PREVALENCE OF *SALMONELLA* SPECIES AND PATHOGENIC *ESCHERICHIA COLI* IN STREET VENDED RAW MEAT IN OFADA, MOKOLOKI, OGUN STATE

BY

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JULY, 2019.

CERTIFICATION

This is to certify that this project was compiled by POPOOLA BEAUTY OREOLUWA a student of the Department of Biological Sciences (Microbiology), Faulty of Basic and Applied Sciences, Mountain Top University Ogun State, under my supervision.

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DEDICATION

I dedicate this project to ALMIGHTY GOD for his mercy, strength, guidance and protection upon my life and enabling me to complete my project successfully and also to my wonderful parents, MR. & MRS POPOOLA E. O. for their support, advice, love and care.

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My profound gratitude goes to ALMIGHTY GOD for his guidance and protection on me, enabling me to successfully complete my project successfully.

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ABSTRACT

Bacterial pathogens are of the greatest concern to customers about meat safety problems. A broad range of pre-slaughtering, post-slaughtering and processing procedures are responsible for human pathogen contamination of raw meat goods. 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 8.05 $\log_{10}$ CFU/g and 8.12 Log_{10} CFU/g for SMAC were found in Offals and meat respectively. *Salmonella* species were found in 25 g of both offals and meat which are 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 hemorrhagic colitis (HC) or hemolytic-uremic syndrome (HUS). There is a need for education on sanitary handling of meat which is possible vehicle for S*almonella* and *E. coli* infections. The responsibility of tracking the hygiene and sanitation of abattoirs and slaughterhouse in Ofada / Mokoloki LCDA should be taken over by government agencies.

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CHAPTER ONE

1.0 INTRODUCTION AND PROBLEM STATEMENT

Bacterial pathogens are of the greatest concern to customers about meat safety problems. Responsible for human pathogen contamination of raw meat products are a wide variety of pre-slaughtering, post-slaughtering and processing processes. While thorough cooking kills pathogens, cooked meat can be re-contaminated during processing or from the setting by food handlers (Tafida *et al.,* 2013). One of the primary sources of food-borne disease globally is contaminated raw meat. Meat is the primary edible component of domesticated animals. The recent definition does, however, include birds and fish, shellfish, poultry and exotic animals such as frogs and alligators (Iroha *et al.,* 2011).

Similarly, meat refers to animal tissue used as food, mostly skeletal muscles and associated fat, but it can also refer to organs including lungs, livers, skin, brains, bone marrow, kidney and a variety of other internal organs as well as blood. Recent growth in meat consumption and its products stems from factors including elevated protein content, vitamins, minerals, lipids, and savory feeling (Iroha *et al.,* 2011). Meat can serve as a vehicle of foodborne disease and many outbreaks have been linked to meat. For example, antibiotic resistant *Salmonella* strains can be transferred from livestock to humans through consumption of contaminated meat (Aslam *et al.,* 2012). *Salmonella* is one of the most significant pathogens reported in foodborne bacterial outbreaks that cause severe gastroenteritis in humans. Different routes of transmission are recognized, but most human infections are caused by the consumption of contaminated products, especially those of animal origin (Hassanein *et al.,* 2011). Gastroenteritis is generally connected with the consumption of poultry and red meat in humans induced by *Salmonella* infection (Lammerding *et al.,* 1988).

Contaminated raw meat has been recognized as one of the major sources of foodborne disease. Contamination of meat by *Salmonella* may happen at slaughterhouses during the removal of the gastrointestinal tract, contact with contaminated slaughterhouse machinery, floors and staff, while the pathogen may have access to meat at any point during the slaughter.

During subsequent handling, processing, preparation and distribution, cross-contamination of carcasses and meat products could continue (Sallam *et al.,* 2014). Likewise, Escherichia coli is a common pathogen associated with meat, with *E. coli* O157 the most common member of a group of pathogenic *E. coli* strains known as organisms producing enterohaemorrhagic, verocytotoxin, or Shiga toxin (Pennington, 2010).

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. 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.

CHAPTER TWO

2.0 LITERATURE REVIEW

Foodborne pathogens are microorganisms (i.e. bacteria, viruses, and fungi) and parasites that can infect people through consumption of unhygienic food or water. Foodborne bacteria includes *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). Foodborne disease has been a severe concern to the government and is still acknowledged as a significant human health issue. One of the primary sources of foodborne disease globally is contaminated raw meat.

Meat is a wealthy source of nutrients, it offers an appropriate atmosphere for spoilage and foodborne pathogens to grow and propagate. Meat spoilage can happen through infection of the living animal by spoilage microorganisms (endogenous illness) or contamination of the postmortem meat (exogenous disease), the latter being more frequently found (Gul *et al.,* 2016).

Meat is one of the most highly perishable foods that by microbial growth and breakdown by endogenous enzymes can become unfit for human consumption and possibly dangerous to health. In latest years, meat safety has been at the forefront of societal issues, and there are signs that meat safety difficulties will remain in the future (Sofos, J.N., 2008). Meat is usually considered to be one of human diet's most common and nutritious products. There are several routes to create biological hazards for meat and poultry products, primarily due to favourable growth conditions. 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. They 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).

Microbial and particularly bacterial pathogens are connected with the most severe meat safety issues arising in instant customer health problems and recalls from the marketplace of possibly contaminated products (Sofos 2008). These dangers may also reduce the suitability of meat for use during processing in certain products, or may result in cross-contamination as a consequence of hygiene failure. Most essential may be that meat-borne risks can cause disease in customers, ranging from subclinical infection to serious disease and even death. If other foods are contaminated, infection pathways may be complicated and lead to indirect infection (Gul *et al.*, 2016). Meat product contamination is mainly correlated with meat exposure during slaughter to faecal matter.

Pathogens such as *Salmonella* spp, once the meat is contaminated incorrect storage or undercooking may proliferate. When *Salmonella* is excreted in faeces; food and water contamination allows the infection to be transmitted to humans. Person-to-person, faecal-oral transmission occurs and was an issue in health care facilities where insufficient hand washing was recognized. Chronic carrier occurrences are uncommon in humans, but are 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 human-only colonization and are not commonly dispersed in nature. Contrary to this, non-typhoidal species of *Salmonella* are widespread in nature and strongly related to both livestock and humans (Percival and Cutting, 2010).

