INCIDENCE OF PATHOGENIC MICROORGANISMS IN CABBAGE AND CARROTS USED FOR COMMERCIAL STREET VENDED COLESLAW

BY

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CERTIFICATION

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……. **…….**

DR. G.B AKANNI **DATE**

PROJECT SUPERVISOR

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. Ambrose and Mrs. Juliana Okunbi for their guidance, understanding and sacrifice. I also dedicate this work to my course-mate and friends for their support in the course of my four years study of Microbiology in Mountain Top University. May the Almighty God bless you all! Amen

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ABSTRACT

There is an increased concern that vegetables might be more important as a carrier for human enteric pathogens like *Escherichia coli* and *Salmonella spp*. Microbes create a niche on the flora of vegetables such as cabbage and carrots and it has now become difficult to control them. *Escherichia coli* is a bacterium found in the environment, food, and intestines of both humans and animals. *Escherichia coli* cause serious food poisoning in their hosts. This study focused on pathogenic *E. coli* and *Salmonella* isolated from cabbage and carrots using SMAC and SS agar. The highest counts of pathogenic *E. coli* were 7.4 Log10 CFU/g and 7.0 Log10 CFU/g for cabbage and carrots respectively. *Salmonella* was detected in 25 g of carrot samples. Genotypic characterization confirmed the presence of this pathogens. In this study, carrots were considered as the major risk factor associated with the occurrence of *Salmonella* and *E. coli*. In addition, the study has demonstrated that Cabbages and carrot may contribute to the prevalence of *Salmonella* and *E. coli* illnesses in Magboro market, ogun state. Vegetables should be stored away from raw animals' products to avoid cross-contamination. Washing your vegetables properly before eating is highly recommended.

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CHAPTER ONE

1.1. INTRODUCTION AND PROBLEM STATEMENT

Vegetables are essential ingredients of most healthy diets, and the demand for vegetables has increased in recent years due to healthy lifestyle recommendations. (CDC 2010). Nutritionists emphasize the importance of vegetables in healthy diets, and researchers and governmental publicity campaigns around the world tend to recommend consumption of at least five servings of fruits, vegetables and salads (coleslaw) per day. In contrast to their health benefits, the consumption of fresh vegetables has also been associated with risk for consumers (Weldezgina and Muleta 2016). Vegetables, carrots and salads (coleslaw) are rich in carbohydrates, antioxidants, minerals, vitamins and fiber (Said 2012) and often consumed uncooked. The food of plant origin can act as effective media for the transmission of pathogens (Khan *et al*. 1992; Abougrain *et al*. 2010).Fruits and vegetables can become contaminated with pathogenic microorganisms, for example *Salmonella, Shigella, Campylobacter, Listeria, Escherichia coli* e.t.c) whilst growing in fields, or during harvesting, post-harvest handling, and distribution. Different agronomic practices can contaminate vegetables at various stages of the food chain. Contamination occurs mostly before harvesting, either by contaminated manure, sewage, irrigation water, and wastewater from livestock operations or directly from wild and domestic animals or during harvesting, transport, distribution, and marketing or even at home (Eraky *et al*. 2014; Maffei *et al*. 2016). Therefore, infections due to consumption of vegetables and salads which are minimally processed pose a great threat to consumers worldwide (Wadamori *et al*., 2017).

Over the past few years, the incidence of foodborne outbreaks from contaminated vegetables has risen. The pathogens most frequently linked to these product contamination and human illness are *Salmonella*, *Listeria monocytogenes* (Davis and Kendall, 2012), Shigella species which are mostly able to survive in shredded cabbage (US Food and Drug Administration, 2012).. Just recently there was an outbreak of *Salmonella l*inked to Karawan brand Tahini sesame seed paste which is often mixed with salad to make it tasty (CDC 2019). Reports on outbreaks from vegetables which is based on approximately 78 scientific papers and reports by CDC (Centers for Disease Control and Prevention), FDA (Food and Drug Administration) and WHO (World Health Organization) on the common pathogens topping the outbreak scale are *Escherichia coli*, *Salmonella*, *shigella*, *campylobacter* e.t.c . The commercial product most associated with outbreaks is the ready-to-eat salads (coleslaw), since it has all kinds of mixed vegetables.. However, there is little or no information on foodborne outbreaks from vegetables in Nigeria. Therefore, this study will investigate the microbial profile of *Salmonella* species and *Escherichia coli* in vegetables, carrots, cabbage and coleslaw in Magboro Mokoloki, Ogun state, Nigeria.

CHAPTER TWO

2.0 LITERATURE REVIEW

Many consumers today are concerned about the risks associated with the consumption of vegetables, carrots and coleslaw due to contamination with *Escherichia coli Salmonella* and other enteric pathogens. (Scallan *et al*,2011). The open nature of the vegetables makes it susceptible to these contamination. Microorganisms that stick to the vegetable surface are predominantly gram-negative saprophytes that can survive even after washing and sanitizing owing to the creation of biofilms on the vegetable surface or the preservation by vegetable cuticles (Seo *et al*. 2010 There has been an growing number of globally recognized foodborne diseases connected with vegetables and salads and attempts are being created to solve these issues of food safety (Denis *et al*. 2016).

The global production of fruit and vegetables grew by 91% from 1999-2010. Given the role of vegetables, carrots and salad in a healthy diet, it is critical that these foods are as safe as possible. In Nigeria, the consumption of minimal processed salads has increased, as they are easily accessible, convenient and cheaper that whole food. (Oranusi, and Olorunfemi, 2011).

TABLE 2.1: Center for Disease Control and Prevention's reported Foodborne Outbreak Online Database.2012-2019

S/N	Types of pathogens	Salad	Leafy Vegetables	Carrot	Total outbreaks
1	*Salmonella*	232	47	17	254
2	*Escherichia coli*	241	51	12	299
3	*Listeria Monocytogenes*	130	32	14	166
4	Norovirus	176	52	21	249
5	*Shigella*	221	29	15	265

2.1 SALMONELLA SPP.

Salmonella spp. are members of the family Enterobacteriaceae. They are Gram-negative, facultatively anaerobic, flagellated, rod-shaped organisms and are responsible for a large number of cases of foodborne illness throughout the world (Quiroz-Santiago *et al*. 2009)**.** Illnesses from food are one of the most important economic and health problems among developed and developing countries. *Salmonella* have circular DNA genomes with a mean length of approximately 4530 kb, although this can vary by up 1000 kb which makes it an ubiquitous and hardy bacterium that can survive several weeks in a dry environment and several months in water(von Ruckert *et al*., 2009). *Salmonella* contain both pathogenic and non-pathogenic strains. The genus *Salmonella* is subdivided into

Salmonella bongori and *Salmonella enterica* species. The table below gives a detailed division of the genus *Salmonella.*

