DETECTION OF *SALMONELLA SPP* AND PATHOGENIC *E. COLI* IN THE STREET VENDED FRESH BEEF AND OFFALS

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A RESEARCH PROJECT SUBMITTED TO THE DEPARTMENT OF BIOLOGICAL SCIENCES, COLLEGE OF BASIC AND APPLIED SCIENCES, MOUNTAIN TOP UNIVERSITY, MAKOGI, IBAFO, OGUN STATE, NIGERIA.

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE (B.Sc) IN MICROBIOLOGY

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CERTIFICATION

This is to certify that this research project titled **"DETECTION OF *SALMONELLA SPP* AND PATHOGENIC *E. COLI* IN THE STREET VENDED FRESH BEEF AND OFFALS"** was carried out by SADIQ, Adebimpe Oluwaseun, with matriculation number 16010101018. This project meets the requirements governing the award of Bachelor of Science (B.Sc) Degree in Microbiology department of biological sciences of Mountain Top University, Ogun State, Nigeria and is approved for its contribution to knowledge and literary presentation.

SADIQ ADEBIMPE O. Date

MR O.E. FAYEMI
(Project Supervisor) Date

MR O.E. FAYEMI
(Head of Department) Date

DECLARATION

I hereby declare that this project report written under the supervision of Dr. O.E. Fayemi is a product of my own research work. Information derived from various sources has been duly acknowledged in the text and a list of references provided. This research project report has not been previously presented anywhere for the award of any degree or certificate.

_____ _____

SADIQ ADEBIMPE O. Date

DEDICATION

I dedicate this project to God Almighty for giving me life, good health and all I needed to make this work a success and secondly to my dear parents, Mr Adebayo and Omowunmi Sadiq for their guidance, understanding and sacrifice. I also dedicate this work to my course mates and friends for their support in the course of my four year study of Microbiology in Mountain Top University. May the Almighty God bless you all! Amen.

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May God in His infinite mercies continue to bless you all, Amen!

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ABSTRACT

Bacterial pathogens are of the utmost concern to consumers about the protection of meat. A wide variety of pre-slaughter, post-slaughter and processing methods are responsible for human pathogenic contamination of raw meat products. Pathogenic *Escherichia coli* is a common pathogen associated with meat, with Shiga-toxin or verocytoxin producing *E. coli* O157 the most common member of a group of pathogenic strains. In this study, *E. coli* O157 was isolated using sorbitol containing MacConkey agar (SMAC medium) while Salmonella-Shigella (SS) agar were used for the isolation *Salmonella* species. The highest counts of 6.1 log10 CFU/g and 6.3 Log10 CFU/g for SMAC were found in Offals and meat respectively. *Salmonella* species were found in 25 g of offals which is contrary to meat safety standards. Thus, such microorganisms pose a potential danger to humans, particularly from the consumption of these products, which may lead to other diseases such as haemorrhagic colitis (HC) or haemolytic uremic syndrome (HUS). There is a need for education on sanitary handling of meat which is possible vehicle for *Salmonella* and *E. coli* infections. The responsibility of tracking the hygiene and sanitation of abattoirs and slaughterhouse in Magboro, Ogun state, should be taken over by government agencies.

CHAPTER ONE

1.1 INTRODUCTION

Animal flesh consumed as food is referred to as meat; while raw meat is the uncooked muscle tissue of animals that is intended for food (Duffy *et al*., 2006). Goat and cattle are popular sources of beef all over the world, including Nigeria. Meat and meat items have become some portion of the day by day human eating regimen, because of their rich and nutritious structure. Beef has been reported to contain high quality proteins, minerals, vitamins and fat (Iroha et al., 2011; Francis et al., 2015). The increase in demand for meat and its products has led to increased sale and slaughtering of livestock (Warris, 2010). The exceptionally nutritious nature of meat gives a suitable climate to the development of both pathogenic, non-pathogenic and spoilage organisms (Steinkraus, 1994). The high consumption rate of meat and its popularity makes meat contamination and its consequences an issue of concern. Raw meat and meat products have been identified as important foodborne disease vehicles and are involved in food poisoning outbreaks (Macrae et al., 1993; Bhandare et al., 2007).

Microbial contamination of carcasses occurs mainly during processing and handling, such as skinning, evisceration, storage and distribution at slaughterhouses and retail establishments (Abdallah *et al.*, 2009). Any treatment completed on a food animal body from the point of butchering until it is prepared for utilization, including remains arrangement, transport and taking care of, will add to the bacterial load of its meat (Ali, 1992). The external contamination of meat constitutes a constant problem in most developing countries, in the abattoir itself where there are large numbers of potential sources of contamination by microorganisms (Davis *et al.*, 2002).

Diseases caused by foodborne pathogens constitute a worldwide public health problem (Maha et al., 2014). *Escherichia coli, Clostrudium perfringens, faecal Streptococci, Klebsiella pneumoniae* as well as species of *Salmonella, Shigella, Bacillus, proteus, Staphylococcus, Salmonella* and *Listeria* have been reported as contaminants of raw meat in various studies within and outside Nigeria (Eze and Ivuoma, 2012).

The high rate of foodborne outbreaks related to meat and meat products have recently become a matter of public health concern. Therefore, there is a need for rapid and precise identification of pathogens responsible for the foodborne outbreaks, thus; the

use of molecular techniques to identify pathogens up to subspecies or strain level is paramount. This study will endeavour to investigate the molecular diversity in the pathogens found in meats and edible offals sold at Magboro market.

CHAPTER TWO

2 LITERATURE REVIEW

Foodborne diseases, in particular those related to meat and meat products, have recently become a matter of great public concern. Microbial food safety is an increasing public health concern worldwide (Kabir, 2010). Also, animal edible offal such as liver and kidneys has great importance as foods for Nigerians.

The state of health of animals prior to slaughtering and the prevailing circumstances in the slaughter house can contribute to the quality of meat from such animals (Whyte et al., 2004; Nnachi et al., 2014). Reports have shown that the butchering of animals in provincial areas inside Nigeria is generally completed under unhygienic conditions. By and large, consumable water is inaccessible, leaving butchers with unhygienic water hotspots for use. In addition to high ambient temperature, humidity and poor handling practices, these reasons dispose of raw meat for deterioration and contamination (Nnachi et al., 2014; Raji, 2006). Animal skin, hide and legs, faecal material and hands, clothing and slaughtering equipment have been identified as pathways of contamination (Adetunde et al., 2011). Meat is usually considered to be one of human diet's most common and nutritious products. There are several paths for meat and poultry products to develop biological hazards, mainly due to favorable circumstances of growth. Healthy animal tissues are contaminated by the employees and the environment during slaughter and dressing by the animal itself (Umoh et al., 2006). Control of such risks is vital because global meat trade implies that outbreaks can impact many nations quickly. Pathogens can trigger subclinical or clinical diseases in livestock and subsequent losses owing to suboptimal development or manufacturing, depending on the biology and epidemiology of the hazard (Gul et al., 2016). Meat product contamination is mainly correlated with exposing meat to faecal matter during slaughter. Once the meat is contaminated, pathogens such as *Salmonella spp.* can proliferate during incorrect storage or undercooking. *Salmonella* is excreted in faeces; food and water contamination and can be transmitted to humans. Person-to-person, faecal-oral transmission can occur and it is an issue in health care centres where insufficient hand washing facilities were limited. Chronic carrier of *Salmonella* are uncommon in humans, prevalent in birds and livestock. *Salmonella* infection happens by ingestion of contaminated food, milk, or water from infected hosts or by ingestion of infected meat products. *Salmonella typhi* and *S. paratyphi* are

transmitted to human host only and not commonly dispersed in nature. Contrary to this, nontyphoidal species of *Salmonella* are widespread in nature and strongly related to both livestock and humans (Percival and Cutting, 2010).

