PREVALENCE OF *S*HIGA TOXIN PRODUCING *ESCHERICHIA COLI* (STEC) *IN* THE STREET VENDED FRESH PRODUCE IN OGUN AND LAGOS STATES, NIGERIA.

GERRY-BURAIMOH OLUWASEGUN VICTOR

17010101019

A RESEARCH PROJECT SUBMITTED TO THE DEPARTMENT OF BIOLOGICAL SCIENCES, COLLEGE OF BASIC AND APPLIED SCIENCES, MOUNTAIN TOP UNIVERSITY, MAKOGI, IBAFO, OGUN STATE, NIGERIA. IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE (B.Sc.) IN MICROBIOLOGY

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DECLARATION

I hereby declare that this project report written under the supervision of Dr. O.E. Fayemi is a product of my own research work. Information derived from various sources has been duly acknowledged in the text and a list of references provided. This research project report has not been previously presented anywhere for the award of any degree or certificate.

_____   _____

GERRY-BURAIMOH OLUWASEGUN   Date

CERTIFICATION

This is to certify that this research project titled **"PREVALENCE OF PATHOGENIC *E. COLI* IN THE STREET VENDED FRESH PRODUCE" IN LAGOS AND OGUN STATES NIGERIA was** carried out by Gerry-Buraimoh Oluwasegun Victor, with matriculation number 17010101019. This project meets the requirements governing the award of Bachelor of Science (B.Sc.) Degree in Microbiology department of biological sciences of Mountain Top University, Ogun State, Nigeria and is approved for its contribution to knowledge and literary presentation.

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DR. O.E. FAYEMI   Date

(Project Supervisor)

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DR. O.E. FAYEMI   Date

(Head of Department)

DEDICATION

I dedicate this project to God Almighty for giving me life, good health and all I needed to make this work a success and secondly to my dear parents, Mr Gerry-Buraimoh Oluwafemi and Mrs Gerry-Buraimoh Omonike for their guidance, understanding and sacrifice. I also dedicate this work to my course mates and friends for their support in the course of my four-year study of Microbiology in Mountain Top University. May the Almighty God bless you all! Amen.

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ABSTRACT

Safety of fresh produce is a key issue of public health concern. Street vended fresh produce is readily available to people in Nigeria, but the safety and consistency of such fruits is always unknown and can be affected either by physical, biological and chemicals hazards. However, street vended fresh produce can be a source of transmission of food-borne diseases. The aim of this study was to determine the prevalence of Shiga toxin producing *Escherichia coli* in street- vended fruits in different areas of Lagos and Ogun state in NIGERIA. Isolation and Identification of associated pathogen with street-vended fresh produce (Lettuce watermelon, carrot, pawpaw, cabbage and cucumber) were performed using culture based (Plate method and Pour plate method) and molecular methods (Simple and multiplex PCR) to test the microbial load in the road sided fresh produce if they are saved for consumption. A total of 64 (n=64) samples were tested for the presence of STEC.It was observed that the microbial counts was very high in all the fresh produce sampled in different areas in Lagos and Ogun state, The existence of Shiga toxin-producing *E. coli* was confirmed by the presence of the stx1 virulence gene only in Magboro Watermelon, as validated by Multiplex PCR. The presence of virulence genes (stx1, stx2, and eae) were identified molecularly in twenty-one isolates (n = 21). The presumptive STEC total viable count (TVC) in the samples ranged from 4.5 to 7.8 log10cfu/g. The lettuce sampled from ibafo had the highest total viable counts. The presence of STEC, in fresh produce in Lagos and Ogun states is a public health concern which could lead to foodborne illnesses such as hemolytic uremic syndrome (HUS), food spoilage, food poisoning and a lack of safe and nutritious food.
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Keywords: Fresh-cut produce, HUS, Public health, STEC

CHAPTER ONE

1.0   INTRODUCTION

Fresh fruits and vegetables are essential for a well-balanced diet and a healthy lifestyle. However Contamination of fresh vegetables, is becoming a major food safety issue. Contaminated produce has been linked to a number of foodborne outbreaks around the world in recent years. Foodborne outbreaks are not only dangerous to people's health, but they are also a cause of economic loss (Hussain and Dawson 2013). According to a recent analysis by the Center for Science in the Public Interest (CSPI), fresh produce was the source of the most outbreaks in the United States from 2002 to 2011. Fresh produce has been reported to be the source of the most diseases with the highest average number of illness per outbreak. In the quest to improve fresh fruit consumption for healthy living. Farmers are being encouraged to promote good agricultural practices (GAP) in order to reduce food-borne illness. There is a high risk of the fresh produce to be contaminated by physical, chemical or biological hazards particularly when grown on the farm and when supplied for consumption. Microbiological contamination of fresh produce with *E. coli* and *Samonella* are examples of biological risks. Also, manufacturer and farmers tend not to wash -off chemical/physical contamination such as the pesticide, manure etc. off the produce which is an example of a chemical risk. Many developed countries have policies and procedures in place to train and educate farmers and manufacturers on how to reduce these risks by using good farming practices and food safety management systems. At any point in the food supply chain, a wide range of microorganisms can colonize or infect fruits and vegetables. E. coli O157:H7, *Samonella*, *Listeria monocytogenes, and norovirus* are all common pathogens found in contaminated fresh fruit. Potential transmission vehicles include cantaloupe, strawberries, mangoes, leafy green vegetables, lettuce, salad mixes, sprouts, cabbage, cut celery, and radishes, of these human pathogens because they are minimally processed. Globally, many foodborne disease outbreaks linked to fresh produce in recent years includes *E. coli* O157:H7 from packaged baby spinach in EU countries and cucumber in Germany and other EU countries (2011); *Cryptosporidium* in bagged mixed vegetable salads in the UK (2012); *L. monocytogenes* in prepacked salad products in the USA (2016); and *Samonella* in pre-packaged lettuce salads in Australia (2016).Bacterial contamination of fresh produce could be attributed to improper handling practices and poor storage conditions. The microbiological contamination of fresh- cut fruits and vegetables is higher than

that of intact products. Therefore, a high microbial load of pathogenic microorganism in fresh produce is indicative of lack of proper hygiene. Street vended fresh fruit and vegetables in Nigeria is of concerns because the microbial safety and hygiene status cannot be ascertained by consumers.

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1.2   STATEMENT OF PROBLEM

1) Fresh cut fruits and vegetables can be contaminated from different sources of contamination such as the food handlers, exposure to the environment(water) and poor hygiene.
2) Humans that consume contaminated fresh produce may tend to have foodborne illness
3) Cross contamination i.e., a good fresh produce that makes contact with the contaminated fresh produce will result in to food contamination.
4) The presence of pathogenic *E. coli* fresh produce and associated risk such Hus, Renal failure and diarrhea etc.

1.3   AIMS AND OBJECTIVES TO THE STUDY

1. To isolate and identify the various pathogenic species of *Escherichia coli* in street vended fresh cut fruits in Lagos and Ogun state.
2. To identify the virulence genes and factors in the Shiga toxin producing *E. coli* (STEC) associated with fresh produce.

CHAPTER TWO

LITERATURE REVIEW

2.1 SIGNIFICANCE OF FRESH PRODUCE

Fresh produce is an important raw resource for food manufacturers because it provides a wide range of vitamins, nutrients, and fiber to health-conscious consumers (Carlin, 2007). Fruits, vegetables, herbs, seeds, and nuts are examples of fresh produce. They can be whole, prepared (pre-cut or reduced in size), ready to eat (no preparation required before consumption), and/or dressed (pH controlled or not) (Goldburn, 2009). It also provides humans with vital nutrients, vitamins, and fiber. Between 1980 and 2004, global fruit and vegetable production increased by 94%. Fruit and vegetable imports in the United States increased from 1994 to 2004 to $12.7 billion (Aruscavage *et al*., 2006), and daily sales of cut produce in North America reached 6 million packages by 2005 (Aruscavage *et al*., 2006). (Jongen, 2005). Fresh produce consumption has increased over the last two decades for a variety of reasons. For example, people are more concerned about keeping healthy and eating properly, and as a result. To meet this demand, diverse range of domestic and imported produce has to be available all year (Warriner et al., 2009). Between 1990 and 2004, global fruit and vegetable consumption increased by 4.5 percent per year on average. (2007, EU) Fruit and vegetable consumption in the United States increased by 25% between 1977 and 1999, compared to 1977 and 1979. The Food and Drug Administration (FDA) issued a warning in 2001. Fruit and vegetable consumption in Canada increased by 56% and 26%, respectively, between 1963 and 2010. (Statistics Canada, 2002, 2011). At the same time, incidences of foodborne illnesses linked to fresh vegetable intake have grown (Warriner *et al*., 2009). This increase could be attributable to a variety of factors, including changes in personal consumption, increased livestock production near areas of intensive (extensive) produce production, and increased livestock production near areas of intensive (extensive) produce output., increased availability of produce globally (some of which comes from countries with questionable hygienic measures), and a growth in the number of immunocompromised people (Beuchat, 2002). *Samonella* and *Escherichia coli* O157:H7 are the most common pathogens linked to outbreaks of foodborne disease linked to fresh vegetables, according to academic journals (Buck *et al*., 2003; FDA, 1998; Warriner *et al*., 2009).

