BACTERIAL FOOD CONTAMINANTS IN VENDED FAST FOODS, SOIL AND WATER IN EMBU TOWN, KENYA

SUSAN WAIRIMU MURIUKI

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DECLARATION

This thesis is my original work and has not been submitted elsewhere for a degree or any other award.

Signature…… Date……

Muriuki Susan Wairimu

Department of Biological Sciences,

B523/1148/2017

This research thesis has been submitted for examination with my approval as the University Supervisor

Signature……. Date……

Prof. Nancy Budambula,

Department of Biological Sciences,

University of Embu.

DEDICATION

I dedicate this work to all those who stood with me during the research journey. I also dedicate this work to my family members who have offered both emotional and physical support throughout the journey, their endless support will forever remain in my heart.

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ACRONYMS/ABBREVIATIONS

ANOSIM	Analysis of Similarity
ANOVA	Analysis of Variance
ASV	Amplicon Sequence Variants
CFU	Colony Forming Units
CLSI	Clinical Laboratory Standard Institute
DNTPs	Deoxyribonucleotide triphosphate
DGGE	Denaturing gel electrophoresis
DMSO	Dimethyl sulfoxide
EDTA	Ethylenediaminetetra-acetic acid
ECDC	European Center for Diseases Control
EFSA	European Food Safety Authority
EWASCO	Embu Water and Sanitation Company
FAO	Food and Agriculture Organization
GLM	General linear model
HSD	Honest Significant Difference
ISO	International Organization for Standardization
LAMP	Loop mediated isothermal amplification
LoD	Limit of Detection
LB	Luria broth
MHA	Muller Hinton agar
NGS	Next Generation Sequencing
NMDS	Non metric Multidimensional Scaling
NaCl	Sodium chloride
NIH	National Institutes of Health
OTUs	Operational Taxonomic Unit
PERMANOVA	Permutational Multivariate Analysis of Variance
PCI	Principal Co-ordinate Axis
PCoA	Principal Coordinate Analysis
PCR	Polymerase chain reaction
QPCR	Quantitative polymerase chain reaction

RDA	Redundancy Analysis
SE	Standard Error
Tri-HCL	Tris hydrochloride
USDA	US Department of Agriculture
WHO	World Health Organization
WGS	Whole Genome Sequencing

ABSTRACT

Ready to eat fast food vending business has grown exponentially however, the microbiological safety of most of these fast foods is questionable. Despite the cholera outbreak in Embu in 2017, diminutive information exists on waterborne and foodborne illness outbreaks resulting from bacterial pathogens in Embu Town and the nearby Kangaru Market. Thus this study aimed to isolate and characterize bacterial food contaminants in the vended fast foods and their surrounding environment in Embu Town and nearby Kangaru Market. Bacterial isolates were isolated from; fried fish, African sausages, roasted meat, samosa, fries, vegetable salads, soil and water. Antibiotic susceptibility testing was done using the Kirby-Bauer method. DNA was extracted from pure isolates using the phenol-chloroform method. The 16S rRNA and resistant genes were amplified with the respective primers. Taxonomy was assigned to each Operational Taxonomic Units using BLAST. Sequence alignment and construction of the phylogenetic tree were done using MEGA X. Total community DNA was extracted from samples using the phenol-chloroform method. The 16S rRNA gene variable region (V4-V7) of the extracted DNA was amplified and library construction was performed according to Illumina sequencing protocol. Sequence analysis was done using QIIME2 while Analysis of similarity (ANOSIM) test, based upon Bray-Curtis distance, Non-metric Multidimensional Scaling (NMDS), redundancy analysis (RDA) as well as Hierarchical clustering of the samples, were carried out using the R programming language. Taxonomic groups were derived from the number of reads assigned to each taxon at all ranks from domain to species using the taxa summary.txt output from the QIIME2 pipeline. Antibiotic resistance of the isolates was; chloramphenicol 90%, cefotaxime 84.29%, nalidixic acid 81.43%, tetracycline 77.14%, amoxicillin 72.86%, gentamycin 48.57%, streptomycin 32.86% and trimethoprim + sulphamethoxazole 30%. Isolate KMP337, exhibited significantly high antibiosis against S. aureus recording an average inhibition of diameter mean and standard error (SE)16.33±0.88mm respectively at P = 0.001. Polymerase chain reaction detected the presence of resistance genes; *tet* (A), *bla TEM*, *str*B, *dfr* (1), *bla Amp*, and *Flor* genes. The isolates clustered in five main genera namely *Bacillus*, *Paraclostridium*, *Lysinibacillus*, *Virgibacillus*, and *Serratia* all having similarity of above 90%. The OTUs were distributed among twenty-three bacterial phyla across the samples with the most abundant belonging to *Proteobacteria* (52.81%), *Firmicutes* (31.16%), *Bacteroidetes* (8.00%) and *Lentisphaerae* (0.001%). The OTUs were shared among archaea as follows; *Candidatus nitrososphaera* (83.89%) and *Nitrososphaera* (11.43%). *Lactobacillus perolens*, *Pseudomonas* spp., *Clostridium* spp., and *Bacillus cereus* were detected in abundance across the samples. Potential pathogens in risk group 2 including *Rickettsia* spp. and *Brucella* spp., *Coxiella* spp. in risk group 3 were detected. Uncultured *Candidatus koribacter* sp, *Candidatus microthrix* sp. and *Candidatus solibacter* sp. were detected in food. This study confirmed the presence of antibiotic-resistant foodborne potential pathogens in vended fast foods and the soil in Embu Town and Kangaru Market. The study, therefore calls for continuous monitoring of bacterial status and hygienic handling of vended foods. The detection of potential pathogens calls for stringent hygiene measures in food vending operations, especially in Kangaru Market.

CHAPTER ONE
1.0 INTRODUCTION

1.1 Background information

Urban centers in underdeveloped nations face a myriad of socio-economic challenges that impact negatively on health standards. Foodborne diseases associated with certain microbial pathogens, their toxins, or subsequent chemicals, is a consequential global public health drawback (Lai *et al*., 2016). Street-vended food is preferred among many urban dwellers due to its readiness, convenience and low cost. However, the hygiene aspects of vending operations are a major source of concern especially the health of both handlers and consumers (Jahan *et al*., 2018). Running surface water, inadequate sanitation and open disposal of untreated sewage provide a conducive environment for the proliferation of pathogenic microbes. Furthermore, street foods are prone to cross-contamination due to poor personal hygiene, sharing of contaminated utensils and free movement of flies that sporadically land on food.

In countries like Canada and Australia, incidences of food poisoning range between 3.1 to 5 million and 5.4 million each year (McLinden *et al*., 2014). Food poisoning has been associated with economic loss as more funds are directed to fighting the outbreaks instead of development. The U.S. Department of Agriculture (USDA), spend 10-83 billion USD annually on cases resulting from food poisoning while in New Zealand, it is 86 million USD (McLinden *et al*., 2014). Over 200 diseases occurring in developing countries are associated with foodborne infections in individuals including the elderly, infants, and immunocompromised persons (WHO, 2015). WHO, in partnership with other African nations, spearheaded the formation of "Integrated Disease, Surveillance & Response" (IDSR) to control cholera outbreak and diarrhea cases associated with foodborne pathogens (WHO/AFRO, 2013). Despite these efforts, it has been reported that diseases resulting from foodborne illness are still rampant (Akhtar *et al*., 2014).

Food and environmental samples have been reported to be reservoirs of foodborne pathogens (Muhonja *et al.*, 2012). The contamination of food is often a result of contact with contaminated environments where they are processed (El-Zamkan & Hameed, 2016; Denis *et al*., 2016). Thus, it is evident that food contamination with antibiotic-resistant bacteria threatens public health since the chances of these

resistance determinants to be transferred to some pathogenic bacteria are high thus complicating the treatment of an infection arising from infections by these bacteria. The release of animal and human waste into the environment results in the subsequent release of antibiotics into the water and soil thus encouraging further dissemination of antibiotic resistance (Walsh & Duffy, 2013).

Food and food production units have been reported to be transmitters of antibiotic-resistant foodborne pathogens and their respective genes to humans (Bengtsson-Palme *et al*., 2018). There has been increasing pressure to describe the respective mechanisms, recurrence, reservoirs and vectors that facilitate the movement of this resistome among the foodborne pathogens (Bengtsson-Palme *et al*., 2018). Bacterial resistance to antibiotics has been reported in pathogens such as *Escherichia coli* (STEC) that produces Shiga toxin, *Shigella* spp., non-typhoidal *Salmonella*, *Vibrio cholerae* and *Klebsiella* spp (Okeke *et al*., 2004; Majowicz *et al*., 2014). Disease associated with the above pathogens are reported to arise from food. Cases of *Salmonella* outbreaks have been reported to arise from the consumption of poultry, chocolate, fresh produce, and eggs that are contaminated. STEC outbreaks are reported to result from consumption of beef and fresh produce that are contaminated with these pathogens (Herman *et al*., 2015).

Approximately 600 million reported cases of disease outbreaks associated with foodborne pathogens and as high as 420, 000 deaths occur yearly https://www.who.int/news-room/fact-sheets/detail/food-safety:year-2020. Therefore, the assessment of food matrices for the presence of contamination by microbial organisms is essential to ensure food safety (Hoffmann *et al*., 2016). Conventional methods that have been used in detecting foodborne pathogens rely on growing these microorganisms on synthetic media. Nevertheless, these methods are time-consuming and sometimes they may give prejudiced results as most of the uncultured microorganisms are missed out (Law *et al*., 2015). Quantitative PCR (qPCR), PCR, the denaturing gel electrophoresis (DGGE) and loop-mediated isothermal amplification (LAMP), are robust, faster, efficient and more reliable in detecting both cultured and uncultured microorganisms (Mayo *et al*., 2014).

Microbial contaminants that are both culturable and non-culturable in food have been extensively detected using Next-Generation Sequencing (NGS). A plethora of

microorganisms have been shown to have preference for fresh produce but at the same time, few studies have applied next-generation sequencing (Li, 2020; Kim *et al*., 2018). Lewis *et al*., (2020), applied NGS in determining *Salmonella* spp limit of detection and phage MS2 which is surrogate norovirus and they detected *Salmonella* sp at 10^6 CFU reaction^{-1} and phage MS2 at 10^7 PFU reaction^{-1}. In recent times routine surveillance has been achieved using Whole-Genome Sequencing (WGS). It has also been extensively applied in the tracking of transmission routes and determining contamination incidents in the farm-to-fork continuum (Rantsiou *et al*., 2018). Multiplex-PCR based Illumina sequencing approach has been applied in the diagnosis of foodborne pathogens (Ferrario *et al*., 2017). Statistically, robust quantitative comparisons between communities have become practical following the increase in sequencing depth and throughput.

1.2 Statement of the problem

Previous studies (Githuka, 2016; Githuka *et al*., 2016; Njiru *et al*., 2016) have reported foodborne outbreaks associated with microbial contamination of food and water in Embu, Kenya. A cross-sectional study conducted in 2014-2016 in 22 out of 47 counties in Kenya diagnosed 11, 033 people with cholera (Githuka, 2016). The cholera was caused by *V. cholerae* serogroup 01. In Embu County, 234 (2.1%) individuals were diagnosed with this cholera strain (Githuka *et al*., 2016). Out of 7, 958 respondents in a study conducted in Embu County, 2.3% used the bush for human waste disposal. This implies that water sources could be contaminated by pathogenic bacteria (Njiru *et al*., 2016). Kangaru Market in Embu County is highly populated with the students' age 18-27 years, who feed on the fast vended foods that are sold daily in the market. The vendors often do not adhere to the public health standards such as hygienic handling of these foods. Recently, a foodborne outbreak associated with cholera outbreak was reported in Embu (https://reliefweb.int/report/kenya/fear-cholera-outbreak-three-hospitalised-embu). Despite the efforts to report on the prevalence of foodborne and waterborne pathogens in Embu County, data on their antibiotic susceptibility is scanty (BIOHAZ, 2014). Some of the food vending premises are poorly constructed thus predisposing food to bacterial contamination. Given the above, the possibility of food contamination leading to disease outbreak cannot be ruled out.

1.3 Justification of the study

Foodborne diseases result from microbial contamination that constitutes meaningful public health concern throughout the world. Although previous studies reported water and foodborne diseases such as cholera outbreak in Embu, little research work on foodborne diseases has been done (Kariuki & Orago, 2017). Many diseases associated with food contamination are difficult to detect as most of them are grouped together as diarrhea diseases. Whereas metagenomic analysis has been used to reveal prokaryotic diversity inhabiting lakes, gut microbiota and soil in Kenya (Cleary *et al*., 2019; Tang *et al*., 2019; Kambura *et al*., 2016; Karanja *et al*., 2020), its application in food safety has not yet been realized. Therefore, the study isolated and characterized antibiotic-resistant bacteria and determined the prokaryotic diversity in vended foods, water and soil from Embu Town and Kangaru Market, in Embu County.

1.4 Research questions

1. Are there bacterial food contaminants in vended fast foods, soil and water in Embu Town and the nearby Kangaru Market?
2. What is the nature of antibiotic resistance of these bacterial food contaminants?
3. What is the diversity and abundance of prokaryotes in vended fast foods, soil and water in Embu Town and Kangaru Market?

1.4.1 General objective

To profile bacteria food contaminants found in vended fast foods, soil and water in Embu Town and nearby Kangaru Market.

1.4.2 Specific objectives

1. To isolate, characterize and identify culturable bacterial food contaminants in vended fast foods, soil and water in Embu Town and nearby Kangaru Market.

2. To characterize antibiotic resistance genes of bacteria in the vended fast foods, soil and water.

3. To determine the diversity and abundance of prokaryotes in the vended fast foods, soil and water.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Microbial food contamination

Food is a substance that is edible and can provide the nutritional requirements needed for the well-being of the physical body (Rodriguez-Lazaro *et al*., 2012). Food can also serve as a vector for disease transmission. Its safety encompasses many factors such as handling, processing and subsequent storage (Altekruse *et al.*, 1997). Microorganisms are the major causes of foodborne illness and may cause a change in the flavour, appearance, taste and texture of food. Street vended foods may contain microbial contamination from bacteria such as *Clostridium perfringens*, *Salmonella* spp and *Staphylococcus aureus*, which are associated with disease outbreaks (Muleta & Ashenafi, 2001). *S. aureus* an enterotoxigenic strain is characterized by the unexpected onset of symptoms such as vomiting, abdominal pain and stomach cramps when consumed in food (Kadariya *et al.*, 2014). Foodborne disease outbreaks have been reported to result from the consumption of fresh fruits and vegetable salads contaminated with bacterial pathogens (Allende & Monaghan, 2015). Ready to eat foods originating from animals which include milk, dairy products, eggs, raw meats, are identified as a major transmitter of foodborne pathogens (Li *et al*., 2019). The Center for Disease Control and Prevention together with the European Food Safety Authority has put in place rigorous measures that ensure food safety. They have also elaborated fact-finding systems that ensure the availability of data related to foodborne disease outbreak (Mališevs *et al*., 2019).

2.2 Water contamination by bacterial pathogens

The availability of safe and clean water is paramount for the well-being of any population. Whereas it is the right of every person to be provided with safe water, it has not been the case since the majority are exposed to water-borne diseases. In most developing countries, 80% of waterborne disease breakouts is due to contaminated water (Chouhan, 2015). Over 700 million people are not supplied with clean and safe water, mostly in Sub-Saharan African (Bain *et al*., 2014). The WHO approximates that 1.8 billion people globally are infected with the waterborne diseases (Bain *et al*., 2014). Globally, children, the majority living in rural areas have been reported to

succumb to death due to drinking unsafe water, which is associated with poor sanitation (Olowe *et al*., 2017). Water contamination resulting from microorganisms such as bacteria and viruses originates from sewage treatment plants, septic systems, wildlife and livestock production among others. This water later finds its way into food during production, processing and preparation (Sowah *et al*., 2017).

Mycobacteria have been associated with biofilm formation in water pipes which consequently contaminate drinking water (Falkinham *et al*., 2001). Members of this genus including; *M. avium* complex, *M. gordonae* and *M. flavescens*, have been associated with respiratory diseases in people who are immunocompromised (Mazumder *et al*., 2010). *Legionella* spp inhabits the aquatic environment as well as surface water, groundwater and manmade water reservoirs. The bacterium has been associated with a respiratory disease known as legionellosis which occurs in form of Legionnaire's disease as well as Pontiac fever (Fields *et al*., 2002). Legionellosis occurs when *Legionella* sp. are inhaled or aerosolized. *Helicobacter pylori*, a pathogen of the gastrointestinal tract has been shown to inhabit rivers, pond water, cattle feces and house flies. *H. pylori* have been associated with cases of duodenal ulcers, peptic and gastritis (Perez-Perez *et al*., 2004). Water is perceived to act as a reservoir of pathogens occurring occasionally in drinking water that has been exposed to sewage systems or infected animals (Bellack *et al*., 2006).

2.3 Resistome of antibiotic resistance and foodborne pathogens in the soil

The instantaneous victory of antibiotic molecules in the early days of discovery was as a result of pathogen sensitivity. The new medicines drastically reduced the mortality and morbidity of infectious diseases (Herrell, 1945). This success was short-lived as resistance towards these antibiotics evolved. Although the urge to develop new antibiotics has been increasing, little is known about the ecology and natural host of genes encoding resistance to antibiotics. This perspective overlooks the concept of intrinsic resistance or the subsequent resistance genes that naturally occur in the environments (Cox & Write, 2013). Fresh produce has been identified as one of the reservoirs of foodborne pathogens inhabiting the soil, of which some have been reported to be antibiotic-resistant. This mostly occurs during production as the vegetables are cross-contaminated during the "farm and fork" chain. The fresh produce is exposed to all forms of environmental conditions and factors that may

enable pathogenic microorganisms to find their way to final consumers (Holvoet *et al*., 2013). Soil is known to be one of the natural habitats for microorganisms including antibiotic-resistant foodborne pathogens. It has been identified as a pre-harvest factor with microorganisms that contaminate farm produce. Incidences of farm produce being contaminated with microbial pathogens have led to many countries developing guidelines and procedures that enhance hygienic practices in the handling of food, and measures that are applied to curb and manage hazards such as pathogenic microorganisms along the farm produce chain supply (FAO, 2012). Even though these preventive measures are reported, relaxation on implementation varies from country to country.

2.4 Foodborne diseases and pathogens

Foodborne illness constitutes a meaningful public health concern throughout the world. Nearly 600 million disease outbreaks associated with bacterial food contamination were reported in 2010, as well as 350 million cases resulting from pathogenic bacteria (WHO, 2015). Severe cases including Guillain-Barre syndrome associated with *Campylobacter* contamination, and central nervous failures as a result of *Salmonella spp* have been reported (Havelaar *et al.,* 2015). Other medical conditions that have been previously reported due to perennial chronic infection include; arthritis caused by *Campylobacter* spp, *Salmonella* spp, *Shigella* spp, as well as mental abnormality, deafness and blindness resulting from *L. monocytogenes* (Hasan *et al*., 2013). Food contaminated with *Salmonella* spp. results in colon cancer and aggravates other medical conditions (Mughini-Gras *et al.,* 2018). *Campylobacter* spp. and *Salmonella* spp., coagulase-positive *Staphylococcus* spp., *Listeria monocytogenes* and *Vibrio parahaemolyticus* have been previously detected in food (Omulo *et al*., 2015; Schoder *et al*., 2015). These foodborne pathogens including the opportunistic pathogens that amalgamate in water and food have been associated with foodborne diseases. Food that harbours these pathogens includes poultry chicken, undercooked meat products, raw milk, fresh produce such as vegetables and fruits, and retailed meat (Zaulet *et al*., 2016). Entero-pathogenic bacteria which include *Vibrio cholerae, V. parahaemolyticus* and *Staphylococcus aureus* are also common causes of foodborne infections (Oladipo & Adejumobi, 2010).

2.5 Emerging and reemerging bacterial foodborne pathogens spread by foodstuffs

Foodborne Diseases Active Surveillance Network CDC, tracks outbreaks that are identified in laboratory diagnosed infections arising from enteric pathogens that are mostly disseminated through food. The 2016 surveillance data highlights nine pathogens and the shifts in the incidences in comparison to the years between 2013-2015. The highest number of confirmed positive infections in 2016 was reported for STEC (1, 845), *Yersinia* (302), *Cyclospora* (55), *Salmonella* (8, 172), *Listeria* (127), *Shigella* (2, 9130 and *Cryptosporidium* (1, 816). Cases where the infection was present but without culture confirmation, were reported for *Yersinia* (32%), *Campylobacter* (32%), STEC (24%), *Salmonella* (8%) *Vibrio* (13%) and *Shigella* (23%) (Marder *et al*., 2018). *Bacillus cereus*, *Arcobacter* spp., *Staphylococcus* spp., *Clostridium botulinum*, *Clostridium perfringens* and *Escherichia coli* are considered as emerging foodborne pathogens that are of clinical importance (Hänel *et al*., 2016). *Arcobacter* was identified as an emerging zoonotic pathogen and categorized as a momentous pathogen (Rasmussen *et al.*, 2013). It was later isolated from food. Hepatitis E virus is also considered as a reemerging food pathogen that can result in chronic, fulminant, or acute diseases that are difficult to treat (Salines *et al.*, 2017).

2.6 Genetic basis of antibiotic resistance in Bacteria

Antibiotics are widely used in veterinary medicine, agriculture well as human medicine among others (Kümmerer, 2004). Due to widespread usage bacteria have undergone rapid evolution resulting in resistance towards antibiotics to which they were initially susceptible (WHO, 2014). Antibiotic resistance developed from antibiotic overuse, non-medical indiscriminate usage, lack of compliance by patients and transfer by R-plasmids (Chen *et al.*, 2018). Through antibiotic-resistant genes, pathogens can resist antibiotics through; pumping out of antibiotics, developing biochemical pathways to resistance, development of copious mucilage, change in L-form, acetylation, adenylation, phosphorylation of antibiotics and modification of target structures for the antibiotics (Alekshun & Levy, 2007). Bacterial pathogens attenuate the effects of antibiotics, through sensory perception and activation of the respective resistance genes thus ensuring the longtime perpetuation of the resistance genes (Olsen, 2015).

Transcription factors have been known to mediate antibiotic resistance as demonstrated in the Gram-negative tetracycline efflux pump mediated by *tetA* promoter, which results in an impediment of *tetA* expression that leads to antibiotic resistance genes expression (Andersson & Hughes, 2010). Bacterial pathogens employ non-coding RNAs that can detect antibiotics and synchronize resistance genes promptly (Fleisher *et al.,* 2018; Oliva *et al.,* 2015). When *E. coli* susceptibility to ampicillin was tested, the strains became tolerant through mutation. This was supported by the whole genome analysis showing *E. coli* strains have mutations in the promoter of *ampC* which codes for Beta-lactamase enzyme that breakdown the beta-lactam ring (Van den *et al.,* 2016). Amoxicillin and ampicillin belong to the class of beta-lactam antibiotics. Production of beta-lactamase enzyme by plasmid-mediated beta-lactamase commonly found in Enterobacteriaceae is the major cause of resistance to these antibiotics (Ahmed & Shimamoto, 2015). Ribosome engineering is the main cause of streptomycin resistance in the majority of eubacteria (Li *et al*., 2019).

2.7 Antimicrobial resistance and susceptibility in foodborne pathogens

The rise in antibiotic resistance in foodborne pathogens poses a detrimental setback to the health of the public globally (WHO, 2014). Antibiotic resistance has been associated with approximately 700, 000 mortalities globally yearly (O'Neill, 2014). The cases of antibiotic resistance continue to increase as a result of the boundless application of antibiotics in agriculture and disease prevention units. Consequently, this has a negative effect on food production, safety and consequently human health (Watkins *et al*., 2016). Agriculture has been shown to account for 75% of the use of antibiotics in the USA and the EU. In the 24 EU countries, the use of antibiotics reduced significantly by at least 12% between 2011 and 2014 (EFSA and ECDC, 2019). This trend is expected to continue since more farmers have become aware of increasing antibiotic resistance. Foodborne pathogens associated with antibiotic resistance include; *Shigella* spp*., Klebsiella pneumoniae, Salmonella typhi* and *Campylobacter* spp. (Fleming-dutra *et al.,* 2018). A study on antibiotic resistance of enteric pathogens in Kibera, Kenya confirmed that fast food and the environment are a reservoir of antibiotic resistance (Muhonja *et al.,* 2012). The family Enterobacteriaceae comprises some clinically important species such as *Klebsiella* spp, *Serratia* spp, *Proteus* spp and *E. coli* and they are the most commonly isolated antibiotic-resistant strains (Iredell *et al.,* 2016). *E. coli* O157 led to foodborne illness

that resulted in antibiotic resistance to penicillin (Xie *et al.*, 2017). Aerobic and facultative anaerobic pathogens in the family Enterobacteriaceae are associated with meningitis, pneumonia and intra-abdominal infections (Nordmann *et al.*, 2011). Carbapenem resistance in Enterobacteriaceae *S. Kentucky* has been reported from the consumption of contaminated beef and turkey meat (O'Bryan *et al.*, 2017).

2.8 Use of Molecular biology techniques for detection and identification of foodborne pathogens

Food has been identified as one of the transmitters of pathogens, thus the microbiological safety of food is of paramount importance. The rapid identification of microbial contaminants in food is essential in ensuring food safety. Traditional culture methods used in the detection of microorganisms are unreliable as some microorganisms are left out and the methods are time-consuming (Law *et al*., 2015). Rapid detection methods such as qPCR and multiplex PCR are fast, effective, sensitive and cost-efficient. Nucleic acid-based methods target specific RNA sequences in the pathogens by hybridizing the sequences to an oligonucleotide (primer) and base-pair appropriately to its complementary sequences (Zhao *et al*., 2014). Polymerase chain reaction-based methods, amplify specific targeted DNA sequences. DNA sequences from a specific foodborne pathogen such as; *Listeria monocytogene* and *Campylobacter jejuni*, are amplified and analyzed thereafter (Law *et al*., 2015). Quantitative PCR has improved the detection of foodborne pathogens. The risk of cross-contamination witnessed in other detection systems is remarkably reduced and thus high throughput is feasible since no post PCR manipulation is needed (Mackay, 2004). Since traditional methods of pathogen detection arbitrate the theoretical detection of pathogens at low concentrations, they may not be reliable. Next-generation sequencing is reported to allow for both non-targeted multiple spoilage agents as well as culturable and non-culturable microorganisms to be detected and characterized (Calo-Mata *et al*., 2016).

2.9 Detection of microbial food contaminants by metagenomics

Investigation of foodborne pathogens, their source, and the resultant risks can be done using metagenomics. The whole-genome data guides investigators as to whether an outbreak arose from deliberate or natural action (Okumura *et al*., 2012). Metagenomics has been used in the investigation and characterization of food

(Kergourlay *et al*., 2015). The detection of leading foodborne pathogen *Campylobacter jejuni* has been made possible by culture-independent sequence-based metagenomics (Kim *et al.*, 2019). Metagenomic analysis of 16S rRNA in food-grade salts detected halophilic archaea belonging to the *Halobacteriaceae* family and genera including *Halobacterium, Halorubrum* and *Haloarcula* (Henriet *et al*., 2014). Phylogenetic analysis revealed watercress as the main cause of STEC O157 in two concurrent foodborne disease outbreaks although no microbiological link was identified (Jenkins *et al*., 2015). The ability to control foodborne disease outbreaks caused by *Listeria monocytogenes* depends on how fast its source is identified and whole-genome sequencing has been used to reveal this (Wang *et al*., 2015). The microbial diversity of Kimchi (a type of fermented food) and Chinese cabbage were revealed using next-generation sequencing. Bacterial genera detected in Kimchi included *Leuconostoc*, *Weissella* and *Lactobacillus* (Jung *et al*., 2011). In Chinese cabbage, the most abundant genera detected were *Chryseobacterium*, *Sphingomonas*, *Pseudomonas* and *Aurantimonadaceae*, Pathogens such as *Klebsiella*, *Salmonella, Yersinia* and *Staphylococcus* were also detected (Kim *et al.*, 2018). Currently, whole genome sequencing detects antibiotic-resistant bacterial pathogens. Surveillance schemes enable the early detection of outbreaks and subsequent epidemiological investigations.

2.10 Presence of uncultured prokaryotes in food and soil

The significant diversity of the uncultured microorganisms has prompted researchers, to develop techniques for the cultivation of uncultured microorganisms. Some of these microorganisms have not been cultured in the laboratory before and thus their biology is not well understood. In the last decade, metagenomics has been shown to resolve these issues, as pathogenic microorganisms can be detected early enough thus reducing cases of foodborne diseases (Kimura, 2018). Two uncultured pathogenic bacteria with accession numbers FJ210785 and FJ210787 were detected in milk powder for babies using molecular methods (Ahmed *et al*., 2014). Pyrosequencing detected 16 unclassified prokaryotic phyla in soil. With the great diversity being revealed as follows phyla Acidobacteria (43.79%) and Proteobacteria (29.70%). Also, 67 novel strains and 35 uncultured strains were detected (Chaudhary, *et al*., 2019). Ravin *et al*., (2015) using metagenomic analysis were able to detect *Candidatus caldiarchaeum* subterraneum in soil, which was later assigned to *Aligarchaeota*. In the same sample,

uncultured *Candidatus Fervidibacteria* and *Candidatus Calescibacterium* ‘nevadense’ were detected.

CHAPTER THREE

3.0MATERIAL AND METHODS

3.1 Description of the study site

Samples were collected from Embu County (latitude: 0°31’58.80’’N and longitude: 37°27’0.00’’) in Kenya. The samples were collected from the Central Business District of Embu Town and the nearby Kangaru Market which are densely populated (Figure 3.1). Kangaru Market is largely inhabited by students aged 18-27 years and other residents. At the time of study in 2018, there were 324 business entities licensed to sell food in the Central Business District of Embu Town and Kangaru Market.

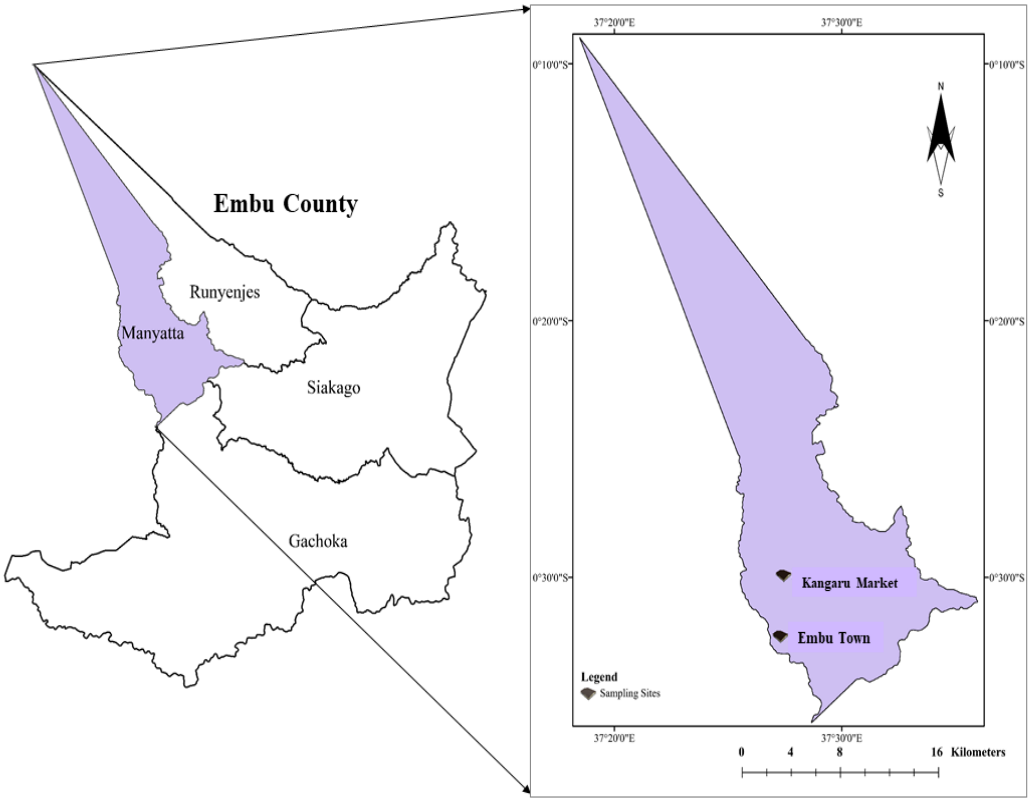


Figure 3.1: Map of Embu County where food, water and soil samples were collected

3.1.1 Sample size determination

A sample size of n = 176 was determined using the formula described by Israel (2009). Samples were purposively collected from 176 sampling points approximately every ½ km distance from each other.

$$s = \frac{x^2\ NP\ (1-P)}{d^2\ (N-1) + \{x^2\ P\ (1-P)\}} \quad (1)$$

$$s = \frac{1.96^2\ \times 324 \times 0.5\ (1-0.5)}{1.96^2\ (324-1) + \{1.96^2\ \times 0.5\ (1-0.5)\}}$$

s=175.98

s~176

S= required sample size, x^2 = the table value of the chi-square for 1 degree of freedom at the desired confidence level (3.841), N = the population size, P = the population proportion, d = the degree of accuracy expressed as a proportion (0.05) and P = the population.

Samples comprising of; fruits, vegetable salad, African sausage, potato fries, fish, roasted meat, samosa, soil, and water. Since the fast foods were not equally distributed in all the sampling points, they were bought based on their availability. All the 176 sampling points were sampled and a total of 21 different sample types were collected. For each of the samples, 10 samples (replicates) were collected and later pooled together. The food samples were carried in sterile disposable bags, water in sterile bottles, and soil in disposable bags that were sterile. To collect soil samples, about I gram of soil was collected from each sampling point by scooping using the spatula. The water samples included the treated Embu Water and Sanitation Company (EWASCO) water and not treated irrigation water. The residents in the Kangaru Market use both irrigation and treated water while in Embu Town residents use only treated water from EWASCO. Water and soil samples were collected at every point where the fast-foods were bought. The water was drawn directly from the taps in the sterile bottles. The collection of samples was done aseptically and taken to the University of Embu Microbiology laboratory using a cool box at 4°C. Preparation of samples was done immediately on arrival and innocula were prepared from each sample aseptically using sterile water.

3.2 Preparation of inoculum and sub-culturing

The samples were incubated in eight selective media namely; macconkey agar, salmonella shigella agar, brain heart infusion agar, phenylalanine agar, pseudomonas fluorescence agar, brilliant green agar, eosin ethidium bromide agar and deoxycholate

agar (manufacture, Legacy limited company). This selective media aided in the identification of the isolates. The standard cultivation method for bacterial food contaminants isolation was performed based on the guidelines described by Hazeleger & Beumer, (2016). The external surfaces of sample packaging were sterilized with 70% (v/v) ethanol. Using a weighing balance, 10g was aseptically weighed. A volume of 10ml of water samples was measured using a measuring cylinder. The food samples were homogenized using 50ml sterile tap water under aseptic conditions using motor and pestle. The inoculum was prepared by adding sterile tap water and stored in a sterile bottle having 100 ml sterile tap water. Serial dilution from the soil, food and water was prepared by transferring 1 ml of the original dilution (10^{-0}) with a sterile pipette into a deep well containing 9 ml sterilized water to prepare further dilutions to (10^{-8}). Using a pipette, 100 µl aliquots were inoculated on the selective media on Petri dishes that had been left to solidify overnight under aseptic conditions and spread equally. The plates were incubated for 24hrs at 37 °C in an inverted position. To obtain pure cultures, distinct colonies following the sub-culturing were grown on fresh media by streaking and incubated for 24hrs at 37 °C. The control experiment included the use of sterile water that was autoclaved and distilled. The inoculum and the plates were cultured and no growth was expected to occur.

3.2.1 Characterization and identification of bacterial isolates

Isolates were characterized based on the shape, color, elevation and margin of the colony. The Gram reaction was performed using the Gram staining technique and their cell morphology was observed under the microscope (Cappuccino & Sherman, 2002). The isolates were subjected to Triple Iron Sugar (TIS) test to determine their ability to ferment lactose, sucrose and glucose as well as produce hydrogen sulfide gas using triple iron sugar as described by Clesceri *et al*. (2005). The capability of an isolate to breakdown and use citrate as the only source of carbon or energy source was tested using simmon citrate agar (Ledeboer & Doern, 2015). The capability of the isolate to produce phenyl-pyruvic acid was tested using phenylalanine agar according to MacFaddin, (1985). The ability of the isolate to produce catalase enzyme was determined by the addition of one drop of 3% hydrogen peroxide on 24-hour cultureas described by Reiner, (2010).

The substrates that were used to screen for the capability of the isolates to release extracellular enzymes were skimmed milk, tween 20, starch and cellulose. For each, 2 g of the substrate and 12 grams of agar were measured and dissolved in 1000 ml of water, sterilized by autoclaving for 15 minutes at a temperature of 121 ºC, allowed to cool, and later poured into sterilized Petri dishes. The media solidified overnight. Serial dilution of workable bacterial inoculum was achieved by transferring 1 ml of the original inoculum (10^{-1}) with a sterile pipette to another tube containing 9 ml of distilled water to prepare further decimal dilution up to 10^{-5} fold. The filter papers were saturated with isolates as disc inoculum. The qualitative screening of the isolates was done by introducing the isolates on cellulose, skimmed milk starch and tween 20 media. The cultured plates were incubated for 48hrs at 37 °C and later assayed. Starch, cellulose, plated were later screened with Congo red dye and the dye washed off with 1M NaCl followed by water that is distilled. Clear halos surrounding the bacterial growth after staining with 0.6% KI (Lugol) solution, revealed that the isolates were able to produce amylase and cellulase enzymes. Observation of a clear zone around the isolates on the skimmed milk indicated the ability of the isolate to produce protease. Observation of precipitation due to calcium laurate around the isolate on tween 20 indicated the ability of the isolate to produce esterase enzyme (Sharma *et al*., 2016; Mulaa, 2018; Torres *et al*., 2016).

3.3 Antibiotic susceptibility

The standardized protocol of Kirby-Bauer's disc diffusion was carried out using Mueller-Hinton agar for the antimicrobial sensitivity screening of the isolates in reference to the Clinical Laboratory Standard Institute (CLSI) guideline (Standard & Testing, 2018). Antibiotics from six classes of commonly used antibiotics were used namely; aminoglycosides (gentamicin and streptomycin), tetracycline, chloramphenicol, quinolones and sulphonamides. *Klebsiella pneumonia* 1792880 and *E. coli* 35218 were used as quality control strains. Antibiotic discs of amoxicillin (20 µg), cefotaxime (30 µg), gentamicin (10 µg), streptomycin (10 µg), tetracycline (30 µg), nalidixic acid (30 µg), trimethoprim + sulfamethoxazole (1.25/23.75 µg) and chloramphenicol (30 µg) were used. Bacterial suspensions of the pure isolates were prepared based on 0.5 McFarland turbidity standard that has nearly 1.5 X 10^8 bacterial cells per ml. A bacterial suspension of 100 µl was introduced and distributed equally in plates with Mueller Hinton Agar (MHA). Selected discs saturated with antibiotics

were aseptically placed on the surface of the agar in triplicate and the plates were replicated three times. The incubation of plates was done in an inverted position at 37 ºC for 24 hrs. The resultant diameter of the zones of inhibition measured in millimeters was recorded. The interpretation of the growth was as per guidelines of CLSI as either susceptible, intermediate, or resistant (Standards & Testing, 2018) as shown in Table 3.1.

Table 3.1: Drug sensitivity interpretation of zones of inhibition diameter based on CLSI

Antibiotics	**Potency**	**Susceptible**	**Intermediate**	**Resistant**
Amoxicillin	20 µg	≥18	14-16	≤8
Cefotaxime	30 µg	≥26	23-25	≤22
Gentamycin	10 µg	≥15	13-14	≤12
Streptomycin	10 µg	≥15	13-14	≤12
Tetracycline	30 µg	≥15	12-14	≤11
Nalidixic	30 µg	≥19	14-18	≤13
Trimethoprim+ sulphamethoxazole	(1.25+23.75)µg	≥16	11-15	≤10
Chloramphenicol	30 µg	≥18	13-17	≤12

3.3.1 Antibiosis test

Standardized bacterial suspensions were prepared as described by Nuneza *et al.*, (2015). Muller Hinton agar, was weighed as per the instructions provided by the manufacturer and dissolved in water, then autoclaved at 121 °C for 15 minutes. The media cooled and was dispensed in the initial plates containing solidified agar as a second layer and left to solidify overnight. Holes of 6 mm were made in the agar using a sterile tip down the first layer. Aliquots of 100 µl of the test microorganism based on McFarland standard, were introduced by swabbing onto Mueller Hinton Agar (MHA) without touching the holes. Using a sterile tip, 50 µl of the obtained pure isolates were introduced in holes. The experiment was repeated three times, incubated at 37 ºC and monitored after every 24 hours for two days. The resulting zones of inhibition were measured. The quality control strains used were, *Staphylococcus aureus* ATCC 1026, *Klebsiella pyrope* 1792880, *E. coli* ATCC 35218, *Klebsiella pneumoniae* ATCC 49619 together with *Staphylococcus aureus* BAA 976.

3.4 The bacterial chromosomal DNA extraction protocol

The bacterial broth that had been inoculated and incubated overnight was aseptically transferred to sterile 2ml Eppendorf microtubes. The bacterial isolates were harvested by centrifuging for 5 minutes at 13200 rpm after which the broth was discarded. The harvested bacterial cells were carefully resuspended in 100 µl of solution A (100 mM Tris-HCL (pH 8.0), 100 mM EDTA (pH 8.0) and 5 µl of lysozyme (from a 20mg/ml solution) and incubated for 15 minutes at 37 °C in a water bath. Lysis buffer 400 µl composed of 400 mM Tris-HCl (pH 8.0), 60 mM, EDTA (pH 8.0), 150 mM NaCl and 1% sodium dodecyl sulfate was added and the microtubes were allowed to stand for 10 minutes. Into the mixture, 10 µl (20mg/ml) of Proteinase K, was introduced after which the composition was allowed to stand at 65 °C for 15 minutes in a water bath. Chloroform was added as an equal volume of the mixture in the vials and centrifuged for 5 minutes at 13200 rpm at 4 °C. The supernatant volume was introduced into a new Eppendorf tube. The volume of supernatant was noted. Sodium acetate 150 µl (pH5.2) and an equal volume of the mixture (supernatant + Sodium Acetate) of isopropyl alcohol was introduced into the mixture. The contents were mixed by gently inverting the tubes. The tubes were centrifuged for 10 minutes at 13, 200 after which the resultant supernatant was removed. The pellet of DNA was carefully cleaned in 300 µl of molecular 70 % ethanol. The extracted pellet of DNA was centrifuged at 13200 rpm for 1 min and later the supernatant was dispensed. The clean DNA was allowed to dry by leaving the tubes in the open air for 1hr and later resuspended in 30 µl PCR water (Sambrook & Russell, 2001b).

3.4.1 Plasmid DNA extraction from antibiotic-resistant bacteria

The bacterial isolates that were phenotypically resistant to antibiotics used were grown overnight in Luria Broth (LB) for 24 hrs. Plasmids were then extracted as previously described by Bimboim & Doly (1979). The bacterial suspension was introduced into 2 ml eppendorf tubes and bacterial cells were harvested by centrifuging at 13200 rpm for 1minute. The supernatant was discarded and the pellet retained. Commercial buffers (Invitrogen Plasmid Extraction Buffer Co) were used according to manufacturer instructions Resuspension buffer (P1), 100 µl was added to the pellet and the mixture vortexed to re-suspend the cells. This was followed by adding 100 µl of lysis buffer (P2). The tubes were gently inverted 5 times to ensure adequate mixing.

Neutralization buffer (P3), 100 µl was added and inverted 5 times gently to allow mixing. The tubes containing the mixture were centrifuged at 13200 rpm for 10 min. The separated volume of supernatant was carefully transferred to another set of Eppendorf tubes without interfering with the white precipitate. The pellet assumed to be DNA was allowed to air dry after which 500 µl Ice cold molecular ethanol was added into the supernatant, mixed by inverting and later incubated at -20 °C for 1 hour. Plasmid DNA was air-dried and re-suspended in 30 µl PCR water and stored at -20 °C.

3.5 Partial characterization of bacterial 16S rRNA gene

The 16S rRNA gene sequence was PCR-amplified using bacterial primer pair 8F (5'-AGCTTTGATCCTGGCTCAG-3') and 1492R (5'-CGGCTACCTTGTTACGACTT-3') for all selected bacterial isolates according to Sambrook *et al*. (1989). Amplification was carried out using an 8F forward primer and 1492R reverse primer. Amplification was carried out using Phusion-High fidelity PCR Kit (New England and Biolabs Inc), 10 µL mixture reaction containing 10X PCR buffer 0.2 µl, 2.5 mM dNTPs 2 µl, 8F forward primer 0.5 µl (5pmol), 1492R reverse primer 0.5µl (5pmol), 5 U/µl Taq polymerase 0.02 µl, DNA template 0.5 µl, $MgCl_2$ 2 µl, DMSO 3 µl and PCR grade water 6 µl (all reagents were products of Bioline Co.). The amplification was performed as follows; hot start 94 °C for 3 mins, start cycle 35 times, denaturation 94 °C for 45 secs, annealing 53.9 °C for 45 secs, elongation 72 °C for 5 mins and storage at 4 °C. The products of PCR following the amplification of the antibiotic resistance genes were loaded in 1% agarose gel and run for 40 minutes using 40V and the quality of the PCR product was checked under UV, labeled and shipped to Inqaba, South Africa for sequencing

3.5.1 PCR amplification of antibiotic resistance genes

The polymerase chain reaction for genes encoding resistance to tetracycline, streptomycin, beta-lactam such as ampicillin, chloramphenicol and trimethoprim was performed as described by Sambrook & Russell, (2001) with some modifications. Amplification was carried out in a mixture of 10 µl containing 10X PCR buffer 02 µl, 2.5mM dNTPs 2 µl, 8F forward primer 0.5 µl (5pmol), reverse primer 0.5 µl (5pmol), 5 U/µl Taq polymerase0.02 µl, DNA and plasmid template 1µl, $MgCl_2$ 2 µl, and DMSO 3 µl and PCR grade water 6 µl (all reagents were products of Bioline Co.).

The amplification was performed as follows; hot start 94 °C for 3 mins, start cycle 35 times, denaturation 94 °C for 45 secs, annealing temperature differed for the respective primers for 45 secs, elongation 72 °C for 5 mins, and storage at 4 °C. The products of PCR following the amplification of the antibiotic resistance genes were loaded in 1% agarose gel and run for 40 minutes using 40V and the quality of the PCR product was checked under UV, labeled and shipped to Inqaba, South Africa for sequencing. Isolates that were phenotypically resistant to the tested antibiotics were screened for the presence of resistance genes using the primers designed in this study (Table 3.2).

Table 3.2: List of primers for PCR amplification of antibiotic resistance genes

Primer	**Sequence (5' to 3')**	**Target gene**	**Amp size (bp)**	**Anne Temp** (°C.)	**Resistance to antibiotic**
Flor-F Flor-R	TTATCTCCCTGTCGTTCCAGCG CCTATGAGCACACGGGGAGC	*Flor*	450	57.5	Chloramphenicol
strB-F strB-R	GGCACCCATAAGCGTACGCC TGCCGAGCACGGCGACTACC	*Str* (B)	400	61	Streptomycin
DfrA1-F DfrA1-R	CGAAGAATGGAGTTATCGGG TGCTGGGGATTTCAGGAAAG	*Dfr*(A1)	200	55	Trimethoprim
TEM-F TEM-R	GCGGTATCATTGCAGCACTG TGCTTAATCAGTGAGGCACC	$_{B}la_{TEM}$	1000	55	Beta-lactam
Amp-F Amp-R	ATGCACACGCTGATCGGATT GCGGACGCAGACTTCACTAA	*bla Amp*	268	65	Ampicillin
Tet-F Tet-R	AACCGGCATTGAGAGCATCA TTGTCTCCTCTCCCTTGGCT	*Tet* (A)	699	60	Tetracycline

Key; Amp (Amplicon), Anne (Annealing)

3.6 Statistical analysis for cultured bacterial cells

In the antibiotic sensitivity test, means of zones of inhibition for susceptible, intermediate and resistant isolates were determined. The experiment was replicated three times. SE (standard error) for the means of inhibition was calculated using descriptive statistics. Data on antibiosis, extracellular enzymatic experiments and antibiotic sensitivity test, was recorded in excel sheets. General linear model (PROC GLM) procedure of SAS software version 9.1 (SAS Institute, Cary.NC) performed the analysis of variance (ANOVA) for all measured data for antibiosis as well as enzymatic activity. The ANOVA tables were presented as mean± standard error (SE). the diameter of zones of inhibition resulting from the antibiotic activity and the zone of clearance resulting from the enzymatic activity were compared using Turkey's

honest significant difference test (HSD). Correlation profiles of zones of clearance on different substrate and zone of inhibition for the selected bacterial isolates were visualized as a heatmap generated by a hierarchical clustering R script using R version 3.3.1 software. The genetic affiliation of the sequenced genes of the isolates was deduced from the phylogenetic tree generated using MEGA X.

3.7 Total DNA extraction protocol from fast food, soil and water environment

The total genome was extracted from water, soil and selected food samples in duplicate according to Sambarook *et al*. (1989) with some modifications. From each soil sample, 0.5g of soil was weighed and introduced in 2ml Eppendorf tubes and dissolved in 1ml of sterile water. From the prepared food inoculum, 1ml was dispensed in 2ml tubes. The samples were dispensed into the tubes and centrifuged for 10 minutes at 13200rpm and the supernatant dispensed off. All samples produced a visible pellet after centrifugation. The harvested samples that contained the microorganisms were re-suspended in 500 µl of solution A comprising of 100mM Tris-HCL (pH 8.0) 100mM EDTA (pH 8.0) and mixed by vortexing, centrifuged for 1 minute after which the supernatant was discarded. The pellets were re-suspended in 200 µl of solution A (100mM Tris-HCL (pH 8.0), 100mM EDTA (pH 8.0) and 5 µl of lysozyme (from a 20mg/ml solution) and suspended in the water bath at 37°C for 30 minutes. Lysis buffer 600 µl constituting 400mM Tris-HCL (pH 8.0), 60 mM, EDTA (pH 8.0), 150 mM NaCl, 1% of sodium dodecyl sulfate) was added and the tubes were allowed to stand for 10 minutes. Proteinase K, 10 µl (20mg/ml) was introduced after which the mixtures were incubated in the water bath at 65°C for 55 minutes. Chloroform: isoamyl alcohol, and an equal volume of the mixtures was introduced and then centrifuged at 13200 rpm, 4°C for 15 minutes. The supernatant was introduced into new tubes and its volume was noted. Sodium acetate 150 µl (pH5.2) and an equal volume of the mixture (supernatant + Sodium Acetate) of isopropyl alcohol was added to the mixture and the tubes mixed by inverting gently and incubating overnight at -20°C. The tubes were centrifuged for 10 minutes at 13200 rpm after which the volume of the suspended supernatant was discarded. The pellets of extracted DNA were cleaned in 300 µl of molecular grade 70% ethanol. The pellets were centrifuged at 13200 rpm for 1 min and later the supernatant was discarded. The DNA pellets were air-dried and stored at -20 °C. The DNA pellets were lyophilized to ensure its stability during the shipping.

3.7.1 Amplicon library preparation

The16S rRNA gene of the total genome extracted, was amplified at the variable region V4-V7. The primers 515F (GTGCCAGCMGCCGCGGTAA) and 806R (GGACTACHVGGGTWTCTAAT) that were barcoded were used (Caporaso *et al*. 2011). The amplification of the variable region was undertaken as follows in 30 cycles. The HotStarTaq Plus Master Mix Kit (Qiagen, USA) used was subjected to the following conditions. The initial heating was done at 94 °C for 3 minutes. This was followed by running 30 cycles of 94 °C for 30 seconds, 53 °C for 40 seconds, and 72 °C for 1 minute repetitively. The final elongation of the PCR products was done at 72 °C for 5 minutes. After PCR products were amplified they were checked for their quality by loading the samples in the wells of 2% agarose. The products were allowed to run for 40 minutes at 40Vthen the quality and the intensity of the bands under the UV was determined. The purified PCR product was used to prepare the DNA library according to the Illumina TruSeq (Yu & Zhang, 2012). Sequencing was performed at Molecular Research DNA (www.mrdalab.com, Shallowater, TX, USA) on a MiSeq 2x300bp Version 3 following the manufacturer's guidelines.

3.8 Sequence analysis and taxonomic classification

The proprietary pipeline was used to deplete the barcodes and primers from the sequences (www.mrdnalab.com, mrDNA, Shallowater, TX) which were designed and developed by the service provider mrDNA. Sequences quality control was undertaken first by trimming and then filtering sequences based on their quality score. The sequences were later clustered into operational taxonomic units (OTUs) that were based on a fixed dissimilarity threshold. Microbiome bioinformatics was performed with QIIME 2 2019.410 according to Bolyen *et al*., (2018). The q2 demux plugin was used in the filtration of the raw sequences and de-multiplexing them and later their denoising was done with DADA2 (Callahan *et al*., 2016) (via q2-dada2). Mafft through q2 phylogeny, were used in the alignment of the sequences variants (ASVs) (Katoh *et al*., 2002) and later phylogeny with fasttree2 was constructed (Price *et al*., 2010).

Diversity metrics which are the unweighted UniFrac were performed according to Lozupone *et al*.,(2007). The Alpha diversity matrices were determined according to Faith (1992). Unweighted UniFrac was performed according to Lozupone & Knight,

(2005). The assigning of the Taxonomy was based on ASVs using the q2 feature classifier (Bokulich *et al*., 2018a). Sklearn naïve Bayes taxonomy classifier which was against the Greengenes 13_8 99% OTUs reference sequences was performed according to McDonald *et al*.,(2012) Jaccard distance, Principle Coordinate Analysis (PCoA) and Bray Curtis dissimilarity were done with reference to q2 diversity. The first principal co-ordinates axis (PC1) change in the direction and the subsequent magnitude was computed using q2 longitudinal according to Bokulich *et al.*, (2018b). The submission of raw sequences was done to NCBI Sequence Read and assigned accession number SUB8376444.

3.9 Statistical analyses of metagenomic data

The R packages which included Vegan, phyloseq, and ampvis2 R packages versions 2.5.6, 1.30.0, 2.6.0 respectively in R version 3.6.3 (2020-02-29), were used to perform diversity indices which include; Richness, Shannon, rarefaction curves, absolute diversity together with the Venn diagram. The above analysis was used in comparing the shared OTUs between the vended food and, soil and water. Hundred interaction of the rarefaction curves were computed in each sample to (20,000 sequences) using QIIME2 pipeline version qiime2-2019.10. The OTUs' richness in the data sets and the sample types was based on Chao1, which is their non-parametric estimation. Vended food and the environmental samples distances were differentiated based on the Analysis of similarity (ANOSIM) test, using Bray-Curtis distance with permutations of 999 measurements. The redundancy analysis (RDA), Non-metric Multidimensional Scaling (NMDS), together with the Hierarchical clustering of the samples according to Bray-Curtis dissimilarity were done using the R programming language according to Team, (2012) and the Vegan package according to Oksanen *et al*., (2007). The correlation of Pearson's coefficient, of the vended food and the consequent structure of the soil and water, was determined using the Mantel test in R programming language (Team, 2012). Data analysis 3D PCoA plots were calculated using unweighted UniFrac and Bray Curtis, which was used to calculate the distance. Based on the samples, taxonomic groups were based on the number of reads that were assigned to taxon at all ranks using the exported OTUs output from QIIME2 pipeline Version 2019.10. Permutational multivariate analysis of variance (PERMANOVA) compared the sites and tested the null hypothesis of the centroids and dispersion of the groups as defined by the measure space.

3.10 Permission to conduct research

A permit to conduct the research was obtained from National Commission for Science, Technology & Innovation (NACOSTI) in Kenya (Appendix 3) and the Public Health Ministry of Embu County (Appendix 4).

CHAPTER FOUR

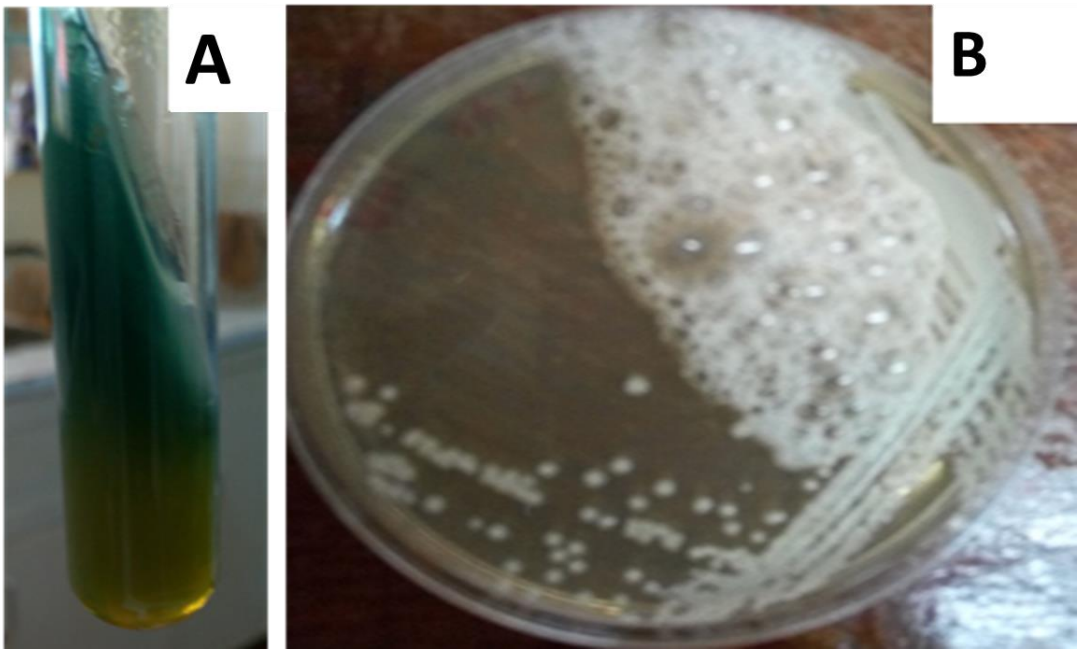
4.0 RESULTS

4.1 Characterization and identification of the antibiotic-resistant bacterial isolates

Out of 345 bacterial isolates isolated from the 210 food samples, 70 bacterial isolates were resistant to at least three antibiotics. The isolates exhibited diverse morphological characteristics. Gram-negative isolates were 47 while Gram-positive isolates were 23. The cocci shaped were seven, rod-shaped were 62 while streptococcus was one. *Bacillus* spp, were 15, *Citrobacter* species were two, *E. coli* were five, *Enterobacter* spp, were three, *Proteus* spp, were two, *Providencia* spp, were two, *Pseudomonas* spp, were 13, *S. pneumoniae* were three, *Salmonella* spp, were six, *Serratia marcescens* were four while *Shigella* spp, were three (Appendix 1).

4.1.1 Biochemical characteristics of the antibiotic-resistant bacterial isolates

Isolates exhibited distinct biochemical reactions characterized by change in the color of the media. Out of the 70 isolates 54.29% utilized lactose 47.17% utilized sucrose, 71.43% utilized glucose utilization, 7.14% produced phenyl-pyruvic acid, 35.71% utilized citrate, 90% broke down hydrogen peroxide by producing catalase enzyme. The catalase reaction was vigorous in 48.57% of the isolates, moderate in 30% and slow in 11.43% of the isolates. Hydrogen peroxide gas was produced by 11.43% of the isolates indicated by a black precipitate and cracks in some cases. *Virgibacillus phasianinus*, *E. coli* and *Bacillus weidmannii* were able to produce bubble cracks (Plate 4.1). The majority of the isolates were from Kangaru Market and a few were from Embu Town (Appendix 2).



(a) Isolate KFR42, was able to utilize citrate indicated by growth on the surface and Prussian blue color

(b) Isolate KF27, was able to produce hydrogen peroxide gas vigorously, following the production of catalase enzyme by this isolate

Plate 4.1: Biochemical reactions of some bacterial isolates from Embu Town and Kangaru Market.

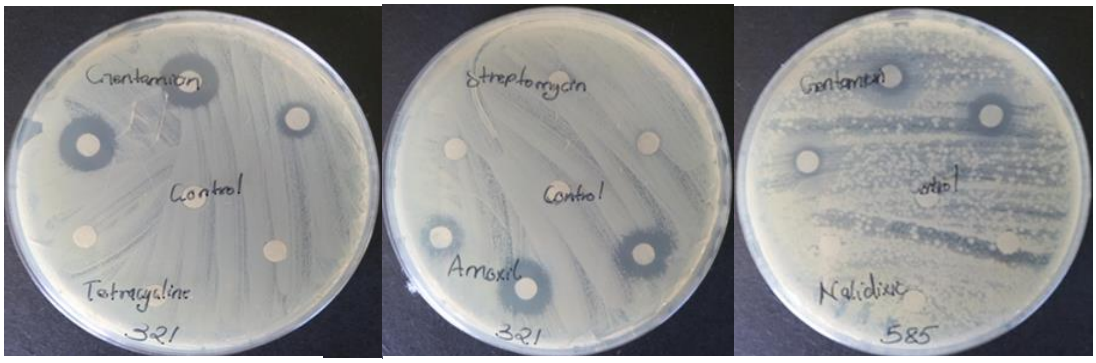
4.1.2 Identification of antibiotic-resistant bacteria

Following morphological and biochemical characterization the probable identities of the isolates were deduced. The most commonly isolated antibiotic-resistant isolates were *Bacillus* spp, (21.43%) followed by *Pseudomonas* spp, (18.57%). Majority of the antibiotic-resistant isolates were from soil (24.29%) followed by fish (20%) and fruits (18.57%), while the least were from samosa (1.42%), chips (5.71%) and roasted meat (4.29%) (Appendix 1 and 2).

4.2 Antibiotic resistance of the bacterial isolates

Antibiotic sensitivity test assays revealed that 70 out of 345 bacterial isolates were resistant to at least three antibiotics (multiple resistant). Isolate KAF253 affiliated to *Salmonella* spp and KAF321 affiliated to *Streptococcus pneumoniae*, were resistant to all antibiotics that were used in this study. Isolate KS65 affiliated to *Paraclostridium* spp., was susceptible to all antibiotics that were used. In this study, Trimethoprim+

sulphamethoxazole was the most effective antibiotic while chloramphenicol and cefotaxime were the least effective antibiotics. Isolate KAF321 affiliated to *Streptococcus pneumoniae* and KS585 affiliated to *Bacillus toyonensis* were both resistant to seven antibiotics. The antibiotic resistance of the two isolates from the African sausage and soil is shown in Plate 4.2.



(a) Isolate KAF321, resistant to gentamycin and tetracycline

(b) Isolate KAF321, resistant to streptomycin and amoxil

(c) isolate KS585, resistant to nalidixic and gentamycin

Plate 4.2: Antibiotic sensitivity test profile of the bacterial isolates from vended foods soil and water in Embu Town and Kangaru Market

The antibiotic resistance of the isolates was as follow; chloramphenicol 90%, cefotaxime 84.29%, nalidixic 81.43%, tetracycline 77.14%, amoxicillin 72.86%, gentamycin 48.57%, streptomycin 32.86% and trimethoprim + sulphamethoxazole 30%. The resistant isolates were mostly (88.57%) isolated from Kangaru Market while the rest (11.3%) were isolated from Embu Town (11.43%). The majority of the resistant isolates were isolated from soil (24.29%) while the least were from samosa (1.42%). The most abundant resistant species were *Bacillus* spp., (21.43%) followed by *Pseudomonas* spp., (18.57%) as shown in Table 4.1.

Table 4.1: Antibiotic resistance profiles of bacteria isolated from vended foods, soil and water in Embu Town and Kangaru Market in 2018

Isolate	Probable Identity	Location	Sample	Strep	Genta	Amox	Tetra	Chlo	Tri-Sul	Cefotax	Nalid	Resistant to (n) antibiotic
KS7	*Providencia* spp.,	Kangaru	Soil	R	I	R	R	I	I	I	R	4
KF27	*Shigella* spp.,	Kangaru	Fish	S	R	R	R	R	R	R	R	7
KRM34	*E.coli*	Kangaru	Roosted meat	S	I	R	R	R	I	R	R	5
KFR38	*Bacillus pacificus*	Kangaru	Fruit	I	S	R	R	R	R	R	R	6
KFR42	*Bacillus amyloliquefaciens*	Kangaru	Fruit	I	R	R	R	R	I	R	R	6
KAF45	*Pseudomonas* spp.,	Kangaru	African sausage	R	I	I	I	R	I	R	S	3
KFR52	*Bacillus megaterium*	Kangaru	Fruit	I	I	R	R	R	I	R	R	5
KSM63	*Shigella sonnei*	Kangaru	Samosa	R	S	I	R	I	I	R	I	3
KS65	*Bacillus wiedmannii*	Kangaru	Soil	I	S	R	S	R	I	R	S	3
KC67	*Pseudomonas aeruginosa*	Kangaru	Chips	S	S	R	R	I	S	R	R	4
KVS68	*Enterobacter* spp.,	Kangaru	Vegetable salad	S	R	R	R	R	R	S	S	5
KVS75	*Bacillus wiedmannii*	Kangaru	Vegetable salad	I	S	S	R	R	S	R	S	3
TVS81	*Bacillus thuringiensis*	Kangaru	Vegetable salad	S	S	R	R	R	I	R	R	5
KVS82	*Serratia marcescens*	Kangaru	Vegetable salad	S	R	R	S	R	S	R	R	5
KVS85	*Serratia marcescens*	Kangaru	Vegetable salad	S	S	R	S	R	S	I	R	3
KAF95	*Serratia marcescens*	Kangaru	African sausage	R	S	I	R	R	R	R	R	6
KF101	*Bacillus velezensis*	Kangaru	Fish	S	S	S	R	R	S	R	R	4
KS104	*Virgibacillus phasianinus*	Kangaru	Soil	S	R	I	S	R	S	S	R	3
KS109	*Lysinibacillus parviboronicapiens*	Kangaru	Soil	R	R	R	R	R	I	R	R	7
KS116	*Providencia* spp.,	Kangaru	Soil	R	R	R	R	R	R	R	R	8
KVS120	*Bacillus subtilis*	Kangaru	Vegetable salad	R	R	R	R	R	R	R	R	8
KC122	*Pseudomonas* spp.,	Kangaru	Chips	S	S	I	I	R	S	R	R	3

KFR131	*Pseudomonas* spp.,	Kangaru	Fruit	R	I	R	R	R	I	R	R	6
KVS147	*Staphylococcus aureus*	Kangaru	Vegetable salad	R	R	R	S	R	S	S	R	5
KF148	*Shigella* spp.,	Kangaru	Fish	I	S	R	S	R	S	S	R	3
TF152	*E. coli*	Town	Fish	R	R	R	R	R	R	R	R	8
KAF159	*Salmonella* spp.,	Kangaru	African sausage	I	R	I	S	R	S	S	R	3
KS160	*Citrobacter* spp.,	Kangaru	Soil	R	R	R	R	R	I	R	R	7
KF169	*Proteus spp.,*	Kangaru	Fish	R	R	R	S	R	S	S	R	5
KA188	*Aerobacter aerogenes*	Kangaru	African sausage	R	R	R	S	R	S	S	R	5
KFR200	*Enterobacter* spp.,	Kangaru	Fruit	S	I	S	R	R	I	R	I	3
KFR204	*Pseudomonas* spp.,	Kangaru	Fruit	S	I	R	R	R	I	R	R	5
KVS214	*S. pneumoniae*	Kangaru	Vegetable salad	R	R	R	R	S	R	R	R	7
KFR217	*Klebsiella* spp.,	Kangaru	Fruit	R	S	S	R	R	S	R	R	5
KFR222	*Bacillus mobilis*	Kangaru	Fruit	S	S	R	R	R	S	R	R	5
KC231	*S. pneumoniae*	Kangaru	Chips	S	S	I	S	R	S	R	R	3
KFR245	*P. aeruginosa*	Kangaru	Fruit	S	S	I	I	R	I	R	R	3
KF246	*S. pneumoniae*	Kangaru	Fish	I	S	I	R	R	I	R	R	4
KVS249	*Pseudomonas* spp.,	Kangaru	Vegetable salad	I	R	R	R	R	R	R	R	7
KAF253	*Salmonella* spp	Kangaru	African sausage	R	R	R	R	R	R	R	R	8
KS260	*E. coli*	Kangaru	Soil	I	R	S	R	R	I	R	R	5
KS267	*Bacillus weidmannii*	Kangaru	Soil	I	R	R	R	R	R	R	R	7
KVS271	*Pseudomonas* spp.,	Kangaru	Vegetable salad	I	R	I	R	R	I	R	R	5
KFR285	*Hafnia* spp.,	Kangaru	Fruit	S	R	I	S	R	S	S	R	3
KFR286	*Pseudomonas* spp.,	Kangaru	Fruit	I	S	R	R	R	S	R	R	5
KFR301	*Salmonella* spp.,	Kangaru	Fruit	I	R	R	R	R	R	R	R	7
KF317	*Streptococcus pneumoniae*	Kangaru	Fish	R	S	I	R	R	I	R	R	5
AF321	*Bacillus wiedmannii*	Kangaru	African sausage	R	R	R	R	R	I	R	R	7
TFR329	*Bacillus proteolyticus*	Town	Fruit	S	R	R	R	R	R	R	R	7

KVS330	*Citrobacter freundii*	Kangaru	Vegetable salad	S	R	I	R	R	R	R	I	5
AF337	*Salmonella* spp.,	Kangaru	African sausage	I	I	I	R	R	I	R	R	4
KRM349	*Pseudomonas* spp.,	Kangaru	Roasted meat	I	I	R	R	R	I	R	R	5
KS376	*E.coli*	Kangaru	Soil	S	R	R	R	R	R	R	R	7
TS378	*Salmonella* spp.,	Town	Soil	I	R	R	R	R	I	R	R	6
TS380	*Pseudomonas* spp.,	Town	Soil	R	S	R	R	R	R	R	R	7
KF381	*Micrococcus spp.,*	Kangaru	Fish	S	R	R	R	R	R	R	R	7
KF389	*Morganella* spp.,	Kangaru	Fish	I	S	R	I	R	I	R	S	3
KF391	*Serratia marcescens*	Kangaru	Fish	R	R	R	R	R	R	R	S	7
KF393	*Yersinia* spp.,	Kangaru	Fish	S	R	R	R	R	I	R	R	6
KF395	*Pseudomonas aeruginosa*	Kangaru	Fish	S	I	R	R	R	S	R	R	5
KF399	*Salmonella enterica*	Kangaru	Fish	I	R	R	R	R	R	R	R	7
TS427	*Pseudomonas* spp.,	Town	Soil	I	R	R	R	R	I	R	S	5
KC430	*Pseudomonas* spp.,	Kangaru	Chips	I	S	R	R	R	R	R	R	6
KRM435	*Bacillus weidmannii*	Kangaru	Roasted meat	I	S	R	R	I	S	R	R	4
KF463	*Bacillus anthracis*	Kangaru	Fish	R	R	R	R	R	I	R	R	7
TS472	*Bacillus cereus*	Town	Soil	S	R	R	R	R	R	R	R	7
TS572	*Bacillus subtilis*	Town	Soil	S	S	R	R	R	I	R	R	5
KS585	*Enterococcus feacalis*	Kangaru	Soil	R	R	R	R	R	R	S	R	7
KS606	*Bacillus anthracis*	Kangaru	Soil	R	I	R	R	R	I	R	S	5
TS621	*Proteus* spp.,	Town	Soil	S	R	R	S	R	I	R	R	5

Key; Strep (Streptomycin), Genta (Gentamycin), Amox (Amoxicillin), Tetra (Tetracycline), Chlo (Chloramphenicol), Tri-Sul (Trimethoprim+ sulphamethoxazole), Cefotax (Cefotaxime), Nalid (Nalidixic).

I (Intermediate), R (Resistant), S (Susceptible)

4.3 Risk groups of antibiotic-resistant bacterial isolates

Two isolates from Kangaru Market; KS606 affiliated to *Bacillus anthracis* and KAF95 affiliated to *Serratia marcescens*, are classified by the National Institutes of Health NIH as Risk Group 3 organisms. Isolate KS606 was isolated from the soil while KAF95 was isolated from the African sausage. The two isolates were resistant to at least five antibiotics used in this study. KS606 was susceptible to only nalidixic acid while KAF95 was susceptible to only gentamycin. KC75 affiliated to *Bacillus wiedmannii* and KFR222 affiliated to *Bacillus mobilis* are classified as risk group 2 organisms. The two were isolated from Kangaru Market in potato chips and fruit respectively (Table 4.2).

4.3.1 Antibiotic activity against antibiotic-resistant bacterial isolates

A hierarchical clustergram was generated using the measured zone of inhibition of the antibiotic-resistant bacteria. Trimethoprim+Sulphamethaxazole was the most effective antibiotic while chloramphenicol was the least effective. KVS120 was the most antibiotic-resistant isolate while KC231 was the most susceptible bacterial isolate (Figure 4.1).

Table 4.2: Risk Groups of antibiotic resistant bacteria from vended foods, soil and water

Isolate No.	**Location**	**Sample**	**Identity**	**% similarity**	**resistant to (n) antibiotics**	**Resistance genes detected**	**Risk group**
KFR42	Kangaru	Fruit	*Bacillus amyloliquefaciens*	99.44	6	-	1
KFR38	Kangaru	Fruit	*Bacillus pacificus*	99.1	6	*Str*(B)	1
KS65	Kangaru	Soil	*Paraclostridium benzoelyticum*	99.57	3	-	1
KC75	Kangaru	Chips	*Bacillus wiedmannii*	99.51	3	-	2
KF101	Kangaru	Fish	*Bacillus velezensis*	97.86	4	-	1
KVS120	Kangaru	Vegetable salad	*Bacillus subtilis*	97.94	8	-	1
KFR222	Kangaru	Fruit	*Bacillus mobilis*	99.83	5	*Bla* TEM	2
KF463	Kangaru	Fish	*Bacillus anthracis*	99.43	7	*Str*(B), *Bla* TEM	1
TFR329	Town	Fruit	*Bacillus proteolyticus*	97.18	7	*Str*(B), *Dfr*(I)	1
KS109	Kangaru	Soil	*Lysinibacillus parviboronicapiens*	90.83	7	*Bla* AMP	1
KS104	Kangaru	Soil	*Virgibacillus phasianinus*	99.31	3	-	1
KAF95	Kangaru	African sausage	*Serratia marcescens*	95.39	6	*Dfr*(I), *Flor*	3
KS606	Kangaru	Soil	*Bacillus anthracis*	100	5	*Tet*(A))	3
TS572	Town	Soil	*Bacillus subtilis*	97.51	5	*Str*(B), *Bla* TEM, *Flor*	1

Key; - (absence of resistant gene)

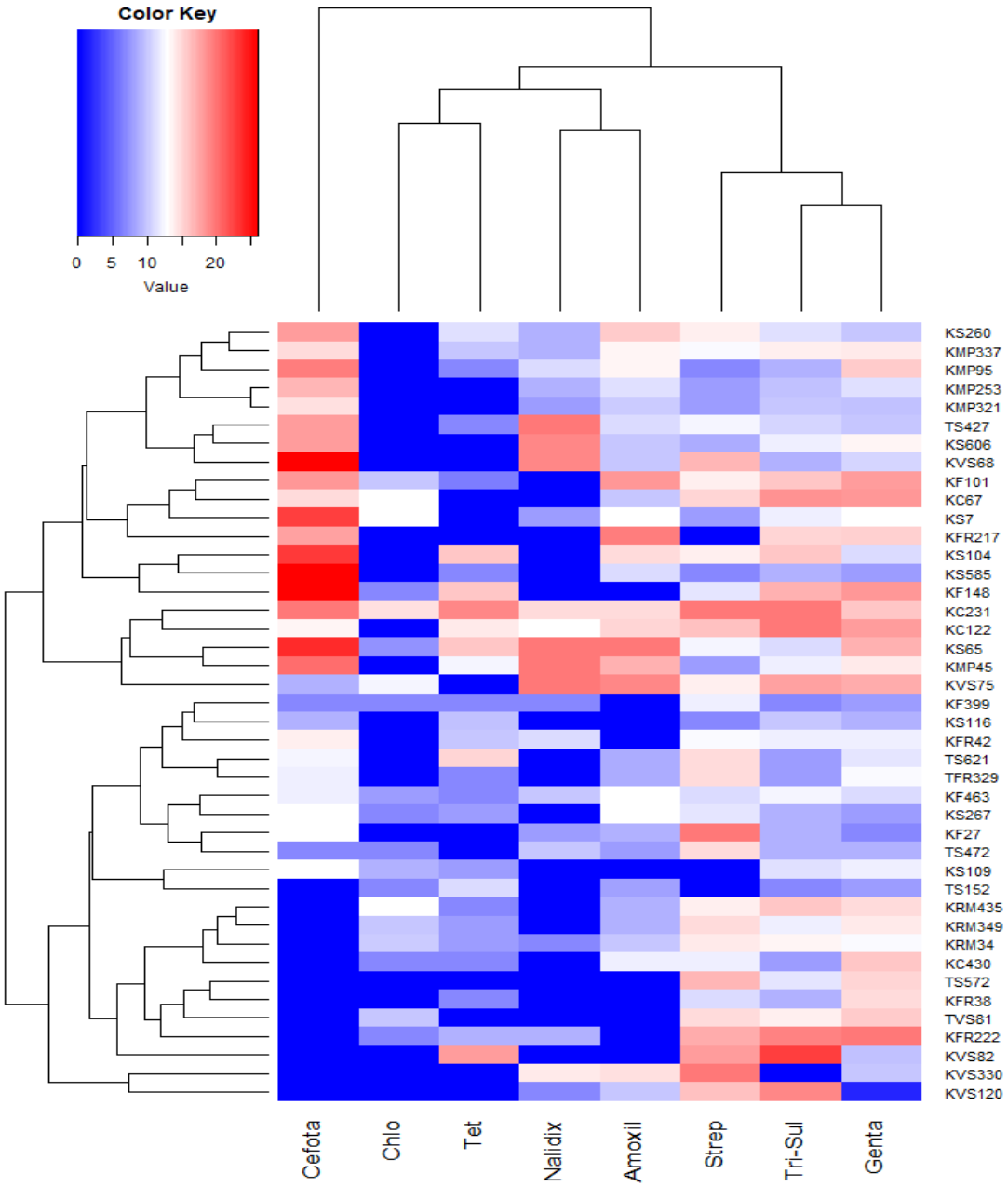


Figure 4.1: Overall performance of eight antibiotics against antibiotic-resistant bacterial isolates from vended food, soil and water in Embu Town and Kangaru Market

Key; Cefota (Cefotaxamine), Chlo (Chloramphenicol), Tet (Tetracycline), Nalidix (Nalidixic), Amoxil (Amoxicillin), Strep (Streptomycin), Genta (Gentamycin), Tri-Sul (Trimethoprim + Sulphamethoxazole).

Blue and red color scale bars in the heat map indicate the least and the highest recorded significant means values respectively at P-value ≤ 0.05 from assayed antibiotics.

The frequency of antibiotic resistance in bacteria varied among different food samples. Smokies from both sites had the lowest number of antibiotic resistant bacterial isolates (1.43%) compared to the rest of the foods in this study. Fish vended in Kangaru Market had the highest proportion of resistant isolates (18.57 %) as shown in Figure 4.2.

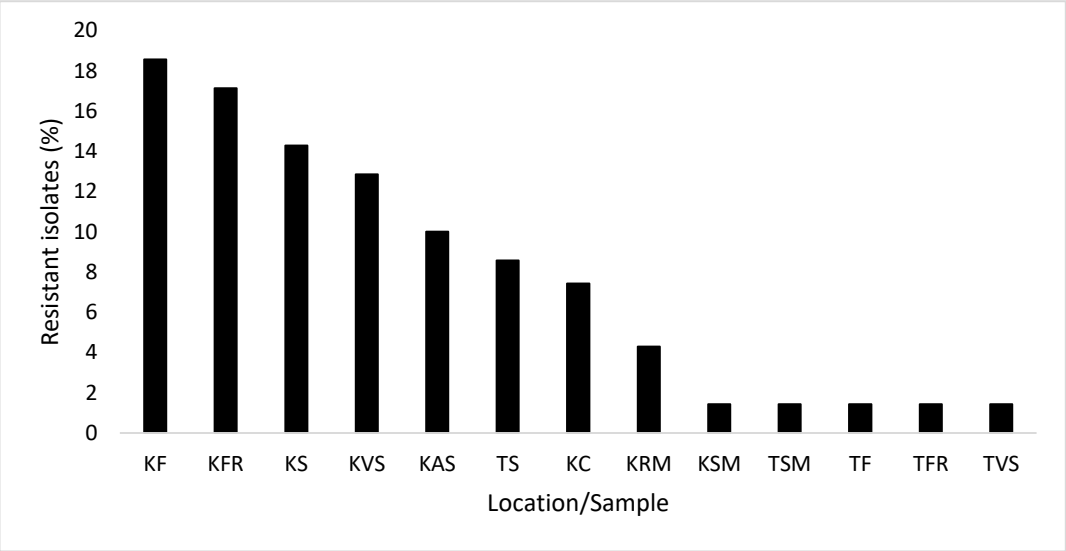


Figure 4.2: Proportion of antibiotic-resistant bacteria isolated from different foods, soil and water in Embu Town and Kangaru Market

Key; KAFS (Kangaru African sausage), KRM (Kangaru roasted meat), KF (Kangaru fish), TVS (Town vegetable salad), KVS (Kangaru vegetable salad), KC (Kangaru chips), TS (Town soil), KSM (Kangaru smokies), KFR (Kangaru fruit), KS (Kangaru soil), TFR (Town fruit), TF (Town fruits), KAFS (Kangaru African sausage).

4.4 Detection of antibiotic-resistant genes by PCR

Resistance genes were detected in 36 out of 70 phenotypically antibiotic-resistant bacterial isolates. *Amp* gene was the most frequently detected gene and was detected in twelve phenotypically resistant isolates while the least detected was *Dfr* A which was detected in seven isolates. More antibiotic resistance genes were detected from Kangaru Market in forty-six isolates as compared to eight isolates from Embu Town. The frequency of resistance genes detected in relation to the sample was as follows; fish (47.22%), soil (25%), vegetable salad (13.89%), African sausage (8.33%), and chips (5.5.60%) as shown in Table 4.3.

Table 4.3: Antibiotic resistance genes in bacterial isolates from vended food and soil in Embu Town and Kangaru Market

				Detected antibiotic resistance genes					
Isolate number	Location	Sample	Probable identity	Dfr A	Str B	*Tet* A	*Bla* TEM	*Amp*	*Flo R*
KFR222	Kangaru	Fruit	*B. mobilis*	-	-	-	+	-	-
KF169	Kangaru	Fish	*Proteus spp.,*	-	-	-	+	-	-
KF463	Kangaru	Fish	*B. parathrancis*	-	+	-	+	-	-
KAF253	Kangaru	African sausage	*Salmonella* spp.,	-	+	-	+	-	-
KFR285	Kangaru	Fruit	*Hafnia* spp.,	-	-	+	+	-	-
KF27	Kangaru	Fish	*Shigella* spp.,	-	-	-	+	-	+
AF188	Kangaru	African sausage	*A. aerogenes*	-	-	-	+	+	-
KF148	Kangaru	Fish	*Shigella* spp.,	-	-	-	+	-	+
KC67	Kangaru	Chips	*P. aeruginosa*	-	-	-	+	-	-
TS572	Town	Soil	*B. subtilis*	-	+	-	+	-	+
KAF95	Kangaru	African sausage	*S. marcescens*	+	-	-	-	-	+
TFR329	Town	Fruit	*B. proteolyticus*	+	-	+	-	-	-
KFR301	Kangaru	Fruit	*Salmonella* spp.,	+	-	-	-	+	-
KVS330	Kangaru	Vegetable salad	*E. coli*	+	-	+	-	-	-
TS380	Town	Soil	*Salmonella* spp.,	+	-	-	-	-	-
TVS81	Town	Vegetable salad	*B. thuringiensis*	+	-	-	-	+	-
KF391	Kangaru	Fish	*Morganella* spp.,	+	+	+	-	-	-
KS160	Kangaru	Soil	*Citrobacter* spp.,	-	+	-	-	-	-
KF169	Kangaru	Fish	*Proteus spp.,*	-	+	-	-	+	-
KF317	Kangaru	Fish	*E. feacalis*	-	+	-	-	-	-
KFR131	Kangaru	Fruit	*Pseudomonas* spp.,	-	+	-	-	-	-
KFR38	Kangaru	Fruit	*B. pacificus*	-	+	-	-	-	-
KVS85	Kangaru	Vegetable salad	*S. marcescens*	-	-	-	-	+	-
KFR204	Kangaru	Fruit	*Pseudomonas* spp.,	-	-	-	-	+	-
KS109	Kangaru	Soil	*L. parviboronicapiens*	-	-	-	-	+	-
KVS214	Kangaru	Vegetable salad	*S. pneumoniae*	-	-	-	-	+	-
KS585	Kangaru	Soil	*B. toyonensis*	-	-	-	-	+	+
KF399	Kangaru	Fish	*P. aeruginosa*	-	-	+	-	+	-
KS267	Kangaru	Soil	*B. weidmannii*	-	-	-	-	+	-
KS116	Kangaru	Soil	*Providencia* spp.,	-	-	-	-	+	-
KC122	Kangaru	Chips	*Pseudomonas* spp.,	-	-	-	-	-	+
KFR245	Kangaru	Fruit	*P. aeruginosa*	-	-	-	-	-	+
KS376	Kangaru	Soil	*Pseudomonas* spp.,	-	-	-	-	-	+
KVS249	Kangaru	Vegetable salad	*Pseudomonas* spp.,	-	-	+	-	-	-
KS606	Kangaru	Soil	*B. anthracis*	-	-	+	-	-	-
KF393	Kangaru	Fish	*S. marcescens*	-	-	+	-	-	-

Key; (+) indicate the presence of the resistance genes following amplification

(-) indicates the absence of the target resistance genes following amplification by PCR.

4.5 Amplification of the antibiotic resistance genes of the selected bacterial isolates.

The *Bla* $_{TEM}$ gene was present in most bacterial isolates (Plate 4.3) while *Dfr* (1) was the least detected resistance gene (Plate 4.4). The resistance genes were detected more frequently in isolates from Kangaru Market as compared to Embu Town. Three resistance genes; *Str* (B), *Bla* Tem, and *Flor* were detected from *Bacillus subtilis* isolated from the soil in Embu Town.

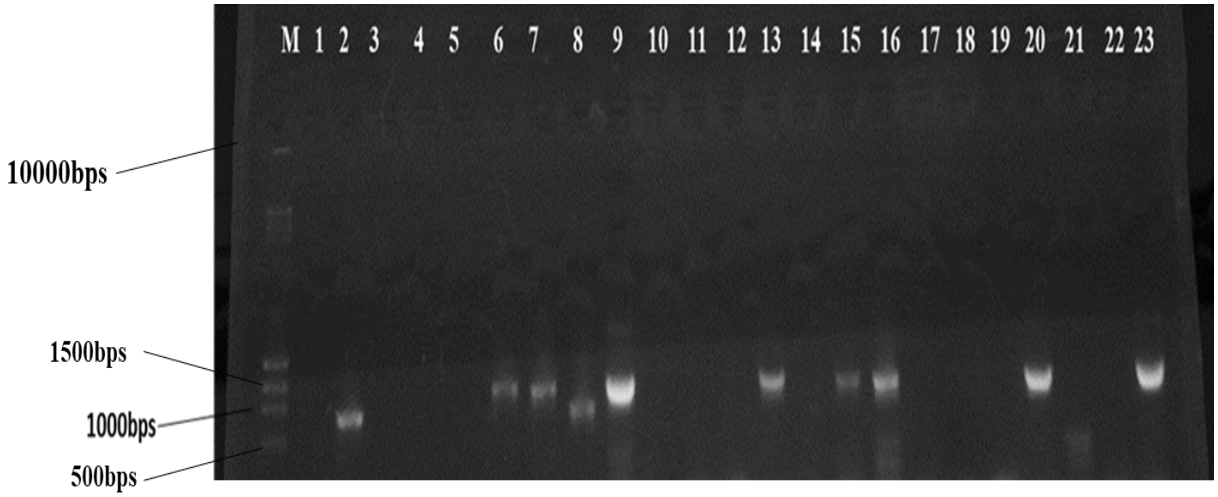


Plate 4.2: Antibiotic resistance determinants *Bla* TEM genes for 10 isolates that were resistant to beta-lactam antibiotics.
M molecular marker, Lane 2 (KFR222) 6 (KF169) 7, (KF463) 8 (KAF253) 9 (KFR285) 13 (KF27) 15 (KAF188) 16 (KF148) 20 (KC67) 23 (TS572). Band size is in base pairs.

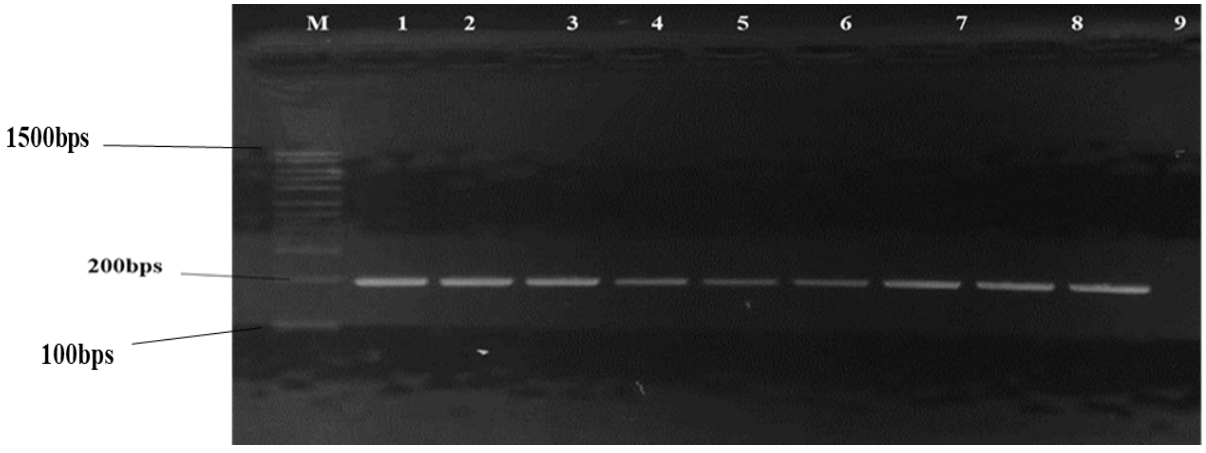


Plate 4.3: Antibiotic resistance determinants of *Dfr* (I) genes for 9 isolates that were resistant to trimethoprim antibiotic.
M molecular marker, Lane 1 (KAF95)2 (TFR329) 3 (KFR301) 4 (KVS330) 5 (TS380) 7 (TVS81) 8 (KF27) 9 (KF391) 10 (KF391). Band size is in base pairs.

4.6 Antibiosis test

Isolate KFR38 affiliated to *E.coli* inhibited most test microorganisms while isolate KVS271 affiliated to *Pseudomonas* spp., inhibited only one test microorganism (Table 4.4). Comparative assessment of bacterial antibiosis showed variation in the ability strengths of 11 out of the 70 isolates to inhibit the growth of test organisms. The majority of the isolates that exhibited antibiosis were from Kangaru Market. Bacterial isolates from the soil in both Embu Town and Kangaru Market exhibited high antibiosis activity. Isolate KMP337, exhibited significantly high antibiosis property against *S. aureus* recording an average inhibition of diameter mean 16.33±0.88mm. The test organism *K. pneumoniae* was inhibited by most of the 11 selected organisms recording an average inhibition of diameter mean 14.33±0.33m 10.00±0.33mm, 9.67±0.33mm, 7.00±0.00mm, 11.00±0.58mm and 14.00±0.58mm with *E. coli*, was only inhibited by KS260 recording an average inhibition diameter means of 7.00±0.00mm. Antibiosis of some bacterial isolates is shown in Plate 4.5.

Table 4.4: Antibiosis effect of antibiotic-resistant bacteria isolated from vended food and soil on laboratory test organisms

Isolate Number	**Probable identity**	**Location**	**Sample**	***E. coli*** ATCC 35218	***S. aureus*** ATCC 1026	***K. pneumoniae*** ATCC 49619
KC430	*Pseudomonas* spp.,	Kangaru	Chips	0.00±0.00^{b}	0.00±0.00^{b}	14.33 ±0.33^{a}
KC67	*P.aeruginosa*	Kangaru	Chips	0.00±0.00^{b}	0.00±0.00^{b}	10.00±0.00^{b}
KF395	*Yersinia* spp.,	Kangaru	Fish	0.00±0.00^{b}	0.00±0.00^{b}	9.67±0.33^{b}
KFR217	*Klebsiella* spp.,	Kangaru	Fruit	0.00±0.00^{b}	0.00±0.00^{b}	7.00±0.00^{c}
KFR38	*B. wiedmannii*	Kangaru	Fruit	0.00±0.00^{b}	8.33±2.33^{b}	13.67±0.33^{a}
KAF337	*C. freundii*	Kangaru	Meat pie	0.00±0.00^{b}	16.33±0.88^{a}	0.00±0.00^{d}
KS260	*E. coli*	Kangaru	Soil	7.00±0.00^{a}	0.00±0.00^{b}	9.00±0.00b^{c}
KSM63	*S. sonnei*	Kangaru	Samosa	0.00±0.00^{b}	0.00±0.00^{b}	10.33±1.33^{b}
KVS271	*Pseudomonas* spp.,	Kangaru	Vegetable salad	0.00±0.00^{b}	0.00±0.00^{b}	7.00±0.00^{c}
TS380	*Salmonella* spp.,	Town	Soil	0.00±0.00^{b}	0.00±0.00^{b}	11.00±0.58^{b}
TS572	*B. subtillis*	Town	Soil	0.00±0.00^{b}	0.00±0.00^{b}	14.00±0.58^{a}
P-value				0.001	0.001	0.001
CV				0.00	76.76	9.03
LSD				0.00	3.80	2.54

Mean with SE for antibiosis activity of the isolates, where the means with different laters are significantly different while those with the same letters are significantly similar as indicated by TurKey's HSD test ($P \leq 0.05$)

Antibiosis test assays revealed that 11 isolates had antibiotic activity against at least one of the test organisms namely; *E. coli* ATCC 35218, *S. aureus* ATCC 1026, and *K. pneumoniae* ATCC 49619 (Plate 4.5).

Isolate KC67 Isolate TS380

Plate 4.4: Antibiosis of some bacterial isolates from vended foods, soil and water in Embu Town and Kangaru Market

4.7 Substrate utilization of antibiotic-resistant bacteria

Different antibiotic-resistant bacteria produced extracellular enzymes in varying magnitude. Isolates KS376, KS606, and KVS147 produced extracellular enzymes on starch, cellulose and tween 20 respectively. Isolate KF148, KF317, KS116, KS7 and KVS249 showed protease activity, recorded the highest zone of clearance with an average diameter and standard error (SE) of 14.67±0.33 mm, 14.00±0.58 mm, 14.00±0.58 mm, 15.00±0.00 mm and 14.67±0.33 mm respectively. Isolate KC67, KFR42, KVS200, TS427 did not show detectable activity with an average diameter and standard error (SE) of 0.00±0.00 mm (P = 0.001) as shown in Table 4.5.

Table 4.5: Enzymatic activity of antibiotic-resistant bacteria from Embu Town and Kangaru Market

Treatment	Protease	Amylase	Cellulase	Esterase
KC122	7.33±0.33^{mnp}	0.00±0.00^{e}	0.00±0.00^{j}	0.00±0.00^{m}
KC231	9.00±0.58^{ijklmnp}	0.00±0.00^{e}	0.00±0.00^{j}	0.00±0.00^{m}
KC430	7.67±0.67^{lmnp}	0.00±0.00^{e}	0.00±0.00^{j}	10.00±0.00^{m}
KC67	0.00±0.00^{q}	0.00±0.00^{e}	0.00±0.00^{j}	0.00±0.00^{m}
KC75	20.67±0.58^{cdfghijk}	11.00±0.00^{a}	22.00±0.00^{b}	0.00±0.00^{m}
KF101	13.67±0.33^{abcd}	13.67±0.67^{a}	0.00±0.00^{b}	0.00±0.00^{m}
KF148	11.00±0.33^{ab}	7.00±0.00^{abcde}	12.00±0.00^{j}	11.00±0.00^{efghi}
KF169	9.00±0.58^{ijklmnp}	0.00±0.00^{e}	0.00±0.00^{j}	15.00±0.67^{bc}
KF246	8.67±0.67^{ijklmnp}	10.00±5.00^{ab}	0.00±0.00^{j}	8.33±0.33^{jk}
KF27	10.33±0.33^{fghijklmn}	0.00±0.00^{e}	0.00±0.00^{j}	9.00±0.00^{ijk}
KF317	14.00±0.58^{abcd}	7.67±3.84^{cd}	10.00±0.00^{cd}	0.00±0.00^{m}
KF381	12.00±0.58^{abcdfghi}	7.00±0.00^{abcde}	9.00±1.00^{def}	0.00±0.00^{m}
KF389	10.67±0.33^{dfghijklm}	0.00±0.00^{e}	7.00±0.00^{hig}	0.00±0.00^{m}
KF391	11.67±0.33^{abcdfghij}	0.00±0.00^{e}	0.00±0.00^{j}	0.00±0.00^{m}
KF393	10.67±0.33^{dfghijklm}	0.00±0.00^{e}	7.00±0.00^{hig}	7.67±0.67^{kl}
KF395	9.67±0.67^{hijklmn}	0.00±0.00^{e}	0.00±0.00^{j}	0.00±0.00^{m}
KF399	9.67±0.33^{higklmn}	10.00±0.00^{ab}	9.33±1.33^{fghijk}	10.00±0.00^{fghijk}
KF463	2.00±2.00^{q}	0.00±0.00e	0.00±0.00^{j}	10.00±0.00^{fghijk}
KFR 131	12.67±0.33^{abcdfgh}	7.00±0.00e	8.00±0.00^{efg}	10.00±0.00^{fghijk}
KFR200	10.67±0.67^{dfghijklm}	7.00±0.00^{abcde}	10.00±0.00^{cd}	0.00±0.00^{m}
KFR204	11.00±0.58^{cdfghijkl}	0.00±0.00^{e}	0.00±0.00^{j}	0.00±0.00^{m}
KFR217	10.00±0.58^{fghijklmn}	0.00±0.00^{e}	0.00±0.00^{j}	0.00±0.00^{m}
KFR222	10.00±0.00^{q}	0.00±0.00^{e}	0.00±0.00^{j}	13.67±1.67^{m}
KFR245	9.00±0.58^{ijklmnp}	0.00±0.00^{e}	7.00±0.00^{ghi}	12.67±1.20^{m}
KFR285	8.00±1.00^{klmnp}	3.67±3.67^{bcde}	0.00±0.00^{j}	0.00±0.00^{m}
KFR286	9.33±0.67^{ijklmnp}	0.00±0.00^{e}	0.00±0.00^{j}	10.00±0.00^{fghikk}
KFR301	11.33±0.33^{bcdfghijk}	0.00±0.00^{e}	0.00±0.00^{j}	11.00±0.00^{efghij}
KFR 38	6.00±0.00^{p}	0.00±0.00^{e}	0.00±0.00^{j}	0.00±0.00^{m}
KFR42	0.00±0.00^{q}	0.00±0.00^{e}	0.00±0.00^{j}	0.00±0.00^{m}
KFR52	7.00±0.00^{np}	0.00±0.00^{e}	6.00±0.00^{j}	11.00±1.00^{efghij}
KMP159	11.33±0.88^{bcdfghijk}	0.00±0.00^{e}	0.00±0.00^{j}	11.67±0.88^{efghij}
KMP188	10.33±0.33^{fghijklmn}	0.00±0.00^{e}	0.00±0.00^{j}	14.67±1.45^{cbd}
KMP253	8.00±0.00^{klmnp}	2.33±2.33d^{e}	0.00±0.00^{j}	9.33±0.67^{hijk}
KMP321	13.33±0.67^{abcdf}	10.33±0.33^{ab}	11.00±0.00^{bc}	0.00±0.00^{m}
KMP337	11.00±1.00^{cdfghijkl}	0.00±0.33^{e}	7.00±0.00^{ghi}	0.00±0.00^{m}
KMP45	11.33±0.88^{bcdfghijk}	0.00±0.00^{e}	0.00±0.00^{j}	0.00±0.00^{m}
KMP95	8.67±0.67^{ijklmnp}	7.00±0.00^{abcde}	0.00±0.00^{j}	12.00±0.00^{defgh}
KRM34	8.00±0.58^{klmnp}	0.00±0.00^{e}	0.00±0.00^{j}	0.00±0.00^{m}
KRM349	9.33±0.88^{hijklmnp}	0.00±0.00^{e}	8.00±0.00^{efg}	0.00±0.00^{m}
KRM435	8.00±1.00^{klmnp}	0.00±0.00^{e}	0.00±0.00^{j}	0.00±0.00^{m}
KS104	7.67±0.33^{lmnp}	7.00±0.00^{abcde}	0.00±0.00^{j}	11.00±0.00^{efghij}
KS109	7.00±0.00^{np}	8.00±0.00^{abcd}	7.00±0.00^{hig}	0.00±0.00^{m}
KS116	14.00±0.58^{abcd}	7.00±0.00^{abcde}	7.00±0.00^{hig}	12.33±0.88^{cdefg}
KS160	9.33±0.88^{hijklmnp}	0.00±0.00^{e}	0.00±0.00^{j}	0.00±0.00^{j}
KS260	12.67±0.33^{abcdfgh}	0.00±0.00^{e}	0.00±0.00^{j}	10.00±0.00^{j}
KS267	12.00±0.58^{abcdfghi}	8.00±4.00^{abcd}	0.00±0.00^{j}	20.00±0.00^{j}

KS376	13.00±0.00^{abcdfg}	12.00±0.00^{a}	11.00±0.00^{bc}	9.00±0.00^{ijk}
KS585	13.67±0.88^{abcd}	0.00±0.00^{e}	0.00±0.00^{j}	0.00±0.00^{m}
KS606	8.33±1.33^{klmnp}	8.00±0.00^{abcd}	14.00±0.58	0.00±0.00^{m}
KS65	9.67±0.33^{ghijklmn}	0.00±0.00^{e}	7.67±0.33^{fgh}	15.33±0.33^{b}
KS7	15.00±0.00^{a}	0.00±0.00^{e}	14.33±0.33^{a}	5.00±0.00l
KSM63	12.00±0.58^{abcdfghi}	0.00±0.00^{e}	11.00±0.00^{a}	0.00±0.00^{m}
KVS120	0.00±0.00^{q}	0.00±0.00^{e}	0.00±0.00^{j}	12.33±1.20^{cdefg}
KVS147	12.67±0.33^{abcdfgh}	9.00±0.00^{abcd}	8.00±0.00^{efg}	15.33±1.86^{b}
KVS214	9.67±0.33^{ghijklmn}	0.00±0.00^{e}	0.00±0.00^{j}	0.00±0.00^{m}
KVS249	14.67±0.33^{ab}	9.67±2.67^{abc}	7.00±0.00^{ghi}	9.67±0.67^{ghijk}
KVS271	12.67±0.33^{abcdfgh}	11.33±0.33^{a}	0.00±0.00^{j}	0.00±0.00^{m}
KVS330	10.67±0.33^{dfghijklm}	0.00±0.00^{e}	0.00±0.00^{j}	0.00±0.00^{m}
KVS68	10.00±0.58^{ghijklmn}	0.00±0.00^{e}	0.00±0.00^{j}	0.00±0.00^{m}
KVS82	8.33±0.33^{jklmnp}	7.00±0.00^{abcde}	0.00±0.00^{j}	0.00±0.00^{m}
KVS85	14.33±0.88^{abc}	0.00±0.00^{e}	11.00±0.00^{bc}	0.00±0.00^{m}
TF152	9.33±0.67^{hijklmnp}	0.00±0.00^{e}	0.00±0.00^{j}	0.00±0.00^{m}
TFR329	13.00±0.58^{abcdfg}	3.33±3.33^{bcde}	0.00±0.00^{j}	0.00±0.00^{m}
TS378	10.33±0.33^{fghijklmn}	0.00±0.00^{e}	6.33±0.33^{hi}	9.00±0.00^{ijk}
TS380	8.33±0.33^{jklmnp}	0.00±0.00^{e}	0.00±0.00^{j}	0.00±0.00^{m}
TS427	0.00±0.00^{q}	0.00±0.00^{e}	0.00±0.00^{j}	0.00±0.00^{m}
TS472	10.67±0.33^{dfghijklm}	2.67±2.67^{cde}	0.00±0.00^{j}	0.00±0.00^{m}
TS572	12.67±0.33^{abcdfgh}	0.00±0.00^{e}	0.00±0.00^{j}	0.00±0.00^{m}
TS621	8.67±0.33^{ijklmnp}	10.00±0.00^{ab}	0.00±0.00^{j}	0.00±0.00^{m}
TVS81	6.67±0.33^{p}	0.00±0.00^{e}	0.00±0.00^{j}	0.00±0.00^{m}
P-value	**0.001**	**0.001**	**0.001**	**0.001**
LSD	**3.56**	**7.21**	**1.33**	**2.85**
CV	**10.67**	**70.35**	**11.81**	**17.64**

Mean with Standard Error (SE) for each extracellular activity of the antibiotic-resistant bacteria. Means with different letters are significantly different while those with the same letters are similar as indicated by Turkey's HSD test ($P \leq 0.05$)

The correlation profile between morphometric descriptors of enzymatic activity and bacterial isolates revealed that isolate KS65 had a unique enzymatic activity correlation profile (Figure 4.3).

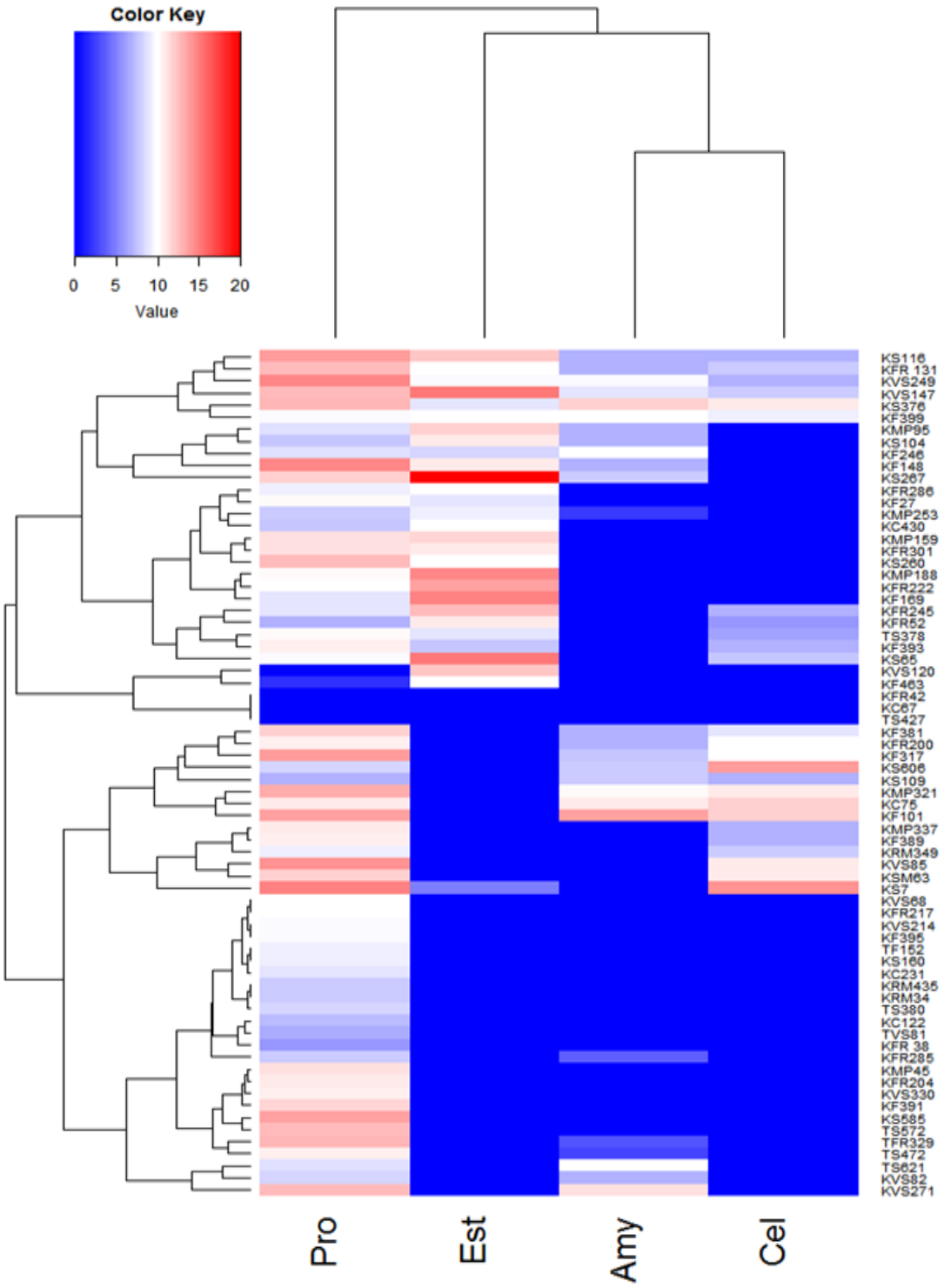


Figure 4.3: Hierarchical clustergram of assayed enzymatic activity of the bacterial isolates

The colored scale bar indicates the quantified strength of the assayed morphometric descriptors. Blue and red color in the heat map indicate the least and the highest recorded significant means values respectively at P-value ≤ 0.05 from assayed treatments.

4.8 Amplification of 16S rRNA genes of antibiotic-resistant bacteria

The 16s rRNA gene was successfully amplified in 47 isolates out of the 70 antibiotic-resistant bacterial isolates from Embu town and nearby Kangaru Market (Plate 4.6 and Plate 4.7).

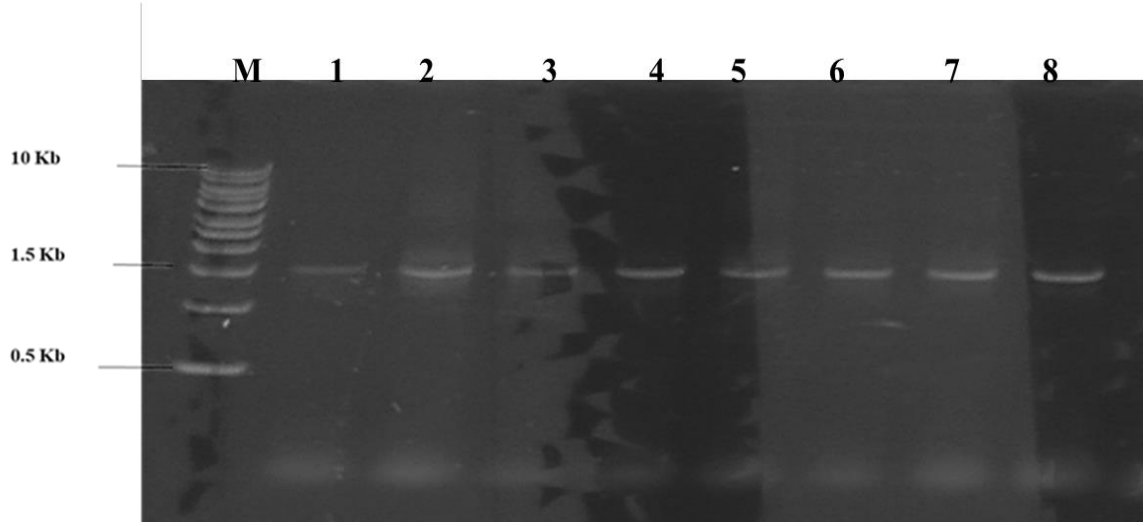


Plate 4.5: Gel photograph of bacterial 16S rRNA gene PCR products

M) molecular marker (10kb plus ladder), 1, (KF463) 2, (KRM34) 3, (KRM349) 4, (KF393) 5, (KS116) 6, (KVS85) 7, (KS7) 8, (KVS330).

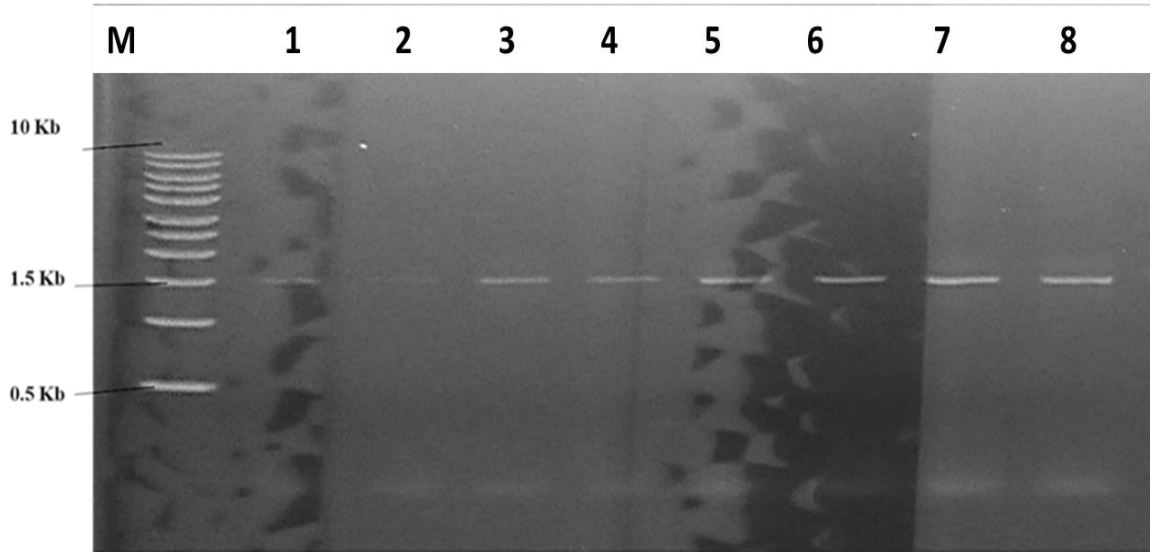


Plate 4.6: Gel photograph of bacterial 16S rRNA gene PCR products

M), molecular marker (10kb plus ladder) 1, (KVS68) 2, (KMP45, was not amplified) 3, (KC75) 4, (TS380) 5, (KVS214) 6, (KFR38) 7, (KS376) 8, (KMP253).

According to the phylogenetic analysis of 14 successfully sequenced antibiotic-resistant bacterial isolates were in five main genus clades namely; *Bacillus*, *Paraclostridium, Lysinibacillus, Virgibacillus* and *Serratia* with a similarity of above 90% (Figure 4.4). *Bacillus* formed the highest number of successfully amplified bacterial isolates distributed in nine subclades with bootstrap values of 98.

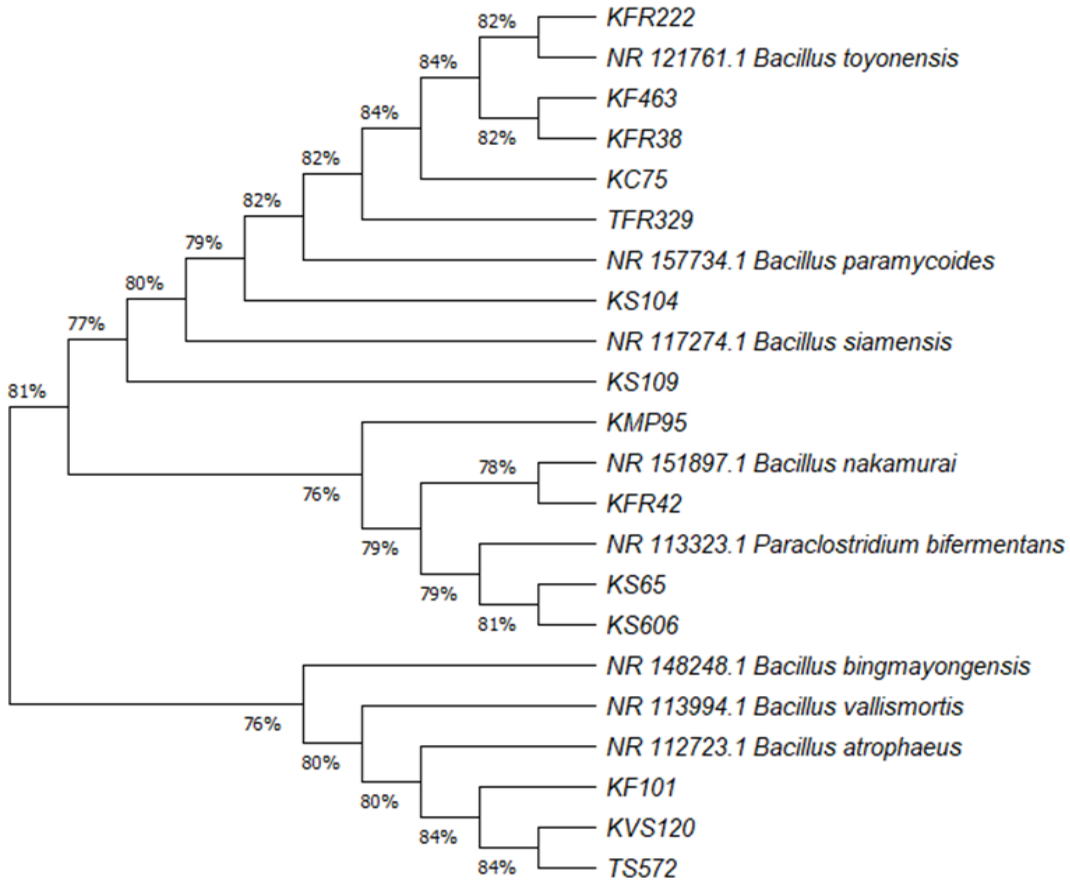


Figure 4.4: Phylogenetic tree of bacterial isolates based on the partial characterization of the 16S rRNA gene sequence

The evolutionary history was inferred through the neighbor-joining method. The optimal tree had a total length of 0.49, the tree was represented to scale and drawn with the branch length in the same units. The analysis involved 20 nucleotide sequences. Ambiguous positions were trimmed. There were a total of 1588 positions in the final dataset. The analysis was conducted in MEGA X. Isolates from the present study are represented by a number and letter codes in the phylogenetic tree.

4.9 Operational taxonomic units (OTUs) of prokaryotic sequences

The sequences read of length (>250 bp) from Illumina sequencing libraries ranged between 210536 and 539981 sequences from both vended foods, soil and water. At the phylum taxonomic level, the reads contained between 0 and 27930 OTUs. Samples from the Kangaru Market had a higher number of observed OTUs compared to samples from Embu Town. The soil had the highest number of observed OTUs while samosa had the least number of observed OTUs (Figure 4.5). When similar samples from different sites were pooled together, soil samples from the Kangaru Market had a higher number of observed OTUs compared to soil samples from Embu Town (Figure 4.6). Samples from the Kangaru Market had a higher number of observed OTUs compared to samples from Embu Town (Figure 4.7).

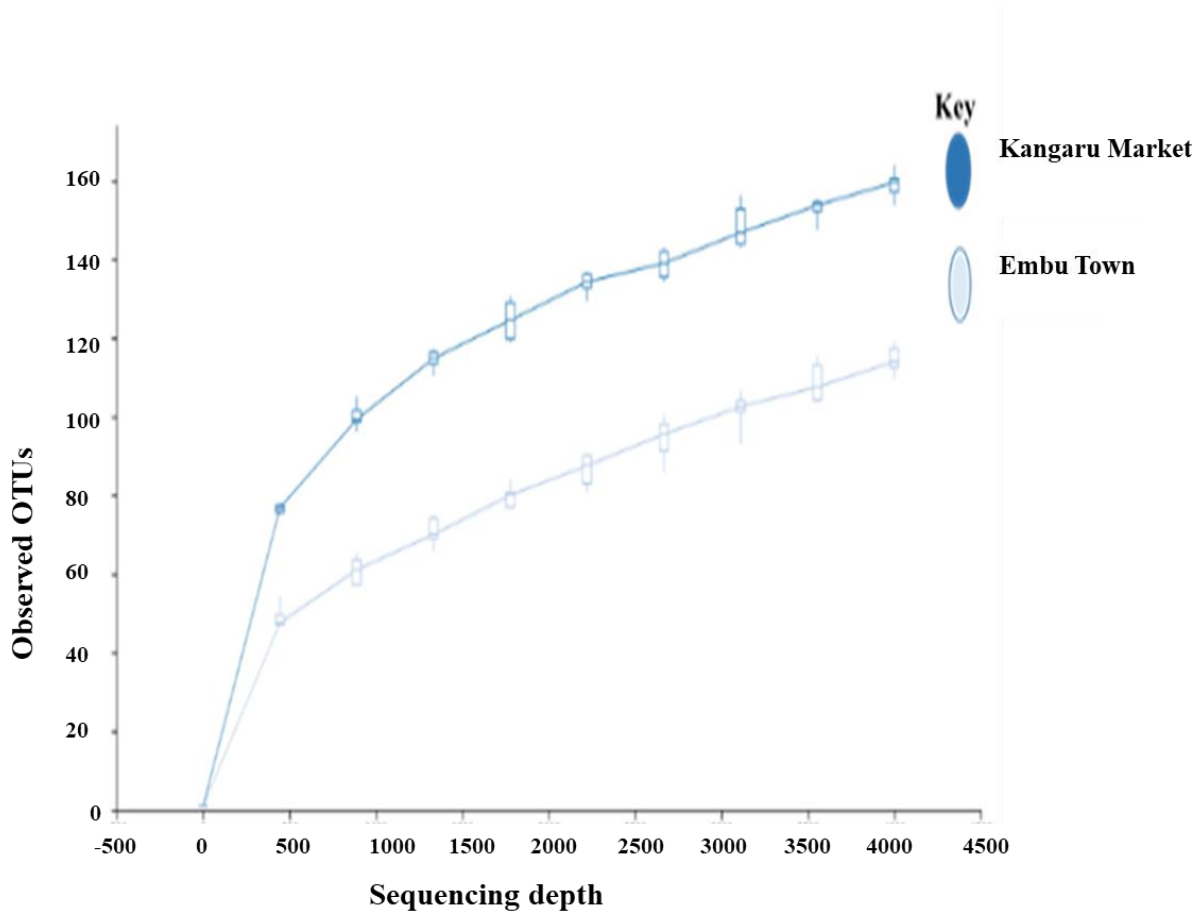


Figure 4.5: Observed OTUs as a function of the number of sequences reads in vended foods and environmental samples from Embu Town and Kangaru Market

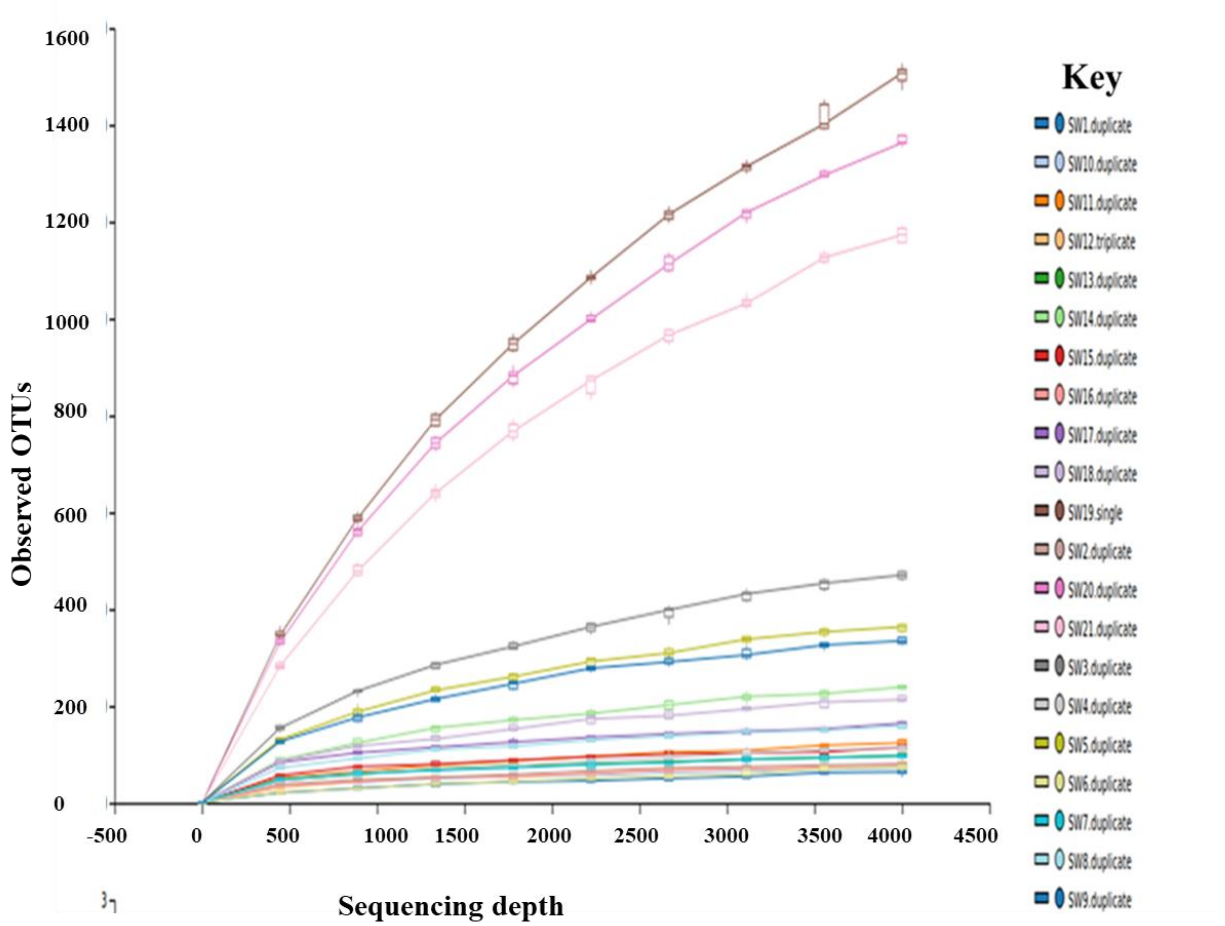


Figure 4.6: Observed OTUs as a function of the number of sequences reads from vended foods, soil and water in Embu Town and Kangaru Market.

Chips Town sw1, African sausage Town sw2, Fruits Kangaru sw3, Fruits Town 1 sw4, Kangaru water sw5, Samosa Kangaru sw6, Fish Kangaru 1 sw7, Roasted meat Kangaru sw8, Vegetable salad Town sw9, Chips Kangaru sw10, Fruits Town sw11, Smokies Town 1 sw12, Fish Town 1 sw13, Vegetable salad Kangaru sw14, African sausage Kangaru sw15, Smokies Town sw16, Fish Kangaru sw17, Fish Town sw18, Kangaru soil 1 sw19, Kangaru soil sw20, Town soil sw21.

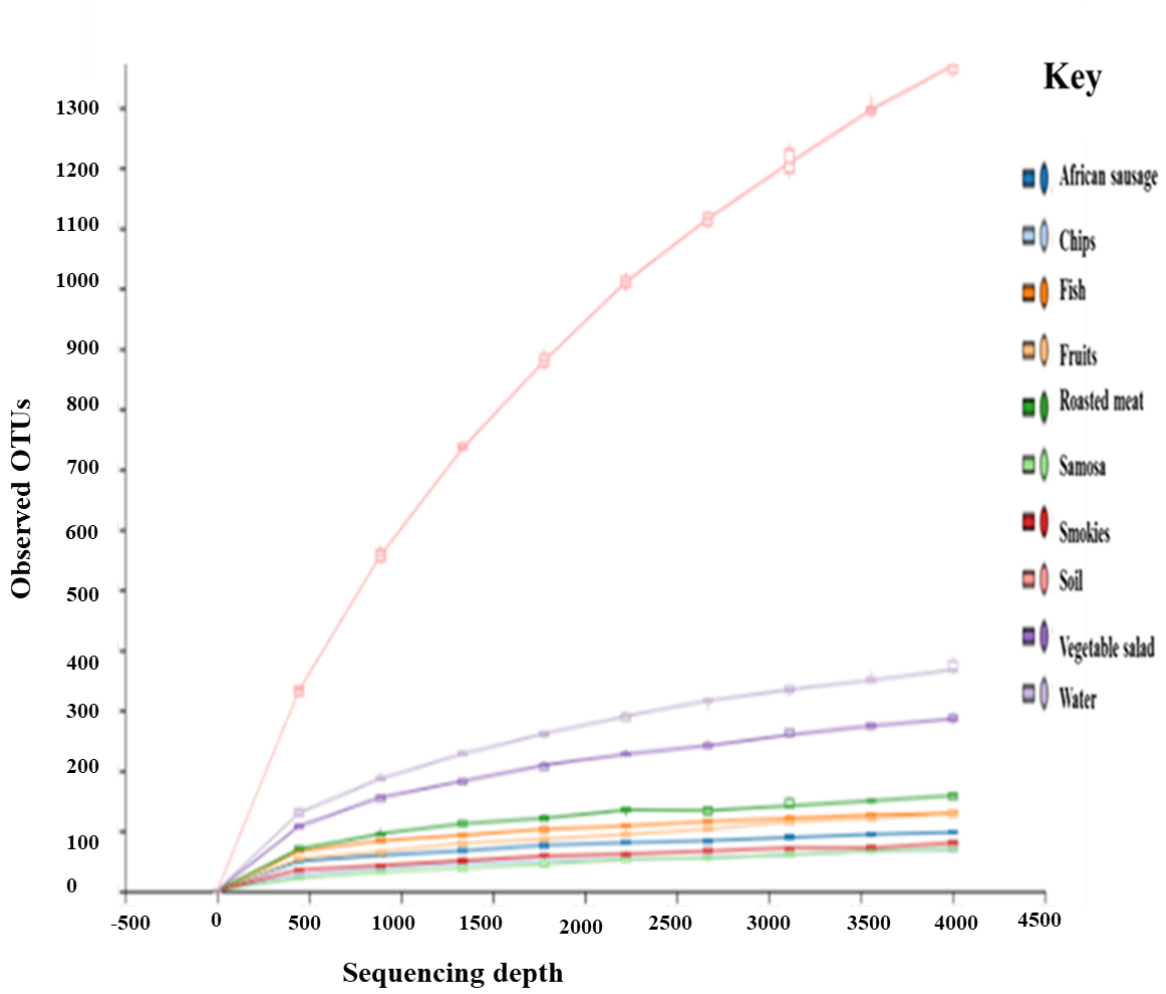


Figure 4.7: Observed OTUs as a function of the number of sequences read from the analyses of samples of vended foods, soil and water samples from Embu Town and Kangaru Market

4.10 Abundance and composition of prokaryotes in vended foods and environmental samples

The OTUs in food and environmental samples were distributed among bacterial phyla as follows; *Proteobacteria* (52.81%), *Firmicutes* (31.16%), and *Bacteroidetes* (8.00%). Hierarchical clustering, of taxa at the phylum rank for bacteria, is shown in Figure 4.8. The most abundant order was *Lactobacillales* which was present in all samples. More bacteria were detected from Kangaru Market (150 OTUs) compared to Embu Town (120 OTUs).

Hierarchical clustering of archaea taxa and abundance in each sample type is shown in Figure 4.9. The most abundant archaea order was *Nitrososphaerales* (Figure 4.10). Vegetable salad from Embu Town and fruits from Kangaru Market had similar archaeal composition. More archaea OTUs were detected from Kangaru Market (150 OTUs) as compared to Embu Town (120 OTUs). The abundance of archaea was higher in the soil compared to the food samples (Figure 4.11).

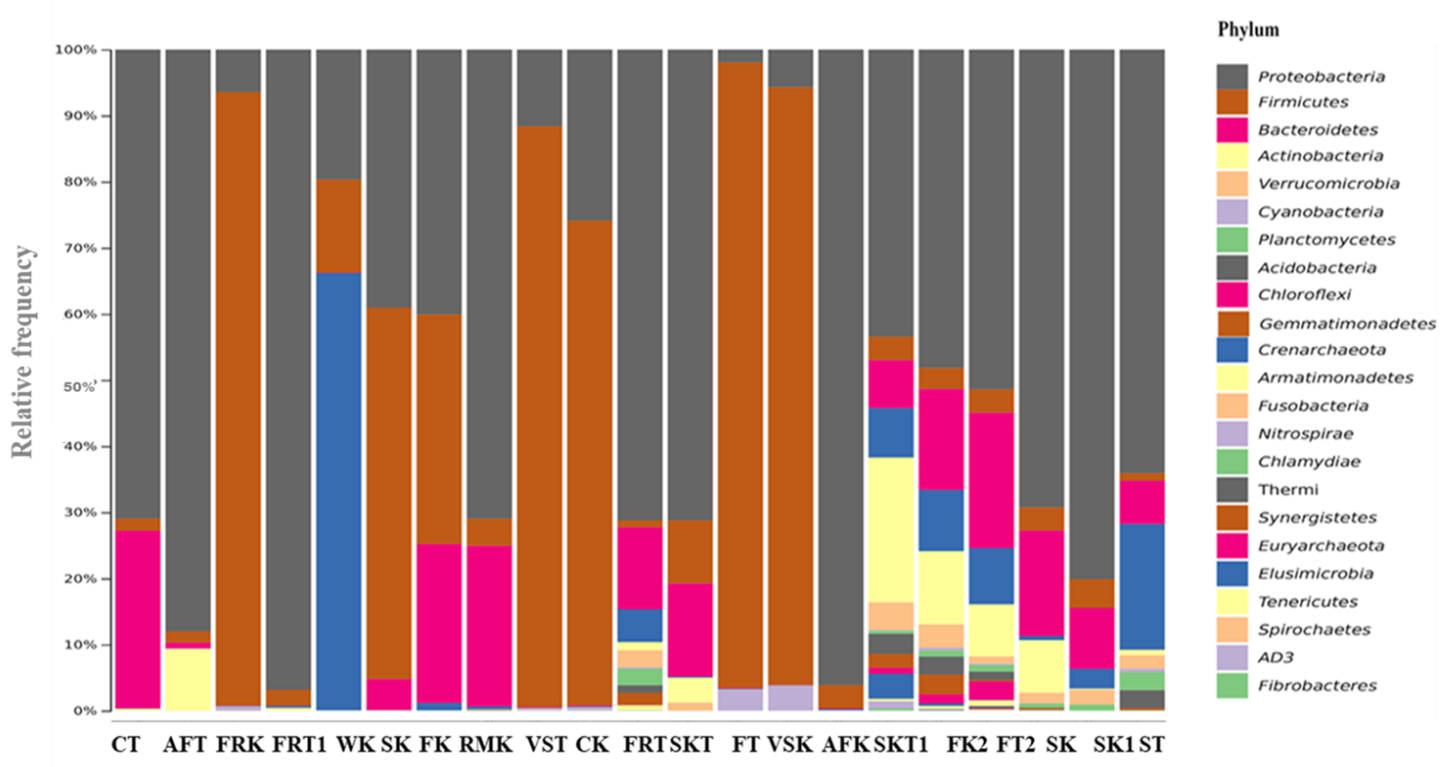


Figure 4.8: Relative abundance of bacteria phyla in food samples from vended food and environmental samples from Kangaru Market and Embu Town

Sample and location: Chips Town (CT), African sausage Town (AFT), Fruits Kangaru (FRK), Fruits Town (FRT), Water Kangaru (WK), Samosa Kangaru (SAK), Fish Kangaru (FK), Roasted meat Kangaru (RMK), Vegetable salad Town (VST), Chips Kangaru (CK), Fruits Town (FRT), Smokies Town (SMT), Fish Town (FT), Vegetable salad Kangaru (VSK), African sausage Kangaru (AFK), Smokies Town (SMT), Fish Kangaru (FK), Fish Town (FT), Kangaru soil (KS), Kangaru soil (KS), Town soil (TS).

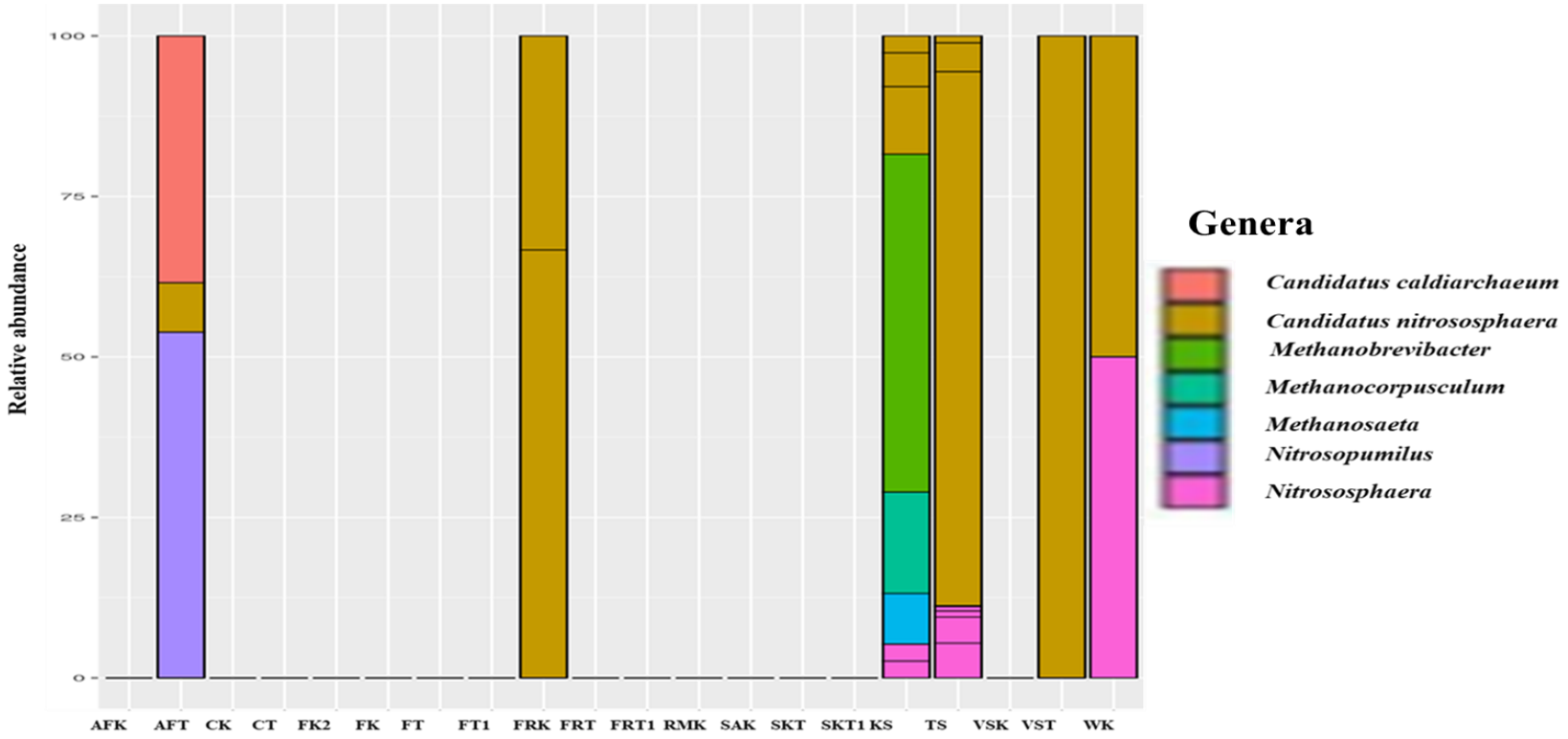


Figure 4.9: Relative abundance of archaea genera in vended food and environmental samples from Kangaru Market and Embu Town.

Sample and location: Chips Town (CT), African sausage Town (AFT), Fruits Kangaru (FRK), Fruits Town (FRT), Water Kangaru (WK), Samosa Kangaru (SAK), Fish Kangaru (FK), Roasted meat Kangaru (RMK), Vegetable salad Town (VST), Chips Kangaru (CK), Fruits Town (FRT), Smokies Town (SMT), Fish Town (FT), Vegetable salad Kangaru (VSK), African sausage Kangaru (AFK), Smokies Town (SMT), Fish Kangaru (FK), Fish Town (FT), Kangaru soil (KS), Kangaru soil (KS), Town soil (TS).

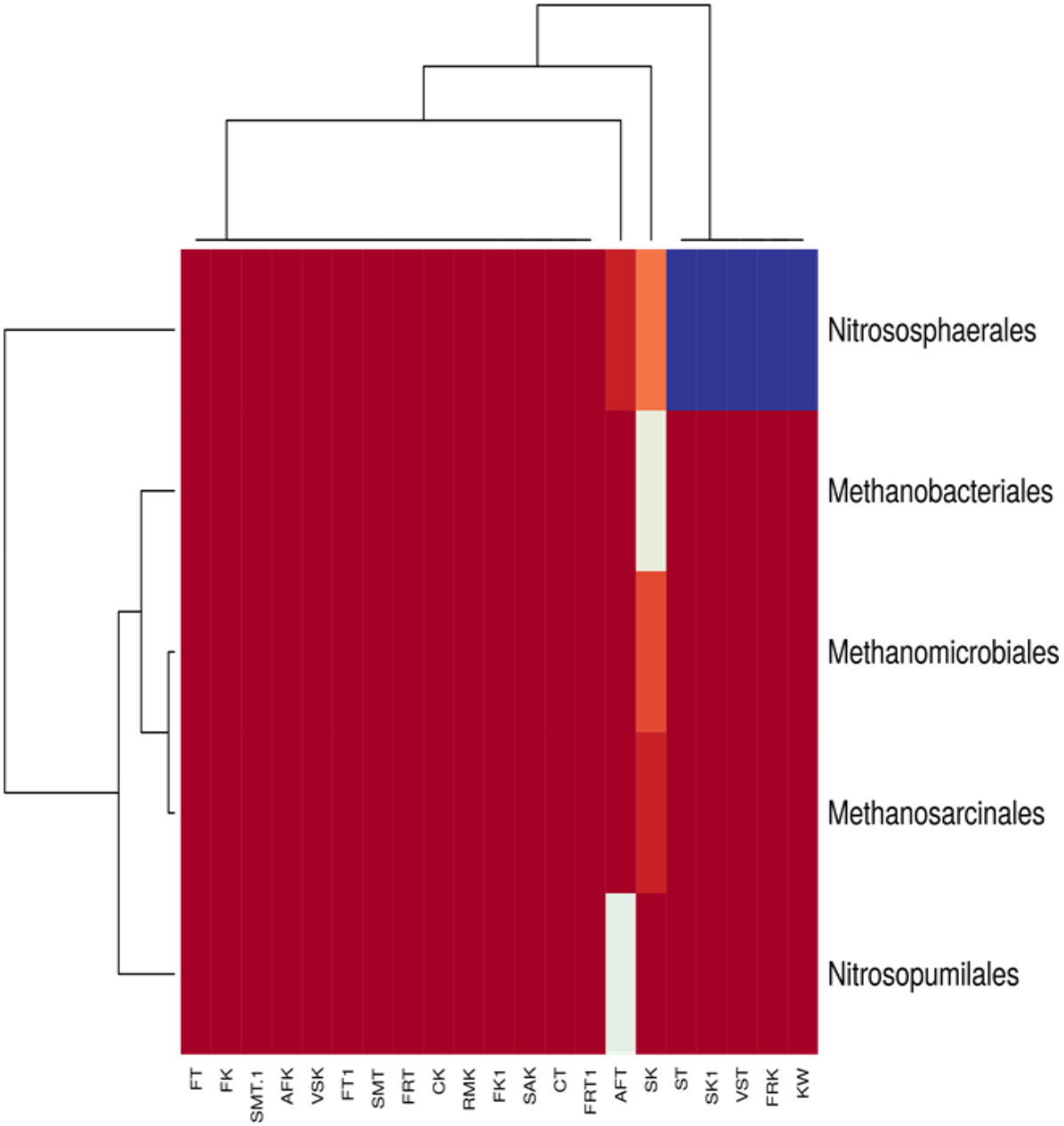


Figure 4.10: Archaea orders from food, soil and water samples collected from Embu Town and Kangaru Market

Sample and location: Chips Town (CT), African sausage Town (AFT), Fruits Kangaru (FRK), Fruits Town (FRT), Water Kangaru (WK), Samosa Kangaru (SAK), Fish Kangaru (FK), Roasted meat Kangaru (RMK), Vegetable salad Town (VST), Chips Kangaru (CK), Fruits Town (FRT), Smokies Town (SMT), Fish Town (FT), Vegetable salad Kangaru (VSK), African sausage Kangaru (AFK), Smokies Town (SMT), Fish Kangaru (FK), Fish Town (FT), Kangaru soil (KS), Kangaru soil (KS), Town soil (TS).

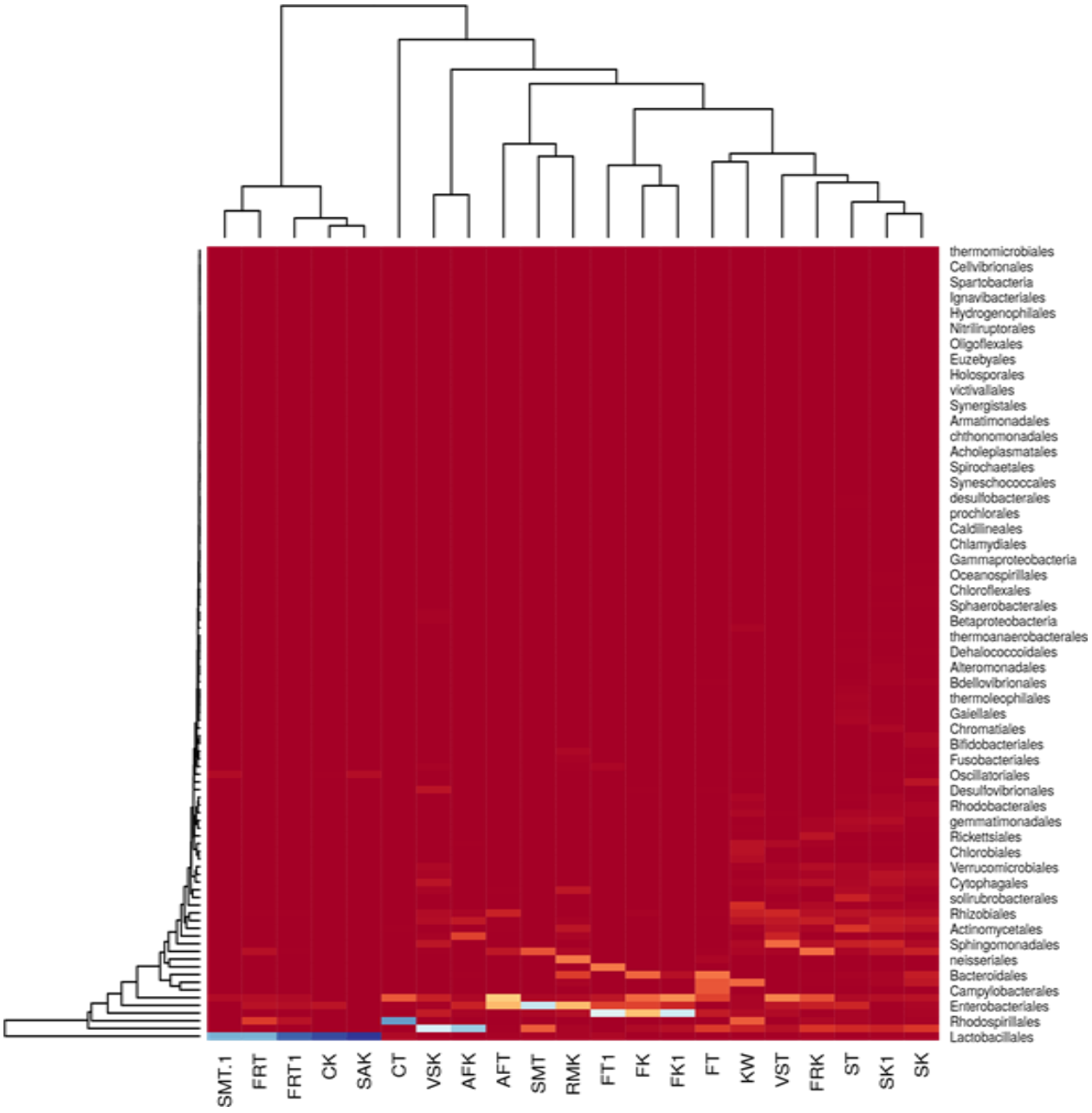


Figure 4.11: Composition of bacteria taxa at order from foods, soil and water samples collected from Embu Town and Kangaru Market.

Sample and location: Chips Town (CT), African sausage Town (AFT), Fruits Kangaru (FRK), Fruits Town (FRT), Water Kangaru (WK), Samosa Kangaru (SAK), Fish Kangaru (FK), Roasted meat Kangaru (RMK), Vegetable salad Town (VST), Chips Kangaru (CK), Fruits Town (FRT), Smokies Town (SMT), Fish Town (FT), Vegetable salad Kangaru (VSK), African sausage Kangaru (AFK), Smokies Town (SMT), Fish Kangaru (FK), Fish Town (FT), Kangaru soil (KS), Kangaru soil (KS), Town soil (TS)

Principle Coordinates Analysis of Jaccard did cluster samples according to those that are closely related (Figure 4.12) with P=0.474, PERMANOVA, 999 permutations in each test. This analysis indicated that both vended foods, soil and water are not distinguishable. When the phylogeny dendrogram was computed by clustering vended foods, soil and water using bray hclust, the difference between the various samples was ambiguous as some samples from the different environments clustered together. The OTUs membership was not significantly different between the vended foods, soil and water samples (P=0.474 PERMANOVA 999 times in each test). Relative abundance of OTUs were not significantly different between the various samples (P=0.458 PERMANOVA 999 times each test). The samples were grouped according to their sample types (Figure 4.13)

Food and environmental samples clustered into four clusters (Figure 4.13) which were significantly different ($P<0.05$). Two of the clusters were from soil and water while the other two clusters were from the vended food samples. This suggests most of the vended food and environmental samples had similar prokaryotic composition. The clusters revealed prokaryotic similarity among the different sample types. Roasted meat from Kangaru Market and smokies from Embu town had similar prokaryotic composition while fish from Embu Town and that from Kangaru Market had dissimilar prokaryotic composition. Fruits from Embu Town had different prokaryotic composition from that of Kangaru Market depicted by the different clusters.

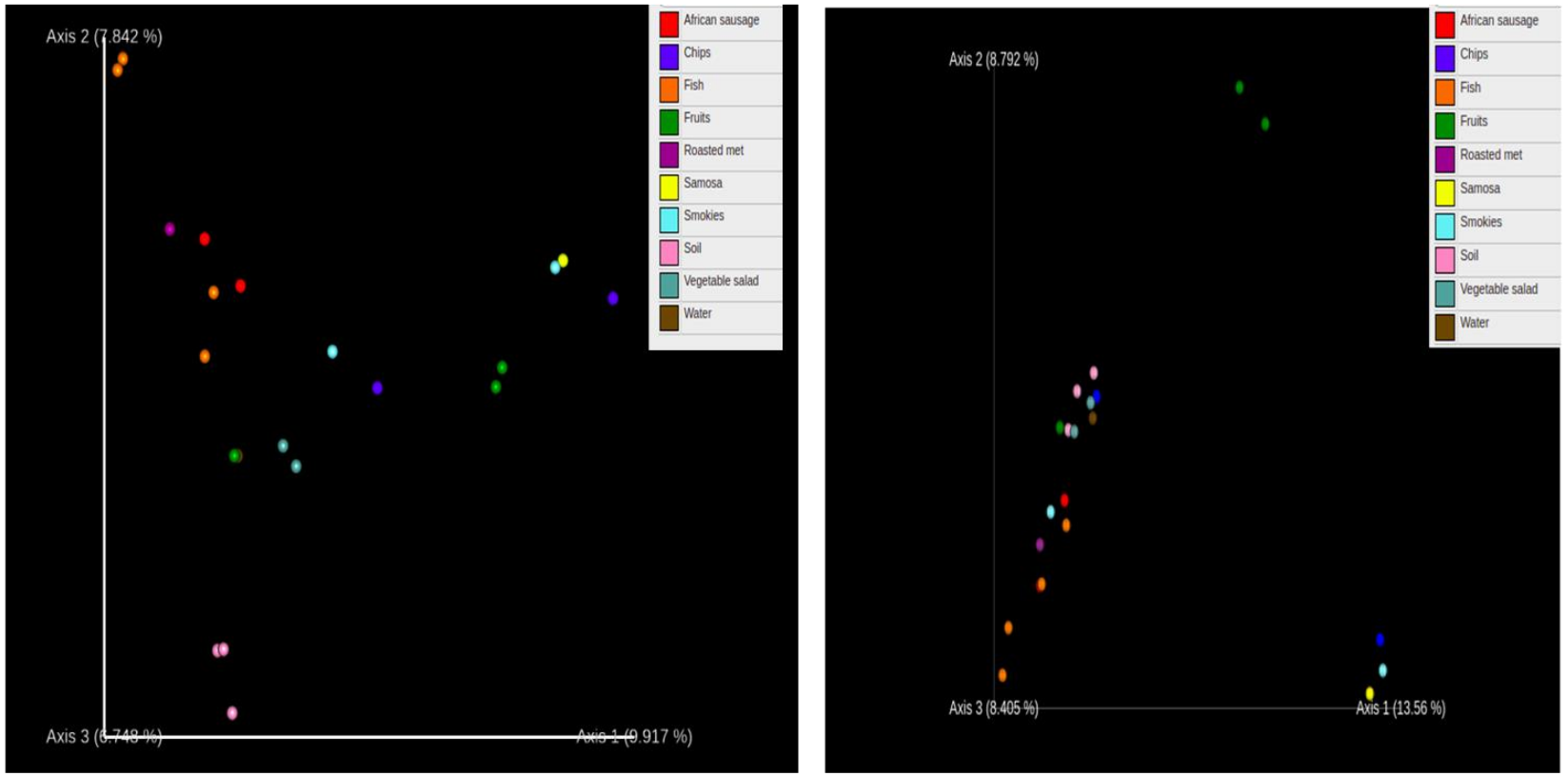


Figure 4.12; Principal Coordinates Analysis of Jaccard did cluster vended food, soil and water samples from Embu Town and Kangaru Market a) PCoA plots of Jaccard distance. b) PCoA of Bray-Curtis distance

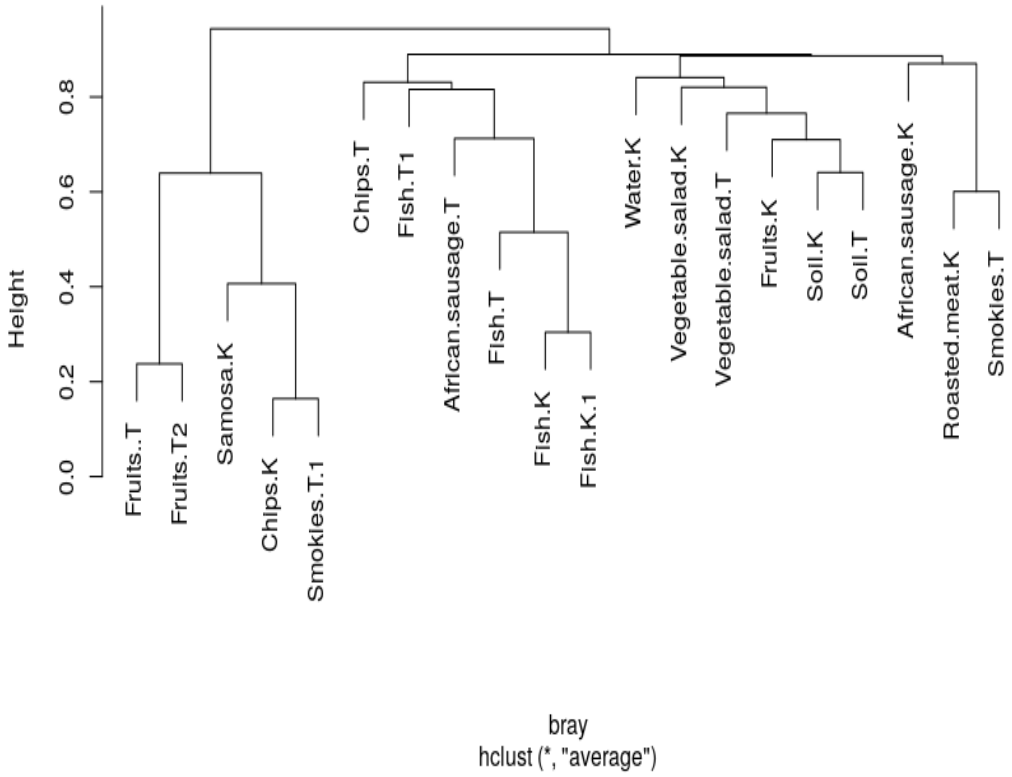


Figure 4.13: Hierarchical clustering of prokaryotes in vended foods, soil and water samples from Kangaru Market and Embu Town.

T indicates samples from Embu Town while K indicates samples from Kangaru Market

4.11 Potentially pathogenic bacteria from vended foods, soil and water

The Illumina sequencing detected 16S rRNA sequences of potentially pathogenic bacteria belonging to *Proteobacteria* and *Firmicutes*. The genera *Legionella, Aeromonas*, *Staphylococcus* and *Erysipelothrix* occurred in almost all the samples. Potentially pathogenic bacteria were in abundance in fruit samples from the Kangaru Market. A water sample from Kangaru had a relative abundance of 3.22% of potentially pathogenic bacteria sequences. Samples from Embu Town had a lower relative abundance with soil having 1.43% abundance of potentially pathogenic bacteria sequences. The most abundant genus of potentially pathogenic bacteria was *Rickettsia* which was most abundant in fruit samples from Kangaru Market. The second most abundant genus was *Legionella* with a high abundance in water from Kangaru Market (Table 4.6). The identification of potentially pathogenic bacteria was done up to the species level (Table 4.7). From the 16 genera considered to be

potentially pathogenic, there were 28 species sequences classified as potential pathogens in Risk Group 2 by NIH Appendix B (Classification of human agents based on hazard 2016). Two species; *Coxiella* spp and *Brucella* spp identified as Risk Group 3 organisms were detected. The *Brucella spp* was detected only in soil samples from Embu Town. *Coxiella spp* was detected in fruit, water and soil samples from the Kangaru Market (Table 4.7).

Table 4.6: Sequences of potentially pathogenic bacteria detected in food, soil and water samples from Embu Town and Kangaru Market

Genus	Chips T	African Sausage T	Fruits K	Fruits T1	Water K	Samosa K	Fish K1	Roasted Meat K	Vegetable Salad T	Chips K	Fruits T	Smokies T1	Fish T1	Vegetable Salad T	African Sausage K	Smokies T	Fish K	Fish T	Soil K1	Soil K	Soil T	Total
Geodermatophilus	1	1	0	1	1	0	0	0	0	0	0	0	0	1	0	0	0	0	49	2	249	**305**
Leptospira	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	**5**
Legionella	1	0	17	1	889	0	3	0	3	0	1	1	2	2	0	0	1	2	7	12	8	**950**
Rickettsia	0	3	1327	1	1	0	2	1	1	2	0	0	1	0	3	0	0	0	12	9	0	**1363**
Mycoplasma	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	**4**
Aeromonas	0	8	1	0	5	1	2	545	4	0	0	2	1	1	1	1	25	40	33	25	4	**699**
Coxiella	0	1	15	0	6	1	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	**27**
Brucella	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	7	**8**
Treponema	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	**3**
Staphylococcus	1	4	28	2	24	13	1	3	52	1	5	1	0	5	2	0	0	0	17	30	27	**216**
Acholeplasma	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	6	49	0	**58**
Erysipelothrix	0	0	3	0	6	0	71	16	0	0	0	0	1	0	0	0	17	0	8	13	1	**136**
Mycobacterium	0	0	4	0	1	0	0	0	1	0	0	0	0	4	0	0	0	0	16	4	9	**39**
Total sequences	**29030**	**26239**	**28732**	**29266**	**29177**	**29260**	**29191**	**28923**	**28297**	**28541**	**28795**	**28908**	**28872**	**29040**	**29314**	**23246**	**27858**	**27886**	**25446**	**26240**	**26195**	
Total potential pathogens	**4**	**17**	**1391**	**5**	**936**	**16**	**79**	**565**	**61**	**3**	**7**	**4**	**5**	**13**	**6**	**1**	**43**	**43**	**141**	**156**	**305**	
Potential pathogens percentages	**0.01**	**0.06**	**4.88**	**0.02**	**3.22**	**0.05**	**0.20**	**1.95**	**0.22**	**0.01**	**0.02**	**0.01**	**0.02**	**0.04**	**0.02**	**0.00**	**0.15**	**0.15**	**0.72**	**0.80**	**1.43**	

Table 4.7: Potentially pathogenic bacteria detected in vended food, soil and water samples

Species	**Sources**	**Risk group***	**Associated disease(s) NIH Appendix: 2016**
Geodermatophilus spp.	Chips T, African Sausage T, Fruits T, Water K, Soil K, Soil T	2	Skin disease
Leptospira idonii	Water K, Soil K	2	*Leptospira*
Legionella spp., Legionella pneumophila, Legionella wadsworthii	Chips T, Fruits K, Fruits T, Water K, Fish K, Vegetable Salad T, Fish K, Fish T, Soil K, Soil T	2	*Pontiac fever* *Legionnaire's Disease*
Rickettsia sp., Rickettsia spp., Rickettsia canadensis	African Sausage T, Fruits K, Fruits T, Water K, Fish K, Roasted Meat K, Vegetable Salad T, Soil K	2	Rocky Mountain spotted fever
Mycoplasma alligatoris	Soil K	2	Mycoplasma pneumonia
Aeromonas salmonicida, Aeromonas sp.	All	2	Septicemia, cellulitis
Thiobacillus spp.	Vegetable Salad T, Soil K, Soil T	2	
Coxiella spp.	African Sausage T, Fruits K, Water K, Samosa K, Soil K	3	Q fever
Brucella spp.	Soil T	3	Brucellosis
Treponema spp.	Soil K	2	Syphilis Congenital syphilis
Staphylococcus pasteuri	Chips T, African Sausage T, Fruits K, Fruits T, Water K, Samosa K, Fish K, Roasted Meat K, Vegetable Salad T, Soil K	2	Necrotizing pneumonia Acute infective endocarditis
Acholeplasma hippikon, Acholeplasma vituli, Acholeplasma oculi *Acholeplasma sp., Acholeplasma laidlawii*	Chips T, Samosa K, Soil K, Soil K, Fish T	2	Acute and chronic respiratory disease
Erysipelothrix spp., Erysipelothrix tonsillarum	Fruits K, Water K, Fish K, Roasted Meat K, Fish K, Soil K, Soil T	2	Erysipelas thread of red disease
Mycobacterium sacrum, Mycobacterium spp.	Fruits K, Water K, Vegetable Salad T, Soil T, Soil K	3	Tuberculosis

4.12 Uncultured prokaryotes detected from vended foods, soil and water

In species rank, uncultured *Candidatus solibacter, Candidatus microthrix, Candidatus protochlamydia* and *Candidatus kuenenia* spp., were detected in both vended foods and environmental samples (Table 4.8). More uncultured isolates were detected from Kangaru Market (9) as compared to Embu Town (7). More uncultured isolates were detected from soil (9) compared to the rest of the samples. The sequences for the uncultured prokaryotes have been archived at NCBI with accession number SUB8376444.

Table 4.8: Uncultured prokaryotes from vended foods and environmental samples

No	Isolate	Sample	Location
1	*Uncultured Candidatus koribacter sp.*	Fruits	Kangaru Market
		Soil	Kangaru Market
		Soil	Embu Town
2	*Uncultured Candidatus kuenenia sp.*	Soil	Kangaru Market
		Soil	Embu Town
3	*Uncultured Candidatus microthrix sp.*	African sausage	Kangaru Market
		Soil	Kangaru Market
		Soil	Embu Town
4	*Uncultured Candidatus protochlamydia sp.*	Soil	Embu Town
5	*Uncultured Candidatus solibacter sp.*	Fruit	Kangaru Market
		Chips	Kangaru Market
		Smokies	Embu Town
		Vegetable salad	Kangaru Market
		Fish	Embu Town
		Soil	Kangaru market
		Soil	Embu Town

CHAPTER FIVE

5.0 DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

This study isolated 70 bacterial isolates that were resistant to at least three antibiotics from vended foods and the environment in Embu Town and Kangaru Market. The isolated antibiotic resistant bacteria exhibited diverse morphological characteristics. Gram-negative isolates were the majority (47) as compared to the Gram-positive were (23). This is expected because the majority of pathogenic bacteria that are antibiotic-resistant belong to Gram-negative group (Bhagirath *et al*., 2019). Food and the environment have previously been reported to harbor bacterial contaminants that are antibiotic-resistant. A study on antibiotic resistance of enteric pathogens in Kibera, Kenya confirmed that fast food and the environment are a reservoir of antibiotic resistance (Muhonja *et al.*, 2012). Consumption of fast food contaminated with antibiotic-resistant bacteria is a drawback to public health as it encourages persistence and dissemination of these resistance determinants. Data from the present study indicate that the sampling sites in Embu Town and Kangaru Market are reservoirs of antibiotic-resistance. The hotspots of these resistant determinants are majorly found in hospitals and the natural environment. Sub-therapeutic concentrations of these antibiotics and contribute to the antibiotic resistance gene bank in the habitat (Berendonk *et al*., 2015).

The distribution of antibiotic resistance varied with the sampling sites. Kangaru Market recorded high resistance as compared to Embu Town. The variation in the distribution of antibiotic resistance between the sites could be due to environmental factors and laxity in the enforcement of public health measures. The occurrence of antibiotic resistance also varied among different food samples. Fish from Kangaru Market had the highest number of antibiotic-resistant isolates compared to fish from Embu Town. The contamination of fish with antibiotic-resistant isolates has been previously attributed to fecal contamination of water due to the unhygienic disposal of human waste (Sifuna & Onyango, 2018). In Lake Naivasha, bacterial fish contamination was attributed to favorable physicochemical properties and temperature that encourage bacterial proliferation (Donde *et al*., 2015). Bacterial contamination of fried fish in the present study could be due to cross-contamination during handling of

cooked and uncooked fish. Contamination of fish could arise from the food they consume or contamination of water that arises from anthropogenic activities around their environment. Vegetable salad and African sausage from Kangaru Market also recorded high antibiotic resistance. Post-harvest contamination of vegetables and cross-contamination of African sausages is reported to be a major risk factor in disseminating antibiotic-resistance (Kutto *et al*., 2011; Karoki *et al*., 2018). Contamination of street vended smokies which are factory-made with antibiotic-resistant isolates was low. Contamination of smokies in previous studies was attributed to the cross-contamination by 'kachumbari' (vegetable salad) or unhygienic handling (Kariuki *et al*., 2017). The level of antibiotic resistance in bacteria isolated was higher compared to those in Embu Town. The difference could be due to varying environmental parameters and adherence to hygiene requirements in Embu Town. Contamination of fruits with antibiotic-resistant bacteria could be ascribed to cross-contamination with environmental microorganisms during production. Fruits in the market may be contaminated with air microorganisms as they are displayed in the open market. Soil samples recorded the highest number of antibiotic-resistant bacteria as compared to food and water samples. In previous studies, the soil has been shown to harbor antibiotic-resistant bacteria. Bacteria from Juja, Kenya isolated from soil were multi-drug resistant to co-trimoxazole, nalidixic, gentamycin and quinolones (Kinyua & Budambula, 2014). The current study also recorded multidrug resistance of soil bacteria to commonly administered antibiotics.

Resistance levels varied with different antibiotics used in the present study. Trimethoprim and sulphamethoxazole a combination known as co-trimoxazole was effective against most bacterial food contaminants in the study. The antibiotic was effective against *E. coli* isolates in the current study. This finding differs from a previous study that reported 75% of the *E. coli* isolated from raw chicken sold in Nairobi Town were resistant to co-trimoxazole (Odwar *et al*., 2014). Antibiotic resistance to gentamycin and streptomycin was low in bacteria from both Embu Town and Kangaru Market. These antibiotics are used in treating severe Gram-negative infections, though their clinical administration is limited by nephrotoxicity. Low-level usage of these antibiotics and the intramuscular mode of administration, have contributed to the preservation of their antimicrobial activity (Muñoz-atienza *et al*.,

2013). The sensitivity of bacteria to streptomycin and gentamycin could also be encouraged by its limited availability which in turn limits its misuse.

Most of the isolates in this study were resistant to amoxicillin. The antibiotic is used to treat chest infections, dental abscesses and urinary infections. Tetracycline was among the least effective antibiotics in the present study. It is one of the most commonly misused antibiotics, due to its availability, broad-spectrum activity and affordability (Schnappinger, 1996). A study in Kisumu, Kenya reported the presence of tetracycline-resistant entero-pathogens in street vended foods (Onyango, 2019). This finding indicates the need to regulate street vended food to curb the spread of antibiotic resistance. The bacterial food contaminants recorded high resistance to nalidixic acid, an antibiotic used to treat typhoid fever. An earlier study in Kenya demonstrated that the antibiotic had lost effectiveness as fever took a longer time to clear (Rahman *et al*., 2014).

No culturable bacteria were isolated from the treated water that is used in Embu Town and Kangaru Market. This was a clear indication that treated water supplied by (EWASCO) which the majority of the population use was free from contamination with culturable antibiotic-resistant bacteria. Studies in Kibera and Kericho, Kenya detected entero-aggregative and enterotoxigenic *E. coli* in treated municipal water that were all multi-drug resistant to; tetracycline, ampicillin, sulfamethoxazole/trimethoprim and ampicillin/sub-lactam (Muhonja *et al.*, 2012; Too, *et al*., 2016).

The current study was able to demonstrate that antibiotic resistance in bacteria that were from food and environmental samples was encoded by streptomycin *str* (B), tetracycline *tet* (A), ampicillin *amp*, trimethoprim *dfr* (I), beta-lactam *bla* TEM, and chloramphenicol *flor*. The contamination of food, water and surrounding environment with bacteria carrying antibiotic resistance genes, has encouraged the dissemination of these resistance genes. These resistance genes could be transferred from the surrounding environment to the bacteria contaminating food as antibiotic resistance genes which include *Amp*, *Tet*, *Flor*, *Bla* Tem, and *srt*B were detected from bacteria isolated from soil. Resistance genes have been previously detected in many natural habitats such as lakes (Zhang *et al*., 2009). Genes encoding streptomycin resistance *str* (B) amplified in only a few isolates. A previous study by Muhonja *et al*., (2012),

detected the genes *tet*, *amp* and *dfr* in bacterial food contaminants. López *et al*. (2008), reported that 39% of *Bacillus cereus* isolated from fried chicken, were resistant to tetracycline and harboured at least one of the resistance determinants; *tet*I, *tet*O, *otr*A, or *otr*B.

Sequencing of the 16S rRNA gene revealed that some of the antibiotic-resistant m bacteria in this study belonged to the genus *Bacillus*. Previous studies associated genus *Bacillus* with food spoilage (Owusu-Kwarteng *et al*., 2017). *Serratia marcescens* isolated in this study is a Gram-negative bacterium that occurs naturally in soil and water. However, in man, it can cause urinary, respiratory, meningitis, pneumonia, septicemia and endocarditis infections. The bacterium is known to colonize catheters thus endangering human health. Previous studies showed that it is difficult to treat infections caused by *S. marcescens* with antibiotics since it is resistant to commonly administered antibiotics (Buckle, 2016). This isolate has also been classified under Risk Group 3 by the NIH (https://my.absa.org/tiki-index-2016). *Bacillus wiedmannii* in this study classified under Risk Group 2, has been grouped as part of *Bacillus cereus* due to its shape and its character and has been reported to contaminate dairy products (Zhao *et al*., 2019). *Bacillus anthracis* isolated from bacteria in the present study is a Risk Group 3 organism which is associated with death in both humans and animals (Erickson & Kornacki, 2003; https://my.absa.org/tiki-index-2016). Isolation of Risk Group 2 and 3 organisms from food is of public health concern.

Microorganisms with antimicrobial activity have been shown to secret metabolites that can antagonize the growth of other microorganisms in the same environment. Isolate TS572 affiliated to *Bacillus subtilis* that was resistant to five antibiotics, was still able to release antimicrobial agents that was able to inhibit the growth of *K. pneumoniae* ATCC49619. *Bacillus subtilis* have been reported to produce antimicrobial agents against Gram-positive foodborne pathogenic bacteria (Torres *et al*., 2015). Cawoy *et al*. (2015) demonstrated the ability of *Bacillus subtilis* to produce an antimicrobial agent that inhibited the growth of fungal phytopathogens. KMP337 affiliated to *Citrobacter freundii* recorded the highest zone of inhibition against *S. aureus* ATCC 1026. *C. freundii* has been associated with multiple antibiotic resistance but not the production of antimicrobial agents (Pepperell *et al*., 2002). Thus the results from the

present study support the need for innovative screening strategies in the search for new antimicrobial agents and modification of the existing ones.

Most of the bacteria in the current study were able to release extracellular enzymes in varying magnitude. Isolate KS606 affiliated to *Bacillus anthracis*, produced a large zone of clearance on cellulose. The isolate was stored carefully preserved in the deep freezer. Cellulase enzymes have attracted a lot of interest in the market due to industrial applications. Thus *Bacillus anthracis* produced a large zone of clearance, though pathogenic can be exploited for the production of cellulase enzymes (Sulman & Rehman, 2013). Isolate KC430, affiliated to *Pseudomonas* spp., had the largest zone of precipitation on tween-20 substrate and still exhibited the highest diameter of zone of inhibition against the test organism *Klebsiella pneumoniae*. Isolate KC430 affiliated to be *Pseudomonas* spp., exhibited the highest zone of inhibition against *K. pneumoniae* and zone of clearance on skimmed milk. Previous studies reported isolates that exhibited high enzymatic activity also exhibited high antibiosis activity, thus these processes could be occurring concurrently (Sicuia *et al*., 2015: Neondo *et al*., 2017).

Vended foods and environmental samples investigated in the present study varied in their microbial community composition. The microbial communities varied depending on the sample type and the site where the samples were collected from. Bacteria formed the most (over 99%) abundant taxa in all the vended foods and environmental samples. This study determined that the dominant phyla were *Proteobacteria* (52.81%), *Firmicutes* (31.16%) and *Bacteroidetes* (8.00%). Previous studies reported the abundance of these phyla in soil and food (Gangwar *et al*., 2009). *Proteobacteria* bacterial pathogens have been detected from drinking water have been associated with biofilm formation in water pipes and leakages (Richards *et al*., 2018). *Firmicutes* have been reported to adapt to solid food which increases their activity thus they can thrive in many solid foods (Hugenholtz *et al*., 2017).

Sequences affiliated to archaeal phyla comprising *Euryarchaeota* (3.36%) and *Thaumarchaeota* (96.64%) were detected in this study. *Thaumarchaeota* sequences were detected in all soil samples, water, fruits and the African sausage. The most abundant archaeal group was *Candidatus* spp., which was recovered predominantly from the soil in Embu Town. *Methanoarcinales*, *Nitrosopumilales*,

Nitrososphaerales, *Methanomicrobiales* and *Methanobacteriales* were recovered from vended foods and soil samples. The order *Nitrososphaerales* is known to inhabit terrestrial ecosystems (Kerou & Schleper, 2015). Despite the ubiquity nature of archaea, no pathogenic order of archaea has been reported. Data from the present study thus suggests there were no pathogenic archaea in the food samples. *Methanomicrobiales* have been shown to inhabit a broad range of anoxic environments such as; marine and freshwater, animal gastrointestinal tract and geothermal habitats. The presence of this order in the animal tract, explains the possibility of it finding its way to food through cross-contamination (Liu, 2010).

The most abundant species groups recovered across all samples were *Alcaligens feacalis, Lactobacillus perolens, Pseudomonas* spp., *Citrobacter freundii*, *Clostridium* spp., *Acetobacte*r spp. and *Pediococcus lactobacillus plantarum*. The majority of the detected microorganisms are potential pathogens and thus may pose a health risk to man. Multidrug resistance has been reported to occur in *C. freundii* thus its presence in food should be of concern (Liu *et al*., 2018). *Bacillus cereus* sequences were recovered from the soil in the surrounding environment, thus may pose a health risk in case of cross-contamination of food. *B. cereus* has been associated with food poisoning following the formation of endospores and toxin production which are known to cause disease that leads to diarrheal and emetic syndrome (Griffiths & Schraft, 2017). *Hafnia* spp., sequences were detected in all samples. Commensal *Hafnia* spp., has been shown to reduce food intake and fat mass in people with obese conditions (Legrand *et al*., 2020). The presence of this bacterium in food could be of benefit. *Pseudomonas spp.* were detected in all samples. *Pseudomonas* spp is considered a clinically important foodborne pathogen. It is a Risk Group 2 organism and was associated with foodborne disease occurrence (Fakhkhari *et al*., 2020). Sequences of *Brucella* spp., belonging to Risk Group 3 were detected in the present study. The isolate has been associated with food poisoning in a previous study (Garcell *et al*., 2016). *Clostridium* spp., whose sequences were recovered from fish samples from Kangaru Market has toxigenic strains and has been associated with intestinal infections in man (Rodriguez *et al*., 2016). Sequences of *Coxiella* spp., which belong to Risk Group 3 were detected and have been associated with dermatitis outbreaks (Raele *et al*., 2018). *Staphylococcus pasteuri*, belonging to Risk Group 2 was detected in almost all samples in the present study. It is known to result in bacteremia in patients

diagnosed with acute leukemia, catheter-associated urinary tract infection and endocarditis (Ramnarain *et al*., 2019). *Legionella pneumophilia* a clinically important pathogen detected in the present study has been associated with severe pneumonia legionnaires disease. It has also been shown to be found in abundance in freshwater where it freely replicates in protozoa (Mendis *et al*., 2015), unlike the present study that detected it in soil. *Orientia tsutsugamushi* detected in the current study has been associated with life-threatening illnesses (Taylor *et al.*, 2015). The detection of potentially pathogenic bacteria in food samples, calls for adherence to public health guidelines in Embu Town and Kangaru Market.

Most of the prokaryotes in this study were detected from the soil. This is expected since the soil is known to harbour millions of microorganisms (Vestergaard *et al*., 2017). Fruits from Kangaru Market (260 OTUs) and vegetable salads from Embu Town (200 OTUs) had the highest number of observed OTUs. Most fresh produce such as fruits and vegetables are exposed to contamination as a result of contact with irrigation water, manure or the soil (Pushpakanth *et al.*, 2019). Microorganisms such as *Lactobacillus pentosaceus*, *Weissella cibaria* and *Lactobacillus plantarum* have been previously detected in the vegetables (Peng *et al*., 2018). Uncultured *Candidatus solibacter*, *Candidatus microthrix*, *Candidatus protochlamydia* and *Candidatus kuenenia* spp., were detected. Most of these uncultured species were detected from soil samples however, *C. solibacter* was detected in soil, water and some of the food samples. A recent study reported the abundance of microorganisms in the soil but not in food samples (Amoo, *et al*., 2020). Uncultured *Candidatus koribacter* sp, *Candidatus microthrix* sp., and *Candidatus solibacter* sp. were detected from food samples. A recent study reported the abundance of the microorganisms in the soil and sludge wastewater but no known study has reported detection in food or pathogenicity (Vestergaard *et al*., 2017). *Candidatus microthrix* has been shown to dominate activated sludge and cause sludge separation problems (Mcllroy *et al*., 2013). These organisms possibly found their way into food through cross-contamination. Cholera outbreak as a result of *V. cholerae* has been previously reported in Embu (Githuka, 2016). However, there no studies including the present study, that have detected *V. cholerae* in food or drinking water using metagenomic techniques.

5.2 Conclusions

Bacterial isolates were mostly Gram-negative and rod shaped. This study showed that vended food and the soil in Embu Town as well as Kangaru Market contained bacterial food contaminants that are resistant to commonly used antibiotics. The study further confirmed that the bacteria harboured antibiotic resistance determinants *Tet*A, ^{Bla}*TEM, StrB, Dfr*A, *Amp,* and *FloR.* Some of the bacterial food contaminants isolated in this study are Risk Group 2 and 3 organisms which is a public health concern. More resistance was recorded in the bacteria originating from Kangaru Market as compared to Embu Town. The prokaryotic diversity varied with the location and sample type. Many of the microorganisms that were detected are linked to foodborne diseases and have been ranked by WHO under NIH guidelines as clinically important pathogens that need special attention. Further, this study was able to detect uncultured prokaryotes including *Candidatus protochlamydia sp.* and *Uandidatus solibacter sp.* Overall, vended food in Kangaru Market may pose a health risk to the public if mitigating measures are not put in place.

5.3 Recommendations

There is a need for enforcement of hygiene measures such as proper handling of food to prevent food contamination and causes of foodborne disease outbreaks by the Public Health Ministry of Embu County. Future research can focus on the development of media with specific growth components that facilitate isolation and cultivation of the uncultured prokaryotes detected in the present study.

REFERENCES

ABSA, “Risk Group Database,” 2016. (Online). Available: https://my.absa.org/tiki-index.php?page=Riskgroups&default%5Bcontent%5D=ascaris. (Accessed: 08-May-2020).

Ahmed, M. M. M., Hafez, E. E., Mkamer, M. A., ElAbdelrrassoul, H. A. A., & Mabrouk, Y. M. A. (2014). Application of DNA technology to detect food infection with some pathogenic bacteria using 16S rDNA gene, PCR-RFLP and sequencing. *Journal of Food, Agriculture and Environment (JFAE), 12*(2), 202-206.

Ahmed, A. M., & Shimamoto, T. (2015). Molecular characterization of multidrug-resistant Shigella spp. of food origin. *International Journal of Food Microbiology*, *194*(January), 78–82. https://doi.org/10.1016/j.ijfoodmicro.2014.11.013

Akhtar, S., Sarker, M. R., & Hossain, A. (2014). Microbiological food safety: a dilemma of developing societies. *Critical Reviews in Microbiology*, *40*(4), 348-359.

Allende, A., & Monaghan, J. (2015). Irrigation water quality for leafy crops: a perspective of risks and potential solutions. *International Journal of Environmental Research and Public Health*, *12*(7), 7457–7477.

Altekruse, S. F., Cohen, M. L., & Swerdlow, D. L. (1997). Emerging foodborne diseases. *Emerging Infectious Diseases*, *3*(3), 285.

Alekshun, M. N., & Levy, S. B. (2007). Molecular mechanisms of antibacterial multidrug resistance. *Cell*, *128*(6), 1037–1050.

Amoo, A. E., Enagbonma, B. J., & Babalola, O. O. (2020). High-throughput sequencing data of soil bacterial communities from Tweefontein indigenous and commercial forests, South Africa. *Data in Brief*, *28*, 104916.

Andersson, D. I., & Hughes, D. (2010). Antibiotic resistance and its cost: is it possible to reverse resistance? *Nature Reviews Microbiology*, *8*(4), 260.

Bain, R., Cronk, R., Hossain, R., Bonjour, S., Onda, K., Wright, J., & Bartram, J. (2014). Global assessment of exposure to faecal contamination through drinking water based on a systematic review. *Tropical Medicine & International Health*, *19*(8), 917-927.

Bellack, N. R., Koehoorn, M. W., MacNab, Y. C., & Morshed, M. G. (2006). A conceptual model of water's role as a reservoir in Helicobacter pylori transmission: a review of the evidence. *Epidemiology & Infection*, *134*(3), 439-449.

Bengtsson-Palme, J., Kristiansson, E., & Larsson, D. J. (2018). Environmental factors influencing the development and spread of antibiotic resistance. *FEMS microbiology Reviews*, *42*(1), fux053.

Berendonk, T. U., Manaia, C. M., Merlin, C., Fatta-Kassinos, D., Cytryn, E., Walsh, F., & Kreuzinger, N. (2015). Tackling antibiotic resistance: the environmental framework. *Nature Reviews Microbiology*, *13*(5), 310-317.

BIOHAZ, E. P. on B. H. (2014). Scientific Opinion on the evaluation of molecular typing methods for major foodborne microbiological hazards and their use for

attribution modelling, outbreak investigation and scanning surveillance: Part 2 (surveillance and data management activities). *EFSA Journal*, *12*(7), 3784.

Bimboim, H. C., & Doly, J. (1979). A rapid alkaline extraction procedure for screening recombinant plasmid DNA. *Nucleic Acids Research*, *7*(6), 1513-1523.

Bokulich, N. A., Dillon, M. R., Zhang, Y., Rideout, J. R., Bolyen, E., Li, H., Caporaso, J. G. (2018a). q2-longitudinal: longitudinal and paired-sample analyses of microbiome data. *MSystems*, *3*(6).

Bokulich, N. A., Kaehler, B. D., Rideout, J. R., Dillon, M., Bolyen, E., Knight, R., Caporaso, J. G. (2018b). Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2's q2-feature-classifier plugin. *Microbiome*, *6*(1), 90.

Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C., Al-Ghalith, G. A., Asnicar, F. (2018). *QIIME 2: Reproducible, Interactive, Scalable, and Extensible Microbiome Data Science*. PeerJ Preprints.

Buckle, J. (2016). *Clinical Aromatherapy: Essential Oils in Healthcare*. Churchill Livingstone.

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). DADA2: high-resolution sample inference from Illumina amplicon data. *Nature Methods*, *13*(7), 581–583.

Calo-Mata, P., Carrera, M., Böhme, K., Caamaño-Antelo, S., Gallardo, J. M., Barros-Velázquez, J., & Cañas, B. (2016). Novel peptide biomarker discovery for detection and identification of bacterial pathogens by LC-ESI-MS/MS.

Chaudhary, D. K., Khulan, A., & Kim, J. (2019). Development of a novel cultivation technique for uncultured soil bacteria. *Scientific Reports*, *9*(1), 1-11.

Cawoy, H., Debois, D., Franzil, L., De Pauw, E., Thonart, P., & Ongena, M. (2015). Lipopeptides as main ingredients for inhibition of fungal phytopathogens by B acillus subtilis/amyloliquefaciens. *Microbial Biotechnology*, *8*(2), 281-295.

Cappuccino, J. G., & Sherman, N. (2002). Techniques for isolation of pure cultures. *Cultural Characteristics of Microorganisms, Microbiology A Laboratory Manual, Pearson Education*, *6*, 13–23.

Cleary, D. F. R., Polónia, A. R. M., Huang, Y. M., & Swierts, T. (2019). Compositional variation between high and low prokaryotic diversity coral reef biotopes translates to different predicted metagenomic gene content. *Antonie van Leeuwenhoek*, 1–25.

Chen, D., Zou, W., Xie, S., Kong, L., Chen, Y., Zhang, X., Qin, Y. (2018). Serotype and Antimicrobial Resistance of Escherichia coli Isolated from Feces of Wild Giant Pandas (Ailuropoda melanoleuca) in Sichuan Province, China. *Journal of Wildlife Diseases*.

Chouhan, S. (2015). Enumeration and identification of standard plate count bacteria in raw water supplies. *IOSR Journal of Environmental Science, Toxicology and Food Technology*, *9*(2), 67–73.

Cox, G., & Wright, G. D. (2013). Intrinsic antibiotic resistance: mechanisms, origins, challenges and solutions. *International Journal of Medical Microbiology*, *303*(6-7), 287-292.

Clesceri, L. S., Greenberg, A. E., Eaton, A. D., Rice, E. W., & Franson, M. A. H. (2005). *Standard Methods for the Examination of Water and Wastewater*.

Denis, N., Helen, Z., Alexander, L., Roger, T. and Henri, B., 2016. Fd. Contr., 67: 225-234.

Donde, O. O., Wairimu, A. M., Shivoga, A. W., Trick, G. C., & Creed, F. I. (2015). Faecal bacterial contamination of borehole water between points-of-access and points-of-use in Naivasha, Kenya; Public health implication. *Egerton Journal of Science & Technology*, *13*.

El-Zamkan, M. A., & Hameed, K. G. A. (2016). Prevalence of Campylobacter jejuni and Campylobacter coli in raw milk and some dairy products. *Veterinary World*, *9*(10), 1147.

European Food Safety Authority and European Centre for Disease Prevention and Control (EFSA and ECDC). (2019). The European Union one health 2018 zoonoses report. *EFSA Journal*, *17*(12), e05926.

Erickson, M. C., & Kornacki, J. L. (2003). Bacillus anthracis: current knowledge in relation to contamination of food. *Journal of Food Protection*, *66*(4), 691-699.

Fakhkhari, P., Tajeddin, E., Azimirad, M., Salmanzadeh-Ahrabi, S., Abdi-Ali, A., Nikmanesh, B., Zali, M. R. (2020). Involvement of Pseudomonas aeruginosa in the occurrence of community and hospital acquired diarrhea, and its virulence diversity among the stool and the environmental samples. *International Journal of Environmental Health Research*, 1–11.

Faith, D. P. (1992). Systematics and conservation: on predicting the feature diversity of subsets of taxa. *Cladistics*, *8*(4), 361–373.

FAO, F. (2012). Agriculture Organization of the United Nations. 2012. *FAO Statistical yearbook*.

Falkinham, J. O., C. D. Norton, and M. W. LeChevallier. 2001. Factors influencing 584 numbers of Mycobacterium avium, Mycobacterium intracellulare, and other 585 mycobacteria in drinking water distribution systems. Appl. Environ. Microbiol. 67:1225- 586 1231.

Ferrario, C., Lugli, G. A., Ossiprandi, M. C., Turroni, F., Milani, C., Duranti, S., & Ventura, M. (2017). Next generation sequencing-based multigene panel for high throughput detection of food-borne pathogens. *International Journal of Food Microbiology*, *256*, 20-29.

Fields, B. S., Benson, R. F., & Besser, R. E. (2002). Legionella and Legionnaires' disease: 25 years of investigation. *Clinical Microbiology Reviews*, *15*(3), 506-526.

Fleming-dutra, K. E., Bartoces, M., & Roberts, R. M. (2018). Characteristics of Primary Care Physicians Associated With High Outpatient Antibiotic Prescribing Volume, (May), 20–23. https://doi.org/10.1093/ofid/ofx279

Fleisher, R. C., Cornish, V. W., & Gonzalez, R. L. (2018). D-amino acid-mediated translation arrest is modulated by the identity of the incoming aminoacyl-tRNA. *bioRxiv*, 323295.

Garcell, H. G., Garcia, E. G., Pueyo, P. V, Martín, I. R., Arias, A. V, & Serrano, R. N. A. (2016). Outbreaks of brucellosis related to the consumption of unpasteurized camel milk. *Journal of Infection and Public Health*, *9*(4), 523–527.

Gangwar, P., Alam, S. I., Bansod, S., & Singh, L. (2009). Bacterial diversity of soil samples from the western Himalayas, India. *Canadian Journal of Microbiology*, *55*(5), 564–577.

Githuka G. Rotich M.D., Kigen J., H., Kiama C., Waweru, M.D Boru B, W., Langat, D. (2016). Notes from the Field.

Githuka, George. (2016). Notes from the field: ongoing cholera outbreak—Kenya, 2014–2016. *MMWR. Morbidity and Mortality Weekly Report*, *65*.

Griffiths, M. W., & Schraft, H. (2017). Bacillus cereus food poisoning. In *Foodborne Diseases* (pp. 395–405). Elsevier.

Hänel, I., Tomaso, H., & Neubauer, H. (2016). Arcobacter-an underestimated zoonotic pathogen? *Bundesgesundheitsblatt, Gesundheitsforschung, Gesundheitsschutz*, *59*(6), 789–794.

Hasan, J., Webb, H. K., Truong, V. K., Pogodin, S., Baulin, V. A., Watson, G. S., Ivanova, E. P. (2013). Selective bactericidal activity of nanopatterned superhydrophobic cicada Psaltoda claripennis wing surfaces. *Applied Microbiology and Biotechnology*, *97*(20), 9257–9262.

Havelaar, A. H., Kirk, M. D., Torgerson, P. R., Gibb, H. J., Hald, T., Lake, R. J., Gargouri, N. (2015). World Health Organization global estimates and regional comparisons of the burden of foodborne disease in 2010. *PLoS Medicine*, *12*(12), e1001923.

Hazeleger, W. C., & Beumer, R. R. (2016). Microbial quality of raw horse milk. *International Dairy Journal*, *63*, 59–61.

Henriet, O., Fourmentin, J., Delincé, B., & Mahillon, J. (2014). Exploring the diversity of extremely halophilic archaea in food-grade salts. *International Journal of Food Microbiology*, *191*, 36–44.

Herrell, W. E. (1945). Penicillin and other antibiotic agents. *Penicillin and other Antibiotic Agents*.

Herman, K. M. (2015). Hall a J, Gould LH. Outbreaks attributed to fresh leafy vegetables, United States, 1973–2012. *Epidemiol Infect*, 1-11.

Hoffmann, M., Luo, Y., Monday, S. R., Gonzalez-Escalona, N., Ottesen, A. R., Muruvanda, T., Janies, D. (2016). Tracing origins of the Salmonella Bareilly strain causing a food-borne outbreak in the United States. *The Journal of Infectious Diseases*, *213*(4), 502–508.

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

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

Hugenholtz, F., Ritari, J., Nylund, L., Davids, M., Satokari, R., & De Vos, W. M. (2017). Feasibility of metatranscriptome analysis from infant gut microbiota: adaptation to solid foods results in increased activity of firmicutes at six months. *International Journal of Microbiology*, *2017*.

Iredell, J., Brown, J., & Tagg, K. (2016). Antibiotic resistance in Enterobacteriaceae: mechanisms and clinical implications. *Bmj*, *352*, h6420.

Israel, D. S. (2009). Using Published Tables Using Formulas To Calculate A Sample Size Using A Census For Small Populations. University of Florida, IFAS extension.

Jahan, M., Rahman, M., Rahman, M., Sikder, T., Uson-Lopez, R. A., Selim, A. S. M., & Kurasaki, M. (2018). Microbiological safety of street-vended foods in Bangladesh. *Journal of Consumer Protection and Food Safety*, *13*(3), 257-269.

Jenkins, C., Dallman, T. J., Launders, N., Willis, C., Byrne, L., Jorgensen, F., Elviss, N. (2015). Public health investigation of two outbreaks of Shiga toxin-producing Escherichia coli O157 associated with consumption of watercress. *Applied and Environmental Microbiology*, *81*(12), 3946–3952.

Jung, J. Y., Lee, S. H., Kim, J. M., Park, M. S., Bae, J.-W., Hahn, Y., Jeon, C. O. (2011). Metagenomic analysis of kimchi, a traditional Korean fermented food. *Applied and Environmental Microbiology*, *77*(7), 2264–2274.

Kadariya, J., Smith, T. C., & Thapaliya, D. (2014). *Staphylococcus aureus* and staphylococcal food-borne disease: an ongoing challenge in public health. *BioMed Research International*, *2014*, 827965. https://doi.org/10.1155/2014/827965

Kariuki, J. G., & Orago, S. S. (2017). Food handling practices and the prevalence of food borne pathogens among food handlers in Embu municipality, Kenya. *International Journal of Applied Research*, *3*(1), 697–698.

Kariuki, E. N., Ng'ang'a, Z. W., & Wanzala, P. (2017). Bacteriological Contamination Of Street Foods Among Street Food Vendors In Githurai And Gikomba Markets-Nairobi County ,. *International Journal of Innovative Research and Advanced Studies*, *4*(1), 337–346.

Katoh, K., Misawa, K., Kuma, K., & Miyata, T. (2002). MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research*, *30*(14), 3059–3066.

Kergourlay, G., Taminiau, B., Daube, G., & Vergès, M.-C. C. (2015). Metagenomic insights into the dynamics of microbial communities in food. *International Journal of Food Microbiology*, *213*, 31–39.

Kim, J., Guk, J.-H., Mun, S.-H., An, J.-U., Song, H., Kim, J., Cho, S. (2019). Metagenomic analysis of isolation methods of a targeted microbe, Campylobacter jejuni, from chicken feces with high microbial contamination. *Microbiome*, *7*(1), 67.

Kinyua, D. M., & Budambula, N. L. (2014). Antibiotic resistance of metal tolerant bacteria isolated from soil in Juja, Kenya. In *Scientific Conference Proceedings*.

Kim, D., Hong, S., Kim, Y.-T., Ryu, S., Kim, H. B., & Lee, J.-H. (2018). Metagenomic approach to identifying foodborne pathogens on Chinese cabbage. *Journal of Microbiology and Biotechnology*, *28*(2), 227–235.

Karoki, W. H., Karanja, D. N., Bebora, L. C., & Njagi, L. W. (2018). Isolation, characterization, and quantification of bacteria from African sausages sold in Nairobi County, Kenya. *International journal of food science*, *2018*.

Kambura, A. K., Mwirichia, R. K., Kasili, R. W., Karanja, E. N., Makonde, H. M., & Boga, H. I. (2016). Bacteria and Archaea diversity within the hot springs of Lake Magadi and Little Magadi in Kenya. *BMC microbiology*, *16*(1), 1-12.

Karanja, E. N., Fliessbach, A., Adamtey, N., Kambura, A. K., Musyoka, M., Fiaboe, K., & Mwirichia, R. (2020). Diversity and structure of prokaryotic communities within organic and conventional farming systems in central highlands of Kenya. *Plos one*, *15*(8), e0236574.

Kerou, M., & Schleper, C. (2015). Nitrososphaera. *Bergey's Manual of Systematics of Archaea and Bacteria*, 1–10.

Kimura, N. (2018). Novel biological resources screened from uncultured bacteria by a metagenomic method. In *Metagenomics* (pp. 273-288). Academic Press.

Kümmerer, K. (2004). Resistance in the environment. *Journal of Antimicrobial Chemotherapy*, *54*(2), 311–320.

Kutto, E. K., Ngigi, M. W., Karanja, N., Kangethe, E., Bebora, L. C., Lagerkvist, C. J. L., & Okello, J. J. (2011). Bacterial contamination of Kale (Brassica oleracea acephala) along the supply chain in Nairobi and its environment. *East African Medical Journal*, *88*(2), 46-53.

Lai, E. P., Iqbal, Z., & Avis, T. J. (2016). Combating antimicrobial resistance in foodborne microorganisms. *Journal of Food Protection*, *79*(2), 321-336.

Law, J. W.-F., Ab Mutalib, N.-S., Chan, K.-G., & Lee, L.-H. (2015). Rapid methods for the detection of foodborne bacterial pathogens: principles, applications, advantages and limitations. *Frontiers in Microbiology*, *5*, 770.

Legrand, R., Lucas, N., Dominique, M., Azhar, S., Deroissart, C., Le Solliec, M.-A., Léon, F. (2020). Commensal Hafnia alvei strain reduces food intake and fat mass in obese mice—A new potential probiotic for appetite and body weight management. *International Journal of Obesity*, *44*(5), 1041–1051.

Ledeboer, N. A., & Doern, G. V. (2015). Haemophilus. In *Manual of Clinical Microbiology, Eleventh Edition* (pp. 667–684). American Society of Microbiology.

Lewis, E., Hudson, J. A., Cook, N., Barnes, J. D., & Haynes, E. (2020). Next-generation sequencing as a screening tool for foodborne pathogens in fresh produce. *Journal of Microbiological Methods*, *171*, 105840.

Li, M., Havelaar, A. H., Hoffmann, S., Hald, T., Kirk, M. D., Torgerson, P. R., & Devleesschauwer, B. (2019). Global disease burden of pathogens in animal source foods, 2010. *PloS one*, *14*(6), e0216545.

Li, S. (2020). *Food Metagenomics: Pathogen Detection, Subtyping, and Microbiome Profiling from Poultry and Fresh Produce* (Doctoral dissertation, University of Georgia).

Liu, Y. (2010). 43 Methanobacteriales.

Liu, L.-H., Wang, N.-Y., Wu, A. Y.-J., Lin, C.-C., Lee, C.-M., & Liu, C.-P. (2018). Citrobacter freundii bacteremia: Risk factors of mortality and prevalence of resistance genes. *Journal of Microbiology, Immunology and Infection*, *51*(4), 565–572.

López, A. C., De Ortuzar, R. V. M., & Alippi, A. M. (2008). Tetracycline and oxytetracycline resistance determinants detected in Bacillus cereus strains isolated from honey samples. *Revista Argentina de Microbiologia*, *40*(4), 231–237.

lozupone, C. A., Hamady, M., Kelley, S. T., & Knight, R. (2007). Quantitative and qualitative β diversity measures lead to different insights into factors that structure microbial communities. *Applied and Environmental Microbiology*, *73*(5), 1576–1585.

lozupone, C., & Knight, R. (2005). UniFrac: a new phylogenetic method for comparing microbial communities. *Applied and Environmental Microbiology*, *71*(12), 8228–8235.

Marder, E. P., Griffin, P. M., Cieslak, P. R., Dunn, J., Hurd, S., Jervis, R., & Tobin-D'Angelo, M. (2018). Preliminary incidence and trends of infections with pathogens transmitted commonly through food—foodborne diseases active

Majowicz, S. E., Scallan, E., Jones-Bitton, A., Sargeant, J. M., Stapleton, J., Angulo, F. J., & Kirk, M. D. (2014). Global incidence of human Shiga toxin–producing Escherichia coli infections and deaths: a systematic review and knowledge synthesis. *Foodborne Pathogens and Disease*, *11*(6), 447-455.

Mayo, B., TCC Rachid, C., Alegría, Á., MO Leite, A., S Peixoto, R., & Delgado, S. (2014). Impact of next generation sequencing techniques in food microbiology. *Current Genomics*, *15*(4), 293–309.

Mališevs, A., Bergšpica, I., Cibrovska, A., Avsejenko, J., Makarova, S., & Grantiņa-Ieviņa, L. (2019, January). Presence of shiga toxin producing Escherichia coli in Latvia in food, veterinary and clinical samples. In *OF THE 77th Scientific Conference OF the University of Latvia* (p. 47).

MacFaddin, J. F. (1985). Media for Isolation-Identification-Cultivation-Maintenance of Medical Bacteria. *Vol. I Williams and Wilkins, Baltimore*.

Mackay, I. M. (2004). Real-time PCR in the microbiology laboratory. *Clinical Microbiology and Infection*, *10*(3), 190-212.

Mazumder, S. A., Hicks, A., & Norwood, J. (2010). Mycobacterium gordonae pulmonary infection in an immunocompetent adult. *North American Journal of Medical sciences*, *2*(4), 205.

McIlroy, S. J., Kristiansen, R., Albertsen, M., Karst, S. M., Rossetti, S., Nielsen, J. L., & Nielsen, P. H. (2013). Metabolic model for the filamentous 'Candidatus Microthrix parvicella'based on genomic and metagenomic analyses. *The ISME Journal*, *7*(6), 1161-1172.

McLinden, T., Sargeant, J. M., Thomas, M. K., Papadopoulos, A., & Fazil, A. (2014). Component costs of foodborne illness: a scoping review. *BMC Public Health*, *14*(1), 509.

McDonald, D., Price, M. N., Goodrich, J., Nawrocki, E. P., DeSantis, T. Z., Probst, A., Hugenholtz, P. (2012). An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. *The ISME Journal*, *6*(3), 610–618.

Mendis, N., McBride, P., & Faucher, S. P. (2015). Short-term and long-term survival and virulence of Legionella pneumophila in the defined freshwater medium Fraquil. *PloS One*, *10*(9), e0139277.

Mughini-Gras, L., Schaapveld, M., Kramers, J., Mooij, S., Neefjes-Borst, E. A., van Pelt, W., & Neefjes, J. (2018). Increased colon cancer risk after severe Salmonella infection. *PloS One*, *13*(1), e0189721.

Muhonja. C, N. Budambula, N., Kiiru, J., & Kariuki, S. (2012). Characterization of antibiotic resistance in environmental enteric pathogens from Kibera slum in, *4*(October), 46–54. https://doi.org/10.5897/JBR12.008.

Mulaa, J. (2018). Kenyan Soda Lake Abstract :, 33–45. https://doi.org/10.2174/1874070701812010033.

Muleta, D., & Ashenafi, M. O. G. E. S. S. I. E. (2001). Salmonella, Shigella and growth potential of other food-borne pathogens in Ethiopian street vended foods. *East African Medical Journal*, *78*(11), 576-580.

Muñoz-atienza, E., Gómez-sala, B., Araújo, C., Campanero, C., Campo, R., Hernández, P. E., Cintas, L. M. (2013). Antimicrobial activity , antibiotic susceptibility and virulence factors of Lactic Acid Bacteria of aquatic origin intended for use as probiotics in aquaculture.

Neondo, J. O., Alakonya, A. E., & Kasili, R. W. (2017). Screening for potential Striga hermonthica fungal and bacterial biocontrol agents from suppressive soils in Western Kenya. *BioControl*, *62*(5), 705–717. https://doi.org/10.1007/s10526-017-9833-9.

Njiru P. K., Omuterema S. O. & Lilechi, D. B. (2016). Sanitation In Relation To Prevalence of Waterborne Diseases in Mbeere, Embu County, Kenya.

Nordmann, P., Naas, T., & Poirel, L. (2011). Global spread of carbapenemaseproducing Enterobacteriaