IDENTIFICATION AND CHARACTERIZATION OF BACTERIAL RESISTOME FROM POTABLE WATER AND ICE POPS IN JUJA, KENYA

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Identification and Characterization of Bacterial Resistome from Potable Water and Ice Pops in Juja, Kenya

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

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DEDICATION

I would like to dedicate this thesis to my beloved parents, James Mwita Chacha and Doris Boke James. They not only raised and nurtured me, but also made many sacrifices over the years to ensure my education and intellectual development. It is hard to put into words how grateful I am to them. Their continuous support on all aspects cannot be underscored. I also extend my dedication to my siblings, Sylvia Boke, Victor Robinson, and Nancy Chacha whose unwavering love and encouragement have been a driving force behind my academic pursuit. Special dedication to my nephew Dre Ledana O'Neil and my niece Ellarie Robinson who are still part of my big family. Their mere presence fills me with profound joy and a sense of purpose, making this thesis a heartfelt tribute to our inseparable bond and collective aspirations.

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ABBREVIATIONS AND ACRONYMS

ARB	Antibiotic Resistant Bacteria
ARG	Antibiotic Resistant Genes
CDC	Center for Disease Control
CFU/ML	Colony Forming Units per Milliliter
ESBL	Extended spectrum beta lactamases
WASREB	Water Service Regulatory Board
WHO	World Health Organization
QIIME	Quantitative Insights into Microbial Ecology
HGT	Horizontal Gene Transfer
PCR	Polymerase Chain Reaction
NGS	Next Generation Sequencing
DNA	Deoxyribonucleic Acid
ZOTUs	Zero-radius Operational Taxonomic Units
SDS	Sodium Dodecyl Sulfate
Mngs	Metagenomic Next Generation Sequencing
µg	Microgram
µl	Microliter

ABSTRACT

Water is essential for life, yet access to safe drinking water remains a challenge in many developing regions where distribution systems and vended products often harbor diverse microbiota. These microbial communities can serve as reservoirs for pathogens and antibiotic resistance determinants, promoting the spread of antibiotic resistance .Antibiotic resistance has emerged as a major global public health threat, reducing the effectiveness of commonly used treatments, leading to many deaths. This study aimed to characterize the bacterial antimicrobial resistance genes (resistome) in potable water and ice pops sold in Juja, Kenya. A total of 45 potable water samples and 10 ice pop samples were collected from vendors in Juja using purposive sampling. Vendor compliance with public hygiene policies was assessed using a survey guided by the Water Services Regulatory Board (WASREB) guidelines. Microbial contamination was evaluated using total coliform counts, bacterial diversity was profiled through metagenomic sequencing, and antibiotic resistance was assessed using disc diffusion technique and the Chan Zuckerberg ID pipeline.The study revealed that most vendors did not comply with the WASREB and public vending hygiene guidelines and policies, which could be a contributing factor to the high levels of bacterial contamination in potable water. Frozen pops and samples from the Gate C region of JKUAT recorded the highest contamination levels (1,073,000 CFU/mL and 505,415 CFU/mL respectively), while Witethie recorded the lowest contamination level (4,950 CFU/mL). Sachet pops showed no detectable contamination.A total of 30 putative microorganisms were identified through culture dependent technique whereas metagenomics identified a total of 113 distinct bacterial species.Metagenomic sequencing results revealed diverse pathogenic bacteria, including *Enterococcus faecium*, *Enterococcus faecalis*, *Burkholderia cepacia*, *Pseudomonas* spp., *Klebsiella* spp., and *Salmonella enterica*. The antibiotic resistant genes (ARGs) detected included *aac(6')-Iae* and *aac(6')-Iai*, found in all samples, *aac(6')-Ia*, *aac(6')-Iaj*, *aac(6')-I33*, *aph(3')-VIIIa* and *mutated rrsB*.These genes primarily confer resistance to aminoglycoside antibiotics. The detected ARGs are associated with pathogenic microbes, further complicating the public health risks posed by contaminated water and ice pops. The detected pathogenic bacteria cause infections, including bacteremia, respiratory tract infections, urinary tract infections, and blood infections. The findings of this study demonstrate that potable water and ice pops sold in Juja can serve as reservoirs of pathogenic bacteria and associated antibiotic-resistant genes. The findings underscore the critical need for enhanced public health interventions and compliance with water vending guidelines to ensure safe drinking water and prevent the spread of waterborne diseases, not only in Juja, Kenya, but also globally.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Water is an essential requirement in all biological processes however, supply clean of water does not meet its ever-growing demands (Curutiu *et al.*, 2019).The high demand and paucity of clean water has made investment in water production and supply very lucrative in developing countries as manifested by rising number of potable water vendors in the peri urban settlement schemes (Mulwa *et al.*, 2021). However, the rapid expansion of informal and small-scale potable water vending, often operating under limited regulation and inadequate hygiene controls, increases the risk of microbial contamination during water handling, storage, and distribution. *Escherichia coli* is normally an indication of fecal contamination. Even though they might be non-pathogenic, their presence in water is an indication that the water is susceptible to contamination by other bacterial species that are harmful and disease causing (Some *et al*., 2021). The World Health Organization guidelines require that potable water to have less than 20 CFU/mL heterotrophic bacterial counts with no coliform bacteria, fecal coliforms, *E. coli*, *enterococci,* and *Pseudomonas aeruginosa* (WHO, 2019). The presence of coliforms indicate the possibility of contamination by other viable pathogenic species such as *Shigella* spp., *Salmonella* spp., *or Vibrio cholerae* (Curutiu *et al*., 2019).

Resistome refers to the complete collection of antibiotic resistance genes present within microbial communities including genes harbored by both pathogenic and non-pathogenic bacteria that may be transferred through environmental exposure and consumption.Antibiotic resistance is swiftly emerging as a critical global health concern compromising the effectiveness of antibiotics and exacerbating the burden of infectious diseases. The World Health Organization (WHO) has classified AMR as one of the ten foremost global public health threats (WHO, 2020). A primary factor

in the proliferation of resistance is the environmental spread of antimicrobial resistance genes (ARGs), which are collectively referred to as the resistome. Environmental settings such as aquatic systems, wastewater, and food products are increasingly acknowledged as reservoirs and pathways for the transmission of ARGs. A World Health Organization report has shown substantial bacterial resistance leading to severe infections and a rise in resistance to antibiotics for common infections, commonly associated with antibiotic misuse and overuse.However, some forms of resistance are acquired through the environment. It is possible to acquire resistance through horizontal gene transfer by transduction, transformation or conjugation. Some species of bacteria such as *E. coli* and Multi-Drug Resistant *Staphylococcus aureus* have been studied to transfer their resistance using these mechanisms (Habboush & Guzman, 2022). In peri-urban settings, these mechanisms are particularly relevant because *E. coli* is a common indicator of fecal contamination in potable water systems, while multidrug-resistant *Staphylococcus aureus* can be introduced through human handling, poor hygiene, and contaminated water contact during vending and distribution.

The presence of antibiotic resistance genes (ARGs) in potable water and ready-to-eat foods such as ice pops has far-reaching implications for public health. Globally, environmental resistomes act as reservoirs of resistance, facilitating the spread of multidrug-resistant pathogens and compromising the effectiveness of critical antibiotics (Manesh & Vaghese, 2021). In Africa, studies have reported high prevalence of ARGs in water systems, street-vended foods, and other consumer-accessible products, reflecting inadequate water treatment, poor hygiene, and limited regulation (Nnadozie & Odume,2019;Makori *et al*., 2024). Locally, in rapidly urbanizing areas like Juja, peri-urban water vendors and informal ice pop producers create potential hotspots for ARG dissemination due to frequent contamination and suboptimal handling. Such resistomes not only elevate the risk of waterborne and foodborne infections but also facilitate horizontal gene transfer among bacterial populations, potentially introducing multidrug-resistant strains into the community.

Ice pops are inexpensive, water-based frozen products commonly prepared and sold in informal settings, often using untreated water and under minimal hygiene controls,

making them potential vehicles for microbial contamination (Qin *et al*.,2025).Potable water and ready-to-consume frozen treats like ice pops are widely consumed, especially in urban and peri-urban communities of developing countries. However, their microbial safety is often compromised by insufficient treatment, unsatisfactory hygiene practices during preparation, and contamination during distribution (Nnadozie & Odume, 2019). In Kenya, particularly in rapidly urbanizing areas like Juja, access to clean water and safe food remains unreliable, exposing consumers at risk of exposure to waterborne pathogens and potentially antibiotic-resistant bacteria (Makori *et al*., 2024). Waterborne antibiotic resistance is a growing global concern and is emerging as an increasingly important public health issue (Manesh & Varghese, 2021).

Vectors of waterborne antibiotic resistance can be categorized into human sources which include sewage, wastewater, runoff from agricultural activities; and environmental sources which include aquatic plants and animals, sediments, soil and natural habitats. Through their activities near or in water, humans introduce thousands of different antibiotics into the environment, either directly through their discharge of water and sewage, or indirectly, by leaching outside into the water (Habboush & Guzman, 2022). These vectors play a major role in transferring antibiotic resistant genes between various bacteria and within the same species. It is clear that natural antibiotic resistance within bacterial populations has a lower propensity for transferability than the antibiotic resistance that arises through human activities (Patridge *et al*.,2018; Iskandar *et al*.,2022). This can occur through the horizontal transfer of genetic material between bacteria, or through the mobilization of genetic material embedded into the bacterial chromosome (Zhuang *et al*., 2021).

The genetic mode of action for this resistance includes three levels; bacteriostatic, bacteriosuicidal, and target modification (Zhang & Cheng, 2022).Bacteriostatic resistance occurs when bacteria develop resistance to the antibiotic, but the bacteria remain alive and infectious. Bacteriosuicidal resistance is when bacteria develop resistance to antibiotics that paradoxically leads to their own death. (Zhang & Cheng, 2022). Target modifcation occurs when the bacteria completely shut down their ability

to be affected by an antibiotic. This is mediated by the genes encoding for antibiotic-targeting molecules (Urban-chmiel *et al*, 2022).

The Water Act 2016 provides that the Water Service Regulatory Board (WASREB) regulates all water service providers, which are county entities, to ensure consumer protection and commercial viability (Water Act, 2016).The Water Service Regulatory Board is responsible for water safety planning that encompasses all steps in the water supply system from catchment to point of consumption. The approach is gaining increasing acceptance worldwide as an unparalleled practice for provision of safe drinking water (WASREB, 2019). WASREB possesses the power to regulate water vending in Kenya. Lapses in monitoring, poor treatment, and unhygienic storage at peri-urban vending points promote microbial contamination and the spread of antibiotic-resistant bacteria. Weak regulatory oversight thus increases public exposure to both pathogens and AMR strains, highlighting the need for stricter compliance and targeted surveillance

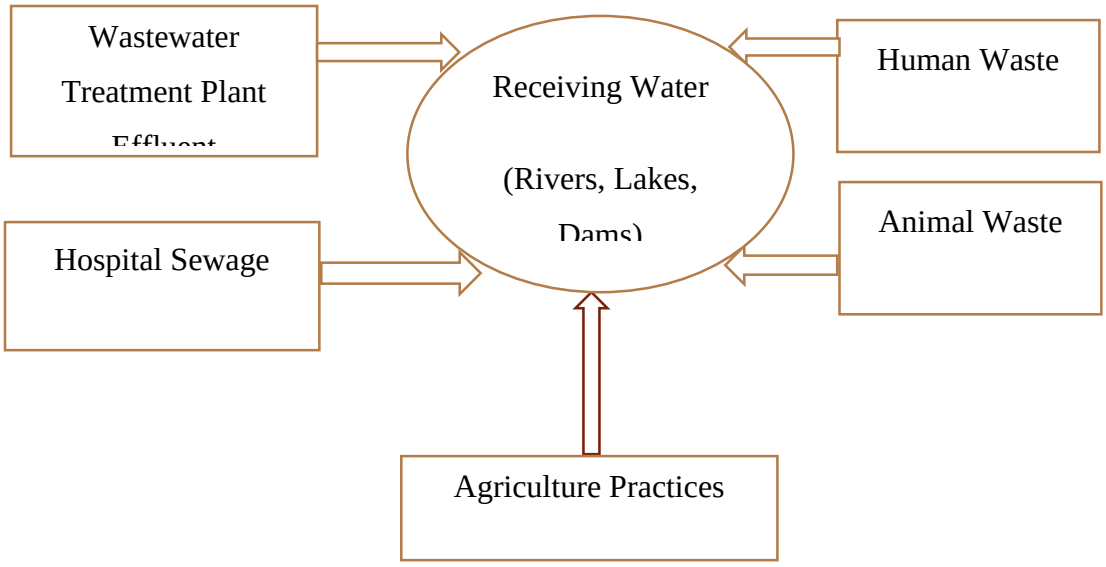

Figure 1.1: Sources of Contamination of Potable Water

Therefore, this study aimed to identify and characterize bacteria and the resistome in potable water and ice pops in Juja, Kenya. Juja is a rapidly growing urban area with high demand for potable water and street foods, making it a critical site for assessing

microbial exposure. Understanding these risks is essential for informing public health interventions, policy development, and AMR mitigation strategies in low- and middle-income countries. The study also aligns with Sustainable Development Goal (SDG) 3 on Good Health and Well-being and SDG 6 on Clean Water and Sanitation, highlighting the link between microbial safety, antibiotic resistance, and national public health capacity.

1.2 Statement of the Problem

Antimicrobial resistance (AMR) has become a critical public health crisis, with an estimated 4.95 million fatalities linked to resistant infections globally in 2019, and approximately 1.27 million deaths directly attributed to AMR (Murray et al., 2022). In sub-Saharan Africa, the burden is particularly severe due to weak surveillance systems, substandard sanitation, and limited access to clean water (WHO, 2020). Environmental reservoirs such as potable water and street-vended foods have increasingly been identified as major contributors to the dissemination of antimicrobial resistance genes (ARGs) within communities (Berendonk et al., 2015).In Kenya, research indicates that numerous sources of drinking water and ready-to-eat foods frequently pose microbiological safety risks. The Kenya Ministry of Health reports that up to 40% of water sources sampled in peri-urban regions such as Juja fail to comply with national microbiological safety standards (Ministry of Health Kenya, 2018).

Ice pops, which are widely consumed by people in the streets and produced in informal settings, often do not adhere to proper hygiene and storage protocols, rendering them potential vectors for pathogenic and antibiotic-resistant bacteria (Guo et al., 2021). Despite ice pops being one of the most consumed products in peri urban areas of Kenya and especially the children, microbial quality of ice pops in Kenya remains underexplored, with research efforts predominantly directed toward ice cream products. There exists a considerable knowledge deficit concerning the presence and variety of ARGs in these commonly consumed items. The majority of surveillance initiatives concentrate on clinical environments, while environmental and food sources of resistance are largely overlooked. Traditional culture-based methodologies are inadequate for identifying most microbial communities and components of the

resistome, as numerous microorganisms cannot be cultured under standard laboratory conditions (Kapinusova et al., 2021). This study sought to fill this gap by characterizing and identifying the resistome in potable water and ice pops in Juja using both culture-dependent and independent methods.

1.3 Justification

Assumptions about the safety of potable water put a lot of unsuspecting individuals at risk of unknowingly introducing harmful microorganisms carrying mutations in the body *(*Carraturo *et al*., 2021). The resistome within a microbiome plays a critical role in the transmission of antimicrobial resistance populations (Partridge et al., 2018). Understanding resistomes in widely consumed products such as drinking water and ice pops is therefore essential for strengthening AMR surveillance, improving knowledge of resistance ecology, and supporting efforts to curb resistance transmission beyond hospital settings.This research addresses existing knowledge deficiencies concerning the presence and spread of ARGs in non-clinical environments within Kenya. Juja is a rapidly urbanizing peri-urban area characterized by high population density, reliance on informal potable water vendors, and widespread consumption of street-vended foods such as ice pops. These conditions create frequent points of human–environment interaction, increasing the potential for microbial contamination and environmental transmission of antibiotic resistance.

The baseline survey of hygiene status of these consumables provides critical baseline data for routine environmental AMR monitoring and support the global effort to limit the spread of resistance from environmental and community-level sources. The findings of baseline survey objective support evidence-based policymaking regarding food safety, street vending practices, and potable water standards. It also strengthens local contributions to Kenya's National Action Plan on AMR, aligned with the Global Action Plan by the World Health Organization (WHO, 2015). The findings from this study can also inform the development of educational programs at the community level aimed at encouraging safe food handling, effective water treatment, and proper hygiene practices, thus minimizing exposure risks. Therefore, the incorporation of culture-independent techniques such as metagenomic sequencing is essential to

achieve a thorough understanding of ARG prevalence and diversity. In the absence of such data, accurately evaluating the risks associated with environmental AMR reservoirs or formulating targeted interventions becomes challenging. Understanding the scope and scale of ARGs in commonly consumed items is essential for informing public health strategies, antimicrobial stewardship, and food safety policies.

1.4 Objectives of the Study

1.4.1 General Objective

To identify and characterize Resistome from Potable Water and Ice pops Juja, Kenya.

1.4.2 Specific Objectives

i. To evaluate water and ice pop vendors compliance to public hygiene policies and the prevalence of contamination in their products
ii. To determine the diversity of pathogenic bacteria from potable water and ice pops in Juja, Kenya using culture dependent technique and metagenomics
iii. To determine the presence of Antibiotic-Resistant Genes in potable water and ice pops sold in Juja, Kenya using culture dependent and independent technique

1.5 Research Questions

i. What is the level of compliance of water and ice pop vendors in Juja with public hygiene policies, and how does this affect contamination in their products?
ii. What is the diversity of pathogenic bacteria present in potable water and ice pops in Juja, Kenya, as revealed by culture-dependent techniques and metagenomics?
iii. What antibiotic-resistant genes (ARGs) are present in potable water and ice pops sold in Juja, as identified through culture-dependent and independent techniques?

1.6 Scope

.This study focused on potable water and ice pops sold in Juja, Kenya, during the study period **(July to September 2023).** Juja was selected as a strategic case study due to its rapid urbanization, growing population density, and heavy reliance on informal potable water vendors and street-vended foods. As a peri-urban area hosting major institutions and commuter populations, Juja experiences high daily consumption of vendor-supplied water and ready-to-eat products, increasing the potential for widespread exposure to microbial and antimicrobial resistance (AMR) risks. The study examined vendor compliance with public hygiene policies, assessed microbial contamination, and identified pathogenic bacteria using both culture-dependent and metagenomic techniques. The study also profiled antibiotic resistance genes to provide baseline data on the resistome in community-consumed water and food products.

1.7 The Limitation of the Study

This study, while illuminating significant trends, is not without limitations. Since the study was limited to Juja, findings may not be generalizable to other regions or seasons. The geographical scope of sampling was confined to Juja, which may not fully capture regional or seasonal variations in microbial contamination and resistome profiles. In addition, although metagenomic analysis provides valuable insights into the diversity of antibiotic resistance genes (ARGs), it does not confirm gene expression or the functional potential for resistance transfer in the absence of transcriptomic or functional assays. The sample size (n = 55) may further constrain the generalizability of the findings, particularly given the heterogeneity of water sources and vending practices in peri-urban settings. Future studies should therefore incorporate larger and more representative sample sizes, longitudinal designs to capture seasonal fluctuations, functional metagenomics or transcriptomics to assess ARG expression, and integrated surveys on antibiotic use, hygiene, and sanitation practices to better elucidate behavioral and environmental drivers of antimicrobial resistance.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

Antibiotic resistance genes confer resistance to specific antibiotics when taken up by a recipient organism. Many times, when antibiotic resistance is mentioned, many people normally conclude this is a result of abuse or misuse of therapeutic antibiotics. However, one of the major ways in which antibiotic-resistance genes are disseminated is through the environment (Barathe *et al.,* 2024). Water treatment aims to remove contaminants such as antibiotic resistant bacteria. However, the presence of these resistant strains in water shows how much public health is being compromised, considering how essential water is for human survival (Bergeron *et al*., 2015;Djordjevic *et al*., 2024). The common use of antibiotics during aquaculture also contributes to the release of antibiotics into the environment (Ahmed *et al.*, 2024).

Drinking water treatment systems represent a critical control point where environmental antibiotic-resistant bacteria and genes are either eliminated or inadvertently allowed to persist. Treatment of drinking water using different techniques has shown irregularities in some instances, with treated water still containing resistance genes. However, evidence suggests that conventional disinfection methods may not be equally effective against antibiotic resistance genes, creating a paradox where microbial cells are inactivated but resistance determinants remain. For instance, according to the study by Bergeron *et al*. (2015), water treated with chlorine tested negative for cultivable bacteria but still tested positive for resistant genes to tetracycline and sulfonamide. This suggests that despite chlorine being an effective disinfectant, it might also be responsible for increasing resistance genes in water environments. The exact mode of action that leads to the release of the genes is not clearly understood, but one possible way may be through chlorine in biofilms. This observation has prompted investigations into the mechanisms by which disinfection processes may facilitate the persistence or release of resistance genes in aquatic environments. Chorine is capable of exerting stress on biofilms, which may result in

biofilm degradation and the subsequent release of the components, which may include resistance genes (Duarte *et al*., 2022).

Beyond drinking water treatment processes, additional environmental pathways further amplify the dissemination of resistance genes. Another avenue through which resistance genes are spread in the environment is through the release of antibiotic wastewater into the environment without proper pretreatment by pharmaceutical companies and healthcare facilities (Duarte *et al*., 2022). Once released into natural water bodies, these resistance genes encounter dense and diverse microbial communities, creating favorable conditions for horizontal gene transfer and the amplification of resistance traits. This has become a huge area of concern, considering the high bacterial density available in the environment and the ability to interact with the antibiotics released (Arnold *et al*., 2024). The genes can freely interact with available bacteria, which develop resistance over time, allowing them to bypass the action of antibiotics. The acquiring and spreading of resistance genes has been observed in gram-positive and gram-negative bacteria (Barathe *et al*., 2024).

2.2 Theoretical Review

This study is guided by the One Health Theory, which recognizes the interconnectedness of human, animal, and environmental health in the spread of antimicrobial resistance. Environmental microbiology theory also supports the idea that water systems act as reservoirs and vectors of pathogens and ARGs. The One Health framework emphasizes that human, animal, and environmental health are intrinsically connected and that the spread of antimicrobial resistance (AMR) cannot be fully understood or mitigated by focusing on one sector alone. One Health recognizes that antibiotic use and resistance selection pressures across human medicine, animal agriculture, aquaculture, and environmental systems interact continuously, creating pathways for resistant microorganisms and resistance genes to circulate among sectors and back into human populations. Such integration underscores that resistant bacteria found in environmental sources like water may originate from clinical, agricultural, veterinary, or community practices, and that these

reservoirs can contribute to human exposure and infection if not properly controlled (Al-Khalaifah *et al*., 2025).

In the context of water vending and ice pop production in peri-urban settings, the One Health approach is particularly relevant. Informal water vendors and ice pop producers operate at the intersection of multiple risk factors: water sources contaminated by human and animal waste, inadequate hygiene practices during preparation and storage, and environmental conditions that support microbial persistence. These practices not only reflect human behavioural determinants of AMR spread (e.g., hygiene compliance among vendors), but also link to environmental reservoirs where resistant bacteria and antimicrobial resistance genes (ARGs) circulate and are amplified by contamination from untreated sewage, livestock and aquaculture runoff, and urban effluents (ILRI., 2024). For instance, antibiotic residues and resistant bacteria released through aquaculture operations can enter surface and groundwater, contributing to the environmental pool of ARGs that may contaminate water used for drinking or ice pop preparation, thereby creating a pathway for resistance to move from animal production systems into human communities.

Environmental microbiology theory further supports the role of water systems as reservoirs and vectors of pathogens and ARGs. Aquatic environments including rivers, wells, and distribution systems host complex microbial communities where horizontal gene transfer can facilitate ARG dissemination across diverse bacteria under selective pressures from antibiotic residues and other co-selective agents. The persistence of ARGs in water, even following conventional treatment processes, reinforces the concept that water bodies are dynamic ecological matrices that both reflect and influence resistance patterns across ecosystems (Sassi *et al*., 2025). Within peri-urban settlements, this suggests that contamination of vending water and ice pops is not merely a matter of hygiene at the point of sale but is tied to broader environmental and anthropogenic drivers of AMR. Integrating One Health and environmental microbiological perspectives, therefore, allows for a more holistic understanding of how human behaviours, animal antibiotic use and environmental contamination

collectively shape the distribution and potential human exposure to antibiotic-resistant microbes in water and street-vended products.

2.3 Water Vending Policy and Regulations

The 2016 WASREB Impact Report suggests that approximately 45% of Kenya's population does not have convenient access to piped water. Given that piped water supply varies from 40% to 80% in most utility areas, the significance of vended water in addressing this demand shortfall should not be underestimated. Formal vendors include water utilities, registered associations, and small-scale informal suppliers (WASREB, 2016). They supply treated water in tankers and water kiosks and may be easier to regulate. Informal vendors tend to supply both treated and untreated water usually in small quantities using containers carried on hand/donkey drawn carts. These may be more challenging to regulate. The inefficiency and general inability to supply the present demand by utilities due to increased population translating to an increased demand with a constant water supply has led to a growing number of un-served consumers (WASREB, 2016).

2.3.1 The Water Act 2016

The Water Act 2016 is key in determining the public health policy and regulation requirement for drinking. The 2016 Water Act provides strict public health policy regulations that must be followed by all drinking water vendors operating in Kenya. This goes a long way to ensure that all Kenyans have access to safe and fit drinking water. According to the 2016 Kenya Water Act, drinking water vendors are subject to rigorous public health policy and regulation requirements. Section 35 of the Act highlights the public health policy guidelines that must be followed by all water vendors in the country (Water Act, 2016).

The relevant local authority must authorize a vendor if they are to engage in the business of providing drinking water. The Authority must assess the vendor and their premises to ensure that they meet the set standards of providing safe and fit water for consumption. The vendor must be provided with the relevant licensing information

and memoranda of understanding that spell out the requirements for the sale and provision of the water. It is important to note that the water vendor must label all containers of drinking water clearly as 'potable drinking water' along with information about its quality. The vendor must keep a record of all water sales, and this should include the volume, quality, and collection points of the water (Water Act, 2016).Additionally, any water that does not meet the required safety standards must be discarded.

The law also requires periodic water tests to ensure the water being sold complies with the standards set out in the Act. Additionally, the vendor must share this information with the relevant local authority. Any water vendor found to be in breach of the public health policy and regulations may be subject to penalties, as outlined in the Act. The law also requires that drinking water vendors regularly provide customers with health and safety information about the dangers of poor quality water and its potential impacts on health (Water Act, 2016).This information must include clear warnings about the dangers posed to vulnerable populations, signifying the importance of compliance with the Water Act.

The Water Act 2016 in Kenya mandates that water vendors must ensure the safe storage and transportation of drinking water to protect consumers from contaminants. This includes maintaining clean storage containers and avoiding any potential contamination during transportation. Vendors are also responsible for preventing adulteration and loss of water quality. The Water Services Regulatory Board (WASREB) regulates the water services sector, ensures fair competition, sets performance standards, determines rates and tariffs, and protects consumers. WASREB also resolves water service complaints and educates consumers on their rights and responsibilities (Water Act, 2016). According to WASREB (2016), despite existing regulatory frameworks for water vending in Kenya, enforcement remains inconsistent, especially among informal vendors. Limited monitoring capacity and weak compliance allow unhygienic handling, use of untreated water, and unsafe storage practices to persist, increasing microbial contamination and creating

conditions that promote the spread of antibiotic-resistant bacteria and resistance genes through vended water.

2.4 Microbial Contamination of Water

Microbial water contamination refers to the existence of harmful microorganisms like bacteria, viruses, and parasites in water sources. This contamination poses substantial health hazards to people and animals that encounter or ingest the contaminated water. It is a prevalent issue, especially in developing nations like Kenya lacking access to clean water and proper sanitation systems (Wen *et al*., 2020). In Kenya, some of the causes of microbial water contamination occur due to factors like aging infrastructure, natural disasters, or inadequate water treatment processes.

One of the main sources of microbial water contamination is faecal matter. Human and animal waste can contain pathogenic microorganisms that can easily enter water sources through inadequate sanitation practices or improper sewage disposal. Contaminated water can then spread diseases such as cholera, typhoid, dysentery, and hepatitis, leading to severe illnesses and even death, especially among vulnerable populations like children and the elderly (Some *et al*., 2021).Poor water treatment practices or malfunctioning treatment plants can fail to effectively remove or kill harmful microorganisms. Agricultural runoff, industrial waste, and improper disposal of chemicals and pharmaceuticals can also introduce contaminants into water bodies, further compromising water quality.

To address microbial water contamination, various preventive measures are essential. Kenya needs to prioritize improving access to clean water and sanitation facilities, implementing proper sewage treatment systems, and promoting hygiene practices such as hand washing with clean water and soap, community based monitoring. Water treatment technologies like chlorination, filtration, and ultraviolet disinfection can effectively remove or inactivate microorganisms in water. Proper water infrastructure maintenance and regular water quality testing are vital to ensure the safety of drinking water supplies. Public education and awareness campaigns about the risks of microbial

water contamination and the importance of clean water practices can also contribute to mitigating this issue (Obaideen *et al*.,2022).

2.5 Water-Borne Pathogenic Bacteria

The common culturable water-borne pathogenic bacteria include *Salmonella enteritidis, Vibrio cholerae, Mycobacterium avium, Escherichia coli, Shigella spp., Yersinia pestis, Legionella pneumophila, Pseudomonas aeruginosa, Streptococcus pneumoniae, Staphylococcus aureus, Helicobacter Pylori, Acinetobacter spp., Citrobacter spp. and C. violaceum* (Ramirez-Castillo *et al*., 2015; Adam Mohamed *et al*., 2020)

Waterborne diseases are responsible for millions of deaths each year globally, primarily affecting children. This includes numerous gastrointestinal and systemic illnesses. Moreover, these illnesses result in an increased cost of treatment. These infections are typically caused by a variety of agents, such as bacteria, viruses, parasitic protozoa, or helminths, which can be transmitted through contaminated water via ingestion or contact (Shayo *et al*., 2023).