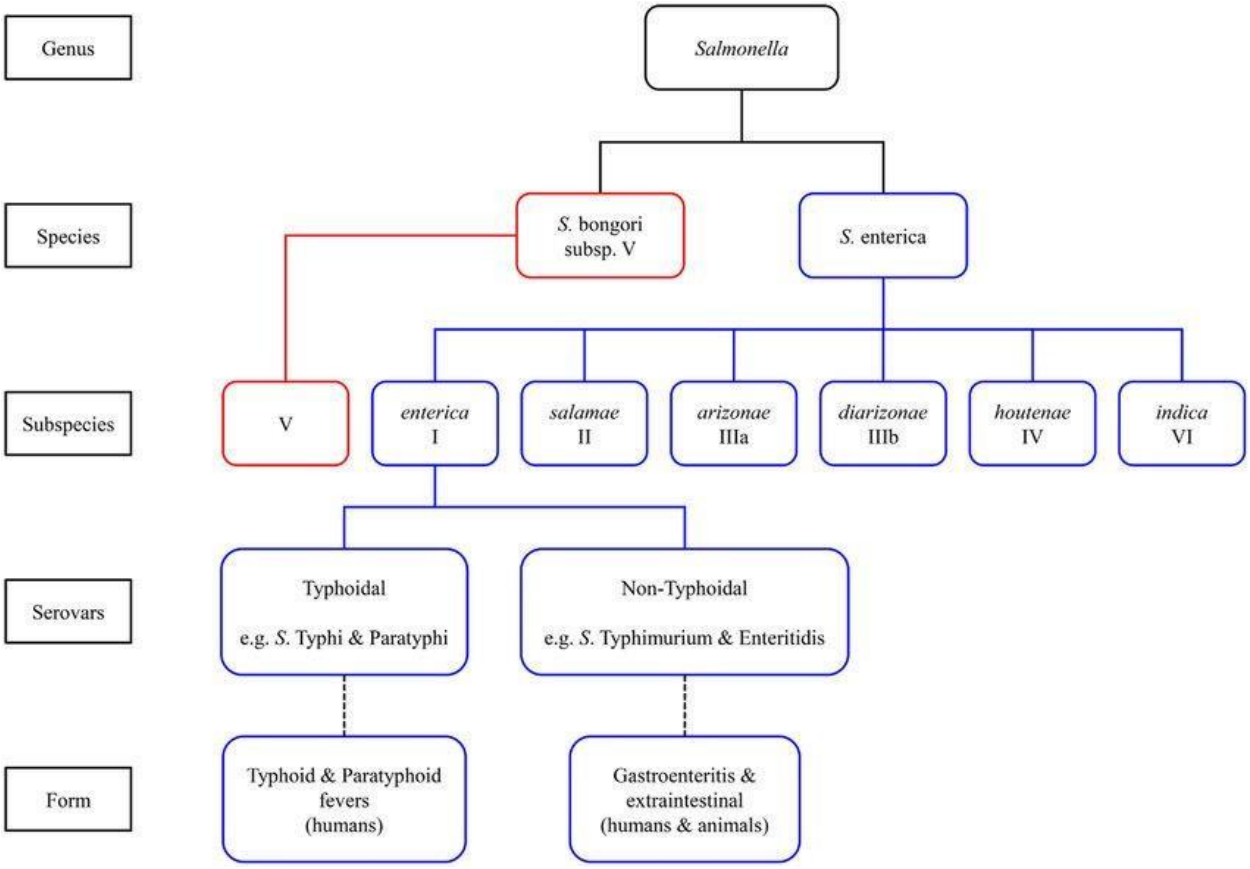

Figure 2.1: *Salmonella species* and subspecies Classification. Source: *Hurley et al 2014*

Salmonella enterica group I is the sub-species of *Salmonella* associated with foodborne illness linked to vegetables. *Salmonella enterica* is estimated to cause 1.5 million illnesses each year globally and to be the leading cause of hospitalizations and deaths from foodborne disease. (Scallan *et al*, 2011).

2.2 *ESCHERICHIA COLI*

Escherichia coli, originally called "*Bacterium coli* commune," was first isolated from the feces of a child in 1885 by the Austrian pediatrician Theodor Escherich (Escherich, 1885). *E. coli* are Gram-negative, motile, non-spore forming bacilli of the family *Enterobacteriaceae*. They are approximately 0.5 µm in diameter and 1.0–3.0 µm in length.

Most strains of *E. coli* are harmless, but a small proportion can cause clinical symptoms in humans and other mammals. (Mushtaq *et al*, 2011). The pathogenic strains of E. coli that cause enteric diseases are grouped into six categories which include; enterohaemorrhagic (EHEC), enterotoxigenic (ETEC), entero-invasive (EIEC), enteropathogenic (EPEC), enteroaggregative (EAEC), and diffuse-adherent E. coli (DAEC). But the *E.coli* strains mainly known to be associated with vegetables is the enterohaemorrhagic (EHEC) including Shiga-toxin *Escherichia coli* due to the major outbreaks that has happened all around the world**.** (López-Gálvez *et al*, 2010). An overview of the various groups of pathogenic E. coli is highlighted in Figure 2.2 below.

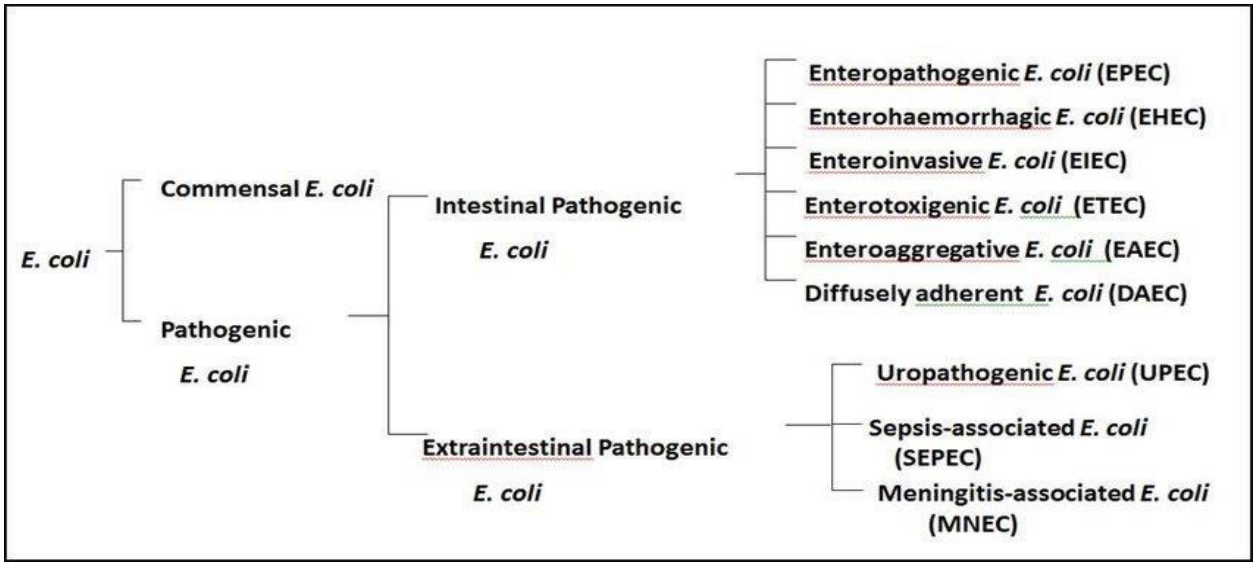

Figure 2.2: *Escherichia coli* species and its sub species classification. *Wakeham D, (2009)*

2.2.1 ENTEROHAEMORRHAGIC *E. COLI* (EHEC)

This category's primary etiological agent is *E. Coli O157:H7*, a public health issue with many significant outbreak involvement worldwide since it was first identified in 1983 (Riley *et al*., 1983). It was concluded by Griffin and Tauxe (1991) that *E. coli O157:H7* is an emerging and new pathogen, because they felt that such a distinctive illness which often has serious consequences (haemolytic uremic syndrome) would have attracted attention at any period. Later, O26:H11, O45:H2 and three non-motile *E. coli* (O4, O111 and O145) were added to this group of organisms. *Escherichia coli* O157:H7 is a non-invasive organism, which produces verotoxin as its primary virulence factor (Doyle, 2000) which are named for their cytotoxicity to African green monkey kidney cells called Vero cells (Meng *et al*., 2001). The Enterohemorrhagic E. Coli (EHEC) is a 60 megadalton virulence plasmid with shiga-like toxins or verotoxins secretion. The Shiga-like toxin was thus named because it is similar both in structure and activity to the toxin produced by Shigella dysenteriae type 1 and is also neutralized by the Shiga toxin antiserum. The shiga like toxins produced by *E. coli O157:H7* are of two types which are shiga-like toxin 1 and shiga-like toxin 2. The two toxins are antigenically different with both toxins known to be cytotoxic, causing fluid accumulation in rabbit ligated ileal loops and paralysis and death in mice and rabbits while the shiga-like toxin 2 produces hemorrhagic colitis in adult rabbits (O'Brien and Holmes, 1987).