Post-harvest deterioration, bacterial soft rot, and microbiological spoilage are all threats to fresh food and processed juices. Consumers consider fresh food (fruits and vegetables) as wholesome and nourishing. because of a large number of scientifically confirmed and documented health benefits of eating fresh fruits and vegetables [Critzer *and Doyle* 2010].

2.2 MICROORGANISMS ASSOCIATED WITH FRESH-PRODUCE SPOILAGE

Human viruses such as *Norovirus and Hepatitis A* can contaminate fresh produce (fruits and vegetables) at any point from farm to table, including during cultivation, harvesting, postharvest handling, storage, processing, transport distribution, retail display, and/or preparation (foodservice or home). Human pathogen-contaminated produce cannot be entirely disinfected by washing or rinsing it in an aqueous solution, and low random levels of human pathogens can be discovered on produce [Leff *and* Dieter 2013]. In 2004, the Alliance for Food and Farming [Round *and* Mazmanian 2009] reviewed and summarized data sets from the Centres for Disease Control and Prevention (CDC) (Tournas.2005). information on foodborne illness outbreaks linked to the eating of fruits and vegetables the most likely source of contamination was during production/growing or postproduction handling. Foodborne illnesses associated with produce in the ''postproduction" category were most likely caused by improper handling at the foodservice, retail, or consumer level, whereas foodborne illnesses associated with produce in the ''grower" category were most likely caused by farm, packing, shipping, or other agricultural postharvest handling.

(Harris *et, al.* 2003) conducted an extensive review of fresh produce outbreaks and reported that the most common human pathogens associated with produce foodborne illness outbreaks are: *E. coli* O157:H7, *Samonella* spp., *Shigella spp., Listeria monocytogenes, Cryptosporidium spp.* [Monge *and Chinchilla* 1996], *Cyclospora spp., Clostridium botulinum, hepatitis A* These microorganisms are classified as follows:
Pathogenic microorganisms found in soil (*Clostridium botulinum, Listeria monocytogenes*).
Pathogenic bacteria found in feces (*Samonella* spp., *Shigella spp., E. coli O157:H7*, and others).
Parasites that cause disease (*Cryptosporidium, Cyclospora*).
Pathogenic viruses (*hepatitis A, enterovirus, Norwalk-like viruses*).

Many of these pathogens are transmitted from human (or domestic animal) to food to human. Risks associated with handling fruits and vegetables by infected field workers or customers include cross contamination, the use of contaminated water, the use of improperly composted manure, and contact with contaminated soil. are just a few examples of how human infections can be transmitted to food. According to data from the CDC's foodborne outbreak surveillance system, the two most commonly reported microorganisms associated with fresh produce foodborne illness outbreaks from 1988 to 1998 were *Samonella* spp. and *E. coli* O157:H7, accounting for 45 percent and 38 percent of the fruit and vegetable linked outbreaks, respectively being attributed to these two microorganisms.

Table 2.1 Major fruit and vegetable pathogens associated with outbreaks (Abadias, Alegre, Oliveira, Altisent, & Viñas, 2012; FAO/WHO, 2008; Olaimat & Holley, 2012; Senorans, Ibanez,& Cifuentes, 2003; Seow, Ágoston, Phua, & Yuk, 2012; Van Boxstael et,al., 2013; War

Pathogen		Product
Bacteria	Clostridium botulinum	Cabbage, pepper, garlic and carrots
	E. coli 0157:H7	Cabbage, Lettuce, Watermelon
	L.monocytogenes	Cabbage, Lettuce and Potatoes
	Samonella spp	Green onions, Cabbage, Lettuce and celery
	Shigella spp	Lettuce, green onions and salad vegetables
	Staphylococcus spp	Lettuce, Parsley and seed sprouts
	Vibrio cholerae	Cabbage and coconut milk
	Yersina entero*coli*tica	Carrots, Cucumbers, lettuce and tomatoes
Viruses	Norovirus	Lettuce, salads, fresh-cut fruits
	Hepatitis A	Lettuce, green onions and strawberries
Protozoa	Cryptosporidium spp	Lettuce and onions
	Cyclospora spp	Lettuce , onions and black berries

2.2.1 CHARACTERISTICS OF SPOILAGE MICROORGANISMS ON FRESH-CUT PRODUCE

Pathogenic bacteria can contaminate fruits and vegetables that come into touch with dirt, insects, animals, or humans. Among all food-borne bacterial human diseases, coliform bacteria such as *E. coli*, *Staphylococcus aureus*, and *Samonella* sp. are among the most commonly isolated species from fresh produce vegetables (Tournas., 2005). Coliforms are Gram-negative, facultative anaerobic, non-spore producing rods that live in the guts of animals and humans. In lactose broth at 35°C, they digest lactose and produce gases in 48 hours. Coliform bacteria are commonly used bacterial markers of food and water quality, and are frequently employed as a sign of fecal pollution. Consumption of infected and damaged fresh-cut vegetables has frequently been linked to outbreaks. *E. coli* is linked to fecal contamination of plant material contaminated water, excrement from humans or animals, sewage, and soil (Sothornvit and Kiatchanapaibul 2009). *Staphylococcus aureus* is the world's third most common cause of confirmed food poisoning (Andrews and harris., 2000). Fresh cut produce can all be spoiled by a variety of bacterial species. Lactic acid bacteria are one of the most common types of bacteria found in plant produce, contributing to the spoilage of fresh cut and juice products. Some of the more well-known lactic acid bacteria genera are *Lactobacillus, Leuconostoc, Pediococcus, Lactococcus*, and *Enterococcus. Pseudomonas aeruginosa* is a particularly important group of bacteria that causes spoilage. is a Gram-negative, strictly aerobic bacterial genus that includes species such as *Pseudomonas marginalis*, *Pseudomonas fluorescens, and Pseudomonas viridiflava*. Soft rot induced by these species at low temperatures is especially dangerous for vegetables in supermarkets and wholesale marketplaces (Liao, 2005). Another typical Gram-negative rotting microbe linked with fresh-cut vegetables is *Pectobacterium carotovorum* (formerly *Erwinia carotovorum*) (Toth *et al*., 2003).

2.2.2 POTENTIAL SOURCES OF PRODUCE CONTAMINATION

Because of the open nature of fresh produce cultivation, it is vulnerable to contamination from a variety of sources. This is due to the fact that each farm has its own unique set of environmental risk factors, including topography, land-use interactions, and climate. The prevalence and transmission of foodborne pathogens, as well as the danger of produce contamination, are

influenced by a combination of these unique environmental risk variables (Strawn *et al.*, 2013). Pathogens can contaminate produce 'on-field' through a different mechanisms, including air deposition, uptake from contaminated soils, and groundwater contamination (Harris *et al.*, 2003) (Lynch *et al.*, 2009)(Mei Soon *et al.*, 2012), use of raw (or poorly treated) manure and compost, exposure to contaminated water (irrigation or flooding), insect transfer, or fecal contamination caused by cattle or wild animals Several researchers have looked at the source of contamination, with soil, water, biological amendments, and wild animal activities all being mentioned as possible routes for human infections to enter. (Warriner *et al.*, 2009) (Olaimat *and holley*, 2012) (Goodburn *et al.*, 2013) (Martínez- Vaz *et al.*, 2014) (Nuesch-Inderbinen and Stephan 2016). In perfect conditions, pathogens would be absent from the soil, water, and biological modifications, preventing contamination. Pathogens, on the other hand, can survive in the environment for long periods of time and become extensively spread (Yang *et al.*, 2010). (Schwarz *et al.*, 2014). Pathogens like *Samonella* can even establish themselves in greenhouse operations that would normally be considered confined (Holvoet *et al.*, 2014).

2.2.1.1 Postharvest Contamination.

In some circumstances, the presence of *E. coli* in vegetables such as alfalfa sprouts, fresh spinach, and raw clover sprouts is much higher at the end of the postharvest process than at the beginning. This could be due to later direct contamination or pathogen proliferation during raw vegetable postharvest operations. The presence of *E. coli* in postharvest packing stages could imply fecal contamination and the presence of enteric pathogens from feces. When *E. coli* O157: H7 was isolated from specific types of fresh vegetables, the prevalence was rather low, according to (Zhang *et, al*), yet these bacteria can cause disease in consumers.

2.2.1.2 **Preharvest contamination**

The main sources of preharvest contamination are soil and inadequately composted animal dung used as organic manure. Due to use of animal feces as manure, the soil is prone to be a natural reservoir for a diversity of human pathogens, including *E. coli* pathogens (Whipps et, al., 2008). *E. coli O157: H7* can live in the soil for 7 to 25 weeks, depending on the soil type, humidity level, and temperature. [Duffy et al., 2005] This bacterium can also survive in the storage and distribution of crops. According to [Launders et al., 2016], the presence of STEC O157 in potatoes poses a risk because it may cause cross contamination *E. coli O157: H7* with other raw foods. Furthermore,

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Table 2.2 Contamination sources during the pre- and postharvest stages in raw vegetables.

