PREVALENCE OF BACTERIA ISOLATED FROM CARROT AND GREEN PEA SAMPLES SOLD IN LOCAL MARKETS IN BENIN CITY.

BY

Chidinma Perpetual CHINWUBA (MISS)

LSC2010074

JANUARY, 2025

PREVALENCE OF BACTERIA ISOLATED FROM CARROT AND GREEN PEA SAMPLES SOLD IN LOCAL MARKETS IN BENIN CITY.

BY

Chidinma Perpetual CHINWUBA (MISS)

(LSC2010074)

A THESIS WRITTEN IN THE DEPARTMENT OF MICROBIOLOGY IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF THE DEGREE OF BACHELOR OF SCIENCE OF THE UNIVERSITY OF BENIN, BENIN CITY.

JANUARY, 2025

CERTIFICATION

We certify that this work was carried out by Chidinma Perpetual CHINWUBA (MISS) in the Department of Microbiology, Faculty of Science University of Benin, Benin City, Nigeria.

_____ _____

Prof. I. S. Obuekwe Date

(Project Supervisor)

_____ _____

Prof. (Mrs.) F. I. Akinnibosun Date

(Head of Department)

DEDICATION

With all privilege, this thesis is dedicated to God Almighty for his grace and wisdom and to my family for their profound support throughout this program.

ACKNOWLEDGEMENTS

My profound gratitude goes to God Almighty for His unfailing love, guidance and protection in the course of this programme.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Fresh vegetables such as green peas and carrots are important components of the human diet but are highly susceptible to microbial contamination, posing significant public health risks. This study aimed to assess the microbial contamination of these vegetables sold in local markets in Benin City, Nigeria, focusing on the isolation and identification of bacteria, the evaluation of their prevalence, and the assessment of antibiotic susceptibility patterns. A total of 20 vegetable samples were collected from New Benin, Adolor, and Ring Road markets. Standard microbiological techniques were employed for bacterial isolation and identification, while the disc diffusion method was used for antibiotic susceptibility testing.

The results revealed high bacterial loads, with Ring Road Market samples showing the highest total heterotrophic bacterial counts (265 ± 77.78 CFU/g for green peas and 115 ± 12.73 CFU/g for carrots), suggesting poor hygiene and handling practices. The bacteria isolated included *Escherichia coli*, *Salmonella* sp., *Shigella* sp., *Staphylococcus aureus*, and *Bacillus* sp. *E. coli* had the highest prevalence in carrots (50%), while *S. aureus* was most prevalent in green peas (40%). Antibiotic susceptibility testing showed multidrug resistance, with many isolates resistant to colistin, erythromycin, tetracycline, metronidazole, and clindamycin. However, ciprofloxacin and gentamicin remained effective against most isolates. These findings highlight the urgent need for improved food safety practices, strict regulation of antibiotic use in agriculture, and regular microbial monitoring to reduce the risks of foodborne illnesses and the spread of antimicrobial resistance in fresh produce.

CHAPTER ONE

INTRODUCTION

1.0 Background of study

Increasing the consumption of fruits and vegetables significantly promotes a healthier and more productive lifestyle (Zheng *et al.*, 2013; Goodburn and Wallace, 2013). However, despite their nutritional benefits, the microbial safety of fresh produce remains a critical concern. Since these foods are often consumed raw, they serve as common carriers for foodborne illnesses, with outbreaks associated with fresh produce becoming increasingly frequent and widespread (Kilonzo-Nthenge *et al.*, 2018).

Vegetable cultivation, including carrots, faces several limitations. Globally, it is estimated that 30–40% of vegetables are lost annually due to spoilage (Barth *et al.*, 2009). According to Owais *et al.* (2018), enzyme activity contributes to the degradation of some vegetables, while chemical processes like oxidation and rancidity damage others. However, the primary culprits of spoilage are microorganisms such as bacteria, molds, and yeasts, as vegetables are particularly vulnerable to microbial deterioration (Morka, 2022). This vulnerability is largely attributed to their physical properties, such as high moisture content and pH levels, which favor the proliferation of spoilage microbes. Contamination by these microorganisms can occur at various stages, including cultivation in fields, greenhouses, or orchards; during harvesting and post-harvesting; and even at distribution and preparation stages (Mritunjay and Kumar, 2015; Bishop and Okwori, 2017).

Vegetables are vital sources of nutrients for humans, particularly vitamins, and play a crucial role in promoting health and balanced diets (Kaur *et al.*, 2017). However, they are also significant carriers of chemical and microbial contaminants (Uzeh *et al.*, 2009). According to Velusamy *et al.* (2010), vegetables are frequently associated with foodborne illnesses due to

the growth of pathogens such as *Staphylococcus aureus, Escherichia coli,* and *Salmonella enterica* on their surfaces (Kim *et al.*, 2013). Despite their nutritional value, vegetables like carrots are often consumed without sufficient consideration of potential contamination by harmful microorganisms (Oranusi *et al.*, 2012).

Pathogens such as *Bacillus cereus, Listeria monocytogenes*, and *Clostridium botulinum* are common soil organisms, while Salmonella, *Campylobacter*, *Shigella*, and *Escherichia* coli are found in the intestines of humans and animals. These microorganisms frequently contaminate vegetables and raw fruits through exposure to feces, untreated irrigation water, surface water, and sewage systems (Kaur *et al.*, 2017).

Many documented outbreaks linked to fresh produce have been attributed to bacteria from the *Enterobacteriaceae* family. These bacteria are common human and opportunistic pathogens, with many species exhibiting increasing resistance to antibiotics frequently used in medicine (Liu and Kilonzo-Nthenge, 2017). In the United States, antimicrobial-resistant bacteria are responsible for approximately 2 million illnesses and 23,000 deaths annually (Kilonzo-Nthenge *et al.*, 2018). The presence of antibiotic-resistant bacteria in fresh produce has been reported (Threlfall, 2002). Contamination often occurs during agricultural production and harvesting, where antibiotic-resistant pathogens or commensals from animals and the environment can transfer to the produce (Holvoet *et al.*, 2013).

Mukherjee *et al.* (2006) highlighted an increasing trend in foodborne outbreaks linked to spoiled fruits and vegetables, emphasizing the need to isolate and identify these spoilage-causing pathogens as a control measure (Akinyele and Akinkunmi, 2012). Recent studies on carrots include research on growth and yield responses to oil palm refuse bunch ash in an ultisol environment (Law-Ogbomo, 2018) and modeling carrot slice drying characteristics using a microwave oven (Nwajinka and Okonjo, 2019). However, these studies have largely

overlooked the impact of handling practices and human actions on exposure to pathogens. Green peas, due to their high moisture content and pod-like structure, provide a favorable environment for the growth of pathogenic bacteria such as *Escherichia coli*, *Salmonella sp.*, and *Shigella sp.*, which can be introduced through contaminated soil, water, or poor handling practices during harvesting, transportation, and market display (Imoni *et al.,* 2023; Kabir *et al.,* 2020). Given the increasing reports of foodborne outbreaks linked to fresh produce (Kilonzo-Nthenge *et al.*, 2018), this research was aimed at investigating the prevalence of enteric bacteria in carrot and green pea samples sold in local markets in Benin City.

1.1 Aim and Objectives

The aim of this study is to investigate the prevalence of enteric bacteria isolated from carrot and green pea samples sold in local markets in Benin City, Nigeria.

The specific objectives were to:

1. isolate and identify bacteria from vegetable samples
2. evaluate the percentage occurrence of bacterial isolates
3. assess the antibiotic sensitivity pattern of the isolates

CHAPTER TWO

LITERATURE REVIEW

2.0 Foodborne Pathogens and Their Public Health Impact

Foodborne illnesses, commonly referred to as food poisoning, arise from consuming food or water contaminated with harmful microorganisms—such as bacteria, viruses, parasites, and fungi—or their toxins and chemicals. An outbreak is typically suspected when two or more individuals experience gastrointestinal or neurological symptoms within 72 hours of sharing a meal. The term "food poisoning" encompasses both foodborne infections and intoxications. Microbial food poisoning is distinct in that the microbes actively multiply in the food before consumption, whereas in foodborne infections, the food acts as a carrier for non-multiplying pathogens (Addis and Sisay, 2015).

Foodborne illnesses are caused by pathogens like bacteria, viruses, molds, worms, and protozoa, although not all microorganisms are harmful; some, such as those used in yogurt and cheese production, are beneficial. Chemicals in food can also lead to illness, either as natural components or contaminants introduced during production and processing. Statistically, bacteria are responsible for 66% of foodborne illnesses, followed by chemicals (26%), viruses (4%), and parasites (4%). The two primary categories of foodborne illness are intoxications and infections: intoxications occur when toxins produced by pathogens cause poisoning, while infections result from consuming food containing pathogens (Adams *et al.*, 2024).

Pathogenic microorganisms often use food as a nutrient source, metabolizing it and producing harmful by-products that render the food unsafe. Contamination can occur at various stages, including contact with plant surfaces, animals, water, sewage, air, soil, or during handling and processing by humans. Symptoms of foodborne illnesses can range from diarrhea, vomiting, and abdominal cramps to more severe issues like headaches, fever, chills,

and difficulty swallowing. Specific conditions, such as mycetism from consuming poisonous mushrooms or mycotoxicosis from toxin-producing fungi, highlight the diverse risks associated with contaminated food (CDC, 2011). Addressing foodborne illnesses is crucial for safeguarding public health, as they not only affect individuals' well-being but also strain healthcare systems and disrupt daily life.

Most foodborne illnesses can be effectively prevented through proper food handling and preparation practices. Key measures include thorough cooking and heat processing to eliminate bacteria, maintaining adequate refrigeration, improving personal hygiene, and avoiding the storage of food at temperatures conducive to bacterial growth. Globally, food safety concerning foodborne illnesses (FBI) remains a significant issue, particularly in developing countries like Ethiopia, where food production often occurs under unsanitary conditions. Despite the widespread public health challenges posed by FBI in Ethiopia, particularly from consuming contaminated food items, there is a lack of comprehensive and well-documented data on its prevalence. Consequently, this seminar paper aims to provide a review and foundational understanding of major bacterial foodborne illnesses (CDC, 2011).

