DETECTION AND MOLECULAR CHARACTERISATION OF PATHOGENIC *ESCHERICHIA COLI* AND *SALMONELLA* SPP IN RAW MEAT AND OFFALS IN MAGBORO, OGUN STATE

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CERTIFICATION

This is to certify that this project was carried out by **OGELEKA, CHINEYE STELLA** with matriculation number 16010104001 of the department of Biotechnology, college of basic and applied sciences in Mountain Top University under the supervision of DR **G.B AKANNI**

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H.O.D

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 Sunday and Mrs Kate Ogeleka 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 Biotechnology 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!

ABSTRACT

Escherichia coli and *Salmonella* are major foodborne pathogen causing severe disease in humans worldwide. The study was conducted to isolate, identify and characterize pathogenic *E. coli* and *Salmonella* from raw meat and offal collected from different meat vendors in Magboro, Ogun state. A total of three (3) samples were subjected to bacteriological isolation and identification, and the isolated bacteria were subjected to molecular typing. *E. coli* and *Salmonella spp* were isolated and identified by culturing on Eosin methylene blue agar, Sorbitol MacConkey agar, Xylose Lysine Deoxycholate agar respectively. Total viable count was higher at 6 Log_{10} Cfu/g in all the samples while the highest count of pathogenic *E. coli* was 6.3 Log_{10} Cfu/g. The *E. coli* isolates were positive to 16S rRNA based polymerase chain reaction (PCR). *Salmonella* species were found in 25 g of offal which is contrary to meat safety standards. The findings of this research project demonstrated the high level of microbial contamination and occurrence of pathogenic bacteria in raw meat and offal sold in Magboro, market, Ogun state.

Keywords: Contamination, *E. coli*, Salmonella, meat and offal, Magboro

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CHAPTER ONE

1.1 INTRODUCTION

The term meat, refers to animal flesh consumed as food while raw meat implies to uncooked muscle tissue of animals that is used for food (Duffy et al., 2006). Edible offal are the internal organs of animals that are used for food like the large intestine, small intestine, liver, heart, liver etc. Goats and cattles are very popular sources of meat and edible offal all over the world, and in Nigeria in particular. Meat and meat products have become part of daily human diet because of its rich and nutritive composition (Hughes et al., 2015). Meat and offal have been reported to contain high quality proteins, minerals, vitamins and fat (Nakai et al., 2000; Iroha et al., 2011). The nutritious nature of meat which is on the high side, provides a suitable environment for the growth of pathogenic, non-pathogenic as well as spoilage organisms (Steinkraus, 1994).

In Nigeria, consumption of meat has increased and slaughtering of livestock increases due to the high demand for meat and its products (Warris, 2010). However, the unsanitary handling of meat during slaughtering and processing has increased the level of meat contamination with pathogens, especially in Nigeria where the practice of food safety and meat standards are not enforced. Raw meat and meat products have been identified as important vehicles of foodborne illnesses and implicated in food poisoning outbreaks (Macrae et al., 1993, Bhandare et al., 2007). The high rate of foodborne outbreaks related to meat and meat products, have recently become a matter of public health concern. The most important pathogen associated with meat are *Salmonella spp,* Shiga toxin *Eschericia coli* (STEC), *Campylobacter jejuni, Staphylococcus aureus, Listeria monocytogenes and Clostridium perfringes.* A study reported 957 STEC outbreaks from 27 countries covering 1998 to 2017 with 16 percent of outbreaks were identified to be attributed to beef, 15 percent to produce including fruits and vegetables, and 6 percent to dairy products (Pires et al., 2019). Therefore, there is a need for rapid and precise identification of pathogens responsible for the foodborne outbreaks, thus; the use of molecular techniques in identifying pathogens up to subspecies or strain level is paramount. There is an impact of food safety on almost all segments of the society which is a complex issue, from the public to the government, industry and academia.

This study will determine the prevalence of foodborne pathogens in raw meat sold in the Ofada LCDA with emphasis on isolation of *Salmonella* species and pathogenic *E. coli* from street vended meat in line with adherence to appropriate food safety standards. This study will endeavour to investigate the molecular detection in the pathogenic *E. coli* found in the meats and edible offal sold at Magboro market.

2 CHAPTER TWO

2.1 LITERATURE REVIEW

2.2 CONTAMINATION OF RAW MEAT AND OFFAL

Foodborne disease is any toxic or infectious nature that is caused by the consumption of contaminated food or water. Foodborne pathogens are microorganisms (i.e., bacteria, viruses, and fungi) and parasites that can infect people through consumption of contaminated food or water. 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 producing *E. coli* strains (non-O157 STEC) causing foodborne diseases that produce high morbidity and mortality rates (Zhao *et al.*, 2014). *Escherichia coli* is a normal part of the intestinal micro floral of many healthy animals, including humans. However, some strains can cause diseases, most especially *E. coli* O157:H7 which is associated with meat contamination and can cause Heamolytic uremic syndrome (HUS) in children. Contamination of meat by *Salmonella* may occur at abattoirs during the removal of the gastrointestinal tract, contact with contaminated abattoir equipment, floors and personnel, while the pathogen can gain access to meat at any stage during butchering to retailing (Adesiyun et al., 1989). Cross-contamination of carcasses and meat products could continue during subsequent handling, processing, preparation and distribution (Adesiyun and Oni 1989). Things that can contribute to the quality of meat from such animals includes the state of health of such animal prior to slaughtering and the circumstance in the slaughtering house (Whyte et al., 2004). Reports have indicated that slaughtering of animals in rural communities within Nigeria is usually done under unhygienic conditions. In most cases, potable water is unavailable, leaving butchers with unhealthy water sources for use (Aleruchi et al., 2006). These earlier mentioned reasons in addition to high ambient temperature, humidity and poor handling practices dispose raw meat to deterioration and contamination (Adegbe et al., 2016). Despite foodborne disease gaining attention globally, there have been no reported cases of foodborne diseases associated with meat products in the Ofada/ Mokoloki local government area, Magboro, Ogun State, Nigeria.

2.3 *ESCHERICHIA COLI*

Escherichia coli belongs to *Domain: Bacteria, Phylum: Proteobacteria, Class; Gammaproteobacteria,* Order*: Enterobacteriales,* Family*: Enterobacteriaceae,* Genus*: Escherichia, Species: coli. Escherichia coli* was named after a German bacteriologist (Theodor Escherich) who isolated it from a faecal sample in 1885 called *Bacterium coli commune,* but later got its new name *Escherichia coli* in 1888. *E. 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. *E. coli* 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 (Kaper et al., 2004)

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. The *E. coli* O157:H7 and other pathogenic *E. coli* is thought to live mainly in the intestines of cattle (Elder, et al., 2000) but has also been found in the intestines of chickens, deer, sheep, and pigs. Shiga toxin-producing *E. coli* does not make the animals that carry it ill. The animals are merely the reservoir for the bacteria.

The *E. coli* O157 serogroup is a highly pathogenic strain of *E. coli.* E. *coli* O157 is of particular public health concern because of its severe consequences of infection (Lukman et al., 2016). 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. Root crops and leafy vegetables have the greatest risk of infection from manure application to soil during direct or indirect contact with cattle or carrier animals. In Nigeria, the only reported *E. coli* O157:H7 illness in vegetables were from the study in South-West Nigeria with minimal outbreak report (Ogunsanya *et al.,* 1994; Olorunshola *et al.,* 2000).

