AFRICA UNIVERSITY

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# ISOLATION, IDENTIFICATION AND ANTIMICROBIAL RESISTANCE PROFILING OF ENTEROBACTERIACEAE (*E. COLI, CITROBACTER SPP., SALMONELLA SPP, AND SHIGELLA SPP)* ASSOCIATED WITH DIARRHEAL DISEASES IN FRUITS AND VEGETABLES SOLD AT SAKUBVA MARKET, MUTARE (2024-2025)

BY

FADZAI JEAN CHIKUNDA

A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF BACHELOR OF MEDICAL LABORATORY SCIENCES IN THE COLLEGE OF HEALTH, AGRICULTURE AND NATURAL SCIENCES.

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# ABSTRACT

The increase in antimicrobial-resistant (AMR) bacteria discovered in fresh produce has become a major public health concern, particularly in resource-constrained countries such as Zimbabwe. Fresh fruits and vegetables sold in open marketplaces, such as Mutare's Sakubva Market, are frequently consumed raw, raising the risk of diarrhoea caused by pathogenic Enterobacteriaceae such as *Escherichia coli*, *Salmonella*, *Citrobacter*, and *Shigella*. Despite frequent monitoring of water sources, there is a scarcity of information on the presence and resistance patterns of these pathogens in fresh produce. This study aimed to isolate, identify, and determine the antibiotic resistance profiles of Enterobacteriaceae that cause diarrhoea in fruits and vegetables sold at Sakubva Market, in order to provide evidence for targeted public health interventions. A descriptive cross-sectional design was used, with 120 fruit and vegetable samples—including apples, mangoes, lettuce, tomatoes, carrots, and cucumbers—collected randomly from the market. Bacteria were cultured and isolated using standard microbiological methods and identified through biochemical techniques including Gram staining, indole, motility, citrate utilization, Kligler iron agar, and lysine decarboxylase tests. Antibiotic susceptibility profiles of the isolates were assessed using the disc diffusion method with routinely prescribed antibiotics. Ethical approval was obtained from both the Africa University Research Ethics Committee (AUREC) and the Mutare City Council. The findings revealed that *E. coli* was the most prevalent pathogen, detected in 45.8% of all samples, with lettuce showing the highest level of contamination. *Salmonella Paratyphi A* was the least prevalent (1.7%), while *Shigella spp.* were not detected in any of the tested samples. Antibiotic susceptibility testing showed high levels of resistance, with *E. coli* and other isolates exhibiting 100% resistance to ampicillin, gentamicin, and cotrimoxazole. Ciprofloxacin was identified as the most effective antibiotic, particularly against *Citrobacter spp.*. Beta-lactamase enzyme production was highest in *E. coli*, correlating with their multidrug resistance. These results underscore the need for improved agricultural practices, vendor hygiene, and community education to curb the transmission of AMR pathogens through fresh produce in open markets.

# DECLARATION

I, Fadzai Jean Chikunda, student number 210583 do hereby declare that this dissertation is my original work except where sources have been cited and acknowledged. The work has never been submitted, nor will it ever be submitted to another university for the award of a Bachelor of Science degree.

Fadzai Jean Chikunda__________________ ___________FJC_________________

Student’s Full Name Student’s Signature (Date)

Zororai Chiwodza

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Main Supervisors Full Name Supervisor’s Signature (Date)

# Copyright

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# Acronyms and Abbreviations

AMR Antimicrobial Resistance

AUREC Africa University Research Ethics Committee

CFU Colony Forming Units

DNA Deoxyribonucleic Acid

E. coli *Escherichia coli*

ESBL Extended-Spectrum Beta-Lactamase

GAP Good Agricultural Practices

$H_2S$ Hydrogen Sulphide

KIA Kligler Iron Agar

LFC Lactose Fermenting Coliforms

MDR Multidrug Resistance

WHO World Health Organization

UV Ultraviolet

## Definition of key terms

Antimicrobial Resistance (AMR): The ability of microorganisms such as bacteria, viruses, fungi, and parasites to resist the effects of medications that once successfully treated them, making standard treatments ineffective and infections harder to control.

Enterobacteriaceae: A large family of Gram-negative bacteria that includes many pathogens such as *Escherichia coli*, *Salmonella*, *Shigella*, and *Citrobacter*, commonly associated with gastrointestinal infections.

Beta-lactamase Enzymes: Enzymes produced by certain bacteria that break down beta-lactam antibiotics (such as penicillins and cephalosporins), rendering them ineffective.

Disc Diffusion Method: A laboratory technique used to evaluate the susceptibility of bacteria to antibiotics by placing antibiotic-impregnated discs on an agar plate inoculated with the bacteria and measuring zones of inhibition.

Coliforms: A group of Gram-negative, rod-shaped bacteria that ferment lactose and are commonly used as indicators of fecal contamination in food and water.

Good Agricultural Practices (GAP): A set of principles and practices applied to on-farm production and post-production processes, aimed at ensuring food safety and environmental sustainability.

Lactose Fermenting Coliforms (LFCs): A subgroup of coliform bacteria that ferment lactose to produce acid and gas, often used as indicators of sanitary quality and possible fecal contamination.

Kligler Iron Agar (KIA): A differential medium used to identify Gram-negative enteric bacteria based on their ability to ferment glucose and lactose, produce gas, and generate hydrogen sulphide.

Sakubva Market: A major open-air market located in Mutare, Zimbabwe, where a variety of fruits and vegetables are sold, often under informal or low-sanitisation conditions.

Cross-sectional Study: A type of observational research that analyzes data from a population at a specific point in time, useful for determining the prevalence of an outcome or condition.

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**CHAPTER 1**

1.1 Introduction

This chapter aimed to provide a complete overview of the isolation and identification of enterobacteriaceae that cause diarrhoea, from fruits and vegetables sold at Sakubva Market. This chapter investigated the significance of these pathogens as possible dangers to public health, particularly in light of antimicrobial resistance (AMR). By exploring the presence of these bacteria in fresh produce, this chapter emphasised the dangers of eating contaminated fruits and vegetables, which are frequently ingested raw.

## 1.2 Background

Significant public health problems are highlighted by the growing awareness of fruits and vegetables as harbouring antimicrobial-resistant (AMR) pathogens, especially Enterobacteriaceae including *Salmonella*, *Shigella*, *Citrobacter*, and *Escherichia coli*. These pathogens are known for causing gastrointestinal diseases, and their presence in fresh produce, commonly consumed raw or little cooked, raises major concerns regarding food safety and public health. Research has shown that vegetables can harbor extended-spectrum betalactamase (ESBL)-producing Enterobacteriaceae and other resistant strains. Studies have shown that ready-to-eat vegetables sold in markets can be infected with multidrug-resistant bacteria, offering major health concerns to consumers who may not fully wash or prepare these foods before consumption ( Schwaiger K et al, 2011). The possibility of foodborne outbreaks associated to AMR pathogens is a major worry. The World Health Organisation (WHO) has observed that foodborne outbreaks caused by AMR infections are becoming more common, with fresh produce usually identified as a source of these pathogens. AMR pathogen contamination of fruits and vegetables can happen at many stages of the food supply chain. The use of contaminated irrigation water, which can bring resistant bacteria into the farming environment, and the application of organic fertilisers and manure, which may contain resistant bacteria and cause contamination during crop growth, are important sources of contamination. Furthermore, unhygienic handling, processing, and distribution procedures can make it easier for AMR bacteria to spread from contaminated surfaces or handlers to produce. Fruits and vegetables are frequently consumed fresh or with little processing, which raises the possibility of coming into contact with AMR bacteria. According to (Raphael, B.H et al, 2011) AMR pathogens in fruits and vegetables present a number of public health issues, such as an increased burden on healthcare systems from infections brought on by these pathogens, which can result in longer hospital stays, more severe illnesses, and higher medical expenses because more sophisticated and costly treatments are needed. Fresh fruits and vegetables contaminated with AMR bacteria makes food safety initiatives more difficult and calls for improved risk assessment and monitoring techniques to safeguard the public's health. Effective interventions, such as bettering agricultural practices, raising standards of hygiene during handling and processing, and teaching consumers about safe food practices, are needed to lower the risk of contamination at various stages of the food supply chain. The importance of fruits and vegetables as possible carriers of AMR entorobacteriaceae draws attention to a crucial public health concern. Given the increasing prevalence of antibiotic resistance worldwide, it is critical to comprehend the mechanisms of infection and put precautionary measures in place to lessen the hazards posed by these infections. In order to solve this urgent issue and guarantee food safety, enhanced farming practices, public education, and ongoing surveillance are essential.

## 1.3 Problem Statement

The growing presence of antimicrobial-resistant Enterobacteriaceae in fresh produce is considered a serious public health risk, particularly for fruits and vegetables sold in local markets. These bacteria have been linked to foodborne illnesses and the spread of antibiotic resistance among consumers. For example, (AL-KHAROUSI. Z et al, 2019) revealed that 62.5% of fresh produce samples exceeded microbiological guidelines, with significant levels of *Escherichia* coli and other Enterobacteriaceae detected. This raises concerns about food safety and the risk of diarrheal illnesses. Zimbabwe has been hit by several cholera outbreaks, (Cuneo.C.N et al, 2017); (Mashe T et al, 2020), typhoid outbreaks (N'cho HS, 2019). Whilst fresh and sewer water are monitored for potential contamination (Li, E.; Saleem, F. et al, 2021); (Vurayai Ruhanya et al.,, 2022), there is a paucity of data for fresh fruit and vegetables and their potential risk of spreading these diseases in Zimbabwe, let alone Manicaland. Additionally, limited access to safe irrigation water and the widespread use of untreated organic manure in small-scale farming can introduce resistant bacteria into the food supply chain.

Foodborne infections caused by pathogenic Enterobacteriaceae, such as E. coli, Citrobacter, Salmonella, and Shigella, remain a major public health concern in Zimbabwe, particularly in resource-limited settings. Open markets like Sakubva Market in Mutare, where produce is frequently handled and displayed without sufficient hygiene or sanitation measures, are likely hotspots for bacterial contamination. This is exacerbated by challenges such as poor infrastructure, limited food safety regulations, and inadequate public health monitoring systems.

Despite this, the incidence and antimicrobial resistance profiles of diarrhea-causing Enterobacteriaceae on fresh produce in Zimbabwe are poorly documented.

Understanding the scope of this problem is critical, as resistant infections increase the burden on healthcare systems and limit treatment options. By isolating and identifying Enterobacteriaceae from fruits and vegetables sold at Sakubva Market and assessing their antimicrobial resistance profiles, this study aims to provide data to address these gaps. The findings will offer critical insights into the microbiological safety of fresh produce in

Zimbabwe and support targeted public health interventions, including improved vendor hygiene practices, strengthened food safety legislation, and public education campaigns to reduce the risks of foodborne illnesses and the spread of antimicrobial resistance.

## 1.4 Study Justification

Foodborne infections resulting from pathogenic bacteria like *Shigella*, *Citrobacter*, *Escherichia coli*, and *Salmonella* pose a serious threat to worldwide public health, especially in impoverished nations. Consuming infected fruits and vegetables which are frequently sold in open markets can spread these intestinal infections. The isolation and identification of these Enterobacteriaceae that cause diarrhea, together with the evaluation of their patterns of antibiotic resistance, are essential for comprehending the possible hazards linked to consuming fruits and vegetables from Sakubva Market. This information can help to inform public health actions and build suitable food safety strategies to protect the local community. There is currently minimal data on the incidence of these infections and their antimicrobial resistance profiles on fruits and vegetables sold in Zimbabwe's open marketplaces. This proposed study seeks to fill this knowledge gap by providing useful insights that may be used to improve food safety and public health outcomes in the Mutare region. The results from this research will contribute to a better understanding of the microbial quality of produce sold at open markets, the potential health risks associated with their consumption, and the antimicrobial resistance profiles of the isolated pathogens. In order to improve food safety standards and lessen the incidence of foodborne diseases in the community, this information can be utilized to tighten food safety rules, educate vendors and customers, and influence policy decisions.

## 1.5 Research objectives

### 1.5.1 Broad Objectives

This study aimed to investigate the prevalence of pathogenic foodborne bacteria and their antimicrobial resistance in fresh produce sold at Sakubva Market during 2024 and 2025.

### 1.5.2 Specific Objectives

1. Assessed the presence of pathogenic Enterobacteriaceae (*E. coli, Citrobacter, Salmonella, and Shigella*) from samples of commonly consumed raw Fruits and Vegetables sold at Sakubva Market, Mutare, Zimbabwe (2024-2025).
2. Accessed the antibiogram isolated Enterobacteriaceace species to establish their antimicrobial resistance patterns against a panel of commonly used antibiotics.
3. Determined the mechanism of drug resistance of the isolated organisms.

## 1.6 Research Questions

1. What is the prevalence of *E. coli, Citrobacter, Salmonella*, and *Shigella* contamination on commonly consumed raw fruits and vegetables sold at Sakubva Market, Mutare, Zimbabwe, during the 2024-2025 period?
2. What are the antimicrobial resistance patterns of the isolated Enterobacterieaceae isolates to a panel of commonly used antibiotics in Zimbabwe, during the 2024-2025 period?
3. What are the mechanisms underlying the antimicrobial resistance of the isolated Enterobacteriaceae isolates?

## 1.7 Study Limitations

For isolation and identification of Enterobacteriaceae, the study used morphological and biochemical approaches. While these approaches are widely used, they may have difficulties in identifying specific microbes or distinguishing closely similar species. The study acknowledged these limitations and provide appropriate interpretations. The investigation was completed within a defined timeframe, seasonal fluctuations in microbial contamination may be missed. At the open market, different seasons or time periods may have varying amounts of microbial presence and diversity on fruits, and vegetables The study shed light on potential hazards, but further epidemiological research may be required to prove direct cause-and-effect links. The study recognized that factors such as handling techniques, storage conditions, and transportation methods used outside of the open market may also contribute to microbial contamination. However, the study largely concentrated on the bacteria found on products bought from Sakubva Market.

## 1.8 Study Delimitations

The study was restricted to a certain geographical place in this case Sakubva Market. Because of differences in environmental factors, vendor practices, and customer behaviour, the findings may not be generalizable to other open markets or areas. The study gathered fruits and vegetables from vendors at Sakubva market using random sampling techniques. However, due to practical constraints, the sample size and duration was limited. As a result, the findings may not represent the whole spectrum of products on the open market.

## 1.9 Summary

This chapter emphasised the importance of fresh fruits and vegetables as potential carriers of enterobacteriaceae that can cause diarrhoeal illness, particularly when consumed uncooked. The chapter discussed the numerous contamination sources, such as farming practices and handling methods, that lead to the presence of AMR bacteria in the fresh produce. The study's objectives are clearly stated in this chapter which are isolating and identifying enterobacteriaceae from fruits and vegetables sold at Sakubva Market, as well as assessing their antibiotic resistance patterns. This fundamental understanding lays the groundwork for investigating the implications of AMR in food safety and public health.

**CHAPTER 2**

2.1 Introduction

This chapter will examine the study's literature review. Our goal is to investigate the current body of literature by reviewing numerous scholarly papers and discoveries linked to our research issue. We can recognize the advantages and disadvantages of various approaches and methodologies employed by other scholars. This enables us to expand on current knowledge while also contributing new insights to the subject.

2.2 Conceptual framework

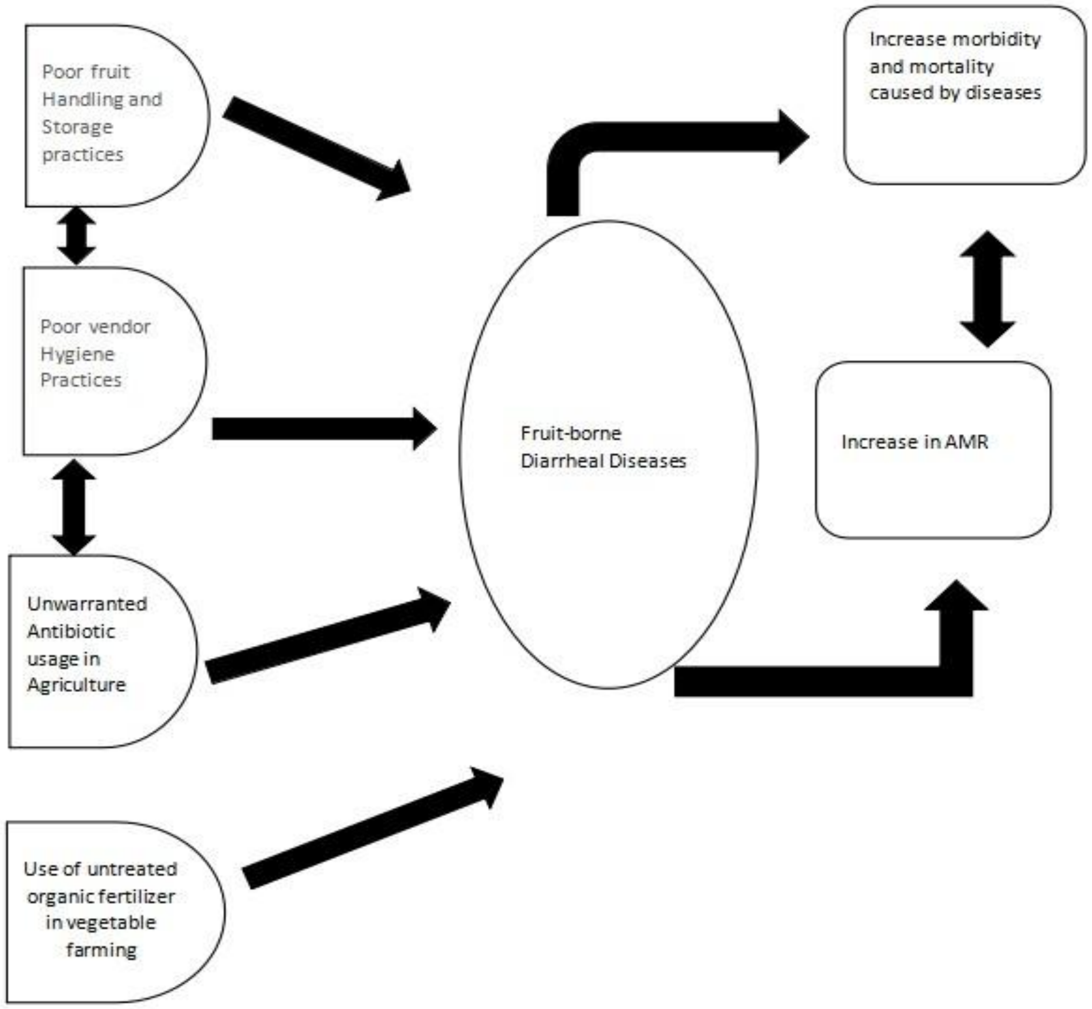


*Figure 1 Conceptual Framework*

The conceptual framework highlights the interplay between poor fruit handling, vendor hygiene, and the use of untreated organic fertilizers in agriculture, which contribute to fruitborne diarrheal diseases. Such infections are exacerbated by antimicrobial resistance (AMR), leading to increased morbidity and mortality, particularly in resource-limited settings like Zimbabwe. Open markets, such as Sakubva Market in Mutare, face challenges with inadequate sanitation, food safety regulation, and public health monitoring. Pathogenic Enterobacteriaceae—*E. coli, Citrobacter spp, Salmonella spp, and Shigella spp*—are known culprits, spreading through contaminated fruits and vegetables. This study aims to isolate these pathogens, assess their antimicrobial resistance profiles, and investigate resistance mechanisms. The findings will provide actionable insights to inform food safety strategies, reduce diarrheal diseases, and mitigate the rise of AMR.

## 2.3 Literature Review

### 2.3.1 The presence of Pathogenic Enterobacteriaceae in Fresh Fruit and Vegetables

Fresh fruits and vegetables are essential components of a healthy diet, however, they can also serve as vectors for foodborne pathogens. Studies have shown that fruits and vegetables can be contaminated with various microorganisms at multiple stages, from cultivation to consumption. For instance, a study conducted in Oman revealed that Enterobacteriaceae were present in 60% of fruit samples and 91% of vegetable samples, highlighting the widespread occurrence of these pathogens in fresh produce. Similarly, research in Thailand indicated that *Salmonella* and *Shigella* were significant contributors to foodborne illnesses linked to raw produce, with numerous outbreaks reported globally (Al-Kharousi ZS et al, 2016). The pathogenic strains of *E. coli* and *Salmonella* are particularly concerning due to their association with severe foodborne illnesses. The World Health Organization estimates that foodborne illnesses affect 600 million people annually, resulting in 420,000 deaths (Jutanat Srisamran et al, 2022).

In Maputo, a study on the microbiological examination of street foods at the point of sale was conducted by (Salamandane, Silva, & Malfeito-Ferreira, 2021)The purpose of this study was to evaluate the microbiological quality and safety of street food sold in Maputo's main streets and informal markets. Traditional hot (76.7%) and cold (75%) cuisines both had high proportions of poor food samples. This survey found no evidence of L. monocytogenes or *Salmonella.* When coagulase-positive staphylococci were utilized as a food safety indicator, however, about 25% (23/83) of the food samples evaluated were classed as unsatisfactory/possibly harmful.

(Vital PG, 2014) Carried out a study and this is the first study in the Philippines to employ culture and molecular approaches to examine the incidence of bacterial pathogens and somatic phages in retailed fresh produce used in salad preparation, specifically bell pepper, cabbage, carrot, lettuce, and tomato. Out of 300 samples collected in the open and in supermarkets, 16.7% were positive for E. coli, 24.7% for *Salmonella spp*., and 47% for somatic phages.

A similar study was carried out in Fiche Town, Oromia, Ethiopia by (Birhanu Degaga et al, 2022). The study looked at the microbiological quality of raw vegetables ingested in and around Fiche town, Central Ethiopia. For the experimental study, 100 samples of five different raw vegetables from two local markets were chosen based on their similarities in overall microbial quality in terms of aerobic mesophilic count, total coliform count, Enterobacteriaceae count, *Staphylococci* count, and yeast and mould levels. Aerobic mesophilic bacteria had the greatest count (5.7 log CFU/g), followed by Enterobacteriaceae (4.7 log CFU/g), with yeasts and moulds having the lowest counts. The highest count of aerobic mesophilic bacteria was found in cabbage (6.4 log CFU/g), while the lowest was found in green pepper samples (4.7 log CFU/gram). S. aureus was found in 11% of the 100 vegetable samples analysed, with cabbage (20%) and lettuce (15%) being the most common carriers. In the current investigation, 15.0% of vegetable samples tested positive for Salmonella, which was discovered in all vegetable varieties.

Research in various regions, including Zimbabwe, is crucial for understanding the local context of food safety. In Zimbabwe, the assessment of raw fruits and vegetables from open markets, such as Sakubva Market in Mutare, will provide valuable data on the prevalence of pathogenic Enterobacteriaceae and contribute to the global understanding of food safety in developing countries.

### 2.3.2 The antimicrobial resistance patterns of Enterobacteriaceae

The presence of antimicrobial-resistant (AMR) Enterobacteriaceae in fruits and vegetables has emerged as a major public health concern, especially given the pathogens' ability to cause foodborne diseases. Several studies have revealed the presence and resistance patterns of these bacteria in fresh produce, underscoring the importance of ongoing monitoring and appropriate food safety measures.

(AL-KHAROUSI. Z et al, 2019) investigated antibiotic resistance in Enterobacteriaceae isolated from 13 different types of native and imported fruits and vegetables. Their findings revealed that 74% of the isolates were resistant to at least one antibiotic, with 16% being multidrug-resistant. The highest rates of resistance were found against ampicillin (66%) and cephalothin (57%). The investigation found AmpC β-lactamases in certain isolates, indicating resistance to many β-lactam drugs, including cephalosporins.

A similar study was conducted by (Moges. M et al, 2024) conducted a cross-sectional study in Ethiopia to investigate the antibiotic resistance patterns of Enterobacteriaceae isolated from street meals, which included fruits and vegetables. The study found substantial resistance rates to routinely used antibiotics, with 78.6% of isolates resistant to erythromycin and 36.4% to amoxicillin. This highlights the possibility for AMR infections to penetrate the food system via contaminated crops.

Research conducted by (Jennifer Lord et al, 2021) in Kenya highlighted that Enterobacteriaceae resistant to third-generation cephalosporins and carbapenems have been identified as critically important for public health because these illnesses have few available treatment options remaining. In this study, the majority of Enterobacteriaceae isolates were resistant to routinely used drugs, including penicillin/β-lactamase inhibitors (91.2%) and folate pathway inhibitors (83.7%). Resistance to extended-spectrum cephalosporins was similarly prevalent (52.9 percent). The AMR and MDR rates for Enterobacteriaceae were 88.5% and 51%, respectively.

Antibiotic-resistant Enterobacteriaceae are a major cause of healthcare-associated infections, leading to increased morbidity, mortality, and healthcare costs. A study in South Korea by (Young Kyun Choi et al, 2021) examined the antibiotic resistance patterns of Enterobacteriaceae isolated from patients with healthcare-associated infections (HCAIs) and community-acquired infections (CAIs). The results showed that the resistance rates to fluoroquinolones and third-generation cephalosporins were significantly higher in HAIs compared to CAIs.The study also found that the resistance rates to fluoroquinolones were 18.8%, 38.5%, and 55.0% in CAIs, HCAIs, and HAIs, respectively. Similarly, the resistance rates to third-generation cephalosporins were 8.3%, 50.0%, and 60.0% in CAIs, HCAIs, and HAIs, respectively. These findings highlight the importance of implementing antibiotic stewardship programs and infection control measures in healthcare settings to prevent the spread of antibiotic-resistant Enterobacteriaceae.

### 2.3.3 Mechanisms of drug resistance of Enterobacteriaceae

Antimicrobial resistance (AMR) has increased dramatically during the last few decades among bacteria in the Enterobacteriaceae family. Enterobacteriaceae are gut flora and major pathogens in both nosocomial and community settings. Enterobacteriaceae are easily transmitted between individuals and can acquire AMR through plasmids or other mobile resistance elements. The introduction and dissemination of multidrug-resistant (MDR) clones has significantly reduced therapy choices. Some infections are not curable with current antimicrobials (Lynch. J. P et al, 2021)

The mechanisms of drug resistance in Enterobacteriaceae, a bacterial family that includes important pathogens such as *Escherichia coli* and *Klebsiella pneumoniae*, are numerous and have developed in response to antibiotic-induced selective pressure. One of the key causes is the formation of β-lactamases, which hydrolyse the β-lactam ring of penicillins and cephalosporins, leaving these antibiotics useless. Extended-spectrum β-lactamases (ESBLs) and carbapenemases are increasingly frequent in clinical isolates (Ruppé. E et al, 2015).

According to (Iredell. J et al, 2016), The mobilisation of resistance genes via mobile genetic elements (MGEs), such as plasmids and transposons, allows for the fast spread of resistance features among bacterial populations, contributing to the establishment of multidrug-resistant strains.

Furthermore, changes in porin channels can reduce the permeability of the bacterial cell wall to antibiotics, while efflux pumps aggressively remove antimicrobial drugs from the cell, further increasing resistance (Lynch. J. P et al, 2021). Notably, aminoglycoside-modifying enzymes (AMEs) have been discovered as critical components in aminoglycoside resistance, which change these antibiotics and inhibit their binding to ribosome targets (Ruppé. E et al, 2015).

The combination of these processes produces a complex landscape of antibiotic resistance, posing significant challenges to treatment options and infection control strategies.
Understanding these pathways is critical for creating effective solutions to combat AMR in Enterobacteriaceae and reduce the impact on public health.

CHAPTER 3

## 3.1 Introduction

This chapter discusses the approach that was utilized to meet the research study's objectives. A descriptive study design was used, with qualitative microbiological techniques being used. Samples were taken from Sakubva open market in Mutare and tested at Africa University's microbiology laboratory. Bacteria were isolated and identified using standard microbiological culturing methods. The use of selective and differential media aided in the detection of probable diarrhoea causing Enterobacteriaceae. The Eneterobacteriaceae present were characterized using biochemical identification of various colonies. To ensure the safety and integrity of the research, proper ethical processes and controls were followed. The findings are expected to aid in identifying possibilities to improve food safety standards in this scenario.

The methodology is discussed in depth in the parts that follow.

## 3.2 Research Design

This study used a descriptive research design. An observational technique is most appropriate because the objectives involve the isolation and identification of naturally occurring Enterobacteriaceae from food samples without experimental manipulation. A cross-sectional sample collection was used in the study to define the Enterobacteriaceae present at one-time point. The use of qualitative microbiological culture methods as well as identification techniques were used. A descriptive study approach includes isolate categorization and any correlational analysis between microbial distribution and food types.

## 3.3 Study site/ setting

This study was conducted at Sakubva Market in Mutare. This area was chosen for examination because it features a large number of vendors who sell various sorts of fruits and vegetables that are potentially contaminated.

## 3.4 Inclusion Criteria

The study only included fresh fruits and vegetables that are frequently sold at Sakubva Market. This guarantees that produce that is likely to be eaten raw or with little processing is the main focus like apples, mangoes, lettuce, carrots, tomatoes and cucumbers. Samples were examined for the presence of Enterobacteriaceae, with a focus on those known to cause diarrhoea (*Salmonella spp, Shigella spp, Citrobacter spp, E.coli*) or their indicators of faecal contamination (*E.coli*). This could include a preliminary screening for bacterial contamination prior to comprehensive investigation. These criteria serve to ensure that the study's findings are reliable, relevant, and pertinent to public health concerns about antibiotic resistance in foodborne pathogens.

## 3.5 Exclusion Criteria

Samples that are not fresh, spoilt, decomposed, or otherwise unsuitable for consumption were excluded since they do not adequately reflect the conditions of products offered in open markets. Fruits that must be peeled before eating were also excluded since they may not be infected inside after peeling, and vegetables prepared before consumption were exempted because cooking may kill the enterobacteriaceae.

## 3.6 Sample size

A well-defined sample size is essential for assuring the credibility and validity of study findings. The sample size can be estimated using the following formula

$$Z^2.P.(1-P) \text{ n=}$$

$$E2$$

Population Size (N) refers to the total number of fruits and vegetables sold at Sakubva Market.

Expected prevalence (P): Based on recent research, the frequency of antimicrobial-resistant Enterobacteriaceae in similar settings is around 20%(0.20). Confidence Level (Z): A typical confidence level is 95%, which results in a Z-value of 1.96. A margin of error (E) of 8% (0.08) is commonly accepted in microbiological studies.

$$n = \frac{(1.96)^2 . 0.20 . (1-0.20)}{(0.08)^2}$$

=96.5

**Adjusting for finite population**

There is a requirement to attain an acceptable sample size; we can change our parameters more or use a finite population correction. Assume we want to account for additional factors by expanding our sample size by approximately 25%. Adjustment factor= 0.25 n = 96.5**.**(1+0.25)

= 120.625

Rounding this off gives a population size of approximately 120 samples.

## 3.7 Sampling Procedure

To assure representative and reliable samples, the sampling technique for isolating and identifying diarrhoea-causing Enterobacteriaceae (*E. coli, Citrobacter, Salmonella*, and *Shigella*) from fruits and vegetables sold at Sakubva Market consists of multiple systematic procedures. To minimise bias, a total of hundred and twenty samples were gathered, consisting of ten apples, ten mangoes, thirty lettuce heads, thirty carrots, thirty tomatoes and ten cucumbers purchased from various market vendors. To avoid cross-contamination, aseptic techniques were used during collection, with sterile gloves and containers, and each sample labelled with pertinent information, such as kind and collection date. To keep the samples fresh, they were carried in a cooler with ice packs and delivered to the laboratory within 24 hours of being collected. Upon receipt, the samples were swabbed and plated on MacConkey Agar to isolate target pathogens. Detailed records of the sampling techniques were kept to help with data analysis and tracing probable contamination sources. This extensive sampling approach intended to give a detailed assessment of the presence of AMR pathogens in fresh produce sold at Sakubva Market.

### 3.7.1 SAMPLE COLLECTION AND SAMPLE PREPARATION

Sterile polythene bags were used to collect and transport the purchased samples on ice to prevent bacteria multiplication and cross multiplication during sample transportation to the laboratory were the analysis was done.

For microbiological analysis of food samples namely apples, mangoes, lettuce, carrots, tomatoes and cucumbers were individually swabbed using sterile swabs and streaked on their respective, labelled plates.

### 3.7.2 Culturing of Samples

All plates were labelled accordingly. After swabbing the samples with a sterile swab, the swab was used to inoculate and then a sterile wire loop was used to streak on MacConkey Agar plates. The plates were incubated at 37°C for 20 hours. The agar plates were examined for growth.

### 3.7.3 IDENTIFICATION OF VARIOUS ISOLATES OBTAINED IN THE CULTURES

The enterobacteriaceae will be characterized and identified using the biochemical tests listed below.

1. Gram’s stain
2. Indole Test
3. Motility Test
4. Citrate Utilization Test

5. Kligler Iron Agar Test
6. Lysine Decarboxylase Test

*3.7.3.1 GRAM'S STAIN*

These tests were carried out in accordance with (Nester, 2007). The Gram stain is by far the most popular method for staining bacteria and classifying them into two primary groups: Gram (+) positive and Gram (-) negative. A thin film of specimen was put over a clean, grease-free slide and air dried. It was fixed by passing it three times over a Bunsen flame. The film was saturated with crystal violet for 60 seconds. The crystal violet was washed off the slide before being stained with lugol's iodine (mordant) for 60 seconds. The iodine was washed away, and the slide was decolorized for a second with acetone (decolorizer), washed again, and then stained with safranin (counter stain) for 60 seconds before being washed off. The back of the slide was dried, and the slide was allowed to air dry. A drop of oil immersion was added to the slide and it was viewed under a microscope at lens at x100 magnification. The color purple represents Gram (+) positive, whereas the color red represents Gram (-) negative.

*3.7.3.2 Indole Test*

According to (Ochei, 2001), this test was performed to separate members of the enterobacteriacea, Escherichia coli is indole positive, and only a few shigella strains are indole positive. In a test tube containing 3ml of sterile peptone water, the test organism was injected and incubated for 37°C for 21 hours. The test for indole was performed by adding 0.5ml of kovac's reagent the following day. The presence of a purple color on the layer's surface after 10 minutes indicates a positive result, while a brown colour change indicates a negative result.

*3.7.3.3 Motility test*

This test is used to identify motile members of the *vibranaceae* and certain *enterobacteriaceae.* A sterile needle was used to choose a well-isolated colony and stab the medium to within 1 cm of the bottom of the tube to test for motility. The needle was removed from the medium in the same line it was placed in. Incubation took place at 37°C for 21 hours. The inoculation line was checked for cloudiness, indicating that the organisms are motile (Chess brough, M., 2000).

*3.7.3.4 Citrate Test*

Citrate agar is used to determine an organism's ability to use citrate as an energy source. The medium's sole carbon source is citrate, while the only nitrogen supply is inorganic ammonium salts. Growth indicates the use of citrate, an intermediate metabolic product in the Krebs cycle. When bacteria metabolise citrate, the ammonium salts are converted down into ammonia, increasing alkalinity. The pH shift causes the bromothymol blue indicator in the medium to become blue, indicating a positive test result at a pH greater than 7.6. This medium is suitable for differentiating the species of Enterobacterales. With the exception of a few species, Salmonella, Citrobacter, Klebsiella, Enterobacter, Serratia, Providencia, Raoultella, and Cronobacter typically produce a positive reaction, while Escherichia, Shigella, Morganella, Plesiomonas, and Yersinia produce a negative response. Proteus is a citrate variable. (Richard Davis & Marie Pezzlo, 2023). This test was carried out by picking a single isolated colony and streaking it on the citrate agar and the plates were incubated at 37° for 21 hours.

*3.7.3.5 Kligler Iron Agar Test*

Kligler Iron Agar (KIA) differentiates gram-negative enteric bacilli through three biochemical reactions, carbohydrate fermentation, hydrogen sulphide ($H_2S$), and gas generation. The medium comprises glucose and lactose, which are fermented by bacteria, creating acid and reducing the pH, as evidenced by the colour change from red to yellow caused by the pH indicator phenol red. Glucose fermentation produces a yellow butt and a red slant, whereas lactose fermentation turns both yellow. Bacteria reduces thiosulphate to $H_2S$, resulting in a black precipitate in the butt. This distinguishes Salmonella from non-$H_2S$ producers like Shigella. Gas production is seen as bubbles or cracks in the agar. The combination of these reactions enables the distinction and presumptive identification of pathogens within the Enterobacteriaceae family, making KIA an important technique in clinical microbiology for detecting intestinal pathogens based on metabolic characteristics. This test was carried out according to (Chess brough, M., 2000), where a sterile straight wire loop was used to stab first the butt and then streak the slope. The tube was then closed with cotton wool and incubated at 37 °C for 21 hours.

*3.7.3.6 Lysine Decarboxylase*

Lysine decarboxylase works by enzymatically decarboxylating lysine to create cadaverine and carbon dioxide, both of which play important roles in bacterial acid stress response and differentiation. Lysine decarboxylase activity is frequently measured using lysine decarboxylase broth, which comprises peptones and yeast extract for bacterial growth, dextrose as a fermentable carbohydrate, L-lysine as a substrate, and bromcresol purple as a pH indicator. Initially, bacteria digest dextrose, producing acids that reduce the pH and transform the medium's colour from purple to yellow. If lysine decarboxylase is present, it decarboxylates lysine into cadaverine, increasing the pH and restoring the medium's purple colour. This process separates enteric bacteria that can produce lysine decarboxylase from those that cannot. CadA, an inducible enzyme, is activated by external variables such as low pH, lysine availability, and oxygen levels, which contribute to acid tolerance in organisms like Escherichia coli and Salmonella enterica. This test was done by using a sterile straight wire loop to stab first the butt and then streak the slope. The tube was then closed with cotton wool and incubated at 37 °C for 21 hours (Chess brough, M., 2000).

3.7.4 Disc diffusion susceptibility testing

Most laboratories employ disc diffusion techniques to regularly screen for antimicrobial susceptibility. A disc of blotting paper is impregnated with a known volume and concentration of an antibiotic, which is then placed on a plate of susceptibility testing agar that has been uniformly infected with the test organism. The antibiotic diffuses from the disc into the media, inhibiting the growth of the test organism at a distance from the disc determined (among other things) by the organism's susceptibility. Strains that are susceptible to the antimicrobial are inhibited away from the disc, whereas resistant strains have narrower inhibition zones or thrive up to the disc's border. (Chess brough, M., 2000). This was done to access the antibiogram isolated Enterobacteriaceace species to establish their antimicrobial resistance patterns.

## 3.8 Pilot Study

The goal was to determine the feasibility of collecting produce samples from Sakubva market, evaluating the effectiveness of sample processing and microbial culture techniques for isolating target pathogens, determining the appropriate sample size for the full-scale study based on preliminary prevalence data, and fine-tuning the study protocol and data collection instruments. The methods that were used were sample collection, sample processing, isolate identification, and antimicrobial susceptibility testing. The expected results are to determine the feasibility of the sample collection and processing methods, estimate the prevalence of target pathogens and their resistance patterns in the pilot samples, inform the sample size calculation for the full study based on the pilot prevalence data, and identify any protocol challenges or limitations that must be addressed. The pilot study provided critical insights into the study design and feasibility, allowing for method optimisation prior to the full-scale investigation on the isolation and antimicrobial resistance of diarrhea-causing Enterobacteriaceae from fruits and vegetables sold at Sakubva market, and guided the development of an effective and robust research protocol.

### 3.9 Data Analysis

After collection, all laboratory results were recorded in structured data sheets. The presence of pathogenic *Enterobacteriaceae* in each sample was determined based on the outcome of biochemical identification tests. Each isolate was logged according to sample type, source, and species identified. Data were entered into Microsoft Excel for statistical analysis. Descriptive statistics were used to calculate the frequency and percentage prevalence of each pathogen identified across the total sample size. Chi-square ($\chi^2$) tests were applied to examine associations between specific fruit or vegetable types and the occurrence of particular pathogens, with a significance level set at $p < 0.05$.

Antibiotic susceptibility results obtained via the disc diffusion method were compiled into an antibiogram. Resistance levels were categorized as sensitive, intermediate, or resistant, and the proportion of resistant isolates for each antibiotic was computed. Cross-tabulations were used to assess resistance patterns across bacterial species. Additionally, correlation analysis was performed to explore relationships between resistance mechanisms (e.g., beta-lactamase production) and multidrug resistance trends observed. This structured statistical approach allowed for meaningful interpretation of the microbiological data, enabling clear identification of contamination trends and antibiotic resistance patterns relevant to public health.

### 3.10 Ethical Considerations

Ethical approval was obtained from the Africa University Research Ethics Committee (AUREC) to ensure that the study adhered to standard ethical guidelines for research involving environmental and public health risks. Additionally, formal permission was sought and granted by the Mutare City Council to conduct the study at Sakubva Market. The council's approval ensured that the research respected local governance protocols and did not interfere with market operations. Both institutions reviewed the study protocol to confirm that the sampling process, data collection, and handling of potentially pathogenic microorganisms posed minimal risk to vendors, consumers, and the broader community. These approvals underscored the study's commitment to ethical integrity, community engagement, and regulatory compliance.

Sample Anonymization

Instead of being directly identifiable, each food sample was coded. Any study data published will not identify the individual vendors or places where positive samples were acquired.

Risk Evaluation

If not managed properly, the identification of possible pathogens poses some risk to public health. To prevent the transmission of viable microorganisms, strict aseptic methods were used throughout laboratory culturing and identification.

Data Privacy

Any isolate identification records or laboratory information generated will be securely stored without personal identifiers. Access will be limited to authorized research personnel.

Waste Disposal

All culture plates, broths and contaminated materials were autoclaved before disposal as biological waste according to institutional guidelines.

Community Engagement

Study results will be shared with market administrators and public health officials. The goal is to provide information that can aid interventions to improve food safety practices, rather than shutter vendors' businesses.

These ethical precautions aim to respect participants, responsibly handle microorganisms, protect privacy and data, minimize risks, and ensure the research benefits the community involved. Approval will be obtained from Mutare City Health Department and AUREC before commencement of the study.

# CHAPTER 4

## 4.1 Introduction

This chapter presented the data analysis and presentation of the investigation the prevalence of pathogenic foodborne bacteria and their antimicrobial resistance in fresh produce sold at Sakubva Market during 2024 and 2025. The results are provided first focusing on the demographic data. The analysis of results are provided. Lastly, the chapter summary is provided.

## 4.2 Characteristics of the samples collected

This section provides the information of fruits that were tested which were sold at Sakubva Market during 2024 and 2025. The data showed that there were 120 samples that were collected for testing fruits (apples and mangoes) and vegetables (carrots, tomatoes, cucumbers and lettuce). However the results provided that the most samples collected were tomatoes, lettuce, and carrots with 25% each and the least samples collected being mangoes, cucumbers, and apples 8.3%. The table 4.2 below is the distribution of the collected samples.

*Table 1* Characteristics of samples collected

| SAMPLE TYPE | NUMBER OF SAMPLES COLLECTED | PERCENTAGE |
|---|---|---|
| Carrots | 30 | 25.0% |
| Mangoes | 10 | 8.3% |
| Cucumbers | 10 | 8.3% |
| Tomatoes | 30 | 25.0% |
| Lettuce | 30 | 25.0% |
| Apples | 10 | 8.3% |

| | | |
|---|---|---|
| Total | 120 | 100% |

**Source: Primary data, (2025).**

4.3 The presence of Pathogenic Enterobacteriaceae from samples of commonly consumed raw Fruits and Vegetables sold at Sakubva Market, Mutare, Zimbabwe (2024-2025).

This section provides the data for the prevalence of pathogenic Enterobacteriaceae (*E. coli*, *Citrobacter spp*, *Salmonella* paratyphi *A* and LFCs that were not speciated) from samples of commonly consumed raw Fruits and Vegetables sold at Sakubva Market, Mutare, Zimbabwe (2024-2025). The table 4.3 below shows the prevalence of Enterobacteriaceae species in fruits (apples and mangoes) and vegetables (tomatoes, cucumbers and lettuce).

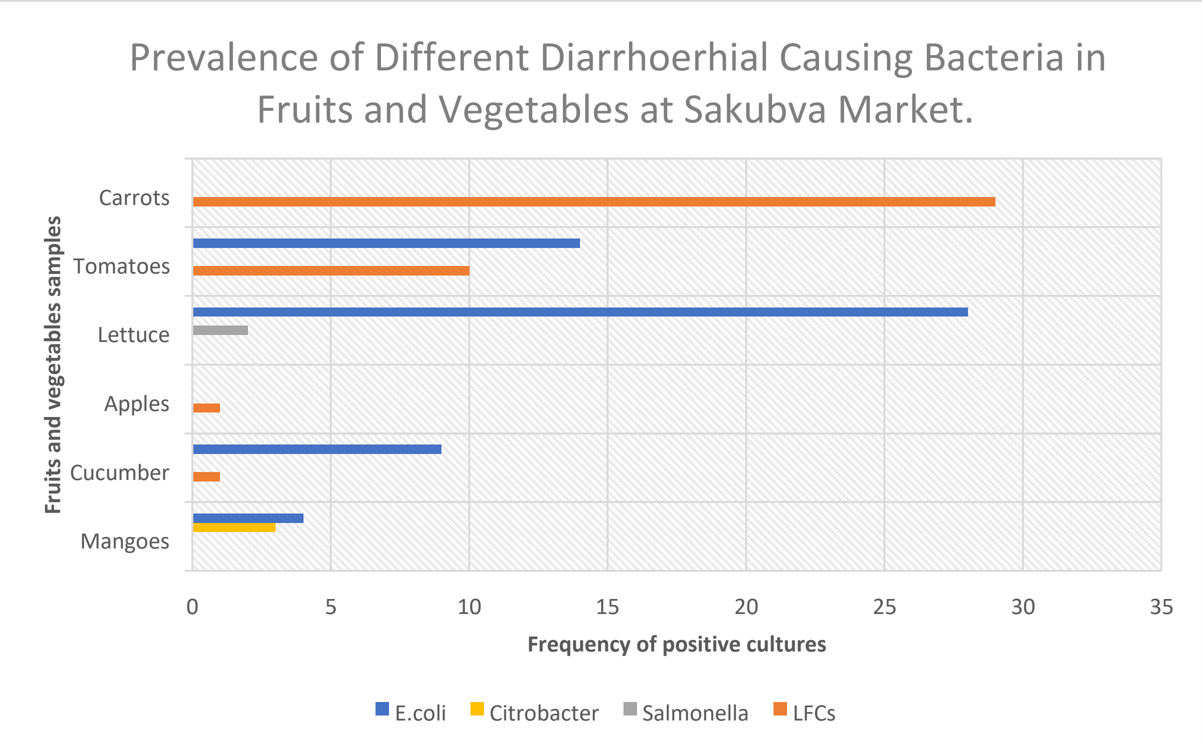


*Figure 2* Prevalence of different diarrhoerhial causing bacteria across the fruit and vegetable samples. Source: Primary data, (2025).

The data showed that *E. coli* had the highest number of positive cultures among the tested samples, with 55 isolates accounting for a prevalence of 45.8%. The calculated mean occurrence for *E. coli* was 0.4583. *Salmonella Paratyphi A* was the least prevalent, found in only 2 samples (1.7%), with a mean of 0.0167. *Citrobacter spp.* was isolated from 3 samples

(2.5%), specifically from mangoes. No isolates of *Shigella spp.* were detected in any of the 120 fruit and vegetable samples tested, despite its inclusion in the study title and objectives. Among all produce types, lettuce showed the highest contamination with *E. coli*, accounting for 28 isolates (23.3%). Apples had the lowest contamination rates, with 9 samples (7.5%) showing no growth of Enterobacteriaceae. *Lactose Fermenting Coliforms* (LFCs) were found in 34.2% of the samples, with carrots exhibiting the highest incidence (29 samples; 24.2%). Although *Enterococcus spp.* (specifically Group D *Streptococcus*) was observed in tomato samples, it was not part of the study's original scope or methodology. Therefore, it will not be further analysed in this report and will be excluded from subsequent interpretations and conclusions. A Chi-squared test was used to assess the association between fruit type and bacterial species. The test returned a statistic of 1.833, which was below the critical value of 2.267 at a 0.05 significance level and degrees of freedom (v) = 9. This suggests that there is insufficient evidence to reject the null hypothesis ($H_0$), indicating no statistically significant relationship between the type of bacteria and the fruit type. However, observational trends in the data suggest that certain fruit types may still influence the likelihood of specific bacterial contamination.

4.4 The results of the antibiogram isolated Enterobacteriaceae species to establish their antimicrobial resistance patterns against a panel of commonly used antibiotics.

This section provides the results of the antibiogram isolated Enterobacteriaceae species to establish their antimicrobial resistance patterns against a panel of commonly used antibiotics. The data shows that the four drugs that were used for the Enterobacteriaceae species are Ciprofloxacin, Ampicillin, Cotrimoxazole, Gentamicin, Cefuroxime, Chloramphenicol, Tetracycline, and Norfloxacin.

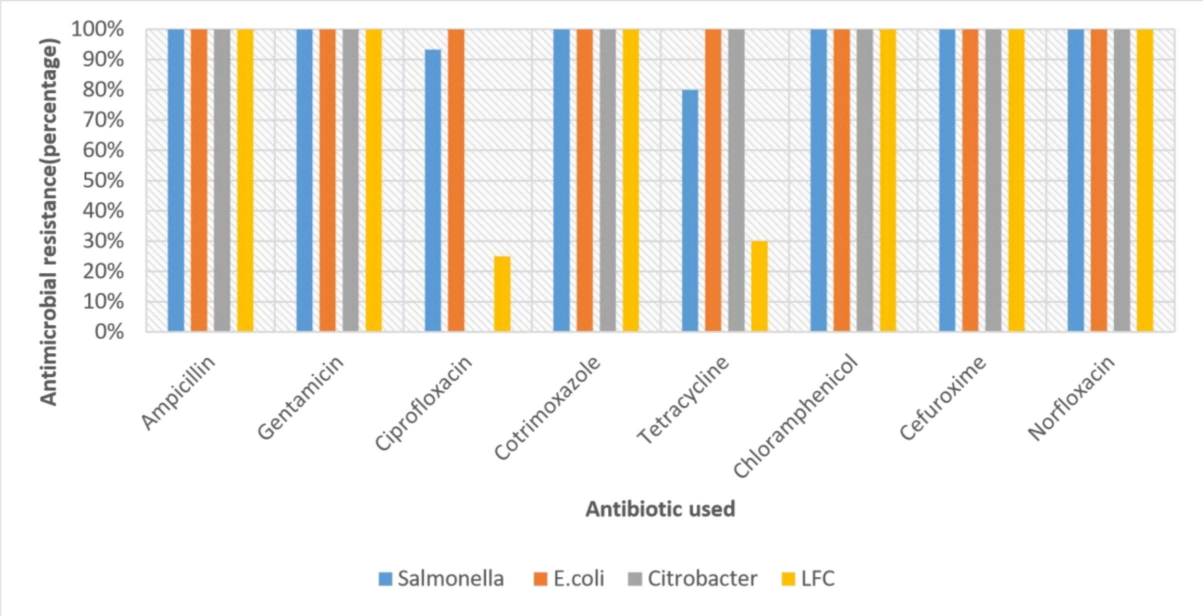


*Figure 3* Resistance profiles of the bacteria isolates

The data above shows that *Salmonella* Paratyphi A, tetracycline and Ciprofloxacin were sensitive with 93.3% and 80% respectively therefore making Tetracycline and Ciprofloxacin the most effective antibiotics on *Salmonella* Paratyphi A. However *E.coli* was resistant to all antibiotics that were used. For all the *Citrobacter spp* tested samples 0% resisted Ciprofloxacin making it the most effective antibiotic over *Citrobacter spp*. Ciprofloxacin and tetracycline were also effective over all LFCs that were isolated with 25% and 30% respectively. The figure below visually presents the resistance profiles.

4.5 The results of the mechanism of drug resistance of the isolated organisms.

The section provides the different types of mechanisms that are used by different Enterobacteriaceae species*;* *Salmonella* Paratyphi A, *Citrobacter spp*, *E.coli* and the nonspceciated LFCs. The table 4.5 below shows the mechanisms. The presence of the mechanism is in the form of production of beta-lactamase enzymes. Though they are other mechanisms these were the only ones carried out due to lack of resources. The data below showed that for beta-lactamase enzymes production, *E.coli* had the most cultures producing those (50%).  The lowest in beta-lactamase enzymes production was seen in *Citrobacter spp* isolates with 10% of the samples.

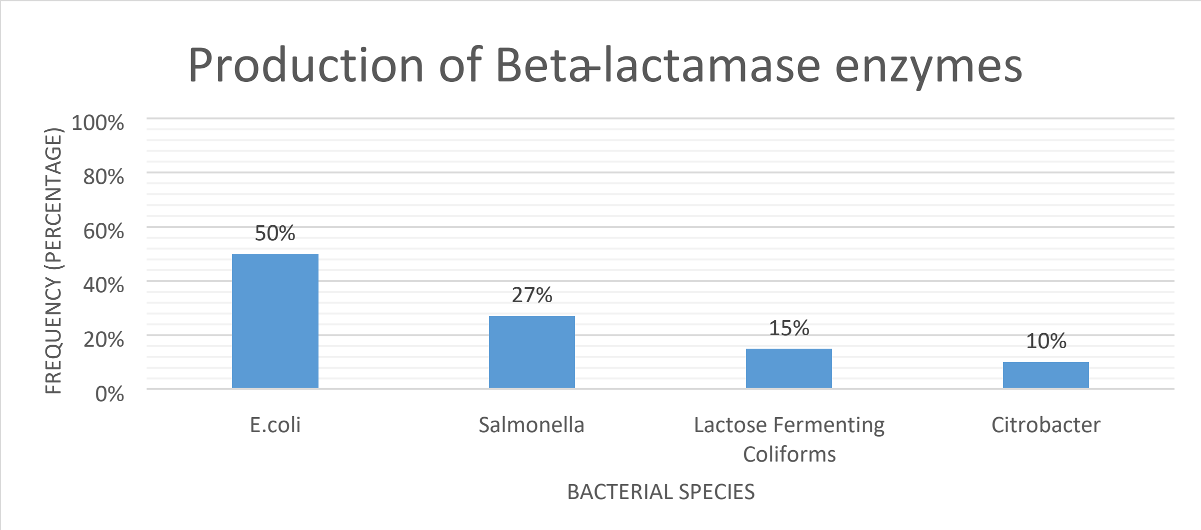


*Figure 4* Mechanisms of drug resistance of isolates

The data above is shown on figure 4. There seems to be a 0.99 correlation between the mechanism of the drug resistance and the antibiotic resistance. The production of betalactamase enzymes correlates with the resistance profiles of the bacteria with *E.coli* having the most resistance profiles than *Citrobacter* spp for the antibiotics that were used. *Salmonella* Paratyphi A is the next most resisting bacteria across the antibiotics used hence had more samples that had production of beta-lactamase enzymes.

## 4.6 Summary

This chapter presented the data analysis and presentation of the investigation and the prevalence of pathogenic foodborne bacteria and their antimicrobial resistance in fresh produce sold at Sakubva Market during 2024 and 2025. The results are provided first focusing on the demographic data. The analysis of results are provided.

# CHAPTER 5: DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

## 5.1 Introduction

This chapter provides the discussion of the investigation the prevalence of pathogenic foodborne bacteria and their antimicrobial resistance in fresh produce sold at Sakubva Market during 2024 and 2025. The summary of the major findings, conclusions and recommendations are provided in this chapter.

## 5.2 The presence of Pathogenic Enterobacteriaceae (*E. coli, Citrobacter spp, Salmonella* Paratyphi A, and LFCs) from samples of commonly consumed raw Fruits and Vegetables sold at Sakubva Market, Mutare, Zimbabwe (2024-2025).

*E. coli* is a common bacteria that can be found in the environment, including soil and water, which may come into contact with these fruits and vegetables during the growing or harvesting process. *E. coli* can also be transferred from contaminated surfaces or through improper handling practices by food workers. Furthermore, certain fruits and vegetables, such as mangoes and cucumbers, have a higher risk of *E. coli* contamination due to their structure and growing conditions. For example, the rough skin of mangoes can harbor bacteria, while cucumbers are grown close to the ground where they may come into contact with contaminated soil or water (Raphael, B.H et al, 2011).

In addition, the Sakubva Market in Mutare may have inadequate hygiene practices or facilities, leading to a higher risk of *E. coli* contamination in the fruits and vegetables sold there. Improper washing of produce, using unclean water, or storing fruits and vegetables at improper temperatures can all contribute to the presence of *E. coli*. It is crucial for vendors and consumers alike to practice proper food safety measures, such as washing fruits and vegetables thoroughly before consumption, to reduce the risk of *E. coli* contamination in raw produce sold at markets like Sakubva Market.

*Salmonella* had the least positive cultures in the samples of fruits and vegetables sold at Sakubva Market in Mutare, which are often eaten raw, due to several factors. One reason is that *Salmonella* is less commonly found in fruits and vegetables compared to other bacteria like *E. coli*. *Salmonella* is more commonly associated with contaminated poultry, eggs, and dairy products (Shaji S et al, 2023). Therefore, the lower prevalence of Salmonella in fruits and vegetables may explain why it had fewer positive cultures in this specific context.

The structure and composition of certain fruits and vegetables may also play a role in the lower presence of *Salmonella*. For example, fruits like mangoes and apples have protective skins that can help prevent bacterial contamination to some extent. Cucumbers, lettuce, tomatoes, and carrots also have smooth surfaces that may be less conducive to bacterial attachment and growth compared to other types of produce (Leff JW et al, 2013). These factors may contribute to the lower incidence of *Salmonella* contamination in these particular fruits and vegetables. The handling practices and hygiene standards at Sakubva Market may also impact the presence of Salmonella in the fruits and vegetables sold there. Proper storage, handling, and sanitation practices can help reduce the risk of bacterial contamination, including *Salmonella*. If vendors at the market adhere to food safety guidelines and regulations, such as washing produce properly and maintaining clean selling areas, the likelihood of *Salmonella* contamination may be reduced. The lower prevalence of *Salmonella* in fruits and vegetables sold at Sakubva Market may be attributed to the nature of the bacteria, the characteristics of the produce, and the adherence to food safety practices by vendors.

Apples are known to have a protective outer skin that acts as a barrier against bacterial contamination. The skin of apples is relatively smooth and impermeable, making it less likely for bacteria to penetrate and contaminate the flesh beneath (Leff JW et al, 2013). This natural barrier may explain why apples showed no growth of *E. coli, Salmonella, or Citrobacter spp* in the samples tested at the market. Mangoes, on the other hand, have a rough and irregular skin surface that can provide crevices and hiding places for bacteria to thrive. *Citrobacter*, a type of bacteria that is commonly found in soil and water, may have been present on the surface of mangoes due to the fruit's susceptibility to bacterial contamination (Raphael, B.H et al, 2011). Improper handling or unsanitary conditions during harvesting and transportation could also contribute to the presence of *Citrobacter spp* in mango samples.

Lettuce is a leafy green vegetable that is often associated with outbreaks of foodborne illnesses caused by pathogens like Salmonella. The structure of lettuce leaves, with their delicate and porous nature, can provide an ideal environment for bacteria to attach and multiply (Leff JW et al, 2013). Contamination of lettuce with *Salmonella* may occur during the growing, harvesting, or processing stages, especially if proper hygiene practices are not followed. Carrots, which had the highest levels of LFCs (presumably indicating bacterial contamination), are root vegetables that grow in soil and may come into direct contact with environmental bacteria during cultivation. The presence of soil particles or organic matter on the surface of carrots could increase the likelihood of bacterial contamination, leading to higher LFC counts in the samples tested.

The differences in bacterial contamination among apples, mangoes, lettuce, tomatoes and carrots at Sakubva Market may be attributed to the inherent characteristics of the produce, their susceptibility to bacterial growth, and the handling and hygiene practices employed throughout the supply chain.

5.3 The antibiogram isolated Enterobacteriaceae species to establish their antimicrobial resistance patterns against a panel of commonly used antibiotics.

*E. coli* is a common bacterium that can be found in the intestines of humans and animals. Over time, multiple strains of *E. coli* have developed resistance to many commonly used antibiotics, making it difficult to treat infections caused by this bacterium. *E. coli's* antibiotic resistance is the acquisition of resistance genes through horizontal gene transfer. This process allows bacteria to exchange genetic material with other bacteria, leading to the spread of antibiotic resistance genes within bacterial populations (Poirel L et al, 2018).

*E. coli* can develop resistance through the accumulation of mutations in its DNA. Mutations can alter the structure of bacterial proteins targeted by antibiotics, making them less susceptible to the drugs' effects. The overuse and misuse of antibiotics in both human medicine and agriculture have contributed to the development of antibiotic resistance in *E. coli*. The selective pressure exerted by the presence of antibiotics in the environment allows for the survival and proliferation of resistant strains of bacteria (Poirel L et al, 2018)**.** The mechanisms behind *E. coli's* resistance to antibiotics are complex and multifaceted, involving both genetic factors and environmental pressures. In order to combat antibiotic resistance in *E. coli* and other bacteria, it is crucial to implement strategies that promote responsible antibiotic use and surveillance of resistant strains in clinical and agricultural settings.

*Salmonella* Paratyphi A and other Lactose Fermenting Coliforms are known to be sensitive to tetracycline and ciprofloxacin due to the mechanisms of action of these antibiotics. Tetracycline inhibits protein synthesis in bacteria by binding to the 30S ribosomal subunit, thereby preventing the attachment of aminoacyl-tRNA to the mRNA-ribosome complex (Shutter MC et al, 2023). Ciprofloxacin, on the other hand, is a fluoroquinolone antibiotic that inhibits DNA replication and transcription by targeting the enzyme DNA gyrase, which is essential for bacterial DNA synthesis (LeBel M, 2018).

*Citrobacter spp* was found to have the least resistance to ciprofloxacin among the studied bacteria. This could be due to the lower prevalence of mutations in the genes encoding DNA gyrase or topoisomerase IV, which are the primary targets of ciprofloxacin in Citrobacter strains (LeBel M, 2018). The differential susceptibility of *Salmonella* Paratyphi A, LFCs, and

*Citrobacter* to tetracycline and ciprofloxacin can be attributed to variations in the mechanisms of action of these antibiotics as well as differences in the genetic makeup of the bacterial strains.

5.4 The results of the mechanism of drug resistance of the isolated organisms.

The study showed that for beta-lactamase enzymes production, *E.coli* had the most cultures producing those (50%). The production of beta-lactamase enzymes in bacteria such as *E. coli* is an important mechanism of resistance to beta-lactam antibiotics, including cephalosporins like cefuroxime. *E. coli* is a common Gram-negative bacterium that is known to produce a variety of beta-lactamases, which can confer resistance to multiple antibiotics. *E. coli* had the highest production of beta-lactamase enzymes compared to other Enterobacteriaceae species could be due to its widespread presence in the environment and in clinical settings (Poirel L et al, 2018). *E. coli* is a versatile bacterium that can adapt to different environments and can acquire resistance mechanisms through horizontal gene transfer, such as plasmids carrying beta-lactamase genes. Also, certain strains of *E. coli* may be under selective pressure due to the widespread use of antibiotics in both human and veterinary medicine. This can lead to the emergence of resistant strains that produce high levels of beta-lactamase enzymes as a defense mechanism against antibiotics like cefuroxime (Nasrollahian S et al, 2024). Enterobacteriaceae species may have different genetic backgrounds and mechanisms of resistance that result in lower production of beta-lactamase enzymes. These differences in resistance mechanisms could be due to variations in the genetic makeup of the bacteria, the presence of specific resistance genes, or different evolutionary pressures in different species.

The lowest in beta-lactamase enzymes production was seen in *Citrobacter spp* isolates with 10% of the samples. *Citrobacter* species, including *Citrobacter freundii* and *Citrobacter koseri*, are Gram-negative bacteria that are known to possess resistance mechanisms such as beta-lactamase enzymes (Tariq FN et al, 2023). These mechanisms play a key role in conferring resistance to beta-lactam antibiotics, including cephalosporins like cefuroxime.

Beta-lactamase enzyme production in *Citrobacter spp* could be due to their genetic makeup and ability to acquire resistance genes. *Citrobacter* species are known to be genetically diverse and capable of exchanging genetic material with other bacteria through mechanisms such as plasmid transfer. This horizontal gene transfer can lead to the acquisition of resistance genes, including those encoding beta-lactamase enzymes (Sun D et al, 2023).

## 5.5. Conclusions

The study concluded that *E.coli* was the most prevalent in all the fruits and vegetables that are eaten raw, sold at Sakubva market. The least prevalent bacteria was *Salmonella* Paratyphi A. However, Lettuce had the most prevalence of *E.coli* and apples had the least contamination possibility. Ciprofloxacin is the most effective antibiotic over *Citrobacter spp*. However, Ciprofloxacin is the most effective antibiotic that can effectively treat *Salmonella* Paratyphi A, *E.coli*, and *Citrobacter spp*. Beta-lactamase enzymes production mechanisms is highest in *E.coli* cultures and the least of this mechanisms found in *Citrobacter.*

## 5.6 Recommendations

i. It is critical to educate consumers about safe food handling techniques, particularly the proper washing and preparation of raw fruits and vegetables before consumption. Public health initiatives should be initiated to educate the public on simple but effective ways to reduce food-borne illness at the household level. These include thoroughly washing fruit in clean, running water, using light disinfectants such diluted vinegar or salt solutions, and keeping storage containers and preparation surfaces clean.

ii. The Mutare city council and the law enforcement agencies should be cautious of public health by gazzetting the standard infrastructure for the sale of the fruits and vegetables especially proper warehouse and storerooms. Sakubva Market can also implement lowcost communal sterilisation units to assist vendors in adequately cleaning fruits and vegetables before presenting them for sale. These units may contain chlorinated water dip stations or low-cost ultraviolet (UV) sterilisation booths, which are easily maintained with the help of local health authorities or non-governmental organisations (NGOs). Vendors should be instructed on how to use these systems efficiently, and incentives such as lower stall fees or public health certification may encourage regular use. Complementary instructional signage posted around the market can further enhance awareness of the need of food hygiene among both vendors and customers, fostering a culture of safety and responsibility while handling fresh produce.

iii. Constant check of the microbial resistances of the Enterobacteriaceae species that are found in the fruits and vegetables by the laboratories to assess how the species are mutating in order to have antibiotics in place that are effective in case of health emergencies or general treatment.

iv. There is a need to promote Good Agricultural Practices through the strengthening of local agricultural extension services. These services should provide small-scale farmers with targeted training on safe and sanitary farming techniques, such as the use of treated compost or certified manure, proper irrigation methods such as drip systems that reduce water contact with edible plant parts, and field hygiene practices such as regular handwashing and the use of sanitised tools during harvesting. Seasonal workshops led by extension officers in collaboration with public health officials can serve as a platform

for educating and empowering farmers to implement these measures, minimising the entry of antimicrobial-resistant bacteria at their source.

