# PREVALENCE OF PATHOGENIC *ESCHERICHIA COLI* FROM FRESH PRODUCE SOLD IN LAGOS AND OGUN STATE LOCAL STREETS MARKETS.

**By**

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**A PROJECT SUBMITTED TO THE DEPARTMENT OF BIOLOGICAL SCIENCES, COLLEGE OF BASOC AND APPLIED SCIENCES, IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD DEGREE OF BACHELOR OF SCIENCE IN MICROBIOLOGY**

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## DECLARATION

I hereby solemnly declare that this Project has been written by me and is a record of my own research work. It has not been presented in any previous application for a higher degree of this or any other university. All citations and sources of information are clearly acknowledged by means of reference.

…….

**NNANYERE PRECIOUS OLUEBUBE**

……..

**Date**

## CERTIFICATION

This is to certify that the content of this project entitled **"Prevalence of Pathogenic *Escherichia coli* from Fresh Produce sold in Lagos and Ogun States Markets"** was prepared and submitted by **NNANYERE PRECIOUS OLUEBUBE** in partial fulfillment of the requirements for the degree of **BACHELOR OF SCIENCE IN MICROBIOLOGY.**
The original research work was carried out by her under my supervision and is hereby accepted.

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Head of Department

## DEDICATION

I dedicate this project to the God Almighty, who has been my strength, provider and my sustainer, Also, to my family and my supervisor for unending support.

## ACKNOWLEDGEMENT

Firstly, I would like to thank God for His grace and blessings that I am able to succeed in this final year project without any tough obstacle. Now here comes a speech to my parents. I would like to thank them for their support and care on all this while. Without them I would not be able to succeed anything in my life. Also, a very big thanks to my respected supervisor Dr. E. O. Fayemi for his patience, generosity, kindness and guidance on the completion of this study. Also, a special thanks to Dr. G. B. Akanni on correcting and improving my thesis.

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## ABBREVIATIONS

| | |
|---|---|
| **mg** | Milligram |
| **gm** | Gram |
| **ºC** | Degree Celsius |
| **ml** | Millilitre |
| **µl** | Microliter |
| **%** | Percent |
| **µm** | Micrometre |
| ∞ | Infinity |
| **mm** | Millimetre |
| **16s Rrna** | 16s Ribosomal RNA |
| **TAE** | Tris-acetate Buffer |
| **Min** | Minute |
| **et al.,** | And others |
| **pH** | Negative logarithm of hydrogen ion concentration |
| **bp** | Base pair |
| **UV** | Ultra violet |
| **Spp.** | Species |
| ***E. coli*** | *Escherichia coli* |
| **No.** | Number |
| **Stx1** | Shiga toxin 1 |
| **Stx2** | Shiga toxin 2 |
| **BMI** | Body Mass Index |
| **$a_w$** | Water Activity |
| **i.e.** | That is |

## ABSTRACT

*Escherichia coli* is a genetically distinct group of bacteria whose members are not normally pathogens found in the normal microbiome of humans and animals' digestive tracts. Diarrhoea, Haemorrhagic Colitis (HC), Haemolytic Uremic Syndrome (HUS), Urinary Tract Infections (UTIs), Sepsis, and Neonatal Meningitis are forms of bacterial strains that have acquired virulence properties that help them to cause tremendous intestinal and extra-intestinal disorders. *E. coli* has been reported to cause disease outbreaks related to the consumption of fresh fruits and vegetables such as (lettuce, cucumber, carrot, cabbage, pineapple, watermelon and papaya.). These fresh produces are contaminated with *E. coli* during field cultivation or during application of organic manure or during harvest, transport, processing, storage and distribution. In Nigeria, there have been reports of outbreaks involving *E. coli* from fresh produce to date. Fresh fruits and vegetables have been collected around Lagos and Ogun states local street markets to isolate *E. coli.* A total of 64 samples were tested for presence of *E. coli* using Sorbitol MacConkey Agar and MacConkey Agar plates. All sampled had presumptive STEC, molecular identification of selected isolates (n = 21) randomly for STEC virulence genes (*$stx_1$ $stx_2$* and *eae*) using multiplex PCR was done. The total viable count (TVC) for presumptive STEC in the samples was in the range of 4.5-7.8 $\log_{10}$cfu/g. Lettuce from Ibafo had the highest total viable count of 7.8$Log_{10}$CFU/g. Virulence gene (*stx1*) was detected only in watermelon from Magboro using Multiplex PCR. From this result, the presence of STEC poses a threat to public health which could lead to foodborne illnesses including haemorrhagic colitis (HC) or haemolytic uremic syndrome (HUS). Findings on this study call for immediate actions by the Nigerian Food Safety Agency to ensure food safety regulations in the fresh produce supply chain in the local markets nationwide. In conclusion isolating *E. coli* from fresh-cut produce by using selective enrichment broth and selective media and molecular characterizations of the isolates. One isolate was found to carry stx1 gene, which is responsible for haemolytic colitis and haemorrhagic uremic syndrome, based on the risk of the presence of STEC from fresh produce.



**Key words:** STEC, fresh produce, PCR, virulence gene.

# CHAPTER ONE

## 1.0 INTRODUCTION

### 1.1 Background of Study

Fresh produce constitutes a significant component of the world's human diets. Ingested raw or uncooked without heat treatment is usually used. (Liu and Luna, 2003). A fruit- and vegetable food that protects from cancer and chronic illnesses including coronary heart disease has been proven. (Goodburn and Wallace, 2013). Consumption of ready-to-eat vegetables, salads, and fruits are increasing as interest in healthy diets grows (Cordano and Jacquet, 2009). Despite consumption, the lives of many consumers have altered as a consequence of their tough schedules that lead to a lack of time to cook their products at home (Cordano and Jacquet, 2009). However, increased fresh produce intake is related to an increase in foodborne outbreaks (Callejón *et al*.,2015). Consumers consider green salad vegetables safe to eat as a food, since they are RTE products for nutritious and simple foods (De Oliveira *et al*., 2011). Most commonly used vegetables in salads are fresh, leafy greens and fruits like Papaya (*Carica papaya*), also known as papaw, Lettuce (*Lactuca sativa*), Cucumber (*Cucumis sativus*), Carrot (*Daucus carota*), Cabbage (*Brassica oleracea),* Watermelon (*Citrullus lanatus*) and Pineapple (*Ananas comosus*) (Stine *et al*., 2011). Because it is ingested raw or poorly processed, fresh produce is bound to continue to cause foodborne disease epidemics because it has less limits to microbial growth than other conventional foodborne illness vehicles. (Stine *et al*., 2011). The incidence of foodborne disease is now being assessed as a factor of the accessibility with which microbial pathogens may grow and thrive in raw or minimally processed fresh produce (fruits and vegetables). (Stine *et al*., 2011).

Fresh produce, given the immense nutrient content, has become a potential source for bacteria, due to a combination of exposure by untreated sewage fertilizers and poor post-harvest treatment. Foodborne outbreaks linked to fresh produce, however, have become more common, with pathogenic *Escherichia coli* being the most common pathogen implicated. (Chang and Fang, 2007). Diarrheoa, hemorrhagic colitis, hemolytic uremic syndrome (HUS), and other predisposing factors by pathogenic *E. coli* in humans, *E. coli* 0157:H7 has been the main cause of illness which is known as STEC or entero-hemorrhagic *E. coli* bacterium, the source of foodborne infections for decades. (Eribo and Ashenafi, 2003).

Many fresh cuts Ready to eat fruits are becoming more popular in Nigeria because they are more accessible, convenient, and cheaper than whole fruits (Oranusi and Olorunfemi, 2011). Consumers may be exposed to much more ailments as a consequence of rising consumption, which is a major source of concern. People who are sick via foodborne illnesses often incur health fees and are unable to work for a period of time, leading to lower economy. Food companies are facing enormous financial costs of unsafe foods. Farm and commercial sales are now being destroyed, consumer confidence is diminishing, and access to domestic and international markets has been harmed. Foodborne diseases increase food loss and waste, both have severe health impacts. Illness from food varies substantially between places as more than just a result of which the spread of infectious illness and related mortality varies. (Buzby 1997).

As a result, this study's work resolves the most prevalent *E. coli* pathologies associated with food-borne onset from fresh produce ingestion. Everything from the manufacturing process to kitchen hygiene has to be sanitary to ensure safety of fresh produce. Inadequate hygiene, cross-contamination, poor room processing and storage, contaminated equipment, and employee contamination all can lead pathogens to invade fruits and vegetables. It is a core part that contributes to the presence of all pathogens within prepared fruits and vegetables. (Reij and Aantrekker, 2004).

### 1.2 Statement of Problem

Fresh Produce such as fruits and vegetable stand suspected towards be easily contaminated by various strains of pathogenic *E. coli*, as a result of unhygienic practices from farm to fork chain. The implication of pathogens in Fresh Produce is of great public health concern, mostly the health of children under 5 years old and the aged people. Therefore, a microbiological survey of the quality of fresh cut RTE fruits and vegetables vended in Lagos and Ogun state is important.

### 1.3 Aim and Objectives of Study

The aim of this research is to investigate the prevalence of Pathogenic *E. coli* in Fresh Produce from Open markets in different Locations.

In order to achieve the aim, the following objectives have been identified:

i. To isolate and presence detection of pathogenic *E. coli* from fruits and vegetables from 3 different locations in Magboro, Ibafo, Magodo, Yaba, Jakande local streets markets in Lagos and Ogun states.
ii. To determine the presence of STEC isolated by using Sorbitol-MacConkey Agar, Nutrient Agar and MacConkey Agar.
iii. To identify *E. coli* isolates isolated from fresh slice fruits and vegetables through a series of biochemical test and PCR.

**1.4 Significance of Study**

The investigation of Fresh Produce eaten raw or used for salad is essential, to know the particular pathogens that may be present in the Fresh Produce capable of causing ill-health or mortality in individuals. The possible pathogen that may be present in fresh-cut fruits and veggies would be examined and its health implications.

# CHAPTER TWO

## 2.0 LITERATURE REVIEW

### 2.1 Overview of Fresh Produce in Nigeria

Fresh-cut produce is considered as "any fruit or vegetable, or combination thereof, that has been physically manipulated from its natural form but continues in a fresh state," as per the (IFPA) International Fresh-cut Produce Association (IFPA and PMA, 1999). Farming methods may vary significantly depending with where fresh food is consumed and cultivated. (Uyttendaele *et al*., 2015). As a result, there are varying norms and regulatory criteria for the quality of agricultural goods as well as the water management for production and preparation in different nations and/or sellers. (Uyttendaele *et al*., 2015).

