PROFILE OF PATHOGENIC *ESCHERICHIA COLI* ASSOCIATED WITH THE SOIL OF VEGETABLE FARM

BY

OLUGBENRO OYINKANSOLA ELIZABETH

17010101006

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(B.Sc.) Hons DEGREE IN MICROBIOLOGY

AUGUST, 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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OLUGBENRO OYINKANSOLA

_____

DATE

CERTIFICATION

This is to certify that this project report titled "**PROFILE OF PATHOGENIC *ESCHERICHIA COLI* ASSOCIATED WITH THE SOIL OF VEGETABLE FARM"** was carried out by OLUGBENRO, Oyinkansola Elizabeth with matriculation number 17010101006 of Microbiology in the Department of Biological Sciences, in partial fulfillment of the requirement for the award of Bachelor of Science (B.Sc.) Degree in Microbiology.

……. …….

DR OPERE B. DATE

PROJECT SUPERVISOR

…… …….

Dr. O.T. KAYODE 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.

ACKNOWLEDGEMENTS

The success of this work was made possible because of God and his un-dying love and help rendered to me through a number of notable people. To the lord, for his Grace and Mercy over my life and academics which am forever grateful to.

I would like to appreciate the effort of my wonderful supervisor, DR. OPERE who through DR. ABIALA supervised this work. Thank you Mummy OPERE, we appreciate you.

I would like to appreciate Mr. Okunbi, Miss Joy, Miss Ronke and every lecturer who impacted knowledge into me and the entire administration of the Department of Biological Sciences, I would like to express my gratitude to my parents and my lovely siblings for helping me a lot throughout my stay in school.

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ABSTRACT

Escherichia coli is a harmful bacterium found with vegetables that has been linked to illness outbreaks all over the world. *E. coli* may be found in a variety of places, including vegetables. The soil of vegetable fields, on the other hand, has not been tested for the presence of pathogenic *E. coli.* As a result, our research is based on soil from vegetable fields that has been routinely treated with poultry manure. To determine the *E. coli*, a molecular method was used, specifically the utilization of 16S rRNA and multiplex PCR. On SMAC, all five isolates were identified as potential enterobacteriaceae containing *E. coli.* Further identification with 16S rRNA revealed that *E. coli* was present in four of the five possible enterobacteriaceae. Using shiga toxin primers and multiplex PCR, the *E. coli* was found to be non-pathogenic. In conclusion, there are no harmful *E. coli* present, however there are *E. coli* strains that use 16S rRNA but do not possess the shiga toxin gene. This research should be expanded to include additional areas.

CHAPTER ONE

1.0 INTRODUCTION

According to numerous studies, vegetable contamination with Shiga toxin-producing Escherichia coli and other bacteria poses a significant risk to human health. Contamination is recognized as a cause of produce contamination in vegetable production and marketing methods such as raw animal manure usage, contamination and cross contamination by harvesting and packing equipment, and worker health and hygiene. Furthermore, due to frequent microbiological contamination, irrigation water is believed to be a significant route in crop contamination (Liu, 2003).

Although food production standards are widely adopted in developed countries, they are more difficult to implement in developing countries, where clean water is frequently scarce and vegetable production is frequently carried out by farmers with little formal education, on small plots of land, and with limited resources. Vegetable contamination with human diseases can be substantial due to flaws in food safety standards across the value chain. Vegetable-related ailments are becoming a growing public health concern in Nigeria Udo et al (2009)

Fresh vegetable consumption has risen significantly in recent years as a result of many nutrient and functional characteristics contributions. In the previous three decades, the average amount of fresh produce consumed per person in the United States has grown by 25%. (Liu, 2003). A diet rich in fruits and vegetables has been shown to reduce the risk of cancer and chronic illnesses such as coronary heart disease. Fresh produce consumption, on the other hand, has been related to an increase in the incidence of foodborne outbreaks due to bacterial contamination of these products. Castro-Rosas et al (2012). Lettuce, spinach, and fresh herbs are among the leafy greens most frequently connected to bacterial illnesses. The Food Safety Project estimated that at least 713 produce-related outbreaks were linked to foodborne disease in the United States between 1990 and 2005, with fresh fruits and vegetables accounting for 12 percent of all outbreaks. Between 2008 and 2010, the Advisory Committee on the Microbiological Safety of Food (ACMSF) documented 531 cases of reported sickness, including one death, in the United Kingdom. In the same year, an outbreak of Shiga toxin-producing *E. coli* (STEC) serotype