2.1 *SALMONELLA* SPECIES

*Salmonella*e are gram-negative, optionally anaerobic, rod-shaped bacteria of the Enterobacteriaceae family. There are two species in the genus *Salmonella*, which are *Salmonella enterica* and *Salmonella bongori*. The *S. Enterica* has six designated subspecies namely: I (*subsp. enterica*), II (*subsp. salamae*), IIIa (*subsp. arizonae*), IIIb (*subsp. diarizonae*), IV (*subsp. houtenae*) and VI (*subsp. indica*) with more than 2400 serovars (Pusterla *et al.,* 2013). Moreover, 99% of human-pathogenic serovars are found in *S. enterica* subsp. *Enterica* (sub-species I) comprising typhoidial (*S. Typhi and S. Paratyphi*) and non-typhoidial (Gamazo *et al.,* 2009).

In the natural environment, members of the other five subspecies (II – VI) are primarily cold-blooded animal parasites. There are 22 serovars in *Salmonella bongori*. Salmonellosis is a condition caused by an enteric or systemic infection with a *Salmonella* genus bacterium. Figure 2.1 shows a summary of the classification of the *Salmonella* species.

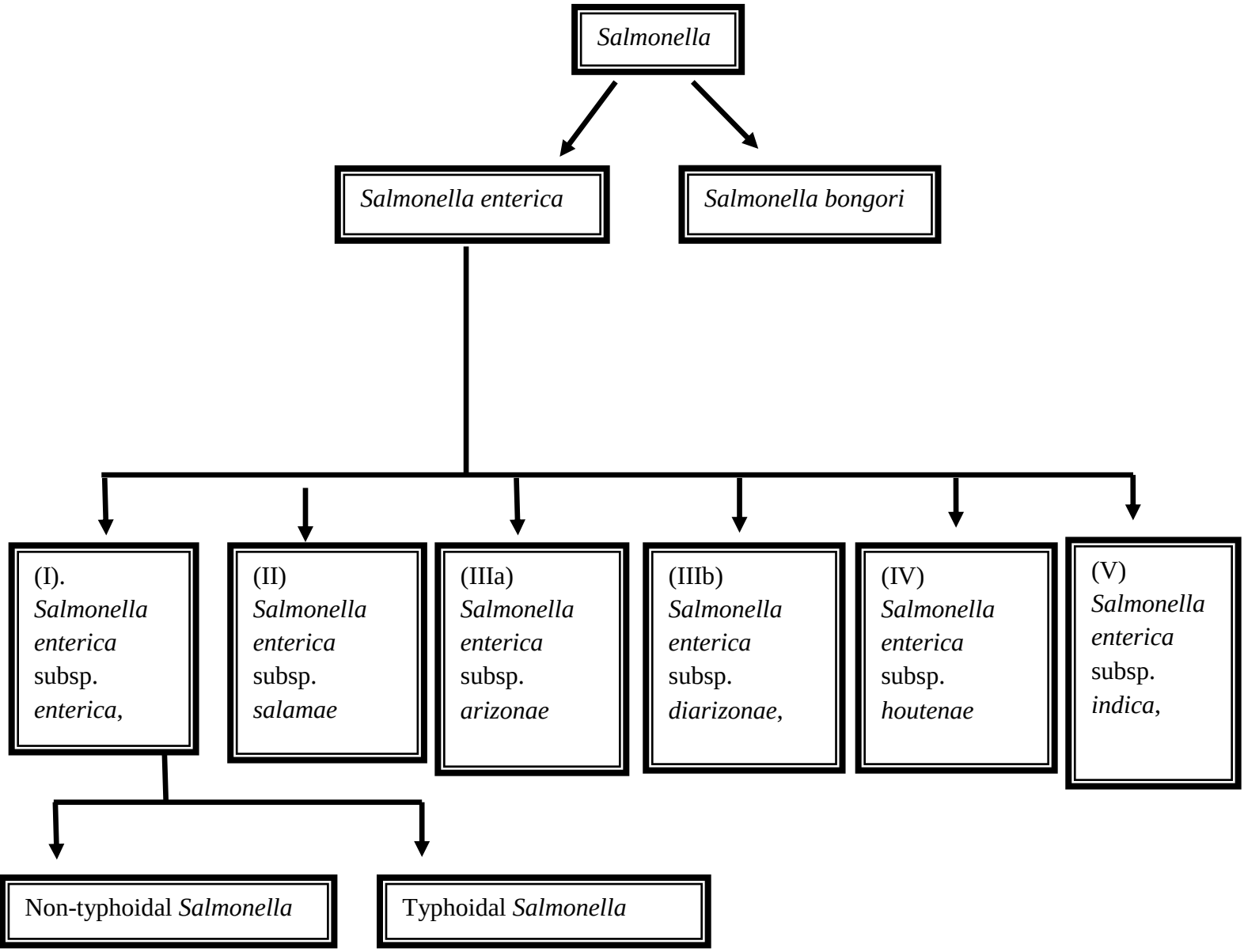

Figure 2.1 - A summary of the classification of the *Salmonella* species. (adapted from pusterla *et al.*, 2013)

2.2 *ESCHERICHIA COLI*

Escherichia coli, a Gram-negative, non-sporulating facultative anaerobic, is an inhabitant of warm-blooded animals and reptiles' intestines and faeces. *E. coli* is a widespread intestinal commensal of vertebrates and a versatile pathogen with the ability to kill more than 2 million people annually through extra-intestinal and intra-intestinal diseases (Tenaillon *et al.,* 2010). *E. coli* is found in the gut microbiota and is a predominant aerobic organism in the gastrointestinal tract that is frequently found in soil, water and food due to fecal contamination or contamination during slaughter. *E. coli* occurs in nature in various forms, ranging from commensal strains to pathogenic ones on hosts of humans or animals (Van *et al.,* 2011). *E. coli O157* is the most common member of a group of pathogenic *E. coli* strains known as organisms producing enterohaemorrhagic, verocytotoxin, or Shiga toxin (Pennington, 2010). There are various groups of pathogenic *E. coli* which are the following:

2.2.1 ENTEROPATHOGENIC E. COLI (EPEC)

This is component of a group of bacteria collectively known as A / E pathogens based on the ability of intestinal cell surfaces (IECs) to form distinctive lesions. EPEC is further categorized as ' typical ' (tEPEC) and ' atypical ' (aEPEC) subtypes based on the *E. coli* attachment factor plasmid (pEAF) accuracy or lack. EPEC is transmitted from host to host via contaminated surfaces, weaving fluids and human carriers (Mondol., 2013).

2.2.2 SHIGA-TOXIN PRODUCING *E. COLI* (STEC)

Organisms producing shiga toxin *E. coli* (STEC), also known as verocytotoxin (VT) producing *E. coli* (VTEC) is a significant cause of gastroenteritis leading to hemorrhagic colitis (HC) or hemolytic-uremic syndrome (HUS) in humans, most notably in kids resulting in acute renal failure (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).

2.2.3 ENTEROHEMORRHAGIC *E. COLI* (EHEC)

E. coli (EHEC) is used by some scientists to denote a subset of STEC that produce severe illness in humans. However, many variants of EHEC definitions exist; none include all human pathogenic strains or exclude all that do not cause human disease in order to avoid confusion, this expression should not be used. The term HUSEC has been proposed for STEC that are associated with acute kidney failure, hemolytic uremic syndrome (HUS) (Mellmann et al. 2008).

2.2.4 ENTEROINVASIVE E. COLI (EIEC)

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

2.2.5 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 traveler diarrhea, endemic diarrhea among kids in industrialized and resource-poor nations, and persistent diarrhea among people with human immunodeficiency virus infections (Boisen *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 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

(Boisen *et al.*, 2011). Results showed that the variety of sequence kinds (STs) connected with EAEC is very wide and disease was connected to ST10, an ST connected with various Pathotypes of *E. coli* only within a particular age group (Chattaway *et al.*, 2014). Figure 2.2 shows a summary of the classification of pathogenic *E. coli* species.

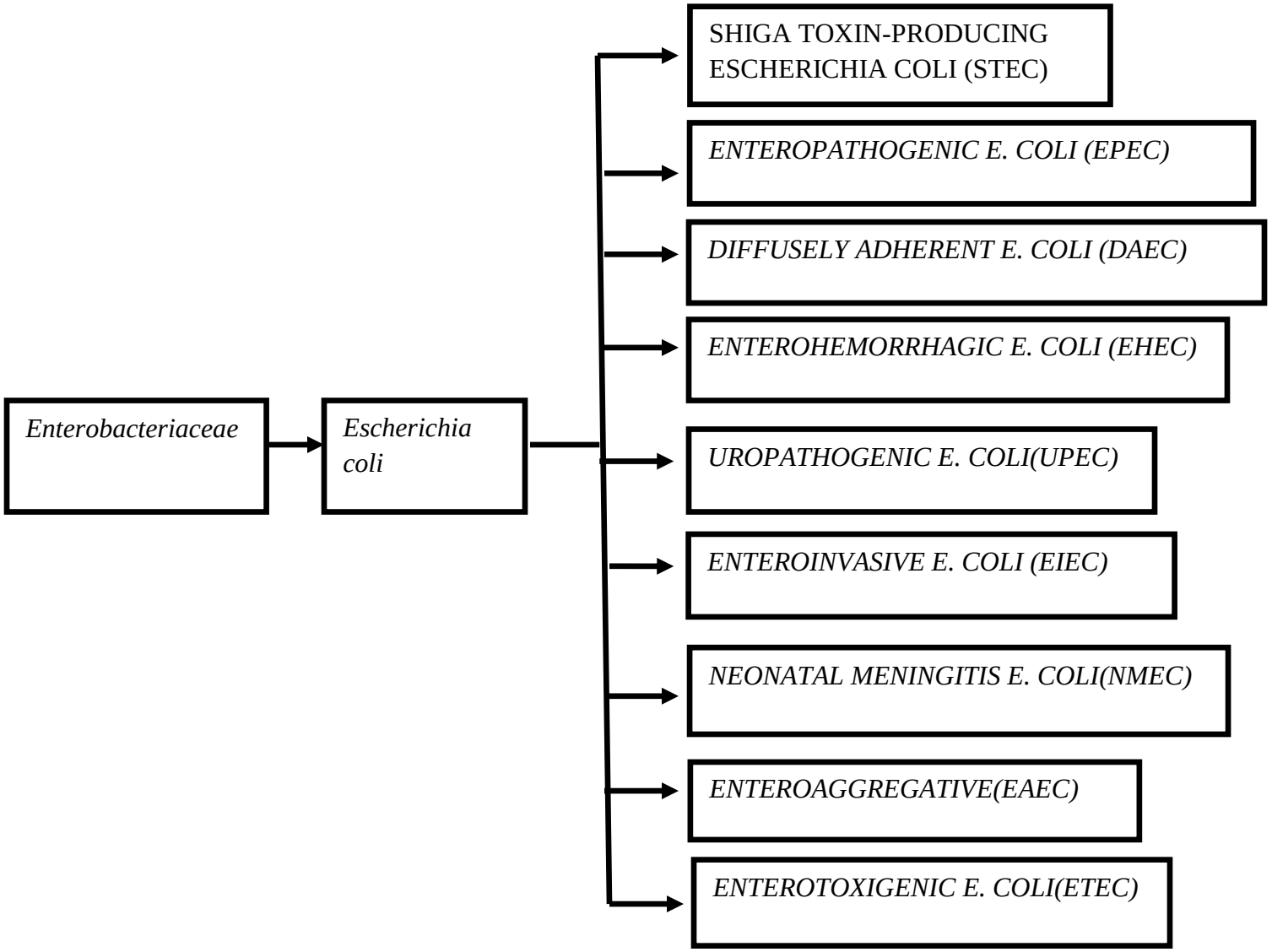

Figure 2.2 – A summary of the classification of pathogenic *E. coli* species (Adapted from mondol., 2013).

2.3 Foodborne outbreaks associated with *E. coli* and *Salmonella* spp. in meat

E. coli O157 outbreak happened in 1982 in Oregon and Michigan, USA, when it was isolated from people who developed bloody diarrhea and serious 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 diseases associated with *Salmonella* pose major public health problems (Hyeon *et al.,* 2011). Salmonellosis is one of many countries' most significant foodborne illnesses.

Few nations have a monitoring scheme that estimates the human population burden of Salmonellosis (Tafur *et al.,* 2010). *Salmonella*, *Campylobacter* and enterohaemorrhagic *E. coli* are microbial pathogens of present concern that need to be controlled in fresh meat. Consumption of contaminated ingredients is the most prevalent cause of human salmonellosis.

Raw and processed meat products, including poultry, beef and pork, are often contaminated with *Salmonella* (Hyeon *et al.,* 2011). 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 big amounts 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 significant pathogenic genera involved in outbreaks and illnesses of foodborne bacteria. Infections with *Salmonella* are worldwide and are a major public health issue in many areas of the globe.

Pork has been recognized in numerous research as a frequent cause of human *Salmonella*. However, the transmission of *Salmonella* was not only connected with pork but also poultry and beef (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 2.1.

Table 2.1- Outbreaks associated with *E. coli* and *Salmonella* spp. in different food sources 2009-2017

Country	**Year**	**Organisms/ Serotype**	**Food source**	**Number of Outbreaks**	**References**
Washington	NA	*E. coli*	Meat	5	CDC
Oregon	NA	*E. coli*	Ground beef, Meatballs	4	CDC
Minnesota	NA	*E. coli*	Meat	11	CDC
Ohio	NA	*E. coli*	Ground beef, Meatballs	5	CDC
New York	2019	*E. coli* O26	Baker's Flour,	NA	FDA
California	2019	*E. coli* O157:H7	Romaine Lettuce	NA	FDA
Yuma Growing Region, California	2018	*E. coli* O157:H7	Romaine Lettuce	NA	FDA
Canada	2017	*E. coli* O157:H7	Leafy Greens	NA	FDA
California	2015	*E. coli* O157	Rotisserie Chicken Salad	NA	FDA
Californa	2009	*Salmonella*	Meat	6	CDC
Californa	2018	*Salmonella*	Shell Eggs	NA	FDA
Californa	2017	*Salmonella*	Frozen Shredded Coconut	NA	FDA
Washington	2009-2010	*Salmonella*	Meats	7	FDA

2.4 Clinical symptoms of *Salmonella* infection.

In mammals and birds, most pathogenic *Salmonella* serotypes are usually subsp. 1. Infections with *Salmonella* can happen in three ways. First, few serotypes that cause systemic disease, instances such as typhoid and paratyphoid bacilli in animals, such as enteric fever with an incubation period of 10-20 days, but outside boundaries are between 3-56 days. Diarrhea, which begins 3-4 days after the start of fever and lasts for at least 6 days, can happen in 50 percent of instances of typhoid fever discovered mostly in younger kids or adults. Second, some other serotypes — Blegdam, Bredeney, Choleraesuis, Dublin, Enteritidis, Panama, Virchow in humans, and Gallinarum in adult fowl — are also invasive, but tend to cause pyaemic diseases and occur in viscera, meninges, bones, joints, and severe cavities.

2.5 Clinical symptoms of *E. coli* O157:H7 (STEC) intoxication

STEC is transferred to individuals through the faecal-oral route by ingestion of contaminated food or water, by interaction with animals that can carry the organism without being sick, or by interaction with a sick individual. 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 disease in all individuals. The clinical spectrum ranges from asymptomatic infection to watery, low-grade fever diarrhea to severe bloody diarrhea, a sign of hemorrhagic colitis; most patients with watery or bloody diarrhea recover spontaneously within approximately one week (Mead and Griffin 1998) but a small percent develop HUS, a condition characterize through microangiopathic hemolytic anemia, 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).

CHAPTER THREE

3.0 MATERIAL AND METHODS

3.0.1 Sample Collection

Fresh meat (beef and offals) samples were collected from various locations of Magboro marketplace, Ogun State, Nigeria. Samples were gathered for evaluation in sterile plastic bags and taken to the laboratory instantly. Sampling was repeated three times.

3.0.2. Methods

Twenty five gram each of meat (beef and offals) were weighed and aseptically transferred in 225 ml of 0.1% sterile buffered peptone water (BPW) to make a primary dilution. The samples were homogenized for 2 mins in a stomacher from the primary dilution of samples, series of 10-fold dilutions up to 10^{-7} were prepared further, by transferring 1ml of samples from each dilution series in triplicates into 16mm Durham tubes containing 9ml of 0.1% buffer peptone water (BPW) and gently rotate to suspend any adhering matter into the liquid.

3.0.3 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 incubated inversely at 37^0C in an incubator for 24h.

3.0.4 Enumeration of *E. coli*

MacConkey 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 MacConkeyAgar and then incubate inversely at 370C in an incubator for 24h.

3.0.5 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 37^{o}C for 24 h. 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 h, 1 ml of the secondary enrichment was transferred to *Salmonella*-Shigella agar for presumptive confirmation of *Salmonella* spp.

Bacterial Count: Colonies of bacteria were counted and expressed by multiplying the dilution factor as the amount of colonies counted. Sub-culturing of distinct colonies selected based on colony color from the Sorbitol MacConkey Agar (SMAC) plates onto fresh Nutrient Agar plates to get a pure culture. Bacterial identification was done using the pure culture on the nutrient agar plates.

3.1 DNA EXTRACTION

Colonies have been resuspended at 0.5 ml of distilled water in eppendorff tubes for preparation of DNA templates for the conventional PCR assay. Tubes containing bacteria were centrifuged at 15,000rpm for 15 min, and the supernatants were aspirated and discarded. The cell pellets were resuspended in 200 ml of distilled water. Samples were boiled for 10 minutes and cooled for 2 minutes at room temperature. After centrifugation at 15,000rpm for 15 min, the supernatants were collected in new tubes for use as DNA templates. An overnight bacterial culture (200 µl) of distilled water was mixed with 800 µl and cooked for 10 min. The resulting solution was centrifuged and used as the DNA template by the supernatant. The DNA template was kept at -20 ° C until it was used.

3.1.1 PCR Protocol

3.1.1.1 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 2 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.	**Sample Code**	**2nd simplex**	**12% Pipette error**
		Component	1 Reaction
1		Mastermix	5
2		fDl	0.4
3		rDl	0.4
4		DNA	1
5		RNaseFreeH_2O	3.2
6		Total	10

TABLE 3.2- PROCEDURE FOR THERMAL CYCLER

ANZ	STEP	T	t
1X	Initial Denaturation	95^oC	5 min.
35X	Denaturation	95^oC	2 min.
	Annealing	42^oC	30 seconds
	Polymerization	72^oC	4 min.
1x	Final Polymerisation	72^oC	10 min
1x Cycler	Hold	4^oC	∞

3.2 STORAGE OF ISOLATES

For long term preservation, a loopful of each isolate was inoculated into 5 ml buffered peptone water (BPS) containing 10% glucose and then incubated for 18 hours. Then, the suspension was added to the eppendorf tubes containing sterile 20% glycerol as cryoprotectant and homogenized then it was stored at -20ºC.

CHAPTER FOUR

4.0 RESULT AND DISCUSSION

The survey of the microbial quality of meat samples in Mokoloki LCDA were analyzed in terms of total viable counts and pathogens. The bacteria isolates were firstly identified using morphological characteristics and this was then followed by biochemical test and pathogens were cultivated on sorbitol macConkey agar. The morphological differences were shown in table 4. While the morphological differences for *Salmonella* spp were summarized in table 5

TABLE 4.1- MORPHOLOGY CHARACTERISTICS OF PATHOGENIC *E. COLI* ISOLATED FROM MEAT AND OFFALS ON SORBITOL MACCONKEY AGAR

LOCATION	**ISOLATE CODE**	**ELEVATION**	**SURFACE**	**OPACITY**	**SHAPE**	**COLOUR**
MEAT						
1	M5	Convex	Rough	Opaque	CIRCULAR	PINK & WHITE
	M6	Raised	Smooth	Opaque	IRREGULAR	PINK & WHITE
2	Mb7	Convex	Rough	Opaque	PUNTIFORM	PINK & WHITE
	Mb8	Raised	Rough	Opaque	IRREGULAR	PINK & WHITE
	Mb9	Convex	Smooth	Opaque	CIRCULAR	PINK & WHITE
	Mb10	convex	Smooth	Opaque	IRREGULAR	PINK & WHITE
3	Mc7	Raised	Smooth	Opaque	CIRCULAR	PINK & WHITE
	Mc8	Raised	Smooth	Opaque	CIRCULAR	PINK & WHITE
	Mc9	Convex	Smooth	Opaque	CIRCULAR	PINK & WHITE
OFFALS						
1	O5	Raised	Rough	Opaque	CIRCULAR	PINK & WHITE
	O6	Convex	Smooth	Opaque	CIRCULAR	PINK & WHITE
2	Ob7	Raised	Rough	Opaque	CIRCULAR	PINK & WHITE
	Ob8	Convex	Smooth	Opaque	CIRCULAR	PINK & WHITE
	Ob9	Convex	Smooth	Opaque	CIRCULAR	PINK & WHITE
	Ob10	Raised	Smooth	Opaque	CIRCULAR	PINK & WHITE
3	Oc7	Raised	Rough	Opaque	CIRCULAR	PINK & WHITE
	Oc8	Raised	Rough	Opaque	CIRCULAR	PINK & WHITE
	Oc9	Convex	Smooth	Opaque	CIRCULAR	PINK & WHITE

TABLE 4.2- MORPHOLOGY CHARACTERISTICS OF *SALMONELLA* SPP ISOLATED FROM MEAT AND OFFALS ON *SALMONELLA*-SHIGELLA AGAR.

ISOLATE CODE	ELEVATION	SURFACE	OPACITY	SHAPE	COLOUR
M7	Convex	Rough	Opaque	CIRCULAR	BLACK
M8	Raised	Smooth	Opaque	IRREGULAR	BLACK
Mb11	Convex	Rough	Opaque	IRREGULAR	BLACK
Mb12	Raised	Rough	Opaque	IRREGULAR	BLACK
Mc10	Convex	Rough	Opaque	IRREGULAR	BLACK
Mc11	Raised	Smooth	Opaque	CIRCULAR	YELLOW
Mc12	Convex	Smooth	Opaque	CIRCULAR	BLACK
Mc13	Convex	Smooth	Opaque	CIRCULAR	BLACK
OFFALS					
O7	Raised	Smooth	Opaque	CIRCULAR	BLACK
O8	Raised	Rough	Opaque	CIRCULAR	BLACK
Ob11	Convex	Smooth	Opaque	CIRCULAR	BLACK
Ob12	Raised	Smooth	Opaque	IRREGULAR	BLACK
Oc10	Convex	Rough	Opaque	CIRCULAR	BLACK
Oc11	Raised	Smooth	Opaque	IRREGULAR	BLACK
Oc12	Raised	Rough	Opaque	IRREGULAR	BLACK
Oc13	Convex	Rough	Opaque	IRREGULAR	BLACK

Table 4.3- BIOCHEMICAL CHARACTERISTICS OF ORGANISMS FROM MEAT AND OFFLAS
MEAT

LOCATION	SAMPLE	ISOLATE CODE	CATALASE	OXIDASE	GRAM STAINING
1	MEAT	M1	+	-	-
		M2	+	-	-
	MAC	M3	+	-	-
		M4	+	-	-
	SMAC	M5	+	-	-
		M6	+	-	-
	SS	M7	+	-	-
		M8	+	-	-
2	NA	Mb1	+	-	-
		Mb2	+	-	-
		Mb3	+	-	-
	MAC	Mb4	+	-	-
		Mb5	+	-	-
		Mb6	+	-	-
	SMAC	Mb7	+	-	-
		Mb8	+	-	-
		Mb9	+	-	-
		Mb10	+	+	-
	SS	Mb11	+	-	-
		Mb12	+	-	-
3	NA	Mc1	+	-	-
		Mc2	+	-	-
		Mc3	+	-	-
	MAC	Mc4	+	-	-
		Mc5	+	-	-
		Mc6	+	-	-
	SMAC	Mc7	+	+	-
		Mc8	+	-	-
		Mc9	+	-	-
	SS	Mc10	+	-	-
		Mc11	+	+	-
		Mc12	+	-	-
		Mc13	+	-	-

