PATHOGENIC *E. COLI* SEROTYPES ISOLATED FROM STREET VENDED FRESH PRODUCE IN IBAFO, OGUN STATE.

By

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A PROJECT SUBMITTED TO THE DEPARTMENT OF BIOTECHNOLOGY COLLEGE OF BASIC AND APPLIED SCIENCES, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF DEGREE OF BACHELOR OF SCIENCE IN BIOTECHNOLOGY.

SEPTEMBER, 2021

DECLARATION

I hereby 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.

…….

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CERTIFICATION

This is to certify that this project report titled **"PATHOGENIC *E. COLI* SEROTYPES ISOLATED FROM STREET VENDED FRESH PRODUCE"** was carried out by ORIAKU, Joy, with matriculation number 17010104002 of Biotechnology in the Department of Biological Sciences, in partial fulfillment of the requirement for the award of Bachelor of Science (B.Sc.) degree in Biotechnology.

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DEDICATION

This project is dedicated to God almighty, the giver of wisdom and understanding, for this love and strength.

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All thanks to Almighty God who gave me an understanding heart and strength all through my stay in school making it a very easy task for me.

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ABSTRACT

Fresh produce, mostly fruits and vegetables, is an important part of the human diet since it contains important vitamins, minerals, and fiber. Increased consumption of fresh food, on the other hand, has contributed to an increase in the frequency of disease outbreaks around the world. Because fresh produce is frequently consumed raw, it is more likely to be contaminated with pathogens such as *E. coli* 0157:H7, which can cause food poisoning and, in extreme circumstances, renal failure and death, especially in children. Fresh produce is purchased in both closed stores and roadside open kiosks in impoverished nations like Nigeria, and most of the time it is not treated hygienically. The prevalence of Shiga toxin-producing *E. coli* in various fresh produce (Lettuce, Watermelon, Pineapple, Cucumber, Cabbage, Carrot, and Pawpaw) from various roadside fruit vendors in different locations around the South-west region of Nigeria, specifically Lagos and Ogun state, is reported in this study. Using SMAC and MAC plates, a total of 64 samples were tested for *E. coli*. STEC virulence genes (stx1, stx2, and eae) were identified using multiplex PCR in selected isolates (n = 21). The presumptive STEC total viable count (TVC) in the samples ranged from 4.5 to 7.8 log10cfu/g. Ibafo lettuce had the greatest total viable count of 7.8 log10cfu/g. The existence of STEC was confirmed by the presence of the stx1 virulence gene detected only in Magboro Watermelon, as determined by Multiplex PCR. Foodborne disorders such as haemorrhagic colitis (HC) or haemolytic uremic syndrome (HUS) are all risks associated with the presence of STEC. The presence of STEC in fresh produce is of public health concerns, Therefore, awareness in terms of hygiene needs to be made for the consumers.

Key words: STEC, fresh produce, food borne disease, virulence gene, HUS.

CHAPTER ONE

Introduction

1.1 Background To The Study

Foods that are readily and easily available on the roadside for immediate consumption in nearly all places across the world are known as street vended foods. These meals are enjoyed and relished all around the world, not only for their distinct taste and flavor, but also because they are readily available. Most of these street vended food are ready-to-eat (RTE) foods and convenient choice for consumers. Fresh fruits and vegetables consumption has risen dramatically in recent years not only for its nutritional and functional contributions but because of convenience (Kljujev *et al*, 2012; Luna-Guevara *et al*., 2019).

In numerous nations, microbial contamination of fresh produce has resulted in foodborne disease epidemics. When raw manure is used as a fertilizer, there is a significant danger of bacterial contamination pathogenic *E. coli* can be found in salads, fruits, and vegetables. *E. coli* contamination of fresh foods can come from water and soil (Chekabab *et al*, 2013; Solomon *et al*, 2002). In most developing countries, such as Nigeria, inadequate control and inspection of *E. coli* levels in foods sold on the market, including salad vegetables and fruits, is usually lacking. This might result in damage, particularly if individuals are unaware of the situation. Several authors have speculated about the prevalence of pathogenic E. coli in fresh fruits and vegetables (Moses et al, 2016).

In Africa, *Escherichia coli* O157:H7 is one of the most common causes of foodborne illness, Leafy vegetables (lettuce and cabbage) have been linked to many significant outbreaks of *E. coli* O157:H7-related foodborne illness. Contamination of raw product with pathogenic microbes can happen at any stage along the food supply chain, from the farm to the market

(Barak *et al*., 2002). Most *E. coli* strains are harmless, but some serotypes can cause serious food poisoning in humans. The innocuous strains are part of the normal gut flora and can help their hosts by generating vitamin K2 and preventing harmful bacteria from establishing themselves in the intestine (Howard, 2002). Some strains, such as Shiga toxin-producing *E. coli* (STEC), can cause severe foodborne disease. It is typically transferred to people by the ingestion of infected foods such as raw or undercooked grounded beef products, raw milk, and contaminated raw vegetables and sprouts. Currently, fruit juice and vegetable salad are widely consumed by most people (wide age group) as meal and dessert. Fruit juices and vegetable salad are also consumed at cafeterias and restaurants. Even if no quantifiable scientific data is available at hand, it is possible to observe that raw fruit juice and vegetable salad consumption has been increasing. It is important to note that the impact of foodborne infections is felt not only by the sick individual, but also by the economy. On one hand, there are expenditures associated with the sick individual, such as medical treatment and missed work or education. Foodborne illness may be the cause of fresh produce contamination by pathogenic bacteria, viruses, and protozoa (Alam *et al*,. 2015; Iturriaga *et al*,.2007; Harris *et al*,. 2003; Bilek and Turantas 2013).It can also happen when you're chopping, washing, packaging, soaking, or making meals (Castro-Rosas *et al*,. 2012). Because of lack of food safety starting from field to fork, microbiological contaminants including human pathogens like E. coli are expected to exist at a heavy load. This is due to unhygienic practices such as poor preparation method, treatment and handling of equipment.

Shiga-toxins are toxins generated by STEC that are comparable to *Shigella dysenteriae* toxins. Temperatures ranging from 7 °C to 50 °C are suitable for STEC growth, with 37 °C being the optimum temperature. Some *E. coli* strains, have developed into pathogenic *E. coli* after acquiring particular virulence factors via mobile genetic components including

plasmids, transposons, bacteriophages, and pathogenicity islands (Sethabutr *et al*,. 1993), EPEC, ETEC, EHEC, EAEC, and EIEC are among the pathogenic strains of *E. coli*.

As a result, a study on the identification and degree of pathogenic *E. coli* contamination from salad vegetables and fruits sold by roadside vendors in Ogun, Nigeria, is required.

1.2 Statement Of The Problem

Foodborne illness is due to fresh produce contamination by pathogenic *E. coli*. It can also occur during slicing, washing, packing, soaking, and food preparation. Food safety standards for fresh produce in Nigeria is lacking, therefore, the level of microbiological contaminants of street vended fresh produce sold in selected areas of Lagos and Ogun state needed to be investigated.

1.3 Aim And Objectives Of The Study

The purpose of this study was to assess the bacteriological quality and safety of widely consumed raw fruit and vegetable salads offered by roadside vendors in Lagos and Ogun state. To examine the bacteriological load and also to isolate and identify the dominant bacteria from paw-paw, cucumber, lettuce, pineapple, watermelon and carrot. To isolate, identify and characterize food-borne pathogenic *E. coli* using molecular techniques.

1.4 Significance Of Study

This research will aid vendors in gaining a better knowledge of the microbiological quality and safety of fresh produce, therefore reducing foodborne illness outbreaks connected to the consumption of street-vended fruits and vegetable salads.