Stage	Contamination sources
Pre-harvest	Insecticides, fungicides, irrigation water, manure that has not been properly composted, human handling, and seasons are all factors to consider (fall, winter, and spring)
Post-harvest	Poor hygiene in harvesting and transporting equipment, contaminated water for washing and distributing equipment, grimy cutlery, and dirty processing equipment

2.3 OUTBREAKS LINKED TO FRESH PRODUCE

Foodborne disease outbreaks connected to fruits and vegetables have increased dramatically (Fig. 2.1) *Samonella* and *E. coli* are the most dangerous diseases. *E.coli O157:H*7, despite the fact that a wide spectrum of dangerous bacteria exists in theory. At any step in the supply chain, microorganisms can taint fresh products. Sprouting seeds, tomatoes, and leafy greens have all been linked to high-profile foodborne disease outbreaks, with sprouted seeds, tomatoes, and leafy greens being the most prevalent. (Doyle and Erickson, 2008). The underlying causes for certain product kinds being linked to the bulk of outbreaks can be explained in part by market volume.
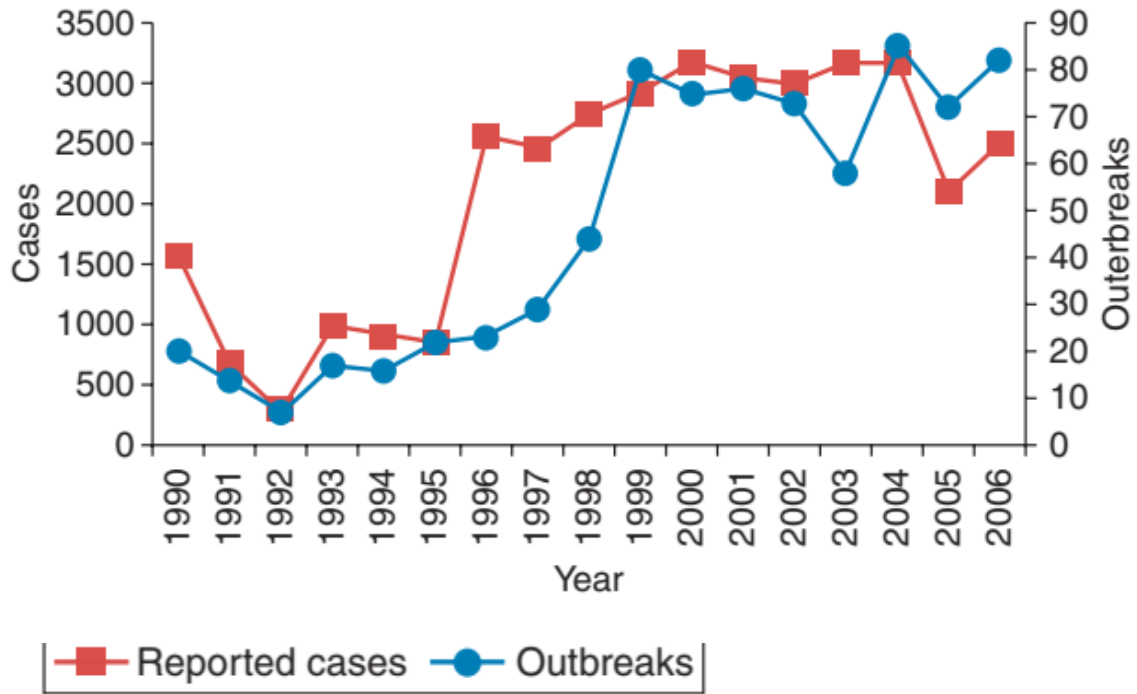
Figure 2.1 Foodborne illness outbreaks linked to fresh produce from 1990 to 2006.

Table 2.3 Outbreaks linked to fresh produce

Date	Pathogen	Produce	Comments
December 2005	*Samonella*	Mung bean sprouts	Canada,618 confirmed cases
February 2006	*Samonella*	Alfalfa sprouts	Canada, sprout recall due to suspected contamination.
February 2006	*Samonella*	Alfalfa sprouts	Australia,100 confirmed cases
June 2006	*E. coli* O121:H9	Lettuce	United states ,4 confirmed cases
July 2006	*Samonella*	Fruit salad	U.S.A and Canada ,41 confirmed cases
September 2006	*E.coli* O157:H7	Spinach	U.S.A ,205 confirmed cases;3 deaths
September 2006	*Clostridium botulinum*	Carrot juice	U.S.A and Canada; 6 cases
October 2006	*E.coli* 0157:H7	Lettuce	U.S.A:81 confirmed
October 2006	*E.coli* 0157:H7	Lettuce	Canada: recall for suspected contamination
October 2006	*Samonella*	Tomatoes	U.S.A:183 cases
August 2007	Shigella sonnei	Carrots	Canada, 4 cases
June 2008	*Samonella*	Tomatoes	United state and Canada 1442 confirmed cases.
September 2008	*E.COLI* 0157:H7	Lettuce	United state and Canada,134 confirmed cases.
September 2008	*Samonella*		United states,14 confirmed cases
November 2008	*Samonella*	Alfalfa sprouts	UK, 32 confirmed cases
December 2008	*Samonella*	Basil	United states, recall for the suspected contamination.
		Alfalfa sprouts	

Despite the fact that outbreaks of foodborne disease connected to fresh fruit have been documented for over 30 years, the number of cases has been rapidly increasing (Fig. 2.1). The biggest salmonellosis epidemic connected to mung bean sprouts occurred in Ontario in 2005. (Table 2.3). The *Samonella* serovar in question was Enteritidis, which is more often seen in poultry

and uncooked eggs. Although one large sprout grower in the region was targeted as the source of the epidemic, it is unclear how the S. Enteritidis strain acquired connected with the sprouts. It is fairly unusual to be unable to link human pathogens implicated in fresh produce foodborne disease outbreaks back to their source, and the "smoking gun" is rarely discovered. This is due to the short shelf life of fresh fruit, which is frequently thrown out by the time an epidemic is discovered. A additional contributing element that makes identifying individual suppliers difficult is the absence of traceability of produce. The *E. coli* O157:H7 outbreak related to baby spinach in America in 2006 was unique in that the pathogen's strain was isolated from affected patients, spinach in unopened bags, and the farm where the outbreak occurred. A crop was grown (Cooley *et,al*., 2007). The source of the spinach contamination was thought to be *E. coli* O157:H7 transmission from a nearby cow ranch by infected wild pigs that gained access to the crop through a damaged fence. However, a survey of the Salinas valley in the summer of 2006 discovered a significant incidence of *E. coli* O157:H7, indicating that the true route might have been via polluted irrigation water (Cooley *et, al.*, 2007).

2.4 Escherichia coli

Escherichia coli is a Gram-negative rod-shaped bacterium that belongs to the Enterobacteriaceae family of the Gammaproteobacterial class. One of the most well-studied bacteria is *Escherichia coli*. Under ideal growth conditions, *Escherichia coli* can grow quickly, replicating in less than 20 minutes. *E. coli* has been used in the development of many gene manipulation systems. As the host bacterium, *E. coli* produces a wide range of enzymes and other industrial products. *E. coli* genome sequence analysis the first case of *E. coli* was discovered in 1997. Since that time, over 4800 E. The genomes of *E. coli* have been sequenced. *E. coli*'s rapid growth characteristics coli makes it ideal for studying microorganism evolution, as well as a long-term experimental evolution research of *E. coli*. More than 50 000 generations of *E. coli* are now being studied (Tenaillon *et, al.*, 2016). While we have a lot of information on *E. coli* under laboratory settings, there is still a lot to learn About bacterium's ecology in the environment. Because The bacteria *E. coli* is commonly employed as a fecal indicator. (FIB) is a tool for determining the quality of water, as well as the survival and reproduction of aquatic organisms. The presence of *E. coli* in the environment causes worry. about the use of this bacteria to detect fecal contamination. Furthermore, since certain

strains of *E. coli* and serotypes may cause human disease is vital. It's crucial to understand this bacterium's ecology in order to avoid it.

2.4.1 *ESHERICHIA coli* AS A HUMAN PATHOGEN

Escherichia coli contains not only commensal strains but also pathogenic ones. including harmful ones that cause a range of human illnesses, killing about 2 million people per year (Kaper *et, al.,* 2004). *E. coli* has six intestinal pathotypes that have been well researched. coli, including those that produce Shiga toxin. Enteropathogenic *E. coli* (STEC), Enterotoxigenic *E. coli* (EPEC), Enteroaggregative *E. coli* (ETEC), and enter invasive *E. coli* are of two types, as well as Shigella strains. The virulence characteristics and pathogenicity mechanisms that cause gastrointestinal disorders like diarrhea are used to classify these bacteria (Nataro and paper 1998; Kaper *et, al.,* 2004). Enterohaemorrhagic *E. coli* (EHEC) is a form of STEC. May induce severe enteric illnesses such hemolytic uremic syndrome and hemorrhagic colitis, which can lead to abrupt renal failure and mortality (Kaper *et, al.*, 2004).

