al*., 2012).

2.7.6 *Escherichia coli* O157:H7 virulence factors

The pathogenicity of *E. coli* O157:H7 is encoded by various plasmids, bacteriophages and chromosomal genes (Kiranmayi, Krishnaiah & Mallika, 2010). *E. coli* O157:H7carry virulence genes which enable it to colonize intestines and induce either intestinal or extra-intestinal disease (Schroeder, White & Meng, 2004). Various virulence factors involved in pathogenic mechanisms include adhesins, invasins, toxins and secretion systems (Bekal *et al*., 2003).

The degree of pathogenicity depends on the combination of virulence genes each pathogenic *E. coli* possesses (Kaplan, Meyers & Schulman, 1998). The shiga toxin family contains two subgroups Stx1 and Stx2 which share approximately 55% amino acid homology (Kaper *et al*., 2004). The presence of other virulence genes includes

eaeA, *ehxA* or *saa* which contribute to the virulence for humans (Paton, Srimanote, Woodrow & Paton, 2001; Boerlin *et al*., 2005).

The significant virulence factor of Shiga Toxin-producing *E. coli* (STEC) is the production of shiga toxin (Stx) (Kaper, Nataro & Mobley, 2004). The production of shiga toxin is significant for the pathogenesis of bloody diarrhea and hemolytic uremic syndrome in humans (Kaper *et al*., 2004). The shiga toxin is produced in the colon and travels by the bloodstream to the kidney where it damages renal endothelial cells and renal inflammation. The shiga toxin also mediates damage in the colon which results in bloody diarrhea, haemorrhagic colitis, necrosis and intestinal perforation (Kaper *et al*., 2004).

2.7.7 *Escherichia coli* O157:H7 detection

E. coli O157:H7 can be detected in foods using various methods and techniques. The culture-based methods are common methods which are used to detect and identify bacterial pathogens, although, they are labor-intensive and time-costing (Food and Drug Administration [FDA], 2012). According to FDA (2012), *E. coli* O157:H7 grows well in selective broth media broth such as chromogenic agars and Sorbitol MacConkey Agar with Cefixime and Tellurite (CT-SMAC) which specifically isolate targeted *E. coli* O157:H7 in the samples. Typical colonies are colorless or neutral/gray with a smoky center and 1-2 mm in diameter on CT-SMAC (FDA, 2012).

Biochemical tests are used to characterize *E. coli* from other members of *Enterobacteriaceae*. Analytical Profile Index (API) is one of the rapid biochemical

system widely used to identify enteric bacteria within 4 hours and serological typing methods are used to confirm presumptive positive isolates (Deisingh & Thompson, 2004). Immunomagnetic Separation (IMS) is another technique that enhances the isolation rate of *E. coli* isolates by targeting micro-organisms directly from the enriched samples prior to the isolation step (Safarik, Safarikova & Forsythe, 1995).

Immunological detection methods are used to detect and enumerate *E. coli* O157:H7 based on the use of monoclonal or polyclonal anti-O157 antibodies (Tokarskyy & Marshall, 2008). According to Tokarskyy and Marshall (2008) the Enzyme-Linked-Immunosorbent-Assay (ELISA) is the common technique widely used in detecting food-borne pathogens because of its specificity, simplicity and sensitivity. There are four kinds of ELISA, namely; direct ELISA, indirect ELISA, direct sandwich ELISA and indirect sandwich ELISA and amongst all, sandwich ELISA is the most common method used to detect pathogens in food (Crowther, 2008).

2.7.8 Prevention of *Escherichia coli* O157:H7 contamination

The important quality assurance practices used in prevention of microbial contamination of meat and meat products includes HACCP and good hygiene practices (USDA, 2012). HACCP is an accepted measure widely used to prevent *E. coli* O157:H7 contamination to decrease the risk of microbial contamination in food processing plants (Pennington, 2010). Moreover, cooking ground beef and vegetable thoroughly before its eaten, avoid consuming raw milk and unpasteurized dairy products and practising hand washing are important measures to prevent cross-contamination in food preparation (Pal, 2007).

There is no specific treatment for *E. coli* O157:H7 infections, although blood transfusion and kidney dialysis have been used for the treatment of hemolytic uremic syndrome (Kaplan *et al*., 1998). Numerous other treatment modalities such as plasma infusion, plasma exchange, intravenous immunoglobulin, shiga toxin 19 inhibitors, prostacyclin and antithrombotic therapy have been tried, although their efficacy was never proved (Besser, Griffin & Slutsker, 1999).

CHAPTER 3: MATERIALS AND METHODS

3.1 Study design

The research work was held in the capital city, Windhoek from February 2015 to May 2015. Microbial analyses were carried out at Central Veterinary Laboratory (CVL) of Ministry of Agriculture, Water and Forestry (MAWF). Windhoek area was chosen for the study because the large proportion of the Namibian population that resides in the city prefers to buy fresh meat from the outlets.

This study was done in order to determine the microbiological quality and safety of beef collected from randomly selected outlets. This study is laboratory based research which was focused on three components namely; 1) total plate counts; 2) coliform counts; and 3) detection of *Salmonella* and *E. coli* O157:H7. Windhoek covers an area of 5133 km^2 with a population of about 322,500 (Fortune of Africa, 2016).

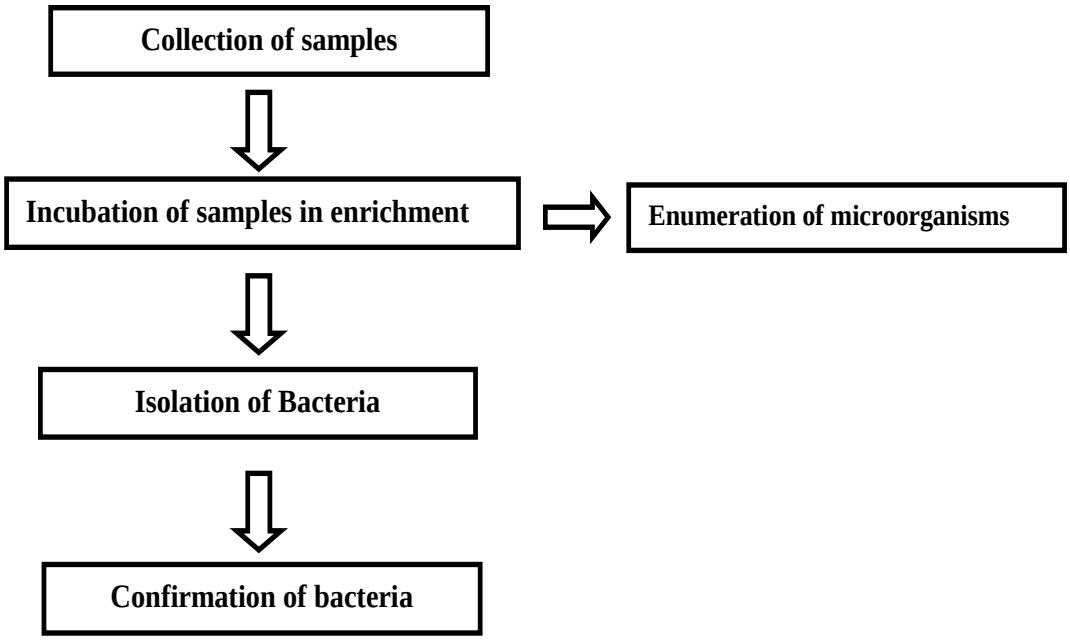

Figure 2. Schematic flow chart of research design that was used during this study.

3.1.1 Determination of sample size

The minimum sample size for this study was calculated as follows; with the confidence level of 95 % and a confidence interval of 5 %, the minimum sample size was calculated using the formula below assuming that the prevalence rate was 5 % for *Salmonella* and *E. coli* O157:H7.

$n = Z^2P(1-p)/d^2$

Where:

n = sample size,

Z = Z statistic for a level of confidence (1.96),

p = expected prevalence or proportion (0.05) and

d = precision (0.05)

From this formula, calculated minimum sample size was 73 samples taken deliberately to maximize the precision of the study.

3.2 Samples

The beef samples were obtained from three different outlets for microbiological analysis. A total of 138 samples of beef trims were randomly purchased from six supermarkets (n=46), single butchery, because large proportion of the population that reside in the city prefer to buy from this abattoir (n=46) and three open markets (n=46) during a period of February 2015 throughout to June 2015. Samples were used to perform total plate counts, coliform counts and *Salmonella* and *E. coli* O157:H7 identification.

3.3 Sample collection

The sample collection was focused on beef trims collected from six different supermarkets, butchery and three different open markets in Windhoek, Namibia. Sample collections from each retail outlet were done once every week. Samples were aseptically collected in sterile plastic bags and kept at between 2°C and 8°C during transportation to the laboratory for bacteriological quality assessment. The samples were analysed immediately upon arrival in the laboratory.

3.4 Microbial analysis

The study was conducted utilizing the method for horizontal enumeration of microorganisms using colony count technique. For total plate count, microorganisms were enumerated according to the spread plate technique standard procedure (International Organization for Standardization [ISO] 4833: 2003). For coliform counts, the coliform bacteria were enumerated according to spread plate technique protocol (Bhandare *et al*., 2007).