2.3.1. PATHOTYPES 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), Enteropathogenic *E. coli* (EPEC),

Enteroaggregative *E. coli* (EAEC), and diffuse-adherent *E. coli* (DAEC) (Simjee, 2007). 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. (ENTEROINVASIVE *E. COLI* (EIEC)

EIEC and shigella share together biochemical, genetic and pathogenic characteristics. EIEC is transmitted from host to host through the faecal-oral route primarily through contaminated water and food or direct individual to spread individual (Mondol, 2013).

2.3.2. ENTEROAGGREGATIVE *E. COLI* (EAEC)

EAEC is the diarrheagenic *E. coli* pathotype identified by displaying the distinctive pattern of AA in culture on epithelial cells. The entero-aggregate *E. coli* (EAEC) pathotype has been associated with traveller diarrhoea, endemic diarrhoea among kids in industrialized and resource-poor nations, and persistent diarrhoea among people with human immunodeficiency virus infections (Rasko *et al.,* 2011). In epidemiological studies and outbreaks, enteroaggregative *E. coli* (EAEC) was connected with diarrhoea. In Nigeria, the most comprehensive EAEC population assessment survey was conducted to identify an association with EAEC complexes and disease in kids under 5-year-old with connections to virulence genes, resistance and plasmid groups (Chattaway *et al.*, 2014).

Molecular epidemiology of EAEC infection remains unclear, largely due to imperfect recognition of the true pathogenic factors within the broadly defined pathotype (Scheutz *et al.,* 2011). Results showed that the variety of sequence kinds (STs) connected with EAEC is very wide and diseases were connected to ST10, an ST connected with various pathotypes of *E. coli* only within a particular age group.

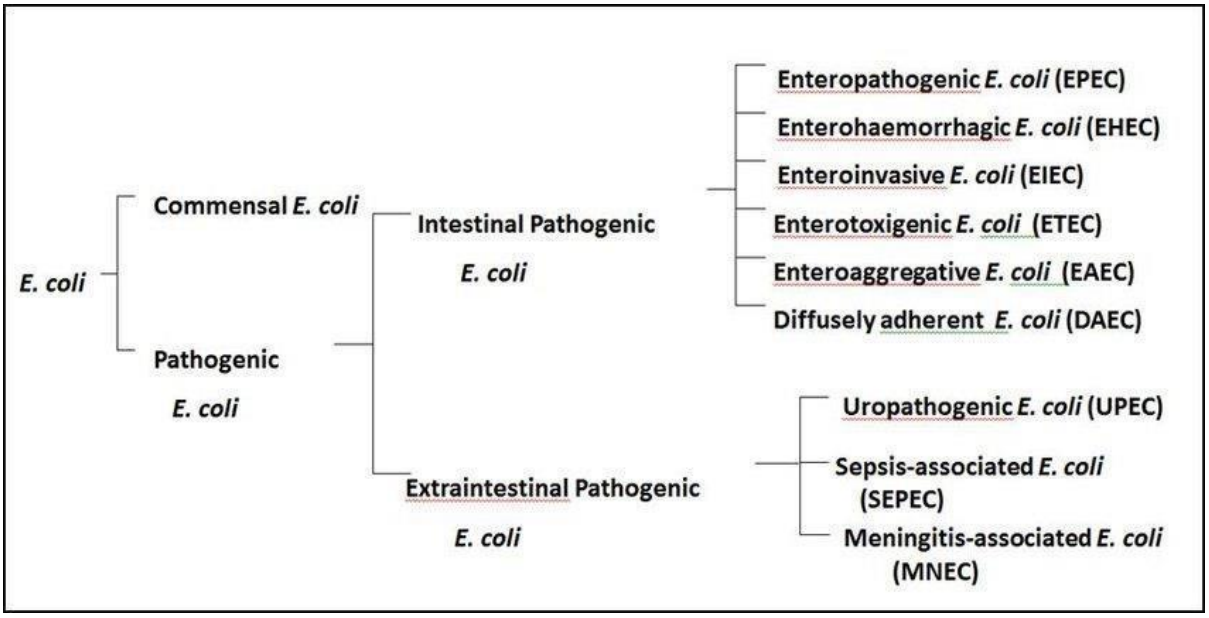


Figure 2. 1: Escherichia coli species and its pathotypes. Adapted from: Wakeham, (2013)

2.3.3. ENTEROHAEMORRHAGIC *ESCHERICHIA COLI* (EHEC)

Escherichia coli O157:H7 is a non-invasive organism, which produces verotoxin as its primary virulence factor (Doyle, 2000) which are named for their cytotoxicity to African green monkey kidney cells called Vero cells (Meng *et al*., 2001).

EHEC is a 60 megadalton virulence plasmid with Shiga-like toxins or verotoxins secretion. The Shiga-like toxin was thus named because it is similar both in structure and activity to the toxin produced by *Shigella dysenteriae* type 1 and is also neutralized by the Shiga toxin antiserum. The shiga like toxins produced by *E. coli O157:H7* are of two types which are shiga-like toxin 1 and shiga-like toxin 2. The two toxins are antigenically different with both toxins known to be cytotoxic, causing fluid accumulation in rabbit ligated ileal loops and paralysis and death in mice and rabbits while the shiga-like toxin 2 produces haemorrhagic colitis in adult rabbits.

2.3.4. SHIGA TOXIN PRODUCING *E. COLI*

The pathogen is transmitted mainly to humans through the ingestion 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). They can grow in temperature ranging from 7 ºC-50

ºC, some can grow in acidic foods down to a pH of 4.4 and in foods with minimum water activities. STEC can be destroyed or killed by thorough cooking of foods until all parts reach a temperature of 70˚ C or higher and most fruits and vegetables are eaten raw or uncooked leading to a high risk of the consumption of Shiga toxin-producing *E. coli* contaminated fruits and vegetables. *E. coli* O157:H7 is the most important STEC serotype in relation to public health (Riley *et al.,* 1983).

2.3.5. *ESCHERICHIA COLI O157*

E. coli O157:H7 is a life-threatening bacterium that produces large quantities of potent toxins that can cause severe damage to the lining of the intestines. Human illness associated with *E. coli* O157:H7 infection may include haemorrhagic colitis, haemolytic uremic syndrome (HUS), or thrombotic thrombocytopenic purpura (TTP). HUS largely affects young children and is the leading cause of acute renal failure in children. *E. coli* O157:H7 outbreaks have been linked to fresh produce such as spinach and leafy greens. *Escherichia coli* O157:H7 is a non-invasive organism, which produces verotoxin as its primary virulence factor (Doyle, 2000The shiga like toxins produced by *E. coli* O157:H7 are of two types which are shiga like toxin 1 and shiga-like toxin 2. The two toxins are antigenically different with both toxins known to be cytotoxic, causing fluid accumulation in rabbit ligated ileal loops and paralysis and death in mice and rabbits while the shiga-like toxin 2 produces haemorrhagic colitis in adult rabbits.

2.3.6. VIRULENCE FACTORS IN *E. COLI*

The ability of *E. coli* to produce toxins enhance its ability to infect a host with disease. It produces α-haemolysin 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.