O104:H4 was detected in Germany; by the end of the outbreak, 3785 instances of sickness had been documented outside of Germany, with contaminated sprouted seeds being blamed for the foodborne outbreak Goodburn and Wallace (2013). It is important to note that the impact of foodborne infections extends beyond the sick person to include significant economic consequences. On the one side, there are expenditures associated with a sick person, such as medical care and missed work or education. On the other side, there are societal costs, such as lower worker productivity, outbreak-related research costs, income loss due to food company closures, legal costs for disease-related litigation, and public medical-service expenditures. The way crops are harvested, processed, and distributed has been found to increase both the availability and variety of products, potentially increasing the danger of more widespread outbreaks. The rise in sickness connected to fresh produce consumption coincides with an increase in food contamination, according to reports. Luna-Guevara and colleagues (2015)

Pathogenic bacteria, viruses, and protozoa may contaminate fresh vegetables, resulting in foodborne disease (Alam et al., 2015; Iturriaga et al., 2007; Harris et al., 2003; Bilek and Turanta, 2013). This contamination can come from manure, soil, sewage, surface water, or wildlife, and it can also happen during food processing such as washing, slicing, soaking, and packaging, according to Liu et al (2013). Among the bacteria associated with foodborne illnesses are *Listeria monocytogenes* Zhu et al (2017), *E. coli* Heiman et al (2015), *Shigella soney* Lynch et al (2009), *Salmonella* Mba-Jonas et al (2011) and *Staphylococcus aureus* Faour-Klingbeil et al (2016). These microorganisms' survival and growth are influenced by a number of variables, including the microorganism's unique characteristics, fruit maturity, environmental circumstances, plant development, bacterial resistance to plant metabolic activities, as well as harvest and postharvest procedures. Some pathogenic bacteria, in particular, can internalize and cling to the plant surface Liu et al (2013).

1.1 JUSTIFICATION OF STUDY

It is hypothesized that soil borne *E. coli* associated with vegetable farm fertilized with organic manure such as poultry manure can be of public health implication of vegetable farmers and

consumers of the fruit. However it has not been established that the soil harbors pathogenic *E.coli*

1.2 OBJECTIVES OF STUDY

On this note the objective of this study

- Enumerate possible *E .coli* and associated enterobacteriaceae in soil of vegetable farms
- To identify the pathogenic *E. coli* using molecular identification

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 ETIOLOGY OF *Escherichia coli*

Escherichia coli, also known as *E. coli*, is a Gram-negative, facultatively anaerobic, rod-shaped coliform bacteria found in the lower intestine of warm-blooded animals (endotherms). This microorganism was first described by Theodor Escherich in 1885. The majority of *E. coli* strains are found as natural flora in the gastrointestinal tracts of humans and animals. However, virulence factors acquired via plasmids, transposons, bacteriophages, and/or pathogenicity islands have resulted in the development of pathogenic *E. coli* strains. Pathogenic *E. coli* can be classified using serogroups, pathogenicity mechanisms, clinical symptoms, and virulence factors. Although the majority of *E.coli* strains are innocuous, certain serotypes (EPEC, ETEC, and others) may cause acute food poisoning in their hosts and are occasionally responsible for food contamination events that result in product recalls. The innocuous strains are part of the normal gut microbiota and can benefit their hosts by generating vitamin K2 (which aids blood clotting) and preventing harmful bacteria from colonizing the intestine, forming a mutualistic connection. E. coli is released into the environment through feces. The bacteria multiplies rapidly in fresh feces under aerobic conditions for three days, but then steadily decreases in numbers Kaper et al (2004). *E. coli* and other facultative anaerobes make up around 0.1 percent of the gut microbiota, and fecal–oral transmission is the most common way for pathogenic *E.coli* strains to spread illness. Because cells can only survive for a short period of time outside the body, they might be used to screen environmental samples for fecal contamination. Environmentally persistent *E. coli*, which can survive for days and develop outside of a host, is the subject of a growing corpus of study. The bacterium may be readily and inexpensively cultivated and cultured in a laboratory setting, and it has been studied extensively for over 60 years. *E. coli* is a chemo heterotroph that requires a carbon and energy source in its chemically specified medium. *E. coli* is the most frequently researched prokaryotic model organism, as well as an important species in biotechnology and microbiology, where it has acted as the host organism for the bulk of recombinant DNA research. It takes as little as 20 minutes to replicate under ideal conditions. Kaper et al., (2004).