CHAPTER TWO

Literature Review

2.1 Overview Of Fresh Produce

Fresh produce means fruits and vegetables that have not been processed in any form. They are a vital element of a healthy diet. They are high in essential vitamins, minerals, fiber, and other nutrients for good health. Indeed, studies have shown that eating a well-balanced diet rich in fruits and vegetables may help to avoid cancer and other chronic diseases.

2.1.1 Key Nutrients In Fruits And Vegetables

Vitamin A (carotenoids) - can be found in cabbage, lettuce, watermelon, carrots and pineapple. It helps for healthy eyes and skin and also protects from infection.

Vitamin C- can be gotten from cabbage, Pineapple and watermelon. It helps for Healthy teeth and gums; helps heal cuts and wounds

Vitamin K- can be gotten from Cabbage, cucumbers. It helps with Synthesis of pro-coagulant factors, osteoporosis

2.2 Fresh Produce And Their Nutritional Value

Cabbage (*Brassica oleracea)*

Cabbage is a fat-free vegetable. Cabbage has a high potassium, folate, and vitamin K content. Cabbage also contains calcium, iron, vitamin A, and vitamin C, among other nutrients.

Cucumber (*Cucurbitaceae*)

Cucumbers contain a lot of water, which can help you stay hydrated. Additionally, the fiber increase they provide helps you maintain your regularity and avoid constipation.Vitamin K aids in the formation of blood clots and the maintenance of bone health. Vitamin A helps with eyesight, the immune system, and reproduction, among other things. It also ensures that vital organs such as the heart, lungs, and kidneys function properly.

Pawpaw (*Carica papaya*)

Pawpaw is higher in vitamin C, Niacin, Calcium and potassium (Snake *et al,*. 1997). Pawpaw is the only fruit that has all of the necessary amino acids and is high in antioxidants. It contains Carotenoids (β-carotene, cryptoxanthin), energy about 163KJ, Carbohydrates, Sugars, Vitamin A and C, Dietary fibre, and minerals such as Calcium, potassium and sodium (Aravind *et al,*. 2013). They also contain significant amounts of riboflavin, niacin, calcium, phosphorus, and zinc.

Carrot (*Daucus carota*)

Carrots can provide in the human diet significant amounts of vitamin A, due to the high bioavailability of carrot carotenoids (Van *et al,*.2000). Other phenols found in them include chlorogenic, caffeic, and p-hydroxybenzoic acids, as well as a variety of cinnamic acid derivatives. Carrots are high in dietary fiber and the trace mineral molybdenum, which assists in artipose tissue and carbohydrate metabolism and is necessary for iron absorption. Magnesium and manganese are also abundant in this fruit. Magnesium is required for bone formation, protein synthesis, B vitamin activation, nerve and muscle relaxation, blood coagulation, and energy generation (Guerrera *et al,*. 2009). Insulin secretion and function also require magnesium (Bartlett *et al,*. 2008; Kim *et al,*. 2010). Manganese is helpful in carbohydrate metabolism, in co-ordination with enzymes in the body (Dias 2012; Dias 2012). Manganese is used by the body as a cofactor for the antioxidant enzyme, superoxide dismutase. Magnesium and potassium in carrots help in functioning of muscles. A deficiency in vitamin A can cause eye's photoreceptors to deteriorate, which leads to vision problems. βcarotene (the carotenoid with the most provitamin A activity) in carrots helps to protect vision, especially night vision and also provides protection against macular degeneration and development of senile cataract, the leading cause of blindness in aged people (Dias 2012; Dias 2012).

Pineapple (*Ananas comosus*)

Pineapple is a tropical fruit having exceptional juiciness, vibrant tropical flavor and immense health benefits. Pineapple contains considerable amount of calcium, potassium, carbohydrates, vitamin C, water, crude fiber and different minerals that is good for the digestive system and helps in maintaining ideal body weight and balanced nutrition. Fresh pineapples are high in bromelain, which acts as an anti-inflammatory, decreasing swelling in inflammatory diseases such as acute sinusitis, sore throat, arthritis, and gout. Food products such as jam, jelly, and pickles are made (Hossain *et al*., 2015).Fresh pineapple contains minerals such as Calcium, Chlorine, Potassium, Phosphorus and Sodium (Dull 1971).

Watermelon (*Citrullus lanatus*)

Watermelon contains more than 91% water and up to 7% of carbohydrates. It is a rich source of lycopene and citrulline. Watermelon also contains antioxidants. These substances can help remove trusted Source molecules known as free radicals, or reactive species, from the body. The body produces free radicals during natural processes, such as metabolism. They can also develop through smoking, air pollution, stress, and other environmental pressures. If too many free radicals stay in the body, oxidative stress can occur. This can result in cell damage and may lead to a range of diseases, such as cancer and heart disease. The body can remove some free radicals naturally, but dietary antioxidants support this process.

Lettuce (*Lactuca sativa*)

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 pro-vitamin A compound, beta-carotene, found in darker green lettuces, such as Romaine (Health Jade 2017).

2.3 Microbial Contamination Of Fresh Produce In Farm-To-Fork

Microbial contamination of fresh food can occur at various points throughout the farm-to-fork chain. Contamination can occur during fresh produce cultivation, harvest,

preparation/washing, distribution networks and transit to stores, and even at the last stage in the consumers' kitchen (Matthews, 2013). Fruits and vegetables can get contaminated at any step in the production process. Contamination sources may be divided into two categories: preharvest and postharvest sources of contamination (Gil *et al*,. 2015).In terms of preharvest contamination sources, studies have shown that the soil in which fruits and vegetables are grown, as well as water used for irrigation, water used to apply insecticides and fungicides, dust, improperly composted manure, feces, and finally human interaction with these vegetables at various points during the production period, can all be sources. Dry season farming and the accompanying microbial contamination of fresh fruits and vegetables in impoverished areas of the world must be studied (Amoah 2014).During the dry season, irrigation methods of farming are widely used throughout Africa.

Furthermore, the use of poultry manure and other incomplete compost to crops might result in contamination with enteric bacteria in feces. Pathogens such as E. coli O157: H7, Listeria monocytogenes, and Salmonella spp. have also been isolated from animal feces, including poultry and cattle (Buck *et al*,. 2003; Soderqvist 2017). It was confirmed few years ago that E. coli O157: H7 may be transferred to lettuce by soil and irrigation water, persist throughout the plant's life cycle, and be transmitted to humans who consume the crop (Bhunia 2018). Postharvest contamination causes include feces, harvesting equipment, human handling, insects, wild and domestic animals, transportation techniques, processing equipment dust, and rinse water (Gil *et al*,. 2015). The use of pond and river water to wash fresh food increases the danger of contamination since these waters are likely to contain harmful microorganisms (Uyttendaele *et al*,. 2015). These same individuals touch the veggies, and the majority of them are already infected with the diseases that act as fomites, and the vegetables are generally stored in contaminated areas. In Africa, vegetables are usually cleaned in conveniently accessible water sources such as rivers and ponds near the manufacturing or

selling location (Acheampong 2015).Containers used to wash vegetables by farmers and fruit and vegetable merchants are seldom cleansed after use, and even when they are, the water is reused for multiple cycles, allowing for microbial cross-contamination with newly washed ones since they are placed in the same water as the initial cycle (Acheampong 2015).

