PREVELANCE OF *SHIGELLA* SPECIES IN STREET-VENDED FRESH PRODUCE FROM LAGOS AND OGUN STATE

BY

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IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF DEGREE OF BACHELOR OF SCIENCE IN MICROBIOLOGY.

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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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OLU-AKINYANJU FAITH TOLUWANIMI

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Date

CERTIFICATION

This is to certify that the content of this project entitled **INCIDENCE OF *SHIGELLA* SPECIES IN STREET-VENDED FRESH PRODUCE FROM LAGOS AND OGUN STATE** was prepared and submitted by Olu-Akinyanju, Faith Toluwanimi with matriculation number 17010101025, in partial fulfillment of the requirements for the degree of Bachelor of science in Microbiology, Department of Biological Sciences of the Mountain Top University, Ogun State, Nigeria. The original research work was carried out by her under my supervision and is hereby accepted.

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DEDICATION

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

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ABBREVIATIONS

PCR POLYMERASE CHAIN REACTION - PCR

DEOXYRIBONUCLEIC ACID- DNA

READY-TO-EAT- RTE

SHIGA TOXIN-PRODUCING *ESCHERICHIA COLI*- STEC

THE FOOD AND AGRICULTURE ORGANIZATION- FAO

WORLD HEALTH ORGANIZATION- WHO

SHIGELLA DYSENTERIAE- S. dysenteriae

SHIGELLA SONNEI- S. sonnei

SHIGELLA FLEXNERI- S. flexneri

SHIGELLA BOYDII- S. boydii

MEGABYTE PER SECOND- MBP

VIRULENCE GENE A- VIRA

INOSITOL PHOSPHATE PHOSPHATASE- IPP

SODIUM CHLORIDE- NACL

MICROFOLD CELLS- M CELLS

INVASION PLASMID ANTIGENS- IPA

LIPOPOLYSACCHARIDE- LPS

POTENTIAL OF HYDROGEN- pH

BRAIN HEART INFUSION- BHI

XYLOSE LYSINE DEOXYCHOLATE AGAR- XLD

NUTRIENT AGAR- NA

HEKTOEN ENTERIC AGAR- HEA

ULTRA VOILET- UV

TOTAL VIABLE COUNT- TVC

16s RIBOSOMAL RIBONUCLEIC ACID- 16s rRNA

16s RIBOSOMAL DEOXYRIBONUCLEIC ACID- 16s rDNA

ABSTRACT

Fresh fruits and vegetables are on an increase demand because of their health benefits but its associated hazards are not taken into consideration. The incidence of food borne outbreaks involving fresh produce is of worldwide concern. Consumer demand would be for bacteriological safe fresh fruits and vegetables, yet cases have proven that consuming these fresh fruits and vegetables is not always safe. A survey of 63 samples of fresh fruits and vegetables were screened for the presence of *Shigella* species consisting of the following: Cucumber (*Cucumis satavum*), Carrot (*Daucus carota*), Pawpaw (*Carica papaya*), Watermelon (*Citrullus lanatus*), Lettuce (*Lactuca sativa*), Cabbage (*Brassica oleracea*) and Pineapple (*Ananas cosmosus*) bought from Magboro or Ibafo markets in Ogun State and Jakande market, Magodo market and Yaba market in Lagos state. Ibafo lettuce (SIL) had the highest total viable count of 7.8 $\log_{10}$ cfu/g. *Shigella* species were identified in 21 samples (33%) of the fresh produce. Lettuce, cabbage, and carrot had the highest number of *Shigella*, revealing that the fresh produce had been highly polluted by pathogenic microbes. The largest incidence of *Shigella* species was found positive in lettuce, followed by carrots, and cabbage. Molecular confirmation by PCR of the *shigella* species carried out using 16S rDNA and *ipa*H gene. Most fruits and vegetables investigated in this study were heavily contaminated with *shigella* species and could be linked to improper hygiene by the food handlers. Adequate awareness should be provided to the general public in terms of safety and hygiene of fresh produce from this locations.

Keywords: *Shigella* species, Pathogens, Fresh produce, Molecular typing

CHAPTER ONE

1.1 INTRODUCTION

Fresh fruits and vegetables are an essential component of a well-balanced diet, as a result of the promotion of a healthy life, their consumption has increased globally in recent years (Betts, 2014). These products are usually ready-to-eat (RTE) (Olaimat & Holley, 2012). Consumers frequently do not treat these foods prior to eating to ensure that contamination such as chemical residues or harmful bacteria are completely destroyed or inactivated. Longer distribution periods and wider distribution distances have resulted from their high consumption, which has been amplified by globalization and large-scale production of RTE meals. This complicates and emphasizes the significance of food safety management (Kirezieva et al., 2015). Notably, the number of outbreaks of produce-related diseases has increased in recent years (Critzer & Doyle, 2010; Hoelzer, Pouillot, Egan, & Dennis, 2012; Olaimat & Holley, 2012).

Food borne disease outbreaks involving virulent bacteria continue to be linked to fresh produce, fresh produce continues to be the dominant source of food borne illness outbreaks involving severe bacteria such as *shigella species* ,Shiga Toxin-Producing *Escherichia coli* (STEC), *Salmonella*, *Listeria monocytogenes*, and increasingly human parasites. Because of the open nature of the fresh produce supply chain, contamination can enter at any stage during cultivation, harvesting, or processing, and subsequently be passed on to the consumer (Nuesch-Inderbinen and Stephan, 2016). Diarrhoea and/or vomiting are common symptoms of foodborne disease, and they usually persist 1 to 7 days. Abdominal cramps, nausea, fever, joint/back aches, and weariness are all possible symptoms. Foodborne diseases that are caused by a pathogen (i.e., virus, bacteria, or parasite) in contaminated food or drink is sometimes referred to as "stomach flu." The incubation period (the time between being introduced to the pathogen and experiencing symptoms) can last from a few hours to a week.

1.2 SHIGELLA SPECIES IN FRESH PRODUCE

Shigella species are grouped into four species, namely, *Shigella dysenteriae, Shigella flexneri, Shigella boydii,* and *Shigella sonnei*. *Shigella* has been isolated with a wide variety of foods, including fruits and raw vegetables and they are present in fresh produce. Ready-to-eat foods such as fruits and raw vegetables are most contaminated with *Shigella* bacteria can be spread by a sick food handler with poor personal hygiene or by products obtained from sewage-polluted areas. (Edwards, B. H.1999). Infection with shigellosis has also been linked to lettuce, cabbage, cucumbers, and fresh produce of several kinds. When traveling to endemic locations, tourists from developed countries are at risk of contracting *shigella flexneri*

and *shigella dysenteriae*, and sporadic food or water-borne epidemics occur in developed countries. In temperate and tropical climates, *Shigella* species are found only. Poor hygiene and sanitation are the most common causes of *Shigella* species transmission. In developed countries, *Shigella sonnei* is the most common cause of shigellosis, whereas in developing countries, *S. flexneri* and *shigella dysenteriae* are more prevalent in impoverished areas with poor sanitation, shigellosis is endemic. Shigellosis is responsible for 10 to 20% of enteric sickness in young children, as well as 50% of bloody diarrhea or dysentery, and the prevalence of these infections drops dramatically after five years of life. Single-source, food- or water-borne epidemics are rare in affluent countries, although pockets of endemic shigellosis can be found in institutions and isolated locations with poor sanitation. (Hale et al., 2005).*S. boydii* and *S. dysenteriae* infections are less prevalent worldwide, although they can account for a significant fraction of *Shigella* species isolated in Sub-Saharan Africa and South Asia. Shigella species is a threat to human beings because shigella causes shigellosis infection which invade the gastrointestinal part of the body when consumed and causes severe disturbance to human health and if care is not properly taken care of the infected patient it may lead to death and *shigella* species is mostly associated with fresh produce and vegetables and majority of Nigerians do not wash their fruits and vegetables prior to eating them. Shigellosis infection is a contagious disease. It is important to look for *shigella* species present in our fresh produce and vegetables to be aware if the fresh produce we consume is safe for consumption and to be able to deal with the likely source of contamination *shigella* species is contaminating the fresh produce and vegetables.