2.2 ESCHERICHIA COLI AS HUMAN PATHOGEN

Escherichia coli contains both commensal and pathogenic strains that cause a wide range of human illnesses, resulting in over 2 million deaths each year (Kaper et al. 2004). Shiga toxin-producing *E.coli* (STEC), Enteropathogenic *E.coli* (EPEC), Enterotoxigenic *E.coli* (ETEC), Enteroaggregative *E. coli* (EAEC), diffusely adherent *E. coli* (EAEC), and Enteroinvasive *E. coli* (EIEC), including Shigella strains, are the six well-studied intestinal pathotypes of *E. coli*. The virulence characteristics and pathogenicity mechanisms that cause gastrointestinal disorders like diarrhea are used to classify these bacteria (Nataro and Kaper 1998; Kaper et al. 2004).

STECs like enterohaemorrhagic *E. coli* (EHEC) can cause serious enteric illnesses including haemolytic uraemic syndrome and haemorrhagic colitis, which can lead to abrupt renal failure and death. The most well-known serotype of EHEC, *Escherichia coli* O157:H7, has been linked to several outbreaks of water- and food-borne illnesses in a variety of countries. Non-O157 STEC, including those caused by serotypes O26, O45, O103, O111, O121, and O145, has been on the rise in recent years (Farrokh et al. 2013). Many waterborne epidemics have been linked to pathogenic *E. coli* strains, and STEC and EPEC have been blamed for numerous outbreaks across the world (Chandran and Mazumder 2015). Manure and other animal wastes, slaughterhouse wastewaters, and effluent from wastewater treatment facilities can all cause pathogenic *E. coli* contamination in the environment, according to Baliere et al (2015). Although several research have been conducted on the clinical features of pathogenic *E. coli* strains, such as pathogenesis, diagnosis, and sources, their presence in the environment has not been well investigated (Kaper et al. 2004; Croxen et al. 2013).

2.3 PLANTS ASSOCIATED WITH *ESCHERICHIA COLI*

Sprouts and green leafy vegetables are the most prevalent vegetables linked to *E. coli* STEC. The seed that is utilized might be a cause of infection in sprouts. Contaminated water (drag water from livestock yards or water contaminated by other sources) appears to be the most prevalent source of contamination in leafy greens. Bacteria can survive and are active in the rhizosphere, or the area around the plant roots, of lettuce and radishes. *E. coli* eventually gets onto the

aboveground surfaces of the plants, where it can live for several weeks Luna-Guevara et al (2019).

Tomato, Chile, Onion, Lettuce, Arugula, Spinach, Cilantro,Ccarrots, and other raw vegetables are used in a variety of fresh meals, including salads and sauces. These foods provide a high nutritional value to the human diet when consumed. However, the frequency of foodborne outbreaks linked to fresh produce is on the rise, with *Escherichia coli* being the most prevalent pathogen involved Luna-Guevara et al (2019).

E. coli can survive for weeks around the roots of produce plants before transferring to the edible parts, but producers can reduce the risk by not harvesting too soon. Luna-Guevara et al (2019).

2.4 PATHOGENIC *ESCHERICHIA COLI* ASSOCIATED WITH FERTILIZED SOIL OF VEGETABLE FARM

Several reports have shown that vegetable contamination with Shiga toxin–producing *Escherichia coli* and other microorganisms are a significant threat to public health De Roever et al (1998). Contamination is acknowledged as a cause of produce contamination in vegetable production and marketing methods such as the use of raw animal manure, contamination and cross contamination by harvesting and packing equipment, and worker health and hygiene (Beuchat and Ryu, 1997). Furthermore, irrigation water is thought to be a significant route in crop contamination due to frequent microbial contamination Umoh et al., (2001). The primary pre-harvest contamination causes are thought to be soil and poorly composted animal dung. Because of the presence of animal feces, soil is a natural repository for a wide range of human diseases, including pathogenic *E. coli*. Depending on the soil type, humidity level, and temperature, *E. coli* O157:H7 can live in the soil for 7 to 25 weeks. Launders et al., (2016). This bacteria can also survive in the storage and distribution of crops. The presence of STEC O157 in vegetables, according to Launders et al., poses a danger since it may promote cross contamination with other raw foods. Furthermore, the use of animal manure is widespread in organic food production; numerous publications link this type of agricultural system to the prevalence of fecal contamination, particularly during the harvest of leafy vegetables. The intake of organic spinach infected with STEC O157 has been linked to outbreaks in many US states, according to the Centers for Disease Control and Prevention (CDC).