2.1 Enteric Bacteria and Foodborne Illness

2.1.1 *Salmonella*

Salmonella has consistently been the leading cause of bacterial foodborne illnesses in both Europe (2007–2011) and the United States (2006–2015). In the U.S., it is also the primary cause of hospitalizations and deaths linked to known foodborne pathogens (Thomas *et al.*, 2024). In the EU, food surveillance sampling revealed that up to 0.84% of ready-to-eat (RTE) fruits and vegetables tested positive for *Salmonella* (EFSA, 2018a).

Salmonella is categorized into two species: *S. bongori*, which rarely causes human disease, and *S. enterica*, the pathogenic species responsible for foodborne illnesses. *S. enterica* comprises over 2,500 serovars distinguished by surface antigens, including lipopolysaccharide O and

flagella H antigens (Thomas *et al.*, 2024). The geographic distribution of these serovars varies; for instance, in the U.S., *S. enterica* serovar Newport is most associated with produce-related outbreaks, while in the EU, serovar Enteritidis is the predominant strain linked to salads (Bennett *et al.*, 2018; Callejón *et al.*, 2015). Notably, the serovars identified on fresh produce differ significantly from those commonly associated with livestock, suggesting certain strains are better adapted to plants (Ferrari *et al.*, 2019). Experimental studies have shown serovars like Enteritidis, Typhimurium, and Senftenberg adhere more strongly to basil, while others like Tennessee prefer lettuce. Additionally, vegetable species influence adhesion; cabbages, for example, support less *Salmonella* adhesion than lettuce. Even within lettuce cultivars, variations exist, with serovar Typhimurium adhering more to "Romaine" than "Iceberg" lettuce and showing higher attachment to "Nelly" than "Cancan" varieties (Thomas *et al.*, 2024).

Sprouted vegetables, papaya (Hassan *et al.*, 2019), melons/cantaloupes (Chan *et al.*, 2023), cucumbers, and tomatoes (Gurtler *et al.*, 2018) are common vehicles for *Salmonella* contamination. In the U.S., a large-scale outbreak of *S. poona* linked to cucumbers affected 907 people across 40 states, resulting in six fatalities (Laughlin *et al.*, 2019). In the EU, *Salmonella* poses a similar public health risk. For example, in 2011, an outbreak of *S. strathcona* traced to 'datterino' tomatoes caused 43 cases in Denmark and 28 additional cases across Germany, Italy, Austria, and Belgium (Thomas *et al.*, 2024). Cucumbers were again implicated in a 2016–2017 outbreak of *S. agona* in Europe, with 147 cases reported across five countries, although microbiological evidence was insufficient to confirm the link conclusively (EFSA, 2018b).

These findings underscore the serovar-specific mechanisms of *Salmonella* attachment to fresh produce and highlight the significant public health risks posed by contaminated fruits and vegetables worldwide.

2.1.2 *Escherichia coli*

Escherichia coli (*E. coli*) is predominantly a non-pathogenic bacterium found in the commensal flora of mammals, playing a vital role in food digestion and the production of vitamin K. However, certain strains are pathogenic and can lead to illnesses in humans, including diarrhea, urinary tract infections, sepsis, and meningitis (Thomas *et al.*, 2024). Diarrheagenic *E. coli* is classified into seven pathotypes: enterotoxigenic (ETEC), enteropathogenic (EPEC), Shiga toxin-producing (STEC), enteroaggregative (EAEC), enteroinvasive (EIEC), adherent-invasive (AIEC), and diffusely adherent *E. coli* (DAEC) (Rojas-Lopez *et al.*, 2018). These pathotypes are distinguished by variations in surface antigens—somatic (O), flagellar (H), and capsular (K)—and by their specific virulence factors.

Foodborne illness outbreaks caused by *E. coli* on fresh produce are more prevalent in the USA compared to the EU, accounting for 12.2% and 3.8% of bacterial foodborne outbreaks, respectively (Thomas *et al.*, 2024). In the USA, STEC is the most common pathotype linked to these outbreaks, with the O157:H7 serogroup responsible for 92% of cases between 1998 and 2013 (Bennett *et al.*, 2018). Notably, a highly virulent strain of O157:H7 emerged in England and Wales in 2015, associated with prepackaged salad leaves and identified in patients (Byrne *et al.*, 2018).

Given the emergence of such pathogenic strains, it is essential to investigate their behavior in the environment and assess their potential to cause disease. This understanding is crucial for developing effective measures to mitigate risks associated with contaminated fresh produce.

2.1.3 *Listeria monocytogenes*

While *Salmonella* and *E. coli* are the leading causes of bacterial outbreaks linked to fresh fruits and vegetables, *Listeria monocytogenes* causes fewer outbreaks but poses a greater financial burden on the food industry. Additionally, listeriosis, the illness caused by *L. monocytogenes*, has the highest fatality rate among these three pathogens and is a significant contributor to deaths from foodborne illnesses (Thomas *et al.*, 2024).

L. monocytogenes is classified into at least 13 serotypes, each varying in pathogenicity. Between 1998 and 2003 in the USA, serotype 1/2a was responsible for eight outbreaks, with a 45% hospitalization rate and a 7% fatality rate. Serotype 1/2b caused two outbreaks with a higher hospitalization rate (60%) but no fatalities. In contrast, serotype 4b, which is the most commonly associated with human listeriosis, caused 10 outbreaks during this period, resulting in a 70% hospitalization rate and a 13% fatality rate (Thomas *et al.*, 2024).

In the USA, several nationwide outbreaks of *L. monocytogenes* have been linked to contaminated fruits and vegetables. A notable example is the 2011 multi-state outbreak traced to cantaloupe melons from a Colorado farm, which resulted in 147 cases across 28 states, 143 hospitalizations, and 33 deaths (Thomas *et al.*, 2024). Similarly, a 2014 outbreak tied to an apple-packing facility led to 35 cases in 12 states, including seven deaths (Angelo *et al.*, 2017).

In Europe, outbreaks associated with *L. monocytogenes* have also been significant. Between 2013 and 2014, 32 cases of listeriosis linked to ready-to-eat salads were reported in Switzerland, caused by serotype 4b (Stephan *et al.*, 2015). Another outbreak of serotype 4b affected five EU countries, resulting in 47 cases and nine deaths, with frozen sweetcorn and other frozen vegetables identified as the source (EFSA, 2018c).

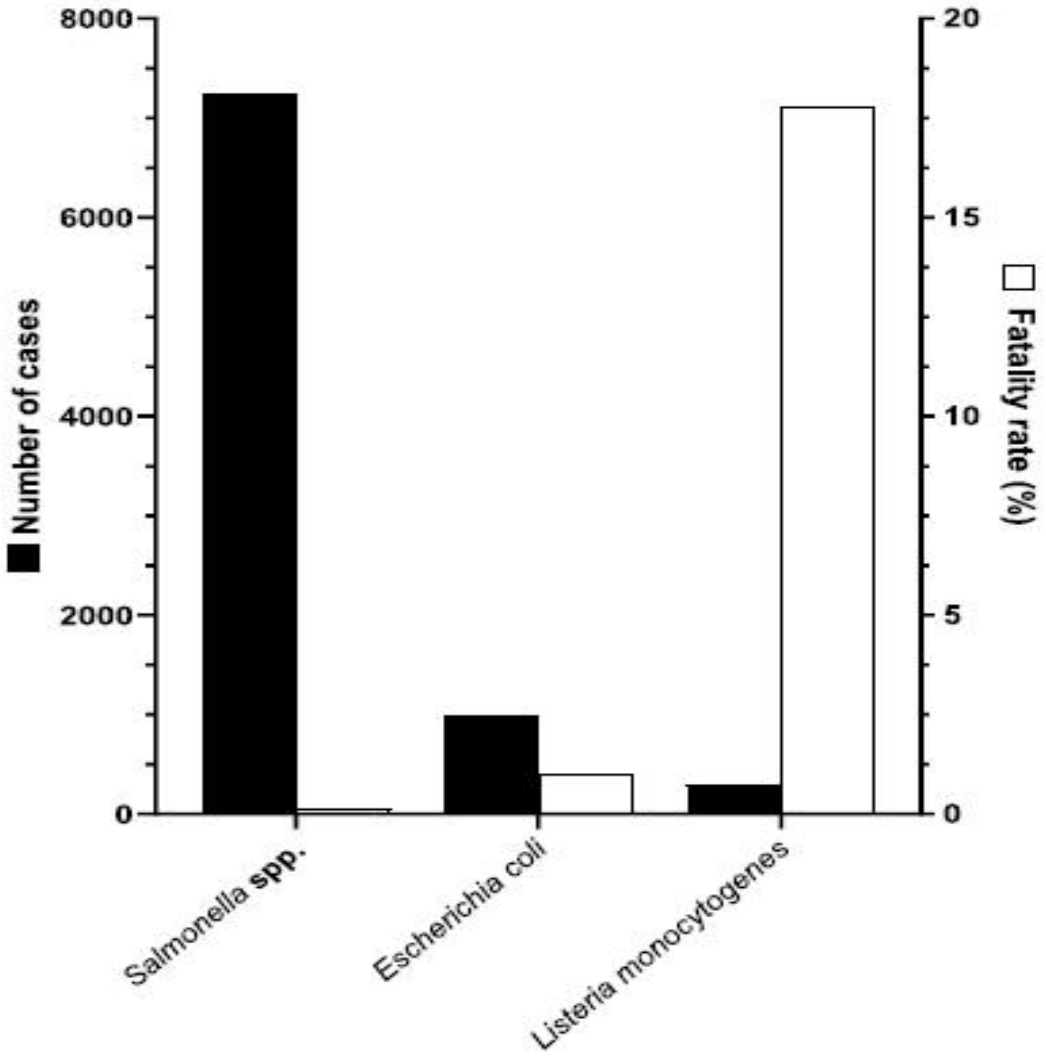

Figure 2.1. Number of cases and associated case fatality rates related to consumption of contaminated fruits and vegetables from CDC data, 2006–2023 (Thomas *et al.,* 2024).