1.3 STATEMENT OF PROBLEM

Fresh fruits and vegetables such as pawpaw, cabbage, lettuce, cucumber, carrot, watermelon, and pineapple (Fresh produce) are thought to be easily infected pathogens during various steps in pre-harvest, harvesting, processing, distribution, and handling practices. All these have contributed to the rise in foodborne illness by pathogenic bacteria due to unsanitary practices (Hedberg, MacDonald & Osterholm, 1999; Beuchat & Ryu, 1997). The Food and Agriculture Organization (FAO) and World Health Organization (WHO) (2006) have reported increased food borne infection/poisoning outbreaks due to international trade; increase in susceptible population, increase in travel and most importantly microbial adaptation.

1.4 OBJECTIVE OF STUDY

1. To identify the pathogenic *Shigella* species present in the fresh produce (fresh fruits and vegetables).
2. To establish that there is a survival of pathogens in the fresh produce at the point of sale and consumption.
3. To establish the potential risk involved in the consumption of fresh produce in (Magboro, Ibafo markets in Ogun State and Jakande, Yaba, Magodo markets in Lagos state). To create awareness on the level of food safety of roadside fruits seller and implications of not washing your fruits and vegetables properly before consumption and its importance.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 PATHOGENS OF FRESH PRODUCE

The minimal processing required for fresh produce and vegetables, which omits any efficient microbial elimination step, resulting in food products that are naturally contaminated with microorganisms, some of which may be detrimental to health. When looking into possible control methods, it's important to look into the nature of the human pathogenic microorganisms that can be found in fruits and vegetables throughout the production chain. This should be factored into risk assessments because bacterial contamination can happen when produce is exposed during harvest, growth, and transportation. Numerous factors influence the incidence, fate, transport, survival, and proliferation of pathogens in the wide variety of sources where they are found, including contaminated manure, irrigation water, soil, livestock/wildlife, and numerous factors influence the incidence, fate, transport, survival, and proliferation of pathogens in the wide variety of sources where they are found. (Alegbeleye, O. O, Singleton, I, & Sant'Ana, A. S. 2018). Once pathogenic bacteria have been introduced into the growing environment, they can colonize and persist on fresh produce using a variety of mechanisms. The protective barriers (peels, husks, and rinds) of fruits and vegetables may be dissolved by washing, heat treatment, allowing microbial pathogens to enter the product and gain access to nutrients necessary for their growth of cells. (Harris, L. J et al., 2003). The characteristics of the most common pathogenic bacteria associated with food produce and vegetables are *Bacillus aureus*, *Campylobacter jejuni*, *Clostridium botulinum*, *Clostridium perfringens*, *Cronobacter sazakii*, *Escherichia coli*, *Listeria monocytogens*, *Salmonella species*, *Shigella species*, *Staphylococcus aureus*, *Vibrio species* and *Yersinia enterocolitis*. Pathogens that affect fresh produce and vegetables when consumed it causes damage to human health thereby altering human health, increasing mortality rate, and causing disease

2.2 *SHIGELLA* SPECIES

Shigella is a genus of rod-shaped bacteria in the Enterobacteriaceae family, some of which are common in the human intestine and can cause dysentery or shigellosis infection. *Shigella* bacteria are gram-negative, non-spore-forming, and non-motile bacteria, according to microbiologic classification. Their cells have a diameter of 0.4 to 0.6 micrometers and a length of 1 to 3 micrometers. *S. dysenteriae*, which is spread through contaminated water and food, is the most severe cause of dysentery, but *S. sonnei* and *S. flexneri* have also been identified as dysentery causes.

Shigella are Gram-negative intracellular bacterial pathogens that live in human epithelial ducts and are the cause of shigellosis, a potentially fatal diarrheal disease with symptoms ranging from mild abdominal pain to death depending on severity. Shigellosis is an endemic disease that affects between 80 and 165 million people every year. Ninety-nine percent of Shigella infections happen in developing nations, with children accounting for the majority of mortality. (Kotloff et al., 1999, WHO, 2000). *Shigella* is extremely infectious, requiring only ten *Shigella* colonies to cause disease.

It is primarily due to visits to developing countries and the consumption of contaminated food materials in industrialized countries. Children under the age of five years old had the highest mortality and morbidity rates due to shigellosis worldwide. *Shigella* causes 80-165 million cases of disease and 600,000 deaths each year around the world, with 1.5 million cases reported in developed countries and 163 million in developing countries. Approximately 500,000 shigellosis cases are reported each year in the United States.

Shigellosis is a complicated pathogenic process that includes an enterotoxic and cytotoxic diarrheal prodrome, cytokine-mediated colon inflammation, and colonic epithelial necrosis. The invasion of *Shigella* into the colonic epithelium and lamina propria is the underlying physiological injury that sets off this inflammatory cascade. Bloody, mucoid stools and/or febrile diarrhea are the outcome of colitis and mucosa ulcers. There are four major species of *Shigella*: namely, *Shigella flexneri*, *Shigella boydii*, *Shigella sonnei and Shigella dysenteriae*

2.2.1 *Shigella sonnei*

Shigella sonnei is a Gram-negative intracellular pathogen with a rod-shaped structure. After Carl Olaf Sonne, who described it as the causative agent of shigellosis; it was given the name ‘Sonne's bacillus'. In Europe, North America, and Australia, *S.sonnei* is the most common cause of shigellosis. It is currently expanding across Asia, the geographic region, and the geographical area in middle-income countries. *S. sonnei* was the parent species of *S. sonnei*. coli's genome contains a 4.99 Mbp circular chromosome and a 216 kbp invasion plasmid (pINV) required for virulence, and it focuses on intracellular infection of the human gut epithelium. The chromosome is 6% smaller than those found in other E. coli strains. The genome of E. coli is punctuated by >300 copies of insertion sequence (IS) elements, whose expansion has degraded the genome through gene disruption and deletion. S. Bloody diarrhea, fever, and abdominal pain are all symptoms of *Shigella sonnei*, which is a self-limiting disease. Infections in children under the age of five can be dangerous, and they can also cause growth issues. *Shigella* bacteria can survive gastric acidity and be released in stool, *Shigella sonnei* spreads through the fecal–oral route. *S. sonnei* is

monoclonal. All circulating strains originated from a common ancestor in Europe ~1500 AD. There is only one serotype in one serogroup (*Shigella* serogroup D). Whole-genome sequencing has found five different subtypes (lineages I–V); the common laboratory strain 53G belongs to lineage II, but most clinical isolates now belong to lineage III.(Torraca, V., Holt, K., & Mostowy, S. 2020).