Domestic animals and wildlife can also be a source of pathogenic bacteria, especially for lettuce and leafy greens at pre-harvest stages along the California coast and in Yuma, where Bergeretal discovered that wild animal feces are involved in vegetable contamination and can cause *E. coli* O157:H7 outbreaks. Jay-Russell et al looked at a possible reservoir for pathogenic *E. coli* in coyote and dog feces. Plant contamination might also come from insects. *E. coli* has been found to be transferred from contaminated flies to plant leaves and fruit. Lynch et al. also discovered that intensive farming methods had pushed crop fields too near to animal producing zones. The ecological consequences of this proximity have increased the likelihood of contamination by *E. coli* O157.H7 in wildlife Berger et al (2010).

CHAPTER THREE

3.0 MATERIALS AND METHODOLOGY

3.1 SAMPLING

Soil samples were collected from (Owode, Opic, Sango-ota) Ogun State, (Iwo road) Ibadan Nigeria shown in table 3. Samples were collected in sterile plastic bags and taken immediately to the laboratory for analysis.

3.2 MATERIALS

Materials used include: petri-dishes, beakers, conical flasks, glass spreader, 70% ethanol, scotch bottles, Eppendorf tubes, micro pipette (with their tips), test tubes (with their racks), PCR tubes

3.3 REAGENTS AND EQUIPMENT USED

Equipment used: Autoclave, weighing balance, distiller, wash bottles, water bath (set at 50°C and 100°C), incubator (set at 37°C), Bunsen burner, oven, inoculating loop, gel electrophoresis tanks, UV Transilluminator

3.4 MEDIA USED

Media used for *Escherichia coli*: Nutrient Agar, MacConkey Agar and Sorbitol-MacConkey Agar (SMAC), Buffer peptone water, Eosine Methylene Blue agar (EMB).

3.5 ISOLATION OF *E. coli*

3.5.1 Sample preparation

Exactly 1g of the samples were weighed and poured aseptically into a sterile stomacher bag containing 9ml of 1% BPW (enrichment broth) and then homogenized using the stomacher at

180 rpm for 2 minutes after which serial dilutions were performed and appropriate dilutions were plated on EMB, SMAC and MacConkey agar plates.

3.5.2 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} to the dilution factor $10^{-7.}$ The test tubes were labeled for easy identification.

3.5.3 Plating

After labelling the agar plates accordingly, 0.1 ml from the appropriate dilutions (10^{-1}-10^{-7}), 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.

3.5.4 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) 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^{O}C for 18- 24 hours.

3.5.5 Cryopreservation of isolate

A loopful of each isolate (white) 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. 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 ºC.

3.6 BIOCHEMICAL TEST

3.6.1 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. Drops of crystal violet were poured and held for 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 percent 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.

3.6.2 Catalase Test

A little amount of the isolate was put to the surface of a clean, dry glass slide, a drop of 3 percent H2O2 was added, and the evolution of oxygen bubbles was monitored using an inoculating loop.

3.6.3 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.

3.7 MOLECULAR IDENTIFICATION

3.7.1 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^{o}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^{o}C for further analysis.

3.7.2 Polymerase chain reaction (PCR)

The components of the PCR used for *E. coli* identification is shown in table 3.2 and 3.4 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 in table 3.3 below. 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.

3.7.3 Agarose gel electrophoresis

The agarose was prepared using dry agarose powder, 1g of the agarose powder was then dissolved in 50ml of 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 swirled and left to cool but not solidify, the content of the flask is then transferred into the gel cast with the combs in place, after, it is left to solidify and the gel is gently removed and put in an electrophoresis tank containing TAE buffer. 4ul of the PCR products are then pipetted into each well that was formed after removing the comb. The tank is connected to the power pack and left to run till it gets to one-third of the gel and then it is turned off and the gel is viewed under the UV transilluminator.

Table 3.1: showing location and soil samples.