3.4.1 Preparation of samples

Beef sample (25 g) was weighed carefully and mixed with 225 ml of 0.85 % saline solution (Merck, Darmstadt, Germany) in a blender for 2 minutes. This mixture was considered to be a 10^{-1} dilution. The mixture (1ml) was transferred to a tube containing 9 ml of saline diluent to make 10^{-2} dilution. Further dilutions were made by transferring 1 ml of the succeeding dilutions to the tubes containing 9 ml diluent to achieve six-fold dilutions.

3.4.2 The enumeration of microorganisms by spread plate technique

The PCA agar (Biolab, South Africa) and EMB agar (Merck, Darmstadt, Germany) plates were taken and inoculated with 0.1 ml of the initial suspension (10^{-1} dilution). The procedure was repeated with the further dilutions until the last intended test dilution of 10^{-6}. Using a sterile glass rod, the inoculum was spread evenly on the PCA agar (Biolab, South Africa) surface/ EMB agar (Merck, Darmstadt, Germany) surface. The plates were covered to allow broth to soak into the agars for several minutes. The prepared dishes were inverted and placed in incubators. The prepared PCA agar plates for total plate count were incubated at 30°C for 72 hours while EMB agar plates for coliform count were incubated at 37°C for 24 hours. Following incubation, the colonies were counted visually under a subdued light source using a colony counter. Examination of the plates was done as soon as they were removed from the incubator. The bacterial colonies were counted and recorded. The pinpoint colonies were included in the count and the spread colonies were counted as a single colony. The coliforms bacteria were counted as colonies with metallic sheen, non-metallic sheen and dark centers.

Average microbial counts obtained were multiplied by the dilution factor and expressed as Colony Forming Unit per gram (CFU/g) (Fawole & Oso, 2001). The total plate counts and total coliform counts were reported as CFU/g using a standard formula in computation of CFU/g (James, John & Charles, 2014). The final counts were expressed in Log CFU/g and were presented by rounding off to the nearest decimal place.

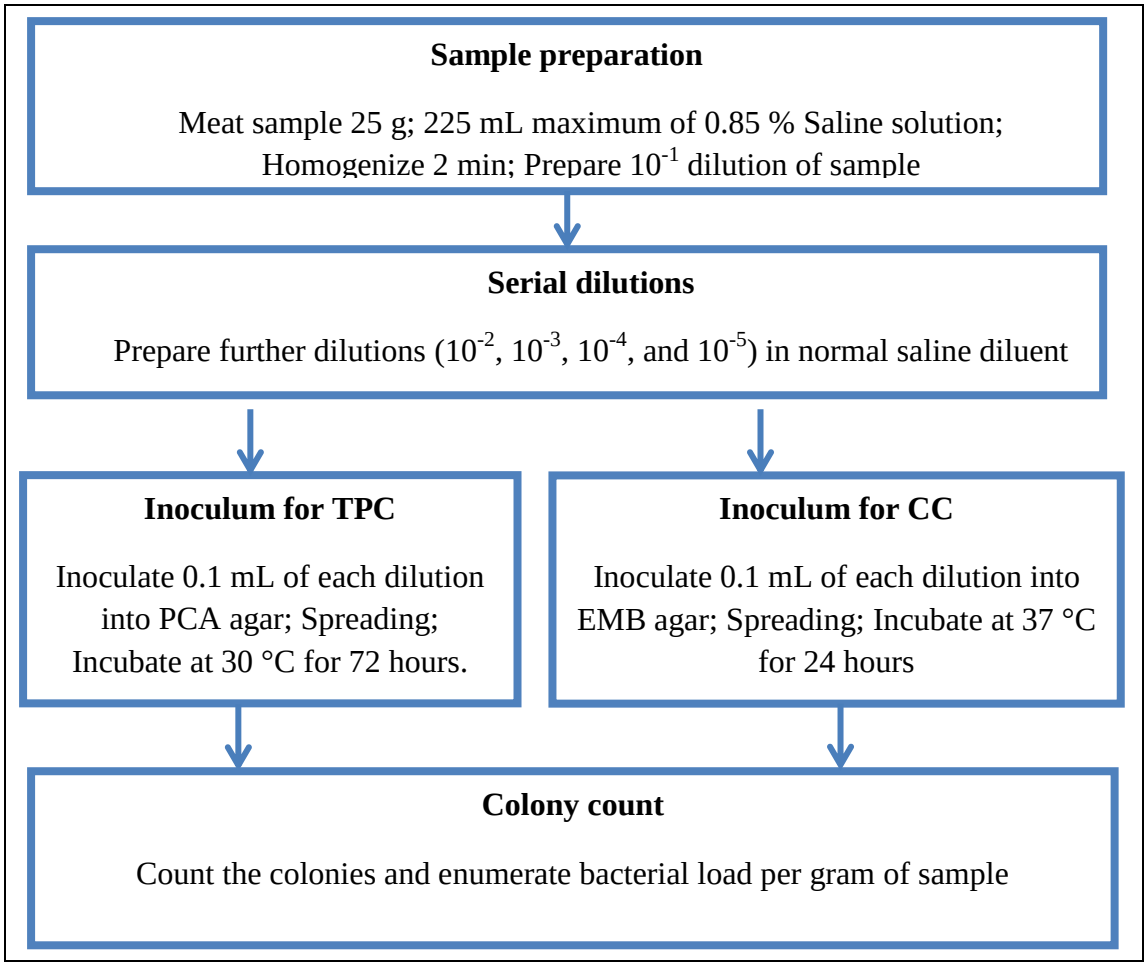

Figure 3: Show the flow chart for the enumeration of total plate counts and coliform counts by spread plate technique.

Salmonella was isolated from beef samples obtained from the outlets. The study was conducted utilizing the conventional methods for the detection of *Salmonella* following the standard guide lines (Microbiology of food and animal feeding stuffs horizontal method for the detection of *Salmonella* spp.) (ISO 6579: 2002). The study was conducted utilizing the conventional methods for the detection of *E. coli* O157:H7 following the standard guide lines from ISO 16654:2001 (Microbiology of food and animal feeding stuffs horizontal method for the detection of *E. coli* O157:H7) (ISO 16654: 2001).

3.4.3.1 Enrichment and culturing of *Salmonella*

Meat sample (25 g) was weighed and transferred into a sterile stomacher bag. A 225 ml of buffered peptone water (BPW) (Merck, Darmstadt, Germany) was added to the sample. The meat sample was homogenized in a stomacher for 2 minutes and then incubated at 37 ºC for 18 h to permit for the detection of low numbers of *Salmonella*. Following incubation, samples were inoculated to Rappaport-Vassiliadis (RVS) and Müller-Kauffmann-Tetrathionate novobiocin (MKTTn) broths, respectively. The culture (0.1 ml) was transferred to 10 ml of the RVS broth (Merck, Darmstadt, Germany) and incubated at 41.5 ºC for 24 h. In parallel to this, 1 ml of the culture was inoculated into 10 ml MKTTn broth (Merck, Darmstadt, Germany) and incubated at 37 ºC for 24 h.

3.4.3.2 Identification of putative *Salmonella*

Following incubation, a loop-full of the enriched cultures of RVS broth and MKTTn broth was streaked separately onto two selective agar plates: Xylose Lysine Deoxycholate (XLD) agar (Merck, Modderfontein, South Africa) and Bismuth Sulphite Agar (BSA) (Merck, Darmstadt, Germany) and incubated at 37 ºC for 24 h. Typical *Salmonella* colonies were black centered and slightly transparent zone of reddish color on XLD agar and black centered, light edges surrounded by a black precipitate with metallic sheen on Bismuth Sulfate Agar (BSA) (Merck, Darmstadt, Germany). The colonies were selected and inoculated on nutrient agar (Merck, Modderfontein, South Africa). The suspected *Salmonella* colonies were sub-cultured on the nutrient agar and incubated at 37 °C for 24 h. Following incubation, typical colonies were picked and cultured in 5 ml nutrient broth at 37 °C for about 6 h. Two

milliliters of the suspicious *Salmonella* culture was dispensed in Eppendorf tubes and kept in nutrient broth stored at -80 °C for further confirmation.

3.4.3.3 *Salmonella* confirmation by BAX® system

Salmonella culture (5 µl) from nutrient broth was transferred to 10 ml of Buffered Peptone Water (BPW) (Merck, Darmstadt, Germany) and incubated at 37 ℃ for 24 h. A negative control (blank BPW) and positive control (spiked BPW with 5 µl of *Salmonella* Typhi and *Salmonella* Typhimurium culture) were incubated together with the samples. Following incubation, the lysis buffer was prepared by adding 150 µL of protease to one bottle of lysis buffer. A 200 µL of lysis reagent was transferred to cluster tubes and 5 µL aliquot of enriched sample was transferred to the corresponding cluster tubes containing lysis reagent. The cluster tubes containing sample solutions were placed on pre-warmed heating block at 37 °C for 20 minutes and later at 95 °C for 10 minutes. Following heating, the cluster tubes were placed on the chilled cooling block at 5 °C for 5 minutes. The appropriate number of PCR strips was placed in chilling block at 5°C and 50 µL of lysate was transferred to the PCR tubes, containing a tablet of all reagents for the PCR. The PCR tubes were placed in the BAX® cycler and the results were analyzed with DuPont BAX® Q7 software (Version 3.0).