2.1 FOODBORNE DISEASES

Foodborne diseases are caused by food contamination and occur at any point of the food processing, distribution and consumption process. They can result from several types of environmental contamination, including water, soil or air pollution, as well as unsafe food storage and processing (WHO, 2020). Foodborne bacteria include *Salmonella spp., Staphylococcus aureus, Campylobacter jejuni, Listeria monocytogenes, Bacillus cereus, Vibrio spp.,* and *Escherichia coli* O157:H7 or other strains of shiga toxin formed by E. coli strains (non-O157 STEC) that cause high morbidity and mortality in foodborne diseases (Zhao et al., 2014). Foodborne disease has been a serious concern to the government and is still regarded as a major human health problem.

2.2 SALMONELLA SPP.

Salmonella spp. are members of the family Enterobacteriaceae. They are Gram-negative, facultatively anaerobic, flagellated, rod-shaped organisms and are responsible for a large number of cases of foodborne illness throughout the world (Quiroz-Santiago *et al*. 2009)**.** Illnesses from food are one of the most important economic and health problems among developed and developing countries. *Salmonella* contain both pathogenic and 9 non-pathogenic strains. The genus *Salmonella* is subdivided into *Salmonella bongori* and *Salmonella enterica* species.

Salmonella enterica contains six sub-species namely; *S. enterica* (subsp) *enterica, S. enterica* (subsp) *salame, S. enterica* (subsp) *arizona, S. enterica* (subsp) *houtenae, Salmonella enterica* (subsp) *indica* (Brenner *et al*., 2000; WHO, 2003). This genus contains 2,463 serovars or serotypes (or approximately 2,500) and it has also been previously researched that more than 99% of *Salmonella* related foodborne diseases are caused by strains from members of *Salmonella enterica* (subspecies) *enterica*, while *S. bongori* accounts for about 1% of clinical isolates (Brenner *et al*., 2000, Pui *et al*., 2011).

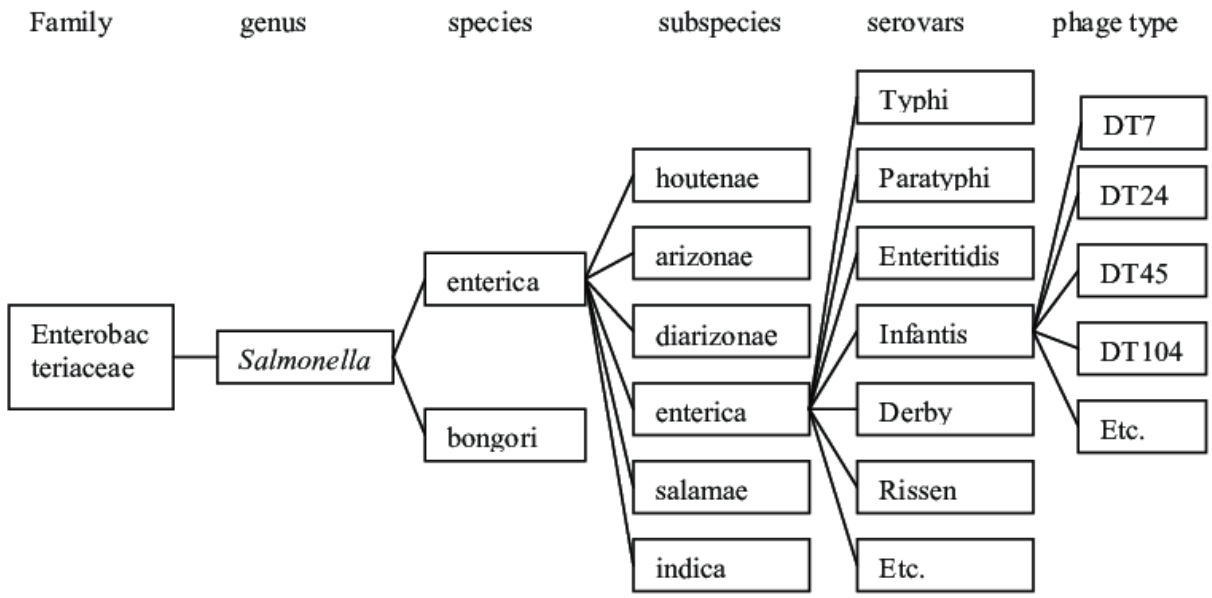

Figure 1: Classification of Salmonella (Source: Hermans, 2007)

2.2.1 SEROVARS OF *SALMONELLA ENTERICA*

Typhoidal *Salmonella*: typhoidal serovars (*Salmonella typhi* and *Salmonella paratyphi* A) cause typhoid fever, the treatment of which is compromised by expanding drug resistance. Our comprehension of *S. typhi* disease in people remains inadequately understood, most likely because of the host limitation of typhoid strains and ensuing prominence of the *S. typhimurium* mouse typhoid model. In any case, there are a few limits to interpreting discoveries with *S. typhimurium* to *S. typhi*. Specifically, *S. typhi* has explicit harmfulness factors, including typhoid poison and Vi antigen, which are engaged with the advancement of manifestations and insusceptible avoidance. (Johnson et al., 2018)

Non-Typhoidal *Salmonella* (NTS) Unlike typhoid fever, which is widespread in the developing world, NTS salmonellosis occurs worldwide. An expected 93.8 million instances of gastroenteritis because of NTS disease are accounted for every year, bringing about around 155,000 deaths (Majowicz et al., 2010). While human NTS contaminations generally cause gastroenteritis, in up to 5 percent of NTS cases, microorganisms cause obtrusive, extra-intestinal sickness prompting bacteremia and central foundational diseases, from this time forward alluded to as intrusive NTS (Mandal and Brennand, 1988).

Table 1:Major Salmonella Serovars and their Host (Adapted from Bhunia, 2018)

Serovar	Pathogen specificity	Diseases
Typhi	Humans	Typhoid fever
Bradenburg	Sheep	Abortion
Dublin	Cattle	Enterocolitis, typhoid fever
Pullorum	Chicken	Bacillary white diarrhea
Cholerasius	Swine	Enterocolitis, Septicemia
Enteritidis	Humans	Gastroenteritis
Paratyphi	Humans	Typhoid fever-like

2.2.2 TRANSMISSION OF *SALMONELLA*

Salmonella transmission to human is through the ingestion of infected foods (Srisawat and Panbangred, 2015) and even the spreading from person to person. Following the ingestion of contaminated food, these bacteria will colonize the intestines by invading dendritic cells and enterocytes of the intestinal epithelium barrier. The transmission of *Salmonella* is at its highest in children (under 5 years), elderly people (older than 70 years old) and immunosuppressed individuals (pregnant women, HIV/AIDS patients)

2.2.3 *SALMONELLA* INFECTION

Salmonella contamination (Salmonellosis) is a typical bacterial infection influencing the intestinal plot. *Salmonella* microorganisms normally live in the digestion tracts of creatures and people and are spread through the dung (Threlfall et al., 2004). Humans can become infected with contaminated water or food. Ordinarily, individuals with *Salmonella* disease may have no side effects while others create looseness of the bowels, fever and stomach cramps inside 8-72 hours (Glynn et al., 1998). Most sound individuals will recuperate inside a couple of days with no particular treatment. At times, the looseness of the bowels related with *Salmonella* disease might be getting dried out and will require brief clinical consideration. Perilous difficulties may likewise create if the disease spreads past the digestion tracts. The danger of *Salmonella* disease is higher in agricultural nations with helpless sterilization.