2.2 Contamination Sources in Vegetables

2.2.1 Pre-harvest contamination

Contamination of crops during their growth cycle can stem from various sources, including soil, seeds, irrigation water, and fecal matter from both domestic and wild animals. These factors enable bacteria to establish themselves on crops, where they can persist and multiply under favorable conditions (Thomas *et al.*, 2024). Soil is one of the primary contamination

sources, particularly if fields used for growing fresh produce were previously utilized for animal husbandry, waste disposal, or fertilized with manure (Uyttendaele *et al.*, 2015). While many enteric pathogens originate from animals, *Listeria monocytogenes* is unique in being a widespread environmental bacterium often found in soil, even in the absence of animal-derived contamination (Smith *et al.*, 2018). Root vegetables, like carrots, are particularly at risk of contamination since they are in direct contact with the soil and may not always be thoroughly washed or peeled before consumption.

Extreme weather events, such as flooding, can exacerbate foodborne illness outbreaks by increasing contamination risks (Castro-Ibanez *et al.*, 2015; Bergholz *et al.*, 2016). Additionally, climate change has introduced new risks, such as dust storms, which can carry pathogens. For instance, a dust storm in Australia in 2018 was implicated in the contamination of rock melons with *L. monocytogenes*. Pathogens can transfer from soil to crop leaves through splash events, a phenomenon demonstrated experimentally with *Salmonella* and *Listeria innocua* (Lee *et al.*, 2019; Thomas *et al.*, 2024).

Enteric pathogens exhibit remarkable persistence in soil, with *Salmonella typhimurium* surviving up to 231 days, *E. coli* O157:H7 for 217 days, and *L. monocytogenes* for 360 days in soil microcosms (Thomas *et al.*, 2024). Their survival rates are influenced by soil properties; for example, *Salmonella* persists longer in loamy soils than in sandy soils (Jechalke *et al.*, 2019). Additionally, microbial diversity in soil plays a role, with lower prokaryotic diversity linked to reduced *Salmonella* survival (Schierstaedt *et al.*, 2020). The colonization of roots by *L. monocytogenes* can also be affected by interactions with other rhizobacteria.

Seed contamination is another critical pathway. Pathogens may be attracted to germinating seeds in contaminated soil or introduced through pre-contaminated seeds. Once seeds germinate, pathogens can spread to edible plant parts, with the sprouting process often

amplifying bacterial growth. This has been linked to several outbreaks of *Salmonella* and *E. coli* associated with sprouted seeds (Thomas *et al.*, 2024).

Irrigation water is a well-documented source of crop contamination during agricultural production. Water sourced from rivers and lakes often carries enteric pathogens, introduced via runoff containing sewage, soil, or animal feces. This issue is worsening due to declining river water quality, driven by urban sewage discharge and agricultural runoff. By 2050, 68% of the global population is expected to live in urban areas, a significant increase from 30% in 1950 (Thomas *et al.*, 2024). This rapid urbanization, coupled with poor management and underinvestment in aging sewage treatment infrastructure, has led to increased environmental waste release. Enteric pathogens, which can persist for extended periods in contaminated water, are increasingly polluting irrigation sources.

In addition to urbanization, climate change is exacerbating water scarcity. Rising global temperatures and dwindling freshwater resources force many lower-income regions to rely on suboptimal water sources, including treated greywater and blackwater, for irrigation. Currently, an estimated 10% of the global population consumes agricultural products irrigated with treated wastewater (Thomas *et al.*, 2024).

Another route of pre-harvest contamination, though harder to control, is the involvement of animals. Both domestic and wild animals serve as reservoirs for enteric pathogens, contributing to contamination through feces deposited in soil, water, or directly on crops. For instance, healthy cattle are a primary reservoir for *E. coli* O157:H7, while *Salmonella* and *Listeria monocytogenes* are also frequently found in livestock. Wild animals present additional challenges, as their movements and behavior are less controllable. In California's Salinas Valley, responsible for producing 91% of the state's salad greens, *S. enterica* has been detected in feces from deer mice, stray dogs, and coyotes, signaling potential risks for

leafy salad contamination. Birds further compound this risk by acting as long-distance carriers of pathogens.

Evidence of animal-related contamination includes outbreaks of *E. coli* O157:H7 linked to strawberries tainted with deer feces in Oregon and spinach contaminated by feral swine feces in Canada. These incidents underscore the ongoing risk of fresh produce contamination by animal-derived pathogens (Thomas *et al.*, 2024).

2.2.2 Post-harvest contamination

Post-harvest processes, such as storage, preparation, and packaging, can introduce enteric pathogens if not properly managed according to good manufacturing practices. Implementing Hazard Analysis and Critical Control Point (HACCP) principles allows for the identification and management of potential contamination risks along the production line (Thomas *et al.*, 2024). Key practices include monitoring the quality of water used for washing produce, ensuring daily sanitation of machinery—especially cutting equipment, which is a critical control point—and maintaining strict temperature controls (Calonico *et al.*, 2019). To support these efforts, companies should develop detailed flowcharts and decision trees outlining their processes for regular assessment.

At the raw material stage, leaf damage is a common issue after harvesting, which can alter the leaf's surface environment and create adhesion sites for pathogens. To mitigate contamination risks, it is essential to minimize both pre- and post-harvest damage. Removing damaged leaves and consuming bagged salads promptly after purchase can further reduce the likelihood of contamination.

Once raw materials are harvested and stored, the preparation phase begins, involving several steps, including washing. Washing leafy produce is vital for removing soil and debris, but it can also become a contamination source. For instance, wash water was identified as the contamination source in a Salmonella outbreak involving melons in the Rio Grande River

Valley. Research has shown how a single contamination point can spread pathogens during washing: when only 5% of lettuce heads in a batch were contaminated with *Salmonella*, washing caused the pathogen to spread uniformly across the entire batch.

Typically, washing includes three stages using separate tanks: the first tank removes soil debris, the second uses a disinfectant treatment to prevent cross-contamination, and the third rinses off the disinfectant with non-chlorinated water (Thomas *et al.*, 2024). Careful management of these steps is crucial to prevent post-harvest contamination of fresh produce.

The preparation of fresh fruit salads presents several contamination risks. Bacteria present on the peel or rind of fruits can inadvertently transfer to the flesh during chopping, as observed in a recent case involving contaminated watermelon imported into the UK (Chan *et al.*, 2023). Melons and watermelons are particularly concerning due to their low acidity, which creates an environment conducive to microbial growth (Luciano *et al.*, 2022). Furthermore, mechanical damage during processing steps such as washing, sanitizing, peeling, and chopping can soften the fruit, further encouraging microbial proliferation (Zhao *et al.*, 2022).

Contaminated surfaces can also introduce pathogens during processing. For example, an outbreak of *Listeria monocytogenes* in Switzerland between 2013 and 2014 was traced to a product-feeding belt that fed produce into a color sorter. Design flaws in the belt made thorough sanitization challenging, allowing pathogens to persist. The ability of bacteria to attach to and colonize surfaces is often linked to biofilm formation. Once adhered, bacteria secrete a matrix of extracellular polymeric substances, including nucleic acids, exopolysaccharides, and proteins. This biofilm not only connects the bacterial colony but also enhances adhesion to surfaces and provides protection from harsh conditions. Biofilm formation depends on factors such as bacterial strain, nutrient availability, and temperature. Research shows that strains of *Salmonella typhimurium* and *L. monocytogenes* can form

biofilms on various surfaces, including polystyrene, polycarbonate, stainless steel, glass, and rubber (Thomas *et al.*, 2024).

These findings underscore the importance of effective surface sanitization during fruit processing. Storage conditions also play a crucial role in pathogen survival and proliferation. Enteric pathogens have been shown to persist on the surfaces of various fruits, such as apples, avocados, strawberries, peaches, nectarines, cantaloupe, mangoes, and papayas (Thomas *et al.*, 2024). The survival of pathogens on fruit is influenced by intrinsic and environmental factors, with pH being a significant intrinsic factor. While the acidic nature of most fruits inhibits microbial growth, melons and watermelons, with their higher pH of around 6, have been found to support greater populations of *L. monocytogenes* compared to other fresh-cut fruits like pears, mangoes, and papayas (Colás-Medà *et al.*, 2017; Luciano *et al.*, 2022).

2.3 Different Ways to Minimizing Contamination of Fruits and Vegetables

Several measures have been suggested by researchers to minimize microbial contamination of fruits and vegetables. These include maintaining strict sanitary conditions during handling and soaking produce in an adequate concentration of vinegar for at least 10 minutes to reduce microbial loads (Nwachukwu and Chukwu, 2013). Mahapatra *et al.* (2015) recommended the development of appropriate and practical disinfection systems by governments. Similarly, Kibitok and Nduko (2016) emphasized the importance of implementing safety control measures and hazard analysis and critical control point (HACCP) principles.

In some studies, the presence of *Staphylococcus aureus*, a bacterium commonly found on human skin and in nasal cavities, was linked to improper hand hygiene by buyers or end-users during the selection process (Iyoha and Agoreyo, 2015). Furthermore, many fruit and vegetable vendors lack adequate education on proper hygiene practices (Orji *et al.*, 2016), highlighting the need for public awareness campaigns by authorities. Oluwatoyin *et al.* (2015) proposed washing sliced fruits packaged in polyethylene with high concentrations of salt or

chlorinated water to effectively remove pathogens. However, while modified atmospheric packaging (MAP) can reduce spoilage by aerobic microorganisms, it may also increase the virulence of pathogens like *Escherichia coli* O157:H7 (Erhirhie *et al.*, 2020).

Iyoha and Agoreyo (2015) suggested laws requiring vendors of ready-to-eat fruits to use temperature-controlled storage systems, while Orji *et al.* (2016) recommended regular inspections by health officials at all stages of production, harvesting, processing, and marketing. These inspections should focus on the quality of washing water, packaging materials, and vendor hygiene.

Contaminated water used for washing, processing, and packaging materials remains a significant source of contamination and should be avoided. While chemical disinfection can moderately reduce bacterial contamination, irradiation has been shown to produce more effective results (Erhirhie *et al.*, 2020). Organic fertilizers, manure, and municipal sludge should be thoroughly decontaminated before use. Additionally, pathogens from human or animal reservoirs, often introduced through unhygienic production practices, underscore the need for strict personal hygiene and education campaigns for consumers and vendors (Kibitok and Nduko, 2016).

To maximize profits, some vendors mix contaminated produce with uncontaminated items during storage, facilitating bacterial spread. Since bacterial contamination is not always visible in the early stages, consumers may unknowingly purchase and consume contaminated products. Educating vendors on the importance of segregating or discarding contaminated produce could significantly reduce the risk of consuming harmful fruits and vegetables (Erhirhie *et al.*, 2020).