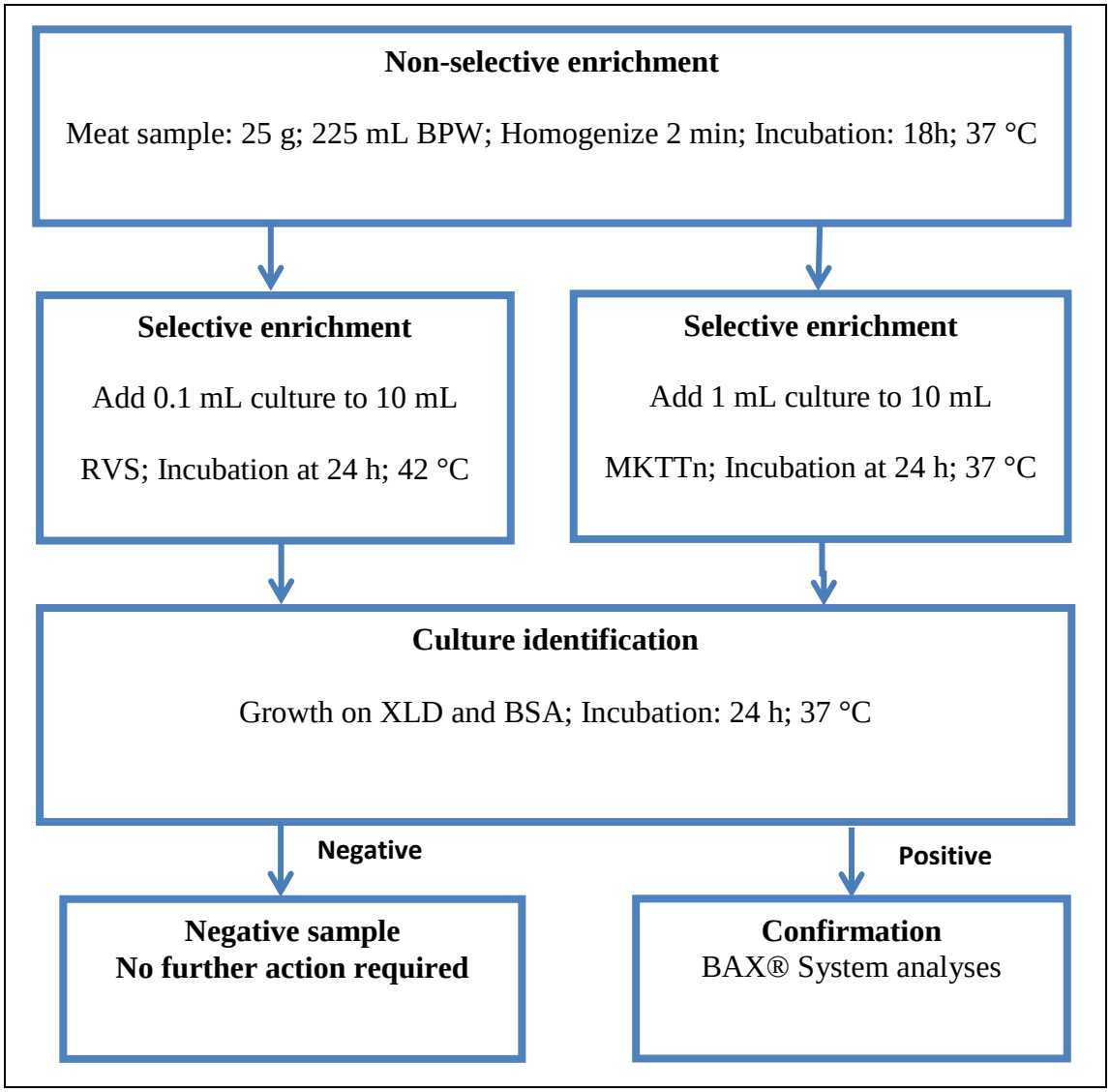

Figure 4 Schematic representation of *Salmonella spp* detection.

The meat sample (25 g) was aseptically transferred to 225 ml of modified Trypticase Soy Broth (mTSB) (Merck, Darmstadt, Germany) containing 0.5 mg/ml novobiocichen for selective enrichment of *E. coli* O157:H7. The sample was homogenized in a stomacher for 3 minutes and incubated at 37 °C for 24 hours. The negative control (blank mTSB) and positive control spiked mTSB with 5 µL of positive of the positive reference culture strain (*E. coli* O157:H7) were incubated together with the samples.

Following incubation, two-fold serial dilution of the non-selective enrichment was performed by adding 1ml of culture samples to 9 ml of 0.85% saline solution. A loop full of materials from the 10^{-1} and 10^{-2} dilutions was streaked onto sorbitol MacConkey medium (SMAC) (Merck, Darmstadt, Germany). The plates were incubated aerobically at 37 °C for 24 hours. After incubation, typical whitish gray colonies on SMAC which depict potential *E. coli* O157:H7 were observed (Kang & Fung, 1999).

The colonies were picked from SMAC agar and then streaked onto SMAC agar supplemented with Cefixime-Tellurite (CT, Difco) (CT-SMAC) (Merck, Darmstadt, Germany) to investigate CT resistance and the purity of the colonies (March & Ratnam, 1986). The cultured CT-SMAC plates were incubated at 37 °C for 24 hours. The colorless or neutral/grey colonies with smoky center which shows *E. coli* O157:H7 were observed on colony counter.

The presumptive colonies were picked from CT-SMAC and sub-cultured on tryptic soy agar (TSA) plates and were incubated at 35 °C for 24 hours (Baran & Gulmez, n.d.). Following incubation, typical CT resistant colonies were picked from the TSA (Merck, Darmstadt, Germany) agar and cultured in 5 ml nutrient broth at 37 °C for about 6 h. Two milliliters of the suspicious *E. coli* O157:H7 culture was dispensed in Eppendorf tubes and kept in nutrient broth stored at -80 °C for further confirmation.

3.4.3.5 *E. coli* O157:H7 confirmation by BAX® System

The culture (5 µL) from the nutrient broth was transferred to 10 mL of *E. coli* broth and incubated at 37 ℃ for 24 h. The negative control (blank *E. coli* broth) and positive control (spiked *E. coli* broth with *E. coli* O157:H7 culture) were incubated together with the samples. Following incubation, the lysis buffer was prepared by adding 150 µL of protease to one bottle of lysis buffer. A 200 µL of the prepared lysis reagent was transferred to cluster tubes and 5 µL aliquot of enriched sample was transferred to the corresponding cluster tubes containing lysis reagent.

The cluster tubes containing sample solutions were placed on pre-warmed heating block at 37 °C for 20 minutes and at 95 °C for 10 minutes. Following heating, the cluster tubes were placed on the chilled cooling block at 5 °C for 5 minutes. The appropriate numbers of PCR strips were placed in chilling block at 5℃ and 50 µL of lysate was transferred to the PCR tubes, containing a tablet of all reagents for the PCR. The PCR tubes were placed in the BAX® cycler and the results were analyzed using DuPont BAX® Q7 software (Version 3.0).

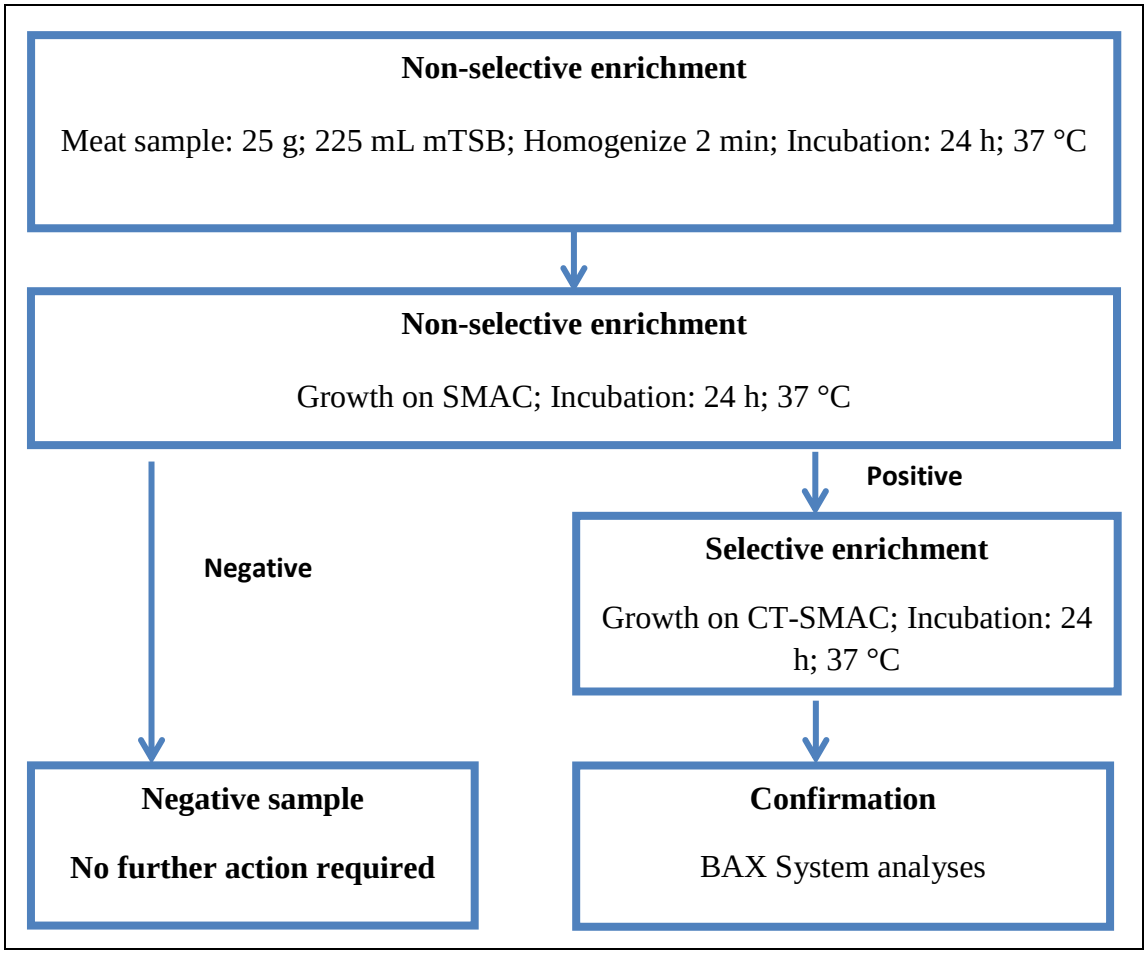

Figure 5: Schematic representation of *E. coli* O157:H7 detection.

3.4 Data analysis

Data were entered and organised in Microsoft excel software. Simple descriptive statistics such as mean, percentage were carried out. The Normality test was used to determine the normal distribution and data were found to be normal distributed. The parametric tests; Chi-squared (χ^2) goodness of fit and ANOVA test was used to analyze data and the differences were considered significant at values of $P < 0.05$. The relationships between microorganisms were compared using correlation analysis at $P<0.01$. The data were presented in the form of tables and graphs

3.5 Research ethics consideration

The following research ethic was taken into consideration. The research did not disclose client's information to protect the privacy and confidentiality of the outlets owners in order to avoid jeopardize the owners businesses. Therefore, the naming of the outlets was done using letters such as A, B, C…. or X, Y, Z.

CHAPTER 4: RESULTS

4.1 Total Plate Count and Coliform Count

The mean total plate count and coliform count of beef samples between the three different outlets are presented in **Figure 6 and Figure 7**. The results obtained indicated that the overall mean TPC of beef samples from three different outlets was 4.01 Log CFU/g within the acceptable range (<5 Log CFU/g). Supermarkets had the highest bacterial count, 4.31Log CFU/g followed by butchery, 3.83Log CFU/g with open markets recording the lowest count of 3.68Log CFU/g. During the study, the mean TPCs of beef samples from three different outlets were compared to establish any statistical significant difference. However, there was no significant difference in the mean total plate count for beef among the three outlets (P=0.188 at P<0.05).

The overall mean CC of beef samples from three outlets was 1.70 Log CFU/g within the acceptable limit (<3 Log CFU/g). Open markets had the highest bacterial count, 2.08 Log CFU/g followed by butchery, 1.71 Log CFU/g with supermarkets recording the lowest count of 1.31 Log CFU/g. During the study, the mean CCs of beef from three outlets were compared to establish any statistical significant difference. However, there was no significant difference in the mean coliform counts on beef samples among the three outlets (P=0.421 at P<0.05).

Quality of beef suitable for consumption based on TPC and CC is illustrated in **Figure 8**. The overall prevalence of TPC on the beef samples was 95 (98.9 %). Of these 95 (98.9 %), 25 (26 %) samples were satisfactory, majority 47 (49 %) samples were within acceptable level and minority of samples 24 (25.0 %) exceeded the

acceptable level. The overall prevalence of CC of beef samples was 49 (56.3 %). Of these 49 (56.3 %), majority of samples 48 (55.2 %) were satisfactory, 16 (18.4 %) were within the acceptable limit and (26.4 %) of samples had CC exceeded the acceptable level.

Limits	TPC Log CFU/g	CC Log CFU/g
Satisfactory	3.5	1.5
Acceptable	<5.0	<2.5
Unacceptable	>5.0	>2.5

Table 2. The microbiological criteria for raw meat according ICMSF

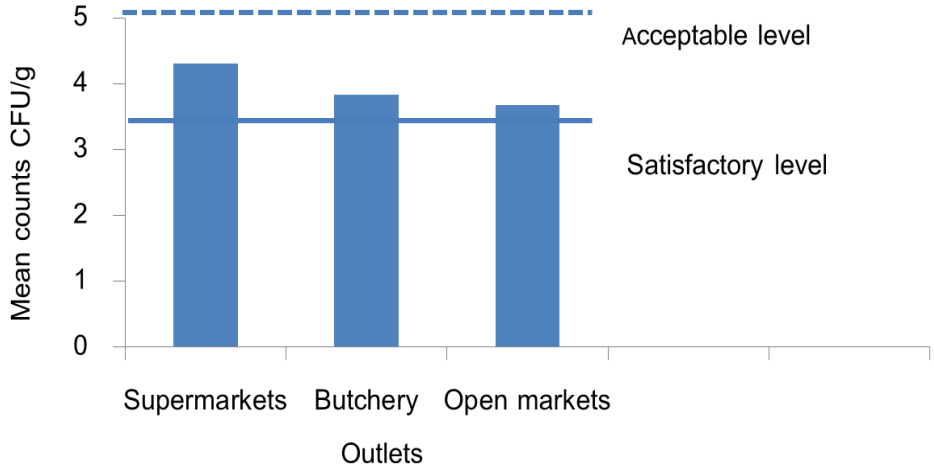

Figure 6. The mean TPC for beef from the three outlets

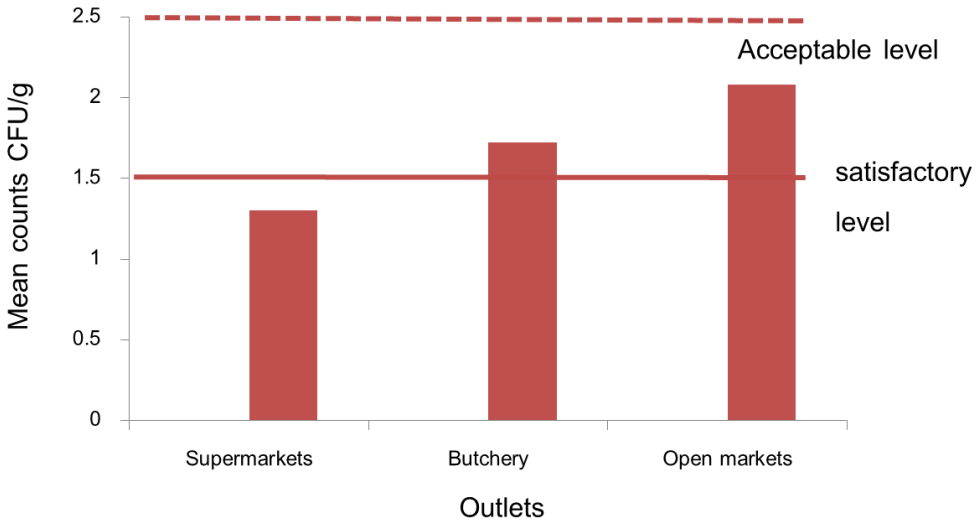

Figure 7. The mean CC for beef from the three outlets

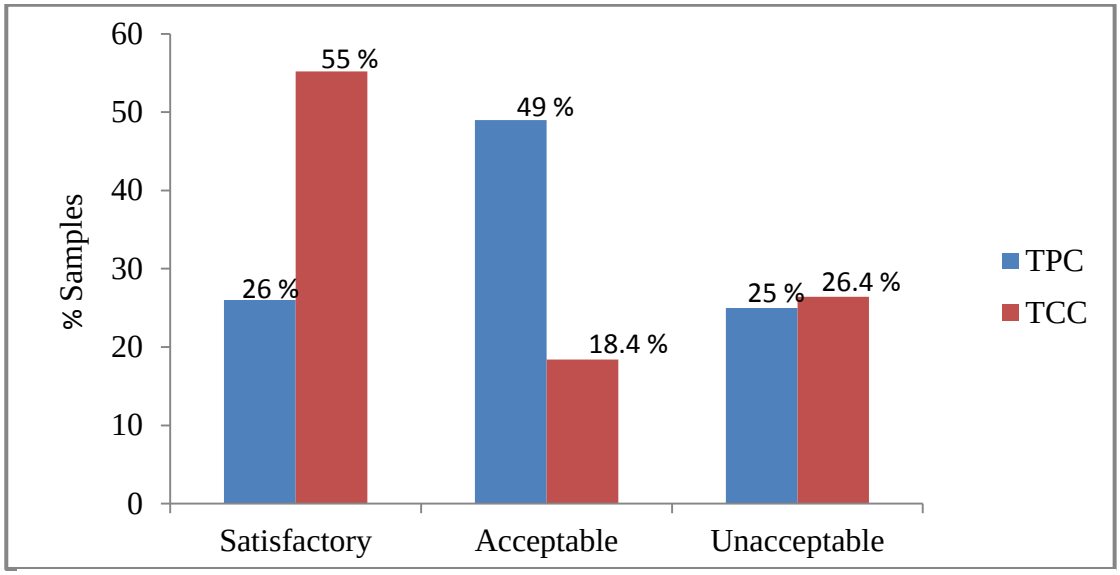

Figure 8. Quality of beef suitable for consumption based on TPC and CC.