The most well-known serotype of *E. coli* is O157:H7. EHEC has been linked to a number of waterborne and foodborne epidemics. In many nations, food-borne illnesses are a problem. The prevalence of it In recent years, non-O157 STEC has been on the rise. Serotypes O26, O45, and O103, for example, are responsible for a variety of diseases. O111, O121, and O145 are all numbers that start with the letter O. (Farrokh *et, al.*, 2013). Some Extraintestinal illnesses can also be caused by *E. coli* strains. Extraintestinal pathogenic E is what it's termed. *E. coli* (ExPEC). The ExPECs, which were identified based on their illness associations, uropathogenic E should be included. *E. coli*, newborn meningitis-associated *E. coli*, neonatal meningitis-associated *E. coli* and the sepsis-causing *E. coli* (Dale and Erickson 2015).

2.5 CLASSIFICATION OF *E. coli*

Escherichia coli are Gram-negative facultative anaerobic rods that are part of the typical gut microbiota in humans and animals. Although the majority are nontoxic, pathogenic variations cause either enteric (diarrhoeagenic *E. coli* (DEC)) or extra-intestinal (extra-intestinal pathogenic *E. coli*) infections in humans (ExPEC). ExPEC cause urinary tract infections, as well as mastitis, septicemia, peritonitis, Gram-negative pneumonia, and meningitis to a lesser extent.

The diarrhoeagenic *E. coli* are divided into seven groups based on virulence traits and mechanism of pathogenicity and include

- Shiga toxin-producing *E. coli* (STEC).

- Enteropathogenic *E. coli* (EPEC).

- Enterotoxigenic *E. coli* (ETEC).

- Enteroinvasive *E. coli* (EIEC).

- Enteroaggregative *E. coli* (EAEC).

- Diffusely Adherent *E. coli* (DAEC).

- Adherent Invasive *E. coli* (AIEC).

2.5.1 DIFFUSELY-ADHERENT *E. COLI*

Diffuse adhering *E. coli* (DAEC) have been recognized as the sixth category and appears as a heterogeneous group (Nataro *et al*., 1998). *E. coli* strains in this category are known to bind to host cells in a pattern of diffuse adhesion in which the bacteria consistently cover the entire surface of the cell. (Scaletsky *et,al*., 1984). The effect of DAEC strains on diarrhea stays dubious, as certain examinations have detailed that these strains are correspondingly found in youngsters with and without the runs (Giron *et, al.*, 1991; Jallat *et, al.*, 1993). (Tacket *et, al.,* 1990) suggested that DAEC may cause disease in immunologically naive or malnourished children due to their inability to conclusively induce diarrhea with DAEC in health adult volunteers. However, in Northern Brazil, Diffusely adherent *E. coli* has been shown in studies to be the cause of acute diarrhea in children (Scaletsky *et, al.*, 2002). Discrepancies between epidemiological studies can be explained by age-dependent susceptibility to diarrhea or the use of an inappropriate technique of identification such as DNA probing (Levine *et, al.*, 1993).

2.5.*2* ENTEROAGGREGRATIVE *E. COLI*

Enteroaggregative *Escherichia coli* (EAEC) Have been found in both developed and developing countries to cause acute and chronic diarrhea (Nataro et, al. 2006). Infections of the urinary tract are possible (Jensen B.H. et, al.,2014) Toxic enterotoxins and cytotoxins are produced by EAEC when it aggregates and adheres to the intestinal mucosa. EAEC is currently perceived as an arising enteric microorganism. Specifically, EAEC are accounted for as the second most normal reason

for traveler's diarrhea, second only to Enterotoxigenic *E. coli,* and a common cause of diarrhea amongst pediatric populations (Huang et*, al.*, 2006; Adachi J.A. *et, al.*, 2001). It has additionally been related with ongoing contaminations in the last mentioned, just as in immunocompromised hosts, for example, HIV-infected people (Huang et*, al.*, 2006)

2.5.*3* ENTEROTOXIGENIC *E. COLI*

The enterotoxigenic *E. coli* (ETEC) strains are a leading cause of diarrhea in both children and travelers (Croxen et, al., 2013). ETEC's virulence factors are heat labile toxin (LT) and heat stable toxin (HST) (ST). Both are involved in the deregulation of ion channels in the epithelial cell membrane (Fleckenstein et, al., 2010). ETEC can grow in a variety of environments, including rivers, drinking water, irrigation water, and fresh produce. (MacDonald *et, al.,* 2015).

2.5.4 ENTEROHAEMORRHAGIC *E. COLI*

The entero-hemorrhagic *E. coli* (EHEC) strains cause bloody and nonblood diarrhea. The most infamous piece of this pathotype is strain O157:H7, which causes bloody diarrhea and no fever. EHEC can cause hemolytic uremic condition and unexpected renal disappointment. It utilizes bacterial fimbriae for connection (*E. coli* basic pilus, ECP), (Rendon *et,al*., 2007) and is tolerably intrusive and has a phage-coded Shiga poison that can cause extraordinary provocative responses.

2.6 Shiga toxin producing *Escherichia coli* (STEC)

The most regular facultative anaerobe identified in the gastrointestinal tracts of warm-blooded animals and humans is *Escherichia coli*. Virulence genes are hardly found in *E. coli* strains. Pathogenic strains distinguished by their ability to generate verotoxins (also known as Shiga toxins) are referred to as verocytotoxigenic *E. coli* (VTEC) or Shiga toxin-producing *E. coli* (STEC). Morbidity and mortality associated with recent major outbreaks of gastrointestinal illness caused by Shiga toxin-producing *Escherichia coli* (STEC) have emphasized the threat these organisms pose to public health. These types of epidemics have the potential to strain acute care services, even in nations with highly developed health-care systems (Ahmed *and cowden* 1997). This pathogen group, the toxin, its structure and function, its interaction with host cell receptors, and signs and symptoms of illness will all receive a lot of attention. The ability to manage STEC illness in people and lower epidemic rates is based on prompt diagnosis and identification of the source of infection. Significant advances in awareness of the pathology of STEC infection have

occurred in recent years, contributing to the development of improved diagnostic tools as well as treatment and preventive efforts. (Konowalchuk *et, al.,*1977). revealed the characteristic which differentiates STEC from other types of pathogenic *E. coli*, namely, the synthesis of a toxin with a severe and permanent cytopathic impact on Vero (African green monkey kidney) cells (Konowalchuk *et, al.,*1977). Verotoxigenic *E. coli* strains were related to instances of hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS) in the early 1980s (Karmali *et, al.,*1983). STEC strains belong to a wide spectrum of serotypes and can cause significant human disease. O157:H7 is a prominent STEC serotype in many regions of the world and has been the type most often connected with major outbreaks.

2.6.1 SEROTYPE OF STEC

Serotype data has been used as a determinant for identifying STEC strains that have the potential to cause major human infections since the discovery of STEC serotype O157:H7 as a prominent foodborne pathogen. When non-O157 STEC strains were linked in outbreaks and other serotypes were recognized as being of health concern, the focus on serotypes remained. However, serotype is not a virulence factor in and of itself, and not all STEC serotypes have been linked to human infections. As a result, some people have devised the term enterohemorrhagic *E. coli* to refer to a subgroup of STEC that contains pathogenic strains, the majority of which have eae. Serotype O157:H7 is the most common EHEC strain, but others from serogroups O26, O111, O103, and O145, to mention a few, have also caused serious human sickness. Alternate EHEC strains, such as those with the serotypes O113:H21, O91:H21, O104:H4, and others, do not contain eae but cause HUS (Karmali *et al*., 1985), indicating that these viruses have other attachment mechanisms. Because many STEC virulence genes are migratory and can be lost or transferred to other bacteria, STEC strains of the same serotype may or may not have the same virulence genes or pose the same threat. A The likelihood of a STEC strain causing severe disease or the severity of STEC-related