CHAPTER THREE

MATERIALS AND METHODS

3.0 Sample collection

The samples used to carry out this study consists of carrots and green peas sold in the local markets of Benin City. All samples were collected by purchase. A total of twenty (20) samples (10 carrots and 10 green peas) were purchased from New Benin Market (3 carrots and 3 green peas), Ring road market (3 carrots and 3 green peas) and Adolor market (4 carrots and 4 green peas) for the purpose of this study.

3.1` Preparation of Media

3.1.1 Preparation of MacConkey Agar

50 g of MacConkey agar powder (as per manufacturer's instructions) was added to 1 litre of distilled water in a clean flask. The mixture was thoroughly stirred to dissolve the powder completely. The solution was gently heated while being stirred continuously. It was allowed to boil to ensure the medium was fully dissolved. The fully dissolved solution was poured into a suitable container, loosely covered, and autoclaved at 121°C for 15 min to sterilize. The sterilized agar was allowed to cool to around 45–50°C before it was poured into sterile petri dishes.

3.1.2 Preparation of Salmonella-Shigella Agar

Dissolve 63g in 1000ml of distilled water. Gently heat to boiling with gentle swirling and dissolve the medium completely. Cool to 45 – 50°C and distribute into sterile petri plates.

3.1.3 Preparation of Eosin Methylene Blue (EMB) Agar

10 g of peptone, 10 g of lactose, 2 g of dipotassium phosphate, 0.4 g of eoain Y, 0.065 of methylene blue and 15 g agar were dissolved in 1 L of distilled water. The mixture was heated and stirred constantly until the agar was completely dissolved. The medium as

sterilized by autoclaving at 121°C for 15 min. It was cooled to 45 – 50°C and poured into sterile petri dishes.

3.1.4 Preparation of Muller Hinton Agar

38g of Muller Hinton agar was dissolved in 1 liter of distilled water in a conical flask covered with cotton wool and aluminum foil paper. It was mixed thoroughly and sterilized by autoclaving at 121^{O}C for 15 min. The medium was cooled to 45-50^{O}C and before dispensing aseptically into Petri dishes in the laminar flow.

3.2 Sterilization of materials

Materials such as Petri dishes, pipettes, glass containers (conical flask, round bottom flask), and bottles were drained and dried. They were wrapped with aluminium foil and sterilized in a hot-air oven at 160^{o}C for an hour. They were allowed to cool after sterilization before usage. An aseptic working environment was achieved using a Bunsen burner flame and the disinfection of work surfaces with alcohol.

3.3 Enumeration of total bacterial count (TBC)

Each vegetable sample was chopped into small sizes with a sterile scissors. One gram of each sample was homogenized in 9.0 mL of peptone water. Thereafter, the samples were serially diluted and 0.1 mL of dilution 10^{-2} was inoculated on MacConkey agar in sterile Petri dishes. The inoculated plates were incubated at 37°C for 24 to 48 h. Colonies were counted and recorded as colony forming unit per gram (cfu/g).

3.4 Phenotypic identification of bacteria from samples

Pure cultures of the bacterial isolates were obtained from the subculture of a single colony from the successful pour plate technique and were characterized using cultural, morphological and biochemical methods. Several tests, such as Gram reaction, catalase, urease, indole, oxidase, citrate utilization and respective reactions of bacteria on triple sugar iron agar, were carried out to identify bacterial isolates presumptively (Cheesbrough, 2000).

3.4.1 Gram staining test

Gram staining test was carried out to classify the isolates into Gram-positive and Gram-negative isolates. Smears of isolates were made into clean, grease-free and sterile-dried microscope slides with labels using a sterilized loop, and the smear was air-dried and heat-fixed over a flaming Bunsen burner. The fixed smear was saturated with drops of crystal violet, left for one minute to react and washed off with distilled water. Lugol's iodine, which serves as a mordant, was added, left for one minute, and washed off with distilled water. The smear was decolourized with 95% ethyl alcohol for 30 seconds and washed off with distilled water. Then, the slide underwent counter staining using safranin solution for one minute and was rinsed with distilled water. Lastly, the smear was allowed to air dry and immersion oil was added for a microscopic view on an immersion objective lens light microscope. Colours, shapes and arrangements were used in identifying the organisms (Cheesbrough, 2000).

3.4.2 Potassium Hydroxide (KOH) test

The potassium hydroxide (KOH) test was used to determine or confirm Gram-negative bacteria. To quickly differentiate between Gram-negative and Gram-positive bacteria as a complement to Gram staining. KOH breaks down the thin peptidoglycan bacterial cell walls of Gram-negative bacteria but does not affect the thick layer of Gram-positive cell walls. Disruption of bacterial cell walls lyses the cell and releases its contents, including the genetic material. A drop of 3% KOH solution was applied on a labelled clean microscope slide and smeared with pure isolated culture using a loop. It was stirred carefully and observed to see if the solution turned to be a viscous or dense suspension, which formed a slimy or mucoid string within 60 seconds (Cheesbrough, 2000).

3.5 Biochemical Tests

For further characterization of the isolates, the following test were conducted.

3.5.1 Indole test

An indole test was carried out to demonstrate the ability of certain bacteria that can decompose amino acid tryptophane to indole. The indole production test is essential in identifying the *Enterobacteriaceae* family that breaks down the amino acid tryptophan by releasing indole in the presence of intracellular enzymes called "tryptophanase." Several drops of Kovac's indole reagent were placed on a filter paper. A portion of a pure isolated colony picked from the Tryptic Soy Agar (TSA) pure culture with an inoculating loop was smeared onto the reagent-saturated area of the filter paper. It was allowed to react and examined to observe for colour development within 2 min (Cheesbrough, 2000).

3.5.2 Oxidase test

The oxidase test was carried out to detect the presence of a cytochrome oxidase or indophenol oxidase that will catalyze electrons between electron donors in the bacteria and a redox dye known as tetramethyl-*p*-phenylene-diamine. The dye would be reduced to deep purple colour if yielded to positive reactions.

Several reagents can be used for this study, but Kovacs oxidase reagent: 1% tetra-methyl-p-phenylenediamine dihydrochloride in water, was used. The filter paper was saturated with a Kovacs oxidase reagent solution, and a speck of the pure culture was smeared on it with a platinum loop. It was allowed to react and observed for colour development within 10 - 60 seconds (Cheesbrough, 2000).

3.5.3 Catalase test

This test was used to distinguish between bacteria that produce the catalase enzyme, such as *Staphylococci*, and bacteria that do not, such as *Streptococci*. Catalase catalyzes the breakdown of hydrogen peroxide (H_2O_2) to oxygen (O_2) and water (H_2O). In this test, 2mL of

hydrogen peroxide solution was poured into a test tube, and some colonies of the test organism were picked and immersed into the H_2O_2 solution using a sterile glass rod. The bacteria that generated catalase (positive result) produced gas bubbles (oxygen), but those that did not possess catalase enzyme had none (negative result) (Cheesbrough, 2000).

3.5.4 Citrate Utilization tests

The citrate utilization test is a part of the test used to differentiate organisms on their ability to utilize citrate as the primary energy source. A citrate test was performed to differentiate members of *Enterobacteriaceae* capable of fermenting citrate in the presence of the enzyme citrate. Simon's citrate agar contained citrate as significant source of energy and was prepared for inoculation on Petri dishes. Well-prepared and sterilized citrate agar plates were inoculated with the pure isolated cultures by streaking the surface with a sterilized loop. The plates were then incubated at 37°C for 24 hours (Cheesbrough, 2000).

3.5.5 Urease test

The urease test is used to identify bacteria capable of producing the urease enzyme. The organisms that secrete urease can hydrolyze urea to ammonia and carbon dioxide. This test was used to distinguish urease-positive bacteria from other *Enterobacteriaceae*. The isolated pure bacteria were inoculated into well-prepared and autoclaved Christensen-modified urea broth and incubated for 24 hours at 37°C (Cheesbrough, 2000).

3.5.6 Sugar fermentation test

Many bacteria species can be differentiated on the basis of the sugars they utilize and ferment. The fermentation medium was prepared by the addition of 0.1g of peptone, 0.1g of sodium chloride and 0.1g of fermentable sugar (glucose, mannitol, lactose, sucrose and mannose) in 10 mL of distilled water. 4 mL of the medium was pipetted into Bijou bottles containing Durham tubes. One (1ml) of phenol red indicator will also be added to the tubes. The Bijou bottles containing the sugar solution was inoculated with the test bacterial isolates and

incubated at 37°C for 24-48h. After incubation, a change of color from red to yellow indicated acid production and the presence of gas in the inverted Durham tubes was indicative of gas production (Cheesbrough, 2000).

3.5.7 Triple sugar iron (TSI) test

The Triple Sugar Iron (TSI) test is used to test an organism's capability to ferment sugars and to produce hydrogen sulphide (H_2S) or gas (O_2), or both. The test was used primarily to differentiate members of the *Enterobacteriaceae* family based on their sugar fermentation patterns from other Gram-negative rods. An agar slant of TSI agar was used in carrying out this test in a sterile test tube at a slanted angle. The slanted medium was inoculated with pure culture of the isolates using a straight inoculation needle by stabbing first through the center to the bottom of the tube and streaking the agar slant's surface. After inoculations, the test tubes were covered with foil paper and left at an ambient temperature of 36°C to incubate for 24 hours. Reactions on test tubes were examined after which the results were interpreted using a standard chart presented in Table 3.1 (Cheesbrough, 2000).