The mean total plate count and coliform count on beef samples from supermarkets are presented in **Figure 9**. The results indicated that the mean TPCs on beef samples from supermarket A, B, C, D, E and F were 4.72, 4.87, 4.38, 4.90, 3.48 and 2.61 Log CFU/g, respectively. All supermarkets had mean TPC within acceptable range except

supermarket F which had mean TPC at the satisfactory level. Supermarket F had lowest mean TPC (2.60 Log CFU/g) whereas supermarket D had highest mean count (4.90 Log CFU/g). During the study, the mean TPC of beef from six supermarkets were compared to establish any statistical significant difference. However, there was no significant difference in the mean TPC between six supermarkets (P=0.174 at P<0.05).

The mean CCs from supermarket A, B, C, D, E and F were 1.49, 2.80, 0.33, 0.00, 2.74 and 0.93 Log CFU/g, respectively. The supermarkets A, C, D and F had mean TCC within the satisfactory level whereas supermarkets B and E had mean CC within the acceptable level. The supermarket D had lowest mean CC (0.00 Log CFU/g) whereas supermarket B (2.80 Log CFU/g) had the highest mean count. During the study, the mean CC of beef from six supermarkets was compared to establish any statistical significant difference. There was a significant difference in the mean CCs between six supermarkets (P=0.031 at P<0.05).

The mean total plate counts and coliform counts on beef samples from open markets are presented in **Figure 10**. The results showed that the mean TPCs on beef samples from open market X, Y and Z were 4.33, 4.28 and 2.95 Log CFU/g, respectively. Open market X and Y had mean TPC within acceptable level whereas open market Z had mean TPC within satisfactory level. During the study, the mean TPC of beef from three open markets were compared to establish any statistical significant difference. The open market Z had lowest mean TPC (2.95 Log CFU/g) whereas open market X

had higher TPC (4.33 Log CFU/g). There was significant difference in the mean TPC on beef samples from three open markets (P=0.022 at P<0.05).

The mean CCs on beef samples from open market X, Y and Z were 2.59, 2.80 and 0.89 Log CFU/g, respectively. Open market X and Y had mean CC within acceptable limit while open market Z had mean CC within satisfactory level. During the study, the mean TCC on beef from three open markets were compared to establish any statistical significant difference. There was significant difference in the mean CC among three open markets (P=0.008 at P<0.05). The open market Z had lowest mean CC (0.89 Log CFU/g) whereas, open market Y had highest mean CC (4.28 Log CFU/g).

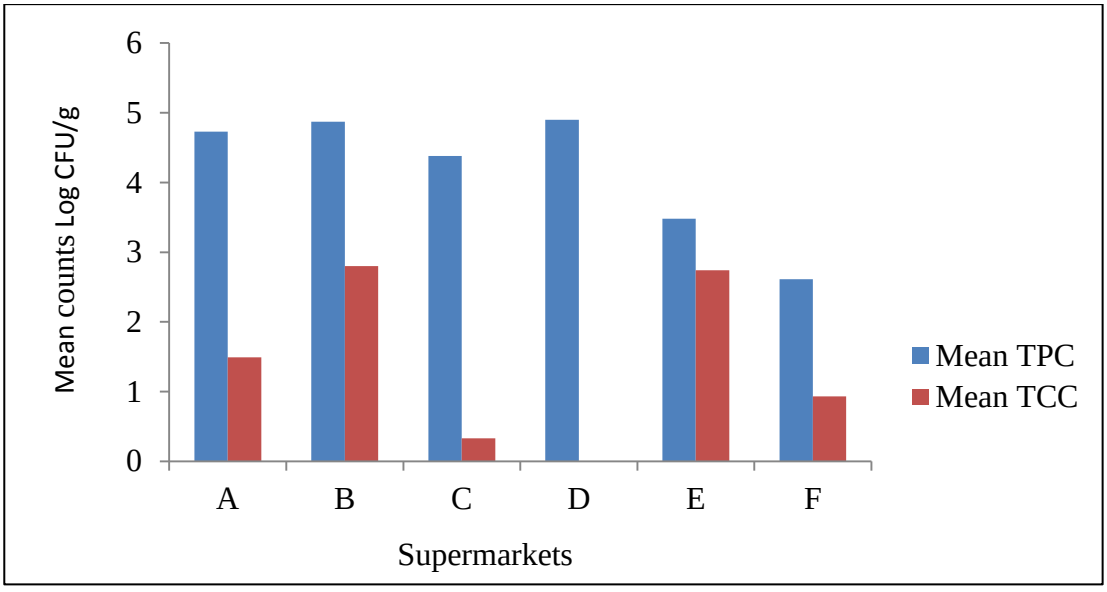

Figure 9. The mean total plate counts and coliform counts on beef samples from supermarkets

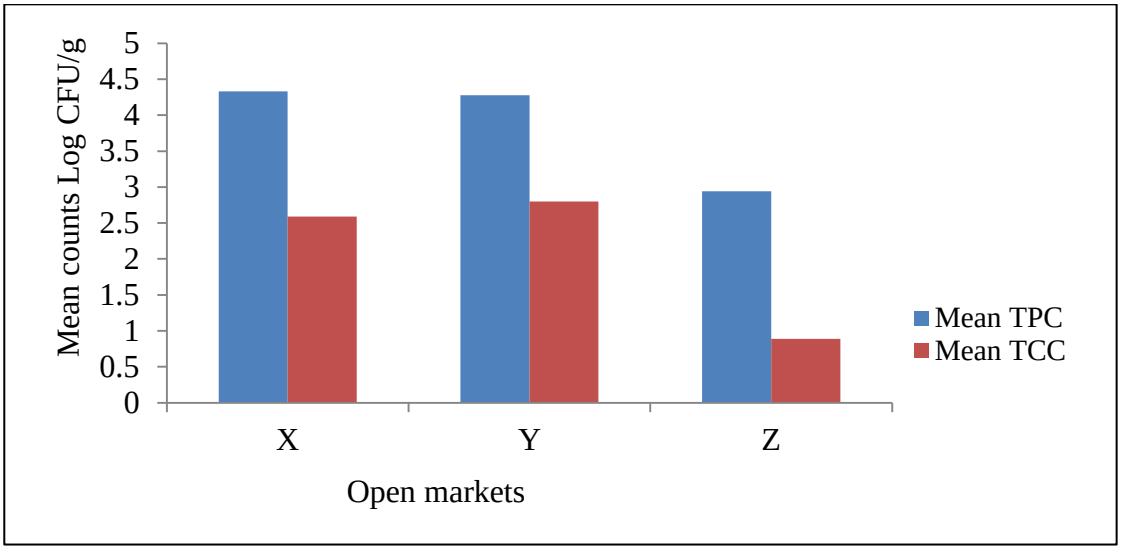

Figure 10. The mean total plate counts and coliform counts on beef samples from open markets.

4.2 Prevalence of *Salmonella* and *Escherichia coli* O157:H7

The comparisons of the prevalence of *Salmonella* and *E. coli* O157:H7 on fresh beef between the three different outlets are presented in **Table 4**. From a total of 138 beef samples collected from three outlets, the overall prevalence of *Salmonella* was 68 (49.3%) for beef samples. Of the 68 (49.3 %), the highest prevalence of *Salmonella* was 31 (67.4 %), followed by the open markets 24 (52.2 %) from the butchery and lowest prevalence was 13 (28.3 %) from the supermarkets. On comparison of the prevalence of *Salmonella* on beef from the three different outlets, there was a significant difference in the prevalence of *Salmonella* on beef samples among the three different outlets (P=0.00000609 at P<0.05).

From a total of 138 beef samples collected from the three outlets, the overall prevalence of *E. coli* O157:H7 was 8 (5.79 %). Of the overall prevalence 8 (5.79 %),

the prevalence of *E. coli* O157:H7 was 5 (10.9 %) from the open markets, 2 (4.35 %) from the butchery and 1 (2.17 %) from the supermarkets. On comparison of prevalence of *E. coli* O157:H7 on beef from the three types of retail outlets, there was no significant difference on the prevalence of *E. coli* O157:H7 on beef samples among the three outlets (P=0.1785 at P<0.05). The open markets were observed with the highest prevalence of *E. coli* O157:H7 for beef followed by butchery and the lowest prevalence was observed in supermarkets.

Table 3. The comparisons of the prevalence of *Salmonella* and *E. coli* O157:H7 for beef samples from three different outlets.