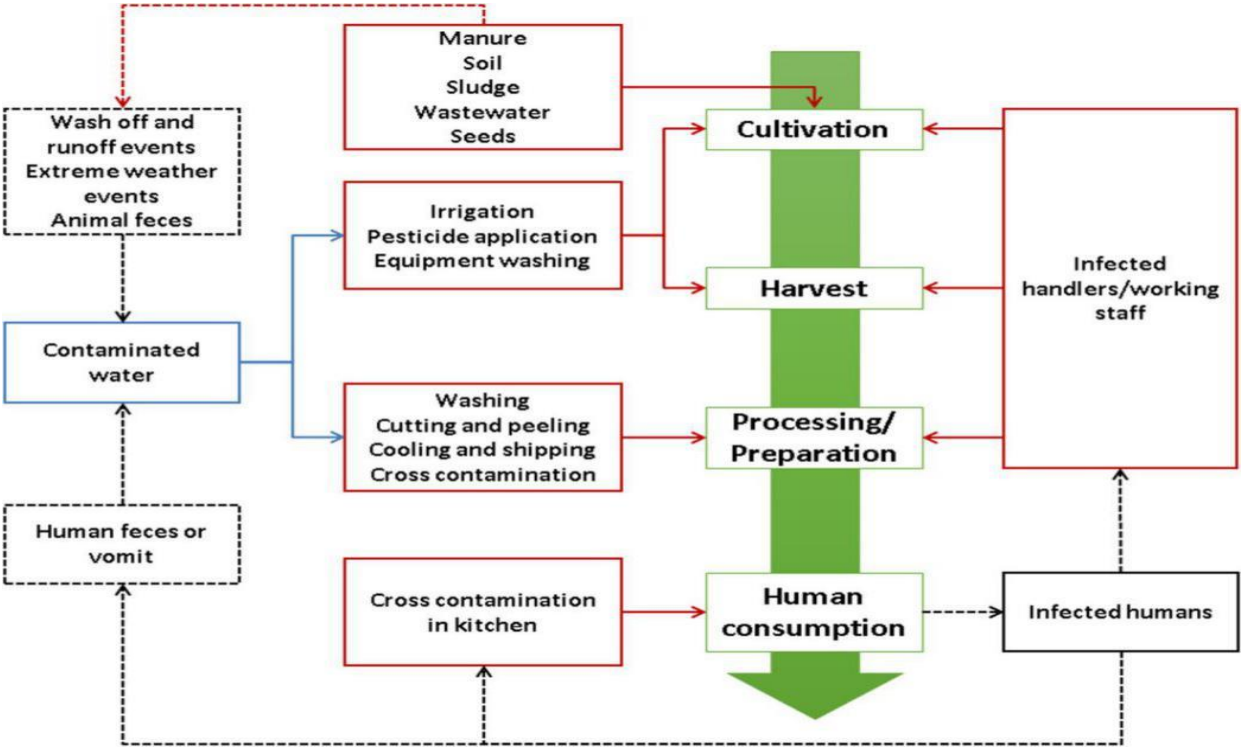

Figure 2. 1 Microbial contamination of RTE foodstuffs in the farm-to-fork chain(Machado-Moreira et al 2019).

2.4 Routes And Chain Of Contamination

Pathogenic microorganisms that often infect fresh fruits and vegetables may be linked back to human pathogens, poultry, and other domestic animals' germs, including soil microbes, and other activities that facilitate microbial colonization of fresh fruit and vegetable surfaces. (Figure 2).

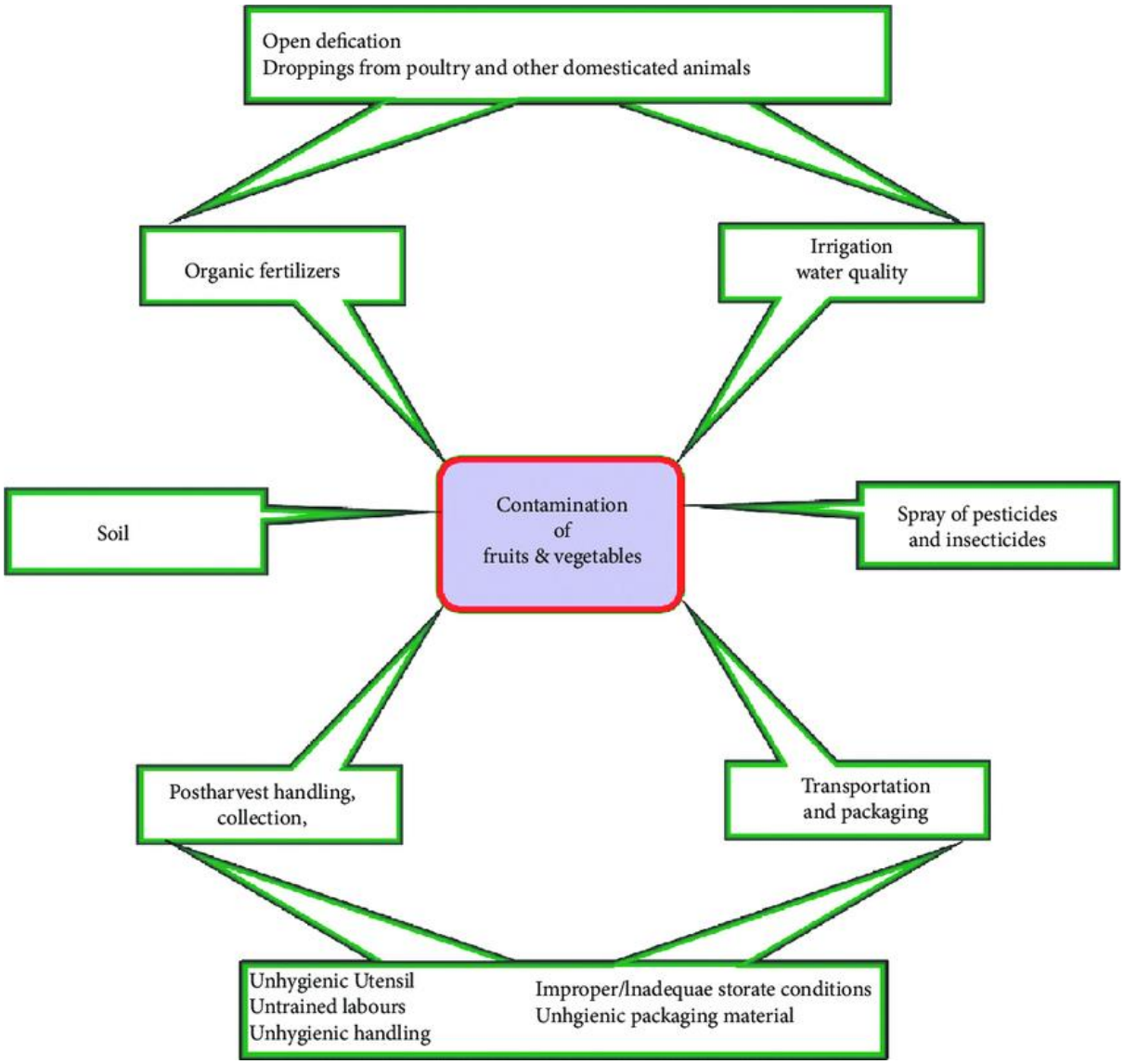

Figure 2. 2 Factors contributing to the contamination of fruits and vegetables.

Contamination can and has occurred:

I. During production or harvest

II. At the retail, food service

III. In the home kitchen.

IV. During processing,

Fruits and vegetables may be contaminated at any point in time during the production chain. Sources of contamination can be grouped into two broader groups, namely, preharvest and postharvest sources of contamination (Gil *et al*,. 2015).

PREHARVEST

According to studies, potential sources include the soil in which fruits and vegetables are grown, as well as water used for irrigation, water used to apply insecticides and fungicides, improperly composted manure, dust, feces, and finally human interaction with these vegetables at various points during the production period. Dry season farming and microbial contamination of fresh fruits and vegetables in disadvantaged parts of the world must be researched (Amoah 2014). The use of irrigation as an agricultural method during the dry season is prevalent throughout Africa. However, in Sub-Saharan Africa, many vegetable crops are produced fresh using irrigation. They usually irrigate their crops with contaminated water or wastewater (Amoah 2014). As a result, some bacteria may contaminate the plants and, as a result, the customers. Similarly, many farmers use fungicides and weedicides with the same water, which can lead to coliform contamination (Amoah 2014). Pathogens found in animal feces include included *E. coli* O157: H7, Listeria monocytogenes, and Salmonella spp (Buck et al,. 2003; Soderqvist 2017). According to studies released a few years ago, *E. coli* O157: H7 can be transferred to lettuce through the soil and also irrigation water and can persist throughout the plant's life cycle as well as to humans who consume the crops (Bhunia

2018). This problem may be prevented by identifying and efficiently managing its origins, not only during farming but also during postharvest processing, such as changing conditions, cleaning contaminated streams before usage, and so on.

POST HARVEST

Feces, human handling, insects, processing equipment, wild and domestic animals, harvesting equipment, transportation techniques, dust, and rinse water are all postharvest contamination sources (Gil *et al*,. 2015). The use of pond and river water to wash fresh food increases the danger of contamination since these waters are likely to contain harmful microorganisms (Uyttendaele *et al*,. 2015).These are the same people that handle the vegetables, and the bulk of them are already sick with illnesses that serve as fomites, and the commodities are stored in filthy settings. Vegetables in Africa are washed utilizing easily available water sources such as rivers and ponds near the production or selling area (Acheampong 2015). Containers used to wash vegetables by farmers and fruit and vegetable merchants are seldom cleaned after use, and even when they are, the water is reused for several cycles, allowing germs to cross-contaminate newly washed produce since they are placed in the same water as the initial cycle (Acheampong 2015). To ensure safety and avoid microbiological contamination, washing containers should be cleaned both before and after use.

2.5 Pathogens Of Fresh Produce

Bacterial pathogens such as Shigella, *E. coli* O157:H7, and Salmonella; the virus Hepatitis A; and parasites Cyclospora and Cryptosporidium have almost become household words in the food industry. These microorganisms are physiologically diverse but they do share some common features. They all originate from enteric habitats, which means they are found in

human or animal feces. These infections can be excreted by humans and animals without causing disease. Illnesses can be serious, especially in vulnerable people (young children, elderly, and immunosuppressed). Finally, infective doses (the number of organisms that cause sickness) might be extremely low. With a low infective dosage, the bacterium just needs to contaminate the meal. Temperature abuse and microbe proliferation, while increasing the probability of disease, are not usually required.

2.6 The Genus *E. coli*

The genus *Escherichia*, which was named after the German pediatrician Theodor Escherich, consists of facultative anaerobic Gram-negative bacilli that belong to the family Enterobacteriaceae (Ewing and Edward 1986).

The Austrian doctor Theodor Escherich isolated *Escherichia coli* from a child's excrement in 1885, under the name "Bacterium coli commune" (Escherich, 1885). *Escherichia coli (E. coli)* is a Gram-negative, facultative anaerobic rod-shaped bacterium of the genus Escherichia that is often found in the lower intestine of warm-blooded organisms (endotherms) (Singleton 1999). Although the majority of *E. coli* strains are safe in the colon and seldom cause disease in healthy people, a few pathogenic strains can cause intestinal and extra intestinal disorders in both healthy and immunocompromised individuals. *E. coli* has evolved the potential to cause diseases in people. *E. coli* (Nataro and Kaper 1987) strains engaged in diarrheal illnesses are one of the most significant of the numerous etiological agents of diarrhea, where strains have developed by acquiring a specific set of traits that have effectively survived in the host through horizontal gene transfer (Nataro and Kaper 1987; Croxen *et al*,. 2013,Nataro and Kaper 1998).*E. coli* strains that cause gastroenteritis in humans are classified into six types: enterohemorrhagic (EHEC), enteroaggregative (EAEC), enteropathogenic (EPEC), enterotoxigenic (ETEC), enteroinvasive (EIEC), and diffuse adherent (DAEC).

2.7 *E. coli* Serotypes

2.7.1 Enteropathogenic *E. coli* (EPEC)

Enteropathogenic *E. coli* (EPEC) is a kind of bacterium classified as an attaching and effacing (A/E) pathogen because of its capacity to produce discrete lesions on the surfaces of intestinal epithelial cells (IECs). EPEC was the first pathotype of diarrheagenic E. coli to be discovered. The name "EPEC" was used in 1955 (Neter et al.1955) to designate a group of E. coli strains that were epidemiologically linked to a series of infantile diarrhea epidemics in the 1940s and 1950s (Robins 1987; Bray 1945). EPEC strains are currently categorized based on pathogenic features rather than serotype (Nataro and Kaper 1998). EPEC strains are currently categorized based on pathogenic features rather than serotype (Nataro and Kaper 1998).EPEC strains are currently characterized as those *E. coli* strains that may cause diarrhea, have a histopathology on the intestinal epithelium known as the attaching and effacing (AE) lesion, and lack the capacity to generate Shiga toxins and heat-labile (LT) or heat-stable (ST) enterotoxins. Over the years, advances in methods that allow for a better knowledge of the virulence mechanisms and genome among EPEC strains have resulted in the sub-classification of EPEC into typical EPEC (tEPEC) and atypical EPEC (aEPEC). (Nataro and Kaper 2004, Trabulsi et al. 2002). Typical EPEC strains that cause human infectious diarrhea include a large virulence plasmid known as the EPEC adherence factor (EAF) plasmid (pEAF), which encodes the type IV fimbriae known as the bundle-forming pilus (BFP), whereas aEPEC do not have this plasmid. (Nataro and Kaper 1998; Trabulsi et al. 2002). EPEC1 and EPEC2 are the two primary lineages of tEPEC strains (rskov et al, 1990; Whittam and McGraw 1996). EPEC1 has widely distributed serotypes such as O55:H6 and O119:H6, whereas EPEC2 contains serotypes with a more limited distribution such as O111:H2 and O114:H2.In turn, aEPEC belong to a large diversity of classical and non-classical serotypes (Trabulsi *et al,*. 20002; Gomes *et al,*. 1996; Hernandes *et al,*. 2009). It has

been found that 35% of the aEPEC strains also belong to the tEPEC lineages (Hazen *et al*,. 2013). As a result, it has been proposed that at least some aEPEC strains may have evolved from tEPEC bacteria that lost pEAF in the host or in the environment. (Hazen et al., 2013; Levine et al., 1985; Vieira et al., 2001).

2.7.2 Enterotoxigenic *E. coli* (ETEC)

ETEC is a diverse pathotype that is a major cause of traveler's diarrhea and endemic in most underdeveloped countries (Dull 1971). ETEC strains are characterized by the production of colonization factors (CFs) and at least one of two enterotoxins: LT and ST. Enterotoxigenic E. coli (ETEC) are estimated to cause 600 million cases of human diarrhea and 800,000 deaths worldwide principally in children under the age of 5 (World Health Organization, 1999).ETEC cause watery diarrhea that can be mild in nature or in some instances can be a severe, cholera-like illness where rapid dehydration can be life threatening. ETEC toxins are released in the terminal small intestine, where ETEC adhere through the production of a complex and varied collection of surface proteins known as "colonization factors" (Gaastra and Svennerholm, 1996).

2.7.3 Diffuse adherent *E. coli* (DAEC).

DAEC comprises of strains that adhere to epithelial cells in a diffused distribution.The epidemiology and pathogenesis of the diffusely adherent *E. coli* (DAEC) are not well understood. DAEC may cause diarrhea in very young children (less than a year old; Scaletsky et al. 2002)

2.7.4 Enterohemorrhagic *E. coli* (EHEC)

EHEC/STEC are a well-known category of foodborne pathogens that are found all over the world. These organisms are both capable of causing A/E lesions, however enterohemorrhagic *E. coli* (EHEC) is distinguished from EPEC by the presence of Shiga-like toxins and the clinical presentation of their disease. The capacity to generate one or more Shiga toxin (Stx)

family cytotoxins is the major virulence feature of this pathogroup of E. coli (Melton 2014).EHEC/STEC causes a wide range of infections, from mild and practically undetectable diarrhea to more serious symptoms such as hemorrhagic colitis (HC) and the development of a potentially fatal condition known as hemolytic uremic syndrome (HUS).EHEC is a subgroup of STEC. EHEC, like EPEC, is LEE positive and produces A/E lesions. However, the term EHEC has also been used in the literature to identify LEE-negative STEC strains that have produced HC and HUS, such as serotypes O91:H21, O104:H4, and O113:H21. HUS involves a triad of hemolytic anemia, thrombocytopenia and renal failure. The most common EHEC serogroup is O157:H7 and has been the subject of many studies, especially for molecular mechanisms of pathogenesis. The *E. coli* O157:H7 serotype was the first to be connected to HC and HUS cases in the early 1980s, and it has since been responsible for countless outbreaks and sporadic instances of severe illness all over the world, making it the prototype of this dangerous group of bacteria (Kaper 2014). Although it is generally known that hundreds of additional *E. coli* serotypes can have the stx genes, epidemiological investigations conducted across the world have shown that only a subset of them is capable of causing human illness. Among those most often associated with human illnesses are serogroups O26, O45, O103, O111, O121, and O145 (Gould et al,. 2013).The majority of *E. coli* strains inhabit the gastrointestinal tracts of people and animals as part of the natural flora. Some strains, however, have developed into pathogenic *E. coli* by gaining virulence factors via plasmids, bacteriophages, transposons, and/or pathogenicity islands. These pathogenic *E. coli* strains can be classified according to their serogroups, pathogenicity mechanisms, clinical signs, or virulence characteristics (Kaper et al,.2004; Nataro and Kaper et al,. 1998).Among them, Enterohemorrhagic *E. coli* (EHEC) is a pathogenic strain of *E. coli* that produces Shiga toxins (Stxs) that causes hemorrhagic colitis (HC) and the potentially fatal sequelae hemolytic uremic syndrome (HUS) in humans. Several EHEC serotypes, including

O26:H11, O91:H21, O111:H8, O157: NM, and O157:H7, are often linked with human diseases (Paton and Paton, 1999).EHEC serotype O157:H7 was originally identified in 1982 as a human pathogen related with epidemics of bloody diarrhea in Oregon and Michigan, USA (Riley et al, 1983; Wells et al, 1983) and is also connected to sporadic instances of HUS in 1983 (Karmali et al,. 1983). Since then, many outbreaks associated with EHEC have been reported and *E. coli* O157:H7 has become one of the most important foodborne pathogens

Escherichia coli is spread by the feces–oral pathway. Because of its flexibility, it is often found in water, soil, and food.

2.8 Pathogenesis of STEC

Many of the clinical characteristics of STEC infection, as well as the life-threatening consequences, require the production of a strong STX. Pathogenesis, on the other hand, is a multistep process involving a complicated interplay between a variety of bacterial and host components. STEC taken orally (typically in very low initial doses) must first withstand the harsh environment of the stomach before competing with other gut bacteria for intestinal colonization. Because STEC organisms persist in the gut, STX generated in the lumen must be absorbed by the intestinal epithelium before being translocated to the bloodstream. This allows transport to particular toxin receptors on target cell surfaces, resulting in both local and systemic effects. Another important step in EHEC/STEC pathogenesis is the capacity to attach to intestinal epithelial cells. The presence of the chromosomal pathogenicity island LEE (Stevens and Frankel 2014), which is also present in EPEC pathotype isolates, is frequent. As a result, it is apparent that EHEC/STEC pathogenesis involves a multistep process that includes, in addition to the synthesis of Stx toxins and the AE lesion. It is also worth noting that, as a pathogen of the human gastrointestinal tract, EHEC/ability STEC's to monitor nutrients in the gut milieu and translate this information to sense the host physiological state in order to program the expression of its virulence markers plays a critical

role in infection development(Pacheco and Sperandio 2015). The capacity of EHEC/STEC to attach, colonize, and develop biofilm on food and other surfaces may be a major source and/or vehicle of transmission. Furthermore, biofilm may shield microorganisms from harmful environmental conditions.

CHAPTER THREE

Materials And Methods

3.1 Sampling

In this study, 7 fresh products were selected for the isolation of pathogenic E. coli, among which 9 samples of lettuce, papaya, cucumber, carrot, pineapple, watermelon and papaya were randomly selected. A total of sixty-three (63) samples. Fresh produce is bought in different open and densely populated markets, such as Ibafo, Magboro, Magodo, Jakande and Yaba. A position is selected at random, once a week, for the next 6 weeks. For each stall in each market, three different samples were purchased. Samples of each product are purchased at different market stalls, individually packaged to avoid cross-contamination, and shipped to the laboratory on the same day for further analysis.

Table 3. 1 Fresh Produce Sampled and Their 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.

3.2 Reagents And Equipment

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

3.3 Media Preparation

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

3.3.1 Mac-Conkey Agar

Mac-Conkey Agar is an agar medium widely used for *E. coli* isolation. It is a selective medium that contains lactose in the form of sugars, peptones, sodium chloride, and bile salts and inhibits the development of other Gram-positive bacteria. To understand the nature of fermentation, Enterobacteriaceae, crystal violet, and neutral red are also utilized.

Preparation;

1. The prepared medium was suspended in the right amount of distilled water i.e. 48.5g of MacConkey in 1 liter of distilled water based on manufacturer's instructions in a conical flask and mixed thoroughly. The conical flask is then closed in cotton wool that is wrapped in aluminum foil.
2. The mixture was heated for a short time to thoroughly dissolve the powder before being autoclaved at 121°C for 15 minutes. Autoclaving is done to sterilize the medium.

3. After cooling to 45-50°C, the agar was placed aseptically onto sterile petri dishes and allowed to harden.

3.3.2 Nutrient Agar

Nutritional Agar is a nutrient medium that may be used to grow a wide range of non-fastidious organisms. Nutrient agar is frequently used because it can support the development of a wide variety of bacteria and fungi and contains various nutrients necessary for bacterial growth.

Preparation;

1. In a conical flask, the dehydrated medium was dissolved in the required volume of distilled water, ie. 28g of Nutrient agar in 1 litre of distilled water, according to the manufacturer's instructions. The cotton wool is then wrapped in aluminum foil and used to seal the conical flask.
2. The mixture was heated for a short time to thoroughly dissolve the powder before being autoclaved at 121°C for 15 minutes.
3. The medium was then cooled to 45-50°C before being aseptically placed onto sterile petri dishes and allowed to harden.

3.3.3 Sorbitol MacConkey Agar (SMAC)

Sorbitol MacConkey agar (SMAC) is a selective and differential media for detecting sorbitol non-fermenting *E. coli* O157:H7. It was made according to the manufacturer's instructions for E. coli O157:H7 isolation and detection. After processing, SMAC has a reddish-purple color.

Preparation;

1. In a conical flask, the dehydrated medium was dissolved in the required volume of distilled water, 51.5g of SMAC in 1000 ml distilled water, according to the

manufacturer's specifications, and well mixed. A foil cork is then used to seal the conical flask.

2. The mixture was swirled for a while on the magnetic stirrer hot plate to thoroughly dissolve the powder, and then autoclaved at 121°C for 15 minutes to sterilize it.
3. The medium was then allowed to cool before being put onto sterile petri dishes and allowed to harden aseptically.

3.3.4 Brain Heart Infusion (BHI) Broth

BHI is recommended for the cultivation of fastidious pathogenic microorganisms.