Salmonella contamination is typically brought about by eating crude or half-cooked meat, poultry, eggs, egg items or products of the soil (Pui et al., 2001). Foods grown from the ground stand a higher danger of conveying *Salmonella* because of the way that they are eaten crude or uncooked. The hatching period goes from a few hours to two days. Most *Salmonella* diseases can be delegated stomach influenza (gastroenteritis). Potential signs and side effects incorporate; retching, stomach cramps, looseness of the bowels, sickness, fever, chills, cerebral pain and blood in the stool. Signs and manifestations of *Salmonella* contamination for the most part last two to seven days. The runs may last as long as 10 days, despite the fact that it might require a while before insides recover to business as usual.

2.2.4 PREVALENCE OF *SALMONELLA*

Salmonella is a prominent cause of diseases and illnesses in both human and animals which has been estimated to cause about 93.8 million cases of human gastroenteritis and 155, 000 deaths worldwide each year (Majowicz *et al*., 2010).

In 1997, the incidence of *Salmonella* reached its highest level when over 32, 000 cases were reported and most were associated with eggs and poultry (PHLS, 1999). Although, the rate declined in the year 2002 due to the introduction of *Salmonella enteritidis* (PT4) vaccine (Wall and Word, 1999) and also improvements in microbiological quality of food in all stages from production point to consumption

point coupled with the adoption of hazard analysis critical control point (HACCP) (O'Brien *et al*., 1998).

Salmonella mostly affects children, elderly and immunosuppressed persons, so outbreaks involving them takes two forms; person-to-person spread and foodborne spread (Gillespie and Hawkey, 2006). Person-to-person is enhanced by poor and inadequate control measures and this may lead to creation of asymptomatic carrier (like nurses and health facility officials) (Meakins *et al.,* 2003). While foodborne spread which is by point source outbreak resulting from large numbers of people consuming food already contaminated by *Salmonella* (Gillespie and Hawkey, 2006).

2.2.5 VIRULENCE OF *SALMONELLA SPP*

The virulence of pathogenic *Salmonella spp.* is associated both with chromosomal and plasmid genes (Chaudhary *et al.,*2015). In bacterial chromosomes, there are enormous gene casettes called pathogenicity islands (SPIs), which encode almost 60 qualities liable for explicit connections with the host living being (Lahiri, et al., 2010). The cycle of infection with *Salmonella spp.* begins after the ingestion of microbes.

2.3 ESCHERICHIA COLI

They belong to the *Enterobacteriaceae* family and are natural colonisers (microflora) of intestinal tracts of warm blooded animals. Its outbreaks have been constantly associated with meat products, dairy products, fruits and vegetables. It belongs to *Domain: Bacteria, Phylum: Proteobacteria, Class; Gammaproteobacteria,* Order*: Enterobacteriales,* Family*: Enterobacteriaceae,* Genus*: Escherichia, Species; Escherichia coli. Escherichia coli* was named after a German bacteriologist (Theodor Escherich) who isolated it from a fecal sample in 1885 (and called it *Bacterium coli commune*, but later got its new name in 1888) (Escherich Theodor, 1885). *Escherichia coli* are fermenters of glucose that produce acid and gas, they have peritrichous flagella which makes them motile and are non-spore formers, they are facultative anaerobes with growth at 37°C (Martinez-Salas *et al.,* 1981).

Escherichia coli is a common inhabitant of the gastrointestinal tract of animals and man. It has the ability to survive for long periods in water and soil, under frozen and refrigerated temperatures, and in dry conditions and they can only be destroyed through thorough cooking or pasteurization. It is a bacterium that is usually found in the lower intestine of warm-blooded organisms. Most strains of *E. coli* are harmless but some of them can cause serious food poisoning and severe food and water-borne

disease outbreaks worldwide. Shiga toxin-producing *E. coli* (STEC) are groups of *E. coli* that causes severe foodborne diseases. Pathogenic *E. coli* is a significant cause of diarrhoea especially in young children and adults in developing countries and localities of poor sanitation, and *E. coli* O157 is a highly pathogenic strain of *E. coli.*

According to Davis *et al.*, (2005) *E. coli* O157 is of particular public health concern because of its severe consequences of infection. Reports of sporadic outbreaks of disease caused by *E. coli* O157 serogroup have been on the increase since it was first identified in the early 1980s as a new group of pathogens implicated in human mortality and morbidity.

2.3.1 SEROTYPES OF *E. COLI*

The pathogenic strains of *E. coli* that cause enteric diseases are grouped into six categories which include; enterohaemorrhagic *E. coli* (EHEC), Enterotoxigenic *E. coli* (ETEC), Entero-invasive *E. coli* (EIEC), (Simjee, 2007) Enteropathogenic *E. coli* (EPEC), Enteroaggregative *E. coli* (EAEC), and diffuse-adherent *E. coli* (DAEC) (Gillespie and Hawkey, 2006). But the *E .coli* strains mainly known to be associated with vegetables is the enterohaemorrhagic (EHEC) including Shiga-toxin *Escherichia coli* due to the major outbreaks that has happened all around the world (López-Gálvez *et al.*, 2010).

2.3.1.1 DIFFUSELY-ADHERENT *E. COLI*

Diffuse adhering *E. coli* (DAEC) have been recognized as the sixth category and appears as a heterogeneous group (Nataro and Kaper, 1998). *E. coli* strains in this category are known to bind to host cells in a pattern of diffuse adhesion in which the bacteria consistently cover the entire surface of the cell. (Scaletsky *et al*., 1984). The effect of DAEC strains on diarrhoea stays dubious, as certain examinations have detailed that these strains are correspondingly found in youngsters with and without the runs (Giron *et al*., 1991; Jallat *et al*., 1993). Tacket *et al*. (1990) suggested that DAEC may cause disease in immunologically naive or malnourished children due to their inability to conclusively induce diarrhoea with DAEC in health adult volunteers. However, in Northern Brazil, Diffusely adherent *E. coli* has been shown in studies to be the cause of acute diarrhoea in children (Scaletsky *et al*., 2002). Discrepancies between epidemiological studies can be explained by age-dependent susceptibility to

diarrhoea or the use of an inappropriate technique of identification such as DNA probing (Levine *et al*., 1993).

2.3.1.2 ENTEROAGGREGRATIVE *E. COLI*

Enteroaggregative *Escherichia coli* (EAEC) are a pathotype of *Escherichia coli* is a cause of acute and chronic diarrhoea in both the developed and developing world (Nataro, J. P. et al., 2006; Jensen B.H. et al., 2014). They may also cause urinary tract infections (Jensen B.H. et al., 2014) The pathogenesis of EAEC involves the aggregation of and adherence of the bacteria to the intestinal mucosa, where they elaborate enterotoxins and cytotoxins that damage host cells and induce inflammation that results in diarrhoea. EAEC is currently perceived as an arising enteric microorganism. Specifically, EAEC are accounted for as the second most normal reason for traveller's diarrhoea, second only to Enterotoxigenic *E. coli*, and a common cause of diarrhoea amongst paediatric populations (Huang D.B. et al., 2006; Adachi J.A. et al., 2001). It has additionally been related with ongoing contaminations in the last mentioned, just as in immunocompromised hosts, for example, HIV-infected people (Huang D.B. et al., 2006)

2.3.1.3 ENTEROTOXIGENIC *E. COLI*

The enterotoxigenic *E. coli* (ETEC) strains are important cause of infantile and travelers' diarrhea (Croxen *et al.,* 2013). ETEC's virulence factors are the heat labile toxin (LT) and the heat stable toxin (ST). Both mediate deregulation of ion channels in the epithelial cell membrane (Fleckenstein *et al.,* 2010). ETEC can survive in a variety of environments, such as rivers, drinking water, irrigation water, and fresh vegetables (MacDonald *et al.,* 2015).