Retail outlets	No. of samples	*Salmonella* n (%)	*E. coli* O157:H7 n (%)
Informal markets	46	31 (67.4)	5 (10.9)
Butchery	46	24 (52.2)	2 (4.35)
Supermarkets	46	13 (28.3)	1 (2.17)
Total	138	68 (49.3)	8 (5.79)

n=number of isolates, %=prevalence of isolates

The prevalence of *Salmonella* and *E. coli* O157:H7 on beef samples collected from supermarkets are presented in **Figure 11**. The prevalence of *Salmonella* on beef samples from supermarket A, B, C, D, E and F were 33.0 %, 22.0 %, 0.00 %, 57.0 %, 50.0 % and 20.0 %, respectively. Supermarket C had lowest prevalence *Salmonella* (0.00 %) whereas supermarket D (57.0 %) had the highest prevalence of *Salmonella*,

followed by supermarket E (50.0 %). On comparison of *Salmonella* prevalence on beef from six supermarkets, the prevalence of *Salmonella* on beef samples was not significantly different among supermarkets (P=0.066 at P<0.05).

The prevalence of *E. coli* O157:H7 on beef samples from six supermarket A, B, C, D, E and F were 0.00 %, 0.00 %, 0.00 %, 0.00 %, 0.00 % and 20.0 %, respectively. Supermarket A, B, C, D and E had the lowest prevalence of *E. coli* O157:H7 (0.00 %) whereas supermarket F (20.0 %) had the highest prevalence of *E. coli* O157:H7. On comparison of *E. coli* O157:H7 prevalence on beef from six supermarkets, there was no significant difference on the prevalence of *E. coli* O157:H7 between six different supermarkets (P=0.057 at P<0.05).

The prevalence of *Salmonella* and *E. coli* O157:H7 on beef samples from three open markets are shown in **Figure 12**. The prevalence of *Salmonella* on beef from open market X, Y and Z were 75.0 %, 63.0 % and 50.0 %, respectively. The open market Z had lowest prevalence (50.0 %) of *Salmonella* and open market X (75.0 %) had the highest prevalence of *Salmonella*. On comparison of *Salmonella* prevalence on beef from three open markets, there was no significant difference in recovery of *Salmonella* among the three different open markets (P=0.214 at P<0.05).

The prevalence of *E. coli* O157:H7 on beef samples were 13.0 %, 0.00 % and 21.0 % from open market X, Y and Z, respectively. The open market Y (0.00 %) had the lowest prevalence of *E. coli* O157:H7 whereas open market Z (21.0 %) had the highest prevalence of *E. coli* O157:H7. On comparison of *E. coli* O157:H7 prevalence

on beef from three open markets, there was no significant difference in recovery of *E. coli* O157:H7 between the three open different markets (P=0.151 at P<0.05).

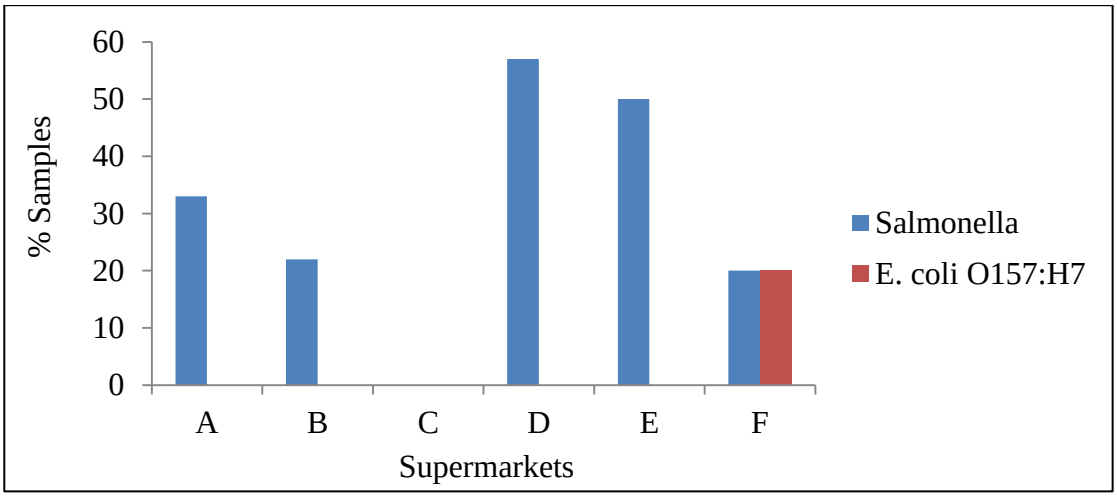

Figure 11. The prevalence of *Salmonella* and *E. coli* O157:H7 on beef samples from six supermarkets.

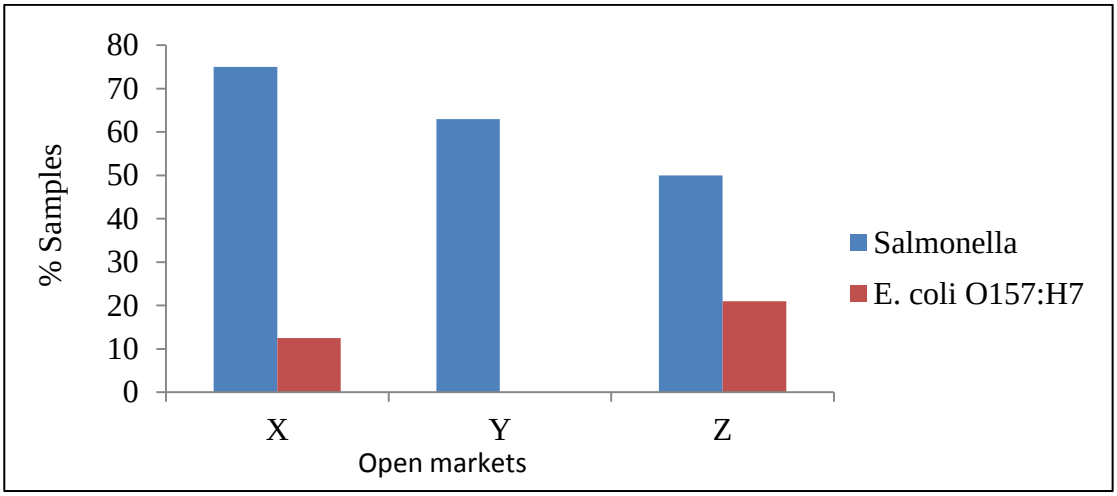

Figure 12. The prevalence of *Salmonella* and *E. coli* O157:H7 on beef samples from three open markets.

4.3 Correlation between TPC, CC, *Salmonella* and *E. coli* O157:H7

Table 5 shows the correlation between microorganisms on beef samples from the three outlets. A significant correlation ($P<0.01$) was found between TPC, CC, *Salmonella* and *E. coli* O157:H7. The results also indicated that there was a strong correlation between CC and *Salmonella*.

Table 4. Correlation matrix between the prevalence of microorganisms for beef from the three different outlets.

Variables	TPC	CC	*Salmonella*	*E. coli* O157:H7
TPC	1			
TCC	0.36	1		
Salmonella	0.27	0.68	1	
E. coli O157:H7	0.22	0.30	0.17	1

CHAPTER 5: DISCUSSION

5.1 Total plate count and total coliform count

Total plate count is one of the indicator microorganisms used to measure the microbial quality of the meat and is a useful tool in monitoring food safety. The overall mean TPC (4.01 $\log_{10}$ CFU/g) of beef from three outlets was within the acceptable standard specified by the International Commission on Microbiological Specifications for Foods (ICMSF) (1986) which indicates good microbial quality of beef and these could be due to proper hygiene practices employed.

In the present study, only 25 % and 26.4% of the beef samples had mean TPC and TCC respectively exceeded the acceptable limit. A study by Ahmad *et al*., 2013 found out that 51% of samples had APC more than 6 log10 CFU/cm2, which indicates highly contaminated meat which suggests that beef offered for sale in the three outlets, is of good hygienic quality.

Lower level of total plate count of the present study did not agree with previous studies (Hassan *et al.*, 2010). This lower microbial contamination in the present study could be due to proper cold storage conditions and good hygiene practices employed at the retail outlets. The availability of refrigerated conditions for beef storage at the outlets suppresses microbial growth and multiplication. This is also supported by Chaubey *et al*. (2004) who stated that fresh meat and meat products stored in cool places had minimum microbial growth that is necessary to safe guard the health of consumers. Delmore (2009) also suggested that the adoption of proper storage temperature and hygienic effectively contribute to improving the safety and quality of raw meat. Another study by Li, Sherwood and Logue (2004) reported that lower

temperatures suppress growth and multiplication of mesophilic bacteria and hence may have accounted for the lower counts in the retail outlets.

In the present study, there was no significant difference (P=0.188 at P<0.05) in the mean TPC among the three outlets. This condition could be explained by similar hygienic practices conducted. This study was not in agreement with a study done by Farhana, Mahbub-E-Elahi and Siddique (2015) who recorded the variations in total plate count between markets. A similar study done by Francis *et al*. (2015) reported the significant differences (P<0.05) in the TPC and TCC between supermarkets and local markets. The condition of the market and the hygienic practice employed by meat sellers and butchers maybe causes the variation. The work done by Ruban and Fairoze (2011) attributed higher microbial levels from non sophisticated outlets compared to the processing units.