Majority of *E. coli* strains are susceptible to most antimicrobial agents that are active against gram-negative bacteria, but resistance has increased due to general and global antibiotic use for the past 50 years. Resistance can be acquired via plasmids and drug efflux systems, also resistance of amoxicillin, cotrimoxazole (due to presence of TEM-1 and TEM-2 betalactamse) and trimethoprim has increased over the years (caused by the frequent carriage on plasmids and integrons of *dhfr* resistance genes) (Yu *et al.,* 2004).

2.3.7. CLINICAL SYMPTOMS OF *E. COLI* O157:H7 (STEC) INTOXICATION

The STEC is transmitted to humans via the faecal-oral route through ingestion of contaminated food or water, contact with animals that may carry the organism without being sick, or through contact with a sick person. The symptoms and diseases caused by STEC are abdominal cramp, diarrhea that may progress to hemorrhagic colitis (bloody diarrhea), fever and vomiting may also occur. When STEC is isolated from a diarrheal stool from a person, the cause of the patient's disease is usually considered established. However, not all STEC are pathogenic to man and not all pathogenic strains cause diseases in all individuals. The clinical spectrum varies from an asymptomatic infection, to watery diarrhoea with no or low grade fever, to serious bloody diarrhoea, a sign of haemorrhagic colitis; most patients with watery or bloody diarrhoea recover spontaneously within approximately one week (Mead and Griffin 1998) but a small percent develop HUS, a condition characterized by microangiopathic haemolytic anaemia, reduced number of circulating platelets and kidney failure and sometimes neurological symptoms (Pennington, 2010), e.g., cognitive impairment or aphasia and epileptic seizures (Magnus et al., 2012).

2.3.8. MOLECULAR SUBTYPING OF *E. COLI*

A variety of molecular subtyping methods have been developed to improve the understanding of the epidemiology of *E. coli* O157:H7 outbreaks. These methods include pulse field gel electrophoresis (PFGE), restriction fragment length polymorphisms (RFLP), amplified fragment length polymorphisms (AFLP), and phage typing (Willshaw et al., 1997, Shima et al., 2006). The PFGE method was standardized by CDC and has been applied successfully to discriminate outbreak-associated, sporadic, or unrelated infections since 1993 (Barrett *et al.,* 1994).

2.4. SALMONELLA SPECIES

Salmonella is a genus of Gram-negative rod-shaped bacteria of the family Enterobacteriaceae, it causes a wide range of human diseases, such as enteric fever, gastroenteritis, endocarditis and bacteraemia (Bennasar *et al.,* 2000). It is a highly resistant pathogen and it is well able to survive outside the intestine, particularly at water activities between 0.43 and 0.52 (Maciorowski *et al.*, 2007). It is usually carried by animals such as pigs or poultry or insects and is passed on to humans when

undercooked meats, eggs or milk are consumed. Salmonella are facultative anaerobes biochemically characterized by their ability to ferment glucose with the production of acid and gas (Hayes 1992). Moreover, they can exist over a diverse range of pH i.e., 4.1 to 9.0 and temperatures of 7 °C to 59 °C.

Salmonella is one of the most frequently isolated foodborne pathogens and are found in poultry, eggs and dairy products (Silva *et al.,* 2011). Other sources that are involved in the transmission of Salmonella includes fresh fruits and vegetables (Pui *et al.,* 2011)

2.4.1. CLASSIFICATION OF *SALMONELLA* SPECIES

Salmonella was first discovered and isolated from the intestines of pigs by Theobald Smith in 1855 (Saba 2012). The bacteria strain was named after Dr Daniel Elmer Salmon, an American pathologist who worked with Smith (Eng et al., 2015). The nomenclature of *Salmonella* is controversial and still changing. Currently, the Centers for Disease Control and Prevention (CDC) is currently using the nomenclature system of *Salmonella* suggested by the World Health Organization (WHO) Collaborating Centre. The genus *Salmonella* is classified into two species, *Salmonella enterica* (type species) and *Salmonella bongori*, based on differences in their 16S rRNA sequence analysis. The type species, *S. enterica*, can be further classified into six subspecies based on their genomic relatedness and biochemical properties (Reeves *et al.* 1989). The genus *Salmonella* contains two species *Salmonella enterica* (2443 serotypes) (initially known as *Salmonella cholerae-suis*) and *Salmonella bongori* (formerly called *S. enterica* (subsp) *bongori*) (20 serotypes). *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).

Typhoidal Salmonella: They are responsible for the causal of typhoid fever and the organism at the centre of this is *S. enterica* serovar typi, their prevalence is enhanced positively by poor food safety practices in the food industry. Typhoidal *Salmonella* are widely known as human parasites that exhibit severe clinical features like. Other groups that cause typhoidal salmonellosis are; *S. Sendai, S. Bublin, S. Enteritidis, S. Typhi, S. Eastbroume, S. Saintpaul* etc. (Todar, 2005; Kumar, 2012).

Non-Typhoidal *Salmonella;* Gastroenteritis is an example and it is caused by non-typhoidal salmonella strains like *S. cholerasius* and *S. typhimirium*, they also cause systemic infections in immune-compromised hosts. This group of salmonella cause diseases in both man and animals. Another example of disease caused by this group is bacteremia (Todar, 2005; Kumar, 2012).