2.3.1.4 ENTEROHAEMORRHAGIC *E. COLI*

The entero-haemorrhagic *E. coli* (EHEC) strains cause bloody and nonbloody diarrhea. The most infamous piece of this pathotype is strain O157:H7, which causes bloody diarrhea and no fever. EHEC can cause hemolyticuremic condition and unexpected renal disappointment. It utilizes bacterial fimbriae for connection (E. coli basic pilus, ECP), (Rendon et al., 2007) and is tolerably intrusive and has a phage-coded shiga poison that can cause extraordinary provocative responses.

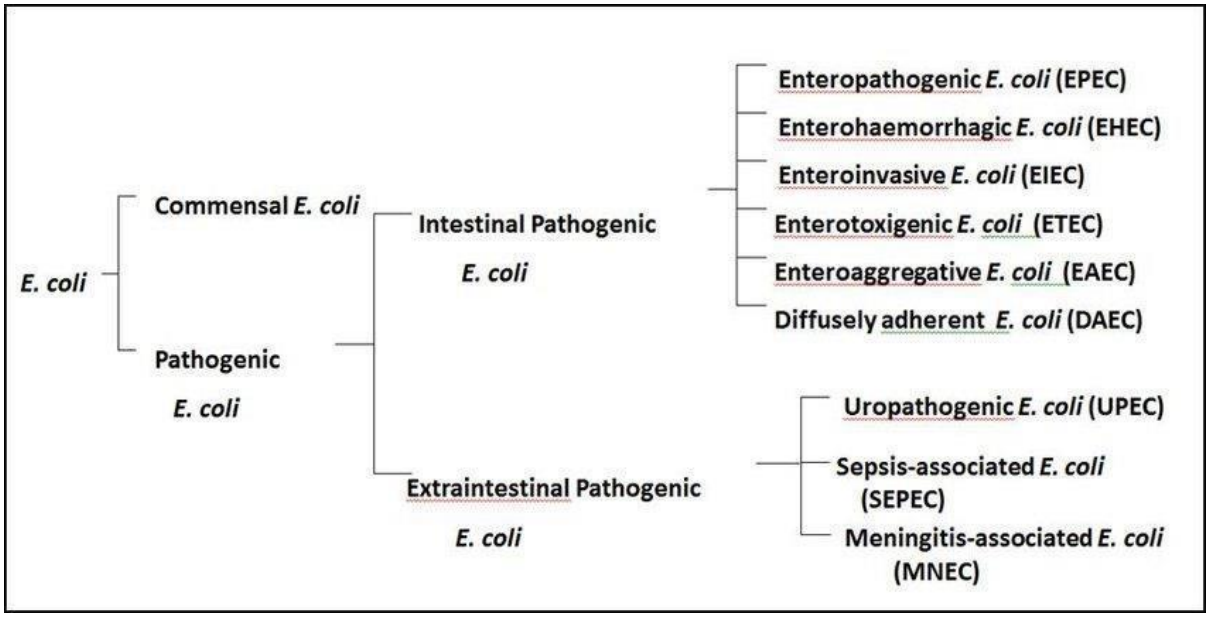

Figure 1: Escherichia coli species and its sub species classification. Adapted from: Wakeham, (2013)

2.3.2 SHIGA TOXIN PRODUCING *E. COLI*

It is transmitted primarily to humans through the consumption of contaminated food such as raw or undercooked meat and contaminated vegetables. STEC produces toxins known as Shiga-toxin because of their similarity to the toxins produced by *Shigella dysenteriae.* (Kapar 2004). *Escherichia coli* serotype 0157:H7, which produces Shiga-like toxin (SLT), also known as verocytoxin, is a known cause of hemorrhagic colitis and hemolytic uremic syndrome (HUS). *E. coli* O157:H7 disease can lead to inflammation of the colon and cause diarrhea and abdominal pain with bloody stools (Liu et al., 2009).

STEC INFECTION

Escherichia coli O157:H7 contamination is a significant general wellbeing worry in North America, Europe, and different pieces of the world. Albeit the absolute number of instances of *E. coli* O157:H7 contaminations is lower than that of other enteric microbes, for example, *Salmonella* or *Campylobacter spp.*, *E. coli* O157:H7 sickness has indicated a lot higher hospitalization and casualty rates (Mead et al., 1999). Human contamination brought about by *E. coli* O157:H7 may have an expansive clinical range, going from asymptomatic cases to death. Most cases start with non-bleeding the runs and self-resolve minus any additional confusion. A few patients, in

any case, progress to bleeding loose bowels or HC inside 1-3 days. In 5–10 percent of HC patients, the infection may advance to hazardous sequelae, HUS, or thrombocytopenic purpura (TTP) (Banatvala et al., 2001). *E. coli* O157:H7 is the most widely recognized reason for HUS infection in the US. Kids and older individuals are at expanded danger of serious clinical side effects, for example, HUS (hemolytic uremic disorder) (Su and Brandt, 1995).

A few treatment procedures, including the utilization of anti-infection agents and immunization, have been examined. Nonetheless, there is no particular treatment for *E. coli* O157:H7 contamination and anti-infection use might be contraindicated. Treatment is along these lines principally strong to lessen the term of manifestations and forestall fundamental difficulties. Exceptionally powerful measures for the avoidance and control of *E. coli* O157:H7 contaminations are fundamental given this status.

2.3.2.1 TRANSMISSION

Escherichia coli O157:H7 disease is transmitted by faecal-oral course through contaminated food or water. Shiga-toxin delivering strains have a high potential for individual to-individual transmission since a low infective portion is required and ingestion of as not many as 10 organisms might be adequate to cause disease (Parry Salmon, 1998). The rate of secondary household transmission for sporadic Shiga-toxin producing strains has been estimated to range from about 4% to 15% (Parry, Salmon., 1998). Institutional outbreaks due to person-to-person transmission have also been reported in nursing homes, schools and day care centres for children (Su and Brandt, 1995). The incubation period is around 3 to 4 days but can range from one to ten days (CDC, 2006). The range of contamination with *E. coli* O157:H7 incorporates asymptomatic fecal shedding of the organism; non-ridiculous or grisly the runs joined by stomach issues, regurgitating and every so often fever; post-diarrheal hemolytic uremic condition (HUS); and thrombotic thrombocytopenic purpura (TTP) (David, 2004; CDC, 2006; WHO, 2005).

2.3.2.2 VIRULENT FACTORS

Toxins; the ability of *Escherichia coli* to produce toxins enhance its ability to infect a host with disease. It produces α-hemolysin toxin which is a pore-forming cytotoxin, it inserts into the plasma membrane of the host cells thereby causing leakage of the

host’s cytoplasmic contents and eventually leading to cell death. Another toxin it produces is one which is similar to the shiga toxin and inhibits protein synthesis by ribosomal binding. Also, it produces labile toxin (LT) (Ryan and Ray, 2004).

CHAPTER THREE

3 MATERIALS AND METHOD

3.1 Sampling

Fresh meat (beef and offal) samples were collected from Magboro marketplace, Ogun State, Nigeria. Samples were collected in sterile plastic bags and taken immediately to the laboratory for analysis.