1. In a conical flask, the dehydrated medium was dissolved in the required volume of distilled water according to the manufacturer's instructions and stirred. A foil cork was then used to seal the conical flask.
2. To thoroughly dissolve the powder, the mixture was agitated for a long time with a magnetic stirrer.
3. 5ml of the medium was then dispensed into different test tubes, capped with foil cork, and autoclaved at 1210C for 15 minutes to sterilize them.

3.4 Isolation of *E. coli* From Samples

3.4.1 Primary Enrichment

All purchased samples were sent directly to the laboratory for direct processing. The work bench was sterilized using 70% ethanol and the collected fruits and vegetables were sliced into smaller pieces by using a sterilized knife and chopping board. Each sample was weighed for 25g aseptically. The Fresh Produce samples were then transferred each into stomacher bags containing 225ml of 1% Buffered Peptone Water (BPW) to homogenize as primary pre-enrichment for *Escherichia coli*.

3.4.2 Secondary Enrichment

The enriched broth after being stomached for two minutes the BPW cultures were incubated at 37 ºC for 24 hours. This was done to detect *E. coli*, and the secondary enrichment media was inoculated with the BPW pre-enrichment media that had been incubated overnight. Each sample was processed and poured into a conical flask separately. 1 mL of each enriched broth was placed in sterile test tubes with 9 mL of 0.1% Buffered Peptone water (BPW) to obtain10^{-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 test tubes were labeled for easy identification.

3.4.3 Inoculation

0.1 ml aliquots of the serially- diluted samples 10^{-1} to 10^{-4}were introduced into plates on NA, MAC and SMAC agar plates and spread uniformly with a sterile hockey stick. For the development of bacteria colonies, inoculated plates were inverted and incubated at 370 C for 24 hours.

3.4.4 Plating

After labelling the agar plates accordingly, 0.1 ml from the appropriate dilutions (10^{-1}-10^{-4}), was plated onto SMAC Agar, MAC Agar, and Nutrient Agar for the isolation of enteropathogenic *E. coli*, and the Total viable count using the spread plate technique (the glass rod was dipped into alcohol and then flamed in the Bunsen burner before spreading so as to maintain aseptic conditions). The plates were incubated at 35^{O}C- 37^{O}C for 18- 24 hours and counted.

3.4.5 Sub culturing

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

physical characteristics. Pure isolates were produced by sub-culturing the isolates from Nutrient Agar, SMAC agar, and MacConkey agar onto another NA plate aseptically using the same medium.

3.4.6 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^{O}C for 18- 24 hour. Following incubation, 750ul of the inoculum was transferred to a sterile Eppendorf tube containing 750ul of sterile 20% glycerol (duplicated) as a cryoprotectant and stored at -4℃.

3.5 Biochemical Test

3.5.1 Gram staining

A smear of suspension was generated with a loopful of the isolate on a clean, grease-free slide. It was allowed to air dry before being heat fastened. Crystal Violet drops were poured and held for around 30 seconds before being washed with water. It was then flooded for 1 minute with gram's iodine and washed with water. After around 10-20 seconds, 95% alcohol was added and washed with water. After about a minute, safranin was added and washed with water. It was then allowed to air dry before being examined under a microscope.

3.5.2 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.

3.5.3 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 Molecular Identification

3.6.1 DNA Extraction

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

200ul of sterile nuclease free 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 to cool for 5 minutes, the content of the Eppendorf tube was then centrifuged finally at 7,000G for 6 minutes, a new set of Eppendorf tubes were labelled and the supernatant containing the extracted DNA was then transferred into the new Eppendorf tubes and they were placed in racks and stored in the freezer at -20^{o}C for further analysis.

3.6.2 Polymerase chain reaction (PCR)

The components of the PCR used to identify *E. coli* are listed in table 3.2 below. The PCR cocktail was then put in the thermocycler once it had been prepared. Initial denaturation was performed at 95°C for 5 minutes, followed by 35 cycles of 95°C for 2 minutes, 42°C for 30 s, and 72°C for 4 minutes, followed by a final elongation step at 72°C for 10 minutes. There were also negative control reactions provided. The template DNA was replaced with sterile water for the negative controls. Electrophoresis was used to validate the PCR products, and a Gel Documentation system was used to see them under UV light.

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 Thermalcycler \

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

Multiplex Pcr For STEC

Table 3. 4 Components used for Multiplex PCR

No.	Reagents	Initial concentration	Final concentration	Volume/rxn
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

Table 3. 5 Components used for multiplex PCR.

Gene	Oligonucleotide sequence (5'-3)	size	Reference
Stx1	GAAGAGTCCGTGGG ATTACGAGCGATGCAGCTATTAATAA	130bp	Paton and Paton, 1999
Stx2	ACCGTTTTTCAGATTTTGACACATA TACACAGGAGCAGTTTCAGACAGT	298bp	Paton and Paton, 1999
EaeA	GTGGCGAATACTGGCGAGACT CCCCATTCTTTTTCACCGTCG	890bp	Paton and Paton, 1999

3.6.3 Agarose Preparation

The agarose was made from dry agarose powder, and only 1.8% of the agarose gel was produced. 1.8g of agarose powder was dissolved in 100ml TAE buffer, which was then

microwaved and agitated every 5 seconds to ensure homogeneity. 3µl of ethidium bromide was then added to the mixture using a micropipette, swirled, and allowed to cool but not harden. The gel is then carefully removed and placed in an electrophoresis tank containing TAE buffer after 50ml of the contents in the bottle is poured into the gel tank cast with the combs in place. The loading buffer (Molecular weight Marker) was pipetted into the first tank in a volume of 3µl. After removing the comb, 4µl of PCR products are pipetted into each well that was created. The tank is connected to the power supply and allowed to run until it reaches one-third of the gel thickness, at which point it is switched off and the gel is examined under the UV trans-illuminator.

3.7 Precautions

I. At every stage of the process, aseptic procedures should be observed.

II. Personal protection equipment, such as covered shoes, a nose cover, gloves, and a lab coat, should also be observed.

III. Ensure that the inoculating loop cools before picking the organism when sub culturing in order not to kill organisms of interest.

IV. Ensure that the petri-dish was inverted during incubation.

V. Ensure proper timing, most especially during autoclaving.

CHAPTER FOUR

Results And Discussion

4.1 Result

This present study was aimed to isolate and characterize pathogenic *E. coli* from fresh produce samples obtained from Lagos and Ogun state. All samples had pink and white, raised, circular and smooth colonies on NA, SMAC and MAC respectively 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.