Escherichia Coli O157:H7 is unlike other strains of *E. Coli* because it is not possible to ferment D sorbitol within 24 hours (Willshaw *et al*., 1997; Meng *et al*., 2001). Differentiation of *E. coli O157:H7* is typically done with Sorbitol MacConkey agar; where positive colonies are clear (Hitchens *et al*., 1995). Also, *Escherichia coli* O157:H7 can not hydrolyze 4-methylumbelliferone glucuronide (MUG) or grow well in *E. coli* broth at temperatures above

44.5 ° C. that distinguishes it from other strains (Meng *et al*., 2001). Dupont *et al*. (1971) determined on the basis of a human study that ingestion of 106 to 108 cells of some pathogenic strains of *E. coli* were needed to cause diarrheal illness in a healthy individual, but for *E. coli O157:H7* the infectious dose was previously unknown. However, from a compilation of outbreak data, it has been reported to be as low as 10 organisms, which indicates that it takes a low number of microorganisms to cause illness in young children, the elderly and immunecompromised people (Meng *et al*., 2001). In Nigeria, the only reported *E. coli O157:H7* illness in vegetables were from the study in SouthWest Nigeria with minimal outbreak report. (Ogunsanya *et al*., 1994; Olorunshola *et al*., 2000).

2.2.2 ENTEROINVASIVE *E. COLI* (EIEC)

This category represents a small group of *E. coli*. Many isolates are non-motile (lacking the H antigen) and they are slow to ferment lactose or are non-lactose fermenting. They are the cause of a disease similar to bacillary dysentery caused by *Shigella* and possess somatic antigens that may cross-react with those of Shigella (DuPont *et al*., 1971). Like *Shigella*, there are no known animal reservoirs; hence the primary source for EIEC seems to be infected humans (DuPont *et al*., 1971). Although the infective dose of Shigella is low and in the range of 10 to few hundred cells, volunteer feeding studies showed that at least 106 EIEC organisms are required to cause illness in healthy adults. EIEC can invade and multiply in the cells of the intestinal mucosa, especially in the colon. The invasion phenotype of EIEC is encoded by a high molecular weight plasmid, which can be detected by invasion assays using HeLa or Hep-2 tissue culture cells

(DuPont *et al*., 1971; Mehlman *et al*., 1982) or by PCR and probes specific for invasion genes. Illnesses as a result of this pathogen occurs within 8 to 24 hrs after ingestion of food or water containing this organism.

Symptoms as a result of Enteroinvasive *E. coli* include abdominal pain, fever, malaise, myalgia, headache and watery faeces containing mucus and blood. In the United States in 1981, an outbreak of this type occurred which was traced to imported French Brie and Camembert cheese. Bacterial counts of the cheese revealed that there were 10^5-10^7 *E. coli* CFU/g (Doyle and Padhye, 1989). These strains are biochemically and culturally different from other strains of E. coli. Unlike typical E. coli, EIEC are non-motile, do not decarboxylate lysine and do not ferment lactose, so they are anaerogenic. EIEC is endemic in the developing countries and accounts for 1% to 5% of all patients with diarrhea who see a doctor (Benenson, 1990).

2.2.3 ENTEROPATHOGENIC *E. COLI* (EPEC)

EPEC was the first pathotype of *E. coli* to be described. The term enteropathogenic *E. coli* (EPEC) was proposed by (Neter *et al*, 1955) to designate certain serotypes of *E. coli* that were associated with outbreaks of infantile diarrhea. In 1987, EPEC serotypes of 12 distinct O serogroups (O26, O55, O86, O111, O114, O119, O125, O126, O127, O128, O142 and O158)were acknowledged by the World Health Organization. The disease occurs primarily in nursing babies under 1 year, in whom it can cause a high mortality rate (Benenson, 1990). The illness is marked by watery, mucous diarrhea but no noticeable blood; fever ; and brief incubation dehydration.

EPEC happens mainly in developing nations and has virtually vanished in Europe and the United States. It occurs mostly in the warm seasons (summer diarrhea). The sources of infection are formula milk and weaning foods that become contaminated due to poor cleaning of bottles and

nipples, or deficient hygiene on the mother ‘s part. Children in poor socioeconomic groups are frequently exposed to EPEC and generally acquire immunity after the first year of life (Benenson, 1990). In epidemic diarrhea in newborns in nurseries, airborne transmission is possible through contaminated dust. Some outbreaks have also been described in adults (Benenson, 1990). Through volunteer feeding studies, the infectious dose of EPEC in healthy adults has been estimated to be 106 organisms. The pathogenesis of EPEC involves intimin protein (encoded by eae gene) that causes attachment and effacing lesions (Hicks *et al*., 1998) and a plasmid-encoded protein referred to as EPEC adherence factor (EAF) that enables localized adherence of bacteria to intestinal cells (Tobe *et al*., 1999). EAF production can be demonstrated in Hep-2 cells and the presence of eae gene can be tested by PCR assays (Nataro and Kaper, 1998).

2.2.4 DIFFUSE-ADHERENT *E. COLI* (DAEC)

Diffuse adhering *E. coli* (DAEC) have been recognized as the sixth category and appears as a heterogeneous group (Nataro and Kaper, 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 implication of DAEC strains in diarrhoea remains controversial, since some studies have reported that these strains are found similarly in children with and without diarrhoea (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 diarrhoea with DAEC in health adult volunteers. However, in Northern Brazil, Diffuse adherent E. coli has been shown in study to be the cause of acute diarrhoea in children (Scaletsky *et al*., 2002). Discrepancies

between epidemiological studies can be explained by age-dependent susceptibility to diarrhoea or the use of an inappropriate technique of identification such as DNA probing (Levine *et al*., 1993).

2.2.5 ENTEROAGGREGATIVE *E. COLI* (EAEC)

This name is given to a group of *E. coli* that shows an aggregative adherence pattern in a HEp-2 assay rather than a localized (as in EPEC) or diffused one. This category is considered to be provisional until it is better defined. Enteroaggregative *Escherichia coli* (EAEC) was first described in 1987 as *E. coli* that adhered to HEp-2 cells in culture in a characteristic, stackedbrick aggregative phenotype (Benenson, 1990). Within the first few years after its description, this pathotype became associated with diarrheal illness in Chile, India and Mexico. A research was carried out on 42 cultures, 40 from kids with diarrhoea in Santiago, Chile, 1 from Peru and 1 from a student from North America who visited Mexico.

All these strains tested negative for enterohaemorrhagic, enterotoxigenic, enteropathogenic and entero-invasive *E. coli* with DNA probes. They also failed to fit in one of these categories on the basis of serotyping. This group causes characteristic lesions in rabbit ligated ileal loops and mice (Levine *et al*., 1988; Vial *et al*., 1988). EAEC causes persistent diarrhoea in nursing babies with an incubation period estimated at one to two days (Benenson, 1990).

2.4 USE OF LIVESTOCK MANURE IN AGRICULTURE AND ITS ASSOCIATED RISK

Livestock manure has been used as fertilizer in farming for centuries because of the benefits derived from it, including the fact that it improves the soil structure (aggregation) so that it holds more nutrients and water and becomes more fertile, it encourages soil microbial activity, thus promoting the soil's trace mineral supply and improving plant nutrition. It is also less costly to generate livestock manure than chemical fertilizers, which can cost as much as four times more than manure (Gagliardi and Karn, 2002).

A study of 48 locations in Asia, Latin America and Africa showed that on their farms, most farmers use animal manure (Baltenweck *et al*., 2002). Overall, approximately 70% of total fertilizer inputs are obtained from animal manure in developing nations (Fresco and Steinfeld, 1998). In Nigeria, it has been estimated that approximately 932,5 metric tons (MT) of manure are generated annually from well-established poultry / livestock sectors that continue to expand at 8% per annum, with bovine manure widely used as fertilizer; either fresh / untreated or after composting (Adejinmi, 2000). It was still involved in several food-borne outbreaks despite the advantages obtained from the use of cattle manure in farming. This is because many potential animal and human pathogens have been discovered in cattle manure. This has raised concerns about health hazards associated with manure handling, including: direct transfer of zoonotic pathogens to farm employees and neighbors, the cause and spread of disease influencing farm animals, water contamination and food plants such as cabbages (Böhm, 2004).These concerns about the health hazards of managing manure can often be the primary reason why manure management procedures are revised.