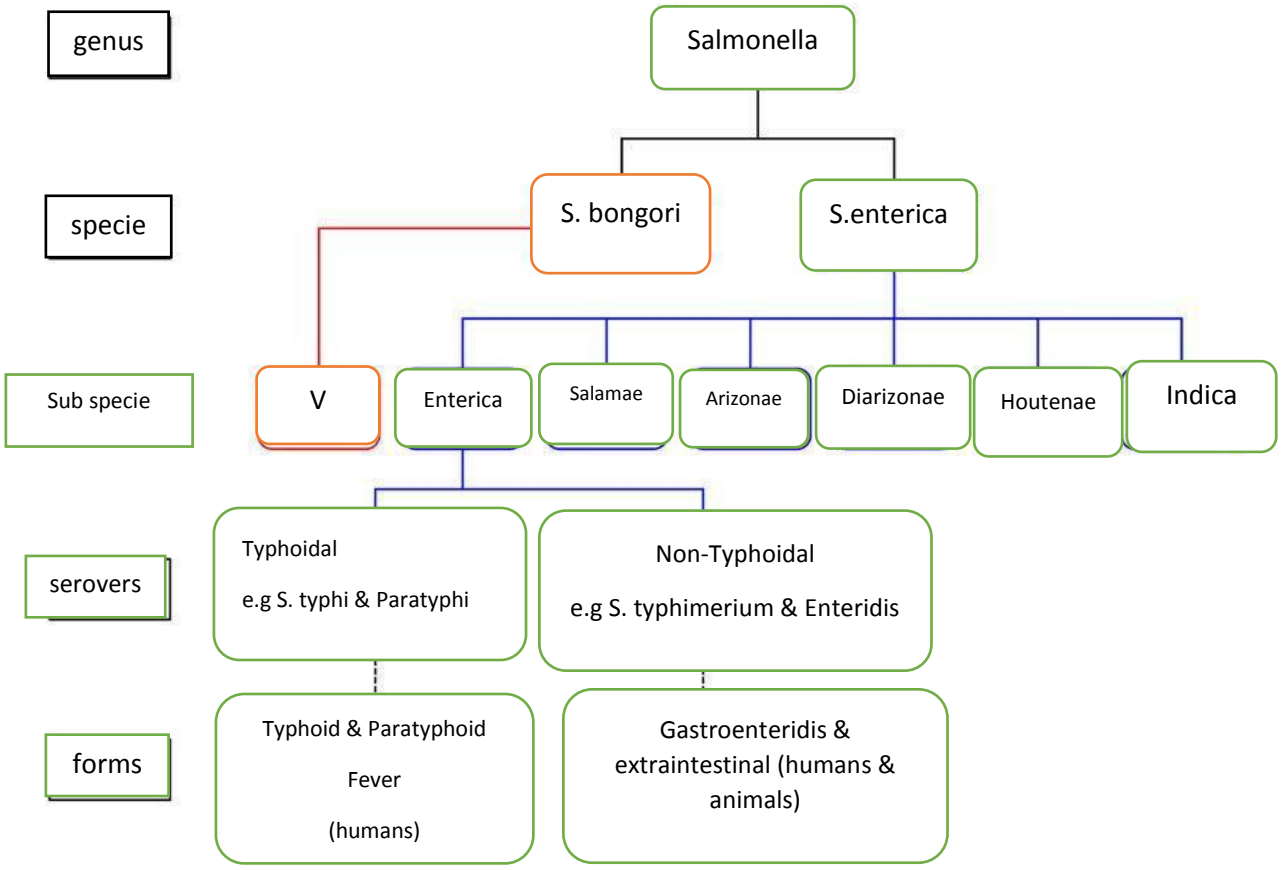


Figure 2. 2: *Salmonella* species and Classification of *Salmonella* serovars. Source: Hurley et al., (2014).

2.4.2. PATHOGENESIS OF SALMONELLA

Around the world, *Salmonella* infection is still a major concern to public health, and it has contributed to the economic burden of both developed and underdeveloped countries through the cost associated with surveillance, prevention and treatment of disease (Crump *et al.,* 2004). *Salmonella* infections in human varies depending on the serotype involved and also on the health status of the human host. Almost all strains of *Salmonella* are pathogenic due to the ability to invade, replicate and survive in human host cells which results in potentially fatal diseases (O'Beirne *et al.,* 2000). Whenever a contaminated food is ingested, *Salmonella* strains (*S. typhi*) pass the intestinal epithelium and spreads to systemic sites like spleen, bone marrow, gall bladder and liver. Symptoms of infection (headache, muscle aches, fever, stomach pain, constipation and diarrhoea) develop within the first 10-14 days of ingestion (Parry *et al.,* 2002). After treatment of infection with appropriate antibiotic treatment, some individuals will continue to shed the microorganism for several months to years (Parry et al., 2002; Gunn *et al.,* 2014). Due to the restriction of typhoidal serovars to humans, carriers are a key reservoir which contribute to the transmission and spread of typhoid (Saul *et al*., 2013; Pitzer *et al*., 2014). The mechanism used to push these *Salmonella*-infected cells into the body cavity resembles the natural mechanism humans use to shed dying or dead epithelial cells from their gut (Knoder et al., 2010). *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). However, *Salmonella* is transmitted through the consumption of contaminated foods (Srisawat and Panbangred, 2015) and also from person-to-person spread. *Salmonella* infection is at its peak in children (less than 5 years old), elderly people (older than 70 years old) and immunosuppressed individuals (pregnant women, HIV/AIDS patients).

2.4.3. *SALMONELLA* INFECTIONS

Salmonella infection (Salmonellosis) is a common bacterial disease affecting the intestinal tract. *Salmonella* bacteria usually lives in the intestine of animal and human and are expelled through faeces. Humans can become infected through contaminated water or food. Typically, people with *Salmonella* infection may have no symptoms

while others develop diarrhoea, fever and abdominal cramps within 8-72 hours (Glynn *et al.*, 1998). Without specific treatment, most healthy people recover within few days. In some cases, the diarrhoea associated with salmonella infection can be so dehydrating and will require quick medical attention. Life-threatening complications also may develop if the infection spreads beyond the intestines. The risk of becoming infected with *Salmonella* is higher in developing countries with poor sanitation (Gersham, 2017).

Salmonella infection is usually caused by eating undercooked or raw meat, poultry, eggs, egg products or vegetables and fruits (Pui *et al*., 2001). The incubation period ranges from several hours to two days. Most *Salmonella* infections can be classified as stomach flu (gastroenteritis) (Gershaman, 2017). Possible signs and symptoms include; vomiting, abdominal cramps, diarrhoea, nausea, fever, chills, headache and blood in the stool. Signs and symptoms of *Salmonella* infection generally last two to seven days (Gershman, 2017). Diarrhoea may last up to 10 days, although it may take several months before bowels return to normal

2.4.4. PREVALENCE OF *SALMONELLA* SPP.

In 1997, *Salmonella* incidence reached a high level when over 32, 000 cases were reported and most were associated with eggs and poultry (PHLS, 1999), although this rate decreased in the year 2002 due to the introduction of *Salmonella enteritidis* (PT4) vaccine and also improvements in microbiological quality of food in all stages from production point to consumption point coupled with the implementation of hazard analysis critical control point (HACCP) .*Salmonella* mostly affects children, elderly and immunosuppressed persons, so outbreaks involving them takes two forms; person-to-person spread and foodborne spread. 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*.

Table 2. 1: Major Salmonella Serovars and their Host. Adapted from Bhunia, (2018)

Serovar	**Pathogen Specificity**	**Disease**
Typhi	Humans	Typhoid Fever
Bradenburg	Sheep	Abortion
Dublin	Cattle	Enterocolitis,Typhoid Fever
Pullorum	Chicken	Bacillary white diarrhea
Cholerasius	Swine	Enteroolitis and Septicemia
Enteritidis	Humans	Gastroenteritis
Paratyphi	Humans	Typhoid fever-like
Gallinarum	Chicken	Fowl Typhoid
Arizonae	Turkeys	Paracolon Infection
Typhimurium	Animals and Humans	Gastroenteritis

2.4.5. MOLECULAR SUBTYPING OF *SALMONELLA* SPP

Molecular subtyping methods such as pulsed-field gel electrophoresis electrophoresis (PFGE), ribotyping, repetitive extragenic palindromic sequence PCR, plasmid profiling, IS*200*-restriction fragment length polymorphism analysis, random amplification of polymorphic DNA, and fluorescent amplified length polymorphism analysis are currently considered to be the serovarspecific typing methods available for certain nontyphoidal serovars, including serovars Enteritidis), Typhimurium and Infantis, and typhoidal serovar Paratyphi. However, there has been no recognized typing method reported for serovar Oranienburg (Johnson et al., 2001)

2.4.6. CLINICAL SYMPTOMS OF *SALMONELLA* INFECTION.

Most pathogenic *Salmonella* serotypes in mammals and birds are generally subsp. 1. *Salmonella* infections can occur in three ways. Firstly, few serotypes that cause systemic disease, cases like typhoid and paratyphoid bacilli in humans, example is the one of enteric fever with an incubation period of 10–20 days, but outside limits is between 3 and 56 days. Diarrhea, starting 3-4 days after the onset of fever and lasting for at least 6 days, may occur in 50% of typhoid fever cases mostly found in younger children or adults. Second, some other serotypes — Blegdam, Bredeney, Choleraesuis, Dublin, Enteritidis, Panama and Virchow in humans, and Gallinarum in adult fowl — are also invasive, but tend to cause pyaemic infections and locate in viscera, meninges, bones, joints, and serous cavities (Steven et al., 2014).