3.2 Materials

Materials used include: petri-dishes, beakers, conical flasks, glass spreader, 70% ethanol, scotch bottles, Eppendorf tubes, micro pipette (with their tips), test tubes (with their racks), PCR tubes

3.2.1 Reagents and Equipment Used

Equipment used: Autoclave, weighing balance, distiller, wash bottles, water bath (set at 50°C and 100°C), incubator (set at 37°C), Bunsen burner, oven, inoculating loop, gel electrophoresis tanks, UV Transilluminator

3.2.2 Media Used

Media used for *Escherichia coli* and *Salmonella*: Nutrient Agar, MacConkey Agar and Sorbitol-MacConkey Agar (SMAC), *Salmonella Shigella* Agar (SS AGAR), Selenite F broth, Buffer peptone water, Eosine Methylene Blue agar (EMB)

3.3 PREPARATION OF CULTURE MEDIA

3.3.1 BUFFER PEPTONE WATER

Peptone water is a microbial growth medium composed of peptic digest of animal tissue and sodium chloride. The pH of the medium is 7.2±0.2 at 25 ℃ and is rich in tryptophan. Peptone water is also a nonselective broth medium which can be used as a primary enrichment medium for the growth of bacteria.

Preparation

1. The dehydrated medium was dissolved in 1 litre of distilled water to make up 0.1% and 1% peptone water based on manufacturer's instructions in a conical flask and was mixed thoroughly. The conical flask is then closed with a cotton wool that is wrapped in aluminium foil.

2. The mixture was heated for a while to dissolve the powder completely and was then sterilized by autoclaving at 121°C for 15mins.

3. 9ml of the 0.1% was then dispensed into various test tubes for serial dilution.

4. 225ml of the 1% was then dispensed into conical flask.

3.3.2 SORBITOL-MACCONKEY AGAR (SMAC)

Sorbitol MacConkey agar was prepared according to the manufacturer's instruction for isolation and detection of *E. coli* O157:H7.

Preparation

1. The dehydrated medium was dissolved in the appropriate volume of distilled water that is 51.5g of SMAC in 1000 ml distilled water based on manufacturer's instructions in a conical flask and mixed thoroughly. The conical flask is then closed with a cotton wool that is wrapped in aluminum foil.

2. The mixture was heated for a while to dissolve the powder completely and was then sterilized by autoclaving at 121°C for 15minutes.

3. The medium was then allowed to cool to a range of 45-50°C and poured aseptically into sterile petri dishes and left to solidify.

Note: This medium is reddish-purple in color.

3.3.3 MACCONKEY AGAR

MacConkey Agar is used for gram-negative enteric bacteria isolation and lactose fermentation differentiation from non-lactose fermenting bacteria and lactose fermenting bacteria but provides pink colonies on MacConkey Agar as *Escherichia coli*.

Preparation

1. The dehydrated medium was dissolved in the appropriate volume of distilled water i.e. 48.5g of MacConkey in 1 liter of distilled water based on manufacturer's instructions in a conical flask and mixed thoroughly. The conical flask is then closed in cotton wool that is wrapped in aluminum foil.

2. The mixture was heated for a while to dissolve the powder completely and was then sterilized by autoclaving at 121°C for 15minutes. Avoid overheating.

3. The medium was then allowed to cool to a range of 45-50°C and poured aseptically into sterile petri dishes and left to solidify.

NOTE: The medium is neutral red in color.

3.3.4 NUTRIENT AGAR

Nutrient agar was prepared according to the manufacturer's instruction for isolation and detection of total count of mesophilic organism.

Preparation

1. The dehydrated medium was dissolved in the appropriate volume of distilled water i.e. 28g of Nutrient agar in 1 liter of distilled water based on manufacturer's instructions in a conical flask and mixed thoroughly. The conical flask is then closed in cotton wool that is wrapped in aluminum foil.
2. The mixture was heated for a while to dissolve the powder completely and was then sterilized by autoclaving at 121°C for 15minutes.

3. The medium was then allowed to cool to a range of 45-50°C and poured aseptically into sterile petri dishes and left to solidify.

Note: The medium appears opalescent and is light amber in color.

3.3.5 SELENITE F BROTH

Selenite F Broth is the medium used for the selective enrichment of *Salmonella* spp from both clinical and food samples. It is a buffered Lactose Peptone Broth to which Sodium Biselenite is added as the selective agent.

Note: Sodium biselenite (sodium hydrogen selenite) is a very toxic, corrosive agent and causes teratogenicity. Handle with great care. If there is contact with skin, wash immediately with lots of water.

Preparation

1. 19g of the dehydrated media of selenite F was dissolved in 750 ml distilled water in a sterile conical flask. (Part A).

2. 4g of sodium biselenite was dissolved in 250ml distilled water in another conical flask. (Part B).

3. Part A and Part B was mixed together. Medium was heated up/ warmed to dissolve conmpletely. Dispense in sterile test tubes.
4. It was sterilized in a boiling water bath or free-flowing steam for 10mins.

3.3.6 POTATO DEXTROSE AGAR

1. The dehydrated medium was dissolved in the appropriate volume of distilled water is 39g in 1000ml distilled water based on manufacturer's instructions in a conical flask and mixed thoroughly. The conical flask was then corked with cotton wool wrapped in aluminum foil.
2. The mixture was then heated for a while to completely dissolve the powder and was then sterilized by autoclaving at 121^0 for 15minutes.
3. The medium is the allowed to cool to a range of 45-50°C and poured aseptically into sterile petri dishes and left to solidify.
4. The potato dextrose agar was modified with 25mg of chloramphenicol.

3.3.7 EOSINE METHYLENE BLUE AGAR

1. The dehydrated medium was dissolved in the appropriate volume of distilled water that is, 36g in 1000ml distilled water based on manufacturer's instructions in a conical flask and mixed thoroughly. The conical flask was then corked with cotton wool wrapped in aluminum foil.
2. The mixture was then heated for a while to completely dissolve the powder and was then sterilized by autoclaving at 121^0C for 15minutes.
3. The medium is the allowed to cool to a range of 45-50°C and poured aseptically into sterile petri dishes and left to solidify.

3.4 Sample Preparation

Twenty-five (25g) of the sample was put in a sterile stomacher bag containing 225ml of 1% peptone water (enrichment broth) and then homogenized using the stomacher at 180 rpm for 4 minutes after which serial dilutions were performed and appropriate dilutions were plated on Nutrient agar, SMAC and MacConkey agar plates.

3.4.1 Primary Enrichment

Another 25g of samples were homogenized with 225ml of 1% peptone water and incubated at 37°C to serve as pre-enrichment for *Salmonella*.

3.4.2 Secondary Enrichment

This was performed for the detection of *Salmonella*, the overnight incubated pre-enrichment media of BPW was used to inoculate the secondary enrichment media. Ten (10) ml of sample pipetted from the peptone water was dispensed into 90mls

selenite F broth in a scotch bottle (Duran bottle) and incubated for 24hrs in 37°C. After incubation 0.1ml of the incubated selenite F broth was transferred into *Salmonella shigella* agar (SS agar) for presumptive confirmation of *Salmonella spp.* They were then incubated inversely at 37°C in an incubator for 24hrs. Colonies counted on plate were sub-cultured from the SS agar on to newly prepared nutrient agar and *Salmonella shigella* agar to get pure culture.

Test tubes were filled with 9ml of peptone water and sterilized using autoclave at 1200C for 15 minutes. The tubes were used to make serial dilutions from 10^{-1} to 10^{-7}. These were plated unto the three (3) culture media; Sorbitol MacConkey agar (SMAC), MacConkey agar (MAC), Nutrient agar (NA) using spread plate method.