2.2.2 *Shigella flexneri*

Shigella flexneri can grow at 28°C in medium containing 7% NaCl and is salt tolerant. Food is sensitive to organic acids, which are routinely employed to preserve it. S. flexneri growth has been demonstrated to be inhibited by carboxylic acid, acid, malic acid, and hydroxy acid, among others (Zaika 2002). *Shigella flexneri* generates cysteine protease-like virulence gene A (VirA) enzymes, which disrupt DNA damage response pathways, as well as inositol phosphate phosphatase D (IpgD) enzymes, which degrade host p53 and disrupt cell cycle (Bergounioux et al., 2012). Shigellosis is a disease caused by the *Shigella flexneri* bacteria, a facultative intracellular organism. *Shigella* IpaB activates caspase 1 in macrophages, causing them to die. *S. flexneri* takes up residence in the human body. It can produce an acid resistance mechanism and make it through the stomach to the large intestine, where cellular invasion occurs (Gorden and Small, 1993). *Shigella* is a letter that begins with the letter S. A 31-kb section of a large pathogenic plasmid contains the *Shigella flexneri* genes essential for invasion. T3SS and various effector proteins, including IpaA, IpaB, IpaC, and IpgD, are all encoded in this area. IpaB is a virulence factor that regulates T3SS secretion, phagosome escape, and macrophage death among these proteins (Guichon et al., 2001, Blocker et al., 2003, Schroeder et al., 2007, Schroeder and Hilbi, 2008, Roehrich et al., 2010). The invasion process is divided into six stages: (1) the path through microfold cells (M cells); (2) immune cell escape; (3) cell invasion; (4) intracellular multiplication; (5) intracellular and intercellular spread; and (6) host cell elimination. Although contaminated food or water can cause *S. flexneri*-induced shigellosis, person-to-person transmission still predominates (Hung et al., 2015). Outbreaks are most common in densely populated settings like nursing homes and schools, and they can last anywhere from a few weeks to several months (Chen et al., 2003,) (Calderon-Margalit et al., 2010).

2.2.3 *Shigella dysenteriae*

Shigellosis is caused by *Shigella dysenteriae*, a gram-negative bacterium. The illness is also divided into two stages. Watery diarrhoea and cramps characterize the first phase, which lasts 1 to 2 days and is caused by an enterotoxin. The second phase, which involves fever, cramps, bloody diarrhoea, and tenesmus, is brought on by the organism's invasion of the large intestine. The endoscopic image, despite its patchwork

appearance, can mimic inflammatory bowel illness since there are multiple ulcers with substantial exudate. The mucosa often appears magenta in colour due to the extensive erythema. *S. dysenteriae* infection is a dangerous condition. Ulcers and bloody diarrhoea, both of which are associated to Shiga toxin assembly, can be caused by *S. dysenteriae*. The illness is diagnosed via stool culture, which is only positive in roughly half of the cases. (Niyogi et al., 2005).

2.2.4 *Shigella boydii*

Another *shigella* species, Shigella boydii, has 19 distinct serotypes. In Central and South America, *Shigella boydii* epidemics are more common than elsewhere. It's difficult to distinguish between isolates of *S. boydii* and *S. flexneri*. *S. boydii* polyvalent group C antiserum and thus corresponding monovalent factor antisera specific for serotypes 1–19 agglutinate isolates of this organism, which differ primarily in that they are typically agglutinated by *S. boydii* polyvalent group C antiserum and thus corresponding monovalent factor antisera specific for serotypes 1–19. The rfb RFLP technique distinguishes them as well (Coimbra et al., 2001), it has proven to be a reliable molecular approach for identifying known Shigella O serotypes as well as discovering new *Shigella* O serotypes.

Fig 2.1 *Shigella species* on Hektoen enteric agar adapted from Medical laboratory by (Hussein et al 2014).

Table 2.1: **Table showing serotypes and group of *shigella* species.**

Subgroup	**Serotypes and Subtypes**
Group A: *Shigella dysenteriae*	15 serotypes
Group B: *Shigella flexneri*	8 serotypes and 9 subtypes
Group C: *Shigella boydii*	19 serotypes
Group D: *Shigella sonnei*	1 serotype

The *Shigella* species are facultative intracellular gram-negative pathogens that cause shigellosis, which remains a significant public health concern. *Shigella* species was adopted as a genus of the family Enterobacteriaceae in the 1950s and sero grouped into the following four species (Sheikh et al., 2019)

2.3 GENETICS OF VIRULENCE

Shigella are precisely adapted to reproduce within the epithelium of the human host's intestine. Many of the bacterial virulence factors that mediate the intricate interactions between these bacteria and mammalian host cells have been discovered using genetic and immunological approaches. These virulence determinants are encoded by large extra - chromosomal elements (plasmids) that are functionally similar in all *Shigella* species including EIEC. Antibodies identify a complex of two plasmid-encoded determinants known as Invasion Plasmid Antigens (Ipa) B and C in the serum of convalescent patients. In conditions identical to those seen in the intestinal lumen, interpretative phenomenological proteins are expressed at their maximum levels (e.g., bile salts, high osmolarity, and human body temperature), and release of the IpaB complex is triggered by contact with the mammalian host cell. This combination stimulates the endocytic absorption of shigella by M cells, epithelial cells, and macrophages. IpaB is also involved in the lysis of endocytic vacuoles in epithelial cells and macrophages. In the latter case, Ipa proteins cause the release of the cytokine IL-1 and macrophage death. Another plasmid-encoded virulence factor is secreted at the poles of *Shigella* daughter cells as they develop within the cytoplasm of infected host cells. The filamentous actin polymerizes due to the Intercellular Spread (IcsA) protein. The creation of this actin tail acts as a driving factor for shigella to impinge on the plasma membrane of the infected cell. As a result of the protrusions that form, the plough is distorted. The filamentous actin polymerizes due to the Intercellular Spread (IcsA) protein. The creation of this actin tail acts as a driving factor for

shigella to impinge on the plasma membrane of the infected cell. Plasma membranes in neighboring cells are distorted as a result of the protrusions. The plasmid-encoded protein IcsB then lyses plasma membranes, allowing bacteria to spread between cells. Biochemical characterization of the link between these *Shigella* virulence factors and host cell components is a research challenge. Characterizing and improving the neutralizing potential of antibodies that detect these protein virulence indicators is a key research priority. (Hale, T. L., & Keusch, G. T. 1996).

2.4 CULTURAL CHARACTERISTICS OF SHIGELLA BACTERIA

Shigella species are aerobic and facultative anaerobes that grow well in culture media at pH 6.4 to 7.8 and temperatures ranging from 10 to 40 degrees Celsius, with a preferred temperature of 37 degrees Celsius. *Shigella* colonies achieve a diameter of around 2 mm after 24 hours of incubation. The colonies are circular, convex, colorless, but somewhat translucent, with a smooth surface and complete edges. At one or more spots on the colony's periphery, little, tangled hair-like projections can be detected. They produce pinkish to reddish colonies on XLD and green to blue green colonies on Hektoen Enteric Agar (HEA). (Chen, W. H., & Kotloff, K. L. 2016).

2.5 SHIGELLA INFECTION

Shigella dysenteriae, Shigella flexneri, Shigella boydii, and *Shigella sonnei* are the bacteria that cause acute diarrhea. Man is Shigella's only known reservoir, and transmission happens through direct contact between individuals, as well as contaminated water and food. Sexual transmission has been observed in homosexual males. Low- and middle-income countries account for the majority of instances in industrialized countries. *Shigella dysentery* is characterized by acute, bloody diarrhea, fever, and abdominal pains and is caused by *Shigella* species. Mucus, pus, and blood in the feces are signs of classic dysentery. Shigellosis is responsible for rectal and colonic ulcers that do not progress beyond the lamina propria. (Lima, I. F., Havt, A., & Lima, A. A. 2015)