2.5. FOODBORNE OUTBREAKS ASSOCIATED WITH *E. COLI* AND *SALMONELLA* SPP. IN MEAT

The first outbreak of *E. coli* O157 occurred in Oregon and Michigan, USA, in 1982, when it was isolated from individuals who developed bloody diarrhoea and severe abdominal cramps after eating hamburgers in a restaurant chain (Pennington, 2010). Research has found *E. coli* strain group known as Shiga-toxin or verocytotoxin (enterohaemorrhagic *E. coli*) which are

diarrheagenic *E. coli* to be one of the most common members of pathogens. *Salmonella enterica* serotypes are important foodborne pathogens, and *Salmonella*-related diseases pose major public health issues. Salmonellosis is one of many countries' most significant foodborne illnesses. Few nations have a surveillance scheme to measure the human population burden of Salmonellosis. *Salmonella*, Campylobacter and enterohaemorrhagic *E. coli* are microbial pathogens of present concern that need to be controlled in fresh meat. The most common cause of human Salmonellosis is the consumption of contaminated ingredients. Fresh and processed meat products are often infected with *Salmonella*, including poultry, beef and pork. Despite advancement in their control, these pathogens continue to be of interest in the future, they have been the object of control attempts for many centuries and are still engaged in a considerable amount of diseases (Sofos 2008). The most prevalent route of *Salmonella* transmission appears to be contaminated poultry and meat products consumption (Percival and Cutting, 2010). *Salmonella* is one of the most important pathogenic genera implicated in foodborne bacterial outbreaks and diseases. *Salmonella* infections are worldwide and

constitute an important public health problem in many parts of the world (Hassanein et al., 2011). Pork has been identified as a frequent source for human Salmonellosis in various studies (Wong et al., 2007). However, not only pork but also poultry and beef have been associated with the transmission of *Salmonella* (Meyer et al., 2010). The summary of outbreak Outbreaks associated with *Salmonella* spp. and *E. coli* in different food sources from 2009-2017 is shown in Table 2

Table 2. 2: Outbreaks associated with *E. coli* and *Salmonella* spp in different food sources 2009-2019 (FDA and CDC 2019)

Country	Year	Microorganisms	Food source	Number of outbreaks	Reference
Washington	NA	*E. coli*	Meat	5	CDC
Oregon	NA	*E. coli*	Ground beef	4	CDC
Minnesota	NA	*E. coli*	Meat	11	CDC
Ohio	NA	*E. coli*	Ground beef, meatballs	5	CDC
New York	2019	*E. coli* O26	Bakers flour	NA	FDA
California	2019	*E. coli* O157:H7	Romaine lettuce	NA	FDA
Washington	2009	*Salmonella*	Meat	7	FDA
California	2009	*Salmonella*	Meat	6	CDC
Canada	2017	*E. coli* O157	Leafy greens	NA	FDA
California	2018	*Salmonella*	Shell eggs	NA	FDA

3. CHAPTER THREE

3.4. MATERIALS AND METHOD

3.4.1. SAMPLING

A total of 3 samples were collected, fresh meat (beef and offal) samples were collected from three different locations of Magboro marketplace, Ogun State 6° 42' 56" North, 3° 24' 9" East, Nigeria. Samples were collected in sterile plastic bags and taken immediately to the laboratory for analysis. Sampling was repeated three times.

3.4.2. MATERIALS

Petri dish, inoculating loop, conical flask, beakers, measuring cylinder, weighing balance, bunsen burner, wash brush, spatula, test tubes, test tube racks, cotton wool, 70% alcohol, hand gloves, cork borer, micropipette, gram staining kit, Eppendorf tubes, sterile tips, distilled water, hydrogen peroxide, autoclave, paper tape, oxidase test strip, glass slide, immersion oil, microscope, electrophoresis tank, UV transilluminator.

MEDIA USED

3.4.3. BUFFERED PEPTONE WATER

Peptone water is a non-selective broth medium which can be used as a primary enrichment medium for the growth of bacteria, it is composed of peptic digest of animal tissue and sodium chloride is a microbial growth medium. It is rich in tryptophan, having a pH of 7.2-7.4 at 25˚C.

Preparation

The dehydrated medium was dissolved in the appropriate volume of distilled water to make up 0.1% and 1% peptone water based on manufacturer's instruction's instructions in a conical flask and mixed thoroughly. The conical flask is then closed in cotton wool that is wrapped in aluminum foil. The mixture was heated for a while to dissolve the powder completely and was then sterilized by autoclaving at 121°C for 15mins. Then, 9ml of the 0.1% was then dispensed into various test tubes for serial dilution.

225ml of the 1% was then dispensed into conical flask for primary enrichment.

3.4.4. SORBITOL-MACCONKEY AGAR (SMAC)

Preparation: 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 instruction's instructions in a conical flask and mixed thoroughly. The conical flask is then closed in cotton wool that is wrapped in aluminum foil. The mixture was heated for a while to dissolve the powder completely and was then sterilized by autoclaving at 121°C for 15minutes. 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.

3.4.5. NUTRIENT AGAR

The dehydrated medium was dissolved in the appropriate volume of distilled water that is 28g of Nutrient agar in 1000 ml 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. The mixture was heated for a while to dissolve the powder completely and was then sterilized by autoclaving at 121°C for 15minutes. 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. The medium appears opalescent and is light amber in color

3.4.6. PREPARATION OF EOSIN METHYLENE BLUE AGAR (EMB)

The dehydrated medium was dissolved in the appropriate volume of distilled water; 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.

The mixture was then heated for a while to completely dissolve the powder and was then sterilized by autoclaving at 121^0C for 15minutes.

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.

3.4.7. PREPARATION OF POTATO DEXTROSE AGAR

The dehydrated medium was dissolved in the appropriate volume of distilled water based on manufacturer's instructions; for instance, 39g dissolved in 1000ml distilled water in a conical flask and mixed thoroughly. The conical flask was then corked with cotton wool wrapped in aluminum foil.

The mixture was then heated for a while to completely dissolve the powder and was then sterilized by autoclaving at 121^0C for 15minutes. 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. The potato dextrose agar was modified with 25mg of chloramphenicol.

3.4.8. 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

Dissolve 19g dehydrated media of selenite F in 750 ml distilled water in a sterile conical flask. (Part A). Dissolve 4g of sodium biselenite in 250ml distilled water in another conical flask. (Part B). Then, mix PART A and PART B together. Warm to dissolve the medium completely. Distribute in sterile test tubes and sterilize in a boiling water bath or free-flowing steam for 10mins.

3.5. SAMPLE PREPARATION

25 g of meat or offal samples were weighed and dispensed in 225ml of 1% peptone water (primary enrichment broth) in a conical flask. The samples were homogenized for 4 minutes at 180rpm in a stomacher.