illness
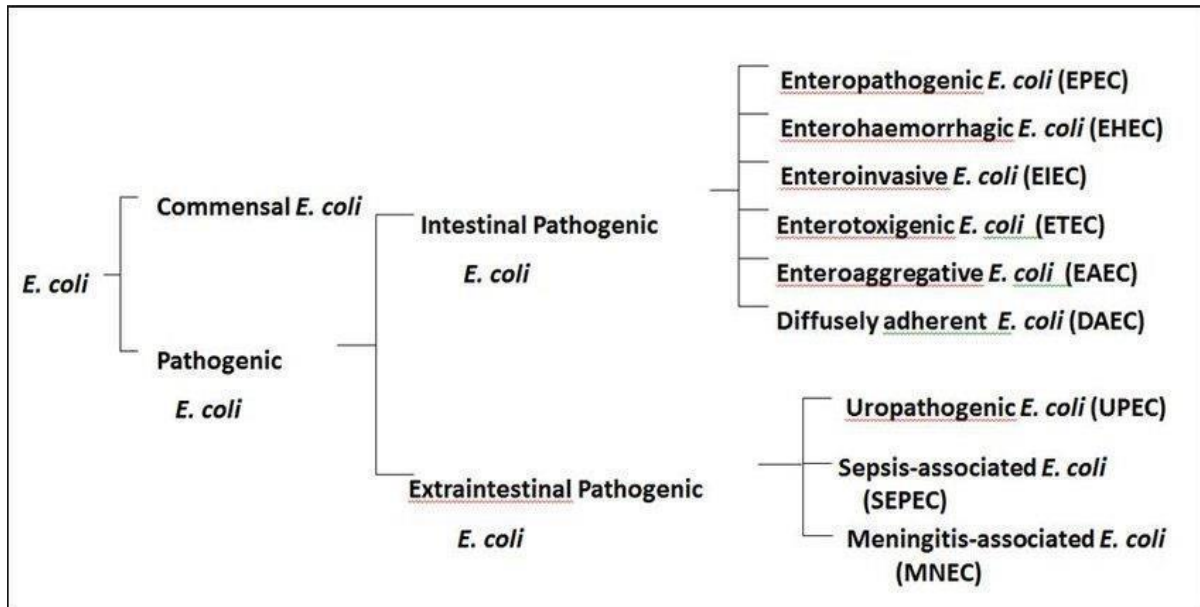
Figure 2.2 *Escherichia coli* species and its sub species classification. Adapted from: (Wakeham, 2013)

Table 2.4 Serological Classification of *Escherichia coli*

CATEGORY	SEROGROUP	SEROTYPE
ETEC-enterotoxigenic *E. coli*	06.08.015.020	06:H16.011:H27
	0.25.080.0115	0128:H7.0149:H10
	0148.0159	0159:H20
EIEC- enteroinvasive *E. coli*	029.0124.0144	028ac:H-0124:H, 0124:H32
	0152.0167	0144:H-.0159:H22
EPEC- Enteropathogenic *E. coli*	055.086.0111.	044:H34.055:H6
	0125.026	0111ab:H12 0126:H7.0127:H9.
EHEC- enterohemorrhagic *E. coli*	0157.0126	0157:H7
EAEC-enteroaggregative *E. coli*		Very little is known

STEC are zoonotic pathogens that enter the human body via contaminated food and water. Individual cases and outbreaks have been linked to direct animal contact (for example, farm visits), environmental contamination, and fecal-oral transmission. Shiga toxins (Stx), named after the toxin produced by *Shigella* dysenteries serotype 1, characterize STEC infections.

However, not all STEC are capable of infecting humans, and only a subset of these is virulent, belonging to the pathovar widely recognized as enterohemorrhagic *E. coli* (EHEC). Most EHEC contain the locus of enterocyte effacement (LEE), a chromosomal pathogenicity island that encodes a type III secretion system, an adhesin called intimin, and its receptor Tir. The eae gene encodes intimin, which allows bacteria to adhere to the epithelia, causing attaching and effacing lesions. It is shared by enteropathogenic *E. coli* (EPEC) strains (Kostal et, al., 2019). Enterohemorrhagic *E. coli* carrying LEE are referred to as typical EHEC, while those that do not are referred to as a typical EHEC.

There are two types of Shiga toxins (Stx1 and Stx2), and the stx toxin genes are carried by lambdoid bacteriophages that have been integrated into the *E. coli* genome. The *E. coli* chromosome the stx1 gene has four subtypes (a, c, d, and e), whereas the stx2 gene has twelve (a to l). There have been no reports of strains with more than one stx1 subtype. A given strain, however, may have both a stx1 and a stx2 subtype gene, or more than one stx2 subtype gene. Many STEC are attaching and detaching (A/E) bacteria, they move the eae gene on the locus of enterocyte effacement (LEE) and form distinguishable lesions on the surfaces of intestinal epithelial cells (Kostal *et, al.*, 2019). The most common STEC serogroup related to human illness is O157, and its molecular pathogenesis has indeed been extensively researched. it is divided into three genetic lineages, *E. coli* O157:H7 (I, II, and I/II) as a result of an ancestral clone's geographical spread and subsequent regional expansion (Kim *et, al.*, 2001).
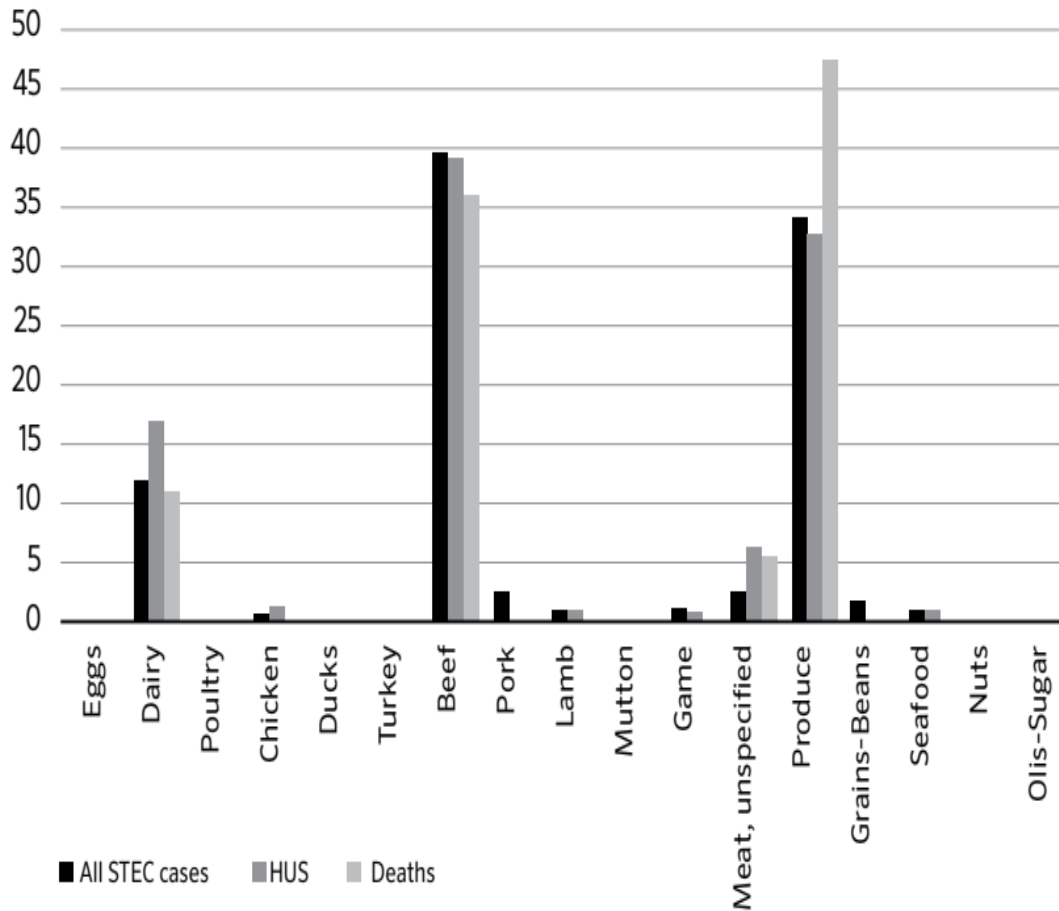
Figure 2.3 Relative contribution of foods categories to STEC cases in WHO regions

2.6.2   TRANSMISSION, SOURCES AND ROUTES OF INFECTION OF STEC

Cattle have long been recognized as the major reservoir of STEC strains, especially those related to serotype O157:H7. However, STEC strains have been found in the gastrointestinal tracts of various domestic animals, including sheep, pigs, cattle, dogs, and cats, according to epidemiological research. (Beutin *et, al.*, 1993). Serological investigations have proven that great majority of cattle had been exposed to STEC at some time throughout their life (Clarke *et, al.*1994).   STEC isolates from animal sources contain the major human disease-causing serotypes, as well as a number of O:H forms that have yet to be linked with human illnesses. STEC can enter the human food chain from numerous animal sources, most often via contamination of meat with feces or intestinal tissues after slaughter.

STEC may be detected samples of beef, lamb, deer, wild boar, ostrich, partridge, antelope, and reindeer (Pierard *et, al.,* 1994). One of the most prevalent sources of human STEC infection is hamburger patties produced from ground beef, and a number of outbreaks of O157:H7 infection have been related to the intake of ground beef (Karmali, 1989). Ground beef may represent a special danger for two reasons. First, the frequency of highly pathogenic STEC strains such as O157:H7 may be greater in cattle than in other animal species. Second, STEC infection on the surface of meat gets equally dispersed throughout the mincing process, and unless it is completely cooked, STEC organisms in the interior may not be exposed to the high temperatures. There is a strong possibility for huge epidemics when hamburgers are offered by fast food restaurant utilizing a common supply of ground-beef patties. Other dietary causes of STEC infection are:

I. Raw or poorly pasteurized dairy products.
II. Marinated or dried meat products such as salami and jerky.
III. Fruit and vegetable items which likely have come into touch with domestic animal excrement at some time during production or processing.