Plating

For the Nutrient agar and MacConkey agar plates, spread plate technique was used for plating of inoculum (samples). About 15-20ml of agar is poured into sterilized petri dishes (observing aseptic methods and conditions), then allowed to cool and solidify. 0.1ml of the inoculum directly from dilutions are plated (using pipettes) onto appropriately labelled agar-containing petri-dishes for Nutrient agar and MacConkey agar, this will suffice for the enumeration, identification and isolation of Total Viable Counts and *Escherichia coli* strains respectively. After dispensing onto respective agar, the glass rod is used to spread or distribute the inoculum all around the agar (the glass rod was dipped into alcohol and then flamed in the Bunsen burner before spreading so as to maintain aseptic conditions). Replicates were made for each dilution for each sample.

However, for the detection of *Salmonella*, 0.1ml of inoculum from the secondary enrichment media incubated overnight at 37°C for 18-24 hours were pipetted onto *Salmonella-Shigella* agar containing plates. Aseptic conditions were observed and replicates were made for each sample and each dilution. After the inoculation with serially diluted samples, the inoculated agar petri-dishes are placed in an inverted fashion and are transferred into the incubator at 37°C for 18-24 hours.

Sub-culturing

Sub culturing is done to purify the isolated bacterial colonies from a mixed culture to a new and single culture, the bacterial isolates transferred or sub-cultured were those which were differentiated on the basis of their colony morphology, shape, color, elevation and other physical characteristics. Colonies differentiated by morphological

characteristics are transferred onto fresh petri dishes containing Nutrient agar. A loopful of preferred isolate was taken using the inoculating loop (the inoculating loop is heated using the Bunsen burner and allowed to cool for like 5 seconds before taking the loop from the original mixed culture and streaked onto the new petri-dish). For sub-culturing, the isolate containing loop is transferred to the new petri-dish using the streaking method procedure.

CRYOPRESERVATION OF ISOLATES

A loopful of each isolate was inoculated into a sterile Eppendorf tube containing 1ml of brain heart infusion and 500ul of 20 % sterile glycerol as cryoprotectant and it was stored in a -4^0c freezer.

3.5 MOLECULAR IDENTIFICATION

3.5.1 DNA Extraction

3.5.2 Boiling method

Each isolate was streaked out on nutrient agar and incubated overnight at 37^0 C. The loopfuls actively dividing cells were emulsified in 500ml double distilled water until it was turbid, it was centrifuged at 14,000 rpm for 5 minutes and the supernatant was decanted, 1ml of sterile water was added to the Eppendorf tube, vortexed and centrifuged again at 10,000 rpm for 2 minutes the process was repeated twice, 200ul of sterile water was pipetted into the Eppendorf tube, vortexed and centrifuged at 14,000rpm for 5 minutes and then it was placed in the heating block to boil for 10-20 minutes, it was then placed in the fridge for a while, the content of the Eppendorf tube was then vortexed and centrifuged finally at 14,000RPM for 5 minutes, a new set of Eppendorf tubes were labelled and the supernatant containing the extracted DNA was then transferred into the new Eppendorf tubes and they were placed in racks and stored in the freezer for further use.

3.5.3 PCR Protocol

16S rRNA amplification

Partial 16S rRNA gene amplification using forward primer fD1 (5´-AGA GTT TGATCC TGG CTC AG-3´) and reverse primer rD1 (5´-AAG GAG GTG ATC CAG CCG CA-3´) (Weisburg *et al*., 1991). The components of the PCR and constituent mixes were summarized in Table 3 below. The PCR was carried with initial denaturation at 95°C for 5 min; 35 cycles of 95°C for 2 min; 42°C for 30 s and 72°C for 4 min; and a final elongation step at 72°C for 10 min. The PCR products were confirmed by electrophoresis and visualized under UV light with a Gel

Doc system (Cleaver Scientific Ltd,Warwickshire, United Kingdom)

PCR REACTION COMPONENTS USED FOR 16S RRNA AMPLIFICATION

Table 2: PCR REACTION COMPONENTS USED FOR 16S RRNA AMPLIFICATION

No.	Component	1 rxn
1	Mastermix	5ul
2	fD1	0.4ul
3	rD1	0.4ul
4	DNA	2ul
5	dH_2O	2.2ul
6	Total	10ul

MULTILEX PCR PROTOCOL

Table 3: PCR REACTION COMPONENTS USED FOR 16S RRNA AMPLIFICATION

No	Component	1 rxn
1	Master mix	7.5ul
2	STX1F	0.186ul
3	STX1R	0.186ul
4	STX2F	0.186ul
5	STX2R	0.186ul
6	EAEF	0.186ul
7	EAER	0.186ul
8	DNA	2ul
9	dH_2O	4.36ul

PROCEDURE FOR THERMALCYCLER

Table 4: PROCEDURE FOR THERMALCYCLER

Analysis	Step	Temperature	Time
1x	Initial denaturation	95^0c	5 min
35x	Denaturation	95^0c	2 min
	Annealing	42^0c	30 sec
	Polymerization	72^0c	4 min
1x	Final polymerization	72^0c	10 min
1x	Hold	4^0c	∞
Cycler			

3.5.4 AGAROSE GEL ELECTROPHORESIS

The agarose was prepared using dry agarose powder, 1g of the agarose powder was then dissolved in 50ml of TAE buffer the mixture was then boiled until a clear solution was gotten 3µl of ethidium bromide was added to the mixture using a micropipette it is swirled and left to cool but not solidify, the content of the flask is then transferred into the gel cast with the combs in place, after, it is left to solidify and the gel is gently removed and put in an electrophoresis tank containing TAE buffer. 4ul of the PCR products are then pipetted into each well that was formed after removing the comb. The tank is connected to the power pack and left to run till it gets to one-third of the gel and then it is turned off and the gel is viewed under the UV transilluminator.

3.5.5 PRECAUTIONS

• Aseptic techniques were observed at every stage of work.

• Personal protective technique was also observed, such as wearing of covered shoe, nose cover, gloves, lab coat, etc.

• Ensured that the inoculating loop cooled before picking the organism when sub-culturing in order not to kill organism of interest.

• Ensured that the petri-dish was incubated inverted.

• Ensured proper timing, most especially during autoclaving.

CHAPTER FOUR

4 RESULTS AND DISCUSSION

The microbial analysis of the meat and offal samples gotten from Magboro market, Ogun state were reported. The morphological characteristics of the isolates are shown in Table 4.1, 4.2, 4.3 and 4.4 for total viable counts, general *E. coli*, pathogenic *E. coli*, moulds and yeasts and *Salmonella* species respectively.

Table 4.1: Morphological Characteristics of bacterial isolates on Eosin Methylene Blue Agar

Isolate	Sample	Isolate ID	Color	Shape	Size	Elevation	Appearance	Surface	opacity
1st sample	Raw beef	RB1 EMB 1	Metallic green sheen	Circular	small	Raised	Shiny	Smooth	Opaque
		RB1 EMB 2	Metallic green sheen	Circular	Small	Raised	Shiny	Smooth	Opaque
2nd sample	Raw beef	RB2 EMB 1	pink	Circular	Small	Raised	Smooth	Smooth	Opaque
		RB2 EMB 2	Green metallic sheen	Circular	Small	Raised	Shiny	Smooth	opaque
		RB2 EMB 3	Metallic green sheen	Circular	Small	Raised	Shiny	Smooth	opaque
		RB2 EMB 4	Metallic green sheen	Circular	Small	Raised	Shiny	Smooth	opaque
3rd sample	Raw offal	OFI EMB 1	Metallic green sheen	Circular	Small	Raised	Shiny	Smooth	opaque
		OF1 EMB 2	Metallic green sheen	Circular	Small	Raised	Shiny	Smooth	opaque
		OF1 EMB 3	Metallic green	Circular	Small	Raised	Shiny	Smooth	opaque

	sheen

Table 4.2: Morphological Characteristics of Isolates on Sorbitol MacConkey Agar

Isolate	Sample	Isolate ID	Color	Shape	Size	Elevation	Appearance	Surface	opacity
1st sample	Raw beef	RB1 SMA C 1	Pink	Circular	Small	Raised	Smooth	Smooth	opaque
		RB1 SMA C 1	White	Circular	Small	Raised	Smooth	Smooth	opaque
2nd sample	Raw beef	RB2 SMA C 1	white	Circular	Small	Raised	Smooth	Smooth	opaque
3rd sample	Raw offal	OF1 SMA C 1	Pink	Circular	Small	Raised	Smooth	Smooth	opaque
		OF1 SMA C 1	White	Circular	Small	Raised	Smooth	Smooth	opaque