Sample	**Location**
Soil samples	Owode (O)
Soil samples	Opic (O)
Soil samples	Sango-ota (O)
Soil samples	Iwo road (I)
Soil samples	Owode (OD)

Key notes: (O)- Ogun state, (I)- Ibadan

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_2O			5.75ul
6	Total			10ul

Table 3.3: PROTOCOL FOR THERMOCYCLER

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	∞

Table 3.4: Components used for Multiplex PCR for STEC

No.	Reagents	Initial concentration	Final concentration	Volume/rxn
1	Master mix	5x	1x	5ul
2	Stx1F	20um	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_20			15.125ul
9	DNA			3ul

Table 3.5: Primers used for detection of STEC

gene	Oligonucleotide sequence (5'-3)	Size	Reference
Stx1	GAAGAGTCCGTGGG ATTACGAGCGATGCAGCTATTAATAA	130bp	Paton and Paton, 1999
Stx2	ACCGTTTTTCAGATTTTGACACATA TACACAGGAGCAGTTTCAGACAGT	298bp	Paton and Paton, 1999

gene	Oligonucleotide sequence (5'-3)	Size	Reference
eaeA	GTGGCGAATACTGGCGAGACT CCCCATTCTTTTTCACCGTCG	890bp	Paton and Paton, 1999

CHAPTER FOUR

4.0 RESULTS

The microbial analysis of the soil samples gotten from Ogun and Ibadan state were reported. All samples had pink and white, raised, circular and smooth colonies on selective media SMAC. Which indicates the presence of *E. coli* in the sample shown in Table 4.1 and 4.2 respectively. The total viable count was highest in Iwo-road and Owode with a count of 6.3 Log_{10}cfu/g for the dilution factor of 10^{-3} and Iwo road has the highest TVC of 7.9 Log_{10}cfu/g for the dilution factor of 10^{-5} and Opic has the highest TVC of 9.2 Log_{10}cfu/g for the dilution factor of 10^{-7}.

The result for the biochemical test in Table 4.2 shows that gram staining for all isolates was negative indicating its gram negative bacteria, for catalase all isolates were positive and oxidase all isolates were negative.

This study reports on the isolates of *Escherichia coli* from soil of vegetable farm with organic manure (poultry dung) using selective medium SMAC, PCR amplification using 16s, stx1, stx2, eaeA forward and reverse primers. Owode soil (SOS) had the highest total viable count of 7.8 log_{10}cfu/g. According to the results, four isolates had *E. coli* (using 16s rRNA) in them shown in Figure 4.2. The four isolates were picked for STEC genes identification by PCR and none of the 4 *E. coli* isolates was positive for stx1 gene at 100bp shown in Figure 4.3. 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 antimicrobials agent if the infection is identified early it is fully curable.

This study represented a systematic approach to examine the soil value chain in western Nigeria (Ogun state and Ibadan) for the magnitude and frequency of vegetable fruits with contamination of total coliforms and *E. coli* from organic manure (poultry manure). This study demonstrated that vegetable farm fertilized with organic manure contamination with total coliforms and *E. coli* is endemic in western Nigeria, with a dramatic increase in contamination as vegetables progressed from farm to market.

Table 4.1: The dilution factor plated on SORBITOL MACCONKEY AGAR and number of colonies observed.

ISOLATES LOCATION	SMAC (WHITE AND PINK COLONIES) 10-3	SMAC (WHITE AND PINK COLONIES) 10-5	SMAC (WHITE AND PINK COLONIES) 10-7
SIK(IWO-ROAD)	200 80(P)120(W)	81 43(P)38(W)	12 12(P)
SOS(SANGO-OTA)	164 85(P)79(W)	52 30(P)22(W)	11 7(P)4(W)
SOW(OWODE)	190 40(P)50(W)	74 49(P)25(W)	15 5(P)
SOP(OPIC)	105 60(P)45(W)	54 32(P)22(W)	16 16(P)
SOWD(OWODE)	130 75(P55)W)	58 30(P)28(W)	10 6(P)4(W)

Note: (W) white colonies on SMAC, (P) pink colonies on SMAC.