As this fresh produce are abundant in dietary fibre, vitamins, minerals, and especially electrolytes, they are included in the nutritional advice. Phytochemicals, particularly "antioxidants," are also found. Insufficient eating of fruit and vegetable have been associated to cardiovascular disease, high blood sugar, hypercholesterolemia, osteoporosis, various tumours, chronic obstructive pulmonary disease, respiratory disease, mental wellbeing, and much more, as according to different studies. (Slavin and Lloyd 2012).

Fruits and vegetables have a high fibre content, which lessens the "acid load" of a meal, which aids calcium absorption. BMI and vegetable intake were found to have a substantial association, with overweight persons eating fewer vegetables (Dehgan *et al.,* 2011)

The need for fresh produce is increasing due to recommendations from nutritionists and researchers, or by promoting consumption through government campaigns (IFPA and PMA, 1999). Minimally processed or freshly cut produce assist in meeting the requirement of these consumers (IFPA and PMA, 1999). Fruits and vegetables that have been peeled, cut, chipped, trimmed, or washed can be considered fresh cut produce (Francis *et al*., 2012).

Most samples of Fresh Produce used in this study are Fresh cut Lettuce and Cabbage, Pineapple, Watermelon, Cucumber, Carrot and Papaya bought from local streets markets in Different locations in Ogun and Lagos State.

## 2.2 SELECTED TYPES OF FRESH PRODUCE AND THEIR PLANTING SEASONS IN NIGERIA

Under this study, there are several kinds of fresh produce in Nigeria that are most likely to be contaminated by pathogen such as *Escherichia coli* is being studied as it can grow with a minimum of 0.95.

### 2.2.1 Lettuce

Lettuce (*Lactuca sativa*) a prominent fresh plant, and is often found in combinations of salads and sandwiches. The tree is taken instead of leaves of lettuce, either cooked, raw, pickled, dried or as a sauce, in certain eastern nations such as China and Egypt (Ryder, 1986)

**Figure 2.1:** Diagram of fresh Lettuce
(Adapted from Markon, 2021).

### 2.2.2 Cucumber

Cucumber (Cucumis sativus) is one among the most significant vegetable in the Cucurbitaceae family (Lower and Edwards, 1986; Thoa, 1998). The fruits are eaten and widely used as salad. The fruits help also for human constipation elimination and are excellent for digestion. The fruit is often utilized as a refreshing snack throughout the summer. The salads and the curries are utilized for cooking.

**Figure 2.2:** Diagram of fresh Cucumber
(Adapted from Pixia and Xiangdong 2013).

### 2.2.3 Papaya

Papaya (Carica papaya) is popularly referred for its quality and dietary values all across the globe. In the indigenous medical system, the therapeutic qualities of papaya fruits and other plant areas are very universally acknowledged. Since the economic worth of each portion of the papaya tree is that of commercial cultivation (Krishna and Patel 2008).

**Figure 2.3:** Diagram of fresh Papaya
(Adapted from Caez Ramirez, *et al.*, 2017).

### 2.2.4 Carrot

Carrot (*Daucus carota*) is an essential root vegetable rich in bioactive substances such as carotenoids besides dietary fibre with substantial health boosters for several other

components. Carrot consumption and their products have constantly increased since they are recognized as a significant source of naturally occurring antioxidants (Sharma *et al.*, 2012).

**Figure 2.4:** Diagram of fresh Carrot
(Adapted from Que, F. *et al.*, 2019).

### 2.2.5 Cabbage

Cabbage (*Brassica oleracea)* is a foliage vegetable in the plant family, (*Brassica oleracea).* Leafy in the sense of leaves. It is mainly grown or cultivated for consumption of its stems or leaves. Humans are primarily attracted to eating the stem, also known as the head, but the leaves are used for some other purpose (Business Plan 2021).

**Figure 2.5:** Diagram of fresh Cabbage
(Adapted from Zhang, and Jing, 2021).

### 2.2.6 Watermelon

Watermelon (*Citrullus lanatus*) is a flowering plant species of the (*Cucurbitaceae*) family. It is cultivated commercially in places with long period of warmth without frost (Wehner, 2008). it is a good source of hydration on sunny days and source of water. Watermelon is one of the most consumed fresh and tender fruits in Nigeria and is in great demand.

**Figure 2.6:** Diagram of fresh Watermelon
(Adapted from Naz, A. *et al.*, 2014).

### 2.2.7 Pineapple

Pineapple (*Ananas comosus*), Pineapple is among the most significant commercial fruit crops in the world, from the Family *Bromeliacea*e (Baruwa, 2013). Pineapples are fresh, cooked, juiced, or are eaten and it can be maintained. The fruit is very peregrinated and seasonal (Bartholomew, 2003).

**Figure 2.7:** Diagram of a fresh pineapple

(Adapted from Daily health post editorial 2018).

## 2.3 NUTRITIONAL VALUES OF THE FRESH PRODUCE LETTUCE

Depending on the variety, lettuce is an excellent source (20% of the Daily Value, DV, or higher) of vitamin K (97% DV) and vitamin A (21% DV), with higher concentrations of the provitamin A compound, beta-carotene, found in darker green lettuces, such as Romaine (Health Jade 2017).

Lettuce is a rich source of vitamin K and vitamin A, and a moderate source of folate and iron (Ogo, 2020). Although lettuce is not usually acknowledged as being a rich source of beneficial phytochemicals, it does contain phenolic compounds, vitamins C and E, and carotenoids (Lorach *et al.*, 2008).

### 2.3.1 Cucumber

Phytonutrients are abundant in cucumbers (plant compounds that have defensive or disease preventive properties) such flavonoids, lignans and triterpenes, which have antioxidant, anti-inflammatory and anti-cancer benefits, according to World's Healthiest Foods (Navitha, 2019). They are also rich in vitamin A, B1, B6, C and D, Folate, Calcium, Magnesium, and Potassium (Jeffery, 2014).

### 2.3.2 Papaya

It has high vitamin C content and a single medium fruit provides 224 percent of recommended daily intake. They also have B vitamins, alpha and beta-carotene, lutein and zeaxanthin, vitamin Calcium, potassium, vitamin K, and lycopene, the powerful antioxidant most commonly associated with tomatoes. (Megan, 2017).

### 2.3.3 Carrot

Carrots' water content ranges from 86–95%, and the edible portion consists of around 10% carbohydrate, Carrots contain very little fat and protein.

### 2.3.4 Cabbage

A cup (89g) of raw chopped cabbage: contains 22g of calories, 0.1g of fat, 16mg sodium, 5.2g carbohydrates, 2.2g of Fibre, 2.9g sugars and 1.1g protein.

### 2.3.5 Watermelon

It is a source of vitamin C. Watermelon contains vitamins A, C, B6 and potassium. It is high in energy from fat. Watermelon is 92% water by weight. It is also a mild diuretic and

contains high amounts of beta-carotene. Red-fleshed watermelon is an important source of lycopene, containing about 6% sugar by weight, with most of the rest being water. It also contains more lycopene than other fruits and vegetables (TFNET news compilation 2016).

### 2.3.6 Pineapple

It contains the proteolytic enzyme bromelain, which helps digest proteins and prevents blood clot formation. Pineapple nutrition includes calcium, potassium, dietary fibre, and vitamin C. It has a minimal fat and cholesterol. It is also a good source of vitamin B1, vitamin B6, copper, and dietary fibre. Pineapple is a rich source of ascorbic acid supplements in our diet.

## 2.4 MICROBIAL CONTAMINATION OF FRESH PRODUCE FROM FARM-TO FORK CHAIN

### 2.4.1 PRE-HARVEST CONTAMINATION

#### 2.4.1.1 Soil

Contaminated soil could contribute to the contamination during cultivation. Soil used during agricultural production is frequently altered with treated or untreated animal vermicomposting biosolids applied as fertilizers, which provide a cost-effective quantity of nutrition while also harbouring harmful microorganisms. (Gutierrez-Rodriguez and Adhikari, 2018; Julien-Javaux *et al*., 2019).

#### 2.4.1.2 Fertilizer

Direct contact of plant surfaces with manure is also a source of contamination (Alegbeleye *et al*., 2018).

#### 2.4.1.3 Water

It can be a significant source of microbial contamination. The water used to irrigate agricultural products can be sourced from municipal water supplies, groundwater, etc. (Jongman and Korsten, 2018). Surface water and recycled wastewater are forms of water sources with low microbiological quality that could infect fresh crops. (Alegbeleye *et al*., 2018).

#### 2.4.1.4 Meteorological Conditions

Heavy rains can lead to incidents that can flood the terrain and contaminate crops. On the other hand, severe drought conditions can increase the likelihood of contamination by using water of low microbiological quality due to lack of drinking water (Yeni and Alpas, 2017). Microbial contamination of agricultural products it has also been demonstrated to depend on the growing season (Marine *et al*., 2015). The survival rate of microorganisms in agricultural

products may vary depending on factors temperature is one factor, amount of solar radiation and humidity (Marine *et al*., 2015).

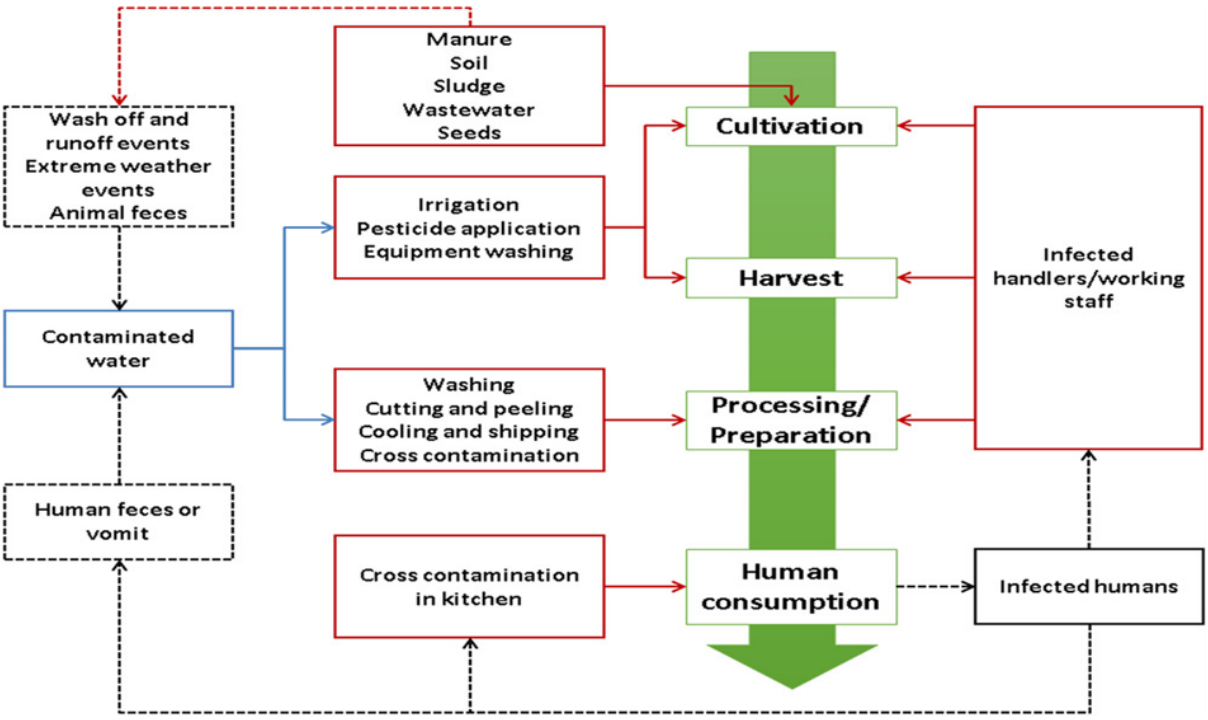


**Figure 2.8:** Microbial contamination of fruits and vegetables from Farm to fork chain (Adapted from Machado-Moreira *et al*., 2019).