In the present study, the mean total plate count was found be 4.31Log CFU/g for supermarkets, 3.83Log CFU/g for butchery and 3.68Log CFU/g for open markets. These findings are comparable with other studies done in the region and elsewhere outside the region. A study done in Ghana on the TPC from beef in open markets found the mean TPC to be 6.36-8.47 Log CFU/g (Francis, Abraham & Victoria, 2015). Another study done in Ghana supermarkets found the mean total plate counts to be 5.01-8.32 Log CFU/g (Francis *et al.*, 2015). A study done in Ethiopia found the mean count to be 4.00-5.00 Log CFU/g on the microbial flora of fresh beef from butcheries in Awasa, Ethiopia (Ashenafi, 1994). Variations in total plate counts

among studies may be due to differences in numbers of samples collected, the manner in which they were collected, the season in which the samples were collected.

The coliform count is a good indicator of faecal contamination of food animals and indicates greater risk of exposure to pathogenic organisms. The overall mean CC (1.70 $\log_{10}$ CFU/g) of beef from the three outlets was within the acceptable standard specified by the International Commission on Microbiological Specifications for Foods (ICMSF) (1986) which indicates low faecal contamination. These could results from proper cold storage conditions employed which influence growth of bacteria. The insignificant difference in the mean CCs (P=0.421 at P<0.05) of beef samples among the three outlets could be due to similarity of storage conditions employed

In the present study, the mean coliform count was found to be 2.08 Log CFU/g for open markets 1.71 Log CFU/g for butchery and 1.31 Log CFU/g for supermarkets. These findings are comparable with other studies done in the region and elsewhere outside the region. A study done in Ethiopia on the CC from beef found the mean TCC to be 3.00-5.00 Log CFU/g on the microbial flora of fresh beef from supermarkets (Ashenafi, 1994). Another study done in Ghana found total coliform count to be 6.14-8.35 Log CFU/g of raw beef in open markets of Ghana (Francis, Abraham & Victoria, 2015). Variations in coliform counts among studies may be due to differences in storage conditions and season in which samples were collected.

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5.2 Total plate count and coliform count among six supermarkets

When the mean TPC was compared among six supermarkets, the insignificant difference (P=0.174 at P<0.05) in the mean TPC on beef samples from supermarkets could explained by the similar processing methods and hygiene practices employed by food handlers in different supermarkets. The higher mean TPC observed on beef samples from one supermarket maybe due to excessive handling of beef carcasses during processing. The pressure from the workload by handling large quantities of beef results in cross contamination. The meat handlers could not adhere to handling practices during processing and hence contaminate the meat. The reported lower mean TPC on beef from one supermarket could be explained by proper hygiene, training and supervision during processing and marketing.

When the mean CC on beef was compared among six supermarkets, the significant difference (P=0.031 at P<0.05) in the mean CC between supermarkets can be explained by differences in storage conditions employed. The observed higher mean CC on beef samples from one supermarket is attributed to incorrect storage conditions for beef preservation. The observed lower mean CC on beef from one supermarket could be explained by proper cold conditions during beef storage.

5.3 Total plate count and coliform count among three open markets

When the mean TPC was compared among three open markets, the significant difference (P=0.022 at P<0.05) in the mean TPC for beef samples between the open markets could be explained by the different handling, processing and hygiene practices employed at open markets. The present report is also in agreement with

Haileselassie *et al*. (2013) who reported a statistically significant difference (P<0.0075) in the microbial load between street sale. The observed higher mean TPC on beef samples from one open market may be attributed to handling beef with unwashed hands and use of unwashed cutting tables and knives. Ali *et al*. (2010) reported that meat sellers at the open markets lack knowledge of disinfecting and sanitizing the working environment to maintain hygienic environment of the shops. The observed lower mean TPC on beef from one open market could be due to cleaning and sanitize their hands, cutting tables and knives when handling beef carcasses.

When the mean CC was compared between open markets, the insignificant differences (P=0.008 at P<0.05) in the mean CC on beef samples between open markets could be due to similar practice of displaying beef in the open air when selling to consumers. The observed higher mean CC on beef samples from one open market could be explained by dirty wrapping materials, poor personal hygiene, flies and dusts. According to Nychas *et al.* (2008), temperature affects the bacterial growth and the composition of the bacterial flora which maybe accounted for the high counts. The lower mean TCC on beef from one open market can be explained by meat sellers washing hands with soaps after using the toilets before they handle beef with their bare hands.

5.4 Prevalence of *Salmonella* and *Escherichia coli* O157:H7

Salmonella is the main cause of food-borne illnesses in humans commonly known as salmonellosis (Patrick *et al.*, 2004). The higher prevalence of *Salmonella spp.*

reported in this study indicates high risk of *salmonella* transmission to consumers and could be due to poor implementation of HACCP plans and improper handling practices. HACCP is a safety tool that is used in food production to prevent, reduce or eliminate contamination risks in the food production chain. According to Chaubey *et al*. (2004), meat and meat products which are not handled under strict hygienic condition often leads to pathogen contamination. However, further research is needed to establish the factors that lead to higher prevalence of *Salmonella* in the outlets.

The significant difference (P=0.00000609 at P<0.05) on the prevalence of *Salmonella* between the three outlets can be explained by different levels of implementing HCCP plan at the retail outlets. The higher prevalence of *Salmonella* on beef samples collected from the open markets as compared to butchery and open markets may be primarily due to poor hygienic and sanitary practices employed at the open markets. This condition is due to the absence of HCCP plans and GHP procedures at the retail points. Filimon, Borozan, Radu and Popescu (2010) suggested that poor personal hygiene practices such as failure to practice regular and effective handwashing after visiting the toilet leads to *Salmonella* contamination. The lower prevalence of *Salmonella* in the beef samples from supermarkets could be due to proper cleaning and sanitizing cutting boards and equipment surfaces such as knives, grinders and mincers conducted at the abattoirs supplying the beef.

The overall prevalence of *Salmonella* (49.3%) obtained in the present study was compared to a similar study done on beef from commercial slaughterhouses in Namibia. The prevalence of *Salmonella* of the present study from three outlets was

higher than the prevalence of 0.85 % on beef samples collected from commercial abattoirs in Namibia (Shilangale, Chimwamurombe & Kaaya, 2015). This could be due to bacterial contamination of meat that can occur during the transport of bovine carcasses from the slaughterhouse to the meat processing units, during cutting and mincing operations and marketing of beef in retail outlets. Niyonzima, Bora & Ongol (2013) reported an increase in *Salmonella* load between the slaughtering and marketing of beef at a commercial abattoir in Kigali city (Rwanda).

The prevalence of *Salmonella* of the present study did not agree with prevalence done in the region and elsewhere outside the region. A study done in Addis Ababa on the *Salmonella* prevalence was 7.9 % on minced beef from local market (Nyeleti, Molla, Hildebrandt & Kleer, 2000). Another study done in Ethiopia found *Salmonella* prevalence to be 14.4 % on minced beef to be from retail outlets (Ejeta, Molla, Alemayehu & Muckle, 2004). A study done Southern Ethiopia found the *Salmonella* prevalence on beef samples to be 35.6 % from butcheries in Awasa, Southern Ethiopia (Ashenafi, 1994). The prevalence of *Salmonella* on beef samples from the open markets was found 0.00 % on beef samples from open markets in Thailand (Atsuka *et al*., 2010). Another study reported prevalence of *Salmonella* to be 15.1 % on beef samples collected from retail stores and supermarkets in Mexico (Miranda, Mondragón, Martinez, Guarddon & Rodriguez, 2009). There was a wide variation in *Salmonella* spp.in fresh beef in the different countries as reflected by the above-mentioned results. The differences in the prevalence of *Salmonella* between studies could be due to slaughtering practices, post slaughter handling of meat and general hygiene at different stage of meat chain which differs from one country to another.

E. coli O157:H7 is a human life threatening pathogenic enteric bacterium that cause bloody diarrhea. The presence of *E. coli* O157H7 on beef samples from the outlets indicates risk of *E. coli* O157:H7 transmission to consumers and could be associated with improper HACCP practices and bad handling. Nkanga and Uraih (1981) stated that unhygienic handling practices during evisceration increases the rate of carcasses contamination with *E. coli* O157:H7. Delmore (2009) emphasized that improving hygienic handling practices in the abattoirs contribute effectively to improving the quality and safety of raw meat.

The insignificant difference (P=0.1785 at P<0.05) on prevalence of *E. coli* O157:H7 between the open markets, butchery and supermarkets could be explained by similar handling practices employed at the outlets. According to Obeng, Johnson and Appenteng (2013), beef contamination occurs from lack of process control under which beef carcasses are processed and handled in the abattoirs. According to Roberts and de Jager (2004), abattoirs greatly contribute to the problem of possible food-borne diseases and potential health hazards associated with foods due to poor handling of meat.

In the present study, *E. coli* O157:H7 prevalence (5.79 %) on beef samples collected from three outlets. This did not agree with previous study done in Italy on the *E. coli* O157:H7 prevalence from beef found the prevalence to be 0.43 % on minced beef from retail outlets (Conedera *et al*., 2004). Another study in Egypt found *E. coli* O157:H7 to be 5.00 % on minced beef and 5.26 % on ground beef in Egypt (El-Safey

& Abdul-Raouf, 2003). A study done in Iran found to be 2.80 % in beef from retail outlets (Mehdi, Narjes, Amirhesam & Mohammad, 2013). A study done in Ethiopia found the prevalence of *E. coli* O157:H7 to be 14.6 % on raw beef samples from open markets (Tizeta, Girma, Genene, Aklilu & Kaleab, 2014). Another study done in Ethiopia reported the prevalence of *E. coli* O157:H7 on beef samples to be 9.30 % reported in beef from butcheries (Adem, Daniel & Girma, 2008). In Iran, the prevalence of *E. coli* O157:H7 from supermarkets was 2.80 % reported in beef from supermarkets (Mehdi *et al*., 2013). The reported prevalence in different countries would not be comparable because of differences in the sampling strategy and the analytical methods used.