Waterborne pathogens that are resistant to antimicrobial agents are a global health concern. Inappropriate use of these agents leads to the selection of resistant microorganisms. Even a small portion of these microorganisms, less than one in a billion, are unaffected by the antimicrobial agent and retain mutations that hinder the drug's effectiveness. This subgroup of resistant microorganisms, known as the mutant selection window, can thrive and multiply during treatment (DeNegre *et al*., 2019; Leon-Buitimea *et al*.,2020). Chlorine, commonly used for disinfection in water treatment, has contributed to antibiotic resistance in some studies. There is a concern that chlorination may enhance the presence of antibiotic-resistance genes and contribute to the spread of antimicrobial resistance in waterborne pathogens. However, the exact reasons for this phenomenon and the factors that trigger antimicrobial resistance during chlorination are not well understood (Jin *et al*., 2020).Therefore, the overreliance on chlorine as a common water treatment technique in Kenya also makes it a challenge. Studies in critical urban settings have indicated persistence of ARGs

despite disinfection. This raises concerns about its effectiveness in fully mitigating antimicrobial resistance risks in treated water.

Table 2.1: Bacterial Pathogens in Drinking Water Systems and their Related Diseases

Pathogen	Associated Disease	Health Significance	Persistence in Drinking water supplies
Campylobacter spp., *C. jejuni*	Diarrhea, gastroenteritis	High	Moderate
Yersinia enterocolitica	Diarrhea, reactive arthritis	High	Long
Enterohemorrhagic *E. coli* (EHEC), enteropathogenic (EPEC), enterotoxigenic (ETEC), and enteroinvasive (EIEC)	diarrhea and gastroenteritis	High	Moderate
Burkholderia pseudomallei	Meliodosis	High	May multiply
Legionella pneumophila and related bacteria	Acute respiratory illness, pneumonia (legionellosis)	High	May multiply
Non-tuberculous mycobacteria	Pulmonary disease, skin infection	Low	May multiply
Pseudomonas aeruginosa	Infections on the lungs, urinary tract, and kidney Can cause inflammation and sepsis	Moderate	May multiply
Salmonella enterica serotype Typhi	Typhoid fever, paratyphoid fever, and other serious salmonellosis	High	Moderate
Other salmonellae	Gastroenteritis, reactive arthritis	High	May multiply
Shigella spp.	Bacillary dysentery or shigellosis	High	Short
Vibrio cholera	Gastroenteritis, cholera	High	Short to long
Helicobacter pylori	Chronic gastritis, ulcer disease, and gastric cancer	Low	Moderate

Source: World Health Organization (WHO); Geneva, Switzerland: 2008

2.6 New Emerging and Re-emerging Bacterial and Parasitic Pathogens Spread by Water

Emerging pathogens are new or previously known diseases that either appear for the first time in a human population or are increasing in their occurrence and spreading to new geographic areas. This phenomenon has been observed over the past 20 years.

The new emerging and reemerging bacterial and parasitical pathogens spread by water are *Cholera, Legionnaires' Disease, Typhoid Fever, Giardiasis, Cryptosporidiosis, Salmonellosis, Shigellosis, Leptospirosis, Vibrio parahaemolyticus, Campylobacter, E. coli O157:H7, Leishmaniasis, Helicobacter pylori, Giardia lamblia, Cyclospora cayetanensis, Toxoplasma gondii, and Entamoeba histolytica* (Ademulugun *et al.*, 2024).

In Kenya, informal food and water vending is a documented risk factor for enteric and waterborne infections, with studies showing that poor sanitation and food-handling practices among street food vendors are significantly associated with contaminated foods that can transmit enteric pathogens like *Escherichia coli*, *Salmonella* spp. and other fecal indicators, and that access to running water, hand-washing before food preparation, and basic hygiene practices reduce contamination risk. These findings link vendor practices such as limited access to clean water, poor hand hygiene, and inadequate sanitation directly to the risk of food contamination and consequent disease transmission in urban and peri-urban areas (Kariuki *et al.*, 2017; Ogalo et al.,2025). For protozoan pathogens such as *Entamoeba histolytica*, *Giardia lamblia*, and *Cryptosporidium* spp., national surveillance and epidemiological reviews in Kenya identify these organisms as prevalent causes of diarrhoeal disease, driven by poor water, sanitation, and hygiene (WASH) conditions that are also common in environments where informal food and water vending occurs (Ogalo *et al.*, 2025).

The emergence and spread of drug-resistant parasites and insecticide-resistant vectors have played a significant role in the rise of emerging pathogens. Changes in the environment, like increased development of water resources, urbanization, and shifts in population demographics, have created circumstances conducive to the transmission of vector-borne diseases (Samsing & Barnes, 2024).

2.7 Circulation of Pathogens in the Environmental Reservoirs such as Water

Environmental reservoirs, like water sources, are crucial as they can harbor different pathogen types and serve as potential transmission origins. Grasping the dynamics of pathogen circulation in the environment is a vital measure for prevention and public

health control. Pathogens commonly circulate in water include fungi, viruses, parasites, and bacteria. Each pathogen type has a different transmission mode and can have different effects on human health. For example, infectious bacteria can spread through contaminated water sources, leading to gastrointestinal issues, while viral pathogens can be spread through person-to-person contact or aerosols (Samsing & Barnes, 2024).

Environmental water reservoirs play a critical role not only in the circulation of pathogens but also in the persistence and dissemination of antimicrobial resistance (AMR) and antibiotic-resistance genes (ARGs). Aquatic environments receive ARGs through sewage effluent, hospital and industrial wastewater, agricultural runoff, and poorly treated municipal discharges, creating reservoirs where resistance genes can accumulate and spread. Within these systems, biofilms formed on pipes, sediments, and surfaces provide ideal conditions for horizontal gene transfer, allowing ARGs to move between environmental bacteria and potential human pathogens. Inadequate sanitation, ineffective wastewater treatment, and insufficient water purification enhance the survival of resistant bacteria and free ARGs, facilitating their circulation through drinking water, food production, and informal vending systems. Understanding ARG dynamics in water reservoirs is therefore essential for controlling the environmental spread of AMR and mitigating downstream public health risks (Al-Khalaifah et al., 2025).

2.8 Detection of Water-Borne Bacterial Pathogens by Modern Techniques

The water-borne pathogen detection and identification process has dramatically changed in recent years. With several modern methods available, such as Molecular tools and Biochemical analysis, detecting and identifying water-borne pathogens has become easier and more efficient Molecular tools such as Polymerase Chain Reaction (PCR) and 16S ribosomal RNA sequencing technology provide quick and efficient results. PCR is used to amplify DNA or RNA segments to allow for the detection and identification of pathogens. 16S ribosomal RNA sequencing technology involves using primers to collect unique sequences from the genomic region that is most commonly found in prokaryotes to identify and compare the related species (Church

et al., 2020). PCR is a rapid and reliable technique for detecting even low concentrations of pathogen DNA or RNA in water (Church et al., 2020). It is particularly sensitive compared to other techniques, such as culturing or microscopy. PCR allows for the detection of specific pathogens in a sample using multiple probes with distinct sequences. These probes amplify a small DNA or RNA portion in the sample, enabling the identification of a particular organism (Chen *et al*., 2021).

Metagenomics has emerged as a powerful tool for detecting and identifying waterborne pathogens. It involves direct genetic analysis of environmental samples containing diverse microbial communities (i.e. the "metagenome") without needing to culture individual species. This culture-independent approach provides significant advantages over traditional techniques for monitoring water quality. Metagenomic analysis of water samples typically begins with filtration to capture microbes, followed by DNA extraction and shotgun sequencing. Computational analysis can then identify species present by matching to reference genomes. This enables comprehensive profiling of all bacteria, viruses, protozoa and other microorganisms in a sample, not just those easily grown in culture (Ji *et al*., 2023). Metagenomics can also assess antimicrobial resistance gene prevalence and perform strain typing to support epidemiological investigations. RNA and DNA sequencing form part of the Next Generation Sequencing (NGS) technique that has proved useful in microbial identification in water systems including pathogen detection and monitoring (Garner *et al*., 2021). This technology has the ability to pick several pathogens at once and gives information regarding the makeup of the pathogen and possible virulence factors. As for the drinking water analysis, several studies have shown that NGS has increased sensitivity compared with the methods of culturing the microorganisms and can identify both culturable and nonculturable microorganisms (Hanif *et al*., 2023).The method has been especially useful in the discovery of new pathogens and in gaining insights into microbial consortia in drinking water networks. The application of NGS in water quality surveillance goes beyond focusing on pathogen identification to the analysis of AMR genes and microbial dynamics. The advantages of NGS technology for water quality surveillance are the capacity for sample analysis simultaneously and obtaining high-resolution data (Li *et al.*,2021).

Whole-genome sequencing is another powerful method for detecting and identifying water-borne pathogens. This technique allows researchers to sequence entire genomes of water-borne pathogens and compare them to reference genomes (Prajapati *et al*.,2022).With whole-genome sequencing, researchers can identify genetic markers associated with a particular organism and distinguish between closely related bacteria or viruses. This technique is particularly useful for identifying recently emerging or evolving organisms in water sources (Prajapati *et al*.,2022; Uelze *et al*., 2020). Biochemical analysis provides a wide range of methods for identifying and detecting water-borne pathogens. This method includes tests such as serology and metabolic testing, which identify the presence or absence of certain enzymes or antigens of the target organism. Biochemical analysis methods are beneficial because they help differentiate between different species of similar bacterial organisms. Immunoassays such as enzyme-linked immunosorbent assay (ELISA) are used in detecting and identifying water-borne pathogens. ELISA is used to detect the presence of an antigen-antibody interaction, which can provide rapid results, making it more efficient. Rapid test kits are also useful for detecting and identifying water-borne pathogens (Saravanan *et al*.,2021; Kim & Kim, 2021).Rapid test kits provide results in less than 24 hours, which may be beneficial in environments that require urgent results. Furthermore, using these kits is less labor intensive as the quality control and technical analysis can be automated.

Culture methods are also used for determining waterborne pathogens. These techniques entail examining water samples for particular microorganisms by providing them with a medium conducive to growth. This method is valuable for pinpointing specific waterborne pathogens in a particular setting and assessing their contamination levels. Furthermore, it can be employed to investigate the mechanisms governing the dissemination and development of waterborne pathogens in aquatic settings**.** Culture methods also allow researchers to quickly determine the type of waterborne pathogen present and measure how much of it is present in the environment (Saravanan *et al*.,2021).

The advanced molecular approaches offer high sensitivity, rapid detection, and detailed genetic information, their application in low-resource settings remains constrained by high costs, need for specialized infrastructure, skilled personnel, and consistent reagent supply. In the Kenyan context particularly for ice pop production and informal water vending, where monitoring is often conducted by small laboratories or public health units routine use of NGS-based techniques is limited, making culture-based and basic biochemical methods more feasible and accessible for day-to-day surveillance (Paruch, 2022).However, reliance on culture alone may underestimate contamination risks, as many waterborne pathogens and antimicrobial resistance genes are viable but non-culturable (VBNC). Therefore, integrating culture-dependent methods (to confirm viable pathogens and quantify contamination) with culture-independent molecular techniques (to detect non-culturable organisms and ARGs) provides a balanced, context-appropriate approach for assessing microbiological safety of water and ice pops in informal vending systems, where public health risks are high but resources are limited (Moirongo *et al.*, 2022).

2.9 Treatment and Cleaning of Potable Water

Several studies have shown that potable water treatment does not result in the total elimination of pathogenic bacteria, resistance genes, and other water wastes. Source water containing antibiotic residues and active bacteria is cleaned in several ways. Chlorination, use of activated carbon, biodegradation, membrane separation, and reverse osmosis are some of the most used techniques when treating water. However, the techniques do not give reliable results at times (Serwecińska, 2020). While these treatments can eliminate culturable bacteria, treated water may still have 16S rRNA, which suggests that some common water cleaning techniques, such as filtration, do not entirely remove bacterial genes in water (Bergeron *et al*., 2015). The suggestions that some treatment techniques are compromised have also been supported by studies that show tap water may contain genes that code for resistance (Yu *et al*.,2022). However, apart from the treatment plans, antibiotic resistance genes in tap water could be attributed to the formation and persistence of biofilms in water during the distribution. This has been proven by studies that indicated that in some cases, bacterial genes

increase in drinking water due to water treatment or reemergence during the distribution process. Beta-lactamase genes were found in tap water during the study to suggest water distribution systems may also be responsible for bacterial genes in tap water (Grenni.,2022).

At the community and informal vendor level in Kenya, advanced water treatment technologies such as reverse osmosis, membrane filtration, and activated carbon are largely inaccessible due to high installation, maintenance, and operational costs, as well as the need for stable electricity and technical expertise. Consequently, low-cost methods like chlorination, boiling, simple filtration, and point-of-use treatment are more commonly relied upon by households and water vendors supplying ice pops and drinking water. While these methods are effective at reducing viable pathogens, they are less effective at removing antibiotic residues, ARGs, and DNA fragments, especially where treatment is inconsistently applied or recontamination occurs during storage and distribution. Poorly maintained storage containers, reuse of untreated water, and biofilm formation in vendor containers, pipes, and taps further enable the persistence and possible enrichment of resistance genes even after treatment (Duarte et al.,2022). This contrast highlights that, although advanced technologies offer superior removal efficiency, practical AMR risk reduction in Kenyan vending systems depends on improving basic treatment consistency, hygiene practices, and distribution infrastructure, alongside targeted surveillance using affordable culture-based and selected molecular tools.

2.10 Ice Pop Production

The key components of ice pop production include water, sweeteners, flavoring, and food coloring agents and additives. Water is one of the most essential ingredients in ice pop production as it forms the basis of the texture and taste. Fruit juice is often used as it can add natural sweetness, flavor, and nutritional value. Sweeteners such as sugar, honey, and agave syrup may be added to ice pops to make them even more enjoyable (Contreras *et al*., 2012). Flavoring and food coloring are also incorporated in ice pops to enhance the flavor or make them more visually appealing. Additives like

stabilizers and emulsifiers may be added to give ice pops a better texture and prolong their shelf life**.**

Ice pops are water-based products and are often prepared, handled, and packaged manually, their composition and production process make them highly susceptible to microbial contamination, particularly when contaminated water, poor hygiene, or inadequate freezing and storage conditions are involved. Ice pops can transmit pathogens through contaminated water, inadequate freezing, and poor vendor handling. Untreated water introduces bacteria, viruses, and protozoa, while insufficient freezing preserves them. Frequent handling, unhygienic surfaces, and temperature fluctuations during storage or vending further increase contamination risks, making ice pops a potential source of waterborne pathogens with antibiotic resistant traits. The presence of these pathogens in ice pops may lead to illnesses, some of which may even be life-threatening (Valentino *et al*., 2023). These pathogens are capable of causing a variety of illnesses, including E. coli, salmonella, hepatitis A, and typhoid fever. If ice pops are stored at inappropriate temperatures, bacteria can multiply quickly, further increasing the risk of contamination (Valentino *et al*., 2023).To prevent contamination, manufacturers should ensure that their production facilities are clean and that only food-grade and clean water is used for producing ice pops.

Figure 2.1: Home-made and Commercial Ice Pops

2.11 Control Strategies of Water-Borne Pathogens in Ready-to-Eat Food

Ready-to-eat foods (RTE) pose unique challenges for controlling water-borne pathogens as they may not require further processing before consumption. Good hygiene is essential to controlling water-borne pathogens in RTE foods (Bintsis, 2017).The key control strategy for waterborne pathogens is ensuring adequate treatment of water used in the production process. Water used for primary production, cleaning, and ingredient addition should be treated to ensure it is safe and contamination-free. Treatments include chlorination, filtration, and ozonation. Chemical controls can also ensure the water is free from pathogens Close monitoring of environmental conditions in the food manufacturing facility is also important to prevent contamination from water-borne pathogens (Bintsis, 2017).

Ready-to-eat ice pops pose particular challenges for controlling waterborne pathogens, as they are consumed directly without further processing. Ensuring the safety of water used for making ice pops for mixing, flavoring, or cleaning equipment is critical, with treatments such as chlorination, filtration, or ozonation recommended. Strict hygiene and sanitation protocols are essential: production surfaces, utensils, and molds must be regularly cleaned, and vendors should practice proper hand washing and avoid cross-contamination (Todd, 2020). Ice pops should be stored and frozen at appropriate temperatures to limit microbial growth, and finished products should undergo periodic laboratory testing to detect contamination. Maintaining these controls, along with accurate ingredient tracing and adherence to regulatory guidelines, helps minimize the risk of waterborne illnesses associated with ice pop consumption (Paudyal & Karatzas, 2016).

2.12 Antimicrobial Resistance

Antimicrobial resistance occurs when microorganisms can withstand an antibiotic, making it ineffective in treating an infection. This is becoming a major health concern as antibiotics are less effective in treating bacterial infections (Manesh & Varghese, 2021). Bacteria can develop resistance to antibiotics through natural evolution and by acquiring a gene from another bacterium. Antibiotic resistance develops when bacteria

reproduce, have mutations, and acquire new genetic information. The resistance can then be passed on to future generations, increasing the number of resistant bacteria. Microbes can develop resistance through genetic mutation, natural selection, gene transfer, and drug efflux (Hwang & Yoon, 2019).Mutation is the most basic process of microbial resistance. It involves a change in the DNA sequence, which can cause a gene to produce a different protein. This new protein can be used to resist an antimicrobial agent. Natural selection is a process in which species with advantageous traits are more likely to survive and reproduce than those without advantageous traits. Unexpectedly, natural selection can also be a significant factor in antimicrobial resistance. For example, bacteria may develop mutations that confer resistance to certain antibiotics, allowing them to survive and spread in the presence of the antibiotic (Zhang & Cheng, 2022).

An example of bacteriostatic mechanism of antibiotic resistance is the Beta-lactamase enzyme, produced by certain bacteria, interferes with the activity of some antibiotics such as penicillin, and helps the bacteria survive the antibiotic. Bacteria also have adopted the target modification mechanism to resist antibiotics. This resistance level is mediated primarily by the genes encoding for antibiotic-modifying enzymes and drug transporters. The set of genes that aid in all three levels of antibiotic resistance include the genes coding for antibiotic-modifying enzymes, drug transporters, beta-lactamases, ESBLs, MBLs, and antibiotic-targeting molecules. Antibiotic-modifying enzymes intercept the antibiotic before it can reach its target within the bacteria, which helps to prevent the bacteria from being killed (Zhang & Cheng, 2022). Drug transporters efflux the antibiotic, pushing it out of the cell and preventing it from reaching its target. Beta-lactamases, Extended-spectrum Beta-lactamases, and Metallo-beta-lactamases break down the antibiotic, slowing its activity and allowing the bacteria to survive (Urban-chmiel *et al*, 2022).Antibiotic-targeting molecules prevent the antibiotic from binding to its target site, thus rendering the antibiotic futile and allowing the bacteria to remain alive. These antibiotic-resistance genes (ARGs) and resistant bacteria are not limited to clinical settings they can also persist in environmental water sources and water-based foods such as ice pops. Contaminated

water used in ice pop production or vendor handling practices can introduce resistant bacteria, allowing ARGs to circulate and potentially reach consumers

Antibiotic overuse is one of the main causes of antibiotic resistance observed in human and animal populations. The misuse of antibiotics in treating infections results in viruses and bacteria becoming resistant to these drugs. Other causes for microbial resistance include inadequate dosing, inadequate duration of treatment, improper disposal of antibiotics, and antibiotic use in livestock (Urban-Chmiel *et al*., 2022). The release of antibiotics into the environment through improper disposal or agricultural runoff can contaminate water sources used in ice pop production. This environmental exposure facilitates the emergence and persistence of resistant bacteria in water, which can then be transferred to ready-to-eat products handled by vendors.

2.12.1 Intrinsic Resistance

This is a form of natural resistance that occurs in certain species of bacteria and results in bacteria survival when exposed to certain antimicrobial agents such as antibiotics. This may be due to mutations that result in an effect towards nullifying the desired effects of the antibiotic. Intrinsic resistance is characterized by the expression of a trait/structure or property common within a specific species or group of bacteria and occurs even without prior exposure to an antibiotic (Impey *et al*.,2020). Intrinsic resistance normally encompasses mechanisms that target a pathogen's physiological, structural, or biochemical features; generally, this form of resistance is not easily transferred horizontally (Impey *et al*., 2020). Intrinsic resistance normally is carried out in the following ways: modifications to a drug target, which results in the decreased uptake of the drug, activation of efflux pumps, which eliminate antibiotic molecules from bacterial cells, as well as alterations in the metabolic pathways ((Duarte *et al*., 2022). A good example of intrinsic resistance is shown in the bacteria *Pseudomonas aeruginosa*, which can resist several antibiotics because of the different genes capable of working against antibiotics. Apart from the genes, *Pseudomonas aeruginosa* has low permeability on its external membrane. It also contains some proteins responsible for alteration in cell metabolism, which directly affects cell growth rates. Another good example of intrinsic resistance is gram-negative bacteria, which show reduced

permeability to antibiotics due to their outer layer membrane compared to gram-positive bacteria (Duarte *et al.*, 2022).

2.12.2 Acquired Antibiotic Resistance

It is the most common way of acquiring resistance among bacteria species in water environments. Acquired resistance to antibiotics and other microbial agents by bacteria is also known as horizontal gene transfer. This mainly involves the direct transmission of genetic material responsible for a certain trait from one bacteria to another. This may occur in the same species of bacteria or different bacteria. Bacteria species with acquired genes show more competence and adaptability compared to their wild type counterparts (Girlich *et al*., 2020). Even before the discovery of antibiotics, it is interesting to note that antibiotic-resistance genes were present in the natural environment, indicating that bacteria resistance is an old-age phenomenon (Serwecińska, 2020). This may mainly be because many antibiotics are derivatives of plants, fungi, and other naturally occurring compounds. The interaction between these compounds and bacteria pre-dates the modern era of synthetic antibiotics. However, the discovery of new resistance genes in some bacteria, e.g., Pseudomonas and Clostridium, which are absent from the same species from the older era, suggests that resistance genes exponentially increase with the increase in current antibiotic use (Serwecińska, 2020).

2.12.3 Types of Horizontal Gene Transfer

There are three major ways in which resistance genes and other genetic material are transferred from one bacteria to another: conjugation, transduction, and transformation (Arnold *et al*.,2022). This transfer form is mediated by the presence of genetic mobilomes capable of carrying genetic material from one bacteria to another. Aquatic environments are highly rich in microbiome populations; hence, the reason for these mobile elements' availability is high in water (Serwecińska, 2020). The most common genetic carriers involved in horizontal gene transfer in aquatic environments include prophages, integrons, transposons, and, most importantly, plasmids.

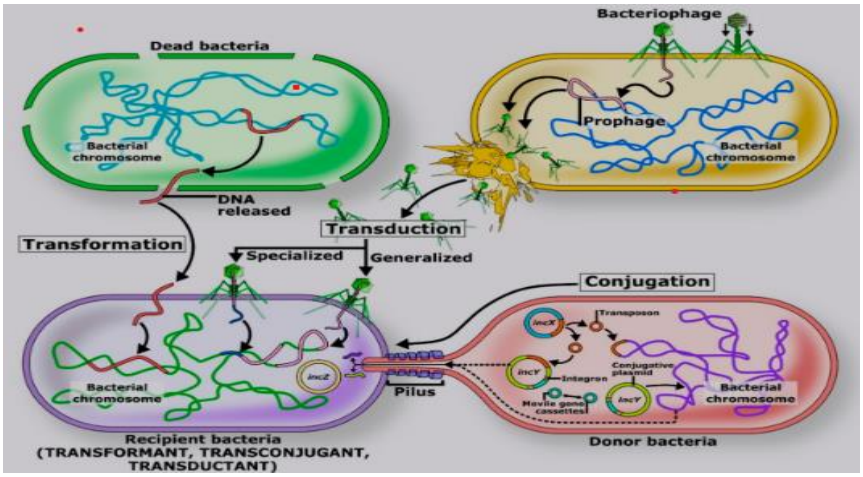

Figure 2.2: Types of Horizontal Gene Transfer

2.12.3.1 Conjugation

This is a type of horizontal gene transfer where genes are carried from a donor bacterium to a recipient bacterium with the aid of plasmids. For conjugation to occur, the two bacteria must be in contact, normally cell to cell (Duarte *et al*., 2022). Gram-Positive bacteria use adhesins for cell-to-cell contact initiation, whereas Gram-Negative bacteria employ conjugative pili for this purpose (Michaelis & Grohmann, 2023). The transfer of genes is normally unidirectional. The bacterial DNA replicates independently from the chromosomal DNA, transferring resistance genes and other genetic material (Duarte et al., 2022). Plasmids have mediated conjugation for a long time, however, in recent times, it has been revealed that other mobilomes, such as Integrative and Conjugative elements (ICEs), are also capable of mediating conjugation (Michaelis & Grohmann, 2023). ICEs can encode genes for their excision, conjugation, and integration. Due to their ability to hide within bacterial chromosomes, ICEs are transferred vertically but also move horizontally when cell-to-cell contact is initiated (Michaelis & Grohmann, 2023). They can only replicate when integrated into host bacteria chromosomes using site-specific recombination, allowing for the transfer of resistance genes.

2.12.3.2 Transduction

Bacteriophages facilitate this form of resistance gene transfer. The host and recipient bacteria normally have phage receptor sites for bacteriophages; hence, transduction occurs between closely related bacteria. The phages hijack the replication machinery of the host bacterium and integrate their DNA into that of the host, which replicates the phage DNA as its own (Michaelis & Grohmann, 2023). The host bacterium ends up gaining antibiotic-resistant genes present during this process.

2.12.3.3 Transformation

This involves the uptake and incorporation of naked DNA by a bacterium from its environment. The DNA is integrated permanently into the competent bacterium though homologous recombination (Michaelis & Grohmann, 2023).

2.12.4 Mechanisms of Acquiring Antibiotic Resistance in Aquatic Environment

When antibiotic and antibiotic-resistance genes are released in water environments, they come in contact with bacteria. The presence of high bacteria communities increases the chances of resistance genes uptake present in a specific ecosystem, for instance, a water ecosystem. Antibiotic-resistant bacteria in water either show intrinsic or acquired resistance/horizontal resistance (Reygaert, 2018). Another form of resistance, however, has been shown through biofilm formation in water distribution systems. This is promoted by the availability of large numbers of bacterial communities and inefficient water-cleaning strategies (Alawi *et al*., 2022).

2.12.5 Tackling Antimicrobial Resistance

As stated by the World Health Organization (2020), addressing antibiotic resistance involves adopting various approaches. One crucial method is boosting the creation of new antibiotics, as combating drug-resistant infections demands fresh drugs and strategies. Several initiatives exist to promote the development of these new antibiotics and treatments including financial incentives, accelerated regulatory processes, and intellectual property incentives. Additionally, using combinations of antibiotic drugs

and reducing the amount of antibiotics used in livestock are also key in managing microbial resistance (WHO, 2020). Public education and awareness are essential to reduce antibiotic misuse and limit the development of resistant bacteria.

Antibiotic stewardship is a collaboration between health professionals, patients, and lab workers to ensure the correct, judicious, and appropriate use of antibiotics (Chinemerem Nwobodo, 2020). Vaccination stands as a top strategy to control the spread of resistant bacteria by lessening the demand for antibiotics since vaccines contain killed or weakened microorganisms that create antibodies in the body, which then fight off the pathogens that cause disease. Infection prevention and control measures, including the sterilization of medical equipment, are effective in reducing disease burden and containing the spread of antibiotic-resistant bacterial strains. (Chinemerem Nwobodo, 2022). Surveillance systems can monitor antibiotic resistance levels and inform public health action, while improved diagnosis can help diagnose infections quickly and reduce the use of antibiotics. Improved diagnostic techniques, such as rapid diagnostics, can help clinicians identify the cause of an infection, diagnose it quickly, and decide on effective antibiotic therapy. This reduces the need for antibiotics and helps reduce the spread of drug-resistant bacteria. Surveillance and stewardship in water and food chains are critical to controlling the spread of antimicrobial resistance. Routine monitoring of water sources, ice pop production water, and vendor-handled foods for resistant bacteria and ARGs can detect contamination early and prevent outbreaks. Coupled with antibiotic stewardship, such as reducing unnecessary antibiotic use and promoting proper disposal, these strategies limit environmental reservoirs of resistance and reduce the risk of ARG transmission to consumers through water-based foods. In the context of this study, antimicrobial stewardship extends beyond clinical settings to include environmental and community-level interventions aimed at limiting the spread of antibiotic-resistant bacteria. By identifying antibiotic resistant bacteria and antibiotic resistance genes presents in potable water and ice pops, this research provides evidence to inform surveillance, guide risk assessment, and support stewardship efforts focused on reducing unnecessary antibiotic exposure resulting from preventable waterborne infections. Improving water quality, enforcing hygiene standards among vendors, and

strengthening routine monitoring can reduce infection rates and, consequently, the demand for antibiotics, thereby contributing to the broader goals of antimicrobial stewardship.

2.13 Detection of Antibiotic Resistant Genes using the Chan Zuckerberg Pipeline

CZ ID pipeline is the latest achievement of the Chan Zuckerberg Initiative in metagenomic pathogen identification and global overview of AMR. As described by Lu et al., (2024) this open source, cloud based system links pathogen identification to the detection of AMR genes in one process. It is therefore the pipelines simultaneous analysis which can be seen as its main novel feature as it eliminates the need for two methods which are often conducted sequentially; pathogen identification and resistance profiling. It is invaluable for clinical metagenomic applications where the speedy and accurate analysis of the large and intricate data is of primary importance thanks to cloud computing resources of the platform (Lu *et al*.,, 2024).

To further validate the pipeline, the researchers investigated the performance using experimental data sets involving both pathogens and their resistance genes. The primary and salient advantage of CZ ID over other similar identification techniques is the fact that it is open-source, thus making it easier for other researchers or clinicians to including it into daily practice. One of the main advantages of the platform is an availability of an easy-to-use graphical interface masking complex computations and data analysis behind it (Lu *et al*.,, 2024).Nevertheless, as with any new technological platform, more work will be needed to replicate the present findings in other settings. CZ ID development could mark a move towards better integration of diagnostic tests that may benefit not only tracking but therapy choices related to antimicrobial utilization.

2.14 Critiques of the Existing Literature

While many studies have investigated microbial contamination in water, most focus on clinical settings or hospital effluents, neglecting informal food and water vending systems common in peri-urban areas like Juja. Traditional culture-based methods,

though useful, miss unculturable organisms and underreport ARG diversity. Moreover, some literature assumes compliance with hygiene regulations, yet evidence shows significant gaps in actual practice. Few studies combine culture-dependent and metagenomic approaches, limiting the depth of analysis on resistome diversity in community water systems.

Although literature has increasingly documented microbial contamination and antimicrobial resistance (AMR) in African water and food systems, significant limitations remain, particularly regarding informal water and food vending environments such as those where ice pops are produced and sold. In Kenya, studies on ready-to-eat foods in informal settlements (e.g., Kibera) have shown high levels of microbial contamination and multidrug-resistant bacteria, including *E. coli*, *Klebsiella spp.*, and ESBL phenotypes, using culture-based isolation and limited PCR screening for β-lactamase genes (e.g., *blaTEM*) but did not comprehensively characterize the broader spectrum of resistance genes present. This approach, though useful for identifying clinically relevant phenotypes, misses non-culturable organisms and underestimates ARG diversity, as acknowledged by the original authors (Maina *et al*.,2021).

Other Kenyan research on street foods (e.g., in Kiambu County) documents high overall microbial loads and identifies *E. coli* and other bacteria, but again relies predominantly on conventional culturing and biochemical tests, without integrating molecular profiling to explore resistance mechanisms or environmental sources of contamination. Additionally, studies of bottled water in Nairobi have revealed the presence of multidrug-resistant indicator bacteria in commercially distributed water, highlighting the limitations of current treatment and surveillance practices (Mwove *et al*.,2021)

At the continental level, systematic reviews and metaanalyses emphasize that ARGs and resistant *E. coli* in water are widespread across Africa, but note the geographic bias toward South Africa, Ethiopia, and Nigeria, with relatively few high-resolution studies from Kenya and similar contexts (Ramatla *et al*., 2023) African work employing metagenomic and whole-genome sequencing (e.g., sewage resistome

studies in South Africa) underscores the power of culture-independent methods to reveal diverse resistance genes across settings, yet such approaches are rarely incorporated in Kenyan food/water studies due to resource and capacity constraints. (Smith *et al*.,, 2024)

The methodological contrast is clear: culture-dependent methods yield viable isolates and phenotypic resistance profiles valuable for public health thresholds but systematically underreport non-culturable taxa and ARG diversity, while culture-independent sequencing approaches offer broader resistome and community insights but require substantial resources often unavailable in peri-urban Kenyan research contexts. This fragmentation limits the ability of current studies to link vendor hygiene practices, informal water handling, and resistome dynamics comprehensively (Smith *et al*.,, 2024).This study is different since it integrates both culture-dependent and metagenomic approaches specifically in potable water and ice pop vending environments. The study addresses these gaps, providing a more complete characterization of microbial communities and resistance profiles and enabling clearer associations between environmental conditions, vendor practices, and AMR dissemination than previous work has achieved.

2.15 Research Gaps

Despite the growing concern over antimicrobial resistance, there are limited studies in Kenya examining the resistome of potable water and ready-to-eat foods such as ice pops, particularly within peri-urban settings. Most existing studies have focused on conventional microbial indicators without exploring the diversity and distribution of antibiotic resistance genes present in these widely consumed products. Few studies have adopted an integrated approach that combines culture-dependent methods with culture-independent techniques such as metagenomics, limiting comprehensive characterization of microbial communities and resistance profiles. There is also insufficient evidence linking vendor hygiene practices and compliance with public health guidelines to levels of microbial contamination and antibiotic resistance gene diversity in vended water and street foods. This gap restricts the ability to establish clear associations between handling practices, environmental conditions, and the

dissemination of resistant pathogens. Consequently, the lack of baseline data hampers informed policy development and the design of targeted community-level interventions aimed at mitigating the spread of antimicrobial resistance through potable water and informal food vending systems.

The literature also points to policy and practice gaps, including weak enforcement of food and water safety regulations and limited system-level surveillance, which are rarely addressed in published work. Vendor-level factors such as knowledge of hygiene standards, adherence to safe handling practices, and training are scarcely documented, despite their likely role in contamination and ARG dissemination. These gaps particularly the lack of integrated monitoring combining culture-dependent and independent methods and the limited attention to compliance and education of vendors represent urgent areas for future research and targeted interventions, as highlighted by recent studies on waterborne AMR in informal food systems.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Ethical Considerations

An Ethical Approval was obtained from the JKUAT Institutional Scientific and Ethics Review Committee (Approval number: JKU/ISERC/02316/1085) before starting the sample collection process. This ensured that the research adhered to ethical guidelines, protected the welfare of participants, and upheld the integrity of the study.

3.2 Study Site

The study was conducted in Juja Subcounty (1.1018° S, 37.0144° E), Kiambu County, located North of Nairobi City, approximately 36 kilometers away.

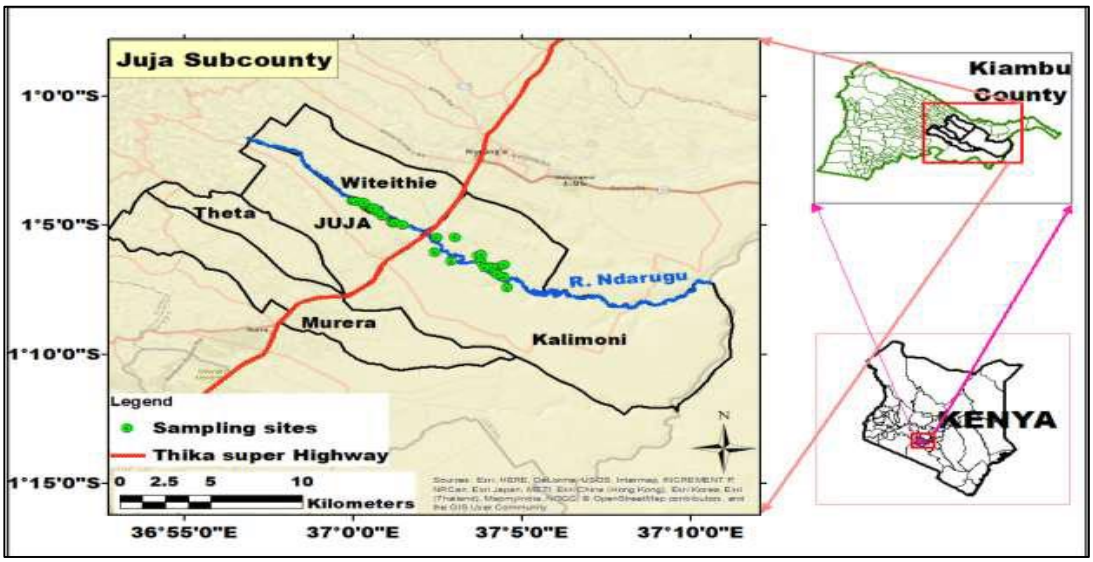

Figure 3.1: The Map of Juja Area

3.3 Study Population

The study population comprised licensed potable water vendors and ice pop vendors operating within Juja subcounty, Kiambu County, Kenya.

3.4 Inclusion and Exclusion Criteria

Participants included in the study were licensed potable water vendors operating within Juja Sub-County who were actively supplying treated water for human consumption and who consented to participate. Ice pop vendors selling ready-to-consume frozen water products within the study area were also included to assess microbiological risks associated with value-added water products. Vendors operating outside Juja Sub-County, unlicensed water suppliers, water sources not intended for direct human consumption, and vendors who declined participation were excluded. Ice pop participants who denied participation were also excluded.

3.5 Study Design

This study adopted a cross-sectional design, whereby potable water and ice pop samples were collected at a single point in time from various vending points to assess microbial contamination, bacterial diversity, and antibiotic resistance profiles.. A total of 55 samples were purposively selected to represent the study area, forming the basis for laboratory analyses. For these analyses, a completely randomized design (CRD) was employed. All samples were randomly assigned to two selective culture media, Salmonella- Shigella Agar (SS, Neogen®, USA) and Eosin Methyl Blue Agar (EMBA, Titan Biotech Limited®,India), for bacterial isolation. The 30 pure isolates obtained were then subjected to nine biochemical tests and antibiotic susceptibility testing against a group of 14 antibiotics comprising eight Himedia and six commercial. Each pure isolate was tested on all media, biochemical tests, and antibiotics. This design minimized bias, ensured uniform treatment of all samples, and facilitated reliable comparison of microbial characteristics, biochemical properties, and resistance patterns.

3.6 Sample Size Determination

The study involved two separate sample size considerations: the survey questionnaire and the total samples collected for laboratory analysis. For the survey, the required

sample size was determined using Cochran's (1963) formula for an infinite population:

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

Where, Z is the Z-score corresponding to the desired confidence level (1.96 for 95%), p is the estimated proportion of the population with the attribute of interest (0.5 was used as a conservative estimate), q=1−p , and e is the desired margin of error (0.05). This gave a survey sample size of 385 participants, who were interviewed to assess vendor practices, knowledge, and compliance with public hygiene guidelines. The distribution of questionnaire respondents into 285 potable water vendors and 100 ice pop vendors was determined through purposive sampling, guided by the actual structure of vending activities within the study area. Potable water vending constituted the dominant practice across Juja and its environs, with a substantially higher number of licensed and operational water vendors compared to ice pop vendors.

For the total samples collected for laboratory analyses, the target population were the licenced water vendors in the study area, estimated at N = 50 based on official Kiambu County data on licenced vendors operating between Juja township and Witethie. Since this is a finite population, the Cochran (1963) formula for finite correction was applied:

$$.n = \frac{\frac{Z^2.P(1-P)}{e^2}}{1+\frac{Z^2.P(1-P)}{e^2.N}}$$

Therefore, n = 45

Where n is the sample size, N is the estimated population size, Z is the Z score, P is the standard deviation, and e is the margin of error. N= 50, Z score=1.96, e=0.05 (5%), P=0.5

The conservative estimate p=0.5 was used because the true proportion of contaminated products among compliant vendors was unknown prior to testing.

A total of 55 samples were collected, comprising 45 water samples from licensed vendors meeting public hygiene standards and 10 ice pop samples to capture product diversity, including five frozen pops from compliant vendors and five sachet pops from different companies. The inclusion of ice pop samples aimed to facilitate a comparative exploratory analysis between treated potable water and value-added water products, which are highly consumed within the Juja community.

3.7 Sample Collection

Samples were purposively collected in triplicate to enhance reliability and reduce random variability, following established WHO and APHA guidelines for water quality sampling. This included samples from water refill dispensing points (n=23), piped water vendors (taps) (n=12), and water hawkers (n=10) (Appendix XIII).Ice pop samples were also collected in replicates of three from various vendors, including both mobile vendors and those operating in fixed locations (n=10). The water samples were collected from 6 different regions in Juja: Juja town (1.1017° S, 37.0144° E), Gachororo (1.0899° S, 37.0199° E), Mung'etho (1.0835° S, 37.0325° E), the Gate C region of JKUAT (1.1010° S, 37.0155° E), Kenyatta Road (1.0900° S, 37.0600° E), and Witeithie (1.0830° S, 36.9890° E) The ice pop samples were collected from Juja Township and Witeithie (Appendix XIII).The water samples were collected using sterile well labelled falcon tubes (50ml) at the various water vending points, while the ice pop samples were collected in sterile zip lock bags stored at 4℃ in cool boxes with frozen ice packs and transported to the Jomo Kenyatta University Government of Kenya Laboratory for microbial analysis.

3.8 Assessment of Public Hygiene Guidelines by Water and Ice Pop Vendors

A questionnaire was used to assess the compliance level to public hygiene policy by potable water vendors in Juja, Kenya (Appendix I). The questionnaire used for the survey was tailor-made based on WASREB Guidelines under the Water Act 2016.Ice-

pop vendors were interviewed to determine their product preparation mode and the water source used for preparation (Appendix II). Purposive sampling was employed to intentionally target licensed and operational vendors who directly influence consumer exposure to drinking water and ice pop products, rather than the general population.

3.9 Isolation of Enterobacteriaceae Bacteria and Coliforms

The study focused on isolation of the Enterobacteriaceae family which are commonly found in water. Selective media, such as Eosin Methylene Blue Agar (EMBA, Titan Biotech Limited®,India), Salmonella Shigella Agar (SS, Neogen®, USA), and Nutrient Agar (NA, Neogen® ,USA), were used for isolation. EMBA was used to isolate coliforms, which are indicators of contamination.EMBA is ideal for targeting coliforms and many Enterobacteriaceae due to its selectivity and differential properties, but it may miss some non-lactose-fermenting or fastidious members, which is why it was complemented with Salmonella Shigella agar. SS agar was used for isolation of both Salmonella and shigella due to its higher selectivity and clearer differential appearance in contaminated samples. The media was prepared by mixing 35.96 grams of EMB agar in 1 litre of distilled water and later sterilized by autoclaving at 121°C for 15 minutes**.** The media was poured into 20ml petri dishes after cooling to 40°C. A tenfold serial dilution of the samples was prepared by transferring 1 mL of the sample to a new sterile universal bottle containing 9 mL of sterile water. 100 µL of the sample were inoculated on the petri plates containing EMB and SS agar and incubated at 37°C for 24 hours. After the incubation period, the petri plates were examined, and the number of colonies exhibiting typical characteristics of coliform bacteria were counted using a colony counter (Infinitek CC-J2, Japan) and then expressed in terms of CFU/ml for each sample. The results were expressed as colony-forming units per milliliter (CFU/mL) using the standard formula:

$$\text{CFU/ML} = \frac{\text{Number of colonies} \times \text{Dilution Factor}}{\text{Volume plated(mL)}}$$

Where the number of colonies is the total count obtained on the plate, the dilution factor corresponds to the dilution of the sample plated, and the volume plated is the

aliquot applied to the agar surface. Counts were recorded for all replicates, and the mean CFU/mL was calculated for each sample to account for variability between replicates. The discrete colonies that were isolated were then subcultured on nutrient agar using the streak plate technique to obtain pure colonies.

3.10 Morphological Characterization

A total of 126 bacterial colonies were initially isolated and subjected to morphological characterization following standard bacteriological principles as described in Bergey's Manual of Systematic Bacteriology (Rainey *et al*., 2015) . Observations included colony size, shape, color, texture, margin, and elevation, which provide preliminary differentiation among bacterial isolates. Morphological assessment was used to identify distinct colony types and phenotypic diversity, ensuring that representative isolates from different morphotypes were included. Based on these criteria, a subset of 30 isolates was purposively selected for further Gram staining and biochemical testing. The selection prioritized morphologically distinct colonies to capture the diversity present among the original 126 isolates, while also reducing redundancy and duplication of similar colonies, which could represent the same bacterial species.

3.11 Gram Stain

The 30 bacterial isolates from pure colony were fixed on sterile slides (in replicates) by picking a drop of sterile water and spreading it on the slide using a sterile loop. The loop was heated on a flame to sterilize and the suspended cells were picked and smeared on the sterile water on the slide. The smear was then passed on the flame for ten seconds to fix the cells on the slide. The heat-fixed smear of cells was flooded with crystal violet staining reagent for 1 minute. The slides were washed in sterile water for 2 seconds. The slides were flooded with the mordant: Gram's iodine for 1 minute. The slides were washed in sterile water for 2 seconds. The slides were then flooded with absolute ethanol (decolorizing agent) for 15 seconds. The slides were flooded with safranin (counterstain) for 30 seconds. The slides were washed in sterile water until no color appeared in the effluent and it was blotted dry with a paper towel. The results of

the staining procedure was observed under oil immersion lens using microscope (Yoshimura *et al*., 2020).

3.12 Biochemical Characterization

Biochemical tests including Citrate utilization test, indole production test, methyl red test, Voges Proskauer test, and sugar fermentation tests were carried out on the 30 pure culture isolates that were obtained in replicates (Akinbankole *et al*., 2015). The nine biochemical tests were selected because they are key for the identification of Enterobacteriaceae. Each test evaluated a specific metabolic or enzymatic trait such as carbohydrate fermentation, enzyme activity, or motility that is crucial for differentiating genera and species within the Enterobacteriaceae family. Christensen's medium was used for the urease test. A loopful of colonies were inoculated in the urea broth and then incubated at 37°C for 24 hours (Anbazhagan *et al.,* 2023). Methyl red (MR) test required Methyl Red Voges Proskauer broth (MR-VP, pH 6.9) which contains 7.0 g of buffered peptone, 5 g of glucose and 5 g of dipotassium phosphate. 0.1 g of methyl red was dissolved in 300ml of ethyl alcohol (95%). The solution was topped up to 500ml using distilled water. The solution was stored in a brown bottle at 4°C (Tripathi & Sapra, 2020).The tubes containing sterile MR-VP Broth were inoculated with freshly prepared cell suspensions in nutrient broth of all the isolates using a sterile loop to pick the suspended cells and transferring them into the media. The tubes were incubated up to four days at 37°C. Five drops of methyl red indicator solution was added to the tubes and the color observed.

Indole test was done using a conventional tube method. The tubes containing tryptophan broth were inoculated with freshly prepared cell suspensions in nutrient broth of all the isolates using a sterile loop to pick the suspended cells and transferring them into the media (Geresu et al., 2016).The tubes were incubated for 24 hours at 37 °C. Approximately 0.5 ml of Kovac's was added to the broth culture in the tubes.

Voges proskauer test utilizes MR/VP broth containing 7 g polypeptone, 5 g of glucose and 5 g dipotassium phosphate. The tubes containing sterile MR-VP Broth were inoculated with freshly prepared cell suspensions in nutrient broth of all the isolates

using a sterile loop to pick the suspended cells and transferring them into the media. The tubes were incubated for 24 hours at 37°C. At the end of this time, aliquots of 1 ml of the broth were transferred to clean test tubes. A total of 0.6mL of 5% α-naphthol, followed by 0.2 mL of 40% dipotassium phosphate was added to the broth. The tubes were shaken gently to expose the medium to atmospheric oxygen and the tubes were allowed to remain undisturbed for 15 minutes.

The lactose fermentation test involved the transfer of a freshly isolated microorganism to a sterile tube containing phenol red lactose broth with a sterile inoculating loop. The tube was inoculated and placed at a temperature 35-37°C for 24 hours to determine the results. Motility test was conducted by inoculating the isolate colony in a tube containing SIM media and then it was incubated for 48 hours after which the results were recorded. Starch hydrolysis test involved picking up the sample bacteria from several fresh colonies using a sterile inoculating loop and streaked the bacteria in the form of short and thick straight lines over the surface of the bacteria. It was then incubated at 35±2°C for at least 48 hours. A few drops of iodine solution was directly added over the colonies and observed for the formation of a clear halo around the colonies. Negative controls were prepared for each test to help with interpretation of the final results.

3.13 Metagenomics and Molecular Identification of Pathogenic Bacteria

3.13.1 Total DNA Extraction

Total DNA was extracted from the 45 potable water and 10 ice pop samples according to Gautam et al. (2022). One milliliter of each sample was transferred into an Eppendorf tube and centrifuged at 13,000 rpm for 10 minutes to harvest the cells. After decanting the supernatant, 200 µL of 0.1 M sodium phosphate buffer (pH 8.0) was added to resuspend the pellet. To facilitate cell lysis, 30 µL of Proteinase K (20 mg/mL) and 30 µL of lysozyme (10 mg/mL) were added, and the mixture was vortexed and incubated at 37°C for 1 hour. 400 µL of 1% (w/v) sodium dodecyl sulfate (SDS) was added to denature cell membranes and proteins. The samples were vortexed, incubated briefly at room temperature for 5 minutes, then placed on ice for 2 minutes.

To further liberate nucleic acids, the mixture was incubated at 65°C for 1 hour, followed by cooling on ice for another 2 minutes. To aid DNA precipitation, 400 µL of 3 M sodium acetate (pH 5.2) was added, vortexed, and centrifuged at 13,000 rpm for 10 minutes; the supernatant was carefully transferred to a new tube. An equal volume of phenol:chloroform:isoamyl alcohol (25:24:1, v/v/v) was added to the supernatant and mixed gently by inversion. After centrifugation at 13,000 rpm for 10 minutes, the upper aqueous phase containing DNA was transferred to a fresh tube. To precipitate DNA, two volumes of ice-cold absolute ethanol and 1/10 volume of 3 M sodium acetate (pH 5.2) were added. The mixture was gently inverted and centrifuged at 13,000 rpm for 10 minutes. The DNA pellet was washed twice with 70% (v/v) ethanol, air-dried to remove residual ethanol, and then dissolved in 100 µL of DNase-free water. Integrity of genomic DNA was confirmed by agarose gel electrophoresis (1.0% w/v agarose in 1× TAE buffer) stained Ethidium Bromide (0.5 µg/mL) and visualized under UV light to confirm the presence of DNA. Sterile distilled water was used as the negative control.

The total genomic DNA obtained from the water and ice was then pooled to six samples based on the location of the source and type of the sample. This included (Sample 1: Juja A; representing Juja township, Sample 2: Juja B; representing Kenyatta Road region, Sample 3: Witeithie, Sample 4: Hawkers samples, Sample 5: Sachet Pops, Sample 6: Frozen pops). The samples were then packaged and shipped to MR DNA Company (USA) for sequencing.

3.13.2 DNA Sequencing and PCR Amplicons Preparation for Illumina MiSeq

The bacterial sequencing process targeted the V4 hypervariable region of the 16S rRNA gene using PCR primers 515F/806R with a unique barcode on the forward primer to allow sample multiplexing (Klindworth et al., 2013). The V4 region was selected due to its optimum amplicon length (≈250–300 bp) compatible with Illumina platforms and its broad coverage of bacterial diversity. PCR amplification was performed using the HotStarTaq Plus Master Mix Kit (Qiagen, USA). Each sample was amplified in triplicate PCR reactions to minimize the effects of stochastic amplification bias. A no-template control (NTC) was included in each PCR batch to

monitor for contamination. Thermocycling conditions were as follows: initial denaturation at 95 °C for 5 minutes, followed by 30 cycles of 95 °C for 30 seconds, 53 °C for 40 seconds, and 72 °C for 1 minute, with a final extension at 72 °C for 10 minutes. Amplification success and product size were confirmed by 2% agarose gel electrophoresis (Whitfield-Cargile et al., 2015).Amplicon products from the triplicate PCRs were, quantified, and purified using calibrated SPRI beads (based on fragment size and DNA concentration) to remove primers, primer dimers, and non-specific products. Purified amplicons were then pooled in equimolar ratios to generate a 16S rRNA gene sequencing library using the Illumina TruSeq DNA library preparation protocol. Sequencing was performed using a paired-end 2 × 300 bp configuration on an Illumina MiSeq platform.To ensure adequate coverage and reliable diversity estimation, a minimum sequencing depth of 25,000 paired reads per sample was targeted during library pooling and sequencing. This depth is consistent with common standards for microbial community profiling of environmental samples, allowing robust detection of both common and rare taxa. Image analysis and base calling were performed by the MiSeq's embedded MiSeq Control Software (MCS). All sequencing procedures were conducted at MR DNA (www.mrdnalab.com, Shallowater, TX, USA) following the manufacturer's guidelines.

3.14 Antibiotic Susceptibility Testing

Pure isolated strains were subjected to the disk diffusion technique of Kirby-Bauer following the Kirby-Bauer Disk Diffusion Susceptibility Test Protocol on Mueller-Hinton agar in replicates (Hudzicki, 2016). The tests were conducted using commercial antibiotics and Himedia Laboratory LLC Antibiotics Disks. Both commercial and Himedia disks to cross-validate results, reduce bias from disk variability, and test a broader range of antibiotics relevant to both clinical and environmental settings. Twenty one bacterial isolates were subjected to antimicrobial susceptibility testing using commercially available antibiotic discs. Although a total of 30 isolates were identified, several represented the same microbial genera and exhibited similar phenotypic characteristics. To avoid redundancy, one isolate was selected as a representative for each repeatedly identified microorganism. For testing

with HiMedia antibiotic discs, six representative genera were selected based on their presumed pathogenicity. This approach was also influenced by the limited availability and high cost of Himedia octate discs.

The commercial antibiotics used included amoxicillin (10 µg/L), tetracycline (10 µg/L), chloramphenicol (30µg/L), gentamicin (10 µg/L), ciprofloxacin (10 µg/L), streptomycin (10 µg/L), penicillin (10 µg/L) (Adam Mohamed *et al*., 2020). The Himedia Laboratory LLC Antibiotic Disks used contained Ampicillin (25 µg/L), Tetracycline (100 µg/L), Nitrofurantoin (200 µg/L), nalidixic acid (30 µg/L), streptomycin (10 µg/L), Sulfamethoxazole (200 µg/L), Co-trimoxazole (25 µg/L) and Gentamicin (10 µg/L). The isolates were inoculated on Mueller-Hinton agar, and discs containing antibiotics at specific concentrations were introduced onto the plates, which were then incubated at 37°C for 24 hours. The diameters of the inhibition zones were measured in millimeters and compared to data from the Clinical Laboratory Standards Institute (CLSI,2020), British Society for Antimicrobial Chemotherapy (BSAC), National Committee for Clinical Laboratory Standards (NCCLS) to determine the susceptibility of the different bacteria to different antibiotics. Using CLSI, BSAC, and NCCLS guidelines allowed us to cross-validate susceptibility results and account for differences in breakpoints. For some antibiotics, breakpoints were not documented in a single guideline, so consulting all three provided the necessary reference data to classify isolates accurately. The discs were compared to the diameters of minimal inhibitory concentrations of known bacterial strains, and the bacteria were classified as either Susceptible (S), Intermediate (I), or Resistant (R).

Table 3.1: Antibiotics Minimum Inhibitory Concentrations and their Spectrum of Activity

Antibiotics	Abbreviation	Concentration	Spectrum of activity
Ampicillin	AMP	10 µg/l	Broad
Streptomycin	S	10 µg/l	Broad
Amoxicillin	AML	10 µg/l	Broad
Gentamycin	CN	10 µg/l	Broad
Sulfamethoxazole	SUL	10µg/l	Broad
Cefotaxime	CTX	10µg/l	Broad
Spectinomycin	SPCM	100 µg/ml	Broad

3.15 Profiling of Antibiotic Resistance Genes

Detection of the Antibiotic-Resistant Genes was conducted using the open source, cloud-based CZ ID pipeline (Chan Zuckerberg ID - Detect & Track Infectious Diseases (czid.org). Sequences from the pooled metagenomics samples were converted to FAST Q format. FASTQ file sequence data were uploaded to the CZ ID web app. The pipeline preprocessed reads by removing low-quality sequences with fast, filtering host reads with Bowtie2/HISAT2, removing duplicates with CZID-dedup, and subsampling to 1M single-end or 2M paired-end reads. For mNGS, duplicate reads were added back to capture low-abundance AMR genes. For AMR detection, two approaches ran in parallel. The contig approach assembled reads into contigs with SPAdes, aligned contigs to the CARD AMR (https://card.mcmaster.ca/) gene database using RGI's main mode and identified AMR genes based on sequence similarity and mutations. To ensure robust identification, results from the contig and read-based approaches were reconciled: only ARGs detected in at least one approach and meeting a minimum similarity threshold of 90% and coverage ≥ 80% were reported. This dual-strategy approach improves confidence in ARG detection by capturing both high-abundance genes (via contigs) and low-abundance genes (via reads).The read approach directly mapped reads against CARD using KMA and RGI's bit mode. In both approaches, RGI's Kmer query mode predicted bacterial hosts

harboring detected AMR genes by matching sequences against CARD's Resistomes & Variants database (Lu *et al*., 2024).

3.16 Data Analysis

The data collected from the questionnaires and microbial contamination of the samples were analyzed using SPSS (version 31) and R software (version 3.5.1). In SPSS, descriptive statistics including frequencies, percentages, means, and standard deviations were computed to summarize the survey responses and contamination data. R software was employed for inferential analyses. ANOVA was conducted to determine significant differences in contamination levels among the different sample types and locations. Where significant differences were observed, Tukey's Honestly Significant Difference (HSD) post hoc test was used to separate the means and determine the least significant differences (LSD) between groups. Correlation analyses were performed to assess the relationship between vendors' public compliance practices and contamination levels. Both a correlation table and graphical representations were generated in R to visualize these relationships. Visualization was performed using R packages such as ggplot2 for general plotting and corrplot for correlation matrices. For the Next Generation Sequencing (NGS) data, the QIIME 2 (2021.4) pipeline was used. Raw sequence reads were quality filtered, denoised, and dereplicated using the DADA2 plugin. Downstream analysis of QIIME 2 outputs was performed in R using specialized packages including phyloseq for handling ASV tables and metadata, vegan for diversity analyses and multivariate statistics, microbiome for community composition analysis, ggplot2 for visualization, and reshape2 for data reshaping.

3.16.1 Sequence Data Analysis

The sequence data, already demultiplexed in Consensus Assessment of Sequence and Variation (CASAVA) 1.8 format, underwent substantial quality assessment. The data were then processed using QIIME 2 version 2021.4 software to determine the diversity of other microbial contigs in the samples (Bolyen *et al*., 2019). Interactive quality plots were generated by importing the sequences into QIIME 2 and analyzing their Phred

scores. The Phred score limit cut off point was at 25. A Phred quality score cutoff of 25 was applied during sequence filtering to balance data quality and retention. While a Q20 cutoff allows a higher error rate (1%), which can artificially inflate rare taxa in microbial community analyses, Q30 is more stringent and can lead to substantial read loss, reducing sequencing depth. The sequences were further subjected to quality control using the Divisive Amplicon Denoising Algorithm 2 (DADA2), a denoising tool designed for Illumina amplicon sequence data. This step involved filtering the sequences to a read length of 220 bp while removing chimeric sequences. Reads were truncated to 220 bp based on platform recommendations to remove low-quality bases at the ends, ensuring high-confidence sequences for denoising and clustering into zOTUs, while preserving enough information for reliable taxonomic assignment. zOTUs were used instead of traditional OTUs to capture error-corrected, unique sequences at 100% similarity, providing higher resolution and reducing false diversity caused by sequencing errors.

3.16.2 Diversity Analysis, Taxonomic Assignment

The samples were statistically grouped and not biologically pooled for comparison without any sample losing its identity. Each sample was sequenced individually and organized by location (Juja and Witeithie) and sample type (Value-Added, Affordable, and Commercialized Water) for diversity analyses. This preserved individual biological variability while allowing accurate comparisons between groups. For diversity analysis, the samples were further grouped into two locations (Juja and Witeithie) and three categories by sample type: Value-Added Water (Sachet and Frozen Pops), Affordable Water (Tap and Refill Dispensers), and Commercialized Water (Hawkers). The samples were rarefied to a sampling depth of 26,900 to ensure comprehensive diversity analysis. Alpha diversity was estimated using Shannon, Simpson aChao1 and Faith Pd metrices while beta diversity was estimated using the Bray-Curtis and Jaccard distance metrics. Dimension reduction on the data was done and graphically represented as PCoA emperor plots (Lozupone *et al*., 2011).Bacterial taxonomy was determined by assigning classifications at a similarity threshold of 97% where a Naïve Bayes classifier was trained using the SILVA 138-99 Reference

Database (Su *et al*., 2024). The 97% similarity threshold was applied during taxonomic assignment against the SILVA reference database because most reference sequences are annotated at this level, which approximates species-level classification. The sequences were screened against the reference sequence and interactive bar plots were generated to show the relative abundances of organisms at different taxonomic levels. A phylogenetic tree illustrating the evolutionary relationships among bacterial taxa was constructed based on molecular sequence data using MEGA version 11. Phylogenetic reconstruction was performed using the neighbor-joining (NJ) method, which is computationally efficient and well suited for exploratory phylogenetic analyses based on short amplicon sequences commonly used in microbial studies. Given that the primary aim of the phylogenetic analysis was to visualize relationships among taxa rather than infer deep evolutionary histories, the neighbor joining method was considered appropriate for this study. The reliability of the inferred clades was assessed using bootstrap analysis with 1,000 replicates, where bootstrap values ≥70% were interpreted as strong support and values ≥90% as very strong support for the phylogenetic relationships.

CHAPTER FOUR

RESEARCH RESULTS AND FINDINGS

4.1 Assessment of Public Hygiene Guidelines by Water and Ice Pop Vendors

Fifty three percent (53.3%) of the respondents in this study were not aware of WASREB guidelines on water vending while seventy percent (68.9%) did not receive training on water vending (Table 4.1). It was also noted that three-quarters of the respondents did not test their water, whereas 11.1% did not clean their vending equipment. Knowledge of antibiotic resistance was at a low 16 percent, while 13.3% confirmed to have had a history of contamination in the past year. Awareness of vending guidelines among the ice pop vendors was recorded at 46.7% of the total respondents (Table 4.1).Thirteen-point three percent (13.3%) of the water vendors confirmed cleaning their storage systems daily, 22.2% on a weekly basis, thirty one percent monthly, and seventeen-point seven percent annually. Only one-fifth of the respondents confirmed they treated their water through chlorination, while 13.3% used UV light sterilization, 4.4% used reverse osmosis, and 17.7% confirmed their water was supplied treated, whereas 46.7% of the vendors confirmed not treating their water.

Table 4.1: Summary of Responses by Water Vendors on Compliance with WASREB Vending Guidelines

Query on aspects of the Water Vending Guideline	Response from vendors (%)	
	Yes	No
Awareness of WASREB guidelines by vendors	46.7	53.3
Training on water vending guidelines	31.1	68.9
Water vending Facility inspection	93.3	6.7
Water testing	22.2	77.8
Cleaning of Vending equipment	88.9	11.1
Knowledge of Antibiotic Resistance	17.7	82.2
History of Contamination	13.3	86.7

n=285

Seventy percent of the ice pop vendors were not aware of the public health guidelines for food handlers, while half of them confirmed to be unaware of the microbial quality of water they use for production of their ice pops. Only 60% of the ice pop vendors confirmed that they test the water used for homemade ice pop production. A total of 20% of the vendors confirmed that they did not clean their cool boxes and freezers for an extended period of time (Table 4.2).

Table 4.2: Summary of Responses by Ice pop Vendors on Compliance with Public Hygiene Guidelines

Ice Pop Vending Guidelines(%)	Responses	
	Yes	No
Awareness of Vending Guidelines	30	70
Awareness of the safety of water used for production	50	50
Testing of water used for Ice pop production	60	40
Cleaning of Ice pop cool boxes and freezers	80	20

n=100

4.2 Isolation of Enterobacteriaceae Bacteria and Coliforms

Water samples were observed to be contaminated due to the coliform growths on Eosin Methylene Blue media (Plate 1). Cultural characteristics observed included pink, colorless, and blue colors.

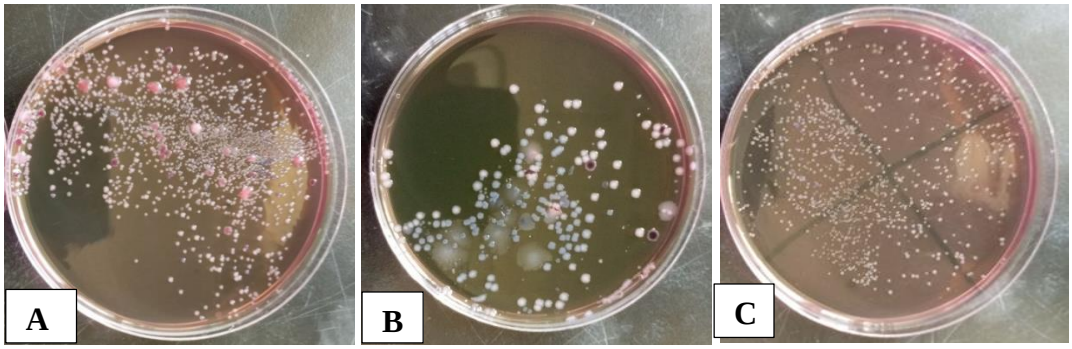

Plate 4.1: Total Coliforms Isolated from the Water and Ice pop Samples

Plate A represents a frozen ice pop sample from Mung'etho (1.0988039° S, 37.0094709° E) ; Plate B represents a water refill dispenser sample from Witeithie (1.0696971° S, 37.0544022° E); Plate C represents a tap water sample from Juja Town (1.1002859° S, 37.0152662° E).

Sachet ice pops were observed not to have contamination, as no coliform growth was detected. Frozen pops were highly contaminated, followed by water from refill dispensers the difference being significant ($p \leq 0.05$). Samples from the Gate C region of JKUAT had the highest level of contamination (505415 CFU/ML), followed by those from Gachororo (478256 CFU/ML), while samples from Witeithie recorded the lowest contamination levels (4950 CFU/ML) though the differences were not significant (Table 4.2).

Table 4.3: The Mean Total Coliform Counts for Water Refill Dispenser, Tap, Hawkers Water, and Ice Pops

Sample Type	Coliforms (CFU/ml)	Region	Coliforms (CFU/ml)
Frozen pops	1073000^{a}	Gate C JKUAT	505415^{a}
Dispenser	11126^{b}	Gachororo	478256^{a}
Tap	6000^{b}	Mung'etho	146346^{a}
Hawkers	5311^{b}	Juja A	80519^{a}
Sachet pops	0^{b}	Juja B	10371^{a}
		Witeithie	4950^{a}
%CV 215.1	FrP 0.01	%CV 284.8	FrP 0.071

4.3 Correlation Analysis between the Survey Variables and Contamination Levels

There was a statistically significant negative correlation ($r = -0.407$, $p \leq 0.05$) between contamination and the adherence to vending guidelines. There were no statistically significant correlations between contamination and the following variables: license ownership, training undergone by vendors, inspection of vendors, non-compliance, vendors' water treatment, contamination report, and vendors' water testing. The correlation coefficients for these variables are relatively small, ranging from -0.167 to 0.055, and their corresponding p-values are greater than the commonly used significance level of 0.05. There was a statistically significant positive correlation ($r = 0.447$, $p \leq 0.05$) between the training undergone by vendors and their water testing practices. There was also a positive significant correlation between water testing and water vendors water treatment ($r = 0.535$, $p \leq 0.001$) (Table 4.4).

Vendors who had not been trained on water vending guidelines had the highest contamination rates as compared to those who had undergone training (Appendix XI). Although most vendors reported no history of contamination, their water samples still tested positive for high levels of total coliforms, exceeding WHO standards (0 CFU per 100 mL for treated drinking water). Contamination was detected in water samples from both vendors who treated their water and those who did not. However, higher contamination levels were observed among those who did not treat their water. Water sources from boreholes, county suppliers, and private suppliers all showed signs of contamination. Borehole water had the highest average contamination, followed by private suppliers, and then county supplied water. Contamination was observed among vendors regardless of their compliance history. Both compliant and non-compliant vendors had contaminated water. All vendors who reported being inspected by local authorities had contaminated water. While contamination was also present among vendors who had not been inspected, their levels were slightly lower compared to those who were inspected (Appendix XI).

Table 4.4: Correlation between the Survey Variables and Contamination

	Contamination	**Vending guidelines**	**License ownership**	**Training undergone by vendors**	**Inspection of vendors**	**Non-compliance**	**vendors water treatment**	**contamination Report**	**Water testing**
Contamination	1								
Vending guidelines	**-.407***	1							
License ownership	0.055	0.291	1						
Training undergone by vendors	-0.115	0.15	0.149	1					
Inspection of Vendors	-0.036	-0.036	-0.089	0.12	1				
Non-compliance vendors	-0.052	0.036	0.089	-0.12	0.071	1			
Water treatment	-0.167	-0.009	-0.134	0.299	0.25	0.018	1		

	Contamination	**Vending guidelines**	**License ownership**	**Training undergone by vendors**	**Inspection of vendors**	**Non-compliance**	**vendors water treatment**	**contamination Report**	**Water testing**
Contamination Report	0.035	0.053	0.131	-0.175	0.105	-0.105	-0.17	1	
Water testing	-0.119	0.067	-0.111	**.447***	0.134	-0.134	**.535****	-0.196	

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

4.4 Gram Stain, Morphological and Biochemical Characterization

It was observed that 82.1% of the isolates were Bacilli shaped while 17.9% were cocci. It was observed that 32.1% of the isolates were gram-positive, whereas 67.90% were gram-negative**.** All isolates (100%) tested positive for catalase and citrate utilization. The methyl red test was positive in 39.3% of isolates, whereas 60.7% tested negative. Indole production was observed in 9.5% of the isolates, whereas 90.4% tested negative. A total of 3.6% of the organisms tested positive for urease test, while 96.4% tested negative. Starch hydrolysis was observed in only 10.7% of the isolates. Fermentation of the sugars varied, with 14.3% fermenting sucrose and lactose and 17.9% fermented glucose. Gas production was noted in 14.3% of the isolates. Hydrogen sulfide production was observed in 10.7% of the samples (Table 4.5; Appendix XIV).

A total of 30 microorganisms were isolated and identified from the water samples. Based on biochemical tests and gram stain techniques, the putative microorganisms identified included E*scherichia coli*, *Shigella*, *Serratia*, *Acinetobacter*, *Bacillus*, *Enterobacter*, *Staphylococcus*, *Listeria*, *Corynebacterium*, *Pseudomonas*, *Klebsiella*, *Streptococcus*, *Enterobacter*, *Salmonella*, *Enterococcus faecalis*, *Moraxella*, *Cedacea*, *Proteus*, *Providencia*, *Proteus mirabilis*, and *Enterococcus faecium*.It was also evident that *Salmonella* was the most prevalent organism, accounting for 10.7% of the isolates. *Escherichia coli*, *Shigella*, *Staphylococcus*, *Enterococcus faecalis*, *Cedacea*, and *Providencia* each represented 7.1% of the isolates. The other microorganisms were unique, appearing only once among the 30 isolates. No differences were observed among the *Salmonella*, *Cedacea* and *Escherichia*. Differences in biochemical tests were observed in *Shigella*, *Staphylococcus*, and *Providencia*. The two *Shigella* isolates differed in their Methyl Red test results with one of the isolate testing positive, while the other isolate testing negative. *Providencia* isolates differed in their Indole test, one of the isolate was indole positive while the other isolate was indole negative. *Staphylococcus* isolates differed in their Methyl Red and H2S production test. One of

the isolate was negative for both Methyl Red and H2S production, while the other one tested positive for both (Table 4.4; Appendix XV).

Table 4.5: Biochemical and Morphological Characteristics of the Isolates

N/B: + Positive Test Negative test

Morphology	Gram Stain	Catalase	Methyl Red	Indole	VP	Urease	Citrate	Starch Hydrolysis	Sucrose fermentation	Lactose Fermentation	Glucose Fermentation	Gas Production	H2S	Putative microorganism
Bacilli	−	+	-	-	-	-	+	-	-	-	-	-	-	*Escherichia coli*
Bacilli	−	+	+	-	-	-	+	-	-	-	-	-	-	*Shigella*
Bacilli	−	+	-	-	-	-	+	-	-	-	-	-	-	*Serratia*
Bacilli	−	+	-	-	-	-	+	-	-	-	-	-	-	*Acinetobacter*
Bacilli	+	+	-	-	-	-	+	+	-	-	-	-	-	*Bacillus*
Bacilli	–	+	+	+	-	-	+	+	-	-	+	-	-	*Enterobacter*
Bacilli	–	+	+	+	-	-	+	+	-	-	+	-	-	*Enterobacter*
Cocci	+	+	-	-	-	-	+	-	-	-	-	-	-	*Staphylococcus*
Bacilli	+	+	-	-	-	-	+	-	-	-	-	-	-	*Listeria*
Bacilli	+	+	+	-	-	-	+	-	-	-	-	-	-	*Corynebacterium*
Bacilli	-	+	-	-	-	-	+	-	-	-	-	-	-	*Pseudomonas*
Bacilli	-	+	-	-	-	-	+	-	+	+	+	+	+	*Klebsiella*
Cocci	+	+	+	-	-	-	+	-	-	-	-	-	-	*Enterococcus faecalis*

Bacilli	-	+	-	-	-	-	+	-	-	-	-	-	-	*Cedacea*
Bacilli	-	+	+	-	-	-	+	-	-	-	-	-	-	*Salmonella*
Cocci	+	+	+	-	-	-	+	-	-	-	-	-	+	*Staphylococcus*
Cocci	-	+	+	-	-	-	+	-	-	-	-	-	-	*Moraxella*
Bacilli	-	+	-	-	-	-	+	-	-	-	-	-	-	*Cedacea*
Bacilli	-	+	-	+	-	+	+	-	+	+	+	+	+	*Proteus*
Bacilli	-	+	-	+	-	-	+	-	-	-	-	-	-	*Providencia*
Bacilli	+	+	-	-	-	-	+	-	+	+	+	+	-	*Streptococcus*
Bacilli	-	+	-	-	-	-	+	-	-	-	-	-	-	*Escherichia coli*
Bacilli	-	+	+	-	-	-	+	-	-	-	-	-	-	*Salmonella*
Cocci	+	+	+	-	-	-	+	-	-	-	-	-	-	*Micrococcus luteus*
Cocci	+	+	+	-	-	-	+	-	+	+	+	+	-	*Enterococcus faecalis*
Bacilli	-	+	+	-	-	-	+	-	-	-	-	-	-	*Providencia*
Bacilli	-	+	-	-	-	-	+	-	-	-	-	-	-	*Shigella*
Bacilli	-	+	+	-	-	-	+	-	-	-	-	-	-	*Salmonella*

4.5 Total DNA Extraction and Screening

Total genomic DNA was extracted and screening for DNA quality was performed through gel electrophoresis using 1% agarose gel to confirm the presence of DNA in the potable water and ice pop samples.

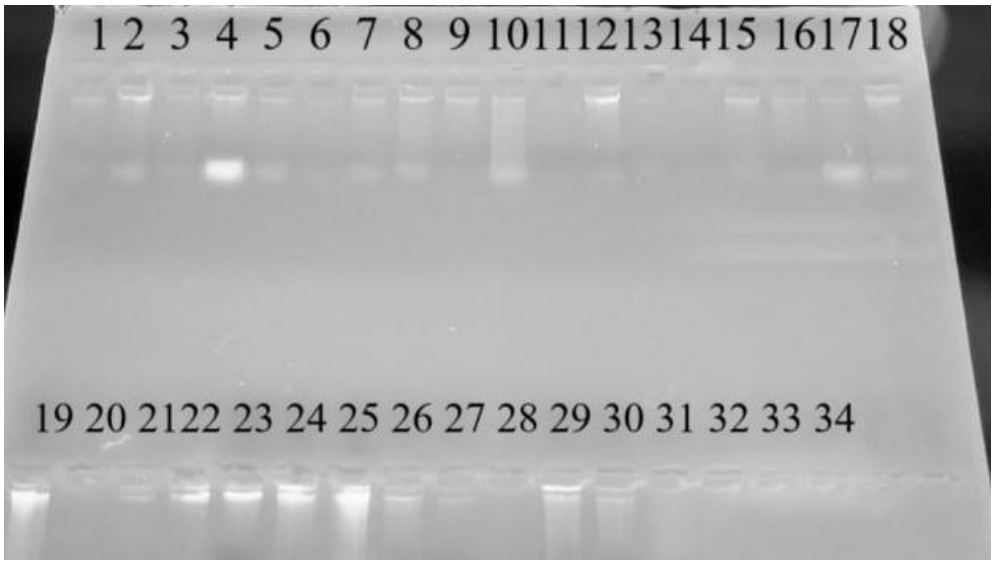

Figure 4.1: Gel Image of Total Genomic DNA from Potable Water and Ice Pop Samples

Lanes: 1) AD1, 2) AD2, 3) AD3, 4) AT1, 5) AT2, 6) BD1, 7) BD2, 8) BD3, 9) BT1, 10) BT2, 11) CD1, 12) CD2, 13) CD3, 14) CT1, 15) CT2, 16) KD1, 17) KD2, 18) KD3, 19) KT1, 20) KT2, 21) WD1, 22) WD2, 23) WD3, 24) WT1, 25) WT2, 26) MD1, 27) MD2, 28) MD3, 29) MT1, 30) MT2, 31) AF1, 32) AS1, 33) BF1

4.6 Diversity Analysis

A total of six samples were analyzed, revealing 238 zOTUs and 171,513 total reads. Both the forward and reverse reads exhibited high quality for the majority of their length with a quality phred score of 25. Juja had a higher zOTUs, 37 (15.5%), while Witeithie had 23 (9.7%) unique zOTUs. Majority of the zOTUs, 178 (74.8%), were shared between both locations (Figure 4.2). A total of 105 zOTUs (44.1%) was shared

among all three water types. Affordable water showed the highest unique zOTUs with 34 (14.3%), followed by Value Added Water with 20 zOTUs (8.4%), and Commercialized water with 16 zOTUs (6.7%). Affordable and Commercialized water shared 48 zOTUs (20.2%) (Figure 4.3).

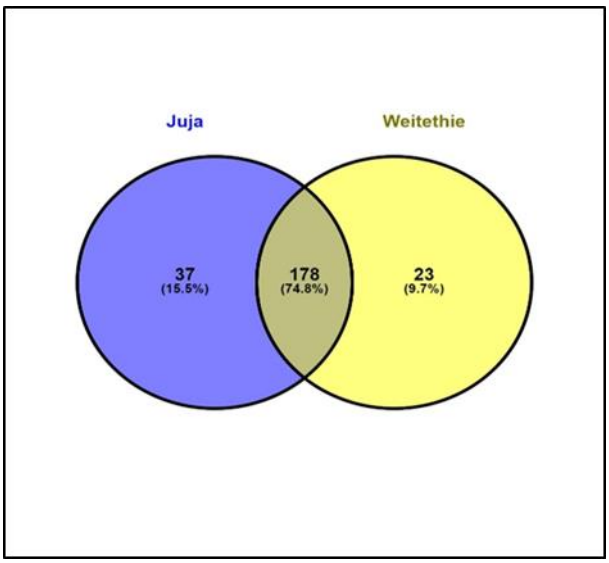

Figure 4.2: Venn Diagram Comparing the zOTUs in Juja and Witeithie. Venn Diagram was done using VENNY

Source: (http://bioinfogp.cnb.csic.es/tools/venny/index.html).

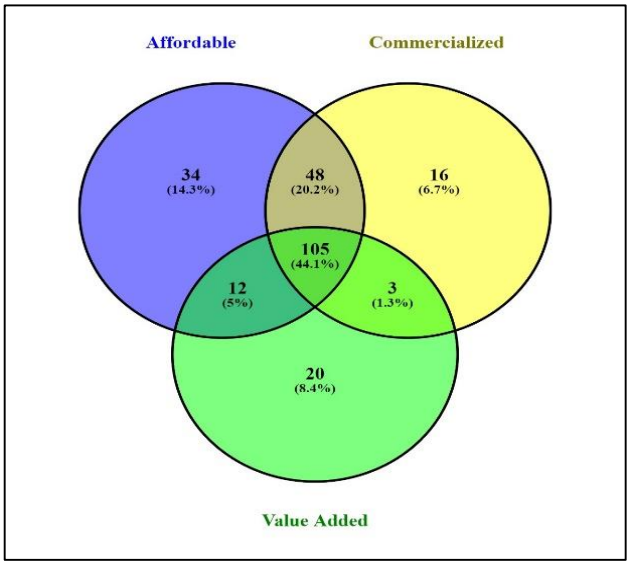

Figure 4.3: Venn Diagram Comparing the zOTUs of the Different Sample Types Venn Diagram was done using VENNY

Source: (http://bioinfogp.cnb.csic.es/tools/venny/index.html).

4.6.1 Alpha Rarefaction

The rarefaction curve levelled off at a sequencing depth of 26,900, which was generally sufficient. Juja indicated a higher observed feature than Witeithie. Both locations showed an increase in observed features with increasing sequencing depth (Figure 4.4). Affordable water indicated the highest observed features followed by Commercialized water while Value Added water recorded the least observed features (Figure 4.5).

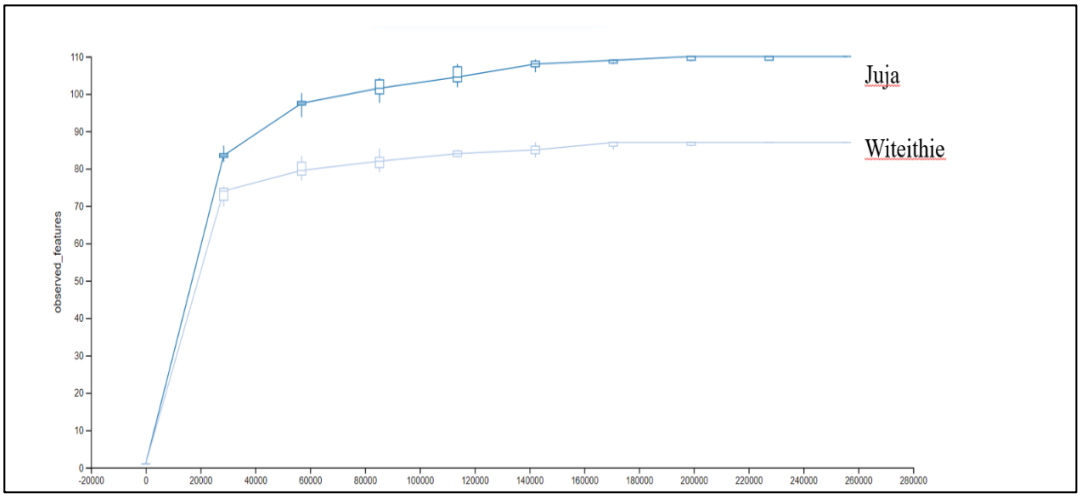

Figure 4.4: Alpha Rarefaction Plot Showing Sample Diversity with Increasing Sequencing Depth Based on the Location

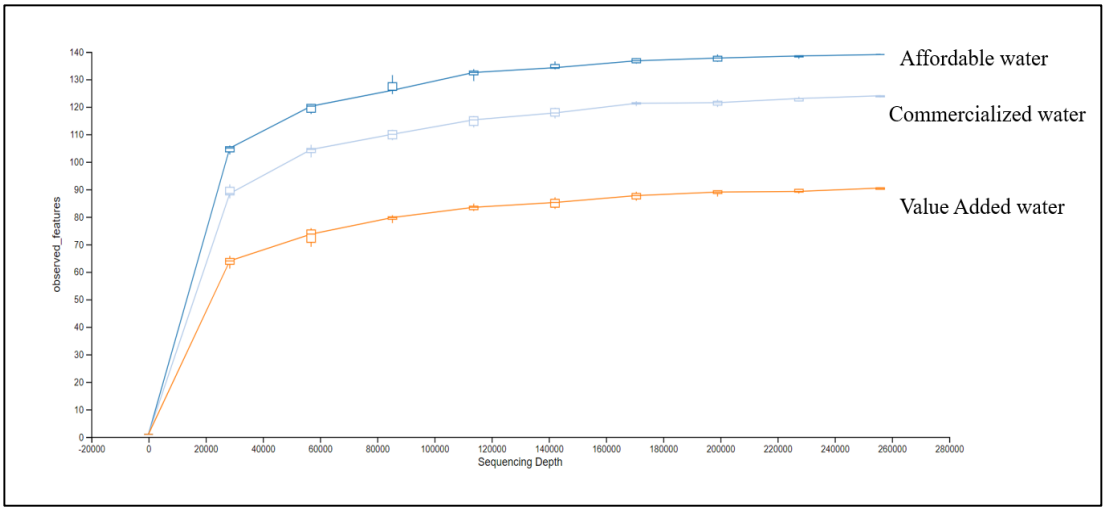

Figure 4.5: Alpha Rarefaction Plot Showing Sample Diversity with Increasing Sequencing Depth Based on the Sample Type

4.6.2 Alpha Diversity Metrics

Samples from Witeithie showed higher species richness compared to Juja, as indicated by the Chao1 index, which estimates the total number of species present. A higher Chao1 value in Witeithie means that more bacterial species were present in these samples compared to those in Juja. However, Witeithie had the lowest Shannon index,

which accounts for both richness and the evenness of species distribution. This indicates that although many species were present, a few species dominated the microbial community, resulting in low evenness. The high Chao1 but low Shannon diversity observed in Witeithie shows that while many bacterial species were present (high richness), the community structure was dominated by a few species, resulting in low evenness. Juja samples had higher Simpson index value (D) compared to Witethie. Simpson metrics (D) measures dominance in a community: higher Simpson values indicate that a few species are numerically dominant, reflecting low overall diversity (Figure 4.6). Juja also recorded a higher Faith's Phylogenetic Diversity (Faith PD) compared to Witethie, which considers the evolutionary relationships among species. This means that bacterial species in Juja were more phylogenetically diverse compared to those in Witethie even if some species were numerically dominant (Figure 4.7).

Affordable water samples had the highest Chao1 and Shannon indices, meaning they had many species that were more evenly distributed, reflecting both high richness and high evenness. Commercialized water showed intermediate richness and evenness. Value-added water had the lowest Chao1 and Shannon indices, indicating fewer species and an uneven community dominated by only a few species. Affordable water also had the highest Simpson index (D), which suggests that, although many species were present, some species were numerically dominant in the community. Value added water had a low simpson index (D) compared to the three samples. This indicated that value-added water had a relatively high community diversity with low species dominance, meaning no single species overwhelmingly dominated the sample (Figure 4.8).Commercialized water recorded the highest Faith PD, meaning its bacterial community had a higher phylogentic diversity, while value-added water had the lowest, reflecting less evolutionary diversity (Figures 4.9).Differences in alpha diversity metrics across sampling locations and water types were evaluated using the non-parametric Kruskal–Wallis test. Although numerical differences in species richness, evenness, and phylogenetic diversity were observed among samples, these differences were not statistically significant for any of the four metrics ($p > 0.05$) (Appendix V).

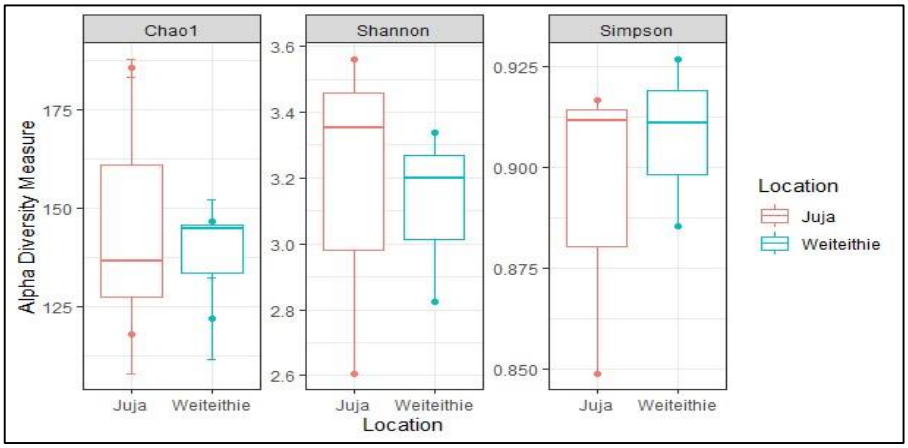

Figure 4.6: Box Plot Indicating Alpha Diversity Metrics for Different Locations

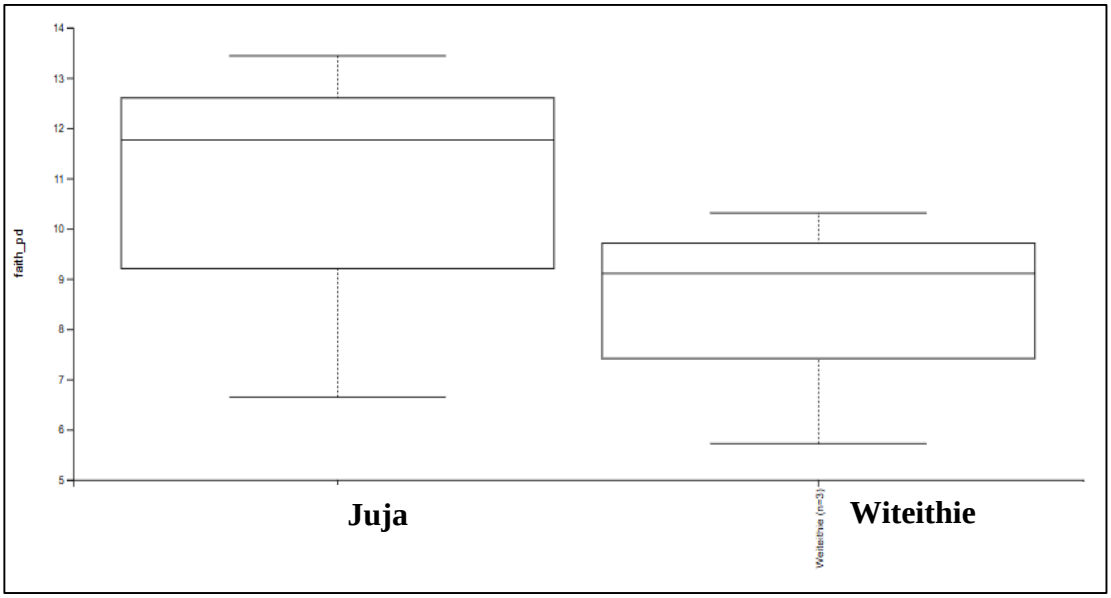

Figure 4.7: Box Plot Showing Faith's Phylogenetic Diversity (Faith_PD) Across Different Locations

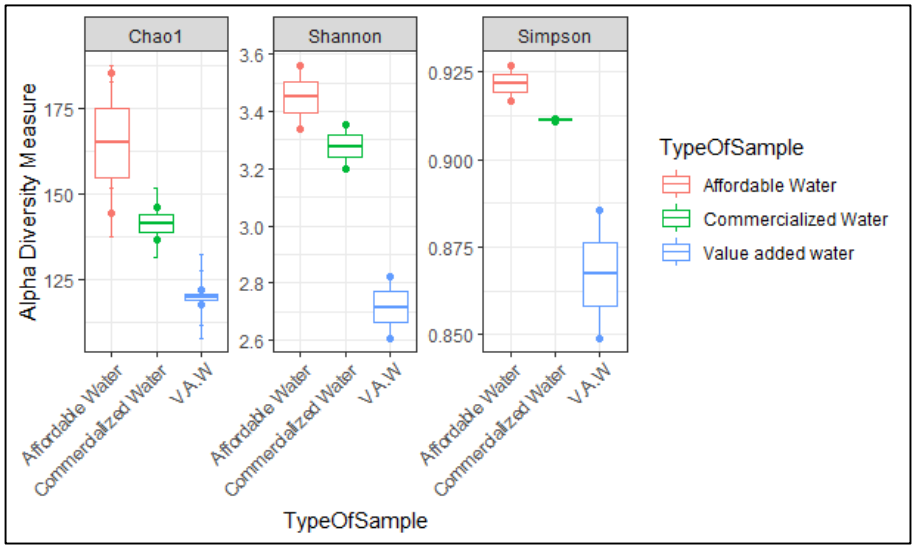

Figure 4.8: Box Plot Indicating Alpha Diversity Metrics for Different Sample Types

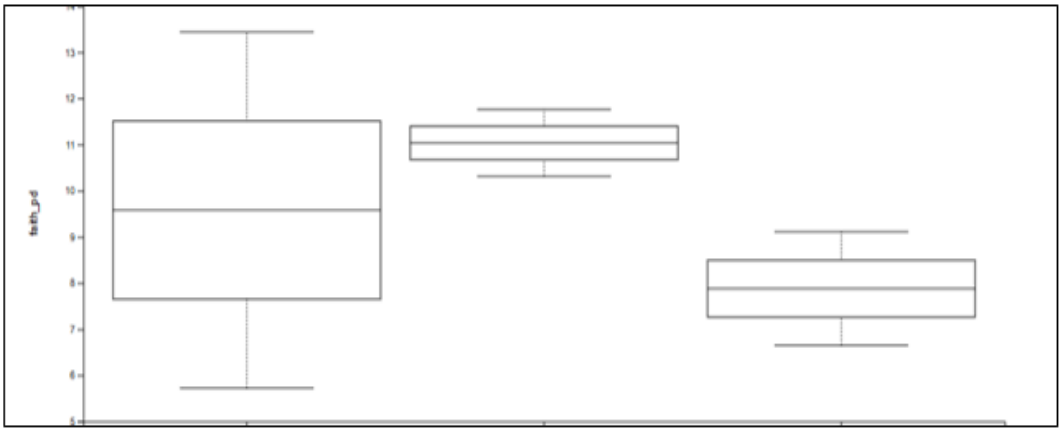

Figure 4.9: Box Plot Showing Faith's Phylogenetic Diversity (Faith_PD) Across Different Sample Types

4.6.3 Beta Diversity Analysis

Dispersion in ordination space reflects differences in community structure between samples.The Bray-Curtis PCoA Analysis indicated 37.8% variation in Axis 1 and 26.8%. Samples from Witeithie exhibited greater dispersion in the ordination space,

indicating high heterogeneity in bacterial community composition across the samples from this region. In contrast, commercialized water samples clustered closely together, reflecting more similar bacterial communities among these samples. Affordable water samples showed moderate dispersion, while value-added water samples displayed the greatest dispersion, suggesting substantial variability in community composition (Figure 4.10).

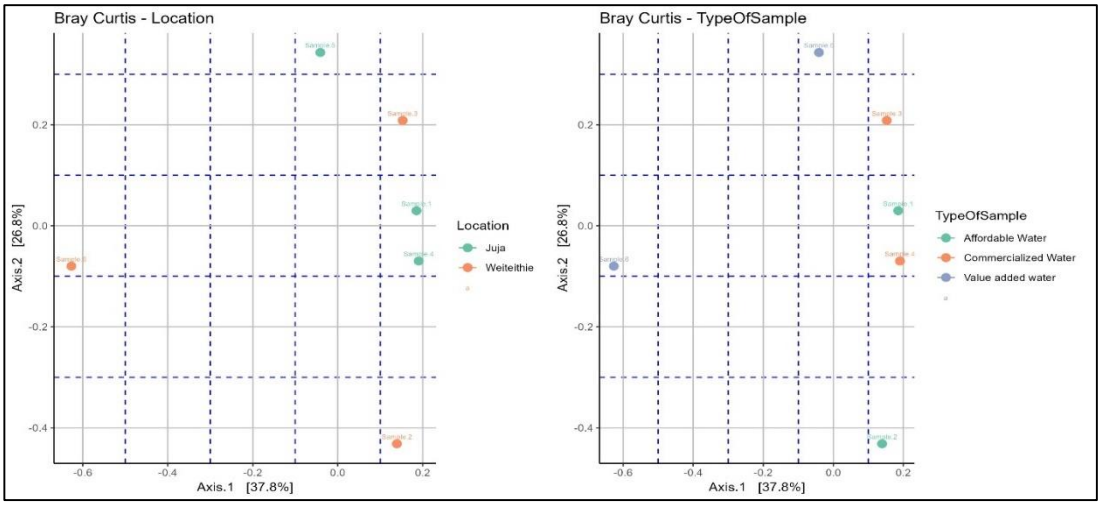

Figure 4.10: Bray-Curtis Plot Indicating Spread of Bacterial Communities among the Different Sample Types and Location

Juja samples were closely clustered, indicating relatively similar bacterial community composition among the samples, although they showed slightly greater separation compared to the Bray-Curtis plot. Witeithie samples exhibited greater dispersion, consistent with the Bray-Curtis results, reflecting higher variability in bacterial community composition across these samples. The sample type-based ordination explained 27.4% of the variation along Axis 1 and 22.9% along Axis 2. Value-added and commercialized water samples were positioned relatively close together, suggesting similar bacterial communities, whereas affordable water samples (green) were more widely dispersed, indicating greater heterogeneity in their community composition (Figure 4.11). Although clustering patterns were visually apparent in the ordination plots, statistical testing was necessary to determine whether these patterns represented significant differences between groups. PERMANOVA analysis based on

Jaccard distance indicated no significant differences in microbial community composition between locations or sample types at the 0.05 significance level.Similarly, Bray-Curtis PERMANOVA showed no significant differences between locations or sample types at the 0.05 significance level (Appendix VI; Appendix VII). The lack of statistically significant differences may be due to the relatively small sample size and the high variability in microbial communities within each group, which could reduce the ability of PERMANOVA to detect differences between locations or sample types.

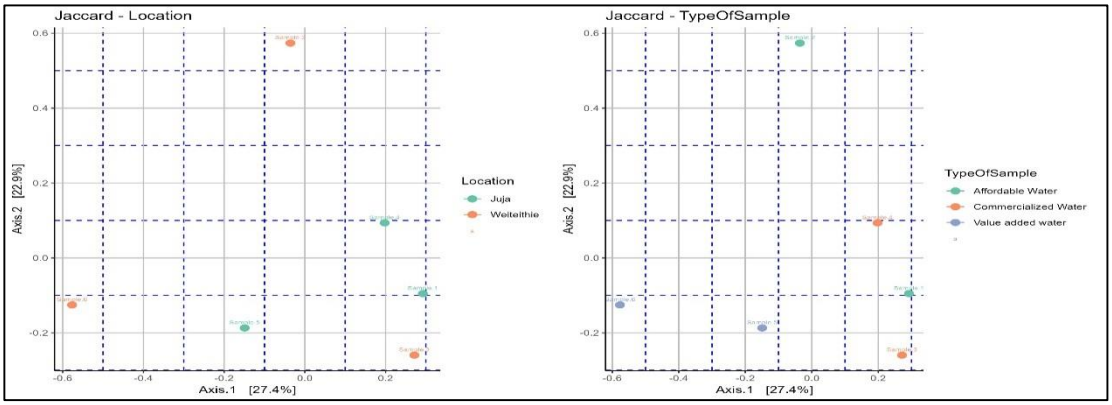

Figure 4.11: Jaccard Combined Plot Indicating Spread of Bacterial Communities among the Different Sample Types and Location

4.7 Taxonomic Characterization of Bacteria

The sequences belonged to 4 phyla, of which Proteobacteria was determined to be the most dominant phylum (82.30%), followed by Firmicutes (15.20%), Bacteriodetes (2.40%) and Actinobacteria (0.01%). Sachet pop samples had the highest number of Proteobacteria, while samples from hawkers had the least. Conversely, hawker's samples recorded the highest number of Firmicutes, with sachet pop samples having the least. Juja B samples had the most Bacteroidetes, whereas Witeithie and hawker's samples had the least. Actinobacteria was only present in frozen pops, with a total of two reads (Figure 4.12).

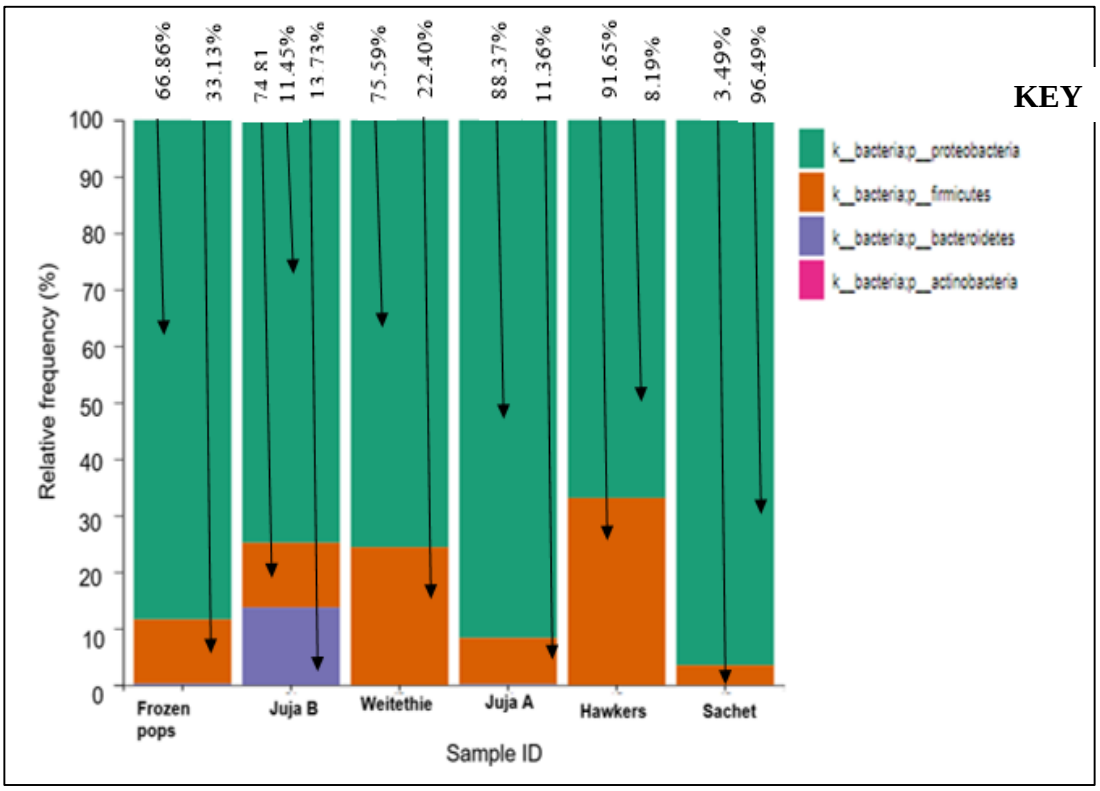

Figure 4.12: Taxonomic Bar-Plots of Bacterial Phyla Present in the Samples

At the genus level, a total of 62 genera were identified across all samples. The overall dominant genus was Pseudomonas (29.7% of total reads), followed by Serratia (8.2%). The least abundant genera included Staphylococcus and Ensifer (0.0006% of total reads). Sachet pop samples were dominated by *Pseudomonas* and *Delftia*, while frozen pop samples were dominated by *Pseudomonas*, *Janthinobacterium*, and *Myroides*. Witeithie samples were dominated by *Pseudomonas*, *Enterococcus*, and *Comamonas*. Juja B samples were dominated by *Pseudomonas*, *Stenotrophomonas*, and *Achromobacter*. Hawker samples were dominated by *Leuconostoc* and *Gluconacetobacter*, whereas Juja A samples were dominated by *Serratia*, *Klebsiella*, and *Gluconobacter*. Genera present in all six sample types included *Pseudomonas*, *Bacillus*, *Klebsiella*, *Delftia*, *Citrobacter*, *Comamonas*, *Stenotrophomonas*, and *Pantoea* (Figure 4.13; Appendix VIII).

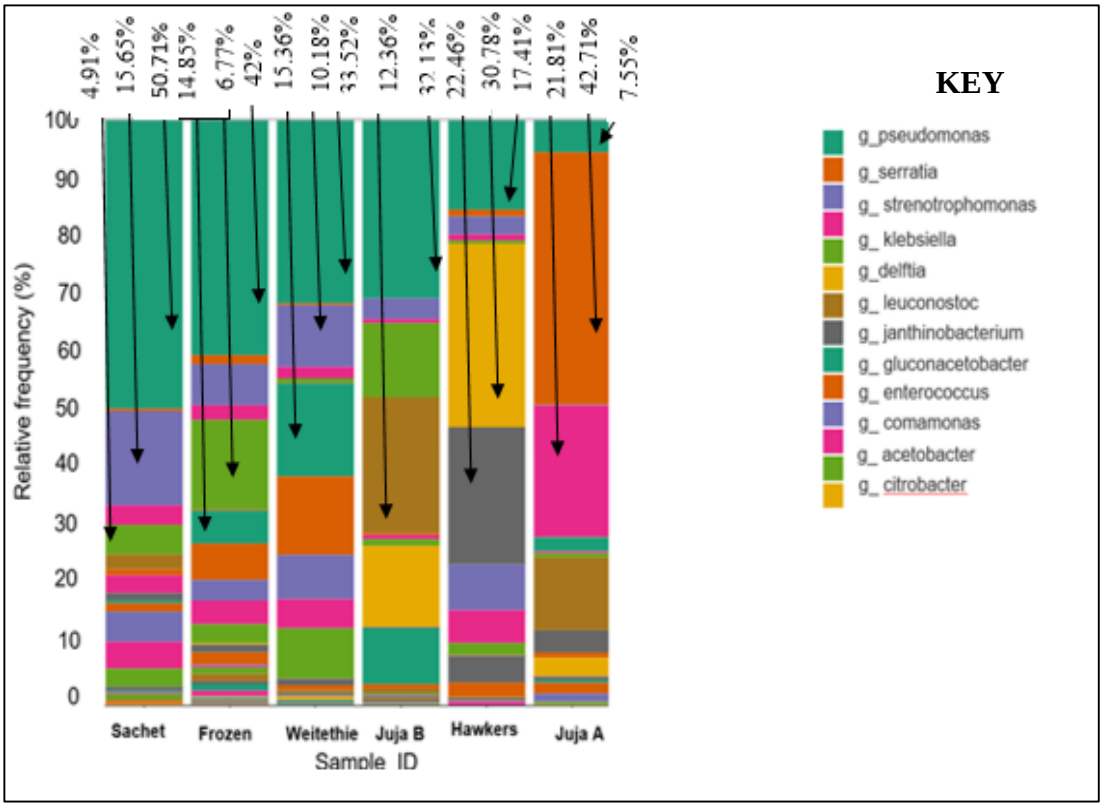

Figure 4.13: Taxonomic Bar-Plots of Bacterial Genus Present in the Samples

At the species level, 113 species were identified. The most dominant species was *Pseudomonas vranovensis* (21.1% of total reads), followed by *Serratia marcescens (7.9%) and Stenotrophomonas maltophilia* (7.3%). The least abundant species included *Staphylococcus pasteuri and Ensifer xinjianensis* (0.0006%). Sachet pop samples were dominated by *Pseudomonas vranovensis, Delftia tsuruhatensis,* and *Stenotrophomonas maltophilia,* while frozen pop samples were dominated by *Janthinobacterium lividum, Pseudomonas vranovensis*, and *Myroides odoratimimus.* Witeithie samples were dominated by *Pseudomonas vranovensis, Enterococcus faecium, and Stenotrophomonas maltophilia.* Juja B samples were dominated by *Pseudomonas vranovensis, Stenotrophomonas maltophilia, and Achromobacter xylosoxidans.* Hawker samples were dominated by *Leuconostoc mesenteroides and Gluconobacter liquefaciens,* whereas Juja A samples were dominated by *Serratia marcescens, Klebsiella oxytoca,* and *Gluconobacter cerinus* (Figure 4.14).

Species present in all six sample types included Pseudomonas vranovensis, Serratia marcescens, Bacillus weihenstephanensis, Klebsiella oxytoca, Delftia tsuruhatensis, Janthinobacterium lividum, Citrobacter werkmanii, Stenotrophomonas maltophilia, Pantoea agglomerans, Pseudomonas plecoglossicida, Pseudomonas straminea, Pseudomonas viridiflava, Pseudomonas rhizosphaerae, Pseudomonas putida, Pseudomonas tarwinensis, Pseudomonas teessidea, Raoultella terrigena, Raoultella planticola, Delftia acidovorans, Klebsiella variicola, Acinetobacter johnsonii, and Methylobacterium thiocyanatum.

Some species were sample-specific: Leuconostoc mesenteroides, Gluconacetobacter liquefaciens, and Acetobacter indonesiensis were unique to hawker samples; Myroides profundi was only found in frozen pop samples; Achromobacter xylosoxidans was found in sachet pop and Juja B samples. Additionally, Bacillus cereus was present in Juja B and Witethie, Bacillus coagulans in Samples Juja A and Witethie, and Serratia quinivorans in Witethie and Sachet pops (Figure 4.14; Appendix IX).

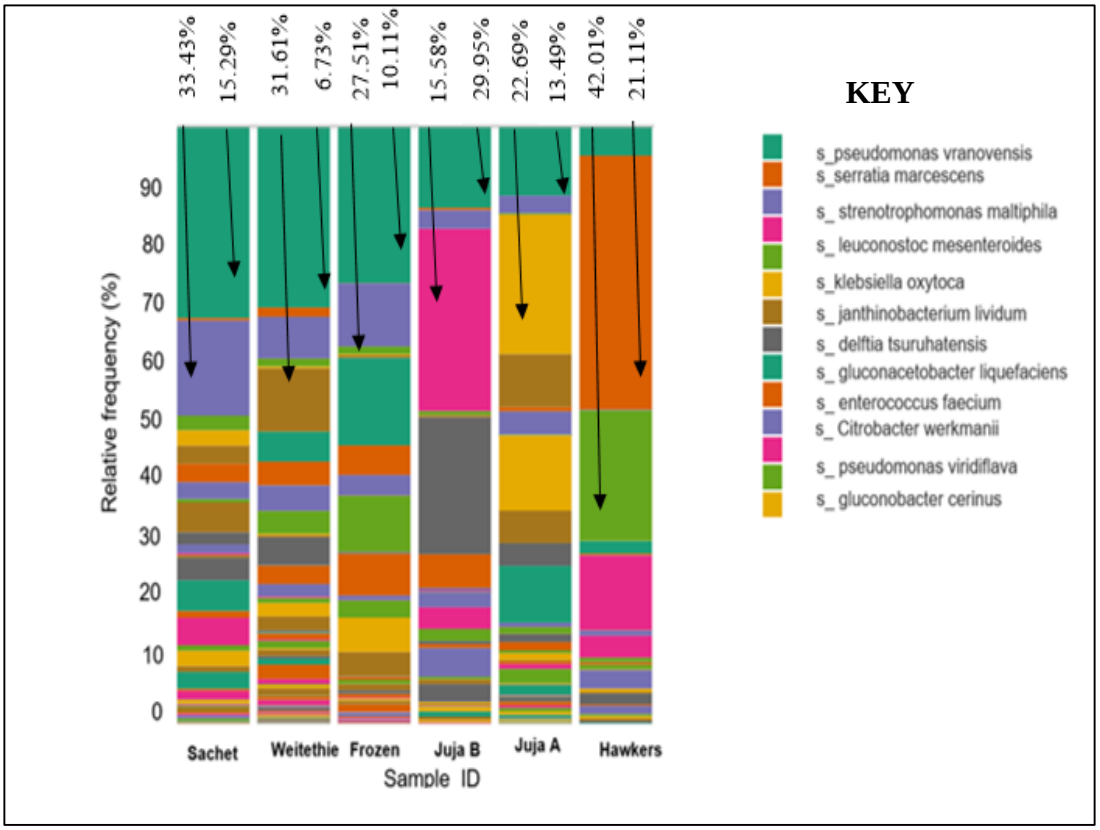

Figure 4.14: Taxonomic Bar-Plots of Bacterial Species Present in the Samples

4.8 Diversity of Pathogenic Bacteria Using Culture Independent Techniques

The heatmap combined with hierarchical clustering was used to examine similarities in bacterial community composition across locations and sample types. Clustering was based on relative abundances, grouping bacteria with similar counts and locations with similar species composition. The analysis revealed that Sachet pop and Juja B samples clustered together, as did Witeithie and Hawker samples, while Juja and Kenyatta Road samples formed another cluster (Figure 4.15). At the species level, *Acinetobacter lwoffii* clustered with *Pantoea vagens*, and *Enterococcus faecium* clustered with *Enterococcus faecalis*, whereas *Raoultella planticola*, *Klebsiella oxytoca*, *Serratia marcescens*, *Cronobacter turicensis*, and *Erwinia pyrifoliae* formed a distinct cluster.

Pathogenic bacteria were detected in all water and ice pop samples, with Sachet pops showing the highest proportion (35%), followed by Hawkers (17.5%) and frozen pops (7.7%). *Serratia marcescens* was the most abundant pathogen overall (20.7%), particularly in sachet pops, followed by *Stenotrophomonas maltophilia* (17.1%), which was present in all samples. Some pathogens were sample-specific: *Myroides odoratimimus* in Witeithie, *Cronobacter turicensis* in sachet pops, *Achromobacter xylosoxidans* in Hawker samples, and *Salmonella enterica* in frozen pops. Others, such as *Klebsiella oxytoca*, *Acinetobacter johnsonii*, *Citrobacter werkmanii*, and *Burkholderia cepacia*, were widely distributed across samples.

Pathogenic bacteria were detected in all water and ice pop samples collected in Juja. Sachet pops exhibited the highest proportion of pathogenic microorganisms (35%), followed by Hawker samples (17.5%), while frozen pops had the lowest proportion (7.7%). Among the pathogens, *Serratia marcescens* was the most abundant overall (20.7%), particularly dominant in sachet pops, followed by *Stenotrophomonas maltophilia* (17.1%), which was consistently present across all sample types. *Klebsiella oxytoca* and *Acinetobacter johnsonii* were also detected in all samples, indicating widespread distribution.

Several pathogens were sample-specific or dominant in certain locations. *Bacillus cereus* was primarily found in Witeithie samples, while *Enterococcus faecalis* dominated Juja A and Juja B but occurred in low abundance in sachet pops. *Enterococcus faecium* was present in Juja A, Juja B, and sachet pops, with highest abundance in Juja B. *Salmonella enterica* was abundant in frozen pops but absent from other samples. Some species were highly localized: *Myroides odoratimimus* in Witeithie, *Cronobacter turicensis* in sachet pops, and *Achromobacter xylosoxidans* in Hawker samples. *Providencia rustigianii* occurred only in Juja and Witeithie, whereas *Citrobacter werkmanii* and *Burkholderia cepacia* were widely distributed across most samples.

The highest pathogen counts were observed for *Stenotrophomonas maltophilia* and *Enterococcus faecium* in Juja B, whereas *Providencia rustigianii* and *Cronobacter turicensis* were least abundant. In Hawker samples, *Stenotrophomonas maltophilia*

dominated, while *Cronobacter turicensis* was the least abundant. In frozen pops, *Citrobacter werkmanii* was most abundant, with *Acinetobacter lwoffii* being the least. *Serratia marcescens* remained dominant in sachet pops, whereas *Enterococcus gallinarum* had the lowest abundance overall (Figure 4.15).

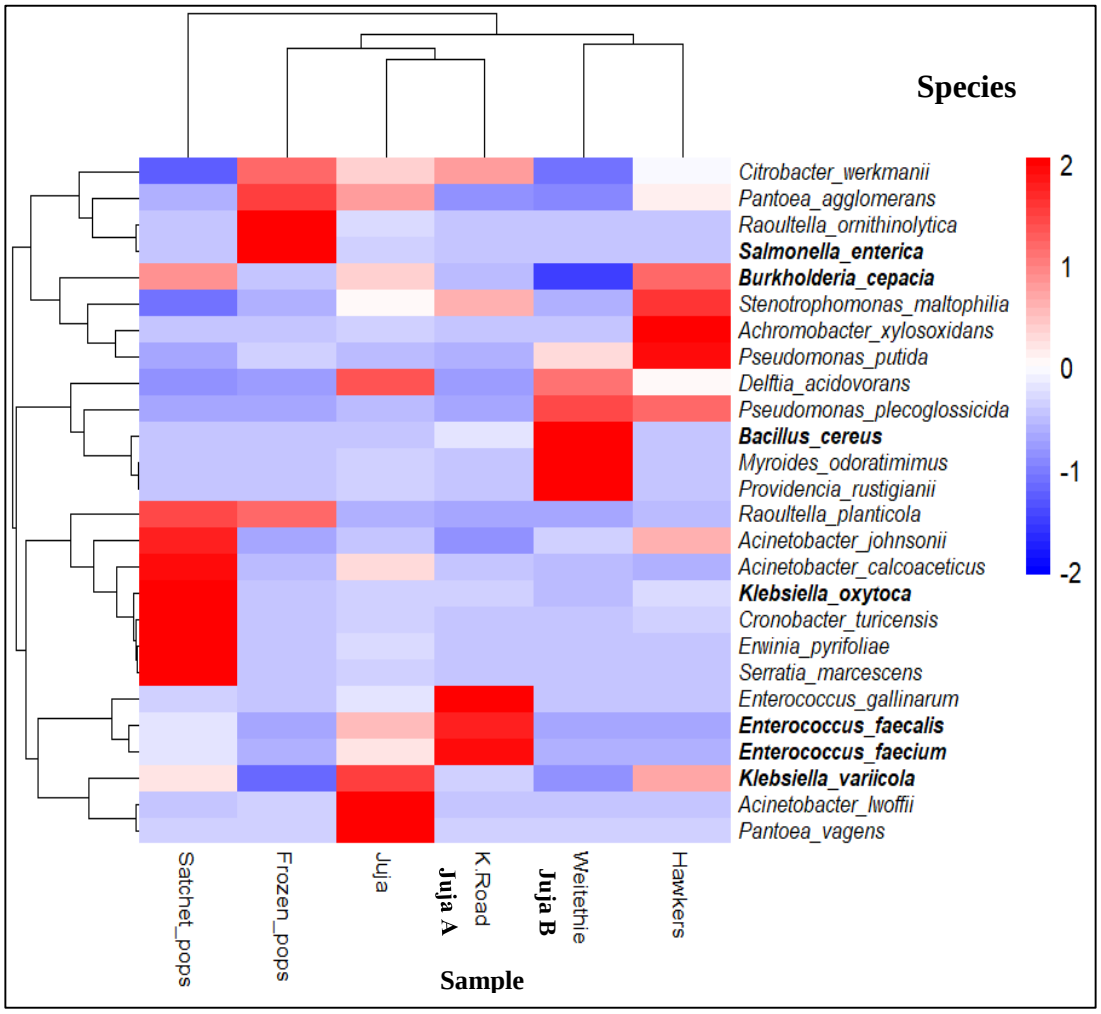

Figure 4.15: Diversity of Pathogenic Bacteria in the Different Sample Types

4.9 Phylogenetic Diversity of Pathogenic Bacteria

The phylogenetic tree reveals a high level of bacterial diversity, with clear clustering of species according to their phylogenetic affiliations. Members of the *Enterobacteriaceae* family, including *Klebsiella oxytoca*, *Klebsiella varicola*, *Erwinia pyrifoliae*, *Serratia marcescens*, and *Cronobacter turicensis*, form a distinct clade,

suggesting close evolutionary relationships likely due to shared ancestry and similar ecological niches.

Gram-positive *Enterococcus* species *E. faecalis*, *E. gallinarum*, and *E. faecium* (bootstrap values of 92,100,88 respectively) indicated a distinct lineage of potential clinical relevance, as these organisms are commonly associated with opportunistic infections.The genus *Acinetobacter* appears in a clades: one containing *A. calcoaceticus* and *A. lwoffii* (bootstrap = 100%). *Stenotrophomonas maltophilia*, *Achromobacter xylosoxidans*, *Burkholderia cepacia*, and *Delftia acidovorans* form another strongly supported group (bootstrap values of 99,98,99,98 respectively), highlighting their close genetic relatedness.A distinct branch is occupied by *Providencia rustigianii*, which separates early from the major clusters, indicating its unique evolutionary lineage within the *Enterobacterales*.The lower part of the tree shows additional separation of important species such as *Pseudomonas putida*, *P. plecoglossicida*, and with the *Pseudomonas* species forming a strongly supported cluster with bootstrap values of 99 and 100 respectively, reflecting their phylogenetic coherence within the *Pseudomonadaceae* family. *Salmonella enterica*, *Pantoea vagans*, *Citrobacter werkmanii*, and *Raoultella* species (*R. ornithinolytica* and *R. planticola*) are grouped into distinct but related branches. *Pantoea agglomerans* appears as a distinct lineage situated between two major clusters. It branches independently and shows a clear separation from *Pantoea vagans*, which is located in a different clade closer to *Salmonella enterica* and *Raoultella* species (Fig 4.16).

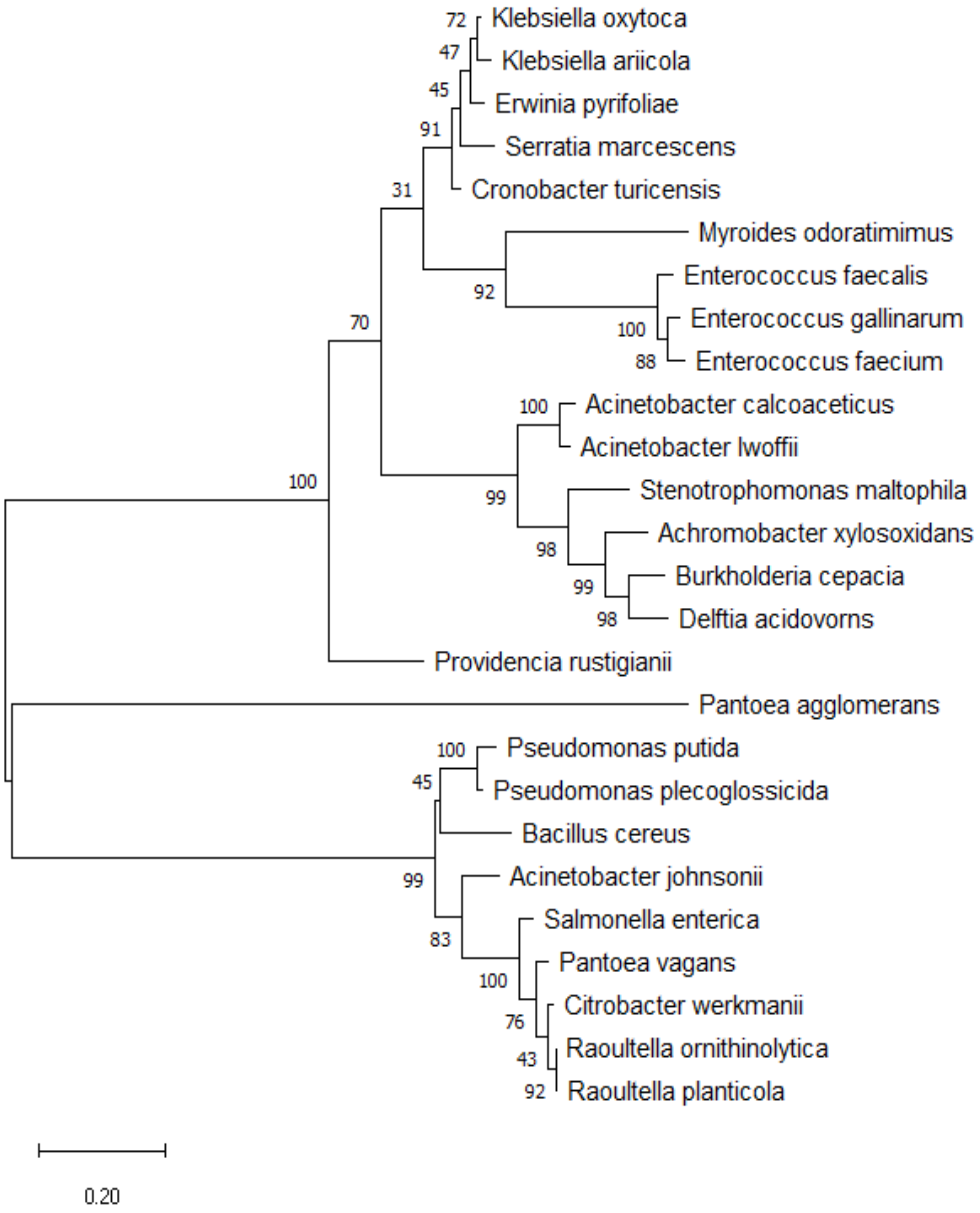

Figure 4.16: Phylogenetic Diversity of Pathogenic Bacteria in the Water and Ice Pop Samples

4.10 Antibiotic Susceptibility Testing

Ciprofloxacin was the most effective antibiotic against the isolated bacteria from the water and ice pop samples. The resistant organism to Ciprofloxacin was *Proteus*

mirabilis. Amoxicillin, Penicillin, and Chloramphenicol were highly resisted, with 95.2% (20 out of 21) of the tested isolates showing resistance to each. Streptomycin and Tetracycline also faced significant resistance, with 13 and 18 resistant isolates, respectively (Appendix XVI) Intermediate resistance was observed in a few cases: Corynebacterium showed intermediate resistance to Amoxicillin and Penicillin, while *Klebsiella* and *Proteus* showed intermediate resistance to Tetracycline (Table 4.5).A total of 20 out the tested 21 putative microorganisms were classified as Multidrug Resistant since they showed resistance to more than three classes of the antibiotics tested.One of the putative microorganisms (Proteus mirabilis) was classified as pandrug resistant since it was able to resist five different antibiotic classes that were tested against it (Appendix XVII).

Table 4.6: Antibiotic Susceptibility Test Results of Different Microorganisms to Commercial Antibiotics

Isolated microorganism	Tetracycline	Amoxicillin	Ciprofloxacin	Penicillin	Chloramphenicol	Streptomycin
Escherichia coli	R	R	S	R	R	R
Shigella spp.	R	R	S	R	R	R
Serratia spp.	R	R	S	R	R	R
Acinetobacter spp.	R	R	S	R	R	S
Bacillus spp.	R	R	S	R	R	R
Enterobacter spp.	R	R	S	R	R	R
Staphylococcus spp.	R	R	S	R	R	R
Listeria spp.	R	R	S	R	R	R
Corynebacterium spp.	R	I	S	I	R	S
Pseudomonas spp.	R	R	S	R	R	R
Klebsiella spp.	I	R	S	R	R	I
Streptococcus spp.	R	R	S	R	R	S
Enterobacter spp.	R	R	S	R	R	R
Salmonella spp.	R	R	S	R	R	S
Enterococcus faecalis	R	R	S	R	R	R
Moraxella spp.	R	R	S	R	R	S
Cedecea spp.	R	R	S	R	R	R
Proteus spp.	I	R	S	R	R	R
Providencia spp.	R	R	S	R	R	S
Proteus mirabilis	R	R	R	R	R	R
Enterococcus faecium	S	R	S	R	R	I

Note: R-Resistant S-Susceptible I-Intermediate

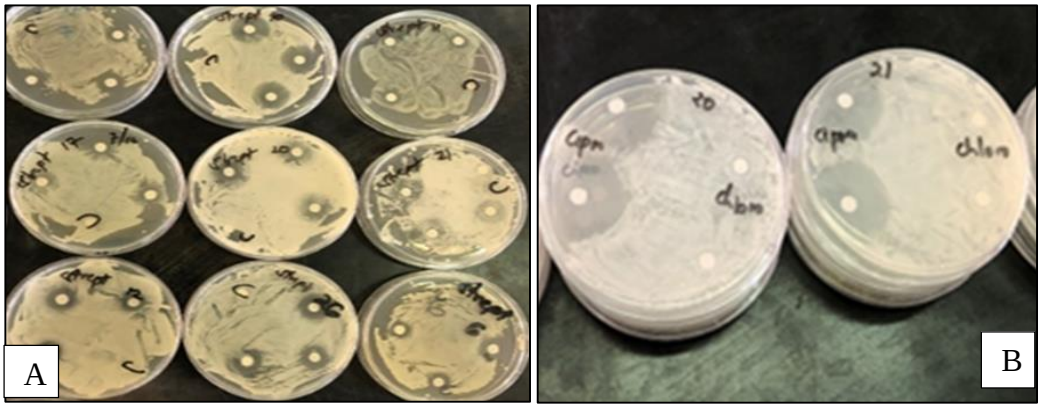

Plate 4.2: Commercial Antibiotics against the Various Bacterial Isolates. A represents Streptomycin, B represents Ciprofloxacin (right side of the petri plate) and Chloramphenicol (left side of the petri plate)

The bacteria isolates also indicated resistance against the commercial Himedia Laboratory antibiotic disks. All the bacterial isolates were resistant to Cotrimoxazole and Sulfamethoxazole. All the bacterial isolates apart from *Streptococcus spp*. were resistant to Ampicillin. Tetracycline, Nitrofurantoin and Nalidixic Acid showed mixed results, with majority of the isolates being resistant while others were either susceptible or intermediate. All the tested isolates were susceptible Gentamicin and Streptomycin (Table 4.6).Plate 2 (B) shows larger inhibition zones for ciprofloxacin compared to chloramphenicol, supporting its higher effectiveness.

Table 4.7: Antibiotic Susceptibility Test Results of Different Microorganisms to Himedia Laboratories LLC Antibiotics Disks

Putative microorganisms	AMP	TET	NIT	NAL	COT	STR	SUL	GEN
Eschericia coli	R	R	R	R	R	S	R	S
Shigella spp.	R	R	R	R	R	S	R	S
Klebsiella spp.	R	S	S	S	R	S	R	S
Streptococcus spp.	I	R	R	I	R	S	R	S
Providencia spp.	R	S	R	I	R	S	R	S
Salmonella spp.	R	R	R	R	R	S	R	S

Note: R-Resistant S-Susceptible I-Intermediate, NIT- Nitrofurantoin, COT-Cotrimoxazole, SUL- Sulfamethaxozole, GEN- Gentamicin , STR- Streptomycin

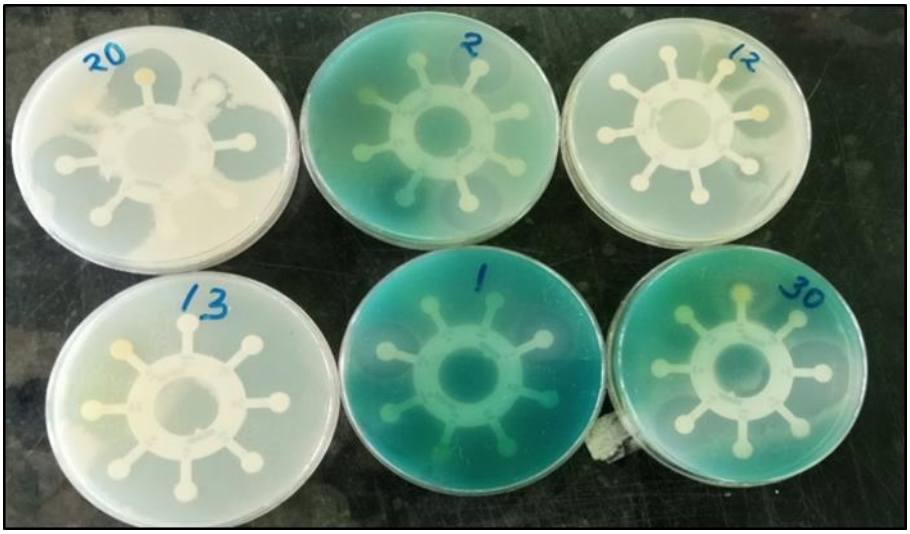

Plate 4.3: Himedia Laboratories LLC antibiotics Disks against Bacterial Isolates (Isolate 20: *Providencia* , Isolate 2: *Shigella* :Isolate 13:*Enterococcus faecalis*, Isolate 30:S*almonella*, Isolate 1:*Eschericia coli)*

4.11 Profiling of Antibiotic Resistance Genes

Antibiotic-resistant genes were detected from the water and ice pop samples. All the identified genes belong to the aminoglycoside drug class. Water samples from hawkers had the highest number of resistance genes (6), while Juja B and Frozen pops had the least number (3). Four antibiotic resistance genes were detected in sachet pops, frozen pops, Witeithie, and Juja A samples.The most prevalent genes were *aac(6')-Iae* and *aac(6')-Iai*, which were present in all samples while *aac(6')-I33* was the least prevalent as it was only present in the frozen pop samples. The gene *aph(3')-VIIIa* was found in Witeithie and hawkers samples. The mutated rrsB gene was present in all the samples apart from the Witeithie samples (Fig 5). Clustering was observed in samples from Juja A, Juja B, and Sachet Pops because they indicated prevalence similar resistant genes (*aac(6')-Ia, aac6')-Iae, aac(6')-Iai*, and Mutated *rrsB*). Hawkers and frozen Pops samples also clustered together because they had unique genes like as *aac(6')-Iaj* in and *aac(6')-I33* in respectively. However, they also shared the *aac(6')-Iae* and *aac(6')-Iai* genes. Juja A and Juja B clustered together because they had almost similar type of resistant genes present in their samples and the same applies to Witeithie and Hawkers samples (Figure 4.21;Appendix IV).Figure 4.21 illustrates the distribution of the antibiotic resistant genes in the different samples using red and blue colors. Red color indicates presence of one ARG whereas blue indicates absence of the ARG in that specific sample.

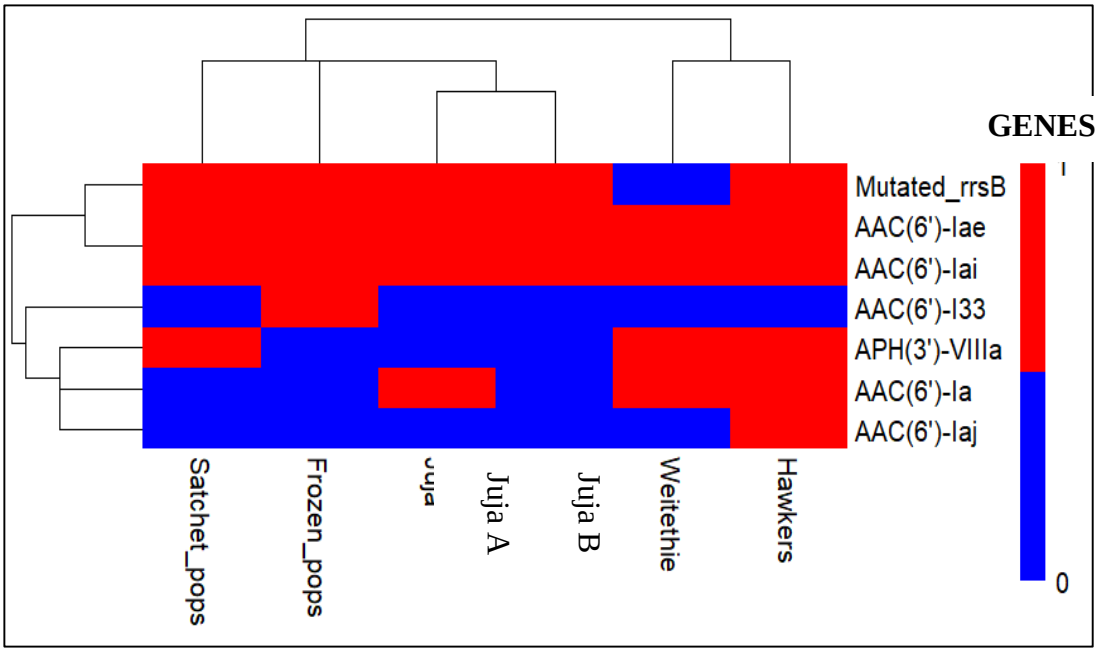

Figure 4.17: Distribution of Antibiotic Resistant Genes among the Various Sample Types

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

5.1.1 Adherence to Water Vending Guidelines

Most of the water vendors did not comply with the WASREB guidelines set by the Kenyan Government to help ensure that safe water is sold to citizens. Despite some vendors trying to abide by the WASREB guidelines, their products were still contaminated and unsafe for consumption. The results of this study contrast with Guhad's (2022) evaluation of water quality at refill dispensers in the Eastern Estates of Nairobi. Guhad reported that most water refill station operators complied with WASREB guidelines, including obtaining the required licenses, conducting regular water quality tests, and applying proper water treatment methods. Despite this compliance, bacterial contamination was still detected in over one-third of the samples collected. In comparison, the present study found that 99% of water samples collected from vendors were contaminated. This higher contamination rate appears to be linked to non-compliance, as the majority of vendors in this study did not adhere to WASREB guidelines, highlighting that proper adherence to regulations is critical in reducing microbial contamination in water intended for human consumption (Guhad, 2022).

According to Morin-Crin *et al*. (2022), compliance with WASREB guidelines often requires capital investments in tools, treatment plants, and reserve tanks, which many vendors cannot afford due to their small capital base. Majority of the vendors prefer operating within the informal sector with minimal regulatory oversight, and they often prioritize immediate profitability over compliance, especially when water sources are already compromised (Morin-Crini *et al*., 2022). Even though water may initially be treated, improper storage, handling, and inadequate hygiene practices can lead to contamination. Most vendors do not regularly test water quality, resulting in lapses in safety standards. Additionally, water may become contaminated while in transit due to

poor transportation infrastructure (Habtu *et al*., 2024; Leslie *et al*., 2021; Salamandane *et al*., 2021)

Affordable water (water from taps and refill dispensers) in Juja is majorly distributed from the water and sanitation company (Ruiru Water and Sanitation Company, Thika Water and Sanitation Company). The companies treat the water and supplies water that has undergone rigorous purification and treatment processes. The Water and Sanitation companies are required to comply with strict regulatory standards set by government authorities. However, the study revealed that some level of contamination was still present in the water provided by the water and sanitation companies. The water and sanitation companies are the major suppliers of water refill dispensers and tap water vendors. The contamination levels in these samples could be due to both the handling practices of the water vendors and the supply infrastructure, specifically the piping systems (Ngasala *et al*., 2021). Faulty pipes with leakages may allow contaminants to enter the water transport system before it reaches the vendors and consumers. The development of biofilms within pipe systems further exacerbates contamination risks (Goraj *et al*., 2021; Learbuch *et al*., 2021). Despite these concerns, many vendors overlook the importance of water purification and treatment, operating under the misconception that supplied water is already purified. Despite the treatment efforts by the Water and Sanitation companies, some pathogenic microbes which are resistant to treatment may still survive in the water supplied to consumers (Guo *et al*., 2021).

Total Coliforms in the potable water samples was an indicator that the water samples and frozen ice pop samples were contaminated, and that they did not meet the WHO standards (Bahrain *et al*., 2019). The highest contamination levels in frozen pops (1073000 CFU/ML) were because they are prepared using unregulated water of unknown microbial quality. The preparation environment may contribute to contamination of the additives used in production thus affecting the quality of the final products. Additionally, the handling of frozen pops also contributed to their rapid contamination. Since the frozen pops are not packaged but stored in freezers in their frozen state, frequent handling during selling increases the likelihood for contamination (Guo *et al*., 2021). The absence of total coliforms in the sachet ice pops

could be as a result of high standards attained during industrial production. The production of sachet pops involves sterilization and preparation is done in controlled environment. They undergo sealed packaging and regulatory compliance hence lowering the chances of contamination (Guo *et al*., 2021).

Bottled water samples from hawkers indicated high levels of contamination due to unreliable and untreated water sources, lack of proper treatment strategies, inadequate storage conditions and improper handling of the water. Majority of water hawkers source their water from avenues that are not safe for human consumption, such as river water, well water or water from boreholes which by then may contain pathogens. Most of the hawkers have no access to proper water treatment facilities. This means that the water they sell undergoes very little to no purification, and thus, microbial contaminants remain present (Akhbarizadeh *et al*., 2020; Bradley *et al*., 2023).

The study found no significant differences in alpha or beta diversity among the samples, indicating a high degree of similarity in microbial community composition. This uniformity is likely attributable to the fact that most water vendors including hawkers and individuals producing homemade ice pops source their water from a common supply, the county water services. As a result, the microbial diversity across these products remains relatively consistent, suggesting that the origin of the water plays a more critical role in shaping microbial communities than subsequent handling or processing practices (Hull et al., 2019). The study found that some water refill dispenser vendors source their water from private suppliers who are not regulated by any authorities. While some suppliers are accredited, the regulations governing them are often less stringent. As a result, these unregulated suppliers may not adhere to strict water treatment and handling guidelines, leading to the supply of water that does not meet safety standards. The reluctance of water refill dispenser vendors to utilize chlorination in water treatment due to concerns about corrosion of their filtration systems is also a major point of concern as it poses an additional challenge in maintaining water quality standards (Costa *et al.*, 2024). Therefore, non-compliance with regulatory guidelines, economic constraints, and poor handling practices are major contributors to microbial contamination. The treatment at the source alone is

insufficient, as inadequate downstream handling and storage by vendors can compromise water safety and pose significant public health risks.

5.1.2 Diversity of Pathogenic Bacteria in Potable Water and Ice Pops

The study highlighted the diverse microbial distribution across different samples revealing insights into microbial community structure and distribution. Affordable water samples had the highest bacterial richness and evenness. This could be attributed to the fact that affordable water samples were sourced from different regions with some not justifiable to be safe for drinking. Some sources have different bacterial communities which can easily be spread to other sources. The water distribution systems for example the piping systems may also contain a community of microorganisms which could easily be transferred to the affordable water sources. The handling of affordable water also matters a lot since this could be an avenue of introduction of more bacterial communities. Many affordable water (water from taps and water refill dispensers) sources undergo minimal or no treatment as it was confirmed by the survey and, allowing a more diverse microbial community to thrive (Mishra *et al*., 2020).

The value-added water products specifically the sachet ice pops recorded a lower diversity due to the fact that water undergoes various stringent processes and treatments that effectively lower microbial counts. Additionally, the fact that the microbial diversity in Juja seems to be higher than that of Witeithie is an evidence that location affects microbial diversity due to varying environmental changes, quality water sources and human interferences (Nguyen *et al*., 2021). This variation brings out the fact that location plays a critical role in determining the bacterial community in water samples (Walters & Martiny, 2020; Zhang *et al*., 2021).

Jaccard and Bray-Curtis indices indicated the patterns of bacterial distribution. The Jaccard index, which measures community similarity based on species presence or absence, indicated that some sample types and locations shared similar bacterial species. However, notable differences were observed between Affordable and Value-added water. Affordable water, typically from taps and refill dispensers, is plain with

no added nutrients. In contrast, Value-added water, such as ice pops, contains additives and nutrients like sugar. These additives create a different environment that supports not only the common waterborne bacteria but also promotes the growth of additional microbial communities that may be specific to that environment due to added nutrients. This explains the distinct bacterial compositions observed between these two types of water samples (Nguyen *et al*., 2021; Walters & Martiny, 2020; Zhang *et al*., 2021).

The phylogenetic tree reflects evolutionary divergence driven by genetic variation, ecological adaptation, and functional specialization among bacterial species. Closely clustered taxa shared higher sequence similarity, indicating recent common ancestry or conserved functional genes, while distant branches reflect greater genetic divergence. Environmental pressures, such as habitat, nutrient availability, and antimicrobial exposure, likely shaped the genetic makeup of these bacteria over time. The tree indicated the ecological and clinical relevance of the isolates, reflecting their potential roles in environmental persistence, pathogenicity, and antimicrobial resistance dissemination. Gram-positive *Enterococcus* species (e.g., *E. faecalis*, *E. gallinarum*, *E. faecium*) cluster tightly, indicating a shared evolutionary lineage and conserved genomic traits typical of Firmicutes. Members of the Gram-negative *Enterobacteriaceae* (e.g., *Klebsiella*, *Serratia*, *Pantoea*, *Cronobacter*) form separate, more diverse clades, reflecting greater diversity and variability (Cattoir, 2022; Huang *et al*., 2021).

The health significance of the observed diversity is further emphasized by the dominance of bacterial groups frequently associated with waterborne and opportunistic infections. The dominance of Proteobacteria in this study coincides with previous studies on by Potgieter *et al*., (2020), Brumfield *et al*., (2021); Jeong *et al*., (2021); Zhang *et al*., (2021) on microbial diversity of drinking water.The studies clearly report that that Proteobacteria was the most dominant phylum in drinking water systems and other large water systems. Firmicutes, Bacteriodetes and Actinobacteria were also present in the samples of this study. Studies by Jiang *et al*., (2021) and Zhou *et al*., (2021) also confirmed that the phylum Firmicutes, Bacteriodetes and Actinobacteria are common in drinking water. A study conducted by Jiang *et al*. (2021)

on microbial diversity on large drinking water sources indicated Actinobacteria as the most abundant phylum while Proteobacteria was also among the dominant bacteria. Despite Actinobacteria being the least dominant phylum in this study, it is clear that there is a relationship between the studies with the variations suggested to be attributed to various factors such as location and environmental factors.

A study conducted by Thom *et al*., (2022) coincides with this study. The study confirmed that *Pseudomonas* was the most dominant genera in drinking water from sources and taps. The genus *Pseudomonas* was observed to be dominant in both untreated and treated water treated by chlorines and chloramines (Thom *et al*., 2022). The presence of *Pseudomonas, Klebsiella, Stentrophomonas* and *Enterococcus* genera in this study is a clear indication of contamination. This is because these bacteria are known to cause opportunistic infections among humans including respiratory tract infection, pneumonia, Urinary Tract Infections, blood infection, Endocarditis (Zhou *et al*., 2021).Previous studies conducted by Brumfield *et al*., (2021) and Zhou *et al*., (2021) have indicated that the genus *Pseudomonas, Bacillus, Klebsiella, Stenotrophomonas* and *Enterococcus* have frequently been reported in drinking water microbiome, aligning with the findings in this research. Additionally, Diniz Rocha *et al*., (2020) reported the abundance of *Stenotrophomonas maltophila* in drinking water systems and some associated with healthcare settings. A study conducted by (Bhat *et al*., 2022) also confirmed the presence of genera *Delftia* in environmental niches including water systems. *Delftia* was also among the dominant genera in most samples in this study. The dominance of genera *Klebsiella* in the study is also consistent with previous studies which have indicated the persistence of genus *Klebsiella* in water systems (Palusiak, 2022).The previous studies provide a supportive context for the microbial profiles identified in this study.

The metagenomic sequencing results revealed a high number of pathogenic bacteria in the potable water and ice pop samples compared to the culture dependent technique. Some of the microorganisms obtained from metagenomics are categorized by WHO as pathogenic while some of them have been reported to cause infections according to the existing literature (Abo-Zed *et al*., 2020; Aguirre-Sánchez *et al*., 2023; Bahrain *et*

al., 2019; Herrera-Hidalgo *et al*., 2020; Jessberger *et al*., 2020, 2020). Some specific microorganism like the*, Serratia marcescens, Enterococcus faecium, Salmonella enterica,* fall under the WHO 2024 priority Bacterial Pathogen List since they cause infections and also pose resistant traits to various groups of antibiotics (Bahrain *et al*., 2019; WHO, 2024). The presence of *Enterococcus faecalis* and *Enterococcus faecium* in the study is a clear indication of water contamination since they are known to be pathogenic and causes opportunistic infections (Zhou *et al*., 2021). They are both human pathogenic microbes with *Enterococcus faecalis* commonly known to cause endocarditis while *Enterococcus faecium* is commonly known to cause nosocomial infections (Fernández-Hidalgo *et al*., 2020; Zhou *et al*., 2021). Both *Enterococcus faecalis* and *Enterococcus faecium* have shown very high levels of antibiotic resistance and World Health Organization has categorized them as critical microbes that need much attention. Studies conducted by Ayobami *et al*., (2020), Bahrain *et al*., (2019); Herrera-Muñoz *et al*., (2024); Korajkic *et al.*, (2020) and Stewart (2022) have also reported the abundance of genus *Enterococcus* in drinking water sources.

Salmonella enterica was dominant among the frozen pops. This could be attributed to the fact that most frozen pops are reportedly homemade. Majority of the vendors do not focus much on the quality of water used during the preparation process since they assume it is of good quality. *Salmonella enterica* is regarded as foodborne and pathogenic bacteria with it being reported to cause serious illness including gastroenteritis which is characterized by symptoms like fever, abdominal cramps and diarrhea (Alvarez *et al*., 2023; Mkangara, 2023). The presence of *Salmonella enterica* in food and water indicate the interconnectedness of water and food safety. *Bacillus weihenstephanensis* was present in all the samples while *Bacillus cereus* was also present but in a small percentage. Both *Bacillus cereus* and *Bacillus weihenstephanensis* are known to contaminate food by releasing spores. The spores grow and release toxins in food that is not well stored and cooked thus causing food spoilage and poisoning. *Bacillus cereus* is known to be highly pathogenic since it causes food poisoning and is associated with symptoms such as vomiting and diarrrhoea (Jessberger *et al*., 2020; El-Arabi & Griffiths, 2021; Kim *et al*., 2021).

Bukholderia cepacia was observed to be present in all the samples. It has also been studied to be a common contaminant of water based pharmaceutical products hence causing more clinical infections instead of performing its intended role. *Bukholderia cepacia* has been associated with infections such as respiratory tract infections, bloodstream infections (Rocha *et al*.,2021; Kwayess *et al*., 2022). Infections caused by *Bukholderia cepacia* can be severe to people who are immunocompromised and more specifically to people with underlying conditions like cystic fibrosis (Tavares *et al*., 2021). *Stenotrophomonas maltophilia* is a ubiquitous microorganism and commonly found in water systems according to Kumar *et al*., (2019).It is regarded as an emerging opportunistic pathogen because of its prevalence in the clinical setting (Brooke, 2021). The bacteria has also been studied to pose inherent multidrug resistance thus making it a serious water contaminant. The bacteria has been observed to cause nosocomical infections to immunocompromised persons and has also been studied to cause a bloodstream infection (Trifonova & Strateva, 2019; Rocha *et al*., 2021).

The abundance of *Serratia marcescens* in the ice pop population could be attributed to the contamination of water used for its production, the environment and the additives used (Tavares-Careon *et al*., 2023).It is known to cause urinary tract infections, wound infections, septicemia, pneumonia (Cristina *et al*., 2019; Zivkovic Zaric *et al.*, 2023). *Serratia marcescens* has also been studied possesses a multidrug resistant trait to various antibiotic classes through efflux pumps and modification of bacterial cell wall. *Klebsiella oxytoca* was present in all the samples in the study. It's a common pathogenic bacteria in the water systems (Dong et al.,2022; Palusiak, 2022). It has been studied to cause various infections including bacteremia, urinary tract infection and pneumonia (Neog *et al*., 2021). Studies have indicated that *Klebsiella oxytoca* possesses various virulence factors contributing to its pathogenicity and antibiotic-resistant mechanisms that has made it a challenge in the clinical treatment (Yang *et al*., 2022; Neog *et al*., 2021).

Leuconostoc mesenteroides was also dominant among the frozen ice pops.The ability of the bacteria being present in both the water and ice pop samples indicate that it is a

common bacteria present in water systems. *Leuconostoc mesenteroides* is a lactic acid bacterium well-known for its ability to thrive in environments with high sugar concentrations thus confiming its abundant population in frozen ice pops which is rich in sweeteners that are produced by sugars (de Paula *et al*., 2015; Lee *et al*., 2016).It is majorly regarded nonpathogenic as it is widely used in the process of food fermentation. It falls under the category of bacteria regarded as safe (GRAS) for human consumption (Lee *et al*., 2016).However, rare cases have been reported of *Leuconostoc mesenteroides* to cause infections among immunocompromised individuals (de Paula et al., 2015; Lee *et al*., 2016). *Gluconobacter liquefaciens* was also dorminant among the ice pops due to its ability and adaptation to thrive in high sugar environments since they can utilize fructose, sucrose and glucose efficiently (Olenski *et al*.,2020). *Jathinobacterium lividum* was also present the six samples hence indicating its dominance. It is regarded nonpathogenic to human and pathogenic to fish. *Jathinobacterium lividum* has been studied to cause mortalities among fish in Red Sea Broomtail Wrasse (Olenski *et al*.,2020). Studies have not indicated whether the bacterial also affects humans. The bacteria has also been studied to possess a unique characteristic of producing violacein , a purple pigment which has been studied to have antimicrobial properties (Lyakhovchenko *et al*.,2022;Park *et al*., 2021). The microbial diversity observed in potable water and ice pop samples represents a significant public health risk, driven by inadequate treatment, poor handling practices, and weak regulatory oversight. The presence of opportunistic and antibiotic-resistant bacteria highlights the need for improved water quality monitoring and stricter enforcement of safety standards.

5.1.3 Antibiotic Resistance Profiles

The findings of this study demonstrate the presence and variety of antimicrobial resistance genes (ARGs) in drinking water and ice pops being sold and consumed in Juja, Kenya. The detection of various resistance determinants using both culture-dependent and independent (metagenomic) approaches suggests that these openly consumed products could be silent reservoirs and vectors of antimicrobial resistance (AMR) in local consumers. Consistent with previous studies carried out in diverse

areas of Kenya (Mutuku et al., 2021) , a culture-based method validated the presence of bacterial species *Escherichia coli*, *Klebsiella spp*., and *Pseudomonas spp*., some of which harbored multiple classes of antibiotic resistance, including beta-lactams, tetracyclines, and sulfonamides. This observation is consistent with the World Health Organization's categorization that positions these organisms as critical or high-priority antimicrobial resistance (AMR), related pathogens (WHO, 2017).Frozen pops, which are most frequently produced under uncontrolled conditions without stringent hygiene or temperature control measures, typically utilize untreated water and are stored at ambient temperaturesconditions that significantly increase the likelihood of microbial contamination and viability (Guo *et al*.,2021). The presence of resistant strains of *Enterococcu*s faecium and *Serratia marcescens* species here suggests an environmental origin of this resistance, likely via fecal contamination of the water sources utilized during their production (Guo *et al*.,2021).

Amoxicillin, penicillin, and chloramphenicol were highly resisted by the microorganisms. The resistance of the microbes to the antibiotics is an indication that the microorganism contained a resistant trait in their genes towards the different antibiotics. Amoxicillin and penicillin are among the frequently used antibiotics for the treatment of both gram-positive and negative bacteria including the infections. Amoxicillin is one of the most used medicine in human and veterinary sector across the globe (Sodhi *et al*.,2021).Therefore, the effects of amoxicillin on the human health and the environment are uncertain, and it is referred to as an emergent pollutant. Amoxicillin does not adequately undergo elimination in wastewater treatment plants (WWTPs), hence ending up in drinking water and water sources thus resulting to microbes with a resistant trait to amoxicillin (Sodhi *et al*.,2021; Georgin *et al*., 2023). Penicillin being a wide spectrum antibiotic, it is commonly used in hospitals and the veterinary sector in treatment of animals and also as an animal feed. They are amongst the most consumed antibiotics (Sodhi *et al*.,2021). The large use in hospitals of broad-spectrum antibiotics promotes intestinal colonization of *bacteria* by greatly increasing the normal gram-negative intestinal microbiota, mutated PBP and the overexpression of β-lactamase enzymes leading to high levels of resistance to β-lactam antibiotics (Pontinen *et al*., 2021). The frequent and unregulated use of chloramphenicol has been

studied to be a driving factor for the emergence of chloramphenicol resistance (Bale *et al*.,2023).According to Bale *et al*., 2023, publications from 2000-2023 have indicated that the mean resistance rates to chloramphenicol from antibiotic susceptibility profiles varied with 86.4% of the studies showing chloramphenicol resistance rates below 50%, and more than half (23 out of 44) of the studies showed resistance rates lower than 20% (Bale *et al*.,2023). A study conducted by Zhao *et al*. (2021) found a high abundance of chloramphenicol antibiotic resistance genes in activated sludge reactors, which are part of wastewater treatment processes that can impact water systems, including sources of drinking water which is an indication of its dominance in water systems. Majority of the microorganisms in this study were susceptible to ciprofloxacin. Ciprofloxacin has been studied to be an outstanding antibiotic and is effective against a large community of microorganisms (Shariati *et al*., 2022; Alsawi *et al*., 2024). This study also confirmed that since no microorganism was resistant to ciprofloxacin.

Previous studies have indicated presence of different antibiotic resistant genes in drinking water. A study conducted in Singapore and China, identified 265 ARGs of 17 distinct types in tap water samples collected from various cities (Ma *et al*., 2019). Multidrug, bacitracin, and aminoglycoside were the most predominant ARGs identified in this study. Tap water sampled from 71 cities in China also contained twenty four ARGs (Zhang *et al*., 2020). From the measured ARGs, the frequency of resistance to sulfonamides was found to be higher followed by aminoglycosides and tetracycline (Zhang *et al*. ,2020).A study conducted in Poland on drinking water indicated high levels of resistance, bacteria resistant to ceftazidime (CAZ) were the most abundant, followed by bacteria resistant to amoxicillin (AML), ciprofloxacin (CIP), and tetracycline (Siedlecka *et al*., 2020).Another study conducted in Nothern Tanzania indicated presence of resistant genes in water with resistance to ampicillin, streptomycin, sulfamethoxazole, tetracycline, and trimethoprim being significantly higher compared to other antibiotics used (Siedlecka *et al*., 2020).Data from the different studies clearly relate to this study which has also indicated microbial resistance to different classes of antibiotics including aminoglycosides, Beta-lactams, tetracyclines, Fluoroquinolones, Nitrofurans, Chloramphenicol. The fact that ARGs

are frequently detected in raw water sources and treated drinking water after treatment indicates that ARGs pollution in drinking water is universal and deserves a high priority.

The exclusive detection of aminoglycoside resistance genes can be attributed to a convergence of environmental selective pressure, biological characteristics of these genes, and methodological limitations of metagenomic analysis. In Kenya, aminoglycosides are widely used in human medicine, veterinary care, and livestock production, creating strong selective pressure that favors the persistence and spread of aminoglycoside resistance genes particularly aminoglycoside-modifying enzymes such as *aac* and *aph* which are frequently located on mobile genetic elements and are therefore highly prevalent in environmental waters (Inda-Diaz *et al.*, 2023). These genes are also relatively conserved, abundant, and diverse, enhancing their detectability in reference-based metagenomic pipelines compared with resistance genes for other antibiotic classes. In contrast, resistance to antibiotics such as β-lactams or fluoroquinolones may occur at lower abundance, be restricted to specific taxa, or arise mainly from chromosomal point mutations rather than well-characterized mobile genes, making them less likely to be detected, particularly under limited sequencing depth and database-dependent annotation approaches (Papp & Solymosi, 2022).

The Antibiotic-Resistant Genes detected in this study have a clinical significance. The aac (6')-Ia, *aac(6')-Iaj*, and *aac(6')-I33*,*aac(6')-Iae* and *aac(6')-Iai*, genes encode acetyltransferase enzyme that alters aminoglycosides by acetylation process. The alteration lowers the binding affinity of the antibiotic to their target hence lowering the effectiveness of the antibiotic leading to resistance (Zhang et al., 2021). The *aph(3')-VIIIa* gene encodes phosphotransferase enzyme which make the antibiotics ineffective through the process of phosphorylation, hence interfering with its effectiveness. The mutated *rrsB* gene indicated resistance specifically to the streptomycins. Mutations in the gene could modify the 16S rRNA structure, the antibiotics bind on the 16S rRNA to inhibit protein synthesis (Zhang *et al.*, 2021). Samples from Juja, Kenyatta Road, and Sachet Pops share a similar pattern of resistance genes. The *aac (6')-Ia, aac(6')-Iae, aac(6')-Iai*, and Mutated *rrsB* genes were dominant among these samples. This is

an indication that the environmental conditions and the water sources in these areas contribute to the spread of the resistance genes. Samples from Hawkers and Frozen pops share unique genes like *aac (6')-Iaj* and *aac(6')-I33*. Despite sharing common genes like *aac (6')-Iae* and *aac (6')-Iai)*, they also had unique resistant profiles that were not present in other samples. Water containing Antibiotic Resistant Genes is regarded contaminated and not safe for drinking (Hu *et al*., 2021). This is because when the ARGs are ingested they can be transferred to other bacteria strains in the gut system through horizontal gene transfer thus leading to existence of bacterial strains that are resistant to antibiotics (Amarasiri *et al*., 2020; Hu *et al*., 2021). If the Antibiotic-Resistant trait is transferred to pathogenic bacterial strains in the body, it could result to complex, expensive and difficult treatment of the infection. Additionally, the ARGs could spread to other ecosystems ,taken up by various environmental microbes and further increasing the resistance traits (Crits-Christoph *et al*., 2022; Kent *et al*., 2020).Antibiotic resistant Antibiotic Resistant genes are regarded contaminants and they could be detected by metagenomics and Next Generation Sequencing of the specific genes present in the total DNA extracted.

Whereas culture-based traditional approaches have provided useful information on culturable bacteria and their phenotypic resistance, metagenomic sequencing has indicated a much broader resistome that contains ARGs for aminoglycoside resistances. The genes *aac(6')-Ia* , *aac(6')-Iae* , *aac(6')-Iai*, *aac(6')-Iaj*, aac(6')-I33 and *aph(3')-VIIIa* were consistently present across different samples. The observation outcomes are consistent with those of other environmental metagenomics studies carried out in urban African settings (Leonard et al., 2018; Riquelme et al., 2021), which reveal the dominating impact of anthropogenic activities, inadequate sanitation strategies, and improper use of antibiotics on the emergence and propagation of antimicrobial resistance genes (ARGs) in urban water and food systems. Juja's swift urban development, combined with inadequate sanitation systems and unregulated food markets, fosters an environment that promotes the spread of antibiotic resistant bacteria. The prevalent use of untreated or inadequately treated water, along with improper food handling practices and informal street vending, heightens the risk of AMR gene transmission. These elements correspond with findings from other peri-

urban regions in sub-Saharan Africa, where high population density and insufficient regulatory oversight intensify the spread of AMR (Okeke et al., 2005; Berendonk et al., 2015).

This study identified Antibiotic Resistant Genes in both water and ice pop samples, therefore calling for effective removal methods. Water treatment processes such as sedimentation, filtration, and coagulation eliminate antibiotic-resistant bacteria but may not effectively remove ARGs according to Zhang *et al*., (2021). Antibiotic resistant genes can be removed by conventional water treatment techniques including sedimentation, filtration, coagulation which target the Antibiotic resistant bacteria rather than the genetic materials (Tan *et al*., 2019; Aguirre-Sánchez *et al*.,2023). The detection of clinically relevant antibiotic resistance genes in drinking water and ice pops consumed in Juja highlights an important environmental pathway for the spread of antimicrobial resistance at the community level. These findings indicate a direct public health risk, as routine consumption of contaminated water and food products may contribute to the dissemination of resistant bacteria in the population. Strengthened regulatory enforcement, routine ARG surveillance in water systems, and improved water treatment and hygiene practices are therefore essential for effective local AMR control.

5.2 Conclusion

There was widespread non-compliance with WASREB guidelines and public hygiene policies among water and ice pop vendors, which was strongly associated with high levels of microbial contamination. Gaps in water treatment, storage, and handling practices contributed to the detection of total coliforms and unsafe products for human consumption.. The study revealed a substantial diversity of pathogenic bacteria in both potable water and ice pops in Juja, Kenya.Clinically significant organisms such as *Enterococcus faecalis, Enterococcus faecium, Salmonella enterica, Burkholderia cepacia, Pseudomonas* spp., and *Klebsiella* spp. were detected across multiple sample types which are capable of causing opportunistic and food- or waterborne infections. The detection of antibiotic resistance genes in water and ice pop samples confirmed they can act as reservoirs and transmission pathways for antimicrobial resistance

within the community. This reinforces the need for integrated, One Health-based surveillance approaches to AMR in food and environmental systems.

5.3 Recommendations

1. WASREB and county public health authorities should strengthen enforcement of water safety and hygiene regulations through regular inspections, licensing checks, and penalties for non-compliance. They should provide practical, low-cost training to vendors on public hygiene policies and WASREB vending guidelines to ensure wider participation and improved compliance.
2. County Public Health Departments should conduct regular microbial testing of water and water related products like ice pops samples from vendors to identify high-risk products and locations, enabling timely interventions to prevent the spread of pathogenic bacteria.
3. The Ministry of Health (MOH), in collaboration with WASREB, should lead awareness campaigns for vendors and consumers on the risks of antimicrobial resistance, emphasizing practical preventive measures to reduce the spread of antibiotic-resistant bacteria.

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APPENDICES

Appendix I: Questionnaire for Water Vendors in Juja Township and its Environs

Instructions: Please answer the following questions to the best of your knowledge. Your responses will be kept confidential and used solely for research purposes.

Section 1: Vendor Information

Question Response Options

Name of Vendor ----------

Type of Water Business

☐Tap Vendor ☐ Borehole Vendor ☐Water Refill Dispenser

Location of Business (GPS Address) ----------

Contact Number ----------

Email Address ----------

Business Operation Period ----------

Section 2: Compliance with Water Vending Guidelines

1. Are you aware of the water vending guidelines outlined by the Water Services Regulatory Board (WASREB)? ☐Yes ☐No
2. Have you undergone any training or received information related to water vending regulations and public hygiene policies? ☐Yes ☐No
3. What training have you undergone? ----------
4. Do you have a valid license/permit to sell water? ☐Yes ☐No
5. If yes, what is the source of the permit number? ☐County Council ☐Public Health ☐WASREB ☐National Government

6. If not, why don't you have a license/permit?

7. Are you familiar with the specific regulations related to water vending in Juja Area? ☐Yes ☐No

8. If yes, which one?

☐Authorization of vendor by local authority

☐Proper labeling of containers with information about its quality

☐Record of water sales kept, including the volume, quality, and collection points of the water

☐Discarding of water that does not meet safety standards

☐Sharing of periodic water tests with relevant local authority

☐Provision of health and safety information about the dangers of poor quality water and its potential impacts on health provided to customers

☐Safe storage and transportation of drinking water

☐Inspection and cleaning of collection points

9. How often do you renew your vending license/permit? ☐ Daily ☐Weekly ☐ Monthly ☐Annually ☐ Never

10. Do you prominently display your license/permit at your vending point? ☐Yes ☐No

11. Do any regulatory authorities inspect your facility? ☐Yes ☐No

12. If yes, how often are the inspections conducted? ☐Daily ☐Weekly ☐ Monthly ☐Annually ☐Other

13. Which authorities inspect your facilities? ☐ WASREB ☐County Council ☐Public Health ☐National Government

14. Do they give any report? ☐Yes ☐No

15. Have you had any non-compliance? ☐Yes ☐No

16. If yes, which one?

☐Authorization of vendor by local authority

☐Proper labeling of containers with information about its quality

☐Record of water sales kept, including the volume, quality, and collection points of the water

☐Discarding of water that does not meet safety standards

☐Sharing of periodic water tests with relevant local authority

☐Provision of health and safety information about the dangers of poorquality water and its potential impacts on health provided to customers

☐Safe storage and transportation of drinking water

☐Inspection and cleaning of collection points

Others----------

17. How many times have you been inspected ☐Once ☐Twice ☐Thrice ☐Four Times ☐Several ☐Others

18. How often have you had non-compliance? ☐Every Inspection ☐Once ☐Twice ☐Several ☐Other

19. What penalties did you face? ☐Business closure ☐Fine ☐Others----------

20. Do you undergo any screening before handling the product? ☐Yes ☐No

21. If yes, after how long do you do the food handler's test?----------

Section 3: Water Quality and Safety

1. What is the source of your water? ☐County suppliers ☐Private Suppliers ☐Boreholes ☐Others----------
2. How often do you receive water from your suppliers? ☐ Daily ☐Weekly ☐Monthly ☐Other
3. Do you treat the water or use any purification methods before vending? ☐Yes ☐ No
4. If yes, how is water purified? ☐Chlorination ☐UV light ☐Supplied Purified ☐Reverse osmosis ☐Boiling
5. Where do you store your water? ☐Tanks ☐Containers ☐Jericans
6. How do you store the water to maintain its quality and safety?.....
7. What is the quantity of your storage tanks/Containers? …….
8. How often do you clean and sanitize the storage containers or tanks? ☐Daily ☐Weekly ☐Monthly ☐Annually
9. Do you clean and sanitize the storage systems before receiving water? ☐Yes ☐No
10. If yes, how often? ☐Before every supply ☐After one supply cycle ☐After two supply cycles ☐4 supply cycles
11. Do you test the water you receive from suppliers or whatever source? ☐Yes ☐No
12. If yes, which laboratory do you use for the water quality tests? ----------
13. From the tests, have you received any reports of water contamination? ☐Yes ☐No
14. What was the contaminant? ☐Bacteria ☐Virus ☐Parasites ☐Chemicals ☐Heavy Metals ☐Other
15. Do you have any test results? ☐Yes ☐No

Section 4: Contamination and Antibiotic Resistance

1. Are you aware of contamination in water? ☐Yes ☐ No
2. Have you ever received any reports of water contamination? ☐Yes ☐ No
3. How often?
4. What contaminants are you aware of? ☐Bacteria ☐Virus ☐Prasites ☐Chemicals ☐Heavy Metals
5. Are you aware of any disease that can be caused by contaminated water? ☐Yes ☐No
6. If yes, which one? ☐Typhoid ☐H.pylori ☐Dysentry☐Cholera ☐Others
7. What measures do you use to prevent contamination?----------
8. Do you clean and sanitize the vending equipment and utensils? ☐Yes ☐No
9. If yes, how often? ☐Daily ☐ Weekly ☐Monthly ☐Annually ☐Other
10. Are you aware of antibiotic resistance? ☐Yes ☐No
11. What about its implications? ☐Yes ☐No
12. Have you heard of antibiotic-resistant genes present in water sources? ☐Yes ☐No
13. Do you have any knowledge of how it can be prevented? ☐Yes ☐No
14. If yes, how?----------

Section 5: General Practices and Hygiene

1. How do you ensure personal hygiene is maintained during vending?---------- --------- -
2. Do you provide disposable bottles for customers? ☐Yes ☐No
3. Where do you source the bottles and lids you give to customers? ---------- ----------
4. How do you store them? ……..
5. Where do you source the bottles you give to customers?---------- ----------

6. Are the bottles and lids cleaned or sanitized before being used? Yes/No
7. If yes, do you ensure they are from approved sources and properly sealed?
8. ☒Yes ☐No
9. What hygiene practices do you follow during vending?----------
10. What hygiene facilities do you have ? ☐Washroom ☐Detergent Dispensers ☐Washing Sinks ☐Other
11. How do you handle any leftover or unsold water at the end of the day?
12. How do you dispose off contaminated water?

Section 6: Additional Comments

Please provide any additional comments or suggestions related to water vending and public health compliance. ----------

Appendix 11: Questionnaire for Ice Pop Vendors in Juja Township and its Environ

Instructions: Please answer the following questions to the best of your knowledge. responses will be kept confidential and used solely for research purposes.

Section 1: Vendor Information

Question	Response Options
Name of Vendor	----------
Gender	☐M ☐F
Type of Business	☐ Fixed ☐ Mobile
Location of Business (Address/GPS Location)	----------
Type of Ice pops	☐Satchet pops ☐Frozen pops ☐Both
Business Operation Period	…….

Section 2: Compliance with Food Vending Guidelines

1. Are there any ice pop vending guidelines outlined by local health authorities? ☐Yes ☐ No
2. Have you undergone any training or received information related to ice pop vending regulations and public hygiene policies? ☐Yes ☐No
3. Which training or information?----------
4. Are you required to have a valid license/permit to sell ice pops? ☐Yes ☐No
5. Are you familiar with the specific regulations of ice pop vending in Juja Area? ☐Yes ☐No
6. Do you pay a levy for selling the ice pops? ☐Yes ☐No

7. Are you ever inspected by any regulatory authorities? ☐Yes ☐ No
8. If yes, how often are the inspections conducted? ☐Daily ☐ Weekly ☐Monthly ☐Annually
9. Which authority conducts the investigations? ☐Public Health ☐County council ☐None ☐Others
10. Have you ever received any warnings or penalties for non-compliance? ☐Yes ☐ No
11. Do you undergo any screening before handling the product? ☐Yes ☐No
12. If yes, after how long do you do the food handler's test?----------

Section 3: Ice Pop Quality and Safety

1. How do you source the ice pops you sell? ☐Homemade ☐Companies ☐Both
2. If Homemade, where do you source your water for ice pop production? ☐Water Dispenser ☐Taps/Boreholes ☐Supermarkets ☐Rivers ☐Boiled water
3. Do you test the water quality? ☐Yes ☐No
4. How do you store the ice pops to maintain their quality and safety? ☐ Cool box ☐Sachets ☐Plastic Tubes ☐Other----------
5. What are the source of the tubes and Sachets? ☐ Homemade ☐ Companies☐ Shops ☐Both
6. If homemade, do you sterilize the tubes/sachets? ☐Yes ☐No
7. Do you sterilize the cool box? ☐Yes ☐No

8. How often do you sterilize the cool box? ☐Daily ☐Weekly☐Monthly ☐Annually
9. What is the source of ice used in the cool box? ☐Homemade ☐Companies/Shops
10. If homemade or from shops, are you aware of the quality of water they use?☐
11. Do you completely sell all the ice pops daily? ☒Yes ☐No
12. If not, how do you store the remaining ice pops? ----------
    ---

Appendix III: Species Count of Pathogenic Bacteria in Water and Ice Pop Samples Based on Illumina Sequencing Technique

Microorganisms	Juja A	Witeithie	Juja B	Hawkers	Frozen pops	Sachet pops	Total Count
Achromobacter xylosoxidans	55	0	0	1421	0	0	1476
Acinetobacter calcoaceticus	9	1	2	0	1	26	39
Acinetobacter johnsonii	42	45	31	76	35	113	342
Acinetobacter lwoffii	52	0	0	0	2	0	54
Bacillus cereus	0	17	2	0	0	0	19
Burkholderia cepacia	142	40	91	187	98	170	728
Citrobacter werkmanii	1049	204	1320	845	1573	88	5079
Cronobacter turicensis	2	0	0	2	0	560	564
Enterococcus faecalis	153	0	315	0	0	65	533
Enterococcus faecium	1306	0	3884	0	3	631	5824
Enterococcus gallinarum	3	0	30	0	0	1	34
Erwinia pyrifoliae	22	0	0	4	0	419	445
Klebsiella oxytoca	353	76	349	676	185	6638	8277
Klebsiella variicola	316	137	172	255	114	218	1212
Myroides odoratimimus	65	3623	0	0	0	0	3688

Pantoea agglomerans	543	204	209	408	689	264	2317
Pantoea vagens	5	0	0	0	0	0	5
Providencia rustigianii	1	138	0	0	0	0	139
Pseudomonas plecoglossicida	83	1574	19	1384	3	3	3066
Pseudomonas putida	85	386	30	1037	143	10	1691
Raoultella ornithinolytica	3	0	0	0	48	0	51
Raoultella planticola	55	9	12	87	1006	1117	2286
Salmonella enterica	2	0	0	0	109	0	111
Stenotrophomonas maltophilia	1827	847	2802	4378	856	32	10742
Delftia acidovorans	1224	1094	70	543	53	11	2995
Serratia marcescens	405	0	14	117	112	12913	13561
Total	7802	8395	9352	11420	5030	23279	65278

Appendix IV: Antibiotic Resistance Genes Screened using the Chan Zuckerberg Pipeline

Sample Name	Gene Name	Gene Family	Drug_class	Resistance Mechanism	ARO Accession
Juja A	AAC(6')-Ia	AAC(6')	aminoglycoside	antibiotic inactivation	ARO:3002545
	AAC(6')-Iae	AAC(6')	aminoglycoside	antibiotic inactivation	ARO:3002573
	AAC(6')-Iai	AAC(6')	aminoglycoside	antibiotic inactivation	ARO:3002575
	Mutated rrsB	16s rRNA	aminoglycoside	antibiotic target alteration	ARO:3003405
Witeithie	AAC(6')-Ia	AAC(6')	aminoglycoside	antibiotic inactivation	ARO:3002545
	AAC(6')-Iae	AAC(6')	aminoglycoside	antibiotic inactivation	ARO:3002573
	AAC(6')-Iai	AAC(6')	aminoglycoside	antibiotic inactivation	ARO:3002575
	APH(3')-VIIIa	APH(3')	aminoglycoside	antibiotic inactivation	ARO:3004680
Juja B	AAC(6')-Iae	AAC(6')	aminoglycoside	antibiotic inactivation	ARO:3002573
	AAC(6')-Iai	AAC(6')	aminoglycoside	antibiotic inactivation	ARO:3002575
	Mutated rrsB	16s rRNA	aminoglycoside	antibiotic target alteration	ARO:3003405
Hawkers	AAC(6')-Ia	AAC(6')	aminoglycoside	antibiotic inactivation	ARO:3002545
	AAC(6')-Iae	AAC(6')	aminoglycoside	antibiotic inactivation	ARO:3002573
	AAC(6')-Iai	AAC(6')	aminoglycoside	antibiotic inactivation	ARO:3002575
	AAC(6')-Iaj	AAC(6')	aminoglycoside	antibiotic inactivation	ARO:3003677
	APH(3')-VIIIa	APH(3')	aminoglycoside	antibiotic inactivation	ARO:3004680
	Mutated rrsB	16s Rrna	aminoglycoside	antibiotic target alteration	ARO:3003405
Frozen pops	AAC(6')-Iae	AAC(6')	aminoglycoside	antibiotic inactivation	ARO:3002573

	AAC(6')-Iai	AAC(6')	aminoglycoside	antibiotic inactivation	ARO:3002575
	Mutated rrsB	16s rRna	aminoglycoside	antibiotic target alteration	ARO:3003405
	AAC(6')-I33	AAC(6')	aminoglycoside	antibiotic inactivation	ARO:3002587
Sachet Pops	AAC(6')-Iae	AAC(6')	aminoglycoside	antibiotic inactivation	ARO:3002573
	AAC(6')-Iai	AAC(6')	aminoglycoside	antibiotic inactivation	ARO:3002575
	APH(3')-VIIIa	APH(3')	aminoglycoside	antibiotic inactivation	ARO:3004680
	Mutated rrsB	16s rRNA	aminoglycoside	Antibiotic target alteration	ARO:3003405

Appendix V: CLSI (2024), BSAC, and NCCLS-Based Breakpoints for Antibiotic Susceptibility Testing using the Disk Diffusion Method

This table compiles the CLSI (2024), BSAC, and NCCLS-based breakpoints for antibiotic susceptibility testing using the disk diffusion method. Each value indicates the diameter of the inhibition zone (in mm) used to categorize bacterial isolates as Susceptible (S), Intermediate (I), or Resistant (R).

Organism Group	Antibiotic	Susceptible (S)	Intermediate (I)	Resistant (R)
Enterobacterales	Ciprofloxacin	≥ 21	16–20	≤ 15
	Tetracycline	≥ 15	12–14	≤ 11
	Amoxicillin/Ampicillin	≥ 17	14–16	≤ 13
	Chloramphenicol	≥ 18	13–17	≤ 12
	Cotrimoxazole	≥ 16	11–15	≤ 10
	Nalidixic acid	≥ 19	14–18	≤ 13
	Nitrofurantoin	≥ 17	15–16	≤ 14
	Gentamicin	≥ 15	13–14	≤ 12
	Sulfamethaxozole	≥ 16	11–15	≤ 10
	Streptomycin	≥ 15	12–14	≤ 11
Pseudomonas spp.	Ciprofloxacin	≥ 26	23–25	≤ 22
	Gentamicin	≥ 15	13–14	≤ 12
	Streptomycin	≥ 18	13-17	≤ 12
	Amoxicillin/Ampicillin	≥ 17	14–16	≤ 13
	Chloramphenicol	≥ 15	13–14	≤ 12

	Tetracycline	≥ 18	15–17	≤ 14
Acinetobacter				
spp.	Tetracycline	≥ 15	12–14	≤ 11
	Gentamicin	≥ 15	13–14	≤ 12
	Streptomycin	≥ 18	13-17	≤ 12
	Ciprofloxacin	≥ 21	16–20	≤ 15
	Chloramhenicol	≥ 15	13–14	≤ 12
	Ammoxicillin	≥ 17	14–16	≤ 13
Staphylococcus				
spp.	Penicillin	≥ 29	26-28	≤ 25
	Chloramphenicol	≥ 18	15–17	≤ 14
	Gentamicin	≥ 15	13–14	≤ 12
	Streptomycin	≥ 15	12–14	≤ 11
	Amoxicillin	≥ 29	25-28	≤ 24
	Tetracycline	≥19	15–18	≤14
	Streptomycin	≥ 15	12–14	≤ 11
Streptococcus				
spp.	Ciprofloxacin	≥ 21	16-20	≤ 15
	Penicillin	≥ 24	21–23	≤ 20
	Gentamicin	≥ 16	13-15	≤ 12
	Chloramphenicol	≥ 18	15–17	≤ 14
	Amoxicillin/Ampic			
	ilin	≥ 28	25-27	≤ 24
	Tetracycline	≥ 23	20–22	≤ 19
	Cotrimaxozole	≥ 23	19–22	≤ 18
	Nalidixic acid	≥ 21	16-20	≤ 15
	Sulfamethaxozole	≥ 23	19–22	≤ 18
	Streptomycin	≥ 15	13-16	≤ 13
Enterococcus				
spp.	Ampicillin	≥ 17	14–16	≤ 13
	Gentamicin	≥ 16	13-15	≤ 12

Streptomycin	≥ 15	13-16	≤ 13
Amoxicilin	≥ 17	14–16	≤ 13
Tetracycline	≥ 23	20–22	≤ 19
Penicillin	≥ 19	16–18	≤ 15
Ciprofloxacin	≥ 21	16–20	≤ 15

Appendix VI: Kruskal –Wallis Test Table for Alpha Diversity Metrics

	Kruskal Wallis Test		
Metric	**Group**	**H-value**	**P-value**
Faith PD	Location	1.19	0.275
	Type of sample	1.142	0.564
Pielou Evenness	Location	0.047	0.827
	Type of sample	3.714	0.156
Shannon	Location	1.19	0.275
	Type of sample	3.714	0.156
Simpson	Location	0.428	0.512
	Type of sample	3.428	0.180
Chao1	Location	1.225	0.268
	Type of sample	1.544	0.462

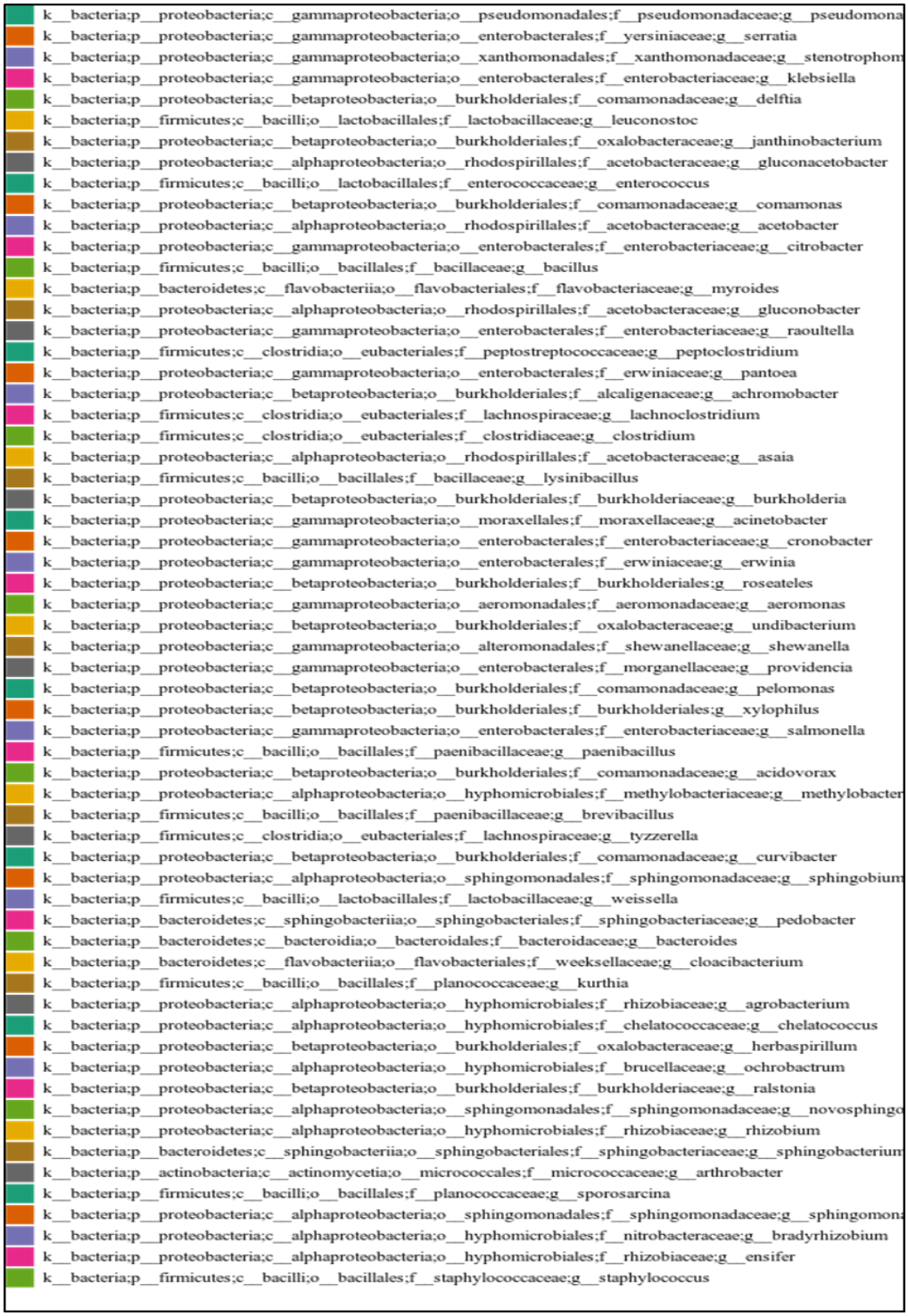
Appendix VII: PERMANOVA Table for Jaccard and Bray-Curtis Distance Metric Based on Sample type (Affordable, Commercialized, Value Added Water)

Parameter	Jaccard	Bray-Curtis
Method Name	PERMANOVA	PERMANOVA
Test Statistic Name	pseudo-F	pseudo-F
Sample Size	6	6
Number of Groups	3	3
Test Statistic	1.039508	1.255
p-value	0.257	0.144
Number of Permutations	999	999

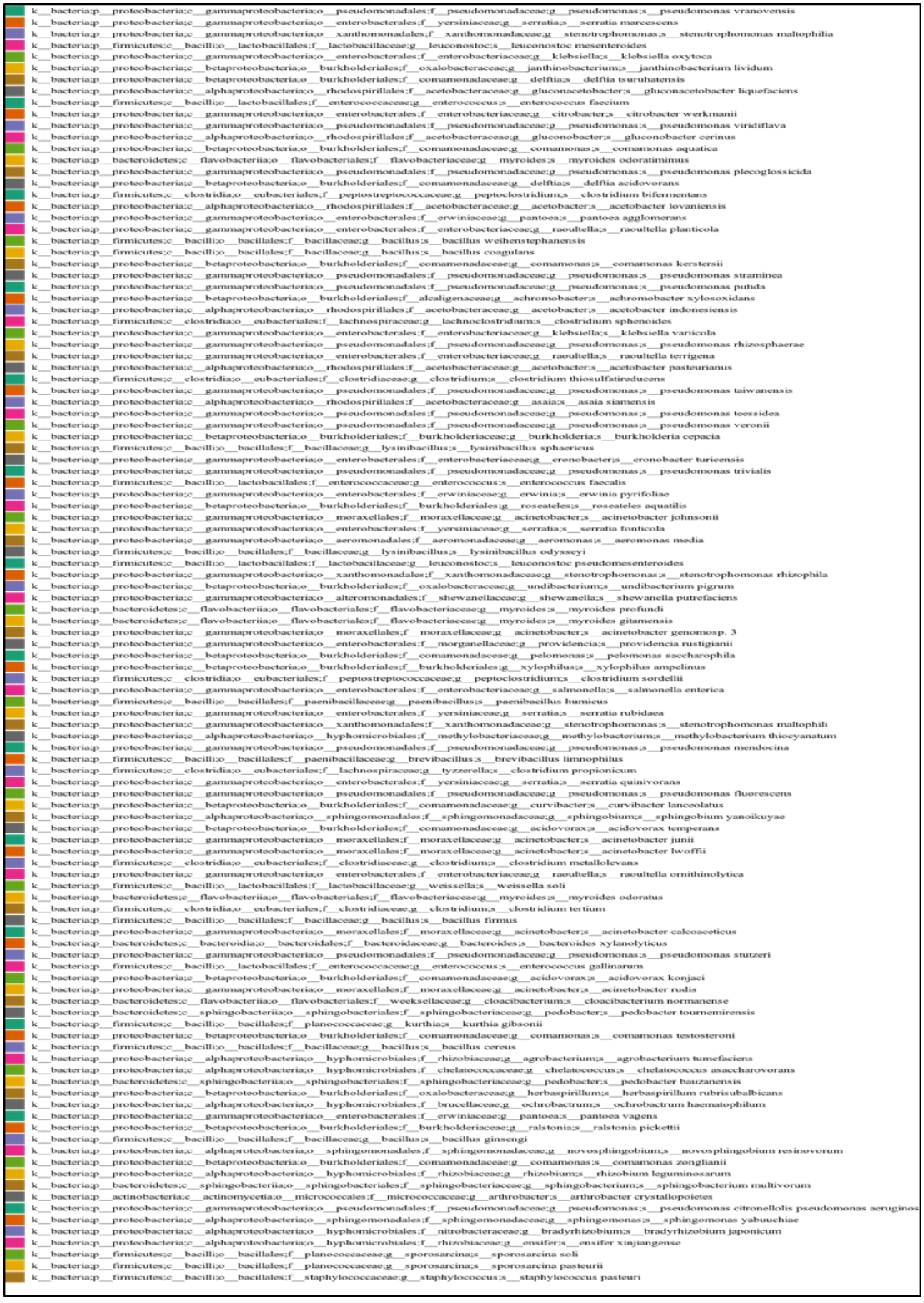
Appendix VIII: PERMANOVA Table for Jaccard and Bray-Curtis Distance Metric Based on Location (Juja and Witeithie)

Parameter	Bray-Curtis	Jaccard
Method Name	PERMANOVA	PERMANOVA
Test Statistic Name	pseudo-F	pseudo-F
Sample Size	6	6
Number of Groups	3	3
Test Statistic	0.73	0.921
p-value	0.902	0.709
Number of Permutations	999	999

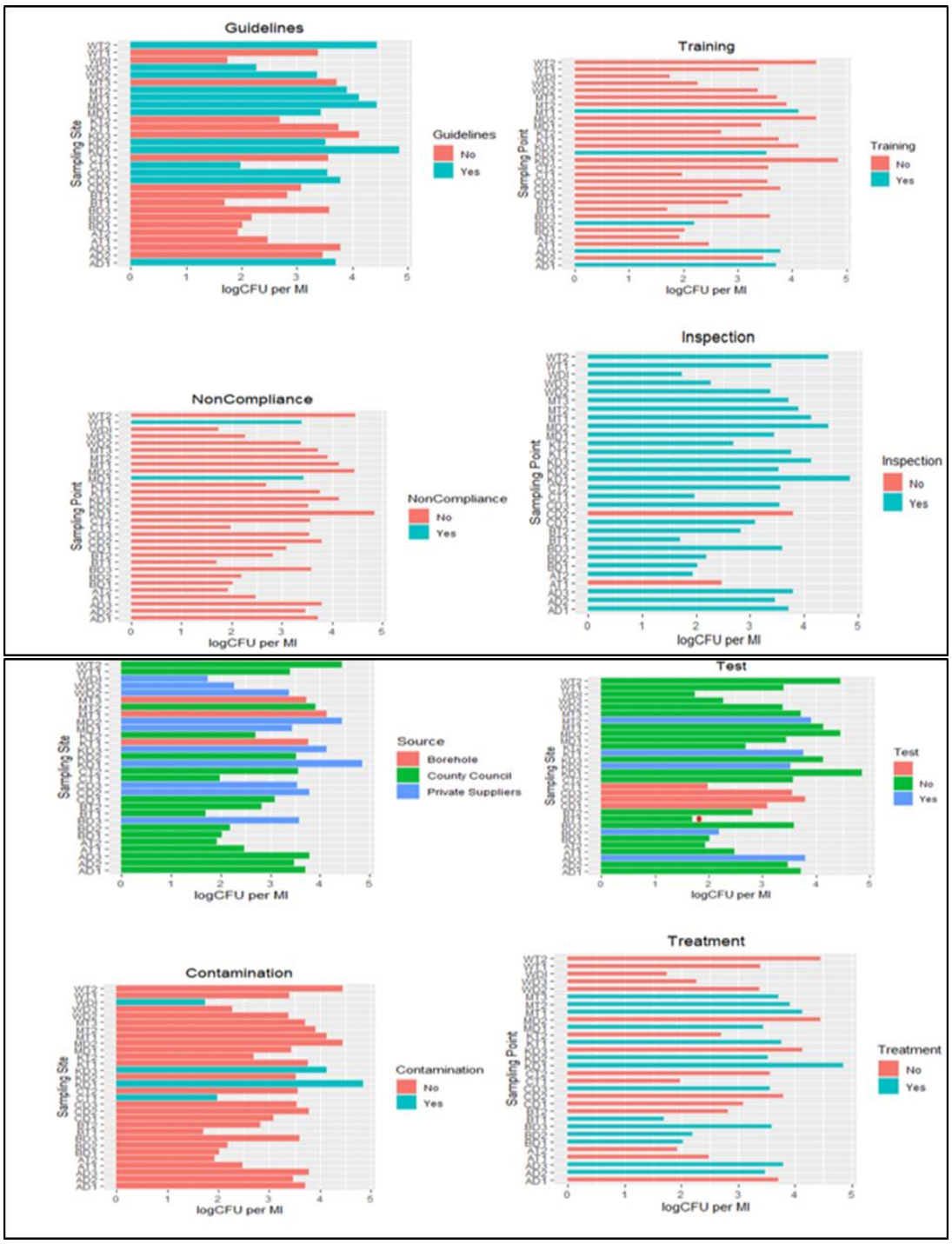
Appendix IX: Legend Representing Taxonomic Groupings (Level 6 – Genus Level)



Appendix X: Legend Representing Taxonomic Groupings (Level 7 – Species Level)



Appendix XI: Correlation Graphs Illustrating the Relationship between Survey Variables and Contamination Levels, as Measured by Total Coliform Count



Appendix XII: GPS Coordinates for the Sampling Points

Sampling points	Latitude	Longitude
AD1	-1.1026339° S	37.0148754° E
AD2	-1.1031276° S	37.0134196° E
AD3	-1.105207° S	37.0139333° E
AD4	-1.102453° S	37.0149321° E
AF1	-1.1046291° S	37.0146392° E
AS1	-1.1038626° S	37.0133489° E
AT1	-1.1002859° S	37.0152662° E
AT2	-1.1028303° S	37.0136284° E
BD1	-1.0928499° S	37.0204912° E
BD2	-1.0955783° S	37.0217774° E
BD3	-1.0960815° S	37.0183115° E
BD4	-1.0920288° S	37.0204909° E
BT2	-1.0888419° S	37.0198746° E
BT1	-1.0897895° S	37.0193701° E
BF1	-1.0882402° S	37.0221465° E
BS1	-1.0881568° S	37.0220183° E
CD1	-1.1021347° S	37.0135302° E
CD2	-1.1026252° S	37.0085919° E
CD3	-1.1018291° S	37.0080436° E
CD4	-1.1002206°S	37.0091254° E
CF1	-1.1002206° S	37.0091254° E
CS1	-1.1027828° S	37.0127074° E
CT1	-1.1025042° S	37.0085065° E
CT2	-1.1005397° S	37.0089449° E
MD1	-1.0988039° S	37.0094709° E
MD2	-1.0945432° S	37.003076° E
MD3	-1.0814998° S	36.9998596° E
MF1	-1.0988039° S	37.0094709° E
MS1	-1.0804344° S	37.0011117° E

MT1	-1.0944517° S	37.0030869° E
MT2	-1.0804696° S	37.0002258° E
KD1	-1.127349° S	37.004815° E
KD2	-1.125880° S	37.004309° E
KD3	-1.12067° S	37.00496° E
KD4	-1.121345° S	37.05387° E
KT1	-1.1204437° S	37.0108449° E
KT2	-1.1217157° S	37.0117653° E
WD1	-1.0696971° S	37.0544022° E
WD2	-1.0697391° S	37.0552254° E
WD3	-1.0668185° S	37.0551812° E
WD4	-1.06578° S	37.0544° E
WT1	-1.0680052° S	37.0531447 ° E
WT2	-1.0665977° S	37.054901° E
WS1	-1.0666346° S	37.0518941° E
WF1	-1.0664521° S	37.0521768° E
WH1	-1.0667758° S	37.0518392 ° E
WH2	-1.0664413° S	37.0522002° E
WH3	-1.06662° S	37.05497° E
JH1	-1.0947868° S	37.0285815° E
JH2	-1.08409° S	36.9996° E
JH3	-1.07397° S	37,05904° E
KH1	-1.127299° S	37.004803° E
KH2	-1.12067° S	37.00496° E
TH1	-1.12986°S	36.98243°E
TH2	–1.102554°S	37.013193°

Appendix XIII: Sample Distribution Criteria

No.	Sample Code	Location Description	Sample Type
1	AD1	Gate A, JKUAT	Dispenser
2	AD2	Gate A, JKUAT	Dispenser
3	AD3	Gate A, JKUAT	Dispenser
4	AD4	Gate A, JKUAT	Dispenser
5	AF1	Gate A, JKUAT	Frozen Pop
6	AS1	Gate A, JKUAT	Sachet Pop
7	AT1	Gate A, JKUAT	Tap Water
8	AT2	Gate A, JKUAT	Tap Water
9	BD1	Gachororo	Dispenser
10	BD2	Gachororo	Dispenser
11	BD3	Gachororo	Dispenser
12	BD4	Gachororo	Dispenser
13	BT1	Gachororo	Tap Water
14	BT2	Gachororo	Tap Water
15	BF1	Gachororo	Frozen Pop
16	BS1	Gachororo	Sachet Pop
17	CD1	Gate C, JKUAT	Dispenser
18	CD2	Gate C, JKUAT	Dispenser
19	CD3	Gate C, JKUAT	Dispenser
20	CD4	Gate C, JKUAT	Dispenser
21	CF1	Gate C, JKUAT	Frozen Pop
22	CS1	Gate C, JKUAT	Sachet Pop
23	CT1	Gate C, JKUAT	Tap Water
24	CT2	Gate C, JKUAT	Tap Water
25	MD1	Mung’etho	Dispenser
26	MD2	Mung’etho	Dispenser
27	MD3	Mung’etho	Dispenser
28	MF1	Mung’etho	Frozen Pop
29	MS1	Mung’etho	Sachet Pop

30	MT1	Mung’etho	Tap Water
31	MT2	Mung’etho	Tap Water
32	KD1	Kenyatta Road	Dispenser
33	KD2	Kenyatta Road	Dispenser
34	KD3	Kenyatta Road	Dispenser
35	KD4	Kenyatta Road	Dispenser
36	KT1	Kenyatta Road	Tap Water
37	KT2	Kenyatta Road	Tap Water
38	WD1	Witethie	Dispenser
39	WD2	Witethie	Dispenser
40	WD3	Witethie	Dispenser
41	WD4	Witethie	Dispenser
42	WT1	Witethie	Tap Water
43	WT2	Witethie	Tap Water
44	WS1	Witethie	Sachet Pop
45	WF1	Witethie	Frozen Pop
46	WH1	Witethie	Hawkers
47	WH2	Witethie	Hawkers
48	WH3	Witethie	Hawkers
49	JH1	Juja	Hawkers
50	JH2	Juja	Hawkers
51	JH3	Witethie	Hawkers
52	KH1	Kenyatta Road	Hawkers
53	KH2	Kenyatta Road	Hawkers
54	TH1	Toll (Kenyatta Road)	Hawkers
55	TH2	Toll (Kenyatta Road)	Hawkers

Appendix XIV: Frequency (%) of Gram Stain, Morphological, and Biochemical Characteristics of Bacterial Isolates

Test / Feature	%Negative	%Positive
Morphology (Bacilli)	17.90%	82.10%
Morphology (Cocci)	82.10%	17.90%
Gram Stain (Gram-positive)	67.90%	32.10%
Gram Stain (Gram-negative)	32.10%	67.90%
Catalase	0%	100%
Methyl Red	60.70%	39.30%
Indole	90.50%	9.50%
Voges-Proskauer (VP)	100%	0%
Urease	96.40%	3.60%
Citrate Utilization	0%	100%
Starch Hydrolysis	89.30%	10.70%
Sucrose Fermentation	85.70%	14.30%
Lactose Fermentation	85.70%	14.30%
Glucose Fermentation	82.10%	17.90%
Gas Production	85.70%	14.30%
Hydrogen Sulfide (H2S)	89.30%	10.70%

Appendix XV: Percentage Distribution of Putative Microorganisms

Putative Microorganism	Frequency (n)	Percentage (%)
Salmonella	3	10.70%
Escherichia coli	2	7.10%
Shigella	2	7.10%
Staphylococcus	2	7.10%
Enterococcus faecalis	2	7.10%
Cedacea	2	7.10%
Providencia	2	7.10%
Serratia	1	3.60%
Acinetobacter	1	3.60%
Bacillus	1	3.60%
Enterobacter	2	7.10%
Listeria	1	3.60%
Corynebacterium	1	3.60%
Pseudomonas	1	3.60%
Klebsiella	1	3.60%
Streptococcus	1	3.60%
Enterococcus faecium	1	3.60%
Moraxella	1	3.60%
Micrococcus luteus	1	3.60%

Appendix XVI: Percentage Frequency of Antibiotics Resisted by the Putative Isolates

Antibiotic	Resistant isolates	Percentage resistance
Tetracycline	17	80.5
Amoxicillin	19	90.4
Ciprofloxacin	1	4.7
Penicillin	20	95.2
Chloramphenicol	19	90.4
Streptomycin	11	52.3

Appendix XVII: Antimicrobial Resistance Profiles and Levels (MDR/XDR/PDR)

Putative Microorganism	Resistance Percentage (antibiotics)	Resistant antibiotic classes (n)	Classification Level
Escherichia coli	83.3% (5/6)	4	MDR
Shigella spp.	83.3% (5/6)	4	MDR
Serratia spp.	83.3% (5/6)	4	MDR
Acinetobacter spp.	66.7% (4/6)	3	MDR
Bacillus spp.	83.3% (5/6)	4	MDR
Enterobacter spp.	83.3% (5/6)	4	MDR
Staphylococcus spp.	83.3% (5/6)	4	MDR
Listeria spp.	83.3% (5/6)	4	MDR
Corynebacterium spp.	50.0% (3/6)	3	MDR
Pseudomonas spp.	83.3% (5/6)	4	MDR
Klebsiella spp.	66.7% (4/6)	3	MDR
Streptococcus spp.	66.7% (4/6)	3	MDR
Salmonella spp.	66.7% (4/6)	3	MDR
Enterococcus faecalis	83.3% (5/6)	4	MDR
Moraxella spp.	66.7% (4/6)	3	MDR
Cedacea spp.	83.3% (5/6)	4	MDR
Proteus spp.	66.7% (4/6)	3	MDR
Providencia spp.	66.7% (4/6)	3	MDR
Proteus mirabilis	100% (6/6)	5	PDR
Enterococcus faecium	50.0% (3/6)	3	MDR

Key: MDR-Multidrug Resistant (Resistance ≥3 antimicrobial classes)

XDR-Extensively Drug Resistant (Resistant to at least one agent in all but one or two antimicrobial classes (only 1–2 classes remain effective)

PDR-Pandrug Resistant (resistance to all antimicrobial classes tested)

Appendix XVIII: Ethical Approval

JOMO KENYATTA UNIVERSITY OF AGRICULTURE AND TECHNOLOGY
P.O BOX 62000(00200) NAIROBI, Tel:(067) 58700001-4
(Office of the Deputy Vice Chancellor, Research Production and Extension Division)

JKUAT INSTITUTIONAL SCIENTIFIC AND ETHICS REVIEW COMMITTEE

REF: JKU/2/4/896B Date: 26th October 2023

RONALDO CHACHA RIOBA
DEPARTMENT OF BOTANY, JKUAT

Dear Rioba,

RE: ASSESSMENT OF PUBLIC HEALTH COMPLIANCE AND MOLECULAR CATALOGUE OF RESISTOME OF PORTABLE WATER AND ICE POPS IN JUJA AREA

This is to inform you that JKUAT Institutional Scientific and Ethical Review Committee has reviewed and approved your above research proposal. Your application approval number is **JKU/ISERC/02316/1085.** The approval period is **26th October 2023 to 27th October 2024.**

i. Only approved documents including (informed consents, study instruments, MTA) will be used.
ii. All changes including (amendments, deviations, and violations) are submitted for review and approval by JKUAT ISERC.
iii. Death and life threatening problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to JKUAT ISERC within 72 hours of notification
iv. Any changes, anticipated or otherwise that may increase the risks or affected safety or welfare of study participants and others or affect the integrity of the research must be reported to JKUAT ISERC within 72 hours
v. Clearance for export of biological specimens must be obtained from relevant institutions.
vi. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. Attach a comprehensive progress report to support the renewal.
vii. Submission of an executive summary report within 90 days upon completion of the study to JKUAT ISERC.

Prior to commencing your study, you will be expected to obtain a research license from National Commission for Science, Technology and Innovation (NACOSTI) https://oris.nacosti.go.ke and also obtain other clearances needed.

Yours sincerely
Dr. Amos Mbugua
CHAIR, JKUAT ISERC

INSTITUTIONAL ETHICS REVIEW COMMITTEE
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