3.5.1. PRIMARY ENRICHMENT

25 g of homogenized sample in 225ml of peptone water was incubated at 37 ºC for 24 hrs.

3.5.2. SECONDARY ENRICHMENT

This enrichment was peculiar to *Salmonella* alone. 1 ml of sample pipetted from the peptone water was dispensed into 9mls selenite F broth in a test tube 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.

3.5.3. SERIAL DILUTION

1 ml of the samples were pipetted using the micro-pipette (set at 1000ul) into test tubes containing 9ml of BPW (0.1%) to obtain 10^{-2}, followed by the transfer of 1ml from 10^{-2} into a new test tube (containing 9ml of BPW) to create 10^{-3} dilution, the test tubes are then put in the vortex mixer for even mixing. The dilution factor was repeated for 10^{-3}, 10^{-4}. The test tubes were labelled for easy identification.

3.5.4. PLATING (SPREAD PLATE TECHNIQUE)

For the Nutrient agar, potato dextrose agar and Eosin methylene blue agar plates, spread plate technique was used for plating of inoculum (samples). About 15-20ml of agar were poured into sterilized petri dishes (observing aseptic methods and conditions), then allowed to cool, set and solidify. 0.1ml of the inoculum directly from dilutions 10^{-2}, 10^{-3} and 10^{-4} were plated (using pipettes) onto appropriately labelled agar-containing petri-dishes for Nutrient agar and Eosin methylene blue agar, this will suffice for the enumeration, identification and isolation of Total Viable Counts and *Escherichia coli* strains respectively. After the dispensing, 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* in the meat and offal samples, 0.1ml of inoculum from the secondary enrichment media (incubated overnight in the scotch bottles) 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 in an inverted fashion, are transferred into the incubator set at 37°C for 18-24 hours

3.6. ENUMERATION OF TOTAL VIABLE COUNT (TVC)

Nutrient agar was used for the enumeration of mesophiles. After mixing, 0.1 ml of each dilution factor was spread onto the surface of sterile Nutrient Agar and then incubate inversely at 37^{o}C in an incubator for 24hrs.

3.6.1. ENUMERATION OF *E. COLI*

Eosin methylene blur agar was used for the enumeration of total *E. coli*. After mixing, 0.1 ml of each dilution factor was spread onto the surface of sterile Eosin methylene blue agar and then incubate inversely at 37ºC in an incubator for 24hrs.

3.6.2. ENUMERATION OF *E. COLI* O157 AND NON-O157

For the enumeration of *E. coli* O157 and non-O157 sorbitol-MacConkey Agar (SMAC) was inoculated with 0.1 ml each of the appropriate dilution and incubated at 37ºC for 24 hrs (ISO 16654:2001).

3.6.3. SUB CULTURING

Sub culturing was carried out to purify the isolated bacterial colonies from a mixed culture. Bacterial isolates transferred or sub-cultured were differentiated based on their colony morphology, shape, colour, elevation and other physical characteristics.

Presumptive colonies obtained after incubation were sub- cultured unto fresh nutrient agar plates by streaking method. The plates were inverted and incubated at 37^{O}C for 18- 24 hrs.

3.6.4. ENUMERATION OF SALMONELLA SPECIES

For *Salmonella spp* enumeration, primary enrichment in buffered peptone water incubated 37oC for 24 h, followed by secondary enrichment in Selenite F broth for 37ºC for 24 hrs, 1 ml of the secondary enrichment was transferred to *Salmonella-Shigella* agar for presumptive confirmation of *Salmonella* spp.(Seow et al., 2012)

3.7. CRYOPRESERVATION OF ISOLATES

A loopful of each isolate was inoculated into a sterile Eppendorf tube containing 1ml of brain heart infusion incubated at 37 ºC for 24h and 500ul mixed with equal volume of sterile 20 % glycerol as cryoprotectant and it was stored at -4 ºC in a freezer.

3.8. MOLECULAR CHARACTERIZATION OF *E. COLI* AND *SALMONELLA* SPP.

3.8.1. DNA EXTRACTION

BOILING METHOD

Each isolate was streaked out on nutrient agar and incubated overnight at 37^0C. The loopful actively dividing cells were emulsified in 500µl 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 14,000 rpm 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.

3.8.2. 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)

Table 3. 1: 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

Table 3. 2: MULTILEX PCR PROTOCOL

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
10	Total	15ul

Table 3. 3: 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

3.8.3. 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 3ul 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.

Statistical Analysis

The number of colonies per plate were converted to colony-forming unit (cfu/g) and log-transformed (log_{10}) to obtain normal distribution for statistical analysis.

4. CHAPTER 4

4.4. RESULTS AND DISCUSSION

The microbial analysis of the meat and offal samples from Magboro market, Ogun state showed high total viable count 6 log_{10} Cfu/g, general *E. coli*, pathogenic *E. coli*, fungi and yeast and *Salmonella* species. The results of the findings were summarized in the tables below

Table 4. 1: Morphological characteristics of isolates on Eosin methylene blue agar for general *E. coli* counts

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

		sheen						
	RB2 EMB 3	Metallic green sheen	Circular	Small	Raised	Shiny	Smooth	opaque
	RB2 EMB 4	Metallic green sheen	Circular	Small	Raised	Shiny	Smooth	opaque
Raw offal 3rd sample	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 sheen	Circular	Small	Raised	Shiny	Smooth	opaque

Table 4. 2: morphological characteristics of isolates on Sorbitol mac Conkey agar for presumptive E. coli O157 and non O157

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

		OF1 SMAC 1	White	Circular	Small	Raised	Smooth	Smooth	opaqu

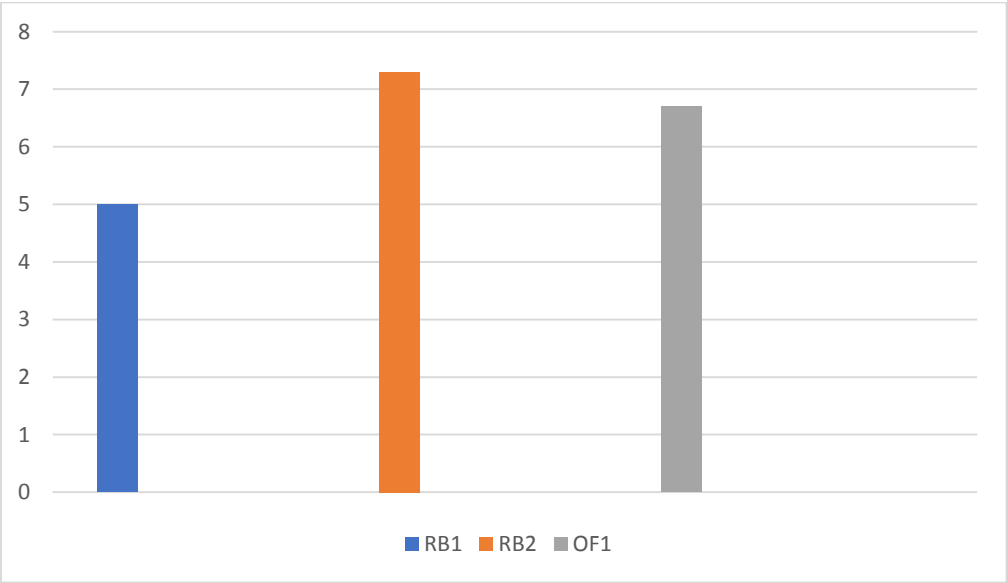
Table 4. 3: Morphological characteristics of isolates on Potato dextrose agar for yeast and mould counts