A rising number of outbreaks are connected with the eating of Fresh produce (fruits and vegetables) including sprouts, spinach, lettuce, coleslaw, and salad which might have been infected due to contact with excrement from animals at some time during its production or processing. STEC can also be found in bodies of water (such as streams and ponds), wells. Waterborne spread has been documented, both from polluted drinking-water and from contaminated swimming pool (Ackman *et, al.* 1997). Another significant method of transmission is individual encounter through the oral-fecal route (Reida *et, al.,* 1994). There is an asymptomatic carrier condition, when individuals exhibit no clinical indications of being sick yet are capable of infecting others. Another recognized health risk for STEC infection is visitation to farms and other places where the general public could come into close contact with farm animals.
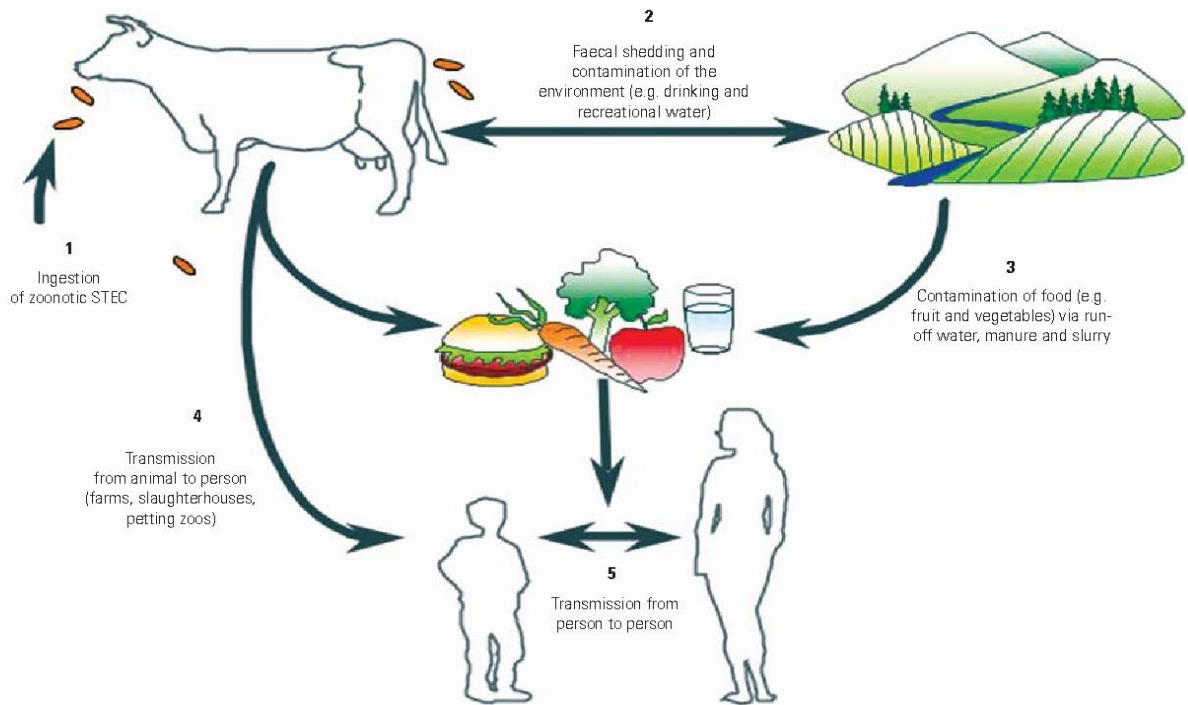
Figure 2.4 Transmission of STEC from farm to fork

2.7 CLINICAL SYMPTOMS OF STEC INFECTION

Symptoms of the illnesses caused by STEC include acid reflux and diarrhea that may in rare circumstances lead to bloody diarrhea (hemorrhagic colitis) (hemorrhagic colitis). Nausea and vomiting could also happen. The incubation time can vary from 3 to 8 days, with a frequency of 3 to 4 days. Most people recover within 10 days, however in a tiny number of patients (especially young children and the elderly), the infection could develop to a life-threatening illness, such as hemolytic uremic syndrome (HUS) (HUS). HUS is distinguished by abrupt renal failure, hemolytic anemia and thrombocytopenia (few blood platelets) (low blood platelets).

2.8 PREVENTION OF STEC

The various ways on how to reduce or eliminate Shiga- toxin producing *E. coli* in our society to prevent food-borne illness. Employees should ensure to wash hands before, during, and after handling any food, especially raw meat and poultry. Sanitize all utensils, cutting boards, and work surfaces before and after use, using an authorized sanitizing solution designed to be used on food or food contact surfaces. Clean food contact surfaces, such as refrigerator shelves, if they come in touch with probable sources of *E. coli* contamination. Wash all vegetables and fruits thoroughly before consumption. Do not use cutting surfaces that have been exposed to raw meat to prepare raw fruits or vegetables such as for salads. Clean liquid spills in the refrigerator, especially spills from items potentially connected with *E. coli*.

CHAPTER THREE

3.0 METHODOLOGY

3.1 SAMPLE COLLECTION

Samples of fresh produce (fruits and vegetables) where collected from different location in Lagos and Ogun state. This fresh produce consists of lettuce(L), cabbage(B), Cucumber(C), Carrots(R), Pawpaw(A), Pineapple(P), Watermelon(W) from roadside/street vendors from different areas in Lagos and Ogun e.g., Jakande, Ibafo, Yaba, Magodo, Magboro, these various fruits were sampled thrice in this location to test for the microbial load.

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.

3.2 Materials and Equipment's

The following material and equipment were used: spatula, weighing balance, cotton wool, 70% ethanol, hockey stick, 90% ethanol, beaker, test tube, conical flask, aluminum foil, test tube rack, measuring cylinder, water bottle, autoclave, Bunsen burner, incubator, pipette, inoculating loop, distiller, gel electrophoresis tank, gel documentation, heating block, vortex mixer, centrifuge

3.3 Culture media and reagent

The following media and reagent were used: Sorbitol MacConkey (SMAC), MacConkey agar (MAC). Nutrient Agar (NA), Buffered peptone water (0.1 and 1%), 20% glycerol, Nuclease free water, 1X TAE buffer, ethidium bromide, distilled water, Brain Heart Infusion (BHI)

3.3.1 Buffer peptone water

Peptone water is a microbial growth medium made up of the peptic digest of animal tissue and NaCl. The medium is alkaline in nature at 25 °C and is rich in tryptophan. Peptone water is also a nonselective A broth medium which could be used as a primary enrichment medium for bacterial growth.

<u>Preparation</u>

1. The dehydrated medium was dissolved in 1 litre of distilled water to make up 0.1% and 1% peptone water based on manufacturer's instructions in a conical flask and was mixed thoroughly. The conical flask is then closed with a 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 15mins.

3. 9ml of the 0.1% was then dispensed into various test tubes for serial dilution.

4. 225ml of the 1% was then dispensed into conical flask.

3.3.2 SORBITOL MACCONKEY AGAR(SMAC):

Sorbitol MacConkey agar was prepared according to the manufacturer's instruction for isolation and detection of *E. coli* O157:H7.

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 cotton wool that is wrapped in aluminum 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.

Note: This medium is reddish-purple in colour.

3.3.3 Nutrient agar

Nutrient agar was prepared according to the manufacturer's instruction for isolation and detection of total count of mesophilic organism.

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.

Note: The medium appears opalescent and is light amber in colour.

3.3.4 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 121^0C for 15minutes.

3.4 Sample Preparation

Twenty-five (25g) of the sample was put in a sterile stomacher bag containing 225ml of 1% peptone water (enrichment broth) and then homogenized using the stomacher at 180 rpm for 4 minutes after which serial dilutions were performed and appropriate dilutions were plated on Nutrient agar, SMAC and MacConkey agar plates.

3.4.1 Serial dilution

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

3.4.2 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°C- 37°C for 18- 24 hours and counted.

3.4.3 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. The colonies gotten from the previously incubated SMAC plates (white and pink) were sub cultured into Nutrient agar.

A loopful of the isolate was taken using the inoculating loop (the inoculating loop is heated using the Bunsen burner and allowed to cool before taking the loop from the original mixed culture and streaked onto the new petri-dish). For sub-culturing, the isolate containing loop is transferred to the new petri-dish using the streaking method procedure. The plates were inverted and incubated at 37^OC for 18- 24 hours.

3.4.4 Cryopreservation of isolate

A loopful of each 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 hour. After incubating, 750ul of the inoculum was added into a sterile Eppendorf tube containing 750ul of sterile 20% glycerol (duplicated) which acts as a cryoprotectant and was stored in a freezer at -4 ℃.