## 2.4.2 POST- HARVEST CONTAMINATION

### 2.4.2.1 Workers

Pathogenic microbes can be transferred into the hands of employees through contaminated soil and agricultural product handling. Furthermore, after being transferred from contaminated agricultural products, a bacterial pathogen such as *E. coli* O157 and *Salmonella* it has been confirmed that it can thrive in nitrile and latex gloves, raising the risk of additional food contamination (Erickson *et al.*, 2018).

### 2.4.2.2 Equipment

The environment in which these foods are handled (such as the food contact surfaces and equipment used for processing) can also affect food contamination. (Gaul *et al*., 2013;

Stephan *et al*., 2015). Disposable crates for instance, have been discovered to be a possible cause of recontamination across multiple batches of product. (Murray *et al.*, 2017).

#### 2.4.2.3 Washing

Washing fresh produce is ineffective at getting rid of microorganisms as they will likely leave them attached to the surface of the plant. (DiCaprio *et al*., 2015), or get absorbed in the plant's edible portions (Franz and van 2008). Therefore, it is not accessible for efficient removal. Likewise, the water being used in washing fresh produce can cause microbial contamination. (CDC, 1989; Hedberg *et al*., 1999).

#### 2.4.2.4 Storage

Maintaining cold storage conditions for fresh produce during processing, transportation, storage, and sales, as low temperatures inhibit the growth of bacterial pathogens in these operations. (Castro-Ibáñez, Gil, and Allende, 2017). Being able to preserve these conditions during storage and/or transportation of these products is advantageous for bacterial growth if they are already present in agricultural products. (Zeng *et al*., 2014)

#### 2.4.2.5 Food Vendors and Consumer

Finally, handling fresh produce in the kitchen, either by final consumers or in a commercial setting such as a canteen or restaurant, poses the risk of cross-contamination with other pathogens such as raw meat or eggs. Which is emphasized as a potentially toxic situation (CDC, 1998a; del Rosario and Beuchat, 1995; de Waal, Alderton and Jacobsen, 2000).

## 2.5 PUBLIC HEALTH HAZARD OF FRESH PRODUCE

Considering fresh fruits and vegetables are a component of the global food system, their safety is also a growing issue. Unfortunately, many of these fresh fruit and vegetable markets are situated in filthy conditions, such as garbage heaps or dumpsters. (Mritunjay and Kumar 2015).

The bulk of foodborne disease cases are caused by *Escherichia coli*, which accounts for 16,416 infections (Michelacci *et al*., 2016; Newitt *et al*., 2016). *E. coli* strains that are not pathogenic are frequent in the gut flora of humans and animals. Some strain, on either contrary, are dangerous and can trigger disease of the digestive, bladder, and neurological systems. The Shiga-toxin-producing *E. coli* O104 and *E. coli* O121 strains can induce gastrointestinal sickness as well as Haemolytic Uremic Syndrome (HUS). *Escherichia coli*

O96 is an enteroinvasive serogroup that is prone to infection in poorly sanitized and underdeveloped nations where there is faecal matter dissemination of water or food contamination. (Michelacci *et al*., 2016; Newitt *et al*., 2016). This strain has been connected to two outbreaks. (Newitt et al., 2016; Escher et al., 2014).

The *E. coli* O157 serotype was detected in 72.1 % and 80.5 % of all *E. coli* infestations. *E. coli* O157:H7 has been found to exist in sewage for up to 21 months in studies, and testing is being carried in lab settings at varying temperatures (Bolton *et al.,* 1999; Franz *et al.,* 2005; Jiang *et al.,* 2002; Kudva *et al.,* 1998) or adjustable environments (Zhang *et al.,* 2009), also in field trials (Hutchison *et al.,* 2004; Islam, *et al.*, 2004, 2005; Kudva *et al.*, 1998). Presence of pathogens by microbes in edible sections of RTE foods includes tomatoes, sprouts, and lettuce has been observed, and the internalized pathogens may not be eradicated by hygienic methods after yield or at the final consumption stage, posing a major health risk. (Franz and van, 2008; Murphy *et al*., 2016).

Microorganisms invade your digestive system when you ingest contaminated food, causing food-borne diseases. These microbes are too small to see without a microscope, so they must be examined under one. Many people eat raw fruits and vegetables and since there is no cooking or "killing" process to eliminate germs that are presumably present. Contamination is difficult to detect since it occurs infrequently and affects just a small portion of the crop. Because germs are difficult to perceive, visual contamination detection is challenging. Many fruits and vegetables have a rough surface, resulting in stem scars where the reduced area provides a place for pathogens to hide. (DuPont, S. T., and L. LaBorde, 2015). Children under the age of five (5) bear 40% of the burden of food borne illness and kill 125,000 people each year. (WHO 2020).

Although everyone is at danger of contaminated food or food poisoning, some persons are at a larger risk than others. People with AIDS, cancer, kidney disease, and other chronic diseases; those with degenerative diseases, such as lupus; those who have had their spleen removed; those taking immune-suppressing medications; children under the age of five; expectant mothers and their unborn or new-born babies; and those with a history of problematic alcoholism use.

## 2.6 THE GENUS *ESCHERICHIA COLI*

Fruit and vegetable eating are commonly acknowledged to have become a risk factor for infection with enteric pathogens such as Pathogenic *Escherichia coli*. The objective of this study is to identify pathogenic *E. coli* in fresh produce that people buy and consume on a daily basis, since fruits and vegetables are known to contain nutrients that help to boost the human immune system. (Heaton and Jones, 2008).

*E. coli* is a ubiquitous bacterium found in the gut microbiota of human and warm-blooded organisms. Pathogenic strains, on the other hand, can cause intestinal and enteric illnesses (Franz *et al.*, 2007).it is a coliform bacterium and one of the six types of *Escherichia spp.* (*E. adecaroxylate, E. blattae, E. fergusonii, E. hermannii, and E. vulneris*). It is among the thirty (30) members of the Enterobacteriaceae family of bacteria (Balpetek, 2010). It is a mesophilic bacterium that grows in the temperature of 7–45°C. It is a gram-negative, non-spore-forming, facultative, anaerobe (it can grow in the presence or absence of oxygen), shaft bacterium that moves with the help of a flagella, it is a mesophilic bacterium that grows in 7–45°C. The bacteria Coliform group are *Citrobacter, Enterobacter, Klebsiella* and *Escherichia* (Uçar et *al.*, 2015).

Some of its pathogenic strains produce toxins that can induce poisoning, induce infectious food poisoning through cell proliferation, and induce gastroenteritis, pathogenic kidney and brain damage (Donnenberg, 2017). Some *E. coli* strains have been isolated from a variety of plants used for food, including plants such as spinach, lettuce, arugula, cruciferous vegetables, beans, arugula, tomatoes and radishes. Considered as a secondary host. (Jablasone *et al.*, 2005). This plant has physical barriers such as waxes, cuticles, cell walls, trichomes and stomata (natural pores). Some bacteria have been found to use stomata at the entrance points inside leaf (Dinu and Bach, 2011). Multiple human pathogenic bacteria can survive in and penetrate the interior of apoplast plants. They can remain low metabolic activity in this environment and survive in rapid changes in temperature, pH, osmolality and undernourishment (Winfield and Groisman, 2003).

For epithelial colony formation in humans, pathogenic *E. coli* has adhesion factors, and some of these factors have been demonstrated to be utilised for attachment to raw vegetables as well (Barak *et al.*, 2005). Plants also offer *E. coli* with a hard environment, including aerobic conditions, low temperatures, low pH, strong UV energy, and an aerial surface (volume of leaves) holding fewer nutrients and secondary compounds that are antimicrobial (Brandl,

2006). However, depending on the strains and plants involved, diarrheagenic *E. coli* has varied plant attachment methods (Berger, 2009).

**Table 2.1:** *Escherichia coli* strains associated with foodborne diseases and their virulence factors (Adapted from Berger, *et al*., 2009, Shaw *et al.*, 2008, Jung *et al.*, 2014).

| PATHOTYPE | SEROTYPE | FOOD OUTBREAK YEAR | VEHICLE | VIRULENCE FACTORS ATTACHED TO RAW VEGETABLES |
|---|---|---|---|---|
| STEC | O157:H7 | 1996 | White Radish | |
| | | 1998 | Sprout | |
| | | 1996 | Lettuce | |
| | | 2006 | Lettuce | |
| | | | Spinach | T3SS (EspA) arugula |
| | | | Iceberg Lettuce | T3SS (Lettuce and spinach) |
| | | | | T3SS, Curli, Flagellum, Enterohemorrhagic *E. coli* |
| | | | | Common pilus (baby spinach leaves) |
| | | | | Curli (alfalfa sprout) |
| | | | | Biofilm sprout and tomato roots |
| | | 2008 | Lettuce | |
| | | 2008 | Spinach | |
| | | 2019 | Spinach | |
| | | 2012 | Romaine Lettuce | |
| | | 2013 | Spring mix and | |
| | O26 | 2012 | spinach | |
| EHEC | O121 | 2014 | Ready-to eat salads | |
| | O104:H4 | | Raw clover sprouts | |
| | | | Raw clover sprouts | |
| | | | Fenugreek sprouts | |
| | | | Sprout and | Colonic acid capsule |
| ETEC | | 2008 | tomato roots | Biofilm 1,5-n-acetyl-D-glucosaminecellulose |
| | | | | Cellulose, colonic acid and Curli |
| | | | | Flagella |
| | | | Lettuce and leafy | |
| EAEC | | 2011 | vegetable | Flagellar adhesion and |

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Contamination by *E. coli* in raw vegetables is important because fresh vegetables are used in the manufacture of fresh food and it has a low dose infection that can cause intestinal disease (*E. coli* 0157: H7; 10-100 cells) (Ackers, 1998).