Table 4.3: Morphological Characteristics of Isolates on Potato Dextrose Agar

Isolate	Sample	Isolate ID	Colour	Shape	Size	Elevation	Appearance	Surface	Opacity
1st sample	Raw beef	RB1 PDA 1	Cream	Circular	Small	Raised	Smooth	Smooth	Opaque
		RB1 PDA 2	Cream	Circular	Small	Raised	Smooth	Smooth	Opaque
		RB1 PDA 2	Cream	Irregular	Medium	Flat	Filamentous	Filamentous	Opaque
		RB1 PDA 3	Cream	Irregular	Medium	Flat	Filamentous	Filamentous	Opaque
		RB1 PDA 3	Cream	Circular	Small	Raised	Smooth	Smooth	Opaque
		RB1 PDA 4	Cream	Circular	Small	Raised	Smooth	Smooth	opaque
2nd sample	Raw beef	RB2 PDA1	Cream	Circular	Small	Raised	Smooth	Smooth	Opaque
		RB2 PDA2	Cream	Circular	Small	Raised	Smooth	Smooth	Opaque
		RB2 PDA 2	White	Irregular	Medium	Flat	Filamentous	Filamentous	opaque
		RB2 PDA 3	Cream	Circular	Small	Raised	Smooth	Smooth	Opaque
3rd sample	Raw offal	OF1 PDA 1	Cream	Circular	Small	Raised	Smooth	Smooth	Opaque
		OF1 PDA 2	Cream	Circular	Small	Raised	Smooth	Smooth	Opaque
		OF1 PDA 4	Cream	Circular	Small	Raised	Smooth	Smooth	Opaque

Table 4.4: MORPHOLOGICAL CHARACTERISTICS OF ISOLATES ON XYLOSE LYSINE DEOXYCHOLATE AGAR

Isolate	Sample	Isolate ID	Colour	Shape	Size	Elevation	Appearance	Surface	Opacity
1st sample	Raw beef	RB1 XLD 1	Pink	Circular	Small	Raised	Smooth	Smooth	Opaque
3rd sample	Raw offal	OF1 XLD 1	Black	Circular	Small	Raised	Smooth	Smooth	opaque

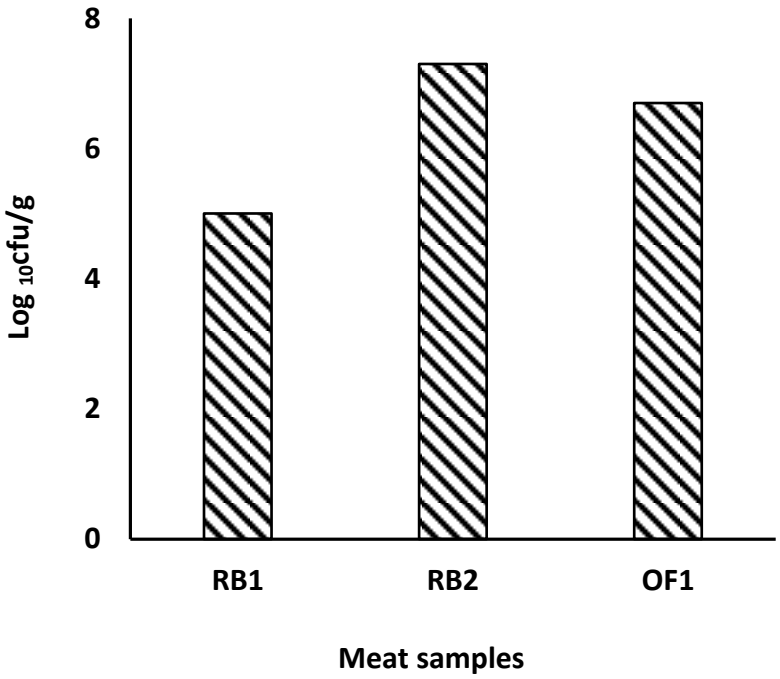

Figure 4.12: The Total Viable Counts in the street vended raw beef and offals sold in Magboro, Ogun State

Keys

RB1 – Raw beef 1

RB2 – Raw beef 2

OF1 – Offals

The total viable count was highest in raw beef sample 2 with a count of 7.3 Log_{10} Cfu/g. The viable count was higher than the range of 4.8 Log10 CFU/g - 6.8 Log10 CFU/g reported for raw meat by Ercolini et al., 2009. However, there have been instances in with the level of mesophilic bacteria are as high as 9 Log10 CFU/g depending on the hygiene and sanitation of the slaughterhouse.

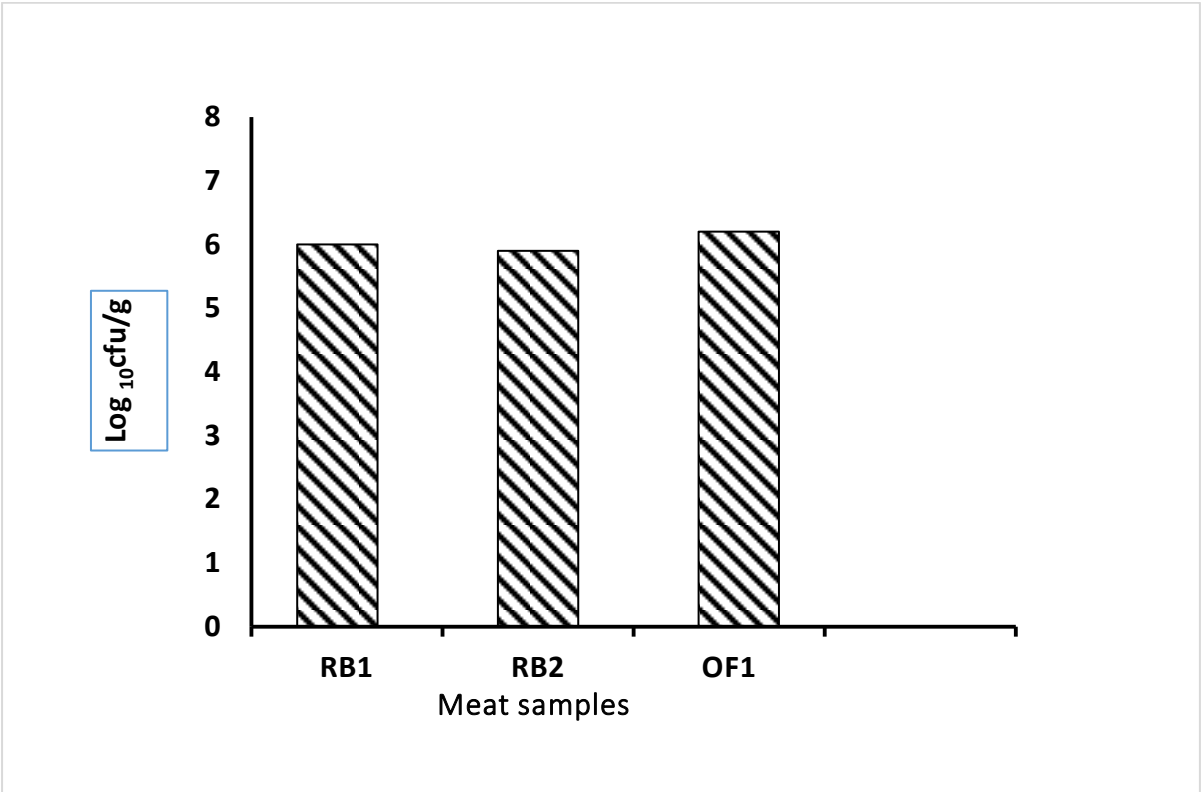

Figure 4.23: The general E. coli counts in the street vended raw beef and offals sold in Magboro, Ogun State.