Isolate	Sample	Isolate ID	Colour	Shape	Size	Elevation	Appearance	Surface	Opacit
1st sample	Raw beef	RB1 PDA 1	Cream	Circular	Small	Raised	Smooth	Smooth	Opaqu
		RB1 PDA 2	Cream	Circular	Small	Raised	Smooth	Smooth	Opaqu
		RB1 PDA 2	Cream	Irregular	Medium	Flat	Filamentous	Filamentous	Opaqu
		RB1 PDA 3	Cream	Irregular	Medium	Flat	Filamentous	Filamentous	Opaqu
		RB1 PDA 3	Cream	Circular	Small	Raised	Smooth	Smooth	Opaqu
		RB1	Cream	Circular	Small	Raised	Smooth	Smooth	opaqu

		PDA 4							
2nd sample	Raw beef	RB2 PDA1	Cream	Circular	Small	Raised	Smooth	Smooth	Opaqu
		RB2 PDA2	Cream	Circular	Small	Raised	Smooth	Smooth	Opaqu
		RB2 PDA 2	White	Irregular	Medium	Flat	Filamentous	Filamentous	opaque
		RB2 PDA 3	Cream	Circular	Small	Raised	Smooth	Smooth	Opaqu
3rd sample	Raw offal	OF1 PDA 1	Cream	Circular	Small	Raised	Smooth	Smooth	Opaqu
		OF1 PDA 2	Cream	Circular	Small	Raised	Smooth	Smooth	Opaqu
		OF1 PDA 4	Cream	Circular	Small	Raised	Smooth	Smooth	Opaqu

Table 4. 4: Morphological characteristics of isolates on Xylose lysine deoxycholate agar for presumptive *Salmonella spp*

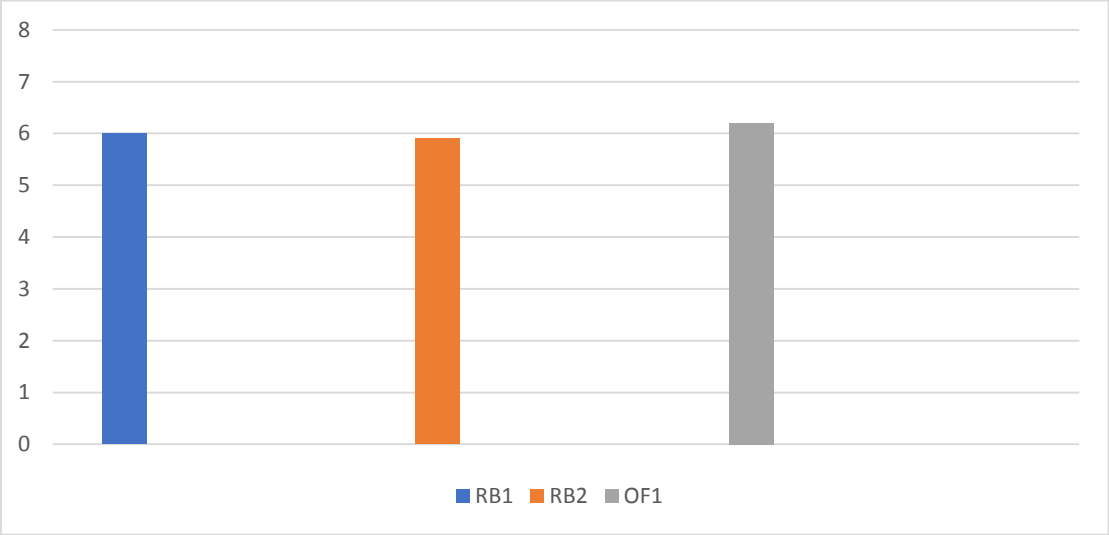
Isolate	Sample	Isolate ID	Colour	Shape	Size	Elevation	Appearance	Surface	Opacity
1st sample	Raw beef	RB1 XLD 1	Pink	Circular	Small	Raised	Smooth	Smooth	Opaque
3rd sample	Raw offal	OF1 XLD 1	Black	Circular	Small	Raised	Smooth	Smooth	opaque



Keys: RB1 – Raw beef 1; RB2 – Raw beef 2, OF1 – Offal

Figure 4. 1: Counts of Total Viable Cells in the two raw beef and one offal samples

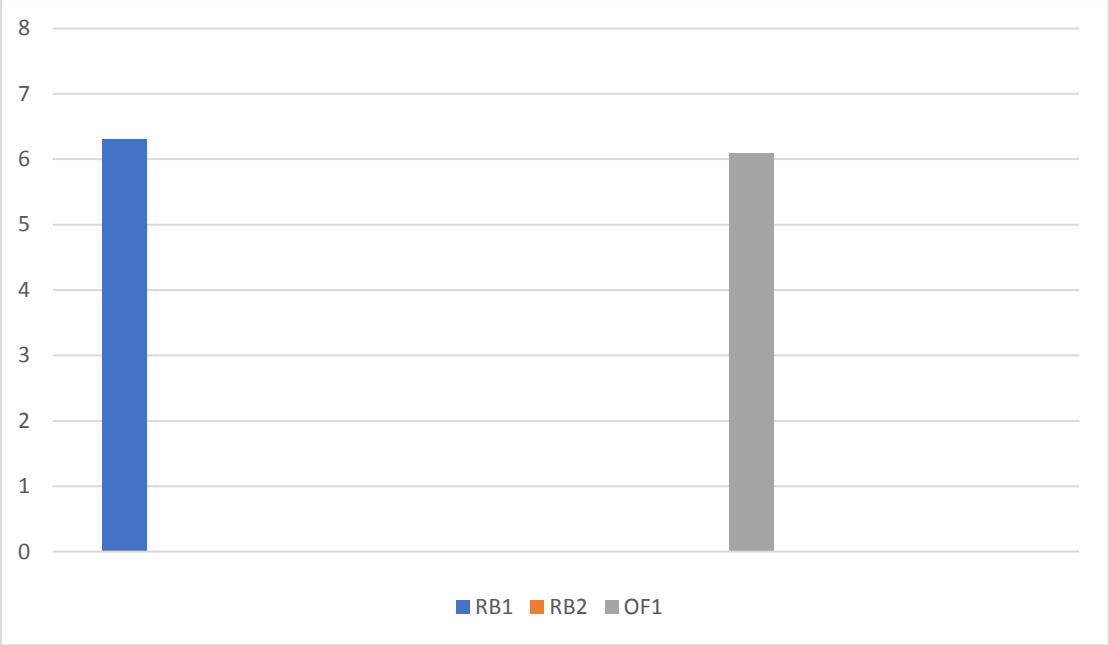
The total viable count was high in raw beef sample 2 with a count of 7.3 Log_{10} Cfu/g. The viable count was higher than the range of 4.8 Log_{10} Cfu/g - 6.8 Log_{10} Cfu/g reported for raw meat by Ercolini et al., (2009). However, there have been instances in with the level of mesophilic bacteria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



Keys: RB1 – Raw beef 1; RB2 – Raw beef 2, OF1 – Offal

Figure 4. 2: Count of E. coli count from two raw beef and one offal samples on EMB agar

The count for General *E. coli* species was high in offal 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 sample. *E. coli* is generally associated with meat at high concentration and is used as a hygiene indicator in the food industry.