OFFALS					
1	NA	O1	+	-	-
		O2	+	-	-
	MAC	O3	+	-	-
		O4	+	-	-
	SMAC	O5	+	-	-
		O6	+	-	-
	SS	O7	+	-	-
		O8	+	-	-
2	NA	Ob1	+	-	-
		Ob2	+	-	-
		Ob3	+	-	-
	MAC	Ob4	+	-	-
		Ob5	+	-	-
		Ob6	+	-	-
	SMAC	Ob7	+	-	-
		Ob8	+	-	-
		Ob9	+	-	-
		Ob10	+	-	-
	SS	Ob11	+	-	-
		Ob12	+	-	-
3	NA	Oc1	+	-	-
		Oc2	+	-	-
		Oc3	+	-	-
	MAC	Oc4	+	-	-
		Oc5	+	-	-
		Oc6	+	-	-
	SMAC	Oc7	+	-	-
		Oc8	+	-	-
		Oc9	+	-	-
	SS	Oc10	+	-	-
		Oc11	-	-	-
		Oc12	+	-	-
		Oc13	-	+	-

TABLE 4.4- OVERVIEW OF THE PRESENCE OF *SALMONELLA* SPP FROM MEAT AND OFFALS

LOCATION	**SAMPLE**	**ISOLATE ID**	***SALMONELLA* SPP**
1	MEAT	M7	+
		M8	+
	OFFALS	O7	+
		O8	+
2	MEAT	Mb11	+
		Mb12	+
	OFFALS	Ob10	+
		Ob11	+
		Ob12	+
3	MEAT	Mc10	-
		Mc11	+
		Mc12	+
	OFFALS	Oc10	+
		Oc11	+
		Oc12	+

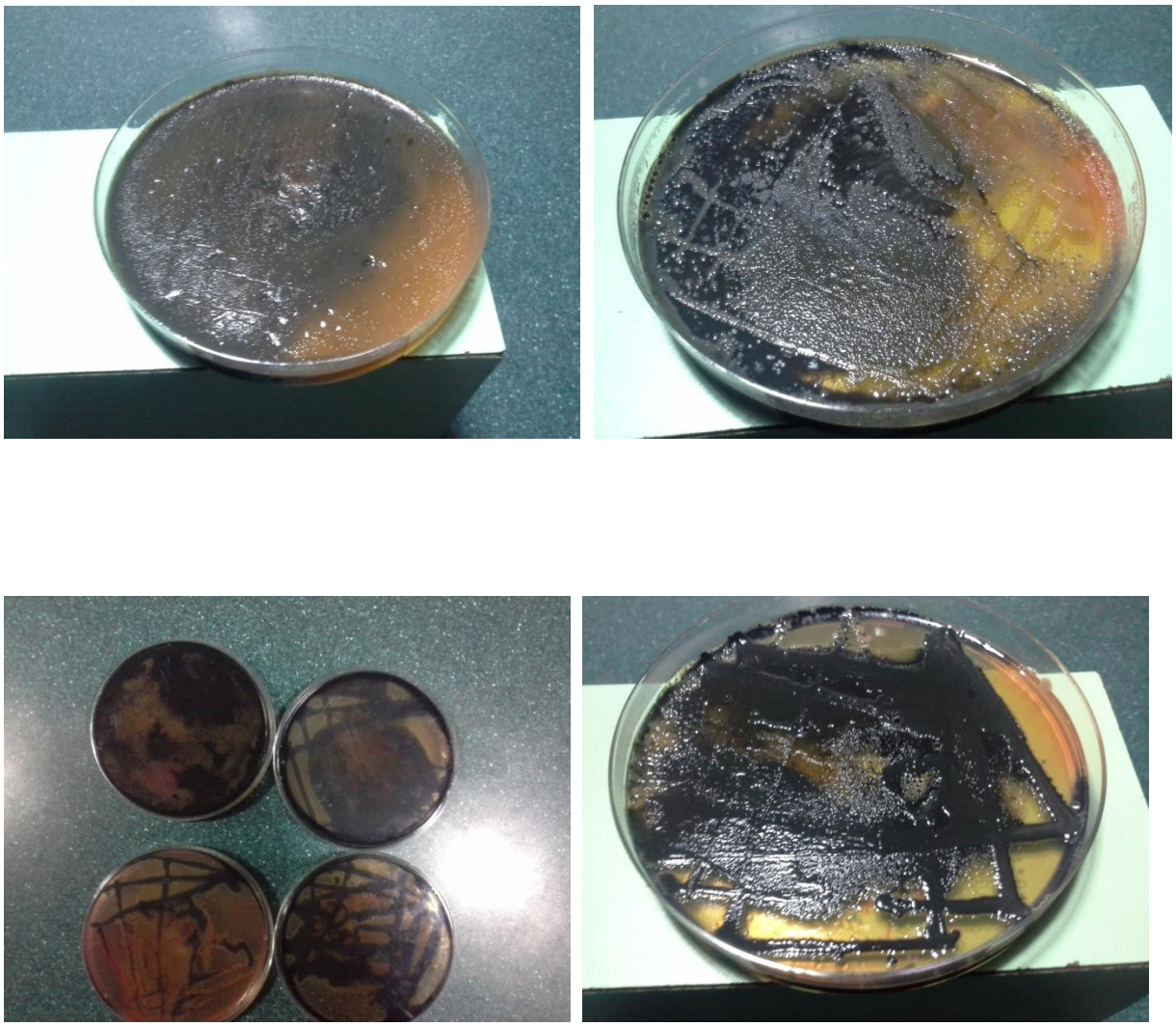

FIGURE 4.1- AN IMAGE DISPICTING THE PRESENCE OF *SALMONELLA* SPP FROM MEAT AND OFFALS

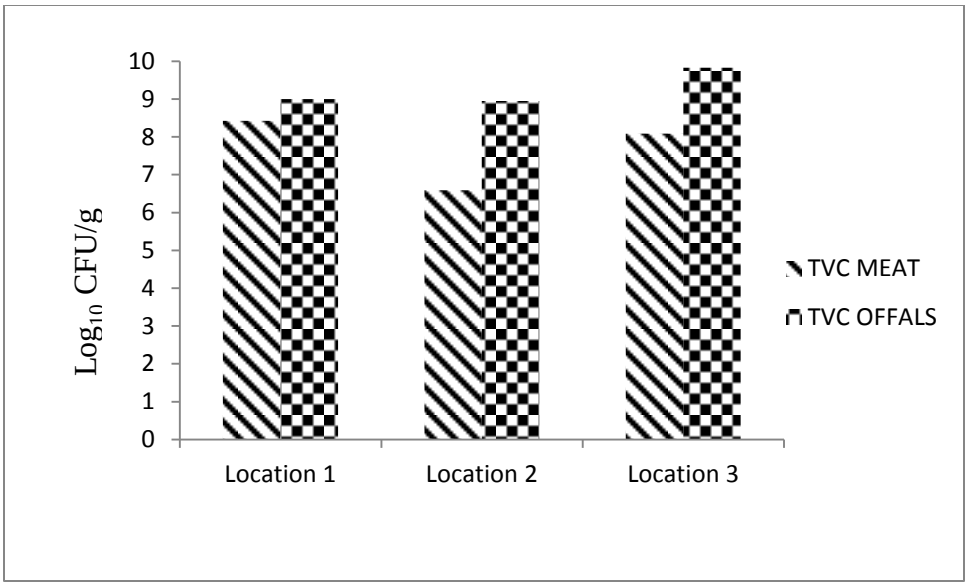

FIGURE 4.2- THE GRAPH OF THE TOTAL VIABLE COUNT OF MEAT AND OFFALS SAMPLES FROM THREE DIFFERENT LOCATIONS.

The total viable count was highest in location 1 with a count of 8.4 log10 CFU/g in meat and decreased in location 2 but location 3 is almost comparable with location 1. While in offals the total viable count was highest in location 3 with a count of 9.8 Log10 CFU/g. The total viable count at locations 1 and 2 is not significantly different. 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, the amount of mesophilic bacteria has been found to be as high as 9 Log10 CFU/g depending on the hygiene and sanitation of the slaughterhouse.

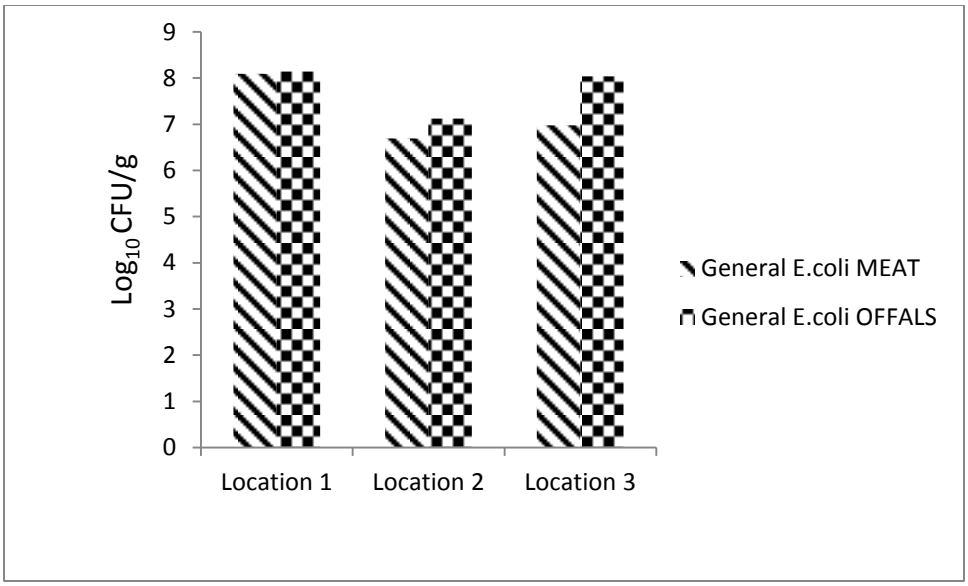

FIGURE 4.3- THE GRAPH OF THE GENERAL *E. COLI* OF MEAT AND OFFALS SAMPLES FROM THREE DIFFERENT LOCATIONS.

The count for General *E. coli* species was high in location 1 with a count of 8.1 log10 and 8.1 Log10 in meat and offals respectively. Locations 2 and 3 do not differ significantly in meat but in offals it decreased in location 2 but increased in location 3. Generally, *E. coli* is associated with meat at high concentration and is used as an hygiene indicator in the food industry.

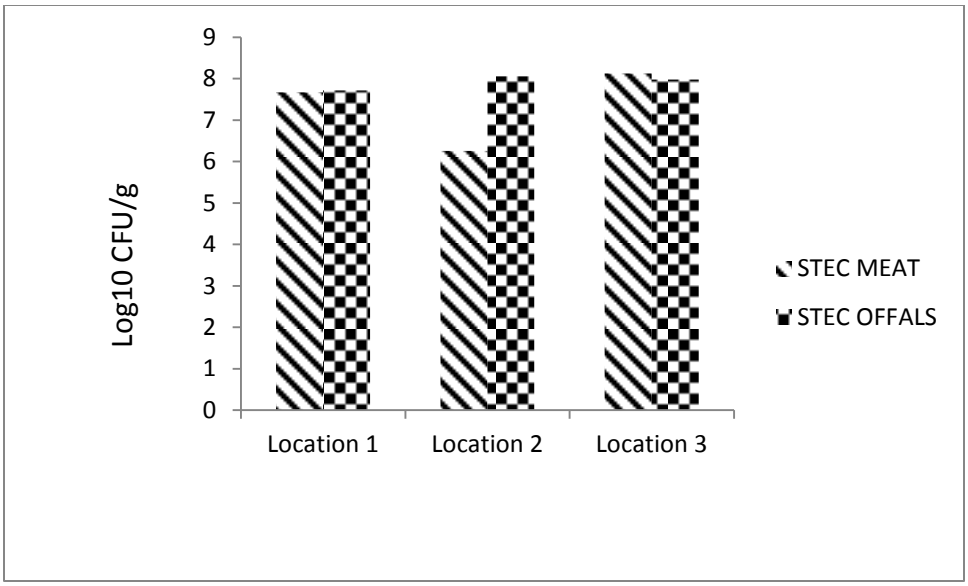

FIGURE 4.4- THE GRAPH OF THE PATHOGENIC *E. COLI* (STEC) OF MEAT AND OFFALS SAMPLES FROM THREE DIFFERENT LOCATIONS.

Pathogenic *E. coli* (STEC) count in locations 1, 2, and 3, has no important difference with a count of 7.7 log10, 8.0 Log10 and 7.9 Log10 in offals while for meat there is also no significant diiference in location 1 and 3 with a count of 7.6 Log10 and 8.1 Log10 respectively. The presumptive pathogenic *E. coli* growth are typically identified as a white colour (non 0157) while pink colour (O157). Illnesses such as hemorrhagic colitis (HC) or hemolytic-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).

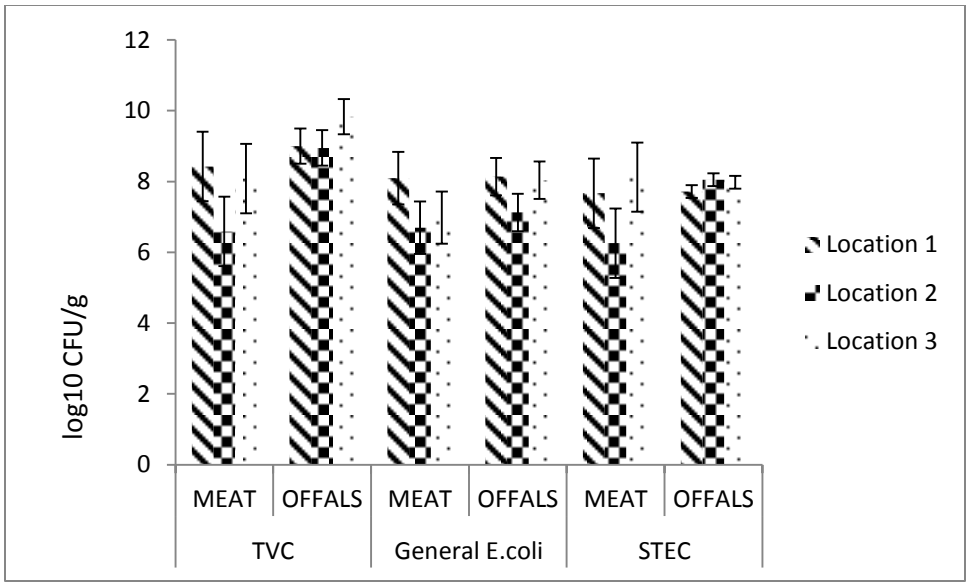

FIGURE 4.5 CHART OF MICROBIAL ANALYSIS SHOWS THE COUNT OF MESOPHILES, *E. COLI*, PATHOGENIC *E. COLI* (STEC) AND *SALMONELLA* IN MEAT AND OFFALS SAMPLES COLLECTED FROM MAGBORO MARKET OGUN STATE.

The genetic characterization shows DNA band on agarose gel of the presumptive *Salmonella* spp and pathogenic *E. coli* after PCR amplification. This confirms that the results from the morphological and biochemical test. The DNA ladder and DNA bands for *Salmonella* and *E. coli* are shown in figure 3.

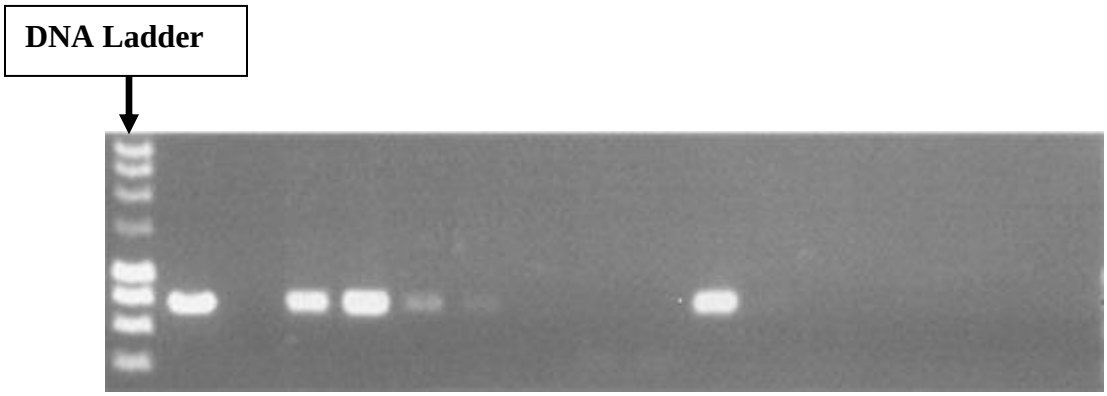

FIGURE 4.6- DNA BANDS SHOWING TARGETED MICROBES OF *E. COLI* AND *SALMONELLA* SPP

CHAPTER 5

5.0 CONCLUSION

The two types of samples namely meat and offals were found to be contaminated with pathogenic bacteria STEC and *Salmonella* spp, result reveals that a high proportion of beef sold in study area Ofada/Mokoloki LCDA for human consumption is contaminated which remains a public health concern. Therefore, there is a possible risk to humans to such microorganisms especially from consumption of these products which can lead to other illnesses such as hemorrhagic colitis (HC) or hemolytic-uremic syndrome (HUS).

5.1 RECOMMENDATION

As a result of this research, it is recommended that meat is cooked thoroughly before consumption. There is a need for education 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 on infections outbreak in organism in local government areas.

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