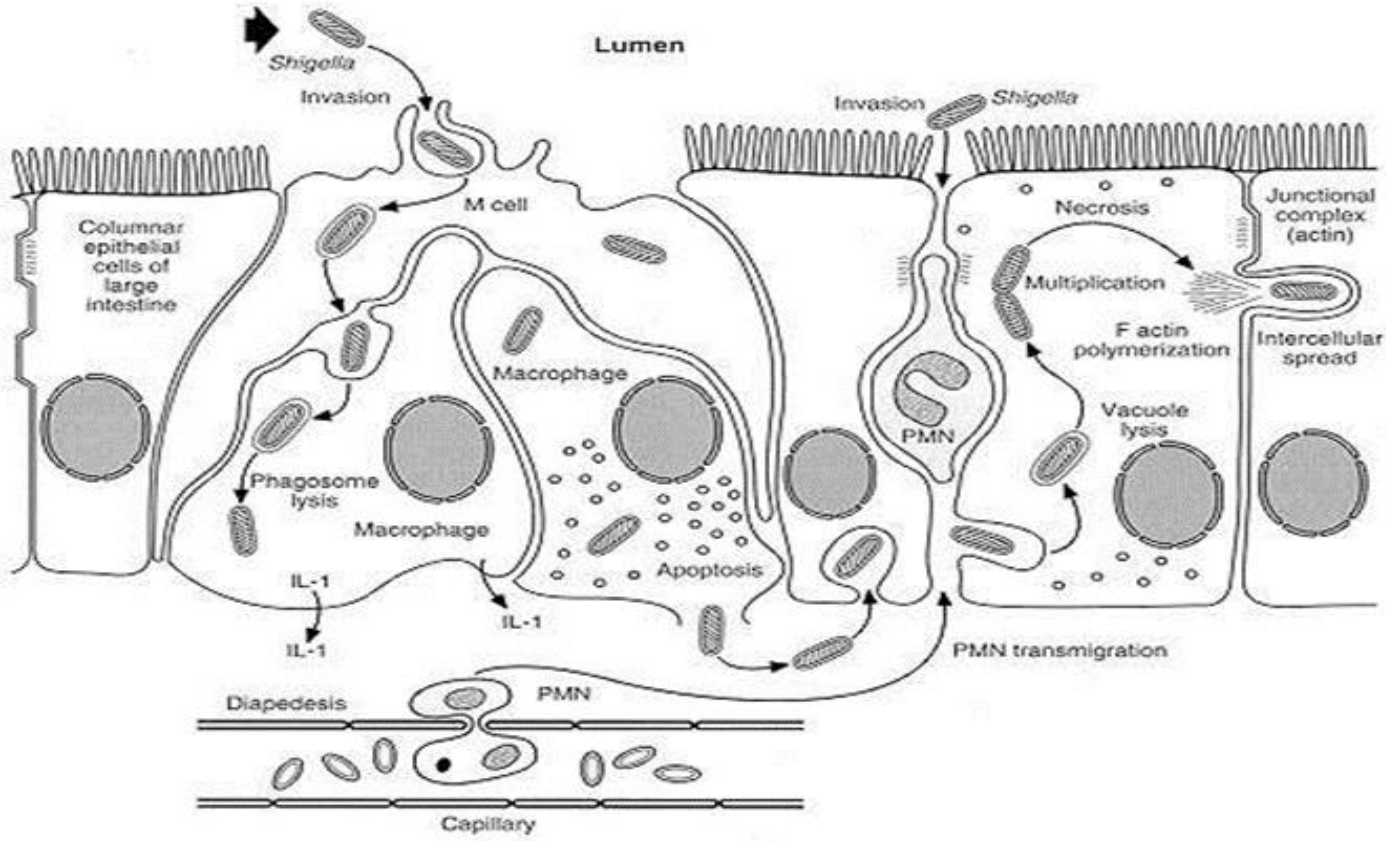

Fig 2.2 Invasion of human epithelial cell by *Shigella* species. (Amir.et al., 2021)

2.5.1 DISEASE AND SYMPTOMS OF SHIGELLOSIS INFECTION

Shigellosis is a disease that affects only one person and is usually spread by polluted and contaminated food and water (Parsot, C., & Sansonetti, P. J. 1996). *Shigella* infections are spread by the oral-fecal channel, and infection can be transmitted with as little as 100 microorganisms (Parsot, C., & Sansonetti, P. J. 1996), owing to the bacterium's capacity to tolerate the stomach's extreme acidity (Small et al., 1994). The lack of unpolluted water, consuming unhealthy fresh produce, inadequate sanitation, starvation, and the value of antibiotic therapy are all factors in the high occurrence of *Shigella* in impoverished nations (Jennison & Verma, 2004). The infection is thought to cause a slew of symptoms, ranging from watery diarrhea to severe dysentery. Fever, stomach aches, and acute, chronic bloody and mucoid diarrhea are all symptoms of severe dysentery (Phalipon & Sansonetti, 2007). Patients with shigellosis can develop secondary problems such as septicemia and pneumonia if there are no appropriate treatments available (Bennish, 1991; Jennison & Verma, 2004). Shigella's type III secretion system (T3SS), a needle-like molecular syringe anchored in the bacterial cell wall, is required for virulence. The T3SS is activated when the needle tip comes into contact with the host plasma membrane, forming a direct route between the bacterial and host cytoplasm. *Shigella* uses this syringe to inject a huge number of bacterial effectors to disrupt a variety of host cell functions and facilitate infection and spread.

Before infecting the host, *Shigella* must first withstand the physiochemical circumstances encountered during its transit through the digestive system, as well as traverse the mucus layer of the colon. *Shigella* penetrates the colon via M (microfold) cells of the specialized epithelium beneath lymphoid follicles. *Shigella* is carried to the epithelium's basolateral side by M cells, which are phagocytosed by antigen-presenting cells such as macrophages and dendritic cells located within or near the M cell pocket. Shigella lyses the macrophage's phagosomal compartment swiftly in a T3SS-dependent way, then leaves the macrophage after limited replication in the cytosol. *Shigella* leaves the macrophage via inducing pyroptosis, a lytic inflammatory cell death. (Schnupf, P., & Sansonetti, P. J. 2019). *Shigella* promotes macrophage pyroptosis by causing caspase-l-mediated proteolytic activation and subsequent release of the proinflammatory cytokines interleukin 1 (IL-1) and IL-18. When infected macrophages die, *Shigella* is discharged at the basolateral side of colonic epithelial cells. By generating epithelial cell macropinocytosis, Shigella interacts with host cell proteins present on the basolateral side of epithelial cells and drives its uptake into epithelial cells. The T3SS and a first wave of effectors are required for invasion to disrupt the host cell cytoskeleton and facilitate vascular escape. Shigella replicates in the cytosol and uses actin-based motility to avoid host cell innate defences like autophagy and spread to

neighbouring cells. *Shigella* is released into its replicative niche, the host cell cytoplasm, by lysing the double membrane of secondary phagosomes created by Shigella physically pushing into surrounding epithelial cells. *Shigella's* cytosolic replication is additionally aided by the injection of a second wave of T3SS effectors, which dampen the host's inflammatory response, enhance host cell survival, and block antimicrobial activities. This protects the integrity of its cytosolic niche and creates favourable circumstances for reproduction and spread. (Schnupf, P., & Sansonetti, P. J. 2019).

Although *Shigella* effectors in infected epithelial cells suppress the inflammatory response, nearby bystander cells play an active role in the inflammatory process by secreting the neutrophil chemoattractant IL-8. Inflammation, the recruitment, and activation of immune cells, including natural killer (NK) cells and particularly neutrophils, and antimicrobial defences are all aided by the release of IL-8 from epithelial cells, in addition to the proinflammatory cytokines IL-1 and IL-18 by pyroptotic macrophages. The instability of the epithelial barrier is aided by neutrophil transmigration to the luminal side, which allows luminal *Shigella* to spread. The instability of the epithelial barrier is aided by neutrophil transmigration to the luminal side, which allows luminal *Shigella* to translocate to the basolateral side. As a result, the inflammatory reaction of the host can help *Shigella* spread. (Schnupf, P., & Sansonetti, P. J. 2019).

In order to prolong survival in the host, *Shigella* has devised a number of tactics to suppress the host's inflammatory response, manipulate the host's innate immune responses, and retain its favoured cytosolic replicative niche. (Schnupf, P., & Sansonetti, P. J. 2019). However, *Shigella* infection is eventually cleared due to inflammation and neutrophil mediated *Shigella* death. Neutrophils are especially crucial in *Shigella* containment and resolution because they are more resistant to *Shigella*-mediated cell death than macrophages and can kill *Shigella* via phagocytosis, reactive oxygen species generation, and the release of microbicidal chemicals from their granules (degranulation). *Shigella* also creates neutrophil extracellular traps, in which the neutrophil dies, and nuclear chromatin and antibacterial proteins are released, immobilizing, and killing *Shigella*. *Shigella* uses its T3SS and effectors to prevent the host from mounting an efficient adaptive immune response by suppressing the innate immune response and directly targeting and regulating the activities of host B and T cells. (Schnupf, P., & Sansonetti, P. J. 2019).

2.5.2 SYMPTOMS OF SHIGELLOSIS INFECTION

Shigella causes diarrhea in those who are infected (occasionally bloody and mucoid) a day or two after swallowing the germs, as well as fever, abdominal pain, and cramps. Dehydration may be present, and in severe situations, seizures in children may develop.

Shigellosis normally lasts 5 to 7 days in those who have a healthy immune system, but it might take months for bowel movements to return to normal. Even if some individuals have minimal symptoms, they can still spread the *Shigella* bacterium to others if they do not take precautions like washing their hands (especially after toileting and changing or handling diapers).

2.6 PATHOGENESIS

Consumption of *shigella* bacteria causes infection, and diarrhea (perhaps induced by enterotoxins and/or cytotoxin) can develop as the organism's transit through the small intestine. Shigellosis is characterized by inflammatory colitis and bacterial invasion of the colonic epithelium. These processes are amplified by the local release of cytokines and the infiltration of inflammatory components. The classic bacillary dysentery symptom of scant, unformed stools colored with blood and mucus is caused by colitis of the rectosigmoid mucosa mixed with malabsorption. (Keusch et al., 1996).

2.7 DEFENSES OF THE HOST

Inflammation, a lot of mucus, and regeneration of the wounded colonic epithelium all contribute to manage colitis and promote self-healing. Serotype-specific immunity is induced after an initial infection, meaning that antibodies that identify the lipopolysaccharide (LPS) somatic antigen play a protective function. *Shigella* antigens that cause bacterial invasion of the epithelium include enterotoxins, cytotoxins, and plasmid-encoded proteins. Immune responses to these antigens do not appear to provide any protection. (Keusch et al., 1996).

2.8 TREATMENT OF SHIGELLOSIS INFECTION

Although severe dehydration is uncommon in shigellosis, the first step in treating any diarrheal illness is to address abnormalities created by isotonic dehydration, metabolic acidosis, and significant potassium loss. As long as the patient is not vomiting or in shock from severe dehydration, the World Health Organization's oral rehydration treatment has been found to be effective and safe in the treatment of acute diarrhea. In the latter case, intravenous fluid replacement is required until the initial fluid and electrolyte deficits are restored. Shigellosis is normally a self-limiting infection that can be treated with excellent hydration, and medications are prescribed based on the severity of the infection transmission. The time it takes for Shigella to be expelled after symptoms have ceased is reduced from 5–7 days to 3 days with effective antibiotic treatment. When the isolate is susceptible, absorbable antibiotics such as ampicillin (2 g/day for 5 days) are likely to be effective. Although resistance to these antibiotics is on the rise, trimethoprim (8 mg/kg/day) and sulfamethoxazole (40 mg/kg/day) kill sensitive bacteria in the intestine

quickly. Ciprofloxacin (1 g/day for 3 days) is effective against a wide range of bacteria that are resistant to antibiotics. However, because of the risk of cartilage injury, the US Food and Drug Administration has not approved it for use in children under the age of 17. Opiates, such as paregoric, produce intestinal stasis, which can lead to bacterial invasion and prolong the fever. (Hale, T. L., & Keusch, G. T 1996).

TABLE 2.2 Sources of pathogenic micro-organism on fresh produce during pre-harvest and post-harvest of the produce

PRE-HARVEST	POST HARVEST
Feces	Wild and domestic animal
Soil	Air (dust)
Irrigation water	Wash and rinse water
Green or inadequately composted manure	Sorting, packing, cutting, and further processing equipment
Air (dust)	Ice
Wild and domestic animals	Transport vehicles
Human handling	Improper storage (temperature, physical environment)
	Improper packaging (includes new packaging technologies)
	Human handling (workers, consumers)
	Cross-contamination (other foods in storage, preparation, and display areas)
	Improper display temperature
	Improper handling after wholesale or retail purchase

2.9 CONTROL OF SHIGELLOSIS INFECTION

Providing safe and ample water, as well as efficient waste disposal, are the most effective techniques for controlling shigellosis and other intestinal disorders. At best, these public health measures in impoverished countries are long-term approaches to managing enteric diseases. More immediate and practical remedies are needed to address the estimated five million deaths from diarrheal disease each year in these countries, as well as survivors' malabsorption and stunting. The most successful intervention method for reducing morbidity and death would be a multi-pronged media and personal outreach campaign that included the following elements: (1) encourage mothers to breast-feed infants; (2) promote the use of oral rehydration therapy to counteract the effects of acute diarrhea; (3) encourage mothers to provide convalescent nutritional care in the form of extra food for children recovering from diarrhea (4) all residents should be educated on how to avoid fecal contamination of food and water, as well as the importance of hand washing after feces. (Hale and, Keusch 1996).

CHAPTER THREE

3.0 MATERIALS AND METHOD

3.1 SAMPLE COLLECTION

A total of 63 fruits and vegetables samples were bought at Ogun state and Lagos state from the area local markets These comprised of cucumbers, watermelon, pawpaw, cabbages, pineapples, and carrots which were placed in food grade bags and then taken to the laboratory and analyzed immediately while others that could not be analyzed immediately were stored at 4^0_C overnight before analysis.

Table 3.1: Table showing the location, and number of times each fresh produce was sampled.

Fresh produce sampled	Location		
Lettuce	Jakande (L)	Ibafo (O)	Magboro (O)
(*n* = 9)	3	3	3
Cabbage	Yaba (L)	Ibafo (O)	Magboro (O)
(*n* = 9)	3	3	3
Pineapple	Magodo (L)	Ibafo (O)	Magboro (O)
(*n* = 9)	3	3	3
Watermelon	Magodo	Yaba (O)	Magboro (O)
(*n* = 9)	3	3	3
Cucumber	Jakande(L)	Ibafo (O)	Magboro (O)
(*n* = 9)	3	3	3
Carrot	Yaba (L)	Ibafo (O)	Magboro (O)
(*n* = 9)	3	3	3
Pawpaw	Yaba (L)	Ibafo (O)	Magboro (O)
(*n* = 9)	3	3	3

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

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 AND REAGENT

3.4.1 FOR ISOLATION OF *SHIGELLA*

Nutrient Agar, Xylose lysine Deoxycholate (XLD), Brain Hearth Infusion Broth (BHI), Buffer peptone water, 20 % Glycerol water, Distill water, Ethanol.

3.4.2 FOR MOLECULAR IDENTIFICATION

Nuclease free water, Master Mix primers, agarose, 1X TAE buffer, Ethidium Bromide.

3.4.3 FOR BIOCHEMICAL TEST

Crystal violet, Iodine, Alcohol (95%), Safranin, 3% Hydrogen peroxide.

3.5 PREPARATION OF CULTURE MEDIA

3.5.1 BUFFER PEPTONE WATER

Peptone water is a microbiological growth medium made up of sodium chloride and peptic digested animal tissue. The medium is rich in tryptophan and has a pH of 7.20.2 at 25 °C. Peptone water can also be utilized as a primary enrichment medium for bacteria growth because it is a nonselective broth medium.

PREPARATION

1. The dehydrated medium was dissolved in 1 liter 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 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. 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.

3.5.2 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 liter of distilled water based on manufacturer's instructions in a conical flask and mixed thoroughly. The conical flask is then closed in cotton wool that is wrapped in aluminum foil.

2. The mixture was heated for a 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 color.

3.5.3 XYLOSE LYSINE DEOXYCHOLATE AGAR (XLD AGAR)

Xylose Lysine Deoxycholate Agar is a selective growth medium used for the isolation of *Shigella species.* From clinical and food samples.

PREPARATION

1. The medium (57 grams) was suspended in 1000 milliliters of distilled water and carefully mixed. To completely dissolve the powder, the mixture was heated with frequent agitation. At 50°C, it was moved to the water bath.
2. It is not to be autoclaved as instructed by the manufacturer. The agar was allowed to cool to 45OC and poured ascetically into sterile Petri dishes and left to solidify.

3.5.4 HEKTOEN ENTERIC AGAR

Hektoen Enteric Agar is a selective and differential medium for isolating and distinguishing Shigella species.

PREPARATION

1. The medium 72.66 grams was suspended in 1000 ml purified/ distilled water and mixed thoroughly. The mixture was heated with frequent agitation to completely dissolve the powder.
2. It is not to be autoclaved as instructed by the manufacturer. The agar was allowed to cool to 45-50°C and poured ascetically into sterile Petri dishes and left to solidify.
3. Spread the inoculum to obtain well-separated colonies. Incubate for 18-24 hours at 37°C.

3.5.5 BRAIN HEART INFUSION

Brain Heart Infusion (BHI) broth is a general-purpose liquid medium for the culture and maintenance of a wide range of fastidious and non-fastidious microorganisms, including aerobic and anaerobic bacteria, yeast, and molds from a variety of clinical and non-clinical specimens.

PREPARATION

1. The dehydrated medium was dissolved in 1 liter of distilled 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 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. 5ml of the 0.1% was then dispensed into various test tubes.

3.6 *SHIGELLA* SPECIES ISOLATION

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 plates.

3.6.1 PRIMARY ENRICHMENT

Another 25g of samples were homogenized with 225ml of 1% peptone water and incubated at 37°C to serve as pre-enrichment for *Shigella.*

3.6.2 SECONDARY ENRICHMENT

This was conducted to see if Shigella could be identified, the overnight incubated pre-enrichment media of BPW was used to inoculate the secondary enrichment media. Ten (1) ml of sample pipetted from the peptone water was dispensed into 9mls Brain heart infusion broth (BHI) in a scotch bottle (Duran bottle) and incubated for 24hrs in 37°C. After incubation 0.1ml of the incubated brain heart infusion broth was transferred into Xylose lysine Deoxycholate agar (XLD) for presumptive confirmation of *Shigella* species. They were then incubated inversely at 37°C in an incubator for 24hrs. Colonies counted on plate were sub-cultured from Xylose lysine Deoxycholate (XLD) agar to the newly prepared nutrient agar and HEA agar to get pure culture.

Test tubes were filled with 9ml of peptone water and sterilized using autoclave at 1200C for 15 minutes. The tubes were used to make serial dilutions from 10-1 to 10-7. These were plated unto the three (3) culture media; Nutrient agar (NA) using spread plate method.

3.6.3 PLATING OF THE AGAR

For the Nutrient agar, spread plate technique was used for plating of inoculum (samples). About 15-20ml of agar is poured into sterilized petri dishes (observing aseptic methods and conditions), then allowed to cool and solidify. 0.1ml of the inoculum directly from dilutions are plated (using pipettes) onto appropriately labelled agar-containing petri-dishes for Nutrient agar, this will suffice for the enumeration, identification and isolation of Total Viable Counts and *Escherichia coli* strains, respectively. After dispensing onto respective agar, the glass rod is used to spread or distribute the inoculum all around the agar (the glass rod was dipped into alcohol and then flamed in the Bunsen burner before spreading to maintain aseptic conditions). Replicates were made for each dilution for each sample.

However, for the detection of *Shigella*, 0.1ml of inoculum from the secondary enrichment media incubated overnight at 37°C for 18-24 hours were pipetted onto Hektoen enteric agar, Aseptic conditions were observed, and replicates were made for each sample and each dilution. After the inoculation with serially diluted samples, the inoculated agar petri-dishes are placed in an inverted fashion and are transferred into the incubator at 37°C for 18-24 hours.

3.6.4 SUB-CULTURING SHIGELLA ORGANISM

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 based on their colony morphology, shape, color, elevation, and other physical characteristics. Colonies

differentiated by morphological characteristics are transferred onto fresh Petri dishes containing Nutrient agar. A loopful of preferred isolate was taken using the inoculating loop (the inoculating loop is heated using the Bunsen burner and allowed to cool for like 5 seconds 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.

- On XLD agar *shigella* organism is color Black.
- On HEA agar *shigella* organism in color Green.
- On Nutrient agar *shigella* organism is color Colorless.

3.6.5 CRYOPRESERVATION OF ISOLATES

A loopful of each isolate(two white, two pink) from the incubated nutrient agar was inoculated into 5ml of Brain heart infusion (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 ^{o}C.

3.7 DNA EXTRACTION

Isolates from the same sample were pulled (1ml of BHI was added to a cryotube and autoclaved. 50ul of each isolate H1-H4 (*Shigell*a1-4) 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.

3.7.1 POLYMERASE CHAIN REACTION (PCR)

The components of the PCR used for *Shigella* 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.

PCR PROTOCOL

PCR REACTION COMPONENTS USED FOR 16S RRNA AMPLIFICATION.

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	0.25ul
3	16sr	20um	0.25um	0.25ul
4	DNA			4ul
5	dH_2O			5.75ul
6	Total			10ul

Table 3.3: PCR PROTOCOL for *ipa*H gene for *Shigella* species

No.	Reagents	Initial concentration	Final concentration	Volume/rxn
1	Master mix	5x	1x	5ul
2	IpaHF	20um	0.25um	0.3125ul
3	IpaHR	20um	0.25um	0.3125ul
6	dH_20			15.125ul
	DNA			3ul

PROCEDURE FOR THERMALCYCLER

Table 3.4: PROCEDURE FOR THERMALCYCLER

Analysis	Step	Temperature	Time
1x	Initial denaturation	950c	5 min
35x	Denaturation	950c	2 min
	Annealing	420c	30 sec
	Polymerization	720c	4 min
1x	Final polymerization	720c	10 min
1x	Hold	40c	∞

3.8 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.

3.8.1 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.

3.9 BIOCHEMICAL TEST FOR *SHIGELLA* ORGANISM

3.9.1 GRAM STAINING

A smear of suspension was formed with a loopful of the isolate on a clean, grease-free slide. Heat was used to repair it after it was air dried. 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 roughly 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.

3.9.2 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.

3.9.3 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.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 RESULTS

This study reports on the isolation of *Shigella* from fresh produce sold by the roadside using selective and differential medium XLD and HEA, PCR amplification using 16s, IpaHF, IpaHR , forward and reverse primers. Ibafo lettuce (**SIL**) had the highest total viable count of 7.0 $\log_{10}$cfu/g. According to the results, some of the samples had *shigella* isolates in them which is not in line with the food safety standard, which explains pathogenic microorganisms should not be found within 25g of food samples; The microbial investigation of pathogens presents in the three different kinds of fresh produce (lettuce, cabbage, and carrot) from some area in Ogun state and Lagos state market was as follows:

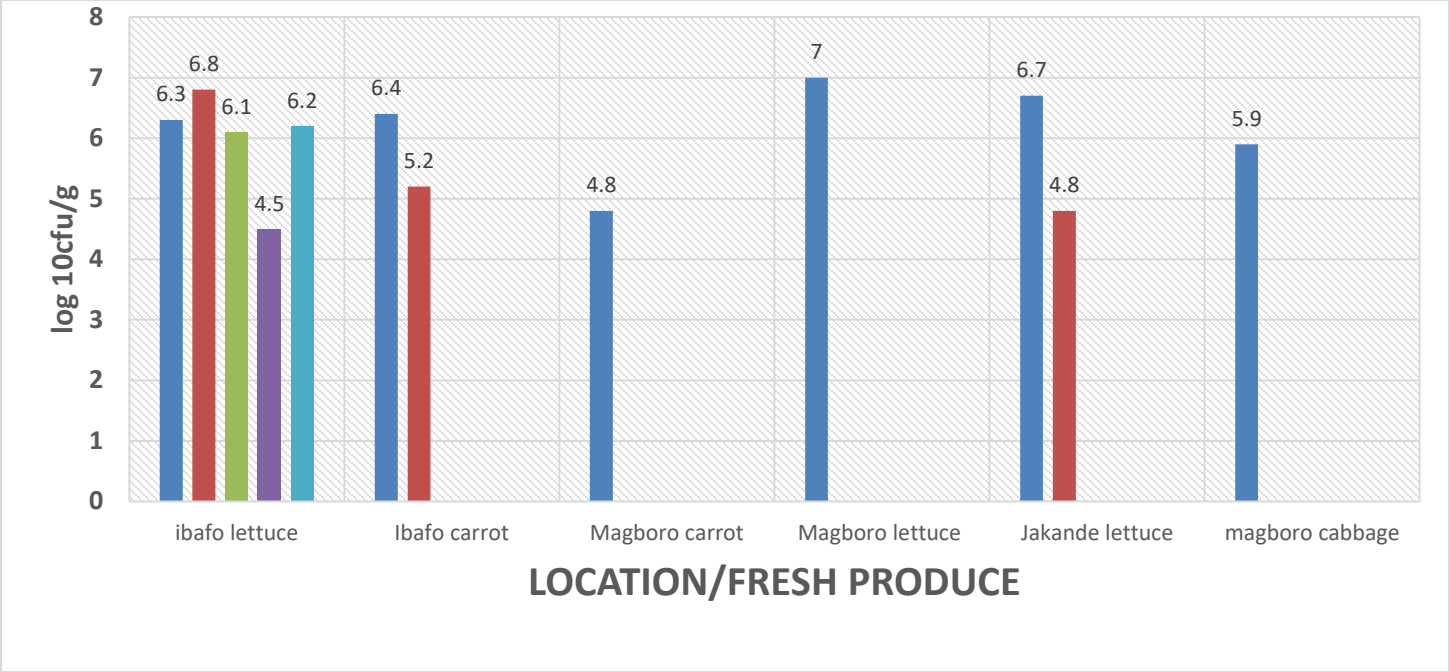

Figure 4.1: Total viable count of *shigella* in different locations

In the histogram above Magboro lettuce had the highest TVC of 6.5- 7.0 log_{10} cfu/g followed by Ibafo Lettuce with a TVC range of 6.3- 6.8log_{10} cfu/g. Jakande lettuce had the third highest TVC range of 4.8-6.7log_{10} cfu/g, followed by Ibafo carrot with a TVC range of 5.2-6.4 log_{10} cfu/g, while Magboro carrot had the lowest TVC range of 4.8 log_{10} cfu/g, followed by Ibafo lettuce (fourth dilution) with a TVC range of 4.8- 6.4log_{10} cfu/g. Carrot the third lowest TVC range of 6.0- 6.9log_{10} cfu/g. while Magboro cabbage had TVC 5.9 log_{10} cfu/g.

4.2 BIOCHEMICAL TEST FOR *SHIGELLA*

Table 4.1 Characteristics of bacterial isolates showing the biochemical test for *shigella* organism.

CODE	GRAM STAINING	CATALASE TEST	OXIDASE TEST
SIL	Negative (-ve)	Positive (+ve)	Negative (-ve)
2SIL	Negative (-ve)	Positive (+ve)	Negative (-ve)
SGB	Negative (-ve)	Positive (+ve)	Negative (-ve)
SIR	Negative (-ve)	Positive (+ve)	Negative (-ve)
SMP	Negative (-ve)	Positive (+ve)	Negative (-ve)
2SIL	Negative (-ve)	Positive (+ve)	Negative (-ve)
2SIL(II)	Negative (-ve)	Positive (+ve)	Negative (-ve)
2SIL(II)	Negative (-ve)	Positive (+ve)	Negative (-ve)
SJL	Negative (-ve)	Positive (+ve)	Negative (-ve)
2SJL	Negative (-ve)	Positive (+ve)	Negative (-ve)
2SIR	Negative (-ve)	Positive (+ve)	Negative (-ve)
SGC	Negative(-ve)	Positive(+ve)	Negative(-ve)

SHIGELLA SPECIES

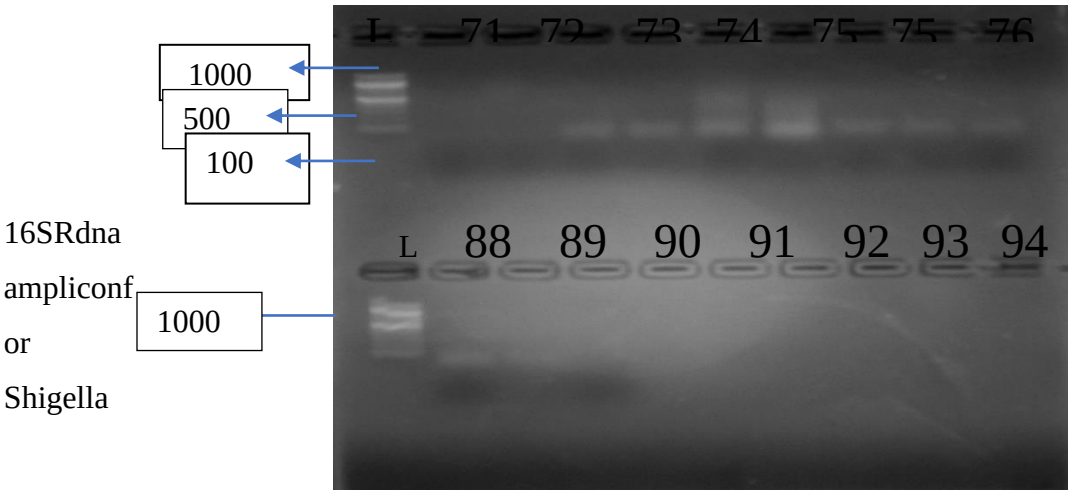

Figure 4.2 - 16S rDNA amplicon for *Shigella species*

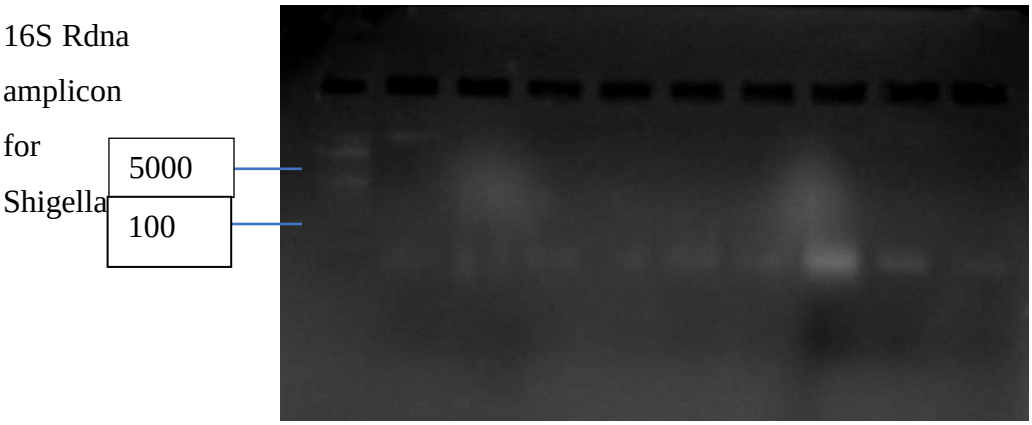

Figure 4.3: Agarose Gel electrophoresis of PCR amplicon for (16 rRNA amplification)

4.3 FOR PCR *ipa*H gene

6 isolates were selected after biochemical test identification for further examination by PCR for the detection of *IpaHF1,* and/or *IpAhR2* genes. It showed that one isolate (Ibafo Lettuce) contained *IpaHf* gene, which makes it toxin producing *shigella* positive. Agarose gel electrophoresis of PCR products for *IpaHF*, *IpaHR* and are shown in figure 4.2.

SPECIFIC

SHIGELLA

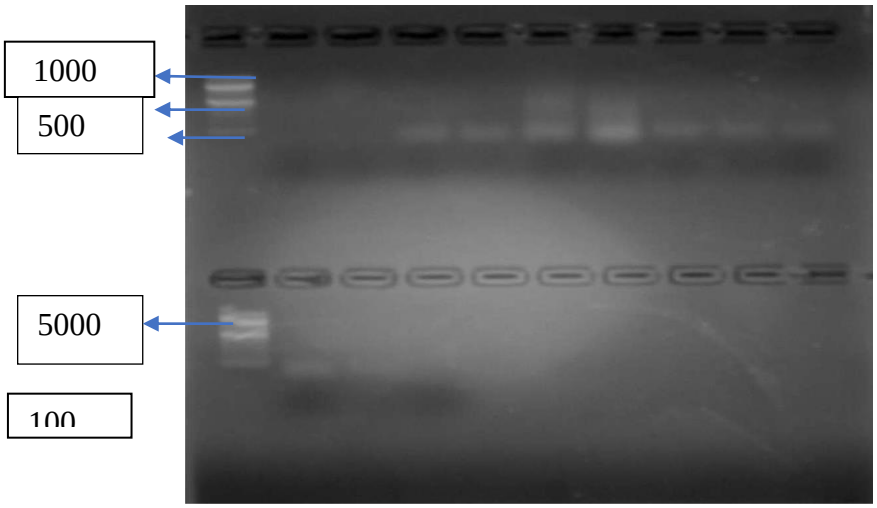

Figure 4.4: Agarose gel electrophoresis for Multiplex PCR

4.4 DISCUSSION

It is difficult to attest to the hygiene of these sellers or the hygienic conditions at stages of processing as well as the packaging materials, eating fresh produce directly from street vendors or hawkers may raise the risk of food-borne diseases caused by a wide variety of pathogens. (Olorunfemi et al., 2011) The considerable differences in contamination levels detected in products from the local market could be due to the level of exposure and handling practices. The products are opened as often as the buyers demand in the market, and the open display of things to attract customers encourages occasional fly visits. (Olorunfemi et al., 2011). The dusty, unsanitary market settings, combined with inadequate vendor handling, all contribute to the high microbial load. The widespread practice of washing all fruits in the same bucket of water, if they are ever washed at all, and cutting with the same equipment, for example using the same knife to cut all the fruits. Cross contamination could potentially be responsible for the microbial loads obtained even from market products. Vendors of ready-to-eat fruits frequently employ basic equipment such as wheelbarrows, trays, tables, and makeshift stalls, increasing the danger of food contamination. (Olorunfemi et al., 2011). During peeling, slicing, trimming, and other processes, pathogens may enter the interior surfaces of the crop. Processes like packaging, handling, and marketing. The risk of contamination is increased by the fact that fruits and vegetables are sold on the street without proper storage, exposing fresh produce to flies and keeping it at optimal temperatures for the invasion and proliferation of contaminants, pathogenic mesophiles, and other disease-causing agents. (Olorunfemi et al., 2011). Fresh produce is processed and sold by unlicensed sellers with low levels of education and food hygiene training]. In impoverished countries, poorly prepared street-vendor produce has been identified as a major source of disease. Fruits have been linked to food-borne disease outbreaks in a number of countries; organisms involved include bacteria. Fungi, viruses, and parasites. It has been reported that fresh produce has been tainted with Norovirus. In the United States of America, shigellosis outbreaks have been linked to the intake of chopped lettuce and cabbage. (Oranusi et al., 2011) Bacteria such as *Shigella species*, *Salmonella species*, *Campylobacter species*, and *E. coli* can contaminate fresh food when it comes into contact with sewage or contaminated water.(Oranusi et al., 2011)*Bacillus, Penicillium,* and *Aspergillus species* had the highest rate of incidence, appearing in 100% to 60% of the samples. (Olorunfemi et al., 2011). This could be because these organisms produce spores and are known prevalent environmental pollutants, but they have also been linked to foodborne diseases their presence in fresh product could be due to the vendor's filthy hands, sewage contact, or contaminated water. Food cross-contamination during preparation has been found as a significant contributor to food-borne disease. The

presence of *Salmonella spp., E. coli, Klebsiella*, and *Enterobacter* should be cause for concern, as these organisms are usually linked to inadequate sanitation and could indicate a risk of foodborne infection. (Olorunfemi et al., 2011)*E. coli and Shigella species* are particularly fecal in origin and have been linked to a variety of foodborne illnesses. Vendors, water, and poor hand- and utensil-washing appear to be the main hazards related with these fruits, and they must be adequately addressed. Fresh fruits should be washed well before peeling, slicing, or cutting; fruits should be handled with clean and disinfected hands, utensils, and surfaces, and stored refrigerated. If any delay prior consumption (Oranusi et al., 2011).Good personal hygiene and effective hazard analysis and critical control point (HACCP) application reduces the chance of contamination of ready to eat fruits. (Oranusi and Olorunfemi 2011).

CHAPTER FIVE

5.0 CONCLUSIONS AND RECOMMENDATIONS

Roadside selling of fresh produce in Lagos and Ogun states markets proved to have pathogenic microorganisms. The presence of *Shigella* in the fresh produce is of great concern as diseases could occur from consumption of fresh produce (lettuce, cabbage, carrot and so on). Roadside fresh produce sold in (Magboro, Ibafo axis) area of Ogun state and (Jakande, Magodo, and Yaba axis) contain presumptive pathogenic *shigella* which can create a public health hazard.

Therefore, fresh food produce such as carrot, watermelon, cabbage and so on should be sold in a supermarket and it must be well packed, washed and germ free. The public should ensure the safety of roadside fresh produce by checking thoroughly washing the fresh produce before consumption. Poor storage condition is also a means that fresh produce get contaminated, proper storage facility should be present and prepared before the transportation and selling of fresh produce.

Farmers producing fresh produce should ensure that proper hygiene is adequately considered, and the transportation of the fresh produce is healthy before, during, and after processing of the fresh produce. As an addendum surveillance system should be set in place by the government and its concerned agencies, to trace the occurrence and prevalence of foodborne diseases occurring as a result of consumption of roadside fresh produce with a high level of microbial load capable of causing diseases and increase mortality rate.

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