# DETECTION OF *SALMONELLA* SPP AND PREVALENCE OF SHIGA TOXIN PRODUCING *ESCHERICHIA COLI* (STEC) IN STREET VENDED CUCUMBER AND GREEN PEPPER

BY

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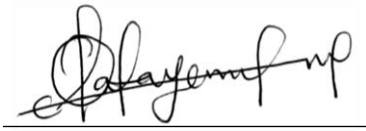
## CERTIFICATION

This is to certify that Dada Olanrewaju Opeoluwa, with matric number 15010101004 carried out this project under the close supervision of Dr. O. E. Fayemi. It was done based on the analysis carried out on street vended fruits in Ofada/Mokoloki LCDA, Ogun state.



**DR. O. E. FAYEMI**
PROJECT SUPERVISOR

DATE

**DR. A. A. ADEIGA**
HOD

DATE

## DEDICATION

I dedicate this work to God the Father, God the Son and God the Holy Spirit who saw me through the completion of this great work. And also to my mummy and sister for their never ending support, care and prayers towards the successful completion of this work.

## ACKNOWLEDGEMENT

My sincere and utmost appreciation goes to my Lord and Redeemer, who in His infinite mercies has given me the wisdom, knowledge, assistance, support and protection to successfully complete this project.

I want to use this medium to appreciate the efforts of my God-given supervisor; Dr. O.E. Fayemi, who stayed with me through thick and thin during the course of this work, I especially want to thank him for his sacrifices in terms of time, sleep, energy, money and other unquantifiable inputs.

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I would love to specially honour the efforts of my mum, who showered me with the necessary physical, financial, spiritual and emotional support required for this work.

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I can't ever forget Pastor Paul Aderoju, for his fatherly care and assistance, I would really like to appreciate him for all the ways he has been helping me achieve my academic career.

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## ABSTRACT

Fruits are a major constituent of human diet, they possess nutrients like vitamins, anti-oxidants and fibres, which makes them essential in our day-to-day nutritional requirements, but their consumption without further processing or heat treatment poses a threat as they may harbor different pathogens ranging from *Salmonella spp* to shiga toxin producing *E. coli* (STEC). This study focused on evaluating the presence of *Salmonella spp* and STEC in street vended green pepper and cucumber in Magboro area of Ofada/Mokoloki LCDA, Ogun state. These samples were collected aseptically from specific locations in the market, target pathogens were isolated using selective media, after which the identity of suspected STEC and *Salmonella* was confirmed using molecular characterization (16S rRNA amplification). S*almonella* was detected in all the cucumber and green pepper samples analysed, however, high microbial counts (7.3 $\log_{10}$ CFU/g) of STEC was recorded in cucumber. Street vended green pepper and cucumber sold in Ofada Mokoloki LCDA (Magboro axis) area of Ogun state contain presumptive pathogenic STEC and *Salmonella* which can create public health hazard.

# CHAPTER ONE

## 1.0 INTRODUCTION

Foods contaminated with pathogenic microorganisms have been associated with outbreaks of foodborne diseases and infections across the world especially in Africa (Mensah et al., 2002). It has been shown that yearly, about 16 million cases of typhoid fever and 1.3 billion cases of gastro enteritis occur due to *Salmonella*, and all these result to 3 million deaths globally (Bhunia, 2008). According to Harvelaar et al. (2015), foodborne diseases cause over 600 million illnesses and 42,000 deaths yearly.

It is noteworthy that a major factor that has influenced this occurrence is the increased sale of street vended foods, which are foods and beverages sold by hawkers on the road side (street) which may be raw or cooked (Ameko et al., 2012). Examples of food materials in this category are; beans, yam, rice, plantain, maize, cassava with fruits and vegetables like; cucumber, carrot, cabbage, lettuce, onions and also beef, meat and fish products (Ameko et al, 2012).

Street vended foods can simply be defined as *ready-to-eat* foods (RTE) sold in the local and informal way, which can be consumed without further processing or treatment (Tsang, 2002). Aside from the dangers of contamination and diseases, street vended foods have numerous advantages which include being sources of foods that are readily available and accessible by numerous individuals in the population (Feglo and Sakyi, 2012). Street vended foods are cheap and they satisfy the need of their many patronisers in towns and cities of developing countries (Adu-Gyamfi and Nketsia-Tabiri, 2007; Tambekar et al., 2008; Feglo and Sakyi, 2012).

In additiona, street vended foods possess a measurable amount of nutritional value, and also they serve as a source of income for the sellers (Adu-Gyamfi and Nketsia- Tabiri, 2007). However,

irrespective of these advantages, street vended foods pose a major threat owing to the implication in foodborne outbreaks and infections in which some of its causative microorganisms such as *Staphylococcus aureus, Bacillus cereus, Escherichia coli* and *Salmonella* have been isolated from these kinds of food (FAO and WHO, 2005).

Despite fruits and vegetables have reported to be associated with frequent foodborne diseases outbreak following their consumption both in Nigeria and in other developing countries, only few studies have been conducted in Nigeria Therefore, this study, evaluated the microbiological safety of minimally processed street vended cucumber and green pepper. These variants of veggies and fruits have been known to have a higher chance of containing food poisoning microorganisms due to their low level of processing (Aycicek et al., 2006) and wide exposure to contaminants at every stage of production from planting, harvesting, transporting, storage, packaging, and selling to its various consumers (Okonko et al., 2008).

# CHAPTER TWO

## 2.0 LITERATURE REVIEW

### 2.1 Foodborne Diseases

Foodborne diseases as defined by World Health Organization (WHO) as any infectious or toxic nature caused by the consumption of contaminated food or water, which can range from mild, self-limiting gastrointestinal upsets through to life-threatening conditions (Adams and Moss, 2008). Foodborne diseases are caused by eating or ingesting food materials that have chemicals materials, heavy metals, parasites, fungi, viruses and bacteria (Jalalpour, 2012). They are illnesses that are related with eating food contaminated by pathogens (bacteria, viruses, parasites) (Feglo and Sakyi, 2012). They can however be classified as; foodborne infections and foodborne intoxications. The former is when the live microorganism present in the food causes the disease or illness, while the latter occurs when the toxins secreted by pathogens like *Campylobacter*, *Salmonella*, *Staphylococcus*, *Listeria*. (Dhama *et al.*, 2013). Previously reported outbreaks of foodborne diseases are shown in Table 2.1.

*Salmonella* is the foodborne pathogen that is generally associated with fresh fruits and vegetables as outbreaks involving Salmonellosis has been traced to variants of fruits like lettuce, apple, cantaloupe, mango, tomato and melon (Pui *et al*., 2011) and all these are commonly sold on the street. Foodborne Salmonellosis occurs mainly by ingestion of contaminated food products like fruits and vegetables owing to the prevalence of poor hygiene cooking practices of individuals and street vended food handlers (Misganaw and Williams, 2013; Faisal *et al*., 2016). It has also been discovered to be the leading causative organism of hospital cases relating to food poisoning all over the world (Hoffman *et al*., 2012).