The overall lower recovery of *E. coli* O157 on beef samples from outlets could be challenges because the bacterium is likely to be present in low numbers and it's usually found in a large population of competent microflora, including other *E. coli*. However, the Immuno-Magnetic Separation (IMS) known to improve the isolation sensitivity of *E. coli* O157 strains was not used in the present study. Immunomagnetic separation (IMS) technique with enrichment in broth culture has been reported to enhance the isolation of STEC from samples with organisms in low concentration (Ojo *et al*., 2010). Instead, the modified trypticase soy broth for enrichment stage was used followed by plating on selective agar to increase the sensitivity of *E. coli* O157:H7 isolation.

5.5 Prevalence of *Salmonella* and *E. coli* O157:H7 among supermarkets

The insignificant difference (P=0.066 at P<0.05) and (P=0.057 at P<0.05) on the prevalence of *Salmonella* and *E.coli* O157:H7 respectively between supermarkets

may be attributed similar practices of implementing HACCP plans conducted at the supermarkets. However, various supermarkets source their beef a similar source. The higher prevalence of *Salmonella* on beef from in supermarket D may be due to unhygienic handling practices employed at the supermarket. The absence of *Salmonella* in supermarket C and the absence of *E. coli* O157:H7 in supermarket A to D could be due to the relatively better hygienic conditions under which sellers in that location sold their meat. Meat sellers in these supermarkets had their tables covered with nets, wear neater clothing and sold meat under a much tidy environment. According to Eze and Ivuoma (2012), the intestinal tract is the cause of pathogenic bacteria contamination due to poor evisceration process employed by the workers.

5.6 Prevalence of *Salmonella* and *E. coli* O157:H7 among open markets

The insignificant difference (P=0.214 at P<0.05) and (P=0.151 at P<0.05) on the prevalence of *Salmonella* and *E. coli* O157:H7 on beef from open markets may be attributed to similar practices of the absence of HACCP plans. The open markets may not have implemented HACCP plans to prevent food-borne pathogens.

The higher prevalence of *Salmonella* and *E. coli* O157:H7 on beef samples from open market X could be due to lower hygienic standards compared to supermarkets with low prevalence. The food handlers at the abattoirs maybe wear dirty clothes and pay little concern on their personal hygiene and handle the meat with unwashed hands. This supported by Adzitey *et al.* (2011) who demonstrated that meat processing can contaminate the meat with microbial pathogens. The lower prevalence of *Salmonella*

on open market Y and absence of *E. coli* O157:H7 on open market Y could be due to good hygienic standards conducted at the open markets.

5.7 The relationship between TPC, CC, *Salmonella* and *Escherichia coli* O157:H7

A correlation analysis indicates positive correlation between TPC, CC, *Salmonella* and *E. coli* O157:H7. However the report of Sankaran *et al.* (1975) did not agree with the present analysis on the correlation between TPC and CC. The report is in good agreement with Cason and Berrang (2002) report of a correlation coefficients of 0.69 and 0.39 between *E. coli* and *coliform* and between AP and *E. coli* ($P<0.0001$) respectively. However, a positive correlation between TPC and *Salmonella* reflects the poor handling and sanitary practices during animal slaughter and transportation of beef carcasses from abattoirs to retail outlets. A positive correlation between CC and *E. coli* O157:H7 may be explained by systematic contamination of beef with the intestinal contents during evisceration at the abattoirs. The coliforms belong to the family *Enterobacteriaceae* and include *E. coli*. Since *E. coli* O157:H7 is the subset of *E. coli*. These organisms originate from intestinal tract of animals.

CHAPTER 6: CONCLUSIONS

The study revealed the total plate count (4.31, 3.90, 3.83 Log CFU/g), coliform count (1.30, 1.72, 2.08 Log CFU/g) and the prevalence of *Salmonella* spp. (28.3 %, 52.2 %, 67.4 %) *E. coli* O157:H7 (2.17 %, 4.35 %, 10.9 %) on beef samples from supermarkets, butcheries and open markets respectively.

There was no significant difference in the mean total plate count, coliform count and *E. coli* O157:H7 prevalence of beef samples among the three outlets but there was a significant difference in the prevalence of *Salmonella* spp. among outlets.

There was no significant difference in the total plate counts and *Salmonella* spp., *E. coli* O157:H7 prevalence of beef samples among supermarkets but there was a significant difference in the mean coliform counts among supermarkets.

There was no significant difference in the *Salmonella* spp. and *E. coli* O157:H7 prevalence on beef samples among open markets but there was a significant difference in the mean total plate count and coliform counts among open markets. A positive correlation was found between prevalence of TPC, CC, *Salmonella* and *E. coli* O157:H7 from the outlets.

CHAPTER 7: RECOMMENDATIONS

The following recommendations are suggested based on the findings obtained in this study:

1) The study recommends training of workers about good handling practices and basic food hygiene i.e. wash hands properly before sale of meat and the use proper clothing such as hand gloves, head covers and nose masks.
2) Proper implementation and maintenance of good handling practices and HACCP approach at the outlets points to help eliminate or reduce significantly food-borne pathogens and the consequent food poisoning in the society.
3) The study also recommends inspections and monitoring of handling practices by health inspectors.
4) Consumers are advised to cook beef appropriately to avoid intoxication due to microbes. Cooking the meat at high temperatures of 100°C helps to eliminate pathogens.

CHAPTER 8: REFERENCES

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APPENDICES

Appendix 1: The total plate counts and total coliform counts of fresh beef from Windhoek retail outlets per week

Total plate counts

Weeks	**Log CFU/g Supermarkets**	**Log CFU/g Butchery**	**Log CFU/g Open market**
1	3.656984	3.503296	3.973092
2	4.570367	3.330862	3.953994
3	3.803276	3.191544	1.965038
4	5.987859	4.889447	4.606201
5	5.271181	3.709535	5.165197
6	1.821435	3.902864	4.278081
7	2.606224	3.217071	4.076144
8	4.895117	2.613753	2.850117
9	3.492459	3.903004	2.92234
10	4.813972	5.503741	4.161088
11	5.953883	4.911365	4.218439

Total coliform counts

Weeks	Log CFU/g Supermarkets	Log CFU/g Butchery	Log CFU/g Open market
1	1.53402	1.343506	2.040442
2	1.44261	0	2.791743
3	0	0	0
4	3.876697	4.202041	1.890201
5	0	0	3.312655
6	2.740744	1.641426	3.706312
7	0	2.716138	1.666673
8	0	0	0.984748
9	0.666667	3.051277	1.444818
10	4.515497	2.407292	2.808595

Appendix 2: *Salmonella* and *E. coli species* confirmed during the entire study from Windhoek retail outlets

Salmonella

Sample numbers	Supermarkets	butchery	Open market
1	+	-	+
2	+	+	+
3	+	+	+
4	-	+	+
5	-	+	-

6	-	-	+
7	-	-	-
8	-	-	+
9	-	+	+
10	+	+	+
11	-	+	+
12	+	+	+
13	-	-	+
14	-	+	+
15	-	-	-
16	+	+	+
17	+	+	+
18	+	+	+
19	-	+	-
20	+	+	+
21	-	-	+
22	-	-	+
23	-	-	+
24	-	+	-
25	-	+	-
26	-	-	-
27	-	+	+
28	-	+	+

29	-	-	-
30	-	-	-
31	-	-	+
32	-	-	+
33	+	-	+
34	+	-	-
35	+	-	+
36	-	+	-
37	-	+	-
38	-	+	-
39	-	+	+
40	+	+	+
41	-	-	+
42	-	-	+
43	-	+	+
44	-	-	+
45	-	-	-
46	-	-	-

***E. coli* O157:H7**

Sample numbers	Supermarkets	Butchery	Open market
1	-	-	-
2	-	-	-

3	-	-	-
4	-	-	-
5	-	-	-
6	-	-	-
7	-	-	-
8	-	-	-
9	-	-	-
10	-	-	-
11	-	-	-
12	-	-	-
13	-	-	-
14	-	-	-
15	-	-	-
16	-	-	-
17	-	-	-
18	-	+	-
19	-	-	-
20	-	-	-
21	-	-	-
22	-	-	-
23	-	-	+
24	-	-	-
25	-	-	-

26	-	-	-
27	-	+	-
28	-	-	-
29	-	-	-
30	-	-	+
31	-	-	-
32	-	-	-
33	-	-	+
34	-	-	-
35	-	-	-
36	-	-	-
37	-	-	-
38	-	-	+
39	-	-	-
40	-	-	-
41	-	-	-
42	-	-	-
43	-	-	+
44	-	-	-
45	+	-	-
46	-	-	-

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