3.5 BIOCHEMICAL TEST

3.5.1 Gram staining

A smear of suspension was made on a clean, grease-free slide using a loopful of the isolate. It was air dried and then heated to fix it. Drops of crystal violet were poured and held for approximately 30 seconds before being rinsed with water. It was then rinsed with water after being flooded with gram's iodine for 1 minute. 95 percent alcohol was added for 10-20 seconds before being rinsed with water. After about 1 minute, safranin was added and rinsed with water. After that, it was air dried and examined under a microscope.

3.5.2 Catalase Test

Using an inoculating loop, a small amount of the isolate was transferred to the surface of a clean, dry glass slide, a drop of 3% H_2O_2 was added and observed for the evolution of oxygen bubbles.

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3.5.3 Oxidase Test

An oxidase disc was used. An isolated colony to be tested was picked and rubbed on the disc. It was observed for color change within 10 seconds.

3.6 MOLECULAR IDENTIFICATION

3.6.1 DNA Extraction

Isolates from the same sample were pulled (1ml of BHI was added to a cryotube and autoclaved. 50ul of each isolate E1-E4 was added into the cryotube to activate). The pulled isolates were centrifuged at 10,000RPM for 5minutes and the supernatant was decanted, 1ml of sterile distilled water was added to the Eppendorf tube, vortexed and centrifuged again at 10,000 RPM 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°C for 15minutes, it was then placed in ice to cool, the content of the Eppendorf tube was then centrifuged finally at 14,000RPM for 5 minutes, a new set of Eppendorf tubes were labelled and the supernatant containing the extracted DNA was then transferred into the new Eppendorf tubes and they were placed in racks and stored in the freezer at -20°C for further analysis.

3.6.2 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	2ul
2	16sf	20um	0.25um	0.125ul
3	16sr	20um	0.25um	0.125ul
4	DNA			2ul
5	dH$_2$O			5.75ul
6	Total			10ul

Table 3.3 PROTOCOOL FOR THERMALCYCLER

Analysis	Step	Temperature	Time
1x	Initial denaturation	95^0c	5 min
35x	Denaturation	95^0c	2 min
	Annealing	55^0c	30 sec
	Elongation	72^0c	4 min
1x	Final Elongation	72^0c	10 min
1x	Hold	4^0c	∞

Table 3.4 Components used for Multiplex PCR

No.	Reagents	Initial concentration	Final concentration	Volume/rxn
1	Master mix	5x	1x	5ul
2	Stx1F	20uµm	0.25um	0.3125ul
3	Stx1R	20um	0.25um	0.3125ul
4	Stx2F	20um	0.25um	0.3125ul
5	Stx2R	20um	0.25um	0.3125ul
6	eaeAF	20um	0.25um	0.3125ul
7	eaeAR	20um	0.25um	0.3125ul
8	dH_2O			15.125ul
9	DNA			3ul
				25

3.6.3 Gel Electrophoresis Using Agarose

The agarose was prepared using dry agarose powder, 1.8g of the agarose powder was dissolved in 100ml of 1x TAE buffer the mixture was then boiled until a clear solution was gotten. 3µl of ethidium bromide was added to the mixture using a micropipette. It is then swirled and left to cool but not solidify, the content of the flask is then transferred into the gel container with the combs in place, after, it is left to solidify and the comb is gently removed. 1x TAE buffer is poured into the gel container. 3ul of DNA ladder as added to the fist well and 4ul of the amplicon (one sample per well) were then pipetted into each well that was formed after removing the comb. The tank is connected to the power pack and left to run at 100 volts for 45mins and the gel is viewed using the gel documentation system for result

3.7 Precaution

1. Ensure you wear lab coat always in the laboratory
2. Ensure you label your sample
3. Ensure you perform aseptic techniques before your experiment
4. Ensure you wash your hand before doing any experiment
5. Ensure u sterilize your equipment's before using them.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 RESULTS

The microbial analysis of the fresh produce samples gotten from Lagos and Ogun state were reported. All samples had pink and white, raised, circular and smooth colonies on SMAC and MAC. Which indicates the presence of *E. coli* in the samples.
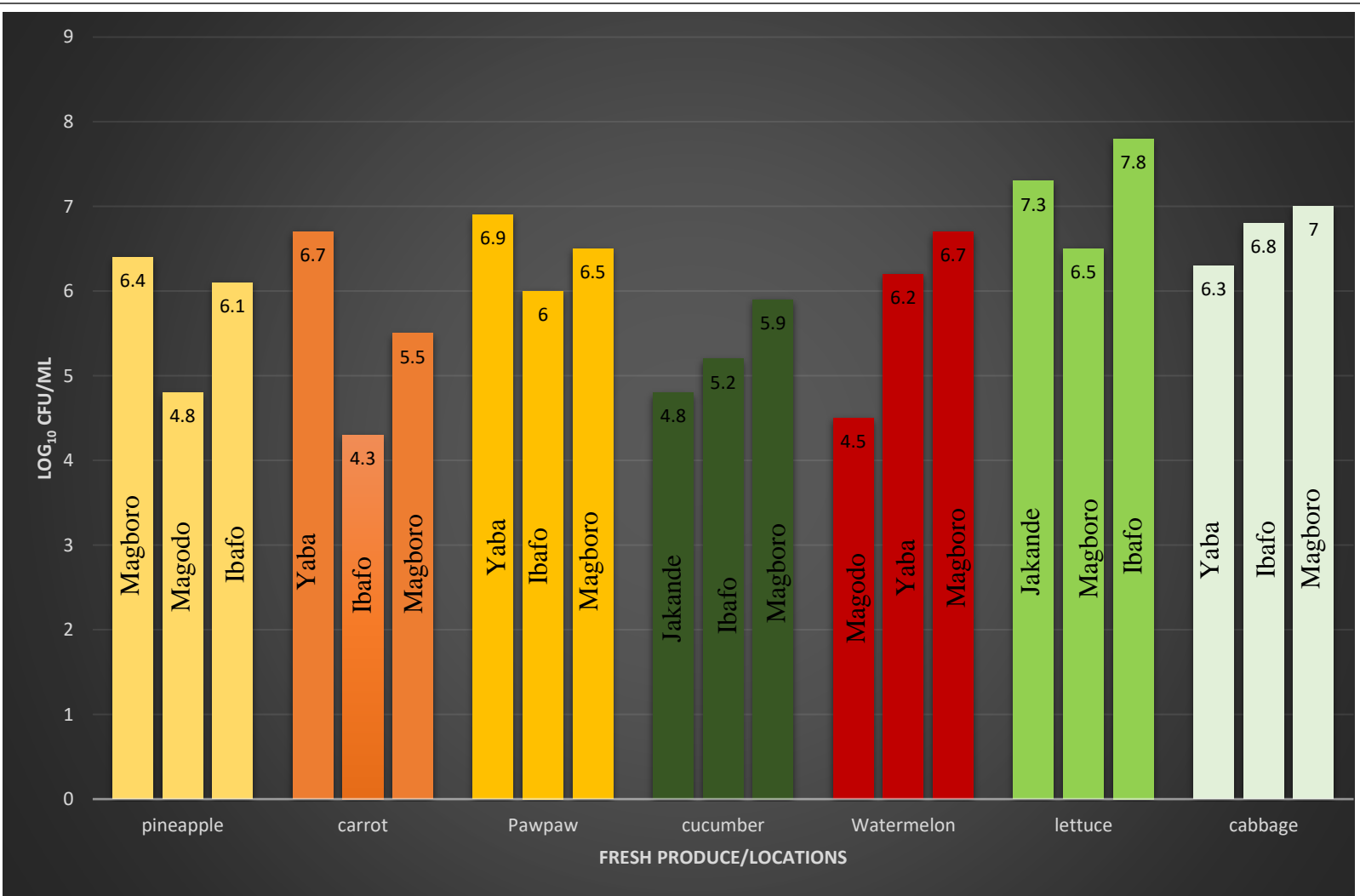
Lettuce had the highest TVC range of 6.5- 7.8 $\log_{10}$ cfu/g followed by cabbage with a TVC range of 6.3- 7.0 $\log_{10}$ cfu/g. The TVC IN Pawpaw range between 6.0- 6.9 $\log_{10}$ cfu/g, while by Water melon had TVC range of 4.5- 6.7 $\log_{10}$ cfu/g, lower TVC was recorded in cucumber which ranged 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.
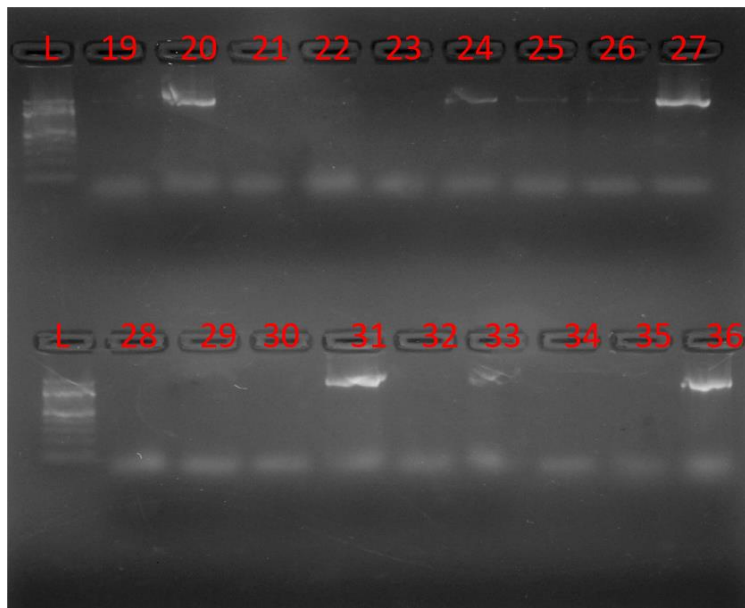
Figure 4.1 Agarose Gel electrophoresis of PCR amplicon for (16 rRNA amplification.