The count for General E. coli species was highest in offals with a count of 6.2 Log10 Cfu/g. Although the difference in count for each sample are not significant with 6.0 Log10 Cfu/g and 5.9 Log10 Cfu/g respectively for the first and second raw beef sanple. *E. coli* is generally associated with meat at high concentration and is used as a hygiene indicator in the food industry.

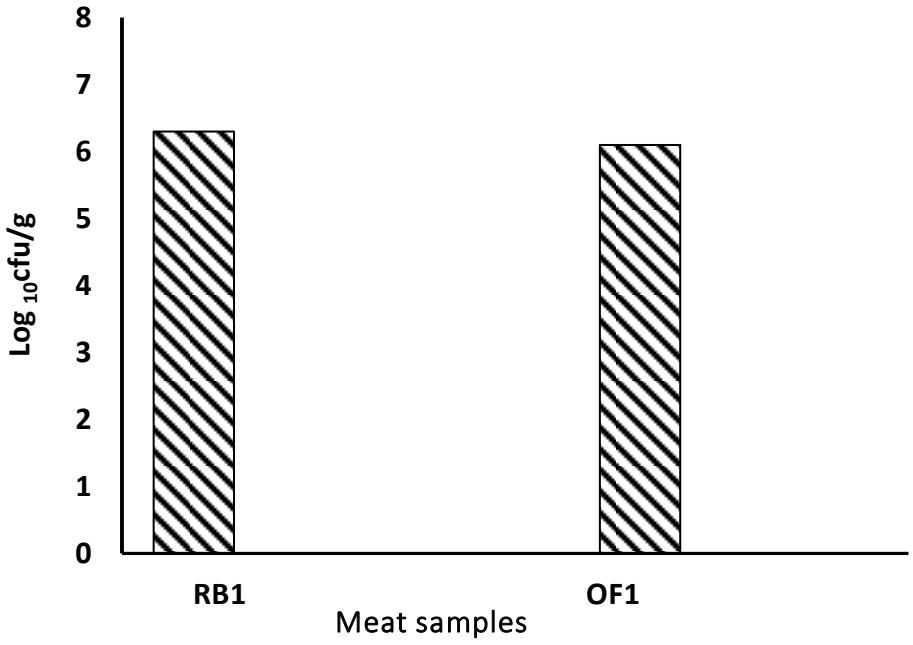

Figure 4.34: The pathogenic E. coli count in the street vended raw beef and offals sold in Magboro, Ogun State.

The count for presumptive pathogenic *E. coli* (STEC) was similar in all the samples with counts of 6.3 Log10 Cfu/g and 6.1 Log10 Cfu/g. The presumptive pathogenic *E. coli* growth are typically identified as a white colour (non 0157) while pink colour (O157). Illnesses such as haemorrhagic colitis (HC) or hemolytic-uremic syndrome (HUS) in humans, most notably in kids under 4 years resulting in acute renal failure occurs when *E. coli* (STEC) is consumed with food such as meat products (Islam et al., 2010).

4.1 DISCUSSION

Contamination of carcass surfaces by foodborne pathogens is a major public health problem, where a healthy animal may harbour pathogenic bacteria on its hide, hair, hooves and intestinal tract (Hussein, 2007). In under-developed countries where contaminated worker supplies were common, refrigeration was rare, foodborne illness might cause billion of illness and over 406 million deaths each year (CDC, 2011). The total viable count was highest in raw beef sample 2 with a count of 7.3 Log_{10} Cfu/g. The viable count was higher than the range of 4.8 Log10 CFU/g - 6.8 Log10 CFU/g reported for raw meat by Ercolini et al., (2009). Daniyan and Unwuchiola, 2013 reported the lower value about 6.6 Log10 CFU/g than this study. The TVC value higher than the maximum limit of microbial contamination might be caused by several factors including contaminated knives, water, slaughterhouse floor and wall, and evisceration table (Bhandare *et al.*, 2009). Moreover, it might also be caused by poor meat handling and poor slaughterhouse environmental conditions. According to Haileselassie et al. (2013), other factors contributed to the high bacteria load were poor standard sanitary operational procedures practiced by the slaughterhouse workers.

The counts for pathogenic *E. coli* (STEC) has no notable difference in both samples with counts of 6.3 Log10 Cfu/g and 6.1 Log10 Cfu/g. Higher *E. coli* prevalence rates have been reported by several researchers, which were about 40% (Ahmad *et al.*, 2013), 44% (Ashraf *et al.*, 2015), and 67.1% (Schlegelová *et al.,* 2004). The high level of *E. coli* in offal might be caused by several factors including *E. coli* which is a normal flora in animal intestine so it is possible that the meat may come in contact with fecal contaminants (Daniyan and Unwuchiola, 2013; Yousuf *et al.*, 2008), the nature of meat which was susceptible to *E. coli* contamination (Fraizer and Westhoff, 2008), high prevalence in developing countries due to large population in temporary shelter and poor hygiene (Nataro *et al.*, 2007), and the worker hands and the slaughtering equipment (Bryant *et al., 2003*).

Based on the Table 4.4, the presence of *Salmonella* was only observed in sample 3 which is raw offals. The prevalence of *Salmonella spp.* is minimal. In related studies

in other nations, contamination of beef carcasses during slaughter has already been observed (Madden *et al.*, 2001; Ruby *et al*., 2007). The lower *Salmonella spp.* prevalence. contamination observed in this study may be due to the successful implementation of high hygiene and sanitation along with the Hazard Analysis and Critical Control Point (HACCP) method in the slaughterhouses. Several factors that may explain the infection of *Salmonella* in raw beef meat at slaughterhouses have been identified, including bovine hide (Cossi *et al.*, 2014), carcass handling and examination (Tadesse and Gebremedhin, 2015), enforcement of slaughterhouse hygienic standards, workers attitude and practice (Haileselassie *et al.*, 2013; Ferede, 2014), and unsanitary slaughterhouse environments (Tadesse and Tessema, 2014).

CHAPTER FIVE

5 CONCLUSIONS AND RECOMMENDATIONS

Conclusions

The samples of raw beef and offal were found to be contaminated with pathogenic bacteria STEC and *Salmonella spp*. Result reveals that a high proportion of beef sold in study area Magboro market for human consumption is contaminated which remains a public health concern. Therefore, there is a possible risk to humans from consumption of these products which can lead to other illnesses such as haemorrhagic colitis (HC) or haemolytic-uremic syndrome (HUS).

5.1 Recommendations

It is recommended that beef and offal are cooked thoroughly before consumption. There is a need to enlighten food handlers on sanitary handling of meat which is possible vehicle for *Salmonella* and *E. coli* infections. Government agencies should take up the responsibility of monitoring of hygiene and sanitation of abattoirs and slaughterhouse. It is recommended that there should be adequate data collection and dissemination of information about infections or outbreak of pathogenic microorganisms in local government areas.