**Table 2.1:** Selected foodborne outbreaks from 2000-2016 (Adapted from Bhunia, 2018)

| Year | Pathogens | Foods | Country | Cases | Deaths |
|---|---|---|---|---|---|
| 2000 | *E. coli* O157:H7 | Dairy farms | USA | 56 | — |
| 2002 | *L. monocytogenes* | Processed chicken | USA | 46 | 7 |
| 2006 | *E. coli* O157:H7 | Spinach | USA | 199 | 3 |
| 2007 | *L. monocytogenes* | Milk products | USA | 5 | 3 |
| 2007 | *Salmonella* | Chicken and turkey | USA | 401 | — |
| 2008 | *S. saintpaul* | Mexican peppers | USA | 1017 | 2 |
| 2009 | *S. Typhimurium* | Peanut butter | USA | 714 | 9 |
| 2009 | *Salmonella* | Alfalfa sprouts | USA | 235 | — |
| 2009–2010 | *S. Montevideo* | Black and red pepper | USA | 272 | — |
| 2010 | *E. coli* O145 | Lettuce | USA | 26 | — |
| 2010 | *L. monocytogenes* | Celery | USA | 10 | 5 |
| 2011 | *Salmonella* | Papaya | USA | 106 | — |
| 2011 | *E. coli* O104:H4 | Sprouts | Germany | 3911 | 48 |
| 2011 | *E. coli* O157:H7 | Strawberry | USA | 15 | 1 |
| 2012 | *S. Bareilly* | Tuna fish | USA | 258 | — |
| 2014 | *S. Heidelberg* | Chicken | USA | 634 | — |
| 2014–2015 | *L. monocytogenes* | Apple | USA | 35 | 7 |
| 2015 | *L. monocytogenes* | Ice cream | USA | 10 | 3 |
| 2016 | *L. monocytogenes* | Frozen vegetables | USA | 9 | 3 |
| 2016 | *Hepatitis A virus* | Sushi | USA | 292 | 2 |
| 2016 | *Salmonella* | Live poultry and flock | USA | 895 | 3 |

## 2.2 IMPLICATION OF FRUITS IN FOODBORNE DISEASES

Fruits are the edible parts formed from leaves of plants. They are rich in vitamins, fibres, anti-oxidants, minerals and carbohydrates (Giugliano and Esposito 2008; Said 2012). Fruits and vegetables have been reported to be vectors of pathogens and other contaminants (Uzeh *et al*., 2009, Weldezgina and Muleta, 2016) and this can be traced to fact that they are always consumed without further processing (or even minor heat processing) and most of the time they contain pathogens from harvesting practices, transportation channel and human handling (Tambekar *et al*., 2006; Itohan *et al*., 2011). However, the frequency of diseases caused by foodborne pathogens has been on the high side during the past few years (Eraky *et al*., 2014)

## 2.3 *Salmonella*

### 2.3.1 Background History

*Salmonella* is widely known as a gram negative anaerobe which is facultative in its mode of growth, flagellated rod shaped bacterium (Yousef and Carlstrom 2003; Montville and Matthews, 2008). Salmonella species are generally motile except Salmonella pullorum and gallinarum. It belongs to the enterobacteriaceae family and is commonly associated with typhoid fever (Prescott, 2002).

*Salmonella* was named after Dr. Elmer Salmon, an American bacteriologist and veterinary surgeon who was the first person to isolate a specie of salmonella from porcine intestine in 1884, although, Scientist Theobald Smith's name is also mentioned in its discovery (News Medical, *Salmonella* History, 2014). *Salmonella* strains are named based on the location of isolation (Bishoftu, 2014).

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; 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). However, Kauffman-White Classification classified *Salmonella* into three major antigenic determinants; which are flagella H antigens, somatic O antigens and virulence capsular K antigens

### 2.3.2 General Characteristics of *Salmonella*

*Salmonella* are widely known to be non-fastidious as they can multiply freely under various conditions of the environment even when outside the living hosts and they can grow in the presence of 0-4% sodium chloride (which they don't need for growth) (Pui *et al.,* 2011). Also most *Salmonella* serotypes grow at a temperature range between 5°C to 47°C (although optimum temperature is between 35°C to 37°c some can still grow at very low temperatures of 2-4°C and very high temperature 54°C), but they are killed at a temperature of 70°C and above due to their sensitivity to heat (Gray and Fedorka-Cray, 2002; Pui *et al.,* 2011). *Salmonella* can grow in a pH range of 4-9 (optimum is between 6.5-7.50; complete inhibition is achieved at a temperature of <7°C, pH of <3.8 and at a water activity of <0.94 (Hanes, 2003; Bhunia, 2008; Pui *et al.,* 2011). A diagrammatic representation of Salmonella is shown in Figure 2.2