Table 3.1 Possible Scenarios for Triple sugar iron test

S.N.	Result (slant/butt)	Symbol	Interpretation
1	Red/Yellow	K/A	Glucose fermentation only, peptone catabolized.
2	Yellow/Yellow	A/A	Glucose and lactose and/or sucrose fermentation.
3	Red/Red	K/K	No fermentation, Peptone catabolized under aerobic and/or anaerobic conditions.
4	Yellow/Yellow with bubbles	A/A,G	Glucose and lactose and/or sucrose fermentation, Gas produced.
5	Red/Yellow with bubbles	K/A,G	Glucose fermentation only, Gas produced.
6	Red/Yellow with bubbles and black precipitate	K/A,G,H_2S	Glucose fermentation only, Gas produced, H_2S produced.
7	Yellow/Yellow with bubbles and black precipitate	A/A,G,H_2S	Glucose and lactose and/or sucrose fermentation, Gas produced, H_2S produced.
8	Red/Yellow with black precipitate	K/A,H_2S	Glucose fermentation only, H_2S produced.
9	Yellow/Yellow with black precipitate	A/A,H_2S	Glucose and lactose and/or sucrose fermentation, H_2S produced.
10	Yellow/Red	A/K	

3.5.8 Coagulase Test

This test is used to identify *S. aureus* which produces the enzyme coagulase. The enzyme coagulase converts fibrinogen to fibrin resulting to blood clothing. The slide method was used. In slide test, a clean slide was divided into two sections, to one section of the slide the test organism was smeared on it using a sterile wire loop while a drop of distilled water was added to the other section which serves as control. Then human plasma was added to both sections and the slides were rocked gently for some minutes. A clumping/agglutination of the plasma was used to indicate the presence of coagulase (Cheesbrough, 2000).

3.6 Antibiotic Susceptibility Test of the Bacterial Isolates

The susceptibility of the test isolates to conventional antibiotics was assessed using the disc diffusion technique on Mueller-Hinton agar. Bacterial cells were standardized to a 0.5 McFarland turbidity standard, and 0.1 ml of the standardized bacterial suspension was spread on the plates using sterile swabs. After allowing the plates to dry, antibiotic discs (Colistin (10 µg), Ciprofloxacin (5 µg), Gentamicin (10 µg), Erythromycin (15 µg), Tetracyclin (30 µg), Metronidazole (5 µg), Clindamycin (2 µg), Augmentin (20 µg)) were aseptically placed. Incubation at 37 °C followed, and the zones of growth inhibition around the antibiotic discs were measured after 24 hours. Results were interpreted following the Clinical and Laboratory Standards Institute guidelines (CLSI, 2020).

3.7 Statistical Analysis

Data from this study was processed using Microsoft Excel and the Statistical Package for the Social Sciences (SPSS) version 22.0 (SPSS Inc., Chicago, IL, USA). Normally distributed data was presented as mean ± standard deviation, and the comparison of means was conducted through analysis of variance (Ogbeibu, 2014).

CHAPTER FOUR

RESULTS

Table 4.1 shows the total bacterial load (in $\times 10^5$ CFU/g) found in green peas and carrots from three markets: New Benin, Adolor, and Ring Road. The results are presented as mean ± standard deviation. Ring Road Market had the highest bacterial load in both green peas (265 ± 77.78) and carrots (115 ± 12.73), suggesting poor hygiene, handling, or environmental conditions. Adolor Market had moderate contamination levels (93.5 ± 86.97 for green peas; 32 ± 21.21 for carrots). New Benin Market showed the lowest bacterial counts (55 ± 19.80 for green peas; 9 ± 8.49 for carrots).

Cultural and morphological characteristics of bacteria isolates were shown in Table 4.2. This table revealed the identification of bacterial isolates based on their cultural, morphological, and biochemical characteristics. The bacterial isolates identified were *Escherichia coli, Salmonella sp., Staphylococcus aureus, Bacillus sp.,* and *Shigella sp.*

Table 4.3 and Figure 4.1 shows the prevalence of isolated enteric bacteria in carrot samples. *E. coli* had the highest prevalence at 50% (5/10 samples), *Salmonella sp.* was found in 30% of samples, *Staphylococcus aureus* in 20%**,** while *Bacillus sp.* and *Shigella sp.* were each found in 10%**.**

The prevalence of isolated enteric bacteria in green pea samples was revealed in Table 4.4 and Figure 4.2. It shows that *Staphylococcus aureus* had the highest prevalence at 40% (4/10 samples), *E. coli* and *Bacillus sp.* were each found in 30% of samples while *Salmonella sp.* and *Shigella sp.* were present in 20% of samples.

Table 4.5 shows the antibiotic susceptibility patterns of the isolated bacteria against eight antibiotics. Many isolates showed resistance to multiple antibiotics, particularly colistin (CS), erythromycin (E), tetracycline (TE), metronidazole (M), and clindamycin (CD). *E. coli* was susceptible to ciprofloxacin but resistant to colistin, erythromycin, and tetracycline. *Staphylococcus aureus* was susceptible to ciprofloxacin, gentamicin, and augmentin. *Bacillus sp*. was susceptible to ciprofloxacin, gentamicin, and tetracycline. *Shigella sp.* was highly susceptible to ciprofloxacin and gentamicin. *Salmonella sp.* was susceptible to ciprofloxacin and augmentin, but resistant to colistin and metronidazole.

Table 4.1: Total heterotrophic bacterial count ($x10^5$)

	Green Peas	**Carrots**
New Benin Market	55 ± 19.80	9 ± 8.49
Adolor Market	93.5 ± 86.97	32 ± 21.21
Ring road Market	265 ± 77.78	115 ± 12.73

Values are recorded as Mean ± Standard deviation

Table 4.2: Cultural and morphological characteristics of bacteria isolates

Cultural characteristics	**1**	**2**	**3**	**4**	**5**
Colour	Cream	Cream	Golden yellow	Cream	Yellow
Shape	Circular	Circular	Circular	Circular	Circular
Elevation	Convex	Convex	Convex	Convex	Convex
Margin	Entire	Entire	Entire	Entire	Entire
Size	Small	Small	Small	Small	Small
Morphological characteristics					
KOH	+	+	-	-	-
Gram stain	-	-	+	+	-
Cell morphology	Rod	Rod	Cocci	Rod	Rod
Cell arrangement	Single	Single	Clusters	Single	Clusters
Biochemical characteristics					
Catalase	+	+	+	+	+
Coagulase	-	-	+	-	-
Indole	+	-	-	-	-
Oxidase	-	-	-	-	-
Citrate	-	-	+	+	-
Urease	-	-	+	-	-
H_2S production	-	+	-	-	-
Glucose	+	+	+	+	+
Lactose	+	-	+	-	-
Sucrose	-	-	+	+	-
Mannitol	+	+	+	+	-
Gr. Diff.	Green metallic Sheen (EMB)	Black (SSA)	Yellow (MSA)	Straw (BCA)	Cream (SSA)
Identity	*Escherichia coli*	*Salmonella* sp	*Staphylococcus aureus*	*Bacillus* sp.	*Shigella* sp

Table 4.3: Prevalence of Isolated Enteric Bacteria from Carrot Samples

	Positive samples (n)	Total samples (N)	Prevalence (%)
E. coli	5	10	50
Salmonella sp	3	10	30
S. aureus	2	10	20
Bacillus sp	1	10	10
Shigella sp	1	10	10

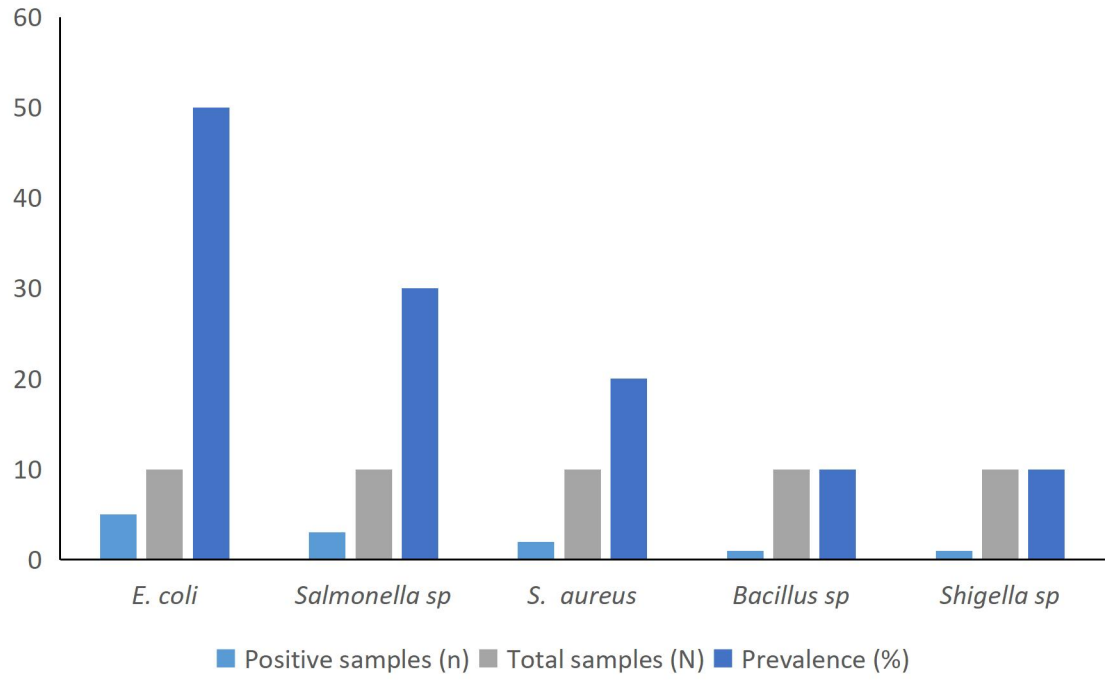

Figure 4.1: Prevalence of Isolated Enteric Bacteria from Carrot Samples

Table 4.4: Prevalence of Isolated Enteric Bacteria from Green Pea Samples

	Positive samples (n)	Total samples (N)	Prevalence (%)
E. coli	3	10	30
Salmonella sp	2	10	20
S. aureus	4	10	40
Bacillus sp	3	10	30
Shigella sp	2	10	20