Keys: RB1 – Raw beef 1; RB2 – Raw beef 2, OF1 – Offal

Figure 4. 3: Count of the pathogenic E. coli count from two raw beef and one offal samples on SMAC agar

The count for pathogenic *E. coli* (STEC) showed no significant difference in both raw beef and offal samples with counts of 6.3 Log_{10} Cfu/g and 6.1 Log_{10} Cfu/g. The presumptive pathogenic *E. coli* growth are typically identified as a colourless/white colour colony as O157 while pink colour non O157 *E. coli* isolates. Illnesses such as haemorrhagic colitis (HC) or haemolytic-uremic syndrome (HUS) in humans, most notably in kids under 4 years resulting in acute renal failure occurs when ingested by *E. coli* (STEC) (Islam et al., 2010). STEC was recognized in 1982 as a pathogen that posed a danger to public health connected mostly with undercooked beef consumption (Schroeder et al., 2002). The STEC is expected to be higher in offal because it is usually found in the intestinal tract of cattle, however, it this case it shows the raw beef had been contaminated which could be from different sources like from the butcher, the butchers knife or table, from the water used by the butcher or from cross contamination.

4.5. MOLECULAR IDENTIFICATION

The amplification of the 16S rRNA of *E. coli* was shown in the gel image (Figure 4.4) showing the amplicons at 1200 bp. Screening of *E. coli* isolates from meat and offal in

this study was carried out by genus specific (16S rRNA gene) polymerase chain reaction (PCR). The PCR assay was able to amplify 1200bp fragment from the targeted gene from the genomic DNA of *E. coli* successfully.

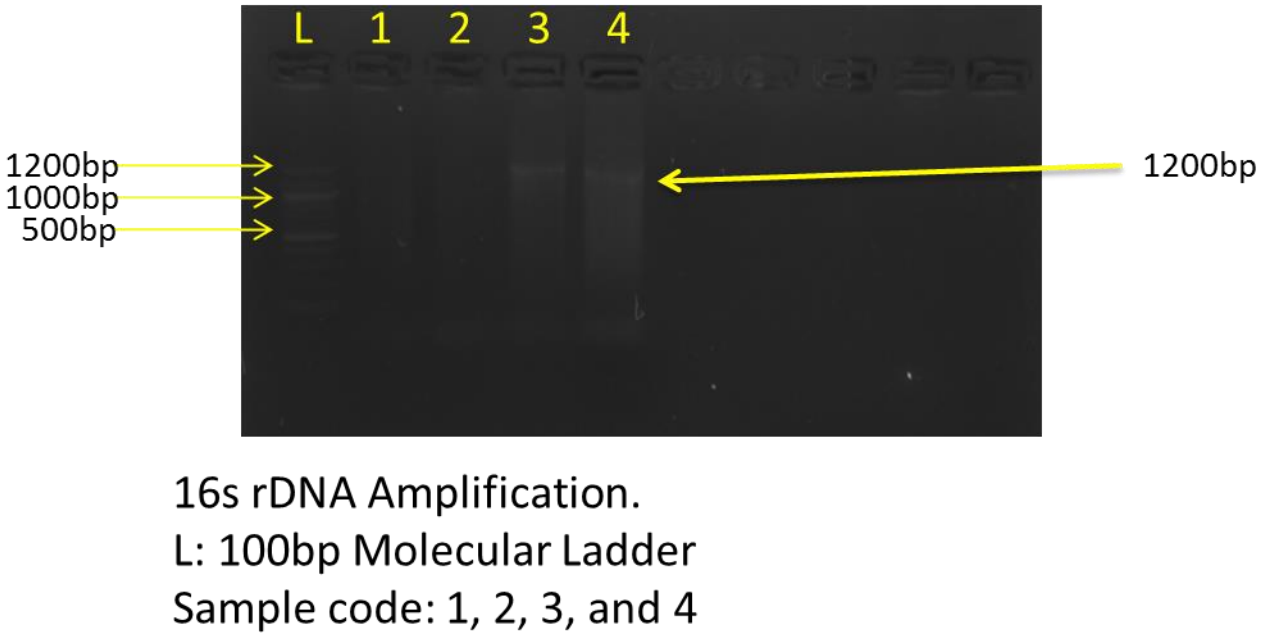


Figure 4. 4: The electrophoresis gel showing the band of E. coli 16S rDNA amplification

Foodborne illness of microbial origin is the most serious food safety problem. These outbreaks are usually caused due to improper handling, poor personal hygiene of the meat sellers which has contributed most to disease incidence. The recorded data in table 6 shows that *E. coli* was detected in all the samples of raw beef and offal, the second sample of raw beef RB2 had the highest isolates of *E. coli*, followed by the first meat sample RB1 and then the raw offal OF1.

The recorded data in Table 4.2 showed that pathogenic *E. coli* was isolated in all the samples of raw beef and raw offal, it shows the occurrence of pathogenic bacteria in the examined samples were high. The presence of *E. coli* and pathogenic *E. coli* is an indication of lack of proper hygiene by butchers and meat vendors in Magboro market. Table 4.3 shows the presence of yeast and fungi in the samples which was on a high side. Finally, the recorded data in table 4.4 clarifies that *Salmonella and Shigella* were detected in just two samples that were examined which were the first raw beef RB1 and raw offal OF1. This shows that the raw beef and offal are not safe for consumption because Salmonella should not be found in 25g of food sample. The beef and offal in

Magboro market is contaminated with pathogens which could have been from the water used in washing the beef carcass, the butchers table, or the butcher's knife.

The DNA extracted were subjected to PCR which amplified the 16S rRNA to detect the presence of the gene for *E. coli.* The specificity and sensitivity of PCR makes this technique a potent tool for the detection of pathogens, including those found in foods. However, only the result of *E. coli* isolates was reported in this study.

5. CHAPTER 5

5.4. CONCLUSIONS AND RECOMMENDATIONS

In this study, pathogenic *E. coli* and *Salmonella* were isolated from 3 samples from different locations in Magboro. The two types of samples analysed meat and offal were

found to be contaminated with pathogenic bacteria; *E. coli* and *Salmonella spp,* yeasts and moulds. This study revealed that a high proportion of pathogenic *E. coli* in both raw beef and offal but *Salmonella* spp were found in offal only. The raw beef and offal sold in the study area are contaminated with pathogens and unsafe human consumption; which remains a public health concern. Therefore, these microorganisms pose a possible risk to humans especially from consumption of these products which can lead to other illnesses. Molecular detection of pathogens will safe time in foodborne pathogen detection.

It is recommended that beef and offal are cooked thoroughly before consumption. There is a need for education on sanitary handling of meat which is a 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 surveillance, data collection and dissemination on foodborne infections outbreak in local government areas.