Table 4.2: Morphological Characteristics of Isolates on Sorbitol MacConkey Agar

Isolate	Sample	Isolate ID	Color	Shape	Size	Elevation	Appearance	Surface	Opacity
1st sample	Soil	SID SMAC 1	Pink and White	Circular	Small	Raised	Smooth	Smooth	Opaque
2nd sample	Soil	SOS SMAC 1	Pink and White	Circular	Small	Raised	Smooth	Smooth	Opaque
3rd sample	Soil	SOW SMAC 1	Pink and White	Circular	Small	Raised	Smooth	Smooth	Opaque
4th sample	Soil	SOP SMAC 1	Pink and White	Circular	Small	Raised	Smooth	Smooth	Opaque

5th sample	Soil	SOWD SMAC 1	Pink and White	Circular	Small	Raised	Smooth	Smooth	Opaque

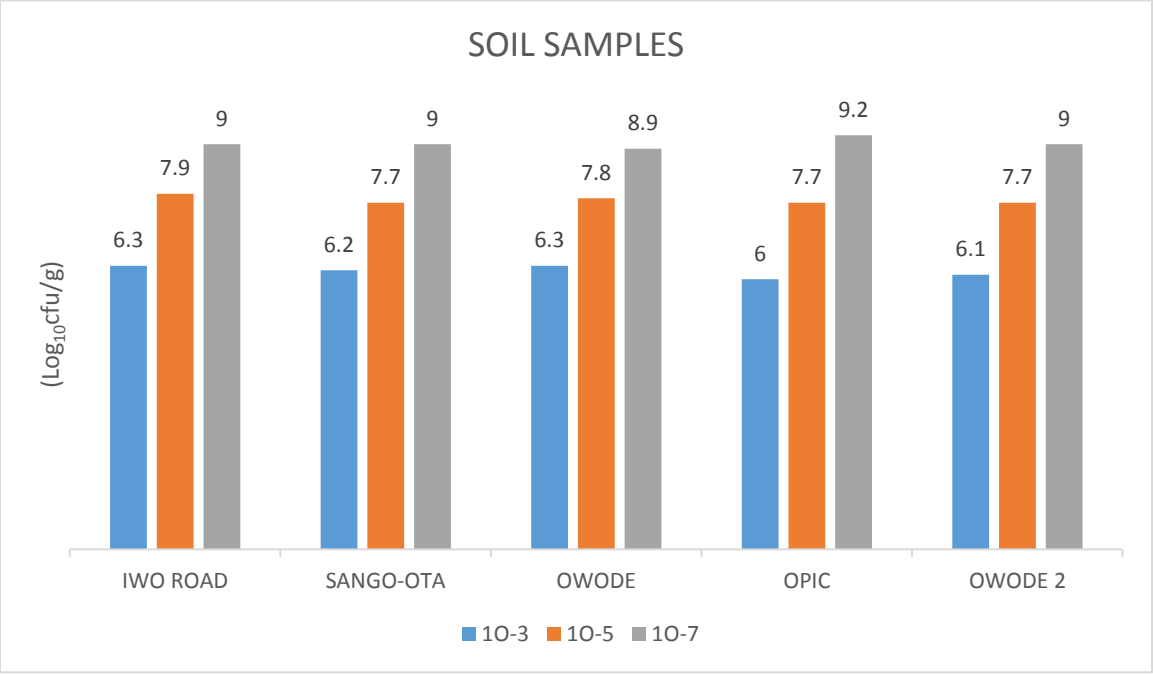

Figure 4.1: Histogram representation of the total viable count (Log_{10}cfu/g) for each sample from different location.

Table 4.2: Biochemical testing for *E. coli* organism.

CODE	**GRAM STAINING**	**CATALASE**	**OXIDASE**
SOW	Negative (-ve)	Positive (+ve)	Negative (-ve)
SOP	Negative (-ve)	Positive (+ve)	Negative (-ve)
SOWD	Negative (-ve)	Positive (+ve)	Negative (-ve)
SOS	Negative (-ve)	Positive (+ve)	Negative (-ve)
SID	Negative (-ve)	Positive (+ve)	Negative (-ve)

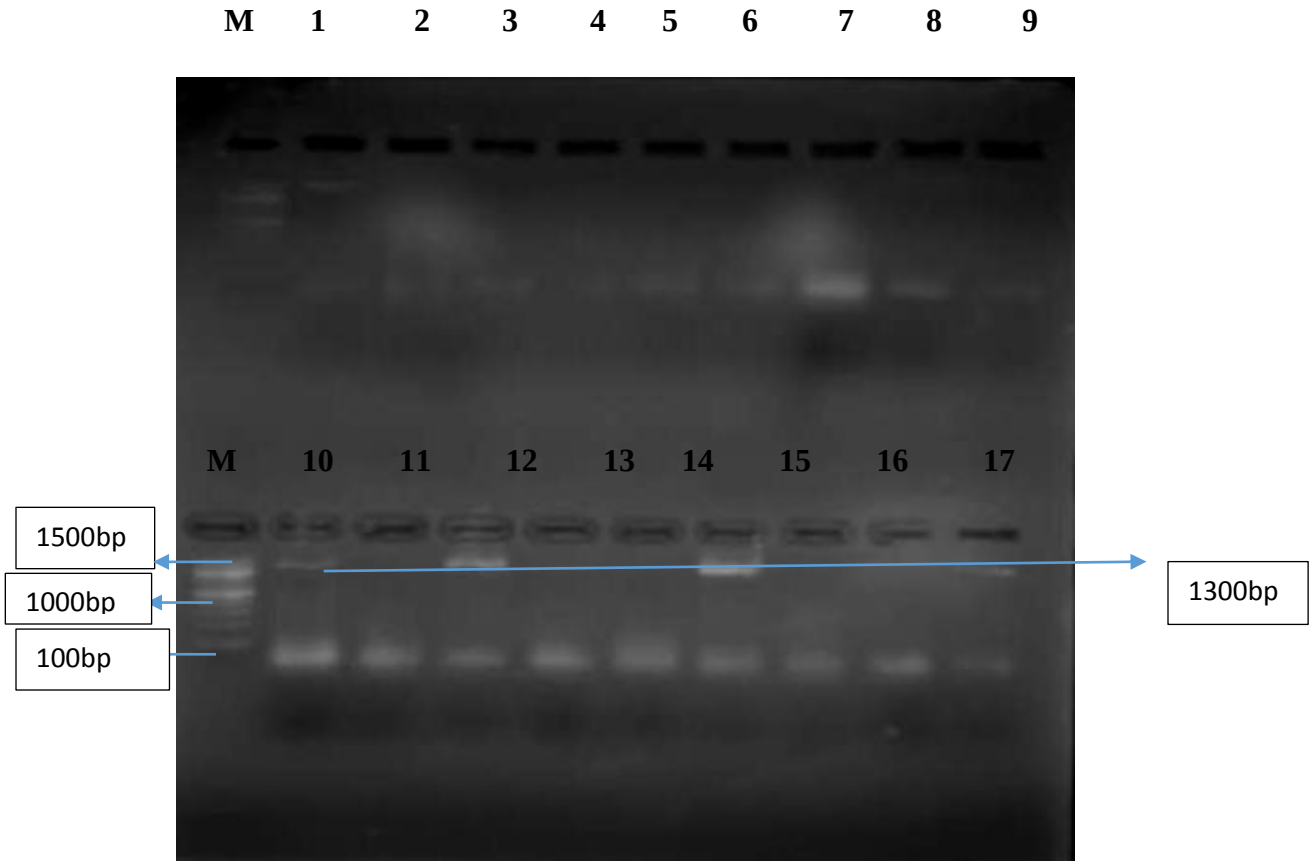

Figure 4.2: Agarose Gel electrophoresis of PCR amplicon for 16 sRNA gene of *E. coli.*
Lane M = DNA marker, lane 10-12 = isolates of probable pathogenic *E. coli* on SMAC, lane 15 and 18 = isolates of probable pathogenic *E. coli* on SMAC.

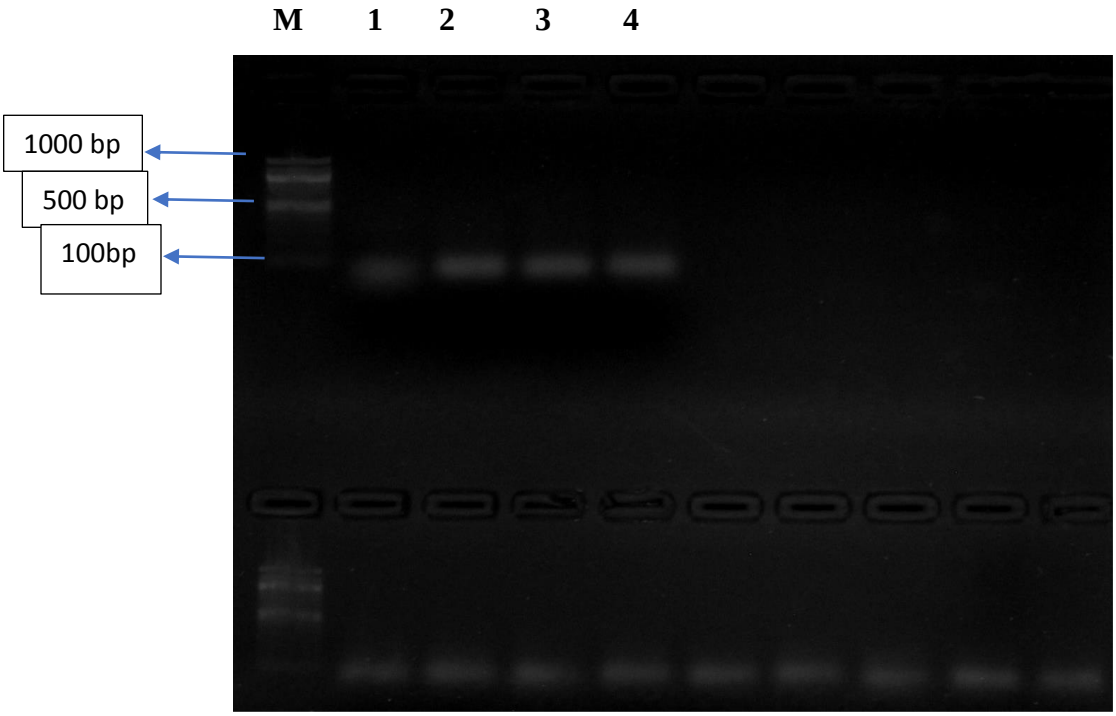

Figure 4.3: Agarose gel electrophoresis Multiplex PCR for detection of STEC

Lane M = DNA marker, lane 1-4 = fragments of positive isolates from 16s rRNA in figure 4.2

4.1 DISCUSSION

According to Kudva et al (1998) This study identified several possible pre-harvest tomato fruit contamination sources. Several reports have linked the use of raw manure in vegetable production to produce contamination with *E. coli*. Although manure was applied before to transplantation or during the first three weeks of transplanting, the duration between application and fruit ripening (80 to 100 days after transplanting) fell well short of the required minimum time limit (120 days (39) or 9 months (41) for pathogens to die off Ibekwe et al (2009). As a result, *E. coli* inoculums could still be present in the manure, contaminating tomato fruits throughout the growing season, according to Islam et al (2004). Furthermore, ruminant animals such as cattle and sheep are known to be *E. coli* reservoirs, shedding the bacteria in their feces Lau et al (2001). Their entry into tomato fields, which some farmers regarded as a sign of good fortune, offers a risk of *E. coli* contamination Erickson et al (2010).

The four E. coli isolates that tested positive for E. coli using 16s rRNA were chosen for STEC gene identification by PCR, but none of the four E. coli isolates tested positive for the stx1 gene. Multiplex PCR investigation of pure cultures of nontoxigenic E. coli O157:H7 strain 3704 found three separate bands of predicted size, corresponding to H7, intimin, and O15, according to Campbell et al (2001). For Shiga-like toxin I and Shiga-like toxin II genes, no PCR amplicons of the expected size were found, supporting prior evidence on the absence of toxin genes (by PCR) in this strain. By spiking different environmental samples with decreasing quantities of stationary phase E. coli O157:H7 and exposing spiked material to a primary and secondary enrichment prior to DNA extraction and PCR amplification, the sensitivity and robustness of the PCR-based assay were examined. In all of the environmental matrices studied, the assay was able to identify initial E. coli O157:H7 populations of 610 cfu g1 or ml1. Indeed, after enrichment, E. coli O157:H7 populations as low as 1 cfu ml1 were detected in private drinking water. The assay

performed well in a variety of soils, despite the fact that soil factors like humic content have been shown to hinder PCR (Tebbe and Vahjen 1993). PCR amplification in the different soils utilized in this investigation could have resulted from secondary enrichment dilution of PCR-inhibiting soil chemicals, while enrichment also served to boost populations of the target bacteria.

CHAPTER FIVE

5.0 CONCLUSION

In conclusion there is no presence of *E. coli* carrying shiga toxin gene but *E. coli* was detected using 16S rRNA.

This study should be furthered extended to other farms because globally food borne diseases caused by pathogenic microbes is inevitable.

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