## 5.7 Area of further studies

It is necessary to evaluate the clinical impact of antimicrobial-resistant Enterobacteriaceae identified in fruits and vegetables on treatment outcomes in affected persons. Future research could look into the link between contaminated produce and real incidences of diarrhoeal sickness, including tracking patient histories and identifying specific resistance genes. This would assist in bridging the gap between microbiological findings and real-world public health outcomes, allowing for more effective interventions and antibiotic stewardship strategies.

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# Appendices

## Appendix 1

Dear Sir/Madam

**Re: Request for permission to conduct research at Sakubva Market.**

I hope this letter finds you well. My name is Fadzai Jean Chikunda and I am a student at Africa University studying Medical Laboratory Sciences.I'm writing to request permission to conduct research at Sakubva Market as part of my study on the isolation and identification of diarrhoeacausing Enterobacteriaceae (*E. coli*, *Citrobacter*, *Salmonella,* and *Shigella*) and their antimicrobial resistance patterns in fruits and vegetables sold there. The goal of my research is to determine the prevalence of these pathogenic bacteria in commonly consumed raw fruits and vegetables, which is critical for understanding the public health hazards connected with foodborne illness. The research will involve gathering samples from several market vendors, and I will guarantee that all data collection procedures adhere to ethical standards.

Mr. Zororai Chiwodza will supervise the project. Upon completion of my studies, I agree to give The Faculty of Health Sciences with a bound copy of my comprehensive research report. If you need any additional information, please don't hesitate to contact me on +263784726521 or chikundaf@africau.edu.

I look forward to hearing from you

Yours sincerely

Fadzai Jean Chikunda

ADDRESS ALL CORRESPONDENCE
TO THE TOWN CLERK

OUR REF: MN/mm

CITY OF MUTARE

CIVIC CENTRE NO.1 QUEENS WAY
P.O.BOX 910, MUTARE, ZIMBABWE
GENERAL LINE : +263202064412
DIRECT LINE :+262202060271
EXT: 309/331

TOWN CLERKS DEPARMENT

Our Ref: MN/mm

22 January 2025

**FADZAI JEAN CHIKUNDA**
Africa University
P. Bag 1320
**MUTARE**

Dear Sir/Madam

**RE: PERMISSION TO CARRYOUT A RESEARCH: DETERMINE THE PREVALENCE OF PATHOGENIC BACTERIA IN COMMONLY CONSUMED RAW FRUITS AND VEGETABLES AND UNDERSTANDING THE PUBLIC HEALTH HAZARDS CONNECTED WITH FOODBORN ILLNESS**

Your letter dated 12 December 2024 on the above matter refers.

I wish to advise that you have been granted permission to carry out a research titled, **"determine the prevalence of pathogenic bacteria in commonly consumed raw fruits and vegetables and understanding the public health hazards connected with foodborne illness".**

Could you please therefore liaise with Acting Health Services Director on the above matter.

Yours faithfully

K.B CHAFESUKA
**TOWN CLERK**

CITY OF MUTARE
HUMAN RESOURCES SECTION
27 JAN 2025
P. O. BOX 910, MUTARE
TEL: 02020 64412

Appendix 2

"Investing in Africa's future"

**AFRICA UNIVERSITY RESEARCH ETHICS COMMITTEE (AUREC)**

***P.O. Box 1320 Mutare, Zimbabwe, Off Nyanga Road, Old Mutare-Tel (+263-20) 60075/60026/61611 Fax: (+263 20) 61785 Website: www.africau.edu***

Ref: AU 3766/25

13 March, 2025

***FADZAI JEAN CHIKUNDA***
C/O Africa University
Box 1320
**MUTARE**

***ISOLATION, IDENTIFICATION AND ANTIMICROBIAL RESISTANCE PROFILING OF ENTEROBACTERIACEAE (E. COLI, CITROBACTER SPP., SALMONELLA SPP, AND SHIGELLA SPP) ASSOCIATED WITH DIARRHEAL DISEASES IN FRUITS AND VEGETABLES SOLD AT SAKUBVA MARKET, MUTARE (2024-2025)***

Thank you for the above-titled proposal you submitted to the Africa University Research Ethics Committee for review. Please be advised that AUREC has reviewed and approved your application to conduct the above research.

The approval is based on the following.

a) Research proposal

- **APPROVAL NUMBER** AUREC 3766/25
  This number should be used on all correspondences, consent forms, and appropriate document
- **AUREC MEETING DATE** NA
- **APPROVAL DATE** March 13, 2025
- **EXPIRATION DATE** March 13, 2026
- **TYPE OF MEETING**: Expedited
  After the expiration date, this research may only continue upon renewal. A progress report on a standard AUREC form should be submitted a month before the expiration date for renewal purposes.
- **SERIOUS ADVERSE EVENTS** All serious problems concerning subject safety must be reported to AUREC within 3 working days on the standard AUREC form.
- **MODIFICATIONS** Prior AUREC approval is required before implementing any changes in the proposal (including changes in the consent documents)
- **TERMINATION OF STUDY** Upon termination of the study a report has to be submitted to AUREC.

AFRICA UNIVERSITY
RESEARCH ETHICS COMMITTEE (AUREC)
APPROVED
P.O. BOX 1320, MUTARE, ZIMBABWE

**Yours Faithfully**

Mhinzou

**MARY CHINZOU**
**FOR CHAIRPERSON**
**AFRICA UNIVERSITY RESEARCH ETHICS COMMITTEE**

## Appendix 3

*Table 2* Timeline of Dissertation

| | Month | August 2024 | | | | September 2024 | | | | October 2024 | | | | January 2025 | | | | February 2025 | | | | March 2025 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Week | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 |
| | | | | | | | | | | | | | | | | | | | | | | | | | |
| Activity | | | | | | | | | | | | | | | | | | | | | | | | | |
| Finalizations of proposal | | → | → | → | → | → | → | → | → | | | | | | | | | | | | | | | | |
| Proposal submission to AUREC | | | | | | | | | | → | → | → | → | | | | | | | | | | | | |
| Data Collection, processing and analysis | | | | | | | | | | | | | | → | → | → | → | | | | | | | | |
| Writing of research findings and antimicrobial resistance patterns | | | | | | | | | | | | | | | | | | → | → | → | → | | | | |
| Project submission | | | | | | | | | | | | | | | | | | | | | | → | → | | |

Appendix 4

*Table 3* Budget

| Item | Cost ($) |
|---|---|
| Fruits and Vegetables | $35 |
| Transport | $30 |
| Sample Transportation tools | $50 |
| Lab equipment | $350 |
| Total Costs | $465 |

Appendix 5

Table 4 Data Collection tool

| DATE COLLECTED | SAMPLE ID | SAMPLE TYPE | NAME OF BACTERIA | SENSITIVE | RESISTANT | MECHANISM OF DRUG RESISTANCE |
|---|---|---|---|---|---|---|
| | | | | | | |
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Appendix 6

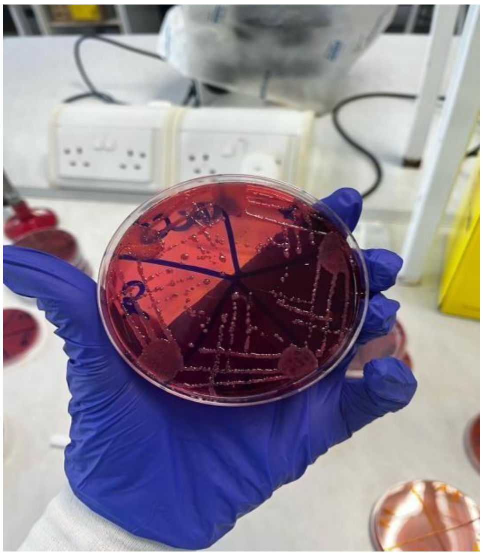

*Figure 5* Colonies


*Figure 6* Antibiotics

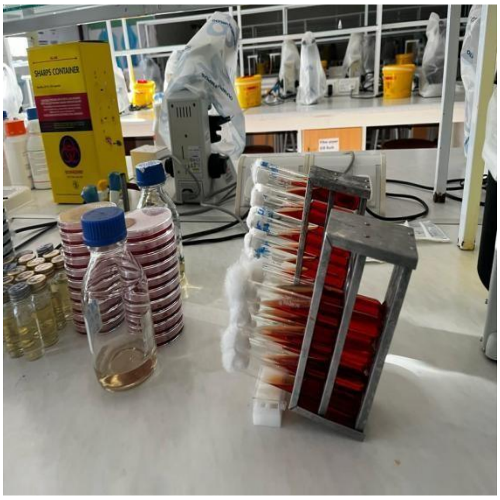


*Figure 7* Further tests