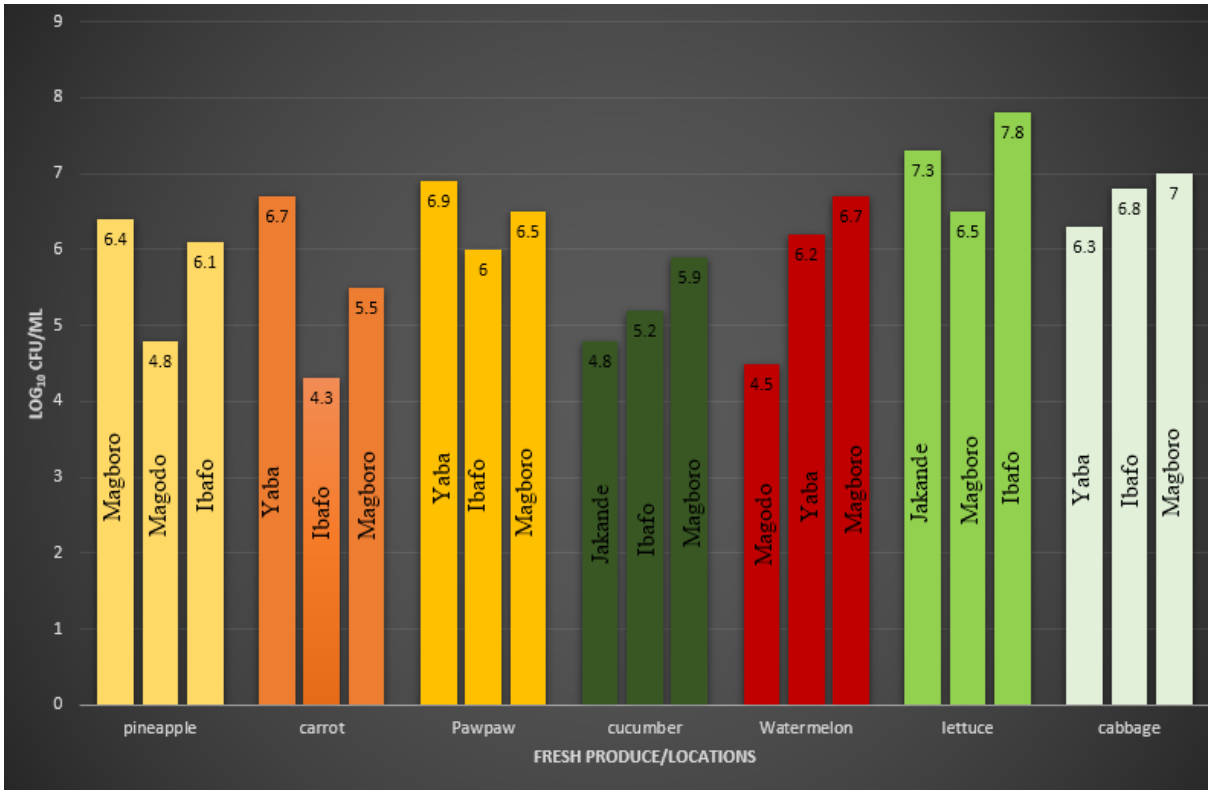

Figure 4.1 The total viable count of fresh produce from various locations in Ogun state and Lagos state.

Lettuce had the highest TVC range of 6.5- 7.8 log_{10} cfu/g followed by cabbage with a TVC range of 6.3- 7.0 log_{10} cfu/g. The TVC in Pawpaw range between 6.0- 6.9 log_{10} cfu/g, while by Water melon had TVC range of 4.5- 6.7 log_{10} cfu/g, lower TVC was recorded in cucumber which ranged between 4.8- 5.9 log_{10} cfu/g, followed by pineapple with a TVC range of 4.8- 6.4 log_{10} cfu/g. Carrot the third lowest TVC range of 6.0- 6.9 log_{10} cfu/g. In Ibafo Lettuce had the highest total viable count with a count of 7.8 log_{10}cfu/g and the lowest was Carrot with a count of 4.3 log_{10}cfu/g.

Biochemical Identification

Isolates which gave pink colors were subjected to different biochemical tests. Isolates showed pattern of biochemical reactions typical for *E. coli* as mentioned in (Table 4.1) were selected for further identification and confirmation.

Result for biochemical test of *E. coli* isolates

Table 4. 1 Result for biochemical test of *E. coli* isolates

TEST	**RESULT**
Catalase	Positive - Presence of bubbles
Oxidase	Negative (red color)
Indole Production	Positive

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.

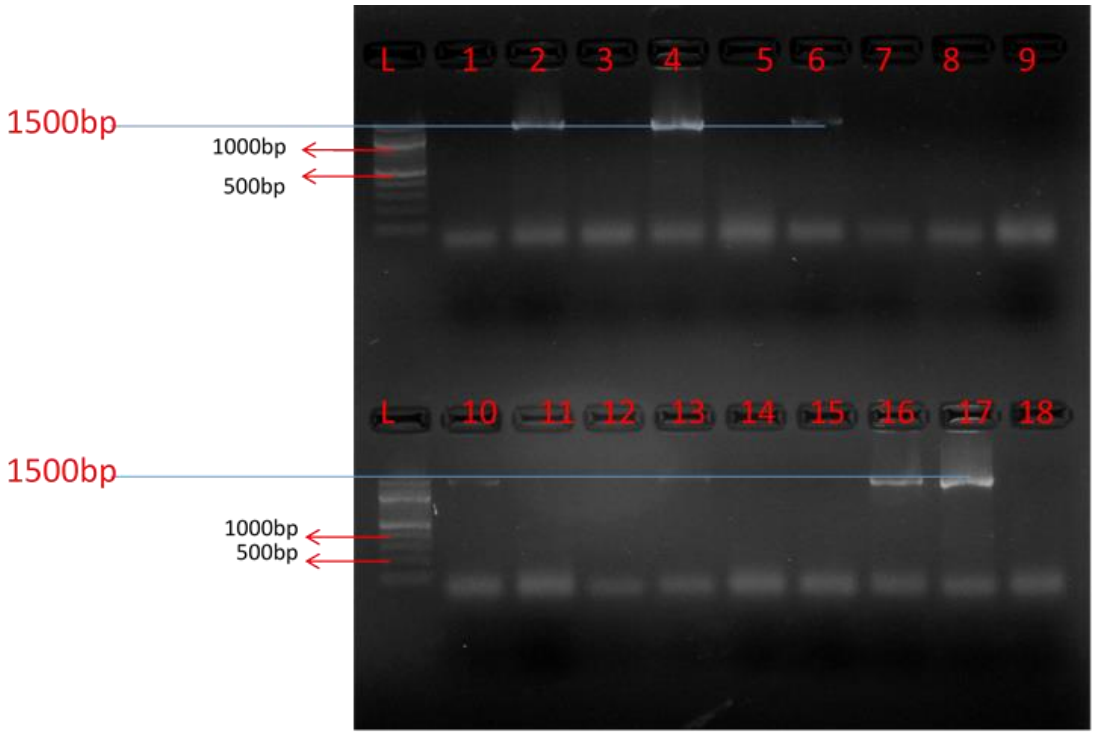

Figure 4.2 Agarose Gel electrophoresis of PCR amplicon for (16SrDNA for E. coli 38-58 No 51, 52, 53)

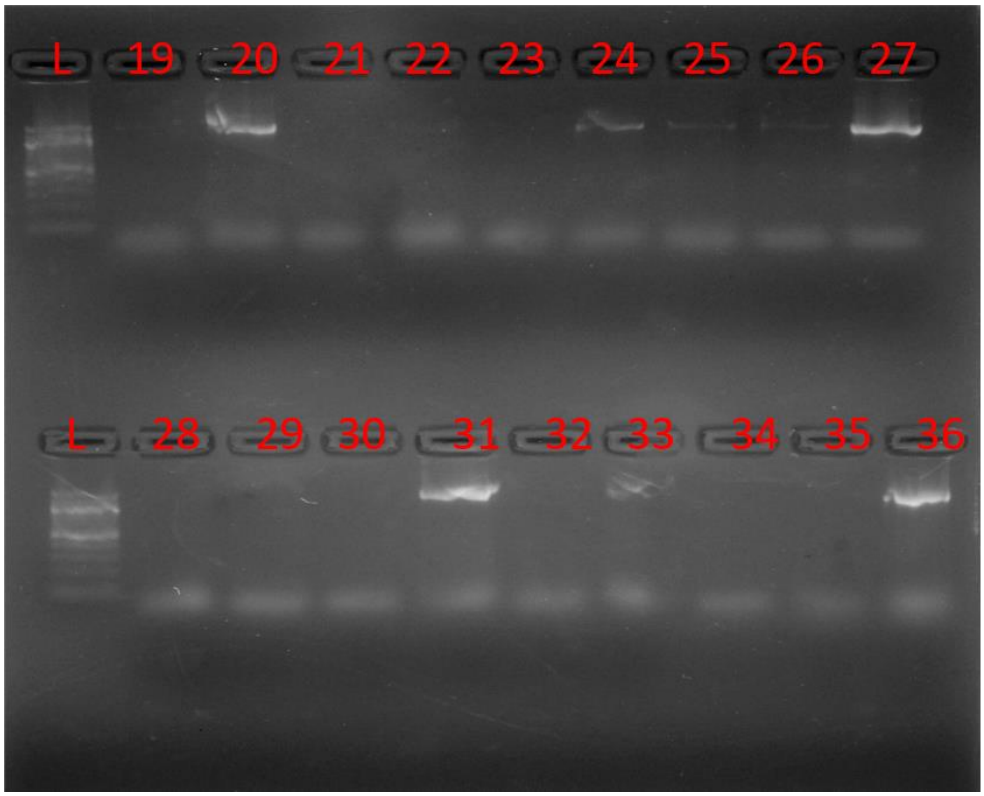

Figure 4.3 Agarose Gel electrophoresis of PCR amplicon for (16SrDNA for E. coli 38-58 no 51, 52, 53)