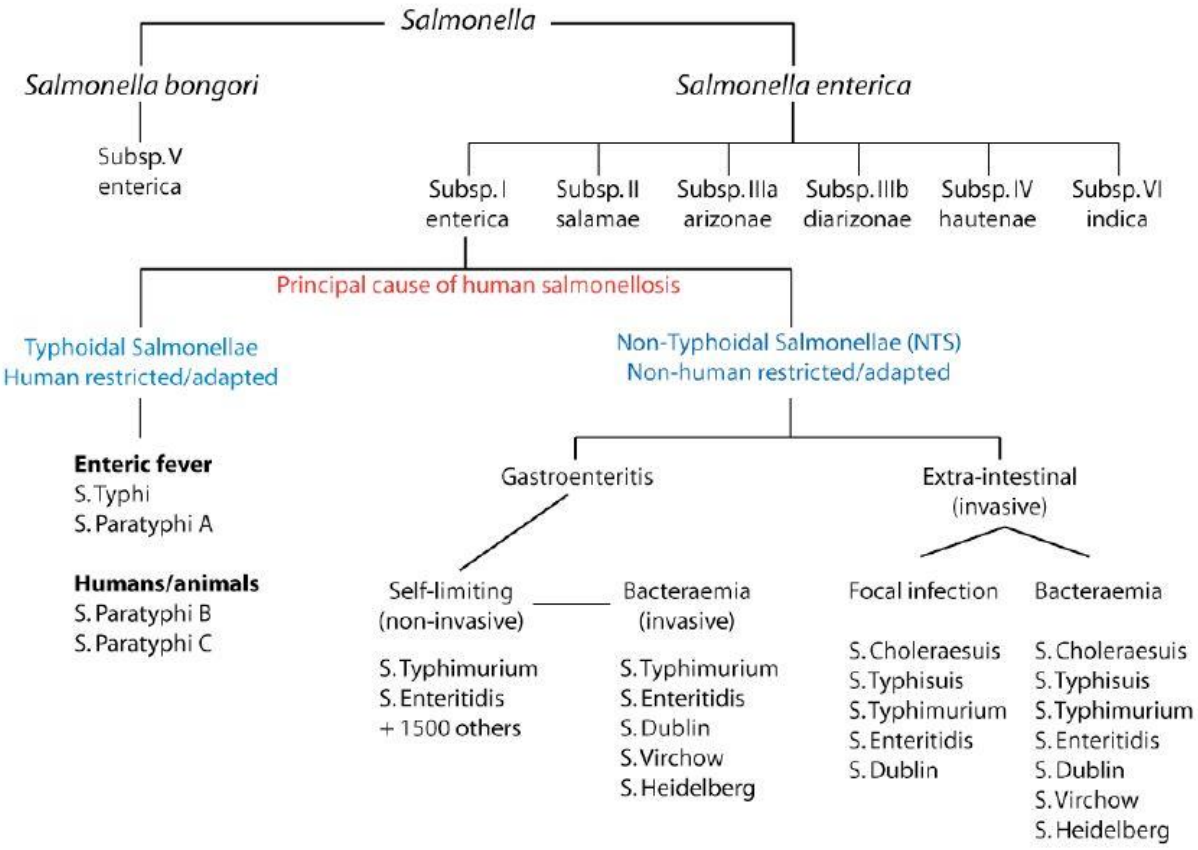



**Figure 2.1:** Classification of *Salmonella* (Longridge *et al*., 2008)

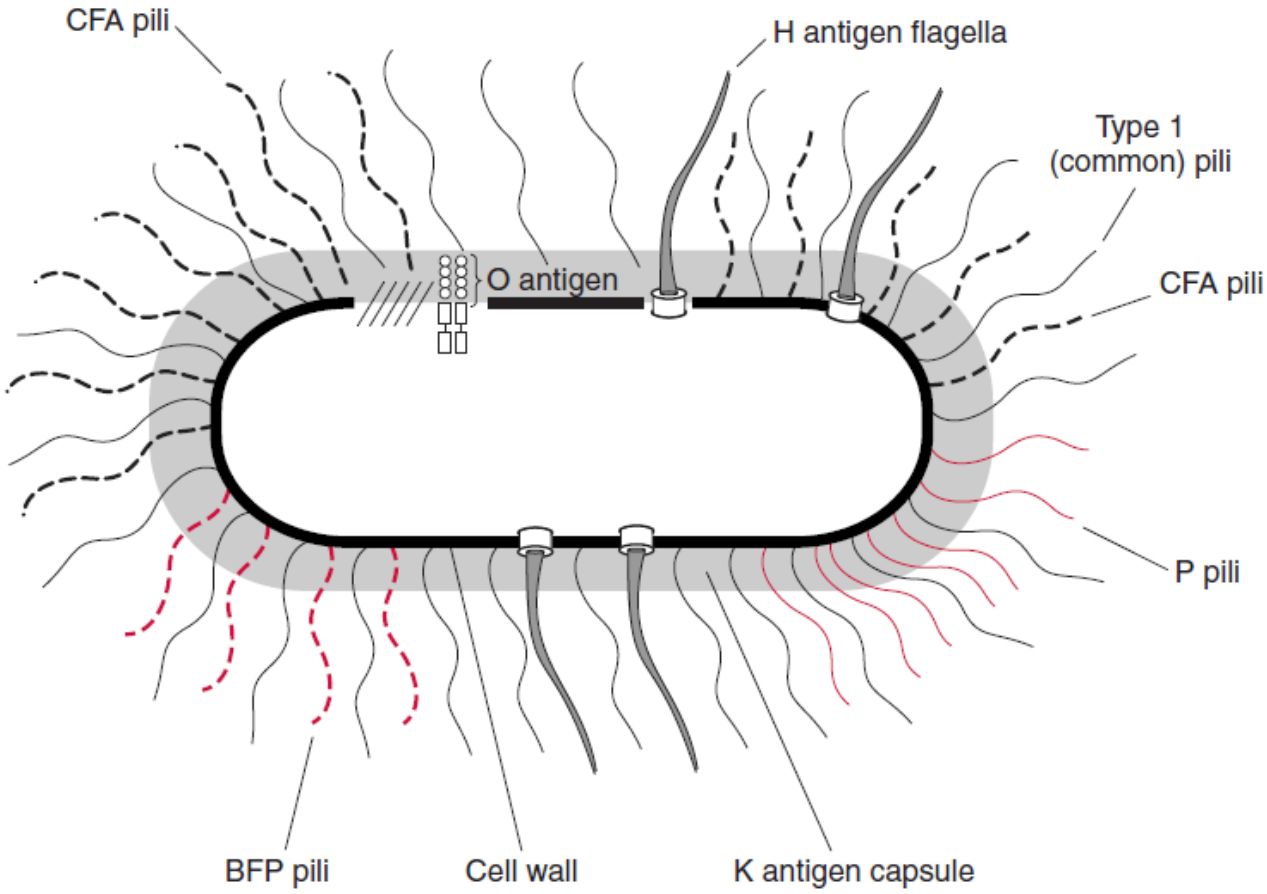



**Figure 2.2:** Typical structure of *Salmonella* and *E. coli*

### 2.3.3 Serovars of *Salmonella*

**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 immunocompromised 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). Some of the serovars of Salmonella are shown in Table 2.2 with some of their hosts.

### 2.3.4 Food Materials Implicated in Foodborne Outbreaks

A summarized analysis of food involved with *Salmonella* is shown in Table 2.3. Poultry has been previously shown to be associated with Salmonella by different disease outbreaks and disease surveillance reports and this has identified *S. pullorum* and *S. gallinarum* as the serovars of interest for *Salmonella* in poultry products like chicken, turkey, ducks, and geese (Pui *et al.*, 2011). This alarming prevalence in poultry has been shown to be as a result of unhygienic food management practices adopted in the rearing and breeding of these poultry animals which has increased the prevalence of *Salmonella* in them and their byproducts (Cox and Pavic, 2010). Eggs are also implicated due to poor food safety practices caused by the presence of

unfavourable and unsuitable conditions in the egg environment (wet environment, low temperature etc.) and

**Table 2.2:** 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 |

damage in cuticle of the egg (in the presence of all these contamination chance is increased) (Pui *et al*., 2011).

Water has been a major medium of typhoid transmission (especially in Africa) and this is due to the consumption of water contaminated with faeces or carcasses (this is caused or influenced by poor sanitary practices, improper sewage disposal and absence of clean water system) (Pui *et al*., 2011). Meat; their presence in fruits and vegetables (fresh plant produce) is attributed to handling practices of the fruit product from farm-to-fork (entrapment of the bacteria into plant tissues during slicing, physical damage to epicarp) (Pui *et al*., 2011)

### 2.3.5 Pathogenesis of *Salmonella* (*Salmonella typhi*)

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 diaarhoea) 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). In carriers, S. typhi and S. paratyphi may persist in an asymptomatic form within the gall bladdee (Dongol *et al*., 2012; Gunn *et al.,* 2014) and 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)

### 2.3.6 Epidemiology 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).

| Year | Country | Source | Serotype | Cases | Reference |
|---|---|---|---|---|---|
| 1994 | Switzerland | Potato salad | Typhi | 10 | Gruner *et al*., 1997 |
| 1999 | Japan | Dried squid | *Salmonella* spp. | <453 | Montville and Matthews, 2008 |
| 2000 | Singapore | Dried anchovy | Typhimurium DT104L | 33 | Ling *et al*., 2002 |
| 2001 | Sweden | Fish | Livingstone | 60 | D'Aoust and Maurer, 2007 |
| 2003 | Germany | Aniseed herbal tea | Agona | 42 | D'Aoust and Maurer, 2007 |
| 2004 | Great Britain | Lettuce | Newport | >350 | Montville and Matthews, 2008 |
| 2005 | Malaysia | Stall food | Typhi | 171 | Nik and Sharifah, 2005 |
| 2005 | Austria | Mixed salad | Enteritidis PT21 | 85 | D'Aoust and Maurer, 2007 |
| 2005 | England | Kebab | Enteritidis PT1 | 195 | D'Aoust and Maurer, 2007 |
| 2006 | United States | Peanut butter | Tennessee | >288 | Montville and Matthews, 2008 |
| 2007 | United States | Dry pet food | Schwarzengrund | 62 | CDC, 2009 |
| 2008 | United States | Cereal | Agona | 28 | CDC, 2009 |
| 2009 | United States | Alfalfa sprouts | Saintpaul | 235 | CDC, 2009 |

**Table 2.3:** Food Products and Salmonella Outbreaks from 1994-2010 (Pui *et al.*, 2011)

| | | | | | |
|---|---|---|---|---|---|
| 2010 | United States | Shell eggs | Enteritidis | 2,752 | CDC, 2009 |
| 2010 | United States | Black & red pepper | Montevideo | 272 | CDC, 2009 |

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 this 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).

Also, *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)

### 2.3.7 Transmission of *Salmonella*

The natural habitat of *Salmonella* is in the gastrointestinal tract of humans and animals, although they may also be found in environment like soil, water and plants through the excreta (waste products) of humans and animals when they contaminate these media. They do not multiply in

natural environments (like soil and water) but can survive weeks or even years if environmental conditions are favourable (pH, humidity, temperature) (Todar, 2005)

However, the transmission of *Salmonella* is through the consumption of contaminated foods (Srisawat and Panbangred, 2015) and also from person-to-person spread. *Salmonella* transmission 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 *Escherichia Coli*

### 2.4.1 Background History of *E. coli*

*Escherichia coli* is a widely known gram negative bacteria which is non-spore forming and a facultative anaerobe which is generally motile (Castellani *et al.*, 1919) also belonging to the family *Enterobacteriaceae,* and it shares similar characteristics with salmonella. Normally, as a human, our bodies are naturally colonized by *E. coli,* because it is a harmless commensal and an inhabitant of the gut of humans and other warm blooded animals, also it is prominent causal microorganism of children's diarrhoea (Adams and Moss, 2008).