### 2.6.1 Pathotypes of Pathogenic *E. coli*

Diarrheagenic *E. coli* or commonly as pathogenic *E. coli*, these groups are classified based on their unique virulence characteristics and can only be identified by these traits. As a result, testing for pathogenic *E. coli* generally necessitates first identifying the isolates as *E. coli* before testing for virulent markers. The pathogenic groups include enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC), enter invasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC), diffusely adherent *E. coli* (DAEC) (Lacher *et al.*, 2016). They all have distinct pathogenic epidemiologic and clinical characteristics.

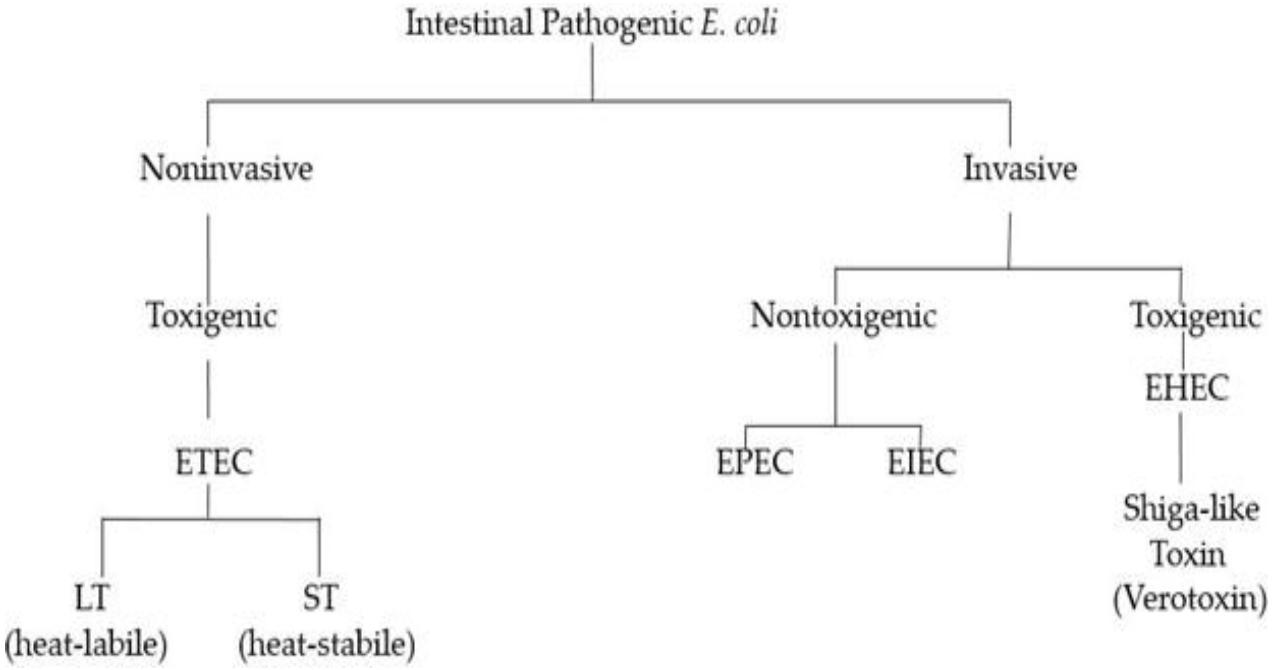



**Figure 2.9:** The Mechanism of Intestinal Pathogenic *E. coli* strains
(Adapted from Ekici and Dümen, 2019).

Pathogenic strains produce all toxins and cause poisoning, causing infectious food poisoning through cell proliferation, causing gastroenteritis, pathogenic kidney and brain damage (Uçar, 2015). Pathogenic strains are also known to cause serious illnesses such as diarrhoea, peritonitis, mastitis, sepsis, pneumonia, and neonatal meningitis (Donnenberg, 2017). Gram-negative bacilli it is the most common pathogen, especially causing neonatal meningitis (Uçar, 2015). It shows serious morbidity and mortality worldwide. The mortality rate of cases of neonatal meningitis is reported to fluctuate between 15-40%, and it is reported that 50% of survivors continue to live with neurological impairment (Donnenberg, 2017).

*E. coli* strains can be serologically characterized based on the detection of specific O (somatic), H (Flagella) and K (capsule) antigens. Most E. coli stocks can be identified with O and H antigens. For example, *E. coli* O157:H7 is the leading cause of STEC infection worldwide (Meng and Schroeder 2007; Gyles 2007).

#### 2.6.1.1 Enteropathogenic *E. coli* (EPEC)

It is known to be the oldest serotype of *E. coli* causing diarrhoea and its most important feature is compliance. In EPEC infection, vomiting and low body temperature are seen in addition to watery diarrhoea (Poole, 2007). It is known to cause diarrhoea in infants and epidemics can occur in neonatal care units (Donnenberg, 2017). Humans, pigs, buffaloes and cows is potential to get infected with this microorganism. However, EPEC is passed from person to person; Rarely, it is also known to be spread through contaminated food and water (Gerba, 2014).

#### 2.6.1.2 Enterotoxigenic *E. coli* (ETEC)

it is often reported that people who live in remote areas have this pathogen in their stool and it has been shown to have developed immunity against this microorganism (Uçar, 2015). As the common risk factor of death in children under 5, the most common microorganism seen in childhood diarrhoea is ETEC and it is also responsible for 30-60% of diarrhoea cases among traveling visitors (Donnenberg, 2017). The infection is characterized by watery diarrhoea and, dependent on the individual, its course can range from normal to difficult defecation with symptoms such as vomiting and high fever (Zhang, 2015).

ETEC strains induce fluid secretion by producing enterotoxins that disrupt fluid and electrolyte homeostasis in epithelial cells of the small intestine, resulting in watery diarrhoea. Without rehydration, moderate or severe diarrhoea can lead to dehydration and acute death (Zhang, 2015).

#### 2.6.1.3 Enteroaggregative *E. coli* (EAEC)

This pathogen is an enteric pathogen observed in acute and persistent diarrhoea in children, immunocompromised patients in developing countries and travellers in endemic areas. In children living in poor counties, growth and intellectual disability is common, resulting from EAEC infections. In the pathogenesis of EAEC, the first step is a strong adhesion of the intestinal mucosa. The second stage leads to the development of enterotoxins and cytotoxins and the third stage is known to potentially cause inflammation of the mucous membranes (Elias, 2016).

Persistent infection and chronic disruption of bowel functions lead to malnutrition and impaired physical and mental development, especially in children. Malnutrition, which is observed due to a lack of micronutrients, causes infections. The development of infection causes malnutrition. This entire cycle increases the burden of acute diarrheal (Okhuysen, 2010).

#### 2.6.1.4 Enteroinvasive *E. coli* (EIEC)

EIEC strains cause inflammatory lesions of the intestinal mucosa and submucosa very similar to those produced by *Shigella.* These microorganisms have the same ability to multiply and reproduce inside epithelial cells. Clinically, however, EIEC is associated with watery diarrhoea which is seen much more frequently than *Shigella* dysentery. EIEC O antigens can cross-react with *Shigella* O antigens. The ailment starts with severe abdominal spasms, weakness, watery stools, dysuria, and fever. This condition rarely gets worse and turns into loose stools that contain blood or mucus. Faecal leukocytes seen in shigellosis can also be seen in the mucus of a person infected with EIEC (Ekici, 2019).

#### 2.6.1.5 Diffusely-adherent *E. coli* (DAEC)

Cultures of Hep2 or HeLa cells are called DAEC because of their diffuse adhesion characteristics (Taddei, 2003). DAEC serotypes are known to cause chronic diarrhoea in children aged 1 to 5 years (Poole, 2007). They induce degeneration of the intestinal epithelium by binding to proteins that accelerate degradation. Mild diarrhoea with white blood cells in the stool is a sign of infection (Taddei, 2003).

#### 2.6.1.6 Enterohemorrhagic (Shiga toxin-producing) *E. coli* (EHEC/STEC)

It is considered a major threat in foodborne illness (Poole, 2007). *E. coli* O157: H7 became the first of several strains known as Enterohaemorrhagic *E. coli* or EHEC, which can produce one or more Shiga toxins (stx1 and stx2) (also called verocytotoxins and formerly known as

verocytotoxins) (Manil, 2005). Strains of STEC can survive in fresh ground beef and on green leafy vegetables, and it is well known that the reservoirs of VTEC are ruminants, which continuously release bacteria into the environment, thus contaminating food and water. (Poole, 2007).

EHEC O157:H7 is phenotypically distinct from *E. coli* in that they express slowly or do not ferment sorbitol and have no glucuronidase activity. (Feng 2000; Itoh, 1998).

**Table 2.2:** Summary of incidence and epidemiology of *E. coli* serotype.

| **Pathogenic *E. coli*** | **Site of infection** | **Associated disease** | **Incidence** | **Target population** | **Significant transmission route** |
|---|---|---|---|---|---|
| ETEC | Small intestine | Traveller's diarrhoea, chronic childhood diarrhoea (in developing countries) | 16 U.S. outbreaks (1996-2003); Prevalence 1.4% in Patients with diarrhoea 79,420 cases of traveller's diarrhoea each year (in the USA) | International travellers and children in developing countries | Food (raw produce, street vendors) and water |
| EPEC | Small intestine | Infant diarrhoea | Hundreds of thousands of deaths worldwide | Children in developing countries | Water, infant formula |

| | | | | | |
|---|---|---|---|---|---|
| EHEC | Large intestine | Haemorrhagic Colitis (HC), Haemolytic uremic syndrome (HUS) | 110,000 cases and 61 deaths annually in the USA | All ages | Food (Beef produce), person-to-person, water, animals |
| EIEC | Large Intestine | Dysentery | Low in developed countries | Children in developing countries | Water(rare), person-to-person |
| EAEC | Intestine | Watery diarrhoea with or without blood in the stool, acute and chronic | Developed and developing countries | Children and adults' travellers | Food, water, person-to-person |

(Adapted from Gerba,2004).