For PCR multiplex

21 isolates were chosen at random for additional PCR testing for the identification of the stx1, stx2, and eae genes. It was discovered that one isolate (Magboro watermelon) had the stx1 gene, indicating that it was a shiga toxin-producing *E. coli*. Figure 4.2 shows agarose gel electrophoresis of PCR products for eaeA, stx1, and stx2.

4.2 Discussion

Consumption of farm-fresh produce has increased over the past two decades due to their intake of important vitamins, nutrients and fiber (Olaimat and Holley, 2012). In addition, consumer awareness of the health aspects of fresh fruits and vegetables has increased and consumers are more interested in good eating habits and a healthy lifestyle. At the same time, however, foodborne illness outbreaks involving fresh produce have increased (Warriner *et al*., 2009).

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 than80 percent. Prior research in many countries have shown a broad variety of data on *E. coli* prevalence in plants. In Brazil, *E. Coli* has been found (Oliveira *et al.,* 2012), 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.

Numerous studies have investigated the potential sources of product contamination in the supply chain both at the pre-harvest (field) and post-harvest stage. During the pre-harvest phase, populations of pathogens can form on the growing crop itself. Risk may be increased after harvest, either from additional direct contamination or from an increase in existing pathogen populations during handling and post-harvest handling. Water can be the main source of pollution in the field. The sources could be running water from nearby livestock pastures and irrigation water from contaminated streams. The risks associated with using water from a variety of microbiological quality sources for agricultural irrigation have been evaluated and recognized the need for improved guidelines (Hamilton *et al*., 2006; Tyrrel and *et al*., 2006).

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 developing and developing countries (Contreras *et al.,* 2010; Estrada-Garcia *et al.,* 2009). 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). 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. Our results showed that a broad range of non-bladed fresh food produced in Nigerian contexts is a source of STECs. The 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 and other diarrheagenic E. coli infections in the community and should thus be seen as a means of transmitting these potentially harmful bacteria. In several stages of food manufacturing, contamination of fresh products can occur.

To this end, we screened more than 63 Fresh-cut Produce raw samples to isolate *E. coli* and determine the phenotypic and genotypic characteristics of the isolates. Microorganisms present in low numbers in food samples and containing many other bacteria in large numbers in addition to the target and desired bacteria can be lost by direct plating. Therefore, the present investigation suggests that food-dwelling organisms prefer enrichment media prior to selective disc isolation. The enrichment medium used in this study for the isolation of *E. coli* was an enrichment medium containing bile salts as a selective agent to suppress the growth of Gram-positive bacteria and other non-group bacteria. After overnight selective enrichment, the diluted cultures were seeded on MacConkey agar (MAC), which is the most commonly used selective medium to isolate *E. coli*. The medium contains bile salts (to inhibit most Gram-positive bacteria), crystal violet dye (also inhibits some Gram-positive bacteria), neutral red dye (stains lactose-fermenting bacteria), lactose and peptones. In this agar, *E. coli* appears as dark pink and specific colonies. More than 60 strains are suspected of isolation. *E.coli* was inoculated on NA agar plates. After incubation, a total of 63 colonies were pink and white on NA, SAMC and MAC agar. All NA plaque-positive isolates undergo biochemical tests widely used to differentiate bacteria from the Enterobacteriaceae family. Trials included indole production, MRVP reaction, citrate use, TSI and fermentation testing. Thirty isolates gave the same positive reaction as that of the control strain *E. coli* O157:H7. After isolation and biochemical identification, all isolates were given detailed genotype and phenotypic characteristics. Genotyping was performed by detecting virulence genes.

The most important components of virulence in *E. coli* infection are production of one or more Shiga toxins (encoded by stx1 and stx2) which inhibit protein synthesis of host cells leading to death and production of intimin protein which is responsible for intestinal colonization and development of attaching-effacing lesions. The stx1 gene is virtually identical to Shiga toxin produced by *Shigella dysenteriae*, while stx2 has only 56% identity to

stx1. The use of PCR to amplify stx genes serves as a highly specific and sensitive method to detect virulent *E. coli.* The PCR amplification using 16s, stx1, stx2, eaeA forward and reverse primers. Ibafo lettuce (SIL) had the highest total viable count of 7.8 $\log_{10}$cfu/g. According to the results, all samples had *E. coli* isolates in them. From the samples, 21 isolates were picked randomly for STEC genes identification by PCR and only 4.8% of the 21 *E. coli* isolates was positive for stx1 gene. However, that doesn't prove that the STEC negative strains are free from other *E. coli* pathotypes and is safe for consumption. Isolation of stx1 gene positive *E. coli* is very alarming for us but as these are sensitive to many antimicrobial agents if the infection is identified early it is fully curable.

We may conclude that these are Shiga toxin producing *E. coli* (STEC).

CHAPTER 5

Conclusions And Recommendations

In the food chain, controllability and traceability are essential to ensure consumer safety and protection of food from biological, physical and chemical hazards from place to time of consumption. The Shiga toxin-producing *Escherichia coli* colony comprises a wide variety of species. Understanding the role, mechanism of action, and animal host of virulence genes is essential for formulating effective prevention strategies. The main way to avoid contamination is to prevent these strains from coming into contact with food throughout the processing chain. The results showed that the salad greens and fruits sold at Ogun were contaminated with pathogenic *E. coli. Escherichia coli* includes: STEC. Although the prevalence of pathogenic E. coli is low, it shows that the presence of pathogenic *E. coli* is evenly distributed in many vegetables and salad fruits. Multiplex PCR detection is a suitable tool to detect the presence of pathogenic *E. coli. E. coli* in vegetable and fruit salads. The results of this study indicate that the vegetables and fruits produced and consumed in Ogun and Lagos states are contaminated with bacteria, which can cause great health hazards because these products are sometimes eaten raw (vegetables) or unwashed (fruits).

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APPENDIX/APPENDICES

LIST OF ABBREVIATIONS

mg	Milligram
g	Gram
℃	Degree Celsius
ml	Milliliter
µl	Microliter
%	Percent
µm	Micrometer
∞	Infinity
mm	Millimeter
16SrRNA	16S Ribosomal RNA
TAE	Tris-acetate Buffer
Min	Minute
et al.,	and others
pH	Negative logarithm of hydrogen ion concentration
bp	Base Pair
UV	Ultraviolet
Spp.	Species
E. coli	Escherichia
EAEC	Enteroaggregative
EHEC	Enterohemorrhagic
EIEC	Enteroinvasive
EPEC	Enteropathogenic
ETEC	Enterotoxigenic
DAEC	Diffuse adherent
No.	Number
Stx 1	Shiga toxin 1
Stx 2	Shiga toxin 2
HUS	Hemolytic uremic syndrome
HC	Hemorrhagic colitis
AE	Attaching and effacing
aEPEC	atypical EPEC

tEPEC	typical EPEC
ST	Heat labile
LT	Heat stable