### 2.4.2 History and Characteristics of *E. coli*

Flashback in history lets us know that *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). Also, *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). 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.*

### 2.4.3 Pathotypes of *E. coli*

Extraintestinal Pathogenic *E. coli (*ExPEC), is a harmless strain of the intestine, but when it enters sensitive places of the body like blood, cerebrospinal fluid (CSF) and the urinary tract. This strain is responsible for urinary tract infections (UTI) neonatal meningitis, bacteraemia, septicemia, pyelonephritis (Carbonetti et al., 1986). They are also classified as uropathogenic *E. coli* (UPEC) or meningitis-associated *E. coli* (MAEC) (Gillespie and Hawkey, 2006). Meningitis-associated *E. coli* contains certain virulence factors that helps them boycott the host's immune response, like adhesins (P, M and S), siderophore aerobactin and haemolysis (Gillespie and Hawkey, 2006)

Enteroinvasive *E. coli* (EIEC) strain are closely related to *Shigella* in biochemical, genetical and pathogenic aspects, as they are non-motile, lysine decarboxylase negative and a higher proportion of them are unable to ferment lactose (Silva *et al.,* 1980). Enterotoxigenic *E. coli* (ETEC) are a group that produce toxins and there are two kinds of enterotoxins they produce: either oligomeric heat-labile enterotoxin (LT) or monomeric heat-stable enterotoxin (ST). It causes diarrhoea in infants in countries like Africa, and also cause of Traveller's diarrhoea (Simjee, 2007)

Enteroaggregative *E. coli* (EAggEC) is a cause of diaarhoeal disease in adults and children in developed countries like Africa and are simply known as *E. coli* strains that do not secrete

enterotoxins, but adhere to HEp-2 cells in a pattern known as aggregative adherence (AA). Although, EAST-1 (enteroaggregative heat-stable toxin-1 and Pet (plasmid-encoded toxin) has been associated with EAggEC it has also been later reported that its prevalence is low (Vila *et al.,* 2000)

Diffusely Adherent *E. coli* (DAEC) is characterized by the ability to adhere to HEp-2 cells (Nataro and Steiner, 2002), presence of Afa/Dr involved in diffuse adherence (Peiffer *et al.,* 2000) and absence of virulence genes and their diffusely adherent phenotype is mediated by fimbriae structures. DAEC can produce EAST-1 enterotoxins and can cause watery diarrhoea without blood (Gillespie and Hawkey, 2006 pp 353). Their pathogenesis is mainly associated with the presence of adhesins (Peiffer *et al.,* 2000). Enteropathogenic *E. coli* (EPEC) are the most extensively studied group of *E. coli* that are the cause of diarrhoe in children from developing countries (Levine *et al,* 1987). It also affects livestock animals like cattles, rabbits, dogs and pigs. They are known to be higly invasive and are potentially fatal to children.

### 2.4.4 Food Materials Implicated in Foodborne Outbreaks

*E. coli* has been attributed to several outbreaks in history with link to different food products which human consume daily and are bought from companies and also from stalls (Nataro and Kaper, 1998). However, a lot of factors have been identified as a function that encourages the prevalence of this pathogen in foods. Cross contamination is one of the factors, poor handling practices, seasonal variations (Nataro and Kaper, 1998, Eblen, 2007, Glyes, 2007.). Examples of foods associated with *E. coli* outbreaks are; Salad, milk, cheese, sausage, lettuce, poultry, fish, ice-cream, shell fish, meat, beef, pork (Doyle and Schoeni, 1987, Brooks *et al.,* 2005, Eblen, 2007, Ethelberg *et al.,* 2009, Perelle *et al.,* 2007.)

### 2.4.5 Virulence Factors in *E. coli*

**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)

### 2.4.6 Resistance and Susceptibility of *E. coli*

Majority of E. coli strains are susceptible to most antibacterial agents who are active against gram-negative bacteria, but resistance has increased due to general and global antibiotic use for the past 50 years (Houndt and Ochman, 2000). 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).

# CHAPTER THREE

## 3.0 MATERIALS AND METHODS

### 3.1 Study Area

The sampling perimeter was Ofada/Mokoloki Local Council Development Area (LCDA), which is located in Ogun state. This LCDA comprises of Makogi, Magboro, Ibafo and Mowe (they are rural-urban settlements). Ofada/Mokoloki LCDA is a minimally civilized area with a critical level of food safety (food safety management system) and this will have a negative impact on the occupants (residents) of this area and state. Ogun state has been nationally identified as the state with the highest numbers of primary, secondary and tertiary institutions, hence the reason for study. In Ofada/Mokoloki LCDA Magboro markets is one central markets. The market in Magboro was used for consecutive samplings due to its size and also the high prevalence of unhygienic and unsafe food safety practices. In this market, fresh farm crops (like vegetables, fruits,) are sold and so are raw food products like (rice, beans, yam), cooked food are also displayed for sale in this market.

### 3.2 Collection of Samples

Fresh cut cumber (*Cucumis sativus*) and green pepper (*Capsicum annuum*) were collected from Magboro market. After buying from the vendors, the samples were collected in stomacher bags which have been ice packed prior to every stage of sampling. The samples were put in stomacher bags to ensure aseptic conditions and to preserve the fruit products and prevent spoilage. The stomacher bag containing samples were then transferred to the laboratory (within 6-10 hours) for further analyses.

### 3.3 Sterilization

To ensure aseptic conditions of working environment and materials, proper sterilization was practiced at every necessary phase of the bench work. Work bench area were also sterilized with 70% ethanol solution applied by the use of cotton balls, and also with the use of bunsen burner to keep the air around the work area sterile and aseptic. Eppendorf tubes, micro pipette tips and test tubes were sterilized in the autoclave at 121°C for 15 minutes, while petri-dishes, beakers, flasks, scotch bottles and McCartney bottles were sterilized in the oven at 160°C for 1 hour.

### 3.4 Reagents and Equipment Used

Materials used include; petri-dishes, beakers, flasks, scotch bottles, Eppendorf tubes, micro pipette (with their tips), test tubes (with their racks), spatula, filter paper,

Equipments used are; Autoclave, weighing balance, distiller, wash bottles, water bath (set at 50°C and 100°C), oven, incubator (set at 37°C), Bunsen burner, inoculating loop,

### 3.5 Preparation of Culture Media

For the enumeration, isolation and identification of *Salmonella, Shigella* and *Escherichia* isolates different selective and differential media were used for enhancement of their viability and isolation. Selective media are made up of ingredients that allow the growth of target microorganisms and inhibit the growth of unwanted microorganisms, they contain sugars, salts, antibiotics and dyes that only the selected microorganism can utilize because of the way it changes the metabolic systems of microorganisms, these ingredients could be the only carbon or nitrogen sources and this results in the inhibition of other unwanted or screened out microorganisms due to their inability to assimilate these sources of nutrients. Also, differential

media are the ones that have the ability to differentiate or group microorganisms based on their varying appearance and patterns of growth and morphology.

Buffer Peptone Water (BPW) was used for primary enrichment of samples for the detection of Salmonella strains (*Salmonella* being a fastidious organism and its presence in foods may go undetected of not enriched). Buffered Peptone Water (0.1% BPW) was used for serial dilution of samples for isolation., To obtain 0.1 % BPW, 1g of peptone powderwas dissolved in 1 litre of water, then autoclaved at 121°C for 15 minutes.

Selenite F broth was used for the secondary enrichment for the detection of *Salmonella* strains which have been incubated in BPW for 24hours, samples were then streaked on SS agar after secondary enrichment. . Selenite F was prepared according to the manufacturer's instruction (TM Media), 19g (Part A) was dissolved in 750ml of distilled water with the addition of 4g of sodium hydrogen selenite (Part B) into 250ml of distilled water. The two parts were then added together to make 1 litre, which was then boiled (sterilized) in boiling water for 10 minutes (preparation does not require autoclaving)

Nutrient Agar was used for identification of Total Viable Count It was prepared according to the manufacturer's instruction (Ritcher). 14g of powdered media was dissolved in 500ml of distilled water, then autoclaved at 121°C for 15minutes. MacConkey Agar was used for the identification and enumeration of Coliforms in the isolate and it was prepared based on the manufacturer's instruction Sorbitol MacConkey Agar was used for the presumptive identification, enumeration and isolation of Escherichia coli strains in the isolate. It was prepared according to the manufacturer's instruction (Lilfilchem ). 51.5 g of powdered media was dissolved in 1 litre of distilled water which was autoclaved at 121°C for 15minutes

*Salmonella-Shigella* agar was used for the selective detection, identification and isolation of *Salmonella* and *Shigella* in the food (fruits) samples plated. It is also prepared according to the manufacturer's instruction (Ritcher). 31.5g of powdered media was dissolved in 500ml of distilled water then boiled with frequent agitation for about 10 minutes (preparation does not require autoclaving). After preparation, the media prepared were put in the water bath set at 50°C for 5 to 10 minutes before pouring into the petri dishes for further use, to prevent condensation.

### 3.6 Sample Preparation

Sample was prepared as previously described by Jane-Francis *et al.,* 2009 and Menghitsu *et al.,* 2011 (with few modifications). 25g of each fruit sample (cucumber and green pepper) was put in a sterile stomacher bag containing 225ml of BPW (0.1%) and then homogenized using the stomacher at 180 RPM (revolutions per minute) for 30 seconds after which serial dilutions were performed and appropriate dilutions were plated on Nutrient agar, SMAC and MacConkey agar plates. Another 25g of samples were homogenized with 225ml of BPW (0.1%) and incubated at 37°C to serve as pre-enrichment for *Salmonella*.

### 3.7 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. The secondary enrichment broth media used was Selenite F broth; 90ml of the broth was aseptically poured into scotch bottles and inoculated with 10ml of solution from overnight incubation). This was then incubated for 24 hours before the next step was taken.

### 3.8 Serial Dilution

One mililitre (1ml) of the samples were pipetted using the micro-pipetter (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 $10^{-3}$, $10^{-4}$ and $10^{-5}$. The test tubes were labelled for easy identification.

### 3.9 Plating (Spread Plate Technique)

For the Nutrient agar and MacConkey 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 $10^{-5}$ were plated (using pipettes) onto appropriately labelled agar-containing petri-dishes for Nutrient agar and Mac Conkey 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 fruit 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

### 3.10 Sub Culturing

The plates were checked after the required duration for the growth a sub-culturing needs to done.Subculturing was 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 were differentiated on the basis of their colony morphology, shape, colour, 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.

### 3.11 Biochemical Tests

#### 3.11.1 Gram Staining

This was done to differentiate the bacterial isolates based on their staining properties based on the function of the properties of their cell wall structure. Gram staining involves the use of dyes to enhance the visibility of bacterial isolates and to differentiate them based on their morphology.

The ability of the bacteria to retain the colour of crystal violet after being treated with alcohol, makes it Gram-positive, while those that lose the colour of the crystal violet but retain the colour of the counter stain (safranin) are Gram – negative.

A smear was prepared by transferring aseptically a loopful of the bacterial isolate to be stained onto a sterile slide containing about one to two drops of water (loopful) and mixed. The smear was then heat fixed by passing it through the flame of the Bunsen burner multiple times. The slide was then flood with crystal violet and left for 1 minute), then rinse with running water, after which iodine was added to the slide (to act as a mordant), followed by decolourization with 70% alcohol and then rinsed with water. The counterstain, safranin was then added to the slide for 30 seconds. The slide was rinsed with water and then dried with blotting paper. The stained slide is observed under the microscope after the application of oil immersion. A pictoral representation is shown in Figure 3.1.

### 3.11.2 Catalase Test

This was also used to differentiate microorganisms that possess enzyme (catalase) that help in the catalysis of hydrogen peroxide, and this is common to aerobic organisms. Drops of 3% hydrogen peroxide The bacteria was smeared onto a slide which was added to a slide (containing a smeared bacterial isolate) and the resultant reaction was observed. Production of bubbles signified the presence of enzyme catalase (Catalase positive) while lack of bubbles signified absence of catalase enzyme.

### 3.11.3 Oxidase Test

A drop of oxidase reagent was added to filter paper, then a loopful of isolate is smeared onto the filter paper. The presence of a purplish blue colouration signifies a positive reaction

### 3.11.4 Preservation of Cultures

The isolates were kept for further use in a 25% solution of glycerol. 0.5µl of the solution was added to the Eppendorf tubes with 0.5 µl of isolates in broth. The Eppendorf tubes were kept in an ultra-low freezer at -85°C after even mixing.

## 3.12 DNA Extraction

### 3.12.1 Sampling

Fresh cut fruits cucumber and green pepper were collected from various locations of Magboro marketplace, Ogun State, Nigeria. Samples were collected in sterile plastic bags and taken immediately to the laboratory for analysis. Sampling was repeated three times.

### 3.12.2 Materials and Methods

Twenty- five gram each of cucumber and green pepper were weighed and aseptically transferred in 225 ml of sterile buffered peptone water. The samples were homogenized for 2 mins in a stomacher. Nutrient agar and MacConkey agar was used for the enumeration of mesophiles incubated at $37^0$ C for 24 h. For the enumeration of E. coli O157 and non-O157 sorbitol-MacConkey Agar (SMAC) was used, incubated at $37^0$ C for 24 h. For *Salmonella spp* enumeration, primary enrichment in buffered peptone water incubated $37^0$ C for 24 h, followed by secondary enrichment in Selenite F and Selenite Z broth for $37^0$ C for 24 h, 1 ml of the secondary enrichment was transferred to Salmonella-Shigella agar for presumptive confirmation of *Salmonella spp.*

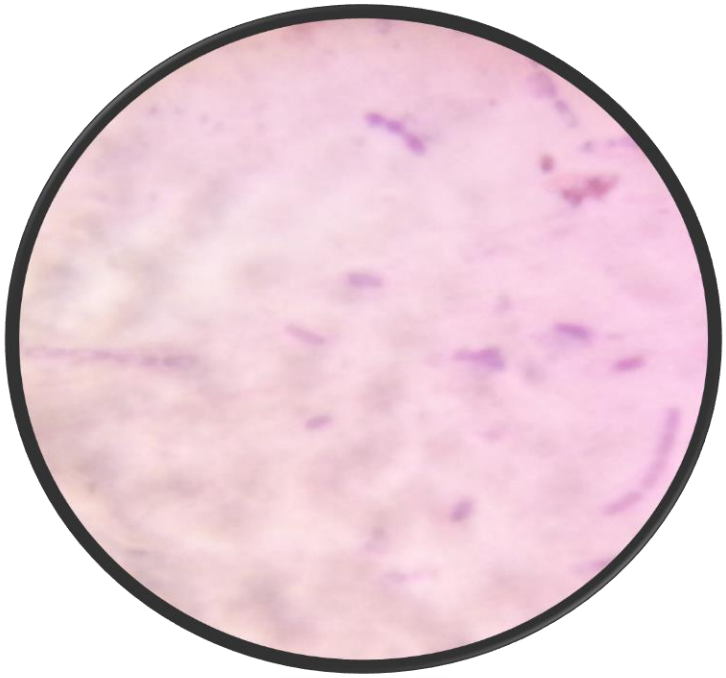


**Figure 3.1:** A microscopic view of the gram stained isolates from cucumber and green pepper samples from Ofada/Mokoloki LCDA

### 3.12.3 DNA Extraction

Each isolate was streaked out on nutrient agar and incubated overnight at $37^0$ C. The loopful actively dividing cells were emulsified in 500ml double distilled water until it was turbid. The cell suspension was then kept in a boiling water bath for 10 minutes. After cooling for 20 minutes, the suspension was centrifuged at 15,000 rpm for 15 minutes and the supernatant was used as template DNA.

## 3.13 PCR Protocol

### 3.13.1 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 4 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. | | |
|---|---|---|
| **1** | **Component** | **1 Reaction** |
| **2** | **Mastermix** | **5** |
| **3** | **fDl** | **0.4** |
| **4** | **rDl** | **0.4** |
| **5** | **DNA** | **1** |
| **6** | **RNaseFree$H_2O$** | **3.2** |
| **7** | **Total** | **10** |

# CHAPTER FOUR

## 4.0 RESULTS AND DISCUSSION

### 4.1 Results

The colonial morphological characteristics of the isolates on the *Salmonella* and SMAC plates revealed the presence of potential Salmonella and STEC strains in all the fruits samples analysed. The morphological characteristics of the isolates were critically observed so as to predict the possible presence of suspected pathogenic microorganisms of interest which are *Salmonella* and Shiga-Toxin producing *E. coli* (STEC) while growth on the MacConkey and Nutrient agar plates indicates the presence of general E. coli and total viable microflora of the fruit samples, respectively.

For further evaluation of the morphological characteristics of the isolates, the suspected *Salmonella* and STEC colonies were sub-cultured onto Nutrient Agar plates (Table 4.1)

Table 4.2 shows the results of the biochemical test performed on all the purified isolates, all the 24 isolates examined were Gram negative as they appeared pink retaining the colour of the counter stain – safranin. This is due to the decolourization of the bacterial cell wall by alcohol because of the low content of peptidoglycan or murein layer. Also, the result of the citrate and oxidase tests further screens the isolate to confirm their identity as pathogenic microorganisms of target leaving *Salmonella spp* and STEC probable organisms of suspicion.

**Table 4.1:** Morphological characteristics of isolates from Location 1, 2 and 3

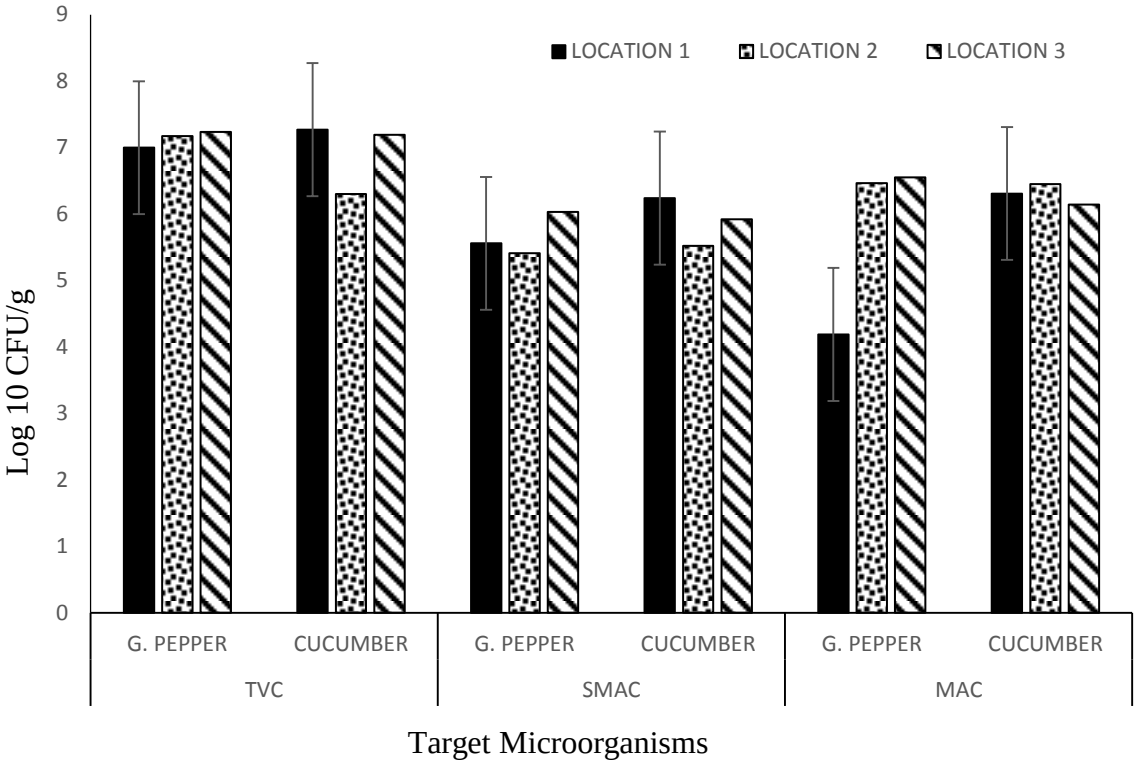
| LOCATION | ISOLATE | COLOUR | SHAPE | ELEVATION |
|---|---|---|---|---|
| **Location 1** | ACUCS$^{-1}$ | White | Circular | Raised |
| | ACUCS$^{-2}$ | White | Circular | Convex |
| | ACUCS$^{-3}$ | Cream | Circular | Raised |
| | AGPS$^{-1}$ | White | Circular | Raised |
| | AGPS$^{-2}$ | White | Circular | Flat |
| | AGPS$^{-3}$ | Grey | Circular | Raised |
| | ACUCSS$^{-1}$ | White | Punctiform | Raised |
| | ACUCSS$^{-2}$ | Yellow | Punctiform | Raised |
| **Location 2** | ACUCS$^{-4}$ | White | Circular | Raised |
| | ACUCS$^{-5}$ | White | Irregular | Convex |
| | ACUCS$^{-6}$ | Cream | Circular | Raised |
| | AGPS$^{-4}$ | White | Punctiform | Flat |
| | AGPS$^{-5}$ | White | Circular | Raised |
| | AGPS$^{-6}$ | Cream | Irregular | Raised |
| | ACUCSS$^{-3}$ | White | Circular | Raised |
| | ACUCSS$^{-4}$ | Yellow | Circular | Flat |
| **Location 3** | ACUCS$^{-7}$ | White | Irregular | Raised |
| | ACUCS$^{-8}$ | White | Punctiform | Raised |
| | ACUCS$^{-9}$ | Cream | Circular | Convex |
| | AGPS$^{-7}$ | White | Circular | Raised |
| | AGPS$^{-8}$ | White | Punctiform | Raised |
| | AGPS$^{-9}$ | White | Circular | Flat |
| | ACUCSS$^{-5}$ | Cream | Circular | Convex |
| | ACUCSS$^{-6}$ | Yellow | Circular | Raised |

**Table 4.2:** Biochemical Test results of isolates from Location 1, 2 a

| ISOLATE | Oxidase | Catalase | Gram Staining |
|---|---|---|---|
| ACUCS$^{-1}$ | - | + | - |
| ACUCS$^{-2}$ | - | + | - |
| ACUCS$^{-3}$ | - | + | - |
| AGPS$^{-1}$ | - | + | - |
| AGPS$^{-2}$ | - | + | - |
| AGPS$^{-3}$ | - | + | - |
| ACUCSS$^{-1}$ | - | + | - |
| ACUCSS$^{-2}$ | - | + | - |
| ACUCS$^{-4}$ | - | + | - |
| ACUCS$^{-5}$ | - | + | - |
| ACUCS$^{-6}$ | - | + | - |
| AGPS$^{-4}$ | - | + | - |
| AGPS$^{-5}$ | - | + | - |
| AGPS$^{-6}$ | - | + | - |
| ACUCSS$^{-3}$ | - | + | - |
| ACUCSS$^{-4}$ | - | + | - |
| ACUCS$^{-7}$ | - | + | - |
| ACUCS$^{-8}$ | - | + | - |
| ACUCS$^{-9}$ | - | + | - |
| AGPS$^{-7}$ | - | + | - |
| AGPS$^{-8}$ | - | + | - |
| AGPS$^{-9}$ | - | + | - |
| ACUCSS$^{-5}$ | - | + | - |
| ACUCSS$^{-6}$ | - | + | - |

Figure 4.1 the total viable counts (TVC) for green pepper from all locations were similar in all locations with 7, 7.1 and 7.2 $Log_{10}$ CFU/g of 7, 7.1 and 7.2, while the TVC for cucumber in locations 1 and 3 showed similarity (7.1 and 7.2 $log_{10}$ CFU/g); leaving the 6.3 $log_{10}$ CFU/g value of location 2.

For SMAC plates, figure 4.1 also shows that cucumber from location 1 had 6.2 $log_{10}$ CFU/g which was the highest for STEC in cucumber followed by green pepper from location 3 had the had 6 $log_{10}$ CFU/g. All the general *E. Coli* counts of cucumber were also similar; 6.3, 6.5, 6.1




$log_{10}$ CFU/g for locations 1, 2 and 3.

**Figure 4.1:** The prevalence of *Salmonella* strains in street vended green pepper and cucumber in Ofada Mokoloki LCDA, Ogun State

Figure 4.2 compares the $\log_{10}$ CFU/g of TVC of both samples (green pepper and cucumber) from the 3 locations.

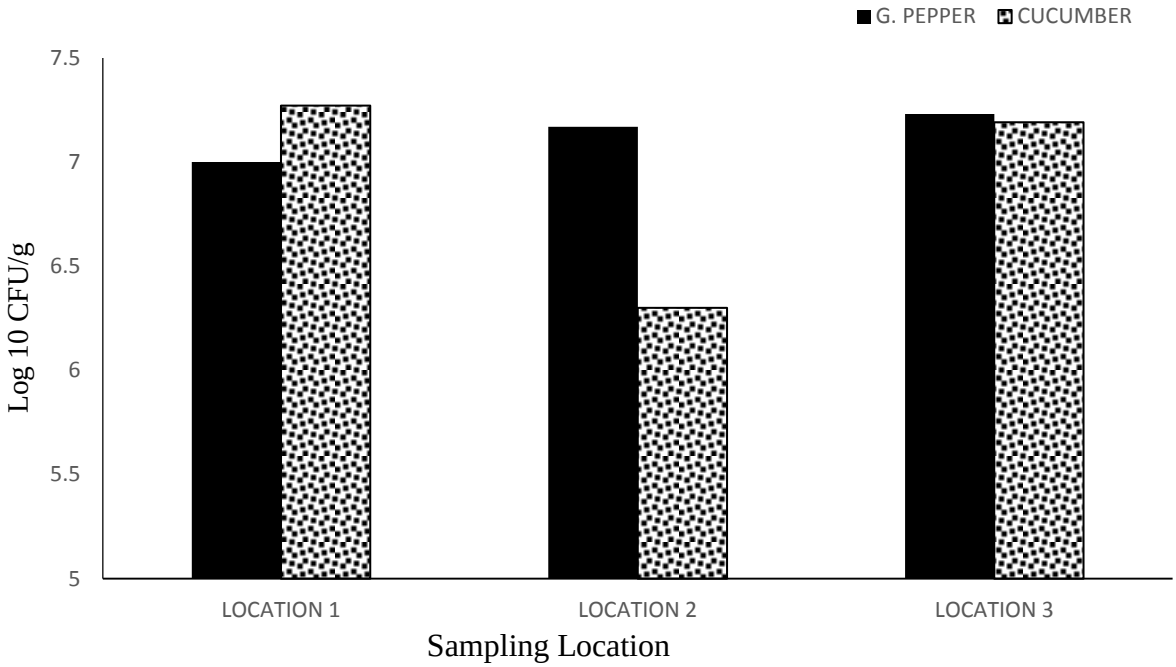



**Figure 4.2:** Total Viable Counts (TVC) of street vended green pepper and cucumber in Ofada Mokoloki LCDA, Ogun State

**Keys:**

G. Pepper – Green Pepper

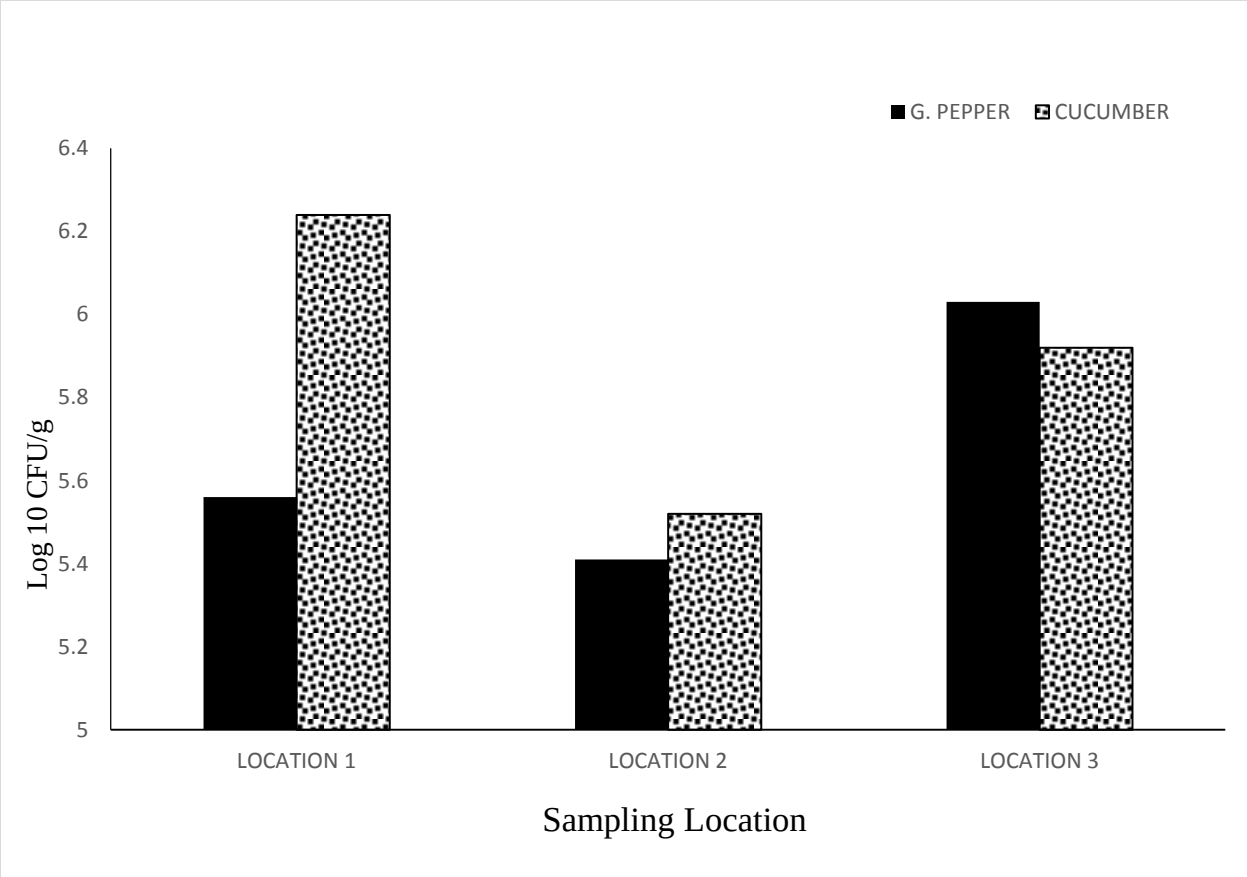
TVC – Total Viable Count

SMAC – Sorbitol MacConkey Agar

MAC – MacConkey Agar

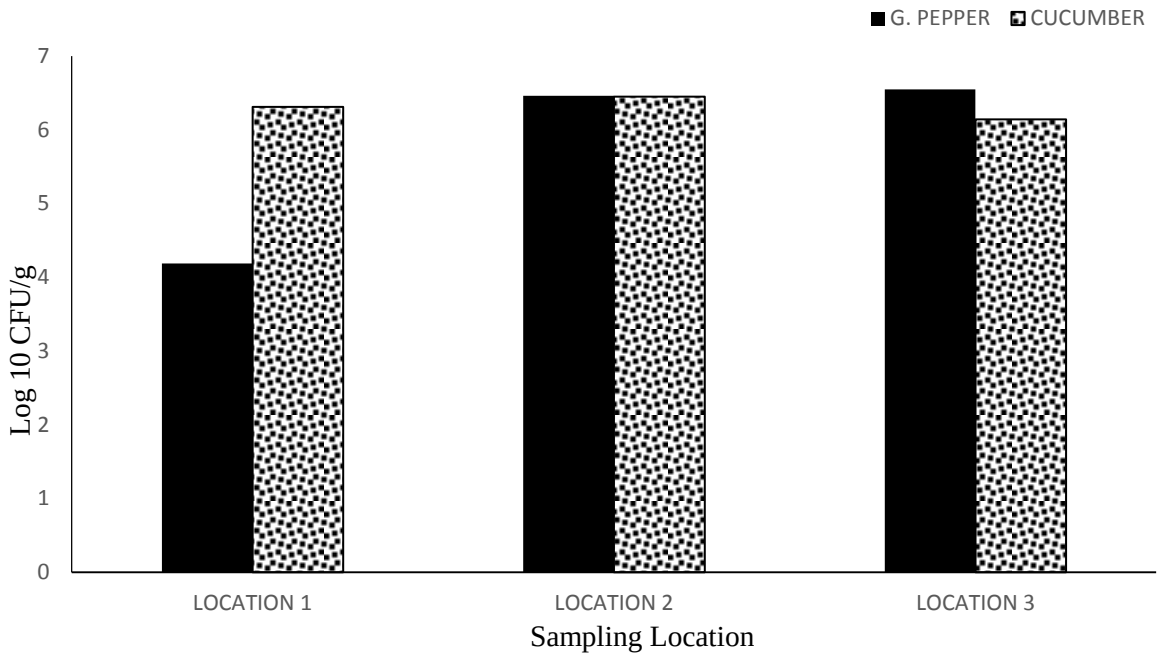
Figure 4.3 shows the Comparism of the $log_{10}$ CFU/g values of STEC (plated on SMAC) in both samples bought from all locations.

**Figure 4.3:** Shiga toxin producing *E. Coli* in street vended green pepper and cucumber in Ofada




Mokoloki LCDA, Ogun State

Figure 4.4 compares the $\log_{10}$ CFU/g of general *E. Coli* found in the samples (green pepper and




cucumber) from the 3 locations where the street vended fruits were bought.

**Figure 4.4:** General *E. coli* in street vended green pepper and cucumber in Ofada Mokoloki LCDA, Ogun State

**Keys:**

G. Pepper – Green Pepper

TVC – Total Viable Count

SMAC – Sorbitol MacConkey Agar

MAC – MacConkey Agar

Table 4.3 shows the suspicion of the presence of *Salmonella* in the fruits analysed (green pepper and cucumber) from all the locations. (based on the growth of colonies on *Salmonella-Shigella* agar, and the biochemical tests conducted).

**Table 4.3:** Detection of *Salmonella* in street vended green pepper and cucumber in Ofada Mokoloki LCDA, Ogun State

| | **Presence of Salmonella** | | |
|---|---|---|---|
| Food Samples | Location 1 | Location 2 | Location 3 |
| Cucumber | + | + | + |
| Green Pepper | + | + | + |

The suspected presence of STEC and *Salmonella* strains in the samples is shown in the DNA amplification image in figure 4.5.

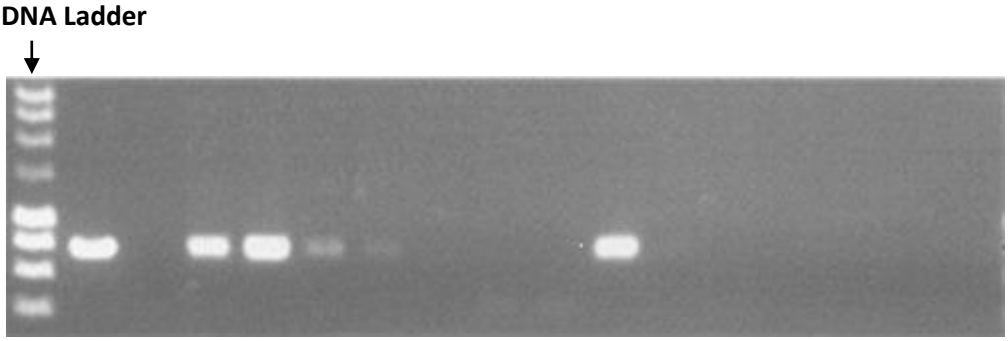



**Figure 4.5:** DNA amplification of cucumber and green pepper samples in Ofada Mokoloki LCDA, Ogun State

Figure 4.6 shows the growth of the suspected colonies of *Salmonella* on *Salmonella-Shigella* agar

**Figure 4.6:** Suspected colonies of *Salmonella* on *Salmonella-Shigella* agar

## 4.2 Discussion

*Salmonella* and *E. coli* are two menace in the world of foodborne diseases (Buck *et al.,* 2003). This study was carried out to identify the prevalence and statistics of foodborne pathogens (*Salmonella* and *E. coli* especially) in fruits (Green pepper and cucumber) sold in Ofada Mokoloki LCDA (Magboro market), the fruits were chosen because they have been observed to be food condiments that do not require special processing before being consumed at home or on the streets. Cucumber is used in cole slaw or salad, while green pepper is used in fried rice preparation or as a spice in other foods. In simple words, the main aim of the study is to know how safety of consumers that consume these fruits in the Magboro area.

*Salmonella* was suspected in the fruits samples examined, and this is a warning signal to the food safety practiced, because Salmonella is a major indicator of poor food safety practices. For all the locations where sampling was done, low strains of *Salmonella* were suspected or identified, maybe because of the low water activity of the fruits (green pepper), *Salmonella* were unable to colonize the fruits and

It is important to have at the back of the mind that the microbiota present in the fruits samples is a direct interpretation and reflection of the storage practices, processing practices, harvesting and agricultural practices which the fruit underwent before sale (Beuchat 1996; Buck et al., 2003; Ray and Bhunia, 2007). The results from Table 7 for cucumber was a bit similar from the study carried out by other researchers on this particular street vended fruit in Ogun state (Eni *et al.,* 2010; Itohan *et al.,* 2011) and this telling us the need for more stringent food safety practices in fruits and foods bought and sold in this part of Nigeria.

*Salmonella* counts for green pepper had the lowest count of $5.5\times10^2$ for location 3 CFU/g and it also had the highest count for general *E coli* with a count of $3.6\times 10^7$ CFU/g. Some of the samples analysed somehow defied the standard limit set by Food and Agricultural Organization FAO, 1979) that the count should be less than $10^5$ CFU/g, the reasons for these could be poor food safety practices by handlers or sellers, contaminated water used for irrigation during planting and rinsing and aerosols from air and dust. (Kawo and Abdulmumin, 2009; Aboloma, 2008; Wada – kura *et al.,* 2009; Sagoo *et al.,* 2003; Nester *et al.,* 2004; Bukar *et al.,* 2010).

Also, Table 7 shows that green pepper had lower CFU/g bacterial count for TVC, STEC and *Salmonella* in all locations of sampling, while cucumber had higher CFU/g counts for TVC, STEC and Salmonella in all locations of sampling, and the counts recorded for cucumber conforms to those recorded by Ajayi *et al.,* 2017

# CHAPTER FIVE

## 5.1 Conclusions

The suspected presence of *Salmonella* in the fruit samples, is of a great concern as typhoid occurrences and other *Salmonella* related diseases will occur from consumption of these fruits. Street vended green pepper and cucumber sold in Ofada Mokoloki LCDA (Magboro axis) area of Ogun state contain presumptive pathogenic STEC and *Salmonella* which can create public health hazard

## 5.2 Recommendations

Fruits sold should be washed and rinsed properly with clean water and other food grade decontaminating chemicals like chlorine, hydrogen peroxide so as to ensure and assure that the fruits sold are free of disease causing microorganisms.

Also, the general public (who are the consumers or buyers of these fruit products should ensure the safety of their fruits products by washing them thoroughly with clean water before consumption. Storage conditions is a means where fruits get contaminated, proper storage facility should be present and prepared before the transportation and selling of fruits. Farmers should monitor their use of contaminated materials like water, fertilizers to nurture their crop produce so as to reduce the incidence of contamination by farm and harvesting practices.

Furthermore, a surveillance system should be set in place by the government and its concerned agencies, so as to trace the occurrence and prevalence of foodborne diseases occurring as a result of consuming contaminated fruits.

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