2.5 SOURCES OF MICROBIAL CONTAMINATION IN THE VEGETABLES

Salmonella species and *E. coli* 0157:H7 (STEC) can come into contact with vegetables in so many ways and can take place at any stage of production, but the most common risk is through the farm. In the farm to sales production, processing and distribution of fruits and vegetables, there are various possible points of contamination which include; irrigation water, manure, handling by workers and contact with contaminated surfaces, wastewater. In an effort to decrease contamination, there are guidelines for testing irrigation water and soil as well as biological modifications. (FDA 2017). *Escherichia coli* was isolated from cabbage roots but not from the edible portion, when cabbage plants were irrigated with contaminated creek water (Watchel *et al*,

2002).The main source of vegetables and salad (lettuce, cabbage, cucumber, carrot e.t.c) contamination are through contaminated manure or compost, contaminated soil, contaminated water and contaminated seeds. (Critzer and Doyle, 2010).

WASTE-WATER CONTAMINATION

In many developing countries, wastewater use in agriculture is common practice and is increasing as a result of the rising water scarcity. Three of 20 carrot samples were positive for *E. coli* when harvested from fields irrigated with feces- contaminated streams (Okafo *et al*, 2003).

Microbes can also be distributed from contaminated areas to other parts during washing and mixing. It has been confirmed that exposing vegetable salad ingredients to various types of cutting has resulted in a six to seven-fold increase in microbial numbers. The cut surfaces expose inaccessible areas and increase the surface area for microbial contamination (Garg *et al*., 1990; O"Beirne, 1999). Enteric pathogens might be found in the aerial plant tissues. How animal pathogens are transported from the roots to these sites is still not clear. Several reports describe the presence of human pathogens in and near xylem vessels in plants, supporting the notion regarding in-planta transport via the transpiration stream (for example, Solomon *et al*., 2002). If the transport of bacteria from the root to the shoot takes place passively with the convection of water in the xylem, then the distribution of the bacteria in the above-ground parts of the plant should be proportional to the volume distribution of the xylem sap. To evaluate this, Analysis of the concentration of *Salmonella* separately in leaves, stems and inflorescences, which have been done are known to differ in the rates of transpiration (Taiz and Zeiger, 2006). The order of volume of transpiration water directed to these organs is: vegetative tissue (leave, stems) > reproductive tissue (flowers) and is based on the density of stomata in the organ and the extent of connections to the transpiration stream.

SOIL CONTAMINATION

In the soil, the human enteric bacteria arrive at the agricultural soils from the excretion of animals and sewage water irrigation. In Ghana, (Amoah *et al*., 2007), found unacceptably high levels of fecal coliforms in lettuce grown in urban cities of Accra and Kumasi. Same strains of enterohemorrhagic *E. coli* and *Salmonella* present in edible parts of vegetables were documented. These observations imply that *E. coli O157:H7* is an ecologically fit microorganism with superior survival and growth capability in non-pathogenic environments, although experimental comparison with non-pathogenic, commensal *E. coli* failed to detect any characteristics that would give the serotype such an advantage (Chung *et al* 2009).

CROSS-CONTAMINATION

Cross-contamination during salad dressing has been identified as an important factor associated with foodborne illnesses (Maffei *et al*., 2016). Cross-contamination from hands or contaminated surfaces to ready-to-eat-foods is common in food service establishments and local restaurants. According to Gerner-Smidt and Whichard (2007), cross contamination in the kitchen accounted for one-third of U.S. foodborne disease outbreaks from 1998 to 2008. (Niemira & Cooke, 2010) also indicated that *E. coli O157:H7* were transferred from hamburger patties to hands, to cutting boards and to lettuce subsequently put on the boards. Dharod (2009) also reported of *Salmonella*, from various food ingredients and contact surfaces during chicken salad preparation in Puerto Rico. The same study indicated that, 13% of the food workers used the same knife for both chicken and vegetables without cleaning the knife between uses. Other researchers have also identified that cleaning food preparation surfaces and hand washing could reduce *Salmonella* contamination (Cogan *et al*., 1999) and E. coli infections (Mugampoza *et al*., 2013). Therefore, it is very necessary to properly instruct and encourage food workers to apply basic hygiene

practices to ensure that transmission of pathogens as result of cross contamination and other related factors are controlled.

FERTILIZERS

At any stage in the manufacturing chain, vegetables can become contaminated: during development, harvesting, processing, delivery and final preparation. Pre-harvest contamination of vegetables can take place directly or indirectly through (wild) animals, insects, water, soil, dirty machinery and human handling. The most significant factors, however, are the application of manure or compost as fertilizer to crop-growing areas and fecal irrigation water contamination. (Ibenyassine, 2007). Cattle is deemed VTEC and *Salmonella*'s main but transient reservoir, generally carries asymptomatic pathogens and sheds them in their feces. Animal manure is used as a plant fertilizer globally, particularly in regions where intensive animal farming co-occurs with arable farming. (van pelt, 2008). This is the case in the Netherlands where, in the lettuce manufacturing industry, roughly 17–35 tons of manure (mostly from livestock) per hectare are applied annually. This involves both organic and conventional manufacturing. A portion of this manure will contain human pathogenic bacteria that, when applied to areas used to generate new vegetables such as Cabbage, will have the ability to enter the food chain. The risk of contamination of vegetables cultivated in contaminated manureenriched soils will rely mainly on the pathogen's survival capacities in manure and manureadjusted soils. Once excreted from the animal intestine, enteric pathogens ' survival requirements are considered unfavorable (Unc *et al*. 2004). Pathogens such as *E.Coli O157* and *Salmonella enterica* can survive in manure for longer periods of time (up to months). (Franz, 2008).

2.6 VEGETABLE SALAD

Salad refers to a food that is made of a mixture of raw vegetables and/or fruits (Uzeh *et al*., 2009; Rajvanshi, 2010). It is usually made up of fresh-cut or minimally processed vegetables and/or 14 fruits with or without salad dressing (addition of mayonnaise). Across the globe, vegetables mostly used in salad preparation include cucumber, pepper, tomatoes, onions, carrots and sometimes addition of spring onions. Some non-vegetable ingredients that may also be used are olives, mushrooms, egg, cheese, nuts, poultry, meat and some sea foods. In Nigeria, vegetables that are mainly used for salad preparation are lettuce, carrots and cabbage. Fresh salads are now part of the normal Nigerian diets due to the information of the high calories and amount of nutrients present in them. Because of these, they are been sold by fast food vendors, canteens and restaurants with about 200,000 consumers every day in Ota alone. (Oranusi *et al*., 2011). In a qualitative study of the microbial flora on some vegetables collected in main market from Akure; Nigeria. (Dada et al, 2015) stated that salad sold by street vendors are usually made of lettuce and a variety of toppings such as eggs, onions, cabbage, tomatoes and other raw vegetables. Whereas most consumers patronize vegetable salads for the perceived nutritional benefits, restaurants and other food service centers usually serve them as appetizers.

Although vegetables are commonly associated with food poisoning, they harbor disease causing organisms (Adebayo-Tayo *et al*., 2012); The processing of raw vegetables into sale salads generates favorable conditions and possibilities for the growth of pathogenic microorganisms in salads (Ameko *et al*., 2012). This is because the salads still maintain sufficient moisture to encourage microbial growth, as well as the natural protective covering on the leaves against the entry of microorganisms during harvesting, storage, transportation and processing. (Samarajeewa, 2005; Ameko *et al*., 2012). The salad may also have undergone some fermentation during sale and the increased acidity may promote the growth of certain microbes

such as *Salmonella* spp. which grow well in optimal pH of 4.2 to 8.2 (Samarajeewa, 2005; Ameko *et al*., 2012).

The production of salads typically involves purchasing and processing of raw materials as well as mixing of ingredients. The main ingredients, vegetables and/or fruits are usually washed, peeled, sliced, chopped and shredded and can be used fresh or partially processed. Some ingredients like poultry, meat, seafood and egg may require some cooking. Other already processed ingredients like canned products and dressings as well as herbs that require no cooking are just obtained for immediate use. Salads are usually served cold with or without dressing depending on consumers" preference.

CHAPTER THREE

MATERIALS AND METHODS

3.1. MATERIALS

Petri dish, glass spreader, inoculating loop, cotton wool, 70% ethanol, latex hand gloves, Bunsen burner, beaker, wash brush, markers, measuring cylinder, gram staining kit, conical flask, test tubes, test tubes racks, Duran(scotch bottles), centrifuge, Eppendorf tubes, cork borer, sterile tips, micropipette, incubator, glass slide, distilled water, hydrogen peroxide, autoclave, paper tape, oxidase test strip, immersion oil, microscope.

3.2 MEDIA USED

PEPTONE WATER

Peptone water is a microbial growth medium composed of peptic digest of animal tissue and sodium chloride. The pH of the medium is 7.2±0.2 at 25 °C and is rich in tryptophan. Peptone water is also a nonselective broth medium which can be used as a primary enrichment medium for the growth of bacteria.

Preparation

1. The dehydrated medium was dissolved in the appropriate volume of distilled water to make up 0.1% peptone water based on manufacturers instruction's instructions in a conical flask and mixed thoroughly. The conical flask is then closed in cotton wool that is wrapped in aluminum foil.
2. The mixture was heated for a while to dissolve the powder completely and was then sterilized by autoclaving at 121°c for 15mins.
3. It was then dispensed by pipetting into various test tubes for serial dilution.

Three types of media were used for the isolation of *Escherichia coli*; MacConkey agar (MAC), Nutrient agar (NA), Sorbitol-MacConkey Agar (SMAC)

SORBITOL-MACCONKEY AGAR (SMAC)

Sorbitol MacConkey agar is a variant of traditional MacConkey agar used in the detection of E. coli O157:H7. It can be used as a selective and differential medium

Preparation

1. The dehydrated medium was dissolved in the appropriate volume of distilled water i.e. 51.5g of SMAC in 1000 ml distilled water based on manufacturers instruction's instructions in a conical flask and mixed thoroughly. The conical flask is then closed in cotton wool that is wrapped in aluminum foil.
2. The mixture was heated for a 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.

This medium is reddish-purple in color.

MACCONKEY AGAR

MacConkey Agar is used for gram-negative enteric bacteria isolation and lactose fermentation differentiation from non-lactose fermenting bacteria and lactose fermenting bacteria. It provides pink colonies on MacConkey Agar as Escherichia coli.

Preparation

1. The dehydrated medium was dissolved in the appropriate volume of distilled water i.e. 48.5g of MacConkey in 1000 ml distilled water based on manufacturers instruction's instructions in a conical flask and mixed thoroughly. The conical flask is then closed in cotton wool that is wrapped in aluminum foil.
2. The mixture was heated for a while to dissolve the powder completely and was then sterilized by autoclaving at 121°c for 15minutes. Avoid overheating.
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.

   The medium is neutral red in color

NUTRIENT AGAR

Nutrient agar is a general- purpose medium supporting growth of a wide range of non-fastidious organisms. it is popular because it can grow a variety of types of bacteria and contains many nutrients needed for bacteria growth Preparation

1. The dehydrated medium was dissolved in the appropriate volume of distilled water i.e. 28g of Nutrient agar in 1000 ml distilled water based on manufacturers instruction's instructions in a conical flask and mixed thoroughly. The conical flask is then closed in cotton wool that is wrapped in aluminum foil.
2. The mixture was heated for a 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.

   The medium appears opalescent and is light amber in color

The following culture medias were used for the characterization of *Salmonella*

SALMONELLA SHIGELLA AGAR (SS AGAR)

Salmonella Shigella Agar is moderately selective and differential for the isolation, cultivation and differentiation of *Salmonella* spp. and some strains of *Shigella* spp. Sodium thiosulfate is added to the medium as a hydrogen sulphide source, and ferric citrate is added as an indicator for hydrogen sulphide production. The inclusion of bile salts, sodium citrate, and brilliant green serve to inhibit gram positive and coliform organisms. It is recommended for testing clinical specimens and food testing for the presence of *Salmonella* spp. and some *Shigella* spp.

Preparation

1. The dehydrated medium was dissolved in the appropriate volume of distilled water i.e., 63g of *Salmonella Shigella* agar in 1000 ml distilled water based on manufacturer's instruction in a conical flask and mixed thoroughly. The conical flask was then closed with cotton wool 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℃ for 15minutes.
3. The medium was then allowed to cool to about 45℃ and poured aseptically into sterile Petri dishes and left to solidify.
   This medium has a reddish orange color.

Selenite F broth

Selenite F Broth is the medium used for the selective enrichment of *Salmonella* spp from both clinical and food samples. It is a buffered Lactose Peptone Broth to which Sodium Biselenite is added as the selective agent.

Note: Sodium biselenite (sodium hydrogen selenite) is a very toxic, corrosive agent and causes teratogenicity. Handle with great care. If there is contact with skin, wash immediately with lots of water

Preparation

1. Dissolve 19g dehydrated media of selenite F in 750 ml distilled water in a sterile conical flask. (Part A)
2. Dissolve 4g of sodium biselenite in 250ml distilled water in another conical flask. (Part B).
3. Mix PART A and PART B together. Warm to dissolve the medium completely. Distribute in sterile test tubes
4. Sterilize in a boiling water bath or free-flowing steam for 10mins.

DO NOT AUTOCLAVE!!!

3.3 STUDY AREA

The study site was done in Magboro market which is located in Obafemi-Owode Local government area in Ogun State and is one of the many towns around the state that share a close proximity with the ever-bustling Lagos. Magboro, a name that is said to mean 'spreading out', has definitely grown in population compared to over a decade ago. A town once occupied by only the natives is now an urban settlement with a population estimated to be over a million inhabitants and still growing. In the market at Magboro, everything needed is mostly sold in these markets because the market is situated closely to Lagos-Ibadan expressway (Link between Ibadan, capital of Oyo state and Lagos) which is a compulsory route for travelers by road.

3.4 Collection of Samples

Cabbage (Brassica oleracea var. capitate) and carrots (Daucus carota subsp. Sativus) were bought from Magboro, market Ogun state and transferred to the laboratory aseptically for identification and microbial analysis.

3.5 SAMPLE PREPARATION

Twenty-five (25) g of cabbage or carrots were taken using a sterile blade and dispensed in 225ml of 0.1% peptone water (enrichment broth) in a conical flask. The samples were homogenized for 2 mins in a stomacher. Then incubated for 24hrs at 37°c.

Primary enrichment

Another (Twenty-five) 25grams of sample was weighed and kept into 225ml of peptone water which was subjected to 7 different dilution factors starting with the original 10^{-1}- 10^{-7} using serial dilution method.

Secondary Enrichment

This enrichment was peculiar to *Salmonella* alone

10mls of sample pipetted from the peptone water was dispensed into 90mls selenite F broth in a scotch bottle (Duran bottle) and incubated for 24hrs in 37°C. After incubation 0.1ml of the incubated selenite F broth was transferred into *Salmonella shigella* agar (SS agar) for presumptive confirmation of *Salmonella* spp. They were then incubated inversely at 37°c in an incubator for 24hrs. Colonies counted on plate were sub cultured from the SS agar on to newly prepared nutrient agar and *Salmonella shigella* agar to get pure culture.

Test tubes were filled with 9ml of peptone water and sterilized using autoclave at 120^0C for 15 minutes. The tubes were used to make serial dilutions from 10^{-1} to 10^{-7}. These were plated unto

the three culture medias; Sorbitol MacConkey (SMAC), MacConkey agar (MAC), Nutrient agar (NA) using spread plate method.

3.6 BIOCHEMICAL TESTS

Gram Staining

- A smear of a suspected colony was made from the culture plate on a clean, grease-free glass slide.
- The smear was heat fixed by passing the slide over a Bunsen burner flame briefly.
- The slide was then covered with the primary stain (crystal violet) and allowed to stay for 60 seconds.
- The stain was decanted, rinsed with a gentle flowing tap water and stained with a mordant (lugol's iodine) for another 60 seconds. The stain was again decanted and also rinsed with a gentle flowing tap water.
- Then decolorized with 70% alcohol for 20 seconds. It was then decanted and also rinsed.
- The secondary/counter stain (safranin) was added for 60 seconds. The stain was decanted, rinsed, and allowed to dry.
- The slide was then observed under the microscope using the oil immersion objective lens.

Catalase Test

This test was used to differentiate those bacteria that produce the enzyme catalase e.g. Staphylococci from non-catalase producing bacteria such as lactic acid bacteria. The catalase enzyme neutralizes the bactericidal effect of hydrogen peroxide and protects them. Catalase mediates the breakdown of hydrogen peroxide into oxygen and water. The absence of catalase is evident by weak or no bubble production.

Procedure: A loopful of hydrogen peroxide was dropped on a clean grease free slide following a loopful of isolate which was then mixed together. The absence of gas bubbles formation showed a negative reaction. *Salmonella* are catalase positive.

$2H_2O_2 \xrightarrow{\text{catalase}} 2H_2O + O_2$

Oxidase Test (using an oxidase strip)

The oxidase test is used in the identification of organisms which produce the enzyme cytochrome oxidase. It detects the presence of a cytochrome oxidase system that will catalyze the transport of electrons between electron donors in the bacteria and a redox dye-tetramethyl-p-phenylene diamine. The dye is reduced to deep purple color (positive reaction).

Procedure:

Using a sterile inoculating loop, a small colony was picked and smeared on the oxidase test strip. Oxidase-positive reaction gives a deep purple coloration while oxidase-negative reaction has no color change (colorless). *Salmonella* are oxidase negative.

3.7 PRESERVATION OF ISOLATES

A loopful of each isolate was inoculated into 5 ml peptone buffered water (BPS) containing 10% glucose for long-term conservation and then incubated for 18 hours. The suspension is then added to the Eppendorf tubes containing sterile 20% glycerol as cryoprotectant and homogenized. It was then stored in a -4°c freezer at -20°C.

Preparation of broth.

Prepare 0.1% of Buffer Peptone Water (i.e. 0.5g in 500ml distilled water).

Prepare 2% glucose in BPW (i.e. Weigh 10g of D-Glucose and add to the 0.1% BPW), allow to dissolve.

Dispense 5ml each of 2% of glucose into test-tubes, then autoclave.

After autoclaving, allow to cool.

Inoculate a loopful of your isolates/pure culture into the test tubes.

Incubate at 37^0c for 18hr.

After 18hrs,

Prepare 75% glycerol (i.e. 225 glycerol in 500ml of distilled water), then autoclave.

Get Eppendorf tube, autoclave them.

Allow to cool.

Add 0.5ml of cultured broth + 0.5ml of glycerol into Eppendorf tube.

Store in deep freezer

3.8 GENOTYPIC CHARACTERIZATION OF THE ISOLATES
DNA EXTRACTION

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

3.9 PCR Protocol

1*6S rRNA amplification*

Partial 16S rRNA gene amplification using forward primer fD1 (5´-AGA GTT TGATCC TGG CTC AG-3´) and reverse primer rD1 (5´-AAG GAG GTG ATC CAG CCG CA-3´) (Weisburg *et al*., 1991). The components of the PCR and constituent mixes were summarized in Table 3 below. The PCR was carried with initial denaturation at 95 °C for 5 min; 35 cycles of 95 °C for 2 min; 42 °C for 30 s and 72 °C for 4 min; and a final elongation step at 72 °C for 10 min. The PCR products were confirmed by electrophoresis and visualized under UV light with a Gel Doc system (Cleaver Scientific Ltd,Warwickshire, United Kingdom)

Table 3.1 – PCR reaction components used for 16S rRNA amplification

No.	Code		
1		**Component**	1 Reaction
2		Mastermix	5
3		fDl	0.4
4		rDl	0.4
5		DNA	1
6		RNaseFreeH_2O	3.2
7		Total	10

Table 3.2 Procedure for thermal cycler

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

PRECAUTIONS

- Aseptic techniques were observed at every stage of work.
- Personal protective technique was also observed, such as wearing of covered shoe, nose cover, gloves, lab coat, etc.

- Ensured that the inoculating loop cooled before picking the organism when sub-culturing in order not to kill organism of interest.
- Ensured that the petri-dish was incubated inverted.
- Ensured proper timing, most especially during autoclaving.

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CHAPTER 4
RESULTS AND DISCUSSIONS

The microbial analysis of the samples gotten from Magboro market, Ogun state. The microbial analysis was carried out for total viable counts, general E. coli, pathogenic E. coli and Salmonella species. The results of the findings were summarized in the table below in Table 4.14.2 showing the morphological characteristics of samples cultured on Sorbitol MacConkey

agar and *Salmonella Shigella* agar using spread plate method with bacterial isolates collected from food sampling from 3 locations in Magboro LCDA, Ogun State. While Table 4.3 shows the results of the biochemical characterization of the twenty (27) isolates using catalase and oxidase tests alongside gram stain results from using spread plate method with bacterial isolates collected from food sampling from 3 locations in Magboro LCDA, Ogun State.

Table 4.1 : Morphological characteristics samples on Sorbitol MacConkey agar

Isolate ID	SAMPLE	Isolate ID	Color	Shape	Size	Elevation	Appearance	Surface	Opacity	Margin
	Cabbage	SM 1 Cb	Creamy Pink	Circular	Medium	Raised Convex	Shiny	Smooth	Opaque	Entire
		SM 2 Cb	white	Circular	Pinpoint		Shiny	Smooth	Opaque	Entire
1st sampling										
		SM 3 Cb	Creamy Pink	Circular	Small	Raised	Shiny	Smooth	Opaque	Entire
	Carrot	SM 1 Cr	PINK	Circular	Pinpoint	Raised	Smooth	rough	Opaque	Entire
		SM 2 Cr	White	Circular	Medium	convex	Shiny	rough	Opaque	Entire
2nd sampling	Cabbage	SM 1 Cb	Creamy Pink	Circular	Small	Convex	Shiny	Smooth	Opaque	Entire
		SM 2 Cb	Creamy Pink	Circular	Medium	Raised	Shiny	Smooth	Opaque	Entire
		SM 3 Cb	Cream Pink	Circular	Small	Shiny	Smooth	Raised	Opaque	Entire
	Carrot	SM 1 Cr	Pink	Circular	Pinpoint	Shiny	Smooth	Raised	Opaque	Entire
		SM 2 Cr	Pink	Round	Medium	Shiny	Smooth	Convex	Opaque	Entire
		SM 3 cr	white	Circular	Medium	Shiny	Smooth	Raised	Opaque	Entire
3rd sampling	Cabbage	SM 1 Cb	Pink	Round	Medium	Shiny	Smooth	Raised	Opaque	Entire
		SM 2 Cb	White	Circular	Pinpoint	Convex	Rough	Convex	Opaque	Entire
		SM 3 Cb	Pink	Circular	Medium	Shiny	Smooth	Convex	Opaque	Entire
	Carrot	SM 1 Cb	Pink	Circular	Small	Shiny	Smooth	Convex	Opaque	Entire
		SM 2 Cb	White	Round	Small	Raised	Rough	Raised	Opaque	Entire
		SM 3 Cb	White	Round	Medium	Raised	Rough	Raised	Opaque	Entire

Table 4.2: Morphological characteristics samples on *Salmonella Shigella* agar

Isolate ID	SAMPLE	Isolate ID	Color	Shape	Size	Elevation	Appearance	Surface	Opacity	Margin
	Cabbage	Ss 1 Cb	Black Yellow	Circular	Medium	Raised Convex	Shiny	Smooth	Opaque	Entire
		Ss 2 Cb		Circular	Pinpoint		Shiny	Smooth	Opaque	Entire
1st sampling										
		Ss 3 Cb	Black	Circular	Small	Raised	Shiny	Smooth	Opaque	Entire
	Carrot	Ss 1 Cr	Black	Circular	Pinpoint	Raised	Smooth	Rough	Opaque	Entire
		Ss 2 Cr	Black	Circular	medium	convex	Shiny	rough	Opaque	Entire
		Ss 3 Cr	Yellow	Circular	Pinpoint	raised	Shiny	smooth	Opaque	Entire
2nd sampling	Cabbage	Ss 1 Cb	Black	Circular	Small	Convex	Shiny	Smooth	Opaque	Entire
		Ss 2 Cb	Yellow	Circular	Medium	Raised	Shiny	Rough	Opaque	Entire
		Ss 3 Cb	Black	Circular	Small	Shiny	Smooth	Raised	Opaque	Entire
	Carrot	Ss 1 Cr	Black	Circular	Pinpoint	Shiny	Rough	Raised	Opaque	Entire
		Ss 2 Cr	Yellow	Round	Medium	Shiny	Smooth	Convex	Opaque	Entire
		Ss 3 Cr	Black	Circular	Medium	Shiny	Smooth	Raised	Opaque	Entire
3rd sampling	Cabbage	Ss 1 Cb	Black	Round	Medium	Shiny	Rough	Raised	Opaque	Entire
		Ss 2 Cb	Yellow	Round	Medium	Convex	Rough	Raised	Opaque	Entire

	Ss 3 Cb	Yellow	Circular	Pinpoint	Convex	Smooth	Raised	Opaque	Entire
Carrot	Ss 1 Cr	Yellow	Circular	Medium	Shiny	Smooth	Convex	Opaque	Entire
	Ss 2 Cr	Black	Circular	Small	Shiny	Rough	Convex	Opaque	Entire
	Ss 3 Cr	Black	Circular	Small	Shiny	Rough	Raised	Opaque	Entire

Table 4.3: Morphological characteristics and biochemical test performed on isolates

S/N	Isolate	Gram reaction	catalase	Oxidase
1	Cab 10^{-5}	-	+	_
2	Cab 1	-	+	_
3	Cab 2	-	+	_
4	Car 1(10^{-2})	-	+	_
5	Cab 3	-	+	_
6	Carrot	-	+	_
7	Cab 4	-	+	_
8	Cab 10^{-4}	-	+	_
9	Cab 1(10^{-2})	-	+	_
10	SM cab	-	_	_
11	SS Cab Y	-	+	_

Based on the biochemical tests results in Table 4.3. Based on the selective media used for identification, isolates were suspected to be *Salmonella* and *Escherichia coli.* All the isolates are Gram negative and oxidase negative. Most of the isolates are catalase positive and only isolate SM cab is catalase negative

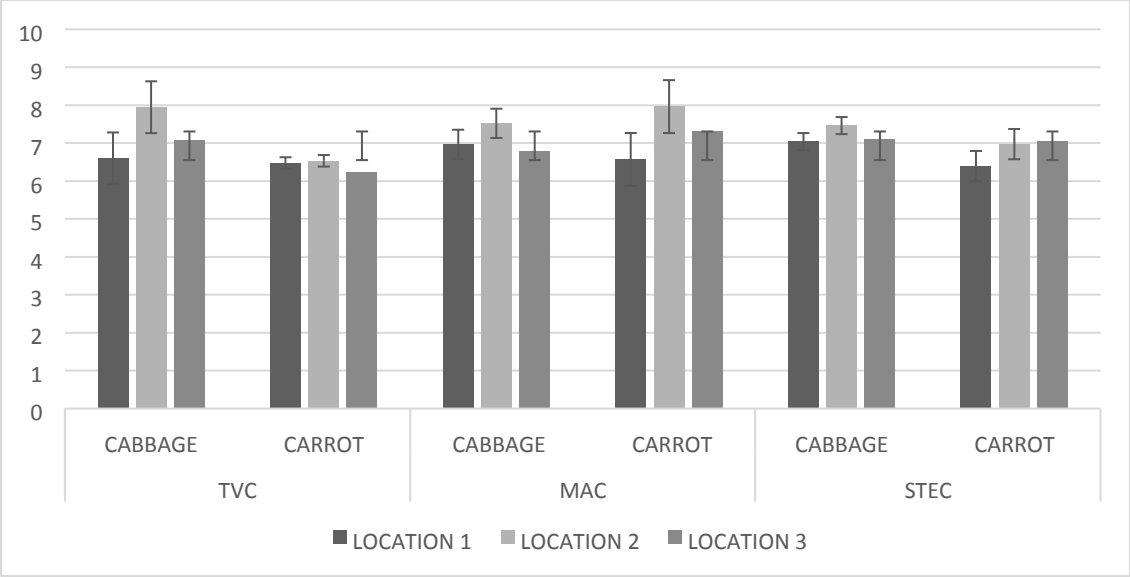

Figure 4.1: Chart of microbial analysis showing the counts of mesophiles, *E. coli* and pathogenic *E. coli* (STEC) in carrot and cabbage samples collected from Magboro market, Ogun state.

According to international regulations (Gilbert *et al*., 2000*), Salmonella* species should be absent in 25g of food sample. The presence of a *Salmonella* in 25g (twenty-five grams) of carrots and cabbages will pose a threat to the consumers thereby resulting to sickness/illness. Also, the presence pathogenic *E. coli* is a major health concern. The incidence of pathogens in vegetables such as cabbage and carrots are of growing concern since these vegetables can be eaten raw without cooking. For example, there have been an outbreak of *E. coli* O157:H7 linked with romaine lettuce which affected the Yuma region in California, USA (CDC, 2019)

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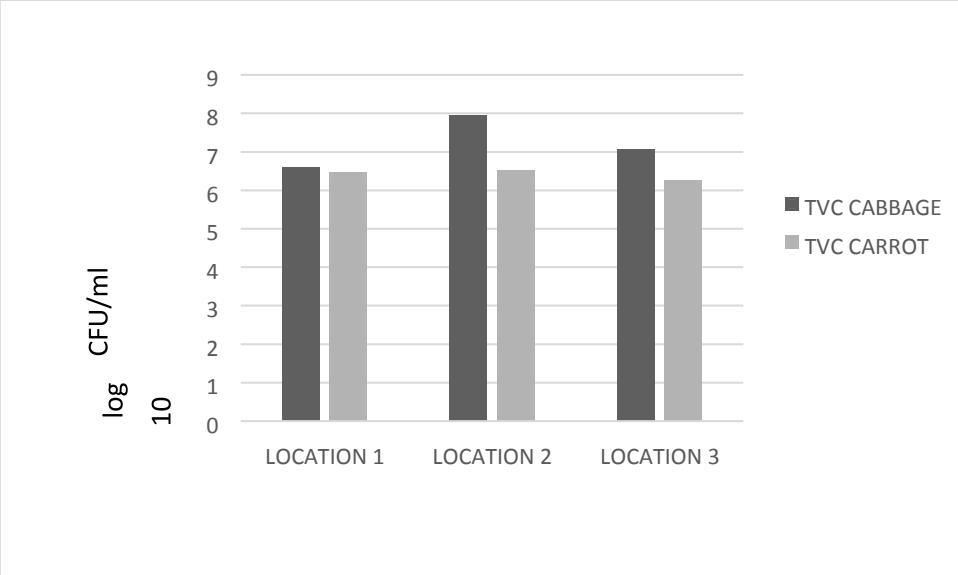

Figure 4.2: Microbial counts in cabbage and Carrots from three (3) different locations

The Total viable count was high in location 2 with a count of 7.9 $\log_{10}$ CFU/g and 6.7 Log_{10} CFU/g Cabbage and Cabbage respectively. There is no significant difference in the microbial concentration in locations 1 and 2. The presumptive microbial growth are typically identified as a smooth colony on the Nutrient agar. In view of the incidence of mesophiles from cabbage and carrots, the soils on which they were cultivated may have high microbial load and cross contamination from the soil may occur.

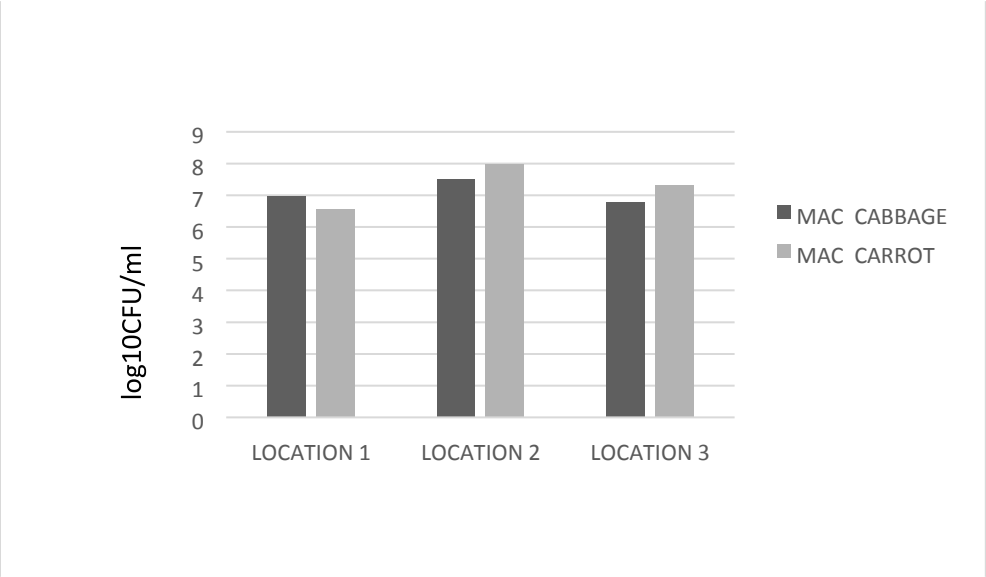

Figure 4.3: **General *E. coli* in cabbage and Carrots from three (3) different locations**

The count for General *Escherichia coli* was high in location 2 with a count of 7.9 $\log_{10}$ CFU/g and 7.3 Log_{10} CFU/g for cabbage and carrot respectively. In locations 1 and 3 there are no significant difference in the *E. coli* concentration in with count of approximately 7 $\log_{10}$ CFU/g. The presumptive *E. coli* growth are typically identified as a pink colony on the MacConkey agar (Gill *et al*., 2014).

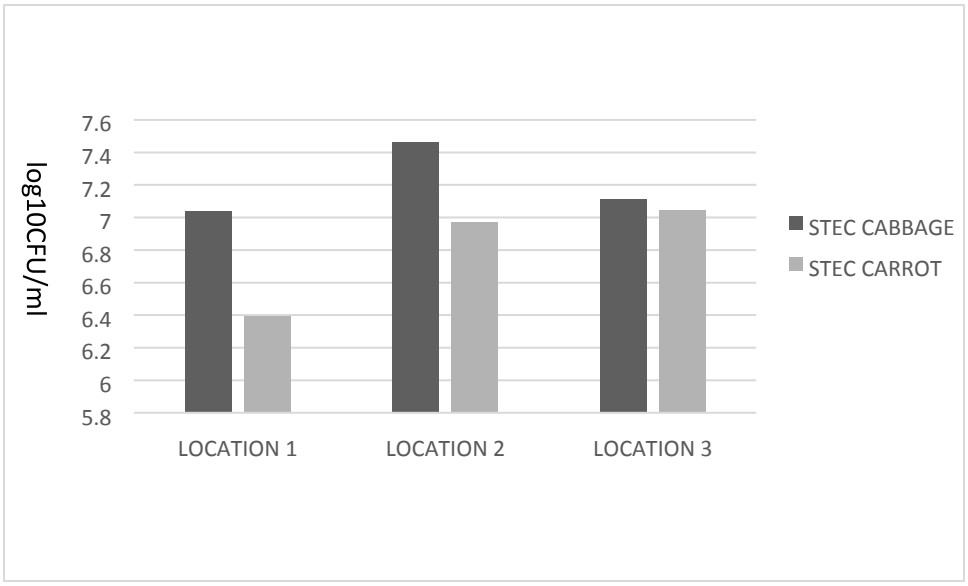

Figure 4.4: Counts of pathogenic *E. coli* (STEC) in cabbage and Carrots from three (3) different locations

The count for STEC was High in location 2 with a count of 7.4 $\log_{10}$ CFU/g and 6.9 Log_{10} CFU/g Cabbage and Carrot respectively. There is no significant difference in the *Salmonella* concentration in locations 1 and 3. The presumptive shiga toxin *Escherichia coli* (STEC) growth are typically identified as a white colony on the sorbitol MacConkey agar while the non-0157 are identified as pink colonies(Sandra and Samuel, 1986). Considering the occurrence of *E. coli O157:H7* from cabbage and carrots, the soils on which they were grown may be contaminated with the pathogen or fecal contamination and there could possibly be cross contamination of these products from the soil.

Table 4.4: Presumptive identification of isolates

Location	Sample	Isolate ID	*Salmonella spp*
1	Cabbage	Cab 1	_
		Cab 4	+
	Carrot	Car 3/6 Y	_
		Ss car b	+
2	Cabbage	Ss cab Y3	_
		Cab 3/6	_
	Carrot	SS Car Y	_
		SS 3/6 Car	_
3	Cabbage	Cab 2	_
		Cab 10^{-4}	+
	Carrot	Car 2	_
		Car 3	_

Note : Positive (+) indicates the presence of *salmonella* while Negative (-) indicates the absence of *salmonella*

4.1 PCR AMPLIFICATION IMAGE

The genetic characterization shows DNA band on agarose gel of the presumptive *Salmonella spp* and pathogenic *E. coli* after PCR amplification. This confirms the results from the morphological and biochemical test.

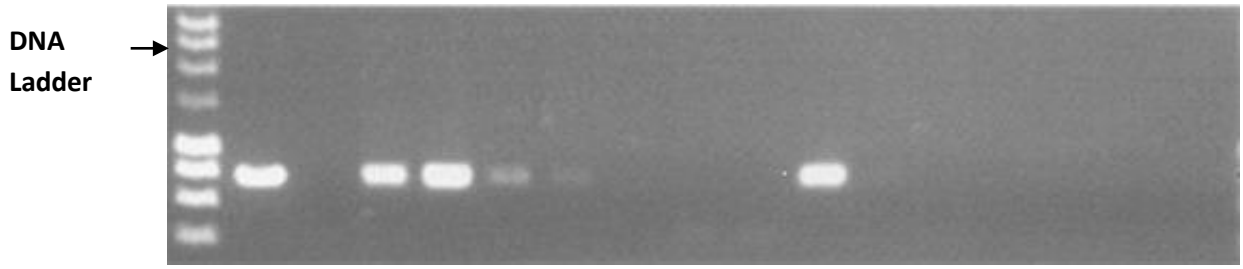

Figure 4.5 : *Salmonella Shigella agar* plate showing growth of *salmonella*

CHAPTER 5
5.0 CONCLUSIONS AND RECOMMENDATION 5.1 CONCLUSIONS

Based on the findings of this study and the deductions derived from there, it could be concluded that cabbage and carrot vended in Magboro are contaminated with strains of pathogenic *E. coli* and *Salmonella* spp, although the frequency of contamination was found to be low. In this study, carrots were considered as the major risk factor associated with the occurrence of *Salmonella* and *E.coli*. In addition, the study has demonstrated that Cabbages and carrot may contribute to the prevalence of *Salmonella* and *E.coli* illnesses in Magboro market, Ogun state

5.2 RECOMMENDATIONS

In light of the findings of the study, the following recommendations were made.

Considering the low infective dose of this organism and the fact that these vegetables can be consumed raw without any form of cooking, the general public need to be educated on the risk involved in the consumption of contaminated vegetables.

Vegetables should be stored away from raw animals' products to avoid cross-contamination.

Finally, washing your vegetables properly before eating is highly recommended.

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