For PCR multiplex

21 isolates were randomly picked for further examination by PCR for the detection of stx_1, and/or stx_2 and eae genes. It showed that one isolate (magboro watermelon) contained stx_1 gene, which makes it Shiga toxin producing *E. coli* positive. Agarose gel electrophoresis of PCR products for *eaeA, stx₁*, stx_2 and are shown in figure 4.2
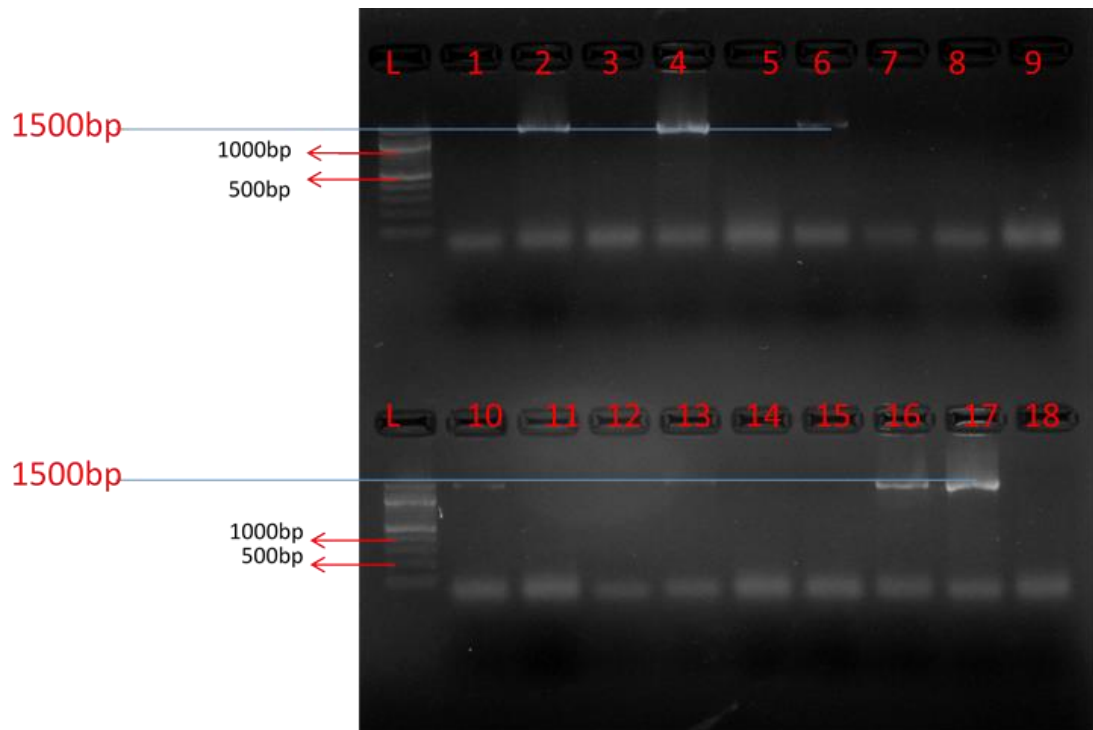
16S RDNA for E.COLI 38-58 no 51
52 53

Figure 4.2 Agarose gel electrophoresis for Multiplex PCR

Table 4.1 All fresh produce samples had the same biochemical tests results.

Biochemical tests	Result and observation
Catalase test	**Positive-** presence of bubbles
Gram staining	Gram negative positive (red colour)
Oxidase test	Negative (no change in color)

4.2 DISCUSSION

Nigeria's microbiological quality, in general, is a major source of concern. Because the infectious dosage of *E. coli* O15:H7 is so low, it can cause infections even at small concentration

that are undetectable (Eric *et al*., 2008; Wilshaw *et al*., 1994). The presence of *E. coli* O157:H7 means recent fecal contamination as well as the presence of other enteric pathogens that are known to cause food-borne gastroenteritis and bacterial diarrhea illness (Jiwa *et al*., 2005; Adebayo-Tayo *et al*., 2012). As a result, the isolation of this bacteria in fresh produce constitutes a food safety concern. The high microbial load, which is more than the standard bacterial counts suggested by the World Health Organization, indicating that all of the samples were contaminated with bacteria (WHO, 2006). The high overall bacterial count is likely due to unsafe methods from farm to fork, as well as exposure to potential microbial contamination at every step, including cultivation, harvesting, shipping, packaging, storing, and selling to ultimate customers (Heaton and Jones, 2008), (Gultie and Sahile,2013; Victor *et al*., 2017).

The presence of these pathogens in ready-to-eat fresh produce can cause gastroenteritis due to the bacteria's release of enterotoxins, and can even cause the fruits and vegetables to spoil if their population is high (Adjrah *et al*., 2013; victor *et al*., 2017). *Escherichia coli* was found in all of the samples, with Ibafo Cabbage and Magboro Cabbage having the highest viable counts of 6.8 and 7.0, respectively, and Magodo watermelon having the lowest (4.5). The presence of *E. coli* in these samples means fecal contamination from manures, as well as poor post-harvest cleaning by handlers to remove soil and debris (Maika *et al*., 2018). It could also be the result of improper hand washing by the handlers, or contamination of utensils and preparation surfaces (Beuchat, 2002). To lessen microbial load, all ready-to-eat   fresh produce should be thoroughly washed before consumption (Gultie *et al*., 2013).

In a microbiological assessment of fresh produce conducted in 2003 and 2004, (Mukherjee *et al*., 2004) failed to isolate *E. coli* O157:H7, which was one of their target harmful bacteria. Another Norwegian investigation that aimed to detect *E. coli* O157:H7 in lettuce was unsuccessful (Loncarevic *et al*., 2005). (Sagoo *et al*., 2001) examined the microbiological quality of ready-to-eat fresh produce, including 86 carrots, mushrooms, cherry, pepper, and alfalfa sprouts, as well as 3200 samples of broc*coli*, cabbage, celery, lettuce, radish, and other vegetables. They found no *E. coli* O157:H7 in any of the fresh produce samples they examined. As a result, the presence of faecal organisms such as *E. coli* is a better indicator of fresh food quality, which may explain why *E. coli* has been added as a hygienic criterion in the new EU legislation (Jeddi *et al*., 2014).

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATION

Fresh produce continues to be responsible for the highest number of foodborne illness outbreaks. Some patients with STEC infection develop hemolytic uremic syndrome (HUS), characterized by renal failure, hemolytic anemia, and thrombocytopenia that can be fatal. The Result revealed that only one was positive for STEC. However, a high proportion of fresh produce sold by the road side food vendors for human consumption was still contaminated by *E. coli* (probably other pathotypes) which remains a public health concern. Therefore, there is a possible risk to humans from consumption of these products which can lead to other illnesses such as hemorrhagic colitis (HC) or hemolytic-uremic syndrome (HUS).

5.1 RECOMMENDATION

This can be eradicated by educating the farmers and all fresh produce handlers in the food chain, and the vendors on the food safety risk involved with unhygienic practices and the consequences. Selling of fresh produce in open places should be eradicated and a fine should be placed on it for those who don't obey the rule. It is also important that fruits and vegetables are wash properly, especially if they are eaten raw. In addition, to prevent foodborne infections linked with fresh produce, it is vital to prevent initial contamination as well as decrease, eliminate, and prevent pathogen amplification. Fresh produce is challenging to regulate because fruits and vegetables are both raw agricultural commodities and ready-to-eat consumables. Proper cleanliness is essential at all stages of the fresh produce cycle, from farm to table. This involves avoiding the use of untreated manure as fertilizer, providing suitable sanitary systems and hand-washing facilities for field workers, using clean equipment and transportation vehicles, and maintaining good hygiene in processing facilities and the kitchen; and precautions to avoid cross-contamination. Persons with diseases that can be transferred through food contamination should avoid handling produce until they are no longer infectious. There is also a need for better foodborne illness surveillance. Regional and national surveillance systems are required for the discovery of diffuse clusters as well as the examination of 'sporadic' occurrences of Fresh produce food-borne disease.

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