## 2.7 PATHOGENESIS OF STEC *E. COLI* O157: H7

The structurally related and biologically similar Shiga toxin family includes Stx1 which is essentially identical to the toxin of *Shigella dysenteriae* with only a single amino acid difference, and Stx2 which has less than 60% amino acid homology to Stx1(Beddoe 2010; Caprioli 2005). Shiga toxins are similar to the heat labile enterotoxins of ETEC in the AB5 toxin family. Globotriaosylceramides (Gb3s) on the surface of human intestinal mucosa and renal epithelial cells, leading to endotoxins where the A subunit is activated, causing cell death. (Croxen, 2010). Among the Stx2 variants, Stx2c is most frequently isolated from HUS patients, but Stx2e and Stx2f are mainly isolated from pigs and birds, and rarely from humans. (Caprioli, 2005).

### 2.7.1 Mode of transmission

STECs is the only common zoonotic pathogen of *E. coli*, with more than 380 different OH serotypes isolated from individuals with gastrointestinal disease, many of which were recovered in animals (Karmali, 2010).

STEC is transmitted through faecal routes, including consumption of contaminated food or water, direct contact with infected animals, or person-to-person contact. It is estimated that 85% of STEC infections are transmitted through food. (Meng and Schroeder 2007; Gyles 2007).

### 2.7.2 Symptoms of disease

Infection with STEC may cause no clinical symptoms (asymptomatic infection) or diarrhoea (which may progress to bloody diarrhoea), abdominal cramps, vomiting, and fever (Meng and Schroeder 2007; WHO 2011). Onset is 3-8 days (average 3-4 days). Most patients recover within 10 days of the first onset of symptoms. (Meng and Schroeder 2007; WHO 2011). In some cases, patients develop haemolytic uremic syndrome (HUS). HUS is characterized by haemolytic anaemia, thrombocytopenia (thrombocytopenia) and renal failure. Children are more susceptible, 15.3% of children younger than 5 years of age develop HUS after STEC infection (Gould *et al.,* 2009).

### 2.7.3 Detection

Laboratory validation of STEC infection can be obtained by isolation and confirmatory testing using culture medium, immunoassays, cytotoxicity assays, and PCR (Smith 1993; Gould 2009). The screening of O157 was based on the strain's inability to utilize sorbitol rapidly, leading to the use of sorbitol MacConkey agar (SMAC) as a differential medium (Smith 1993; Gould 2009). Potential STEC strains should be evaluated by serotype and Shiga Toxin Detection Methods (Gould, 2009). However, PCR provides a fast and reliable method for the detection of STEC, which, similar to immunoassays, can be used directly with stool samples as well as isolated colonies and, depending on the primer used, it is possible to distinguish between stx1 and stx2 and detect the eae and enterohemolysin (hly) genes (Gould, 2009).

## 2.8 TREATMENT OF STEC

Patients with diarrhoea caused by diarrheal *E. coli*, in particular STEC and *E. coli* expressing Intin and HEC- haemolysin, were treated with bovine colostrum, rich in antibodies against Shiga toxin and enteric *E. coli* haemolysin, in a duplicate study blind, placebo-controlled (Huppertz *et al.,* 1999). Anti-lipopolysaccharide (LPS) antibody from *E. coli* also has

potential for therapeutic use because of its effect on blocking the adhesion of STEC to the human intestinal epithelial cell line (Henle 407) (Paton,1998).

In conclusion, Pathogenic *E. coli* are associated with fresh produce and the ingesting of contaminated food may cause related illness. Therefore, it is paramount to investigate the occurrence and prevalence of those pathogens in fresh produce street vended in Ogun state and Lagos state.

# CHAPTER THREE

## 3.0 METHODOLOGY

### 3.1 MATERIALS

Materials used include: Measuring cylinder, Cotton wool, aluminium foil, Petri-dishes, beakers, Conical flasks, Hockey stick, 70% ethanol, Spirit lamp, Eppendorf tubes, micro pipette (with their tips), test tubes (with their racks), PCR tubes.

### 3.2 REAGENTS AND EQUIPMENTS

Autoclave, weighing balance, distiller, wash bottles, water bath (set at 50°C and 100°C), incubator (set at 37°C), Bunsen burner, oven, inoculating loop, gel electrophoresis tanks, UV Transilluminator, Ice machine, Centrifuge machine, Thermal cycler, Refrigerator, Freezer and Vortex mixture.

### 3.3 COLLECTION AND HANDLING OF SAMPLE

In this study, seven types of Fresh produce were selected for the isolation of pathogenic *E. coli* of which 9 samples of Lettuce, Papaya, Cucumber, Carrot, Pineapple, watermelon and Papaya were selected randomly. A total of Sixty-three (63) Samples. The Fresh produce were purchased from different open and well populated markets, like Ibafo, Magboro, Magodo, Jakande and Yaba, one stall was picked randomly and this was done for once in a week for subsequently 6 weeks. For each stall in each market, the three different samples were purchased. Samples of each type of the produce were bought from the different stalls in the market were packed separately to avoid cross contamination in a sterile polythene bag and transported to the laboratory for further analysis the same day.

**Table 3.1:** Fresh produce samples and their corresponding location

| **Fresh produce sample** | **Location** | | |
|---|---|---|---|
| Lettuce (n=9) | Jakande (L) 3 | Ibafo (O) 3 | Magboro (O) 3 |
| Cabbage (n=9) | Yaba (L) 3 | Ibafo (O) 3 | Magboro (O) 3 |
| Pine apple (n=9) | Magodo (L) 3 | Ibafo (O) 3 | Magboro (O) 3 |
| Water melon (n=9) | Magodo 3 | Yaba (O) 3 | Magboro (O) 3 |
| Cucumber (n=9) | Jakande(L) 3 | Ibafo (O) 3 | Magboro (O) 3 |
| Carrot (n=9) | Yaba (L) 3 | Ibafo (O) 3 | Magboro (O) 3 |
| Pawpaw (n=9) | Yaba (L) 3 | Ibafo (O) 3 | Magboro (O) 3 |

Key notes: (L)- Lagos state. (O)- Ogun state.

### 3.4 MEDIA PREPARATION

Media used includes Nutrient agar, for total bacterial count (TBC), MacConkey agar, Buffered Peptone Water, Brain Hear Infusion Broth and Sorbitol MacConkey (SMAC) were prepared according to manufacturer's instructions.

#### 3.4.1 MacConkey Agar

The agar medium most widely used for isolating *E. coli* is MacConkey agar. It is a selective media which contains lactose as sugar, peptone, sodium chloride, bile salt; inhibits the growth of other gram positive Enterobacteriaceae, crystal violet and neutral red is also used to understand the nature of fermentation. Gram-negative organisms from clinical, dairy; food, water, pharmaceutical and industrial sources samples should be detected and isolated using MacConkey agar. Enzymatic digest of gelatin, enzymatic digest of casein, and enzymatic digest of animal tissue are the nitrogen and vitamin sources in MacConkey Agar.

**Preparation:**

1. The prepared medium was suspended in the appropriate volume of distilled water i.e. 48.5g of MacConkey in 1 litre of distilled water based on manufacturer's instructions in a conical

flask and mixed thoroughly. The conical flask is then closed in cotton wool that is wrapped in aluminium foil.

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

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

NOTE: The medium is neutral red in colour.

### 3.4.2 Nutrient Agar

Nutrient Agar is a general purpose, nutrient medium used for the culturing of microbes supporting growth of a wide range of non-fastidious organisms. Nutrient agar is popular because it can grow a variety of types of bacteria and fungi, and contains many nutrients needed for the bacterial growth.

**Preparation:**

1.The dehydrated medium was dissolved in the appropriate volume of distilled water i.e., 28g of Nutrient agar in 1 litre of distilled water based on manufacturer's instructions in a conical flask and mixed thoroughly. The conical flask is then closed in cotton wool that is wrapped in aluminium foil.

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

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

### 3.4.3 Sorbitol MacConkey Agar (SMAC)

(SMAC) Sorbitol MacConkey agar is a selective and differential media used for detecting sorbitol non-fermenting *Escherichia coli* O157: H7. It was prepared according to the manufacturer's instruction for isolation and detection of *E. coli* O157:H7. SMAC is reddish-purple in colour after preparation.

**Preparation:**

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

2. The mixture was stirred for a while using the magnetic stirrer hot plate to dissolve the powder completely and was then sterilized by autoclaving at 121°C for 15mins.

3. The medium was then allowed to cool and poured aseptically into sterile petri dishes and left to solidify.

### 3.4.4 Brain Heart Infusion (BHI) Broth

BHI is recommended for the cultivation of fastidious pathogenic microorganisms.

1. The dehydrated medium was dissolved in the appropriate volume of distilled water based on manufacturer's instructions in a conical flask and mixed. The conical flask was then corked with a foil cork.
2. The mixture was stirred for a while using the magnetic stirrer to completely dissolve the powder.
3. 5ml of the media was then dispensed into various test tubes and covered with foil cork and was then sterilized by autoclaving at $121^0$C for 15minutes.

## 3.5 ISOLATION OF E. COLI FROM SAMPLES

### 3.5.1 Primary Enrichment

All the samples that were purchased were directly transferred to the laboratory for direct processing. Firstly, the work bench was sterilised using 70% ethanol and the selected fruits and vegetable were sliced into smaller pieces by using a sterilized knife and chopping board and they were weighed for 25g aseptically. Then the Fresh Produce samples were transferred into stomacher bags containing 225ml of 1% Buffered Peptone Water (BPW) to homogenize individually for one sample as pre-enrichment for *Escherichia coli.*

### 3.5.2 Secondary Enrichment

This was performed for the detection of *E. coli*, the overnight incubated pre-enrichment media of BPW was used to inoculate the secondary enrichment media. Each sample was processed individually and poured into a conical flask. Then 1ml of each enriched broth was subjected into 9ml of 0.1% Buffered Peptone water (BPW) into sterile test tubes Consequently of four-fold dilution series from $10^{-1}$ to $10^{-4}$ dilutions were spread plated in MacConkey Agar, Nutrient Agar and Sorbitol MacConkey Agar and the plates were incubated at $37^0$C for 24hours. The enriched broth after been stomached for two minutes the BPW cultures were incubated at 37 ºC for 24 hours.

### 3.5.3 Inoculation

Zero-point one millilitre (0.1 ml) aliquots of the serially- diluted samples were introduced into plates on NA, MAC and SMAC agar plates and spread uniformly with a sterile hockey stick. Inoculated plates were incubated at 37ºC for 24hours in an inverted position for the growth of bacteria colonies. Characteristics of colonies which appeared were counted as total aerobic heterotrophic count for the agars. while the colonies on nutrient agar plates were sub cultured repeatedly on fresh sterile petri dish for pure isolates which were later transferred to slant bottles for biochemical test.

### 3.5.4 Sub culturing

The isolates from nutrient, SMAC agar and MacConkey agar were sub-cultured onto another NA plates aseptically using the same medium and pure isolate were obtained. Pure isolates were further sub-cultured into universal slant bottles containing nutrient agar slants.

### 3.5.5 Cryopreservation of isolate

A loopful of each pure isolate (two white, two pink) from the incubated nutrient agar was inoculated into 5ml of BHI broth each in a test tube and incubated at 37 ºC for 18- 24 hours. After incubating, 750µl of the inoculum was added into a sterile Eppendorf tube containing 750µl of sterile 20% glycerol (duplicated) which acts as a cryoprotectant and was stored in a freezer at -4 ºC.

## 3.6 BIOCHEMICAL IDENTIFICATION

Biochemical tests were performed with pink and white producing isolates according to the methods described in Bergey's Manual of bacteriology. The following biochemical test was carried out for the identification of bacterial isolates. Catalase coagulase, urease, citrate, motility, indole, oxidase, Methyl red (MR), Voges Proskauer (VG) sugar fermentation such as glucose, maltose, sucrose mannitol, lactose, fructose starch hydrolysis.

### 3.6.1 Gram staining

On a clean, grease free slide, a smear of suspension was created with a loopful of the isolate. It was air dried and heat fixed. Drops of crystal Violet was poured and kept for about 30 seconds and rinsed with water. It was then flooded with gram's iodine for 1 minute and rinsed with water. 95% alcohol was added for about 10-20 seconds and rinsed with water. Safranin was added for about 1 minute and rinsed with water. It was then air dried and Observed under Microscope.

### 3.6.2 Catalase Test

Place the microscope slide in the Petri dish. Keep the lid of the Petri dish available. Using a sterile inoculation loop or wooden applicator, collect a small amount of the isolate and place it on a microscope slide. Be careful not to collect any agar. Using an eyedropper, place 1 drop of 3% H2O2 on the spacer on the microscope slide. Don't mix up. Immediately cover the petri dish with a lid to limit the aerosol and pay attention to the immediate formation of bubbles (O2 + water = bubbles). Observing bubble formation on a dark background can improve readability.

### 3.6.3 Oxidase Test

Soak a small piece of filter paper in 1% Kovács Oxidase Reagent and let it dry. Use a ring to pick a well-separated colony from a fresh bacterial plate (18-24-hours incubation) and rub it on the treated filter paper. Observe the colour change. When the colour changes to deep purple within 5 to 10 seconds, the microorganism is oxidase positive. When the colour turns purple within 60 to 90 seconds, the microorganisms delay the oxidase positive. If the colour does not change or lasts longer than 2 minutes, the microorganism is oxidase negative.

### 3.6.4 Indole Test

Inoculate a tube of tryptone broth with a small amount of pure culture. Incubate at 37 ° C for 24 to 48 hours. To test indole production, add 5 drops of Kovác's reagent directly to the test tube. Within seconds of adding Reagent, a pink to red colour ("cherry red ring") formed in the reagent layer on top of the medium, indicating a positive indole test. If the culture is indole negative, the reagent layer will remain yellow or slightly cloudy.

## 3.7 MOLECULAR IDENTIFICATION

### 3.7.1 DNA Extraction

Isolates from the same sample were pooled (1ml of BHI was added to a cryotube and autoclaved). Then 50ul of each isolate E1-E4 was added into the cryotube to activate the cell). The next day, the pooled isolates were centrifuged at 5,000G for 3minutes and the supernatant was discarded, 1ml of sterile distilled water was added to the Eppendorf tube, for washing which is then vortexed and centrifuged again at 5,000G for 3 minutes the supernatant was discarded and the process was repeated.

Then 200ul of injection water was then pipetted into the Eppendorf tube containing the pellets, vortexed and then it was placed in the heating block to boil at 100$^{o}$C for 15minutes, it

was then placed in ice pack to cool for 5minutes. the Eppendorf tube containing the content was then centrifuged finally at 7,000G for 6minutes.

A Fresh 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 at -20$^{o}$C for further analysis.

### 3.7.2 Polymerase chain reaction (PCR)

The components of the PCR used for *E. coli* identification is shown in table 3.2 below. After preparing the PCR cocktail It was placed into the thermocycler. 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. negative control reactions was included. For negative controls template DNA was replaced with sterile water. The PCR products were confirmed by electrophoresis and visualized under UV light with a Gel Documentation system.

**Table 3.2:** PCR reaction components used for 16s rRNA amplification

| **No.** | **Component** | **Initial concentration** | **Final concentration** | **Volume/rxn** |
|---|---|---|---|---|
| 1 | Master mix | 5x | 1x | 2µl |
| 2 | 16sf | 20µm | 0.25µm | 0.125µl |
| 3 | 16sr | 20µm | 0.25µm | 0.125µl |
| 4 | DNA | | | 2µl |
| 5 | $dH_2O$ | | | 5.75µl |
| 6 | Total | | | 10µl |

**Table 3.3:** Protocol for Thermal cycler

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

**MULTIPLEX PCR FOR STEC**

**Table 3.4:** Primers used

| No. | Reagents | Initial Concentration | Final Concentration | Volume/Reaction |
|---|---|---|---|---|
| 1 | Master mix | 5x | 1x | 5µl |
| 2 | Stx1F | 20µm | 0.25µm | 0.3125µl |
| 3 | Stx1R | 20µm | 0.25µm | 0.3125µl |

| | | | | |
|---|---|---|---|---|
| 4 | Stx2F | 20µm | 0.25µm | 0.3125µl |
| 5 | Stx2R | 20µm | 0.25µm | 0.3125µl |
| 6 | eaeAF | 20µm | 0.25µm | 0.3125µl |
| 7 | eaeAR | 20µm | 0.25µm | 0.3125µl |
| 8 | $dH_20$ | | | 15.125µl |
| 9 | DNA | | | 3µl |
| 10 | Total | | | 25µl |

**Table 3.5:** Components used for Multiplex PCR

| Gene | Oligonucleotide sequence (5'-3) | Size | Reference |
|---|---|---|---|
| *Stx1* | GAAGAGTCCGTGGG<br>ATTACGAGCGATGCAGCTATTAATAA | 130bp | Paton and Paton, 1999 |
| *Stx2* | ACCGTTTTTCAGATTTTGACACATA<br>TACACAGGAGCAGTTTCAGACAGT | 298bp | Paton and Paton, 1999 |
| *EaeA* | GTGGCGAATACTGGCGAGACT<br>CCCCATTCTTTTTCACCGTCG | 890bp | Paton and Paton, 1999<br>Paton and Paton, 1999 |

### 3.7.3 Agarose Preparation

The agarose was prepared using dry agarose powder, 1.8% of the agarose gel is prepared. 1.8g of the agarose powder was then dissolved in 100ml of TAE buffer the mixture was then microwave and stirred every 5seconds so that it can mix homogenously, 3µl of ethidium bromide was added to the mixture using a micropipette it is swirled and left to cool but not solidify. Then 50ml of the content in the bottle is then transferred into the gel tank 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. 3µl of the loading buffer (Molecular weight Marker) was pipetted into the first tank. 4µl of the PCR products are then pipetted into each well that was formed after removing the comb. The tank is connected to the power pack and left to run till it gets to one-third of the gel and then it is turned off and the gel is viewed under the UV transilluminator.

### 3.7.4 Precautions

I. Aseptic techniques were observed at every stage of work.
II. Personal protective technique was also observed, such as wearing of covered shoe, nose cover, gloves, lab coat, etc.
III. Ensured that the inoculating loop cooled before picking the organism when sub-culturing in order not to kill organism of interest.
IV. Ensured that the petri-dish was incubated inverted.
V. Ensured proper timing, most especially during autoclaving.

# CHAPTER FOUR

# 4.0 RESULTS AND DISCUSSION

## 4.1 RESULTS

### 4.1.1 Isolation of *E. coli* from Samples

This present study was aimed to isolate and characterise *E. coli* from fresh produce samples gotten from Lagos and Ogun state were reported. All samples had pink (O157) and white (non O157), raised, circular and smooth colonies on SMAC and MAC Which indicates the presence of E. coli in the sample. Enrichment, selective plating, biochemical tests and molecular based methods have been applied for isolation and identification of *E. coli* from collected samples. Then the confirmed isolates were genotypically and phenotypically characterized.

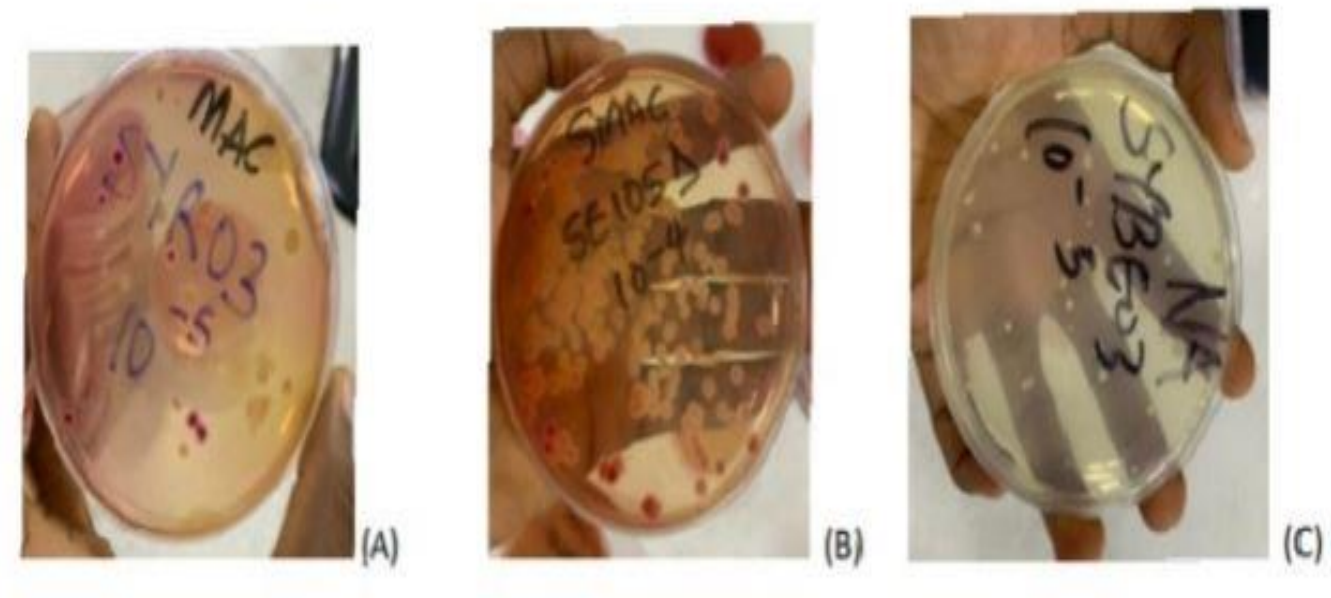



**Figure 4.1:** Image of viable colony counts on agar plates

(A)MacConkey agar plate showing typical white pink lactose fermenting colonies of *E. coli* from carrot. (B) Sorbitol-MacConkey agar plate showing typical white pink lactose fermenting colonies of *E. coli from* Watermelon (C) Nutrient Agar showing total viable counts white

**Table 4.1** Colony characteristics of *E. coli* on plates

| **Colony characteristics** | **Nutrient Agar** | **Sorbitol-MacConkey Agar** | **MacConkey Agar** |
|---|---|---|---|
| Size | Small | Big | Moderate(1-2mm) |
| Shape | Round | Round | Round |
| Elevation | Raised | Raised | Raised |
| Margin | Not Entire | Entire | Entire |
| Colour | White | White and Pink | Pink and white |
| Opacity | Transparent | Opaque | Opaque |

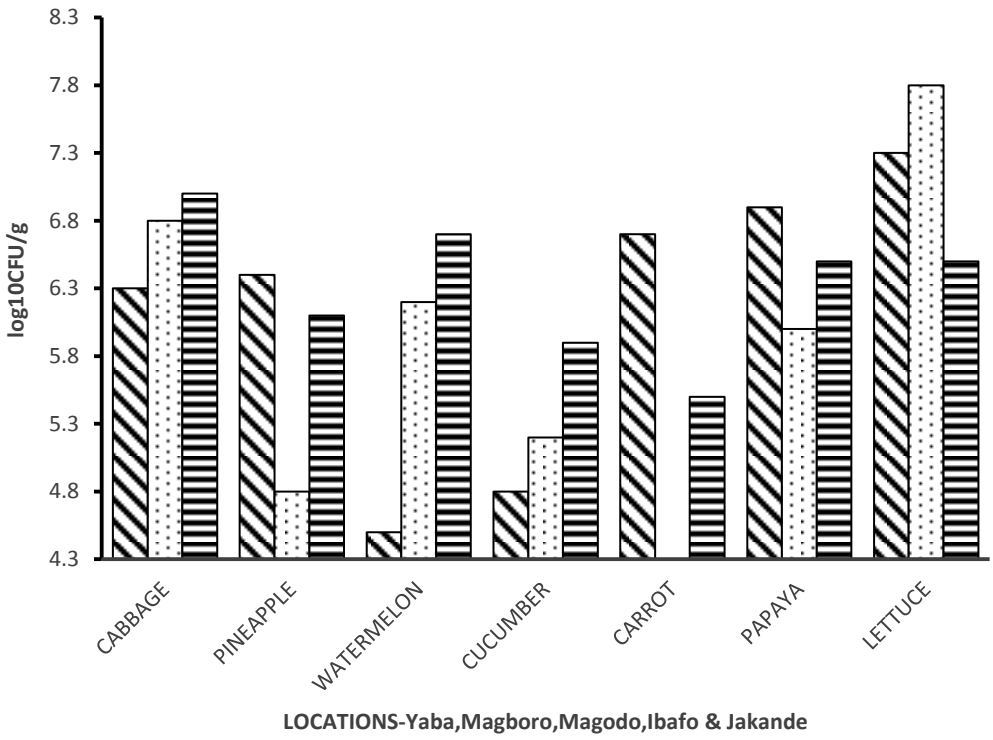


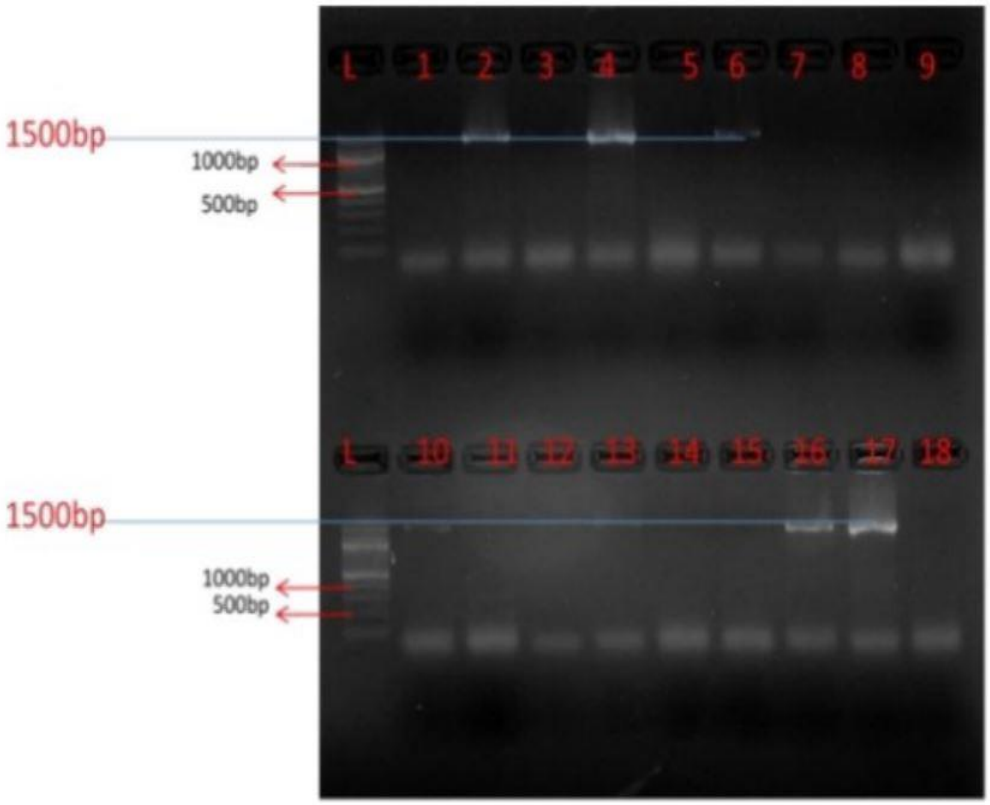

**Figure 4.2:** Total viable count of fresh produce from various locations in Ogun State and Lagos State

## 4.2 BIOCHEMICAL IDENTIFICATION

Isolates which gave pink colours were subjected to different biochemical tests. Isolates showed pattern of biochemical reactions typical for E. coli as mentioned in (Table 4.2) were selected for further identification and confirmation.

## 4.3 GENOTYPIC CHARACTERIZATIONS OF THE ISOLATES

Template DNA was prepared from cellular DNA of biochemically identified isolates by boiling method and 5 µl of template DNA was subjected to PCR for the detection of E. coli specific virulent genes stx1 and stx2 using specific primers. Isolates that gave bands of expected size were suspected to carry these genes in their chromosomes.




(I) Agarose gel electrophoresis of PCR amplicon for of 16S RDNA *E. coli 38-58* no: 51-53

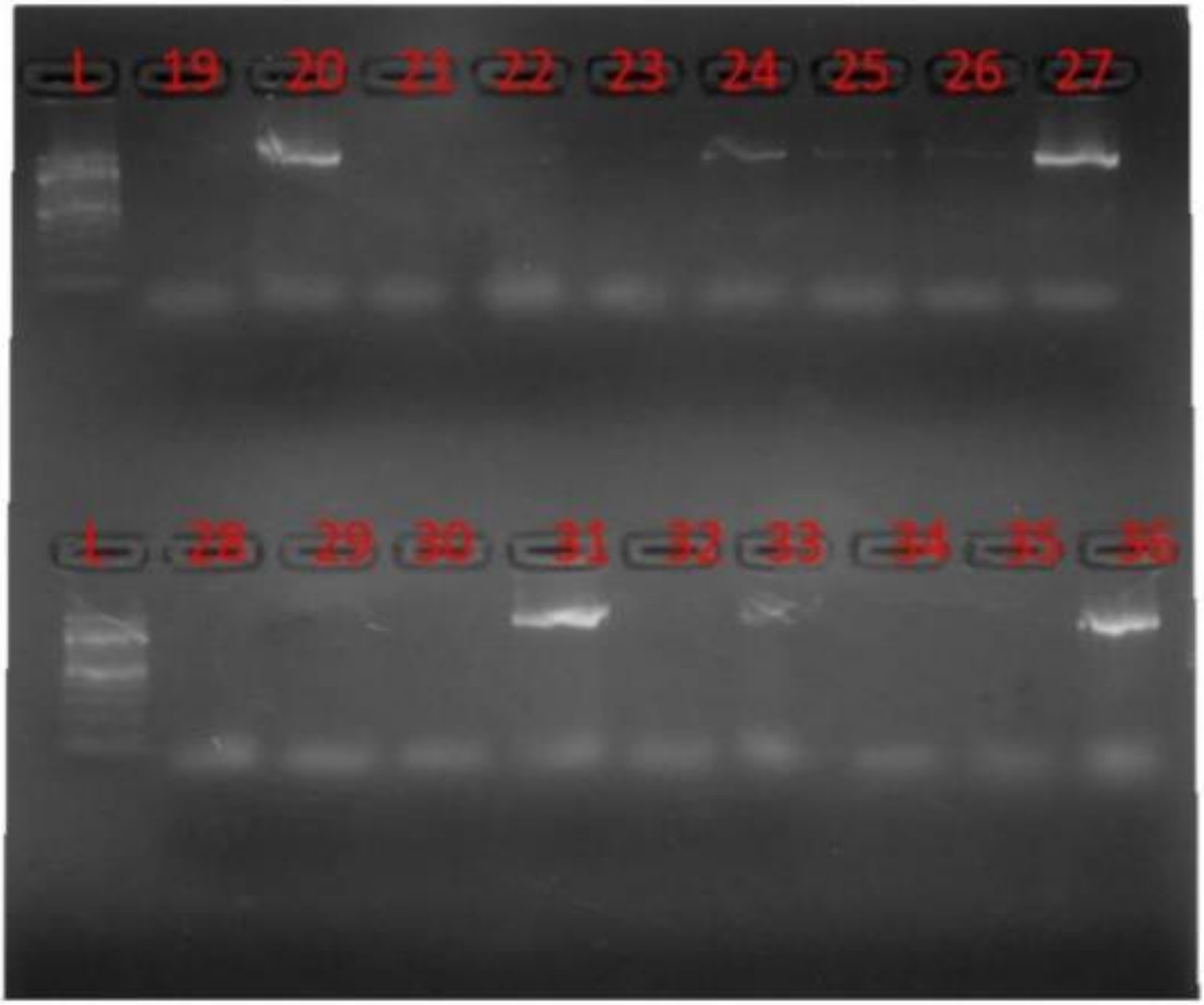



**Figure 4.3:** (II) Agarose Gel electrophoresis of PCR amplicon for (16s rRNA amplification) of 21 samples.

**For 16S rRNA PCR**

Results from 21 specimens showed a high concordance of >50% for 16S rRNA gene PCR and routine bacterial culture, indicating that the diagnostic performance of PCR for acute bacterial infections is comparable to that of bacterial culture, which identify *Escherichia coli* as shown in Fig 4.2.

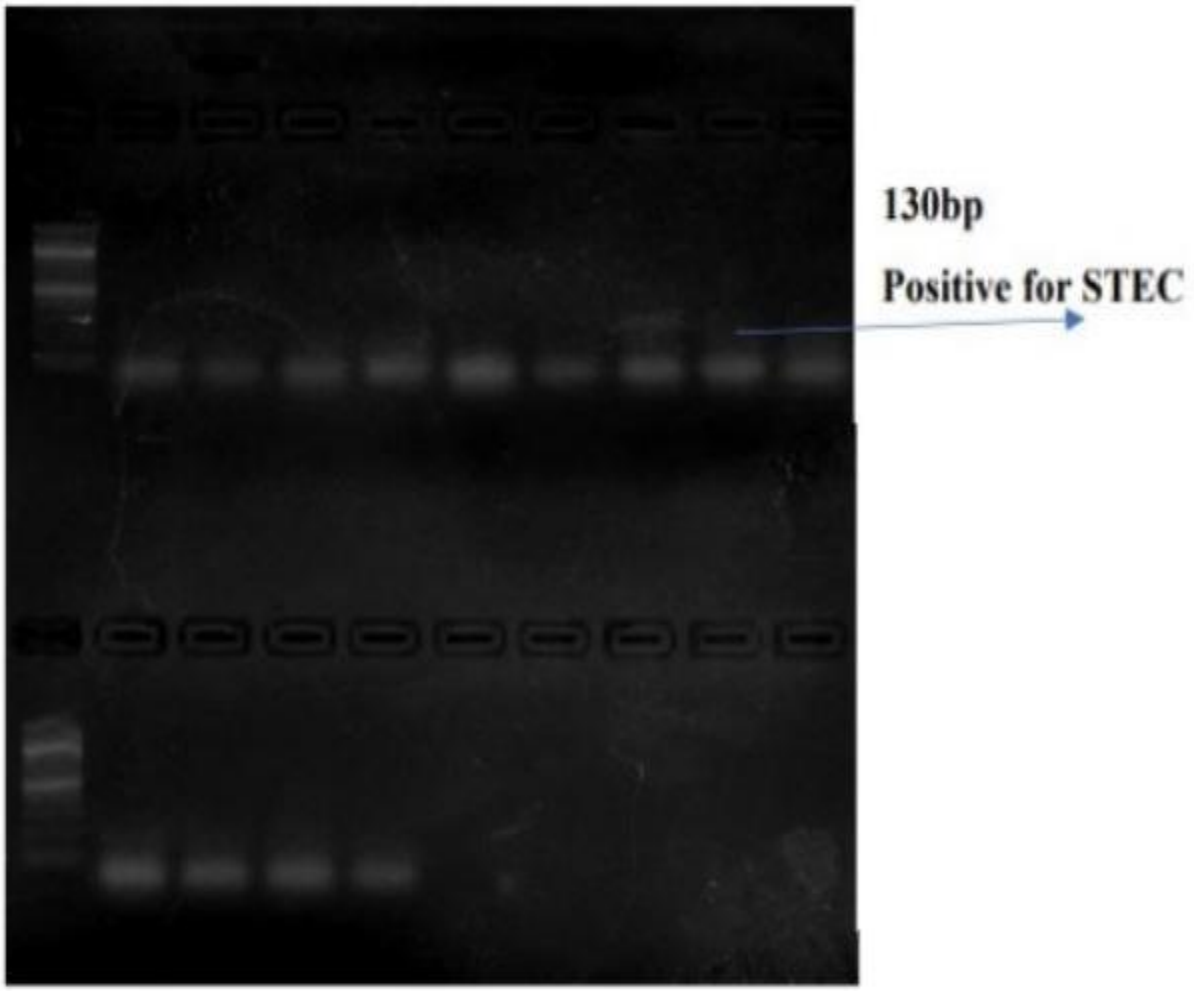



**Figure 4.4:** Agarose gel electrophoresis for Multiplex PCR for the 21 samples

**For PCR multiplex**

21 presumptive STEC isolates were randomly picked for further examination by multiplex PCR for the detection of *stx*$_1$, and/or *stx*$_2$ and eae genes. The result revealed that one isolate from watermelon purchased from Magboro (1SGW) contained *stx*$_1$ gene, which makes it shiga toxin-producing diarrheagenic *E. coli* positive. Agarose gel electrophoresis of PCR products for *eaeA*, *stx*$_1$, *stx*$_2$ as shown in **figure 4.4**

## 4.4 DISCUSSION

*Escherichia coli* is a significant bacterium polluting faeces in food. In the 63 samples taken in Nigeria from Lagos and Ogun countries with substantial populations, the proportion of *E. coli* was more than 80%. Prior research in many countries have shown a broad variety of data on *E. coli* prevalence in plants. Also, because presence of virulence genes presumptively determines the presence of bacteria in the case of STEC, the strain must be isolated to confirm the presence of stx genes in addition to relevant virulence factors in the same live cell while excluding the presence of free DNA or free stx phages in the enrichment culture (EFSA, 2013). Because of the difficulty in establishing culture medium that specifically or differentially allow the development of STEC (EFSA, 2013), this step may delay identification, but it is necessary because molecular approaches may overstate the true STEC contamination.

The level of contamination observed in this study is in line with what has been previously found in other countries such as in Brazil, *E. Coli* has been found (Oliveira *et al.,* 2012) in 53%, in Turkey in 53% and in Pakistan, in 48% of the vegetables sold on the street (Razzaq, Farzana, Mahmood & Murtaza, 2014); and in the Philippines in the 16.7% of the samples sold out on the open-air market. *E. Coli* has been found in 53% of the vegetable samples sold in Pakistan. 2.8 % *E. coli* was found in lettuce from Canada, in contrary, a comparable amount was discovered (Wood *et al.,* 2015). and it was only identified in 2% of lettuce in Switzerland (Althaus *et al.,* 2012).

*E. coli* was recovered from 80% of the samples in this investigation, with concentrations varying significantly not just among produce varieties but also between sellers. The contamination was dominated by STEC. The eae gene is present in one of 21 watermelon samples taken in Magboro Local Street Market in Ogun State, indicating that the eae-positive fruit was contaminated with STEC, an emerging diarrheagenic pathogen in developed and developing countries (Contreras *et al.,* 2010; Estrada-Garcia *et al.,* 2009).

International commission on Microbiological specification for food (Shaltout, 2017). recognized some degree of contamination in food but stated that ready-to-eat food products with plate counts between 0-103 were acceptable, tolerable within 104 –105, and 108 and higher were inacceptable, based on the results obtained, the level of contamination cannot be tolerable on the basis of a plate counting level. The degree of product contamination can indicate the level of exposure and the processes of sales market handling. It is found that

most of these vendor establishments are quite near the main road which exposes the fruit to dust and other contaminants (Chukwu *et al.*, 2010). In a recent Egypt research, 7.9% (34/432) of vegetable samples reported higher incidence of *E. coli* O157:H7 vs 1.4%.

The isolates were mostly serotypes seldom linked in Nigeria with human illnesses. Some of them, however, have been connected with patients in different nations with serotypes and pathotypes. Genetic analysis of 1 *E. coli* virulence gene isolation contains genes encoding additional toxin, Adhesin and type-III-related components that can contribute to its virulence, was aided by the simultaneous detection using a Multiplex PCR method.

The results showed that a broad range of non-bladed fresh food produced in Nigerian contexts is a source of STECs. The sanitary market conditions and the inadequate handling by sellers contribute to the high microbial burden in ready-to-eat fruit (Muinde and Kuria, 2005).Use of the same water bucket to wash all fruit if ever washed, and the use of the same equipment, such as cutting knives, may increase the microbial load (Khali *et al.*,2006). During peeling, slicing, trimming and marketing pathogens may penetrate the product's inner surfaces (Oku, 2020). risk of pollution is increased since the local streets markets are ready to be sold.

This research indicates that in Nigeria fruit and vegetables may cause STEC related foodborne diseases and other diarrheagenic *E. coli* infections in the community. Therefore, fresh produce is potential source of transmitting these potentially harmful pathogens. As watermelon sampled from Magboro contained $stx_1$ gene, which Shiga toxin-producing diarrheagenic *E. coli*. Contamination of fresh produce can occur at any critical points in the supply chain. Pre-harvest contamination with STEC excretion by soil, water and manure is considered to be the most significant fertilizer on the field (Franz and van 2008). Therefore, during transport or by food handlers, fresh product may primarily be contaminated or cross-infected. Their presence in fresh produce indicates that these strains are potentially food-borne viruses and that we must monitor these items carefully and stress additional training efforts on the part of producers, distributors, business owners and consumers.

# CHAPTER FIVE

## 5.0 CONCLUSION AND RECOMMENDATION

### 5.1 CONCLUSION

The present study was aimed at isolating *E. coli* from Fresh-cut Produce by using selective enrichment broth and selective media and Molecular characterizations of the isolates. One isolate was found to carry stx1 gene, which is responsible for haemolytic colitis and haemorrhagic uremic syndrome. Based on the risk of the presence of STEC from fresh produce.

### 5.2 RECOMMENDATION

From this study area people need to wash fruits and vegetables thoroughly before consumption. Contamination of *E. coli* might be due to irrigation of these vegetables with contaminated water or lack hygiene while transport of the fresh produce. Therefore, Prevention strategies may include Good Agricultural Practices, Good Manufacturing Practices, the use of chlorinated water in washing the fresh produce. Also Supervised handling of raw fruits. All these may help to reduce the risk of transmission of this food borne pathogen.

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