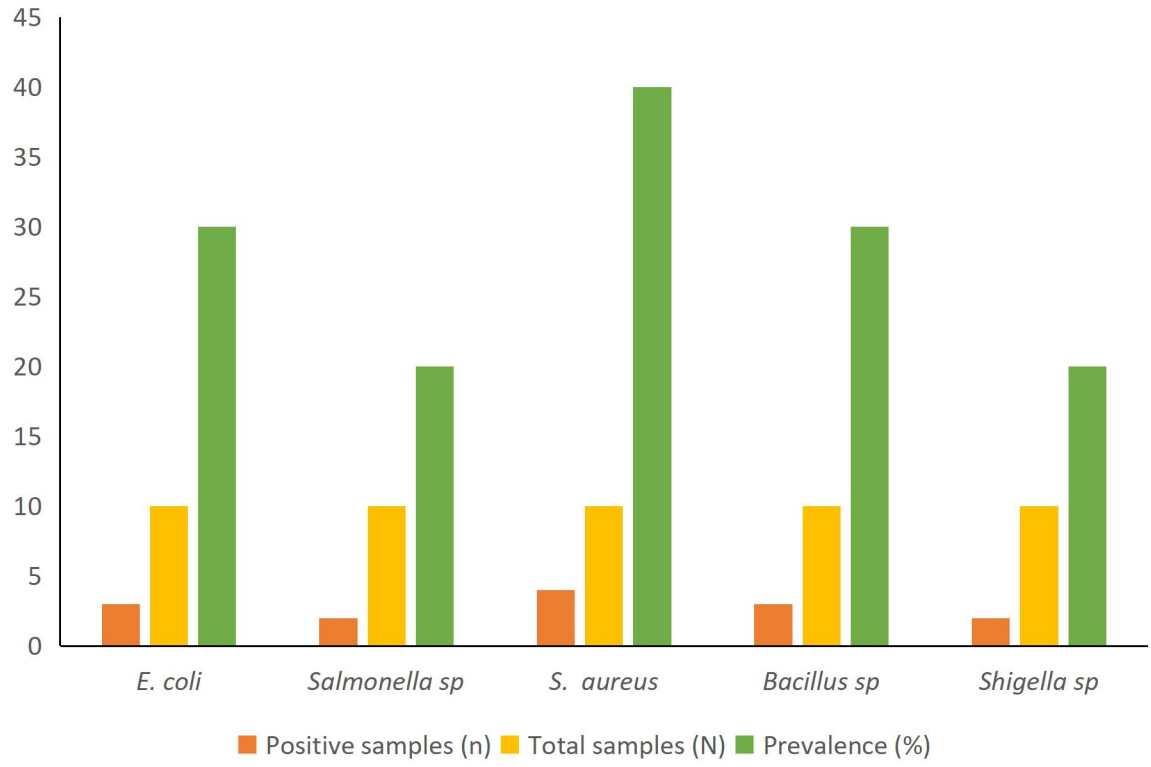

Figure 4.2: Prevalence of Isolated Enteric Bacteria from Green Pea Samples

Table 4.5. Antibiotic sensitivity test

ISOLATES	CS	CIP	GEN	E	TE	M	CD	AG
Escherichia coli	0(R)	17(S)	12(I)	0(R)	0(R)	0(R)	9(R)	12(I)
Staphylococcus aureus	0(R)	14(S)	15(S)	8(R)	0(R)	7(R)	11(I)	14(S)
Bacillus sp	0(R)	18(S)	15(S)	10(I)	14(S)	7(R)	0(R)	9(R)
Shigella sp	7(R)	24(S)	19(S)	10(I)	8(R)	0(R)	10(R)	15(S)
Salmonella sp	0(R)	16(S)	14(S)	0(R)	0(R)	0(R)	0(R)	14(S)

KEY: **R** = RESISTANCE
S = SUSCEPTIBLE
CS = COLISTIN
CIP = CIPROFLOXACIN
GEN = GENTAMICIN
E = ERYTHROMYCIN
TE = TETRACYCLIN
M = METRONIDAZOLE
CD = CLINDAMYCIN
AG = AUGMENTIN

CHAPTER FIVE

DISCUSSION

The total heterotrophic bacterial count (THBC) in green peas and carrots collected from New Benin, Adolor, and Ring Road markets in Benin City revealed significant variations in bacterial load. The highest bacterial counts were recorded in samples from Ring Road Market, with green peas showing 265 ± 77.78 CFU/g and carrots 115 ± 12.73 CFU/g, indicating poor hygiene conditions, inadequate handling, and environmental contamination. Adolor Market showed moderate contamination levels (93.5 ± 86.97 CFU/g for green peas and 32 ± 21.21 CFU/g for carrots), while New Benin Market had the lowest bacterial load (55 ± 19.80 CFU/g for green peas and 9 ± 8.49 CFU/g for carrots).

These results are consistent with the findings of Imoni *et al.* (2023), who reported high bacterial loads in fresh vegetables sold in open markets in Benin City due to factors such as contaminated irrigation water, poor sanitation, and unhygienic post-harvest handling practices. Similarly, Morka (2022) observed significant microbial contamination in spoilt carrots from local markets in Abraka, linking it to environmental exposure and cross-contamination during transportation and storage. Kabir *et al.* (2020) also documented high microbial loads in ready-to-eat fruits and vegetables in Katsina State, attributing this to improper handling throughout the supply chain. Their study emphasized the role of dust exposure, unclean containers, and the absence of cold storage in promoting bacterial growth in fresh produce. The considerably higher bacterial counts in Ring Road Market compared to New Benin Market may reflect disparities in environmental hygiene, vendor practices, and market infrastructure. According to Balali *et al.* (2020), open markets with poor sanitary conditions create favorable environments for microbial proliferation, especially when vegetables are displayed without adequate protection from contamination.

Overall, the elevated bacterial counts observed raise serious public health concerns, as they increase the risk of foodborne illnesses. These findings highlight the urgent need for improved hygiene practices during harvesting, transportation, and marketing, as well as regular microbial monitoring to ensure the safety of fresh produce.

The isolation and identification of bacteria from vegetable samples are critical in assessing food safety, as fresh produce can serve as a vehicle for the transmission of foodborne pathogens. In this study, bacteria were isolated from green peas and carrots obtained from three major markets in Benin City—New Benin, Adolor, and Ring Road. The isolates identified include *Escherichia coli*, *Salmonella* spp., *Staphylococcus aureus*, *Bacillus* spp., and *Shigella* spp., based on their cultural, morphological, and biochemical characteristics. This finding highlights the extent of microbial contamination in vegetables, which poses potential health risks to consumers.

The presence of *E. coli* and *Salmonella* spp. in the samples aligns with the findings of Imoni *et al.* (2023), who reported the prevalence of *Enterobacteriaceae* in fresh vegetables sold in open markets in Benin City. Their study suggested that poor hygiene during harvesting, improper washing, and contaminated water used in irrigation significantly contribute to bacterial contamination. Similarly, Morka (2022) isolated pathogenic microbes, including *E. coli* and *Salmonella* spp., from spoilt carrots in Abraka, Delta State. These pathogens are known to cause severe gastrointestinal infections, indicating that fresh produce can serve as reservoirs for foodborne diseases if not handled properly. Furthermore, the detection of *Staphylococcus aureus* in green peas is consistent with the work of Ojodomo and Ashar (2023), who identified similar bacterial contaminants in carrots sold at community markets in Zaria. This bacterium is commonly associated with post-harvest contamination, often introduced through human contact during handling, transportation, and vending. The isolation

of *Bacillus* spp. also suggests environmental contamination, as this genus is widespread in soil and dust, reflecting inadequate storage conditions. Kabir *et al.* (2020) reported high bioburdens of pathogenic bacteria in ready-to-eat fruits and vegetables in Katsina State, further emphasizing that microbial contamination is a nationwide issue in Nigeria. Their study highlighted that factors such as poor sanitary conditions in markets, lack of cold chain facilities, and inadequate food safety regulations contribute to the proliferation of these pathogens.

All in all, the isolation of multiple bacterial species from vegetable samples in this study underscores the urgent need for improved hygiene practices across the entire food supply chain—from farm to table. Regular monitoring, public health education on safe food handling, and strict enforcement of food safety standards are essential to reduce the risk of foodborne diseases associated with the consumption of contaminated fresh produce.

The evaluation of the percentage occurrence of bacterial isolates from vegetable samples provides critical insight into the prevalence of specific pathogens and the potential risks they pose to public health. In this study, *Escherichia coli* exhibited the highest prevalence in carrot samples at 50%, while *Staphylococcus aureus* was the most prevalent in green pea samples at 40%. Other isolates such as *Salmonella* spp., *Bacillus* spp., and *Shigella* spp. were also detected at varying rates, indicating widespread microbial contamination across the vegetable samples. The high occurrence of *E. coli* in carrots aligns with findings from Liwanag and Soriano (2018), who reported a significant prevalence of *E. coli* in fresh vegetables sold in public markets. Their study attributed this to contamination from fecal matter due to the use of untreated water for irrigation, poor post-harvest handling practices, and unhygienic market environments. Similarly, Kabir *et al.* (2020) reported high bioburdens of enteric bacteria,

including *E. coli*, in ready-to-eat fruits and vegetables in Katsina State, Nigeria, suggesting that contamination is not limited to one region but is a pervasive issue across the country.

The detection of *Staphylococcus aureus* in 40% of green pea samples mirrors the results of Ojodomo and Ashar (2023), who found similar prevalence rates in vegetables sold in Zaria markets. *S. aureus* contamination is often associated with human handling, as it is commonly found on the skin and nasal passages. This indicates inadequate personal hygiene practices among vendors and handlers as a critical factor contributing to contamination. *Salmonella* spp. and *Shigella* spp. were identified in 20% of green pea samples and 30% and 10% of carrot samples, respectively. These findings are consistent with Kilonzo-Nthenge *et al.* (2018), who observed a notable prevalence of *Salmonella* spp. in fresh produce from small-acreage farms in Tennessee, USA, highlighting that fresh vegetables are global vectors for foodborne pathogens. Addis and Sisay (2015) also noted the significant role of these bacteria in foodborne outbreaks, particularly in developing countries where sanitation is often compromised.

The overall prevalence rates observed in this study emphasize the urgent need for improved food safety measures. These include the use of clean water for irrigation, better hygiene practices during harvesting and marketing, and consumer education on proper washing and handling of fresh vegetables. Addressing these issues is crucial in reducing the burden of foodborne illnesses associated with contaminated produce.

The assessment of the antibiotic sensitivity pattern of bacterial isolates from vegetable samples is crucial in understanding the potential public health risks associated with antimicrobial resistance (AMR). In this study, the isolated bacteria—*Escherichia coli*, *Staphylococcus aureus*, *Salmonella* spp., *Bacillus* spp., and *Shigella* spp.—exhibited varying degrees of resistance to commonly used antibiotics. Notably, many isolates showed resistance

to colistin (CS), erythromycin (E), tetracycline (TE), metronidazole (M), and clindamycin (CD), while ciprofloxacin (CIP) remained the most effective antibiotic across most isolates. *E. coli* displayed susceptibility to ciprofloxacin but showed resistance to colistin, erythromycin, and tetracycline. This pattern aligns with the findings of Liwanag and Soriano (2018), who reported multidrug-resistant *E. coli* strains isolated from fresh vegetables in public markets. Their study attributed this resistance to the overuse and misuse of antibiotics in agricultural practices, such as the application of antibiotic-contaminated fertilizers and irrigation water. Similarly, Kilonzo-Nthenge *et al.* (2018) identified antibiotic-resistant *Enterobacteriaceae* in fresh produce from small-acreage farms in Tennessee, USA, highlighting the global nature of AMR in foodborne pathogens. *Staphylococcus aureus* in this study was susceptible to ciprofloxacin, gentamicin, and augmentin but resistant to clindamycin and erythromycin. This resistance pattern is consistent with Osman and Yin (2018), who observed similar antibiotic resistance trends in bacteria isolated from plant environments. The resistance of *S. aureus* to clindamycin is particularly concerning, as this antibiotic is commonly used to treat staphylococcal infections. The presence of resistant strains in food items increases the risk of transferring resistant genes to human pathogens, complicating treatment outcomes.

Salmonella spp. showed susceptibility to ciprofloxacin and augmentin but resistance to colistin and metronidazole, while *Shigella* spp. were highly susceptible to ciprofloxacin and gentamicin. These findings are supported by Addis and Sisay (2015), who highlighted the rising prevalence of multidrug-resistant *Salmonella* and *Shigella* strains in foodborne outbreaks, particularly in low- and middle-income countries. The widespread resistance observed in this study may be linked to the indiscriminate use of antibiotics in agriculture, poor regulatory enforcement, and the lack of antibiotic stewardship programs. The detection of multidrug-resistant bacteria in fresh vegetables poses significant public health concerns, as it increases the risk of treatment failure during foodborne disease outbreaks. Erhirhie *et al.*

(2020) emphasized the urgent need for integrated surveillance systems to monitor AMR trends in foodborne pathogens and implement strategies to curb the spread of resistant strains. This includes stricter regulations on antibiotic use in agriculture, improved hygiene practices during food handling, and public awareness campaigns on the risks associated with AMR.

The high prevalence of pathogenic bacteria and the emergence of multidrug-resistant strains in fresh vegetables underscore a significant public health concern. These findings are consistent with previous studies that have reported widespread microbial contamination and antibiotic resistance in fresh produce due to poor hygiene practices, inadequate food safety measures, and the indiscriminate use of antibiotics in agriculture (Liwanag & Soriano, 2018; Erhirhie *et al.*, 2020). To reduce the risk of foodborne diseases and curb the spread of antimicrobial resistance, there is an urgent need for improved food handling practices, stricter enforcement of food safety regulations, and public health education on proper vegetable washing and storage.

5.1 Conclusion

This study revealed significant microbial contamination in fresh vegetables, particularly green peas and carrots, sold in major markets in Benin City. The high total heterotrophic bacterial counts, especially in samples from Ring Road Market, reflect poor hygiene practices, inadequate handling, and environmental exposure. The isolation of pathogenic bacteria such as *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* spp., *Bacillus* spp., and *Shigella* spp., along with their varying prevalence rates, underscores the potential health risks associated with consuming contaminated vegetables. Furthermore, the detection of multidrug-resistant strains, particularly against commonly used antibiotics like colistin, erythromycin, and tetracycline, raises serious public health concerns. These findings highlight the urgent need for improved hygiene practices across the food supply chain, stricter regulation of antibiotic

use in agriculture, and continuous surveillance to mitigate the risks of foodborne illnesses and the spread of antimicrobial resistance.

REFERENCES

Adams, M.R., McClure, P.J. and Moss, M.O. (2024). *Food Microbiology.* (5th Edn). Royal Society of Chemistry. 582 p.

Addis, M. and Sisay, D. (2015). A review on major food borne bacterial illnesses. *Journal of Tropical Diseases* **3**(4): 1-7.

Akinyele, B.J. and Akinkunmi, C.O. (2012). Fungi associated with the spoilage of berry and their reaction to magnetic field. *Journal of Yeast and Fungal Research* **3**(4): 49-57.

Angelo, K.M., Conrad, A.R., Saupe, A., Dragoo, H., West, N., Sorenson, A., Barnes, A., Doyle, M., Beal, J., Jackson, K.A. and Stroika, S. (2017). Multistate outbreak of Listeria monocytogenes infections linked to whole apples used in commercially produced, prepackaged caramel apples: United States, 2014–2015. *Epidemiology & Infection* **145**(5): 848-856.

Balali, G.I., Yar, D.D., Afua Dela, V.G. and Adjei-Kusi, P. (2020). Microbial contamination, an increasing threat to the consumption of fresh fruits and vegetables in today's world. *International Journal of Microbiology* **2020**(1): 1-13.

Barth, M., Hankinson, T.R., Zhuang, H. and Breidt F. (2009). Microbiological spoilage of fruits and vegetables. **In**: *Compendium of the Microbiological Spoilage of Foods and Beverages*. Springer, New York. pp. 135-183.

Bennett, S.D., Sodha, S.V., Ayers, T.L., Lynch, M.F., Gould, L.H. and Tauxe, R.V. (2018). Produce-associated foodborne disease outbreaks, USA, 1998–2013. *Epidemiology & Infection* **146**(11): 1397-1406.

Bergholz, P.W., Strawn, L.K., Ryan, G.T., Warchocki, S. and Wiedmann, M. (2016). Spatiotemporal analysis of microbiological contamination in New York state produce fields following extensive flooding from Hurricane Irene, August 2011. *Journal of Food Protection* **79**(3): 384-391.

Bishop, H.G. and Okwori, G.O. (2017). *Escherichia coli* and *Staphylococcus aureus* contaminations of carrots sold within Zaria, Nigeria and their antibiotic susceptibility profiles. *Open Access Journal Science* **1**(4): 110–113.

Byrne, L., Dallman, T.J., Adams, N., Mikhail, A.F., McCarthy, N. and Jenkins, C. (2018). Highly pathogenic clone of Shiga toxin–producing Escherichia coli O157: H7, England and Wales. *Emerging Infectious Diseases* **24**(12): 2303.

Callejón, R.M., Rodríguez-Naranjo, M.I., Ubeda, C., Hornedo-Ortega, R., Garcia-Parrilla, M.C. and Troncoso, A.M. (2015). Reported foodborne outbreaks due to fresh produce in the United States and European Union: trends and causes. *Foodborne Pathogens and Disease* **12**(1): 32-38.

Calonico, C., Delfino, V. and LO NOSTRO, A. (2019). Microbiological quality of ready-to-eat salads from processing plant to the consumers. *Journal of Food and Nutrition Research* **7**: 427-434.

Castro-Ibáñez, I., Gil, M.I., Tudela, J.A. and Allende, A. (2015). Microbial safety considerations of flooding in primary production of leafy greens: a case study. *Food Research International* **68**: 62-69.

Center for Disease Control and Prevention (CDC) (2011). Vital signs: incidence and trends of infection with pathogens transmitted commonly through food-foodborne diseases active surveillance network, 10 US sites, 1996-2010. *MMWR Morb Mortal Weekly Report* **60**(22): 749-755.

Chan, Y.W., Hoban, A., Moore, H., Greig, D.R., Painset, A., Jorgensen, F., Chattaway, M.A., Jenkins, C., Balasegaram, S., McCormick, J. and Larkin, L. (2023). Two outbreaks of foodborne gastrointestinal infection linked to consumption of imported melons, United Kingdom, March to August 2021. *Journal of Food Protection* **86**(1): 100027.

Cheesbrough, M. (2000). *District Laboratory Practice in Tropical Countries Part 1.* 2nd Edn. Cambridge University Press. 434 p.

Clinical and Laboratory Standard Institute, (CLSI) (2020). *Performance Standards for Antimicrobial Susceptibility Testing* (30th Edn.). CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute 291 pp.

Colás-Medà, P., Viñas, I., Oliveira, M., Anguera, M., Serrano, J.C. and Abadias, M. (2017). Exposure to minimally processed pear and melon during shelf life could modify the pathogenic potential of Listeria monocytogenes. *Food Microbiology* **62**: 275-281.

Erhirhie, E.O., Omoirri, M.A., Chikodiri, S.C., Ujam, T.N., Kesiena, E.E. and Oseyomon, J.O. (2020). Evaluation of microbial quality of vegetables and fruits in Nigeria: A review. *International Journal of Nutrition Sciences* **5**(3): 99-108.

European Food Safety Authority (EFSA). (2018a). The European Union summary report on trends and sources of zoonoses, zoonotic agents, and food-borne outbreaks in 2017. *EFSA Journal* **16**(12): 05500.

European Food Safety Authority (EFSA). (2018b). Multi-country outbreak of *Salmonella* Agona infections possibly linked to ready-to-eat food. *EFSA Supporting Publications* **15**: 1–15.

European Food Safety Authority (EFSA). (2018c). Multi-country outbreak of *Listeria monocytogenes* serogroup IVb, multi-locus sequence type 6, infections linked to frozen corn and possibly to other frozen vegetables – first update. *EFSA Supporting Publications* **15**: 1–22.

Ferrari, R.G., Rosario, D.K., Cunha-Neto, A., Mano, S.B., Figueiredo, E.E. and Conte-Junior, C.A. (2019). Worldwide epidemiology of Salmonella serovars in animal-based foods: a meta-analysis. *Applied and Environmental Microbiology* **85**(14): 00591-00619.

Goodburn, C. and Wallace, C.A. (2013). The microbiological efficacy of decontamination methodologies for fresh produce: a review. *Food Control* **32**: 418–427.

Gurtler, J.B., Harlee, N.A., Smelser, A.M. and Schneider, K.R. (2018). Salmonella enterica contamination of market fresh tomatoes: a review. *Journal of Food Protection* **81**(7): 1193-1213.

Hassan, R., Whitney, B., Williams, D.L., Holloman, K., Grady, D., Thomas, D., Omoregie, E., Lamba, K., Leeper, M., Gieraltowski, L. and Outbreak Investigation Team, (2019). Multistate outbreaks of Salmonella infections linked to imported Maradol papayas–United States, December 2016–September 2017. *Epidemiology & Infection* **147**: 265.

Holvoet, K., Sampers, I., Callens, B., Dewulf, J. and Uyttendaele, M. (2013). Moderate prevalence of antimicrobial resistance in Escherichia coli isolates from lettuce,

irrigation water, and soil. *Applied and Environmental Microbiology* **79**(21): 6677-6683.

Imoni, A.A., Terin, O.V. and Ogbebor, A.S. (2023). Prevalence of enterobacteriaceae in fresh vegetables sold in open markets in Benin City Nigeria. *African Scientist Volume* **24**(3): 318-322.

Iyoha, O. and Agoreyo, F. (2015). Bacterial contamination of ready to eat fruits sold in and around Ugbowo campus of University of Benin (Uniben), Edo State, Nigeria. *British Journal of Medicine and Medical Research* **7**(2): 155-160.

Jechalke, S., Schierstaedt, J., Becker, M., Flemer, B., Grosch, R., Smalla, K. and Schikora, A. (2019). Salmonella establishment in agricultural soil and colonization of crop plants depend on soil type and plant species. *Frontiers in Microbiology* **10**: 967.

Kabir, M., Riko, Y.Y., Abdullahi, B., Kabir, K., Zubairu, U.D. and Hamza, U.A. (2020). Bioburdens of selected ready-to-eat fruits and vegetables consumed in Katsina Metropolis, Katsina State, Nigeria. *International Journal of Science and Research (IJSR)*, **9**(9): 108-114.

Kaur, A., Sood, A., Kaur, S. and Bhowate, P. (2017). Bacterial population associated with fruits and vegetables and its treatment using antimicrobial rinsing. *International Journal of Current Microbiology Applied Science* **6**(12): 2099-2107.

Kibitok, S.K. and Nduko, J.M. (2016). Evaluation of microbial contamination of consumed fruits and vegetables salad (Kachumbari) around Egerton University, Kenya. *Journal of Food Safety and Hygiene* **2**(1/2): 26-29.

Kilonzo-Nthenge, A., Liu, S., Hashem, F., Millner, P. and Githua, S. (2018). Prevalence of Enterobacteriaceae on fresh produce and food safety practices in small-acreage farms in Tennessee, USA. *Journal of Consumer Protection and Food Safety* **13**: 279-287.

Kim, S.J., Cho, A.R. and Han J. (2013). Antioxidant and antimicrobial activities of leafy green vegetable extracts and their applications to meat product preservation. *Food Control* **29**(1): 112-120.

Laughlin, M., Bottichio, L., Weiss, J., Higa, J., McDonald, E., Sowadsky, R., Fejes, D., Saupe, A., Provo, G., Seelman, S. and Concepción-Acevedo, J. (2019). Multistate

outbreak of Salmonella Poona infections associated with imported cucumbers, 2015–2016. *Epidemiology & Infection* **147**: 270.

Law-Ogbomo, K.E., Osaigbovo, A.U. and Omokaro, K.E. (2018). Growth and yield responses of carrot (Daucus carota L.) to different levels of oil palm refuse bunch ash in an ultisols environment. *Journal of Agriculture and Environment* **14**(1): 67-74.

Lee, D., Tertuliano, M., Harris, C., Vellidis, G., Levy, K. and Coolong, T. (2019). Salmonella survival in soil and transfer onto produce via splash events. *Journal of Food Protection* **82**(12): 2023-2037.

Liu, S. and Kilonzo-Nthenge, A. (2017). Prevalence of multidrug-resistant bacteria from US-grown and imported fresh produce retailed in chain supermarkets and ethnic stores of Davidson County, Tennessee. *Journal of Food Protection* **80**(3): 506-514.

Liwanag, M.C.S. and Soriano, G.P. (2018). Bacterial load of fresh vegetables sold in a selected public market and their susceptibility to commonly used antibiotics. *International Journal of Food Safety, Nutrition, Public Health and Technology* **10**(3): 18-25.

Luciano, W.A., Griffin, S., de Souza Pedrosa, G.T., Alvarenga, V., Valdramidis, V. and Magnani, M. (2022). Growth behavior of low populations of Listeria monocytogenes on fresh-cut mango, melon and papaya under different storage temperatures. *Food Microbiology* **102**: 103930.

Mahapatra, S., Chaly, P.E. and Girija, A.S. (2015). Effectiveness of various disinfection techniques on vegetables and fruits: an in-vitro study. *Archives of Dental and Medical Research* **1**: 1-8.

Morka, E. (2022). Isolation of Some Pathogenic Microbes Associated with Spoilt Carrots (*Daucus carota* L.) obtained from Local Markets in Abraka, Delta State, Nigeria. *Dutse Journal of Pure and Applied Sciences (DUJOPAS)* **8**: 1-8.

Mritunjay, S.K. and Kumar, V. (2015). Fresh farm produce as a source of pathogens: A review. *Research Journal of Environmental Toxicology* **9**(2): 59-70.

Mukherjee, A., Speh, D., Jones, A.T., Buesing, K.M. and Diez-Gonzalez, F. (2006). Longitudinal microbiological survey of fresh produce grown by farmers in the upper Midwest. *Journal of Food Protection* **69**: 1928–1936.

Nwachukwu, E. and Chukwu, C.M. (2013). Effect of chemical treatments on the microbial load of fruits and vegetables. *International Journal of Applied Microbiology and Biotechnology Research* **1**(3): 16-19.

Nwajinka, C.O. and Okonjo, E.O. (2019). Modeling the carrot slices (*Daucus carota* L.) drying characteristics using microwave oven. *CIGR Journal*, **21**(4): 231–235.

Ogbeibu, A.E. (2014). *Biostatistics: A Practical Approach to Research and Data Handling.* 2nd Edn. Mindex Publishing Co. Ltd, Benin City. 285 pp.

Ojodomo, B. and Ashar, S.J. (2023). Isolation and Identification of possible pathogenic bacteria on carrots sold at community market, Ahmadu Bello University, Zaria. *International Journal of Clinical and Medical Education Research* **2**(10): 271–283.

Oluwatoyin, A., Adejare, O., Wasiu, A. and Adeyanju, A. (2015). Microbial safety of polyethylene packaged sliced fruits sold in Abeokuta, South-West Nigeria. *Natural Science Research* **5**: 16-21.

Oranusi, S., Braide, W. and Nwankwo, O.E. (2012). Microbial and geohelminth population in different parts of carrot (*Daucus carota* L.). *Current Trends in Microbiology* **8**(1): 21-27.

Orji, J.O., Orinya, C., Okonkwo, E., Uzoh, C.V., Ekuma, U.O., Ibiam, G.A. and Onuh, N. (2016). The microbial contamination of ready-to-eat vended fruits in Abakpa Main Market, Abakaliki, Ebonyi State, Nigeria. *Journal of Pharmaceutical Biology and Science* **11**: 71-80.

Osman, N.I. and Yin, S. (2018). Isolation and characterization of pea plant *(Pisum sativum L.)* growth promoting rhizobacteria. *African Journal of Microbiology Research* **12**(34): 820–828.

Owais, Y., Iftikhar, A., Mohd, I.B. and Sushree, T. (2018). Assessment of microbial spoilage and techniques to avert the deterioration in fruits and vegetables. *International Journal of Chemical Studies* **6**(3): 2230-2235.

Rojas-Lopez, M., Monterio, R., Pizza, M., Desvaux, M. and Rosini, R. (2018). Intestinal pathogenic *Escherichia coli*: insights for vaccine development. *Frontiers in Microbiology* **9**: 440.

Schierstaedt, J., Jechalke, S., Nesme, J., Neuhaus, K., Sørensen, S.J., Grosch, R., Smalla, K. and Schikora, A. (2020). Salmonella persistence in soil depends on reciprocal interactions with indigenous microorganisms. *Environmental Microbiology* **22**(7): 2639-2652.

Smith, A., Moorhouse, E., Monaghan, J., Taylor, C. and Singleton, I. (2018). Sources and survival of Listeria monocytogenes on fresh, leafy produce. *Journal of Applied Microbiology* **125**(4): 930-942.

Stephan, R., Althaus, D., Kiefer, S., Lehner, A., Hatz, C., Schmutz, C., Jost, M., Gerber, N., Baumgartner, A., Hächler, H. and Mäusezahl-Feuz, M. (2015). Foodborne transmission of Listeria monocytogenes via ready-to-eat salad: A nationwide outbreak in Switzerland, 2013–2014. *Food Control* **57**: 14-17.

Thomas, G.A., Gil, T.P., Müller, C.T., Rogers, J.H. and Berger, C.N. (2024). From field to plate: How do bacterial enteric pathogens interact with ready-to-eat fruit and vegetables, causing disease outbreaks? *Food Microbiology* **117**: 1-19.

Threlfall, E.J. (2002). Antimicrobial drug resistance in Salmonella: problems and perspectives in food-and water-borne infections. *FEMS Microbiology Reviews* **26**(2): 141-148.

Uyttendaele, M., Jaykus, L.A., Amoah, P., Chiodini, A., Cunliffe, D., Jacxsens, L., Holvoet, K., Korsten, L., Lau, M., McClure, P. and Medema, G. (2015). Microbial hazards in irrigation water: standards, norms, and testing to manage use of water in fresh produce primary production. *Comprehensive Reviews in Food Science and Food Safety* **14**(4): 336-356.

Uzeh, R.E., Alade, F.A. and Bankole, M. (2009). The Bacterial quality of pre-packed mixed vegetable in some retail out lets in Lagos, Nigeria. *African Journal of Food Science* **3**(9): 270-272.

Velusamy, V., Arshak,, K., Korostynska, O., Oliwa, K. and Adley, C.(2010). An overview of foodborne pathogen detection: In the perspective of biosensors. *Biotechnology Advance* **28**: 232-254.

Zhao, P., Ndayambaje, J.P., Liu, X. and Xia, X. (2022). Microbial spoilage of fruits: A review on causes and prevention methods. *Food Reviews International* **38**(sup1): 225-246.

Zheng, L., Bae, Y.M., Jung, K.S., Heu, S. and Lee, S.Y. (2013). Antimicrobial activity of natural antimicrobial substances against spoilage bacteria isolated from fresh produce. *Food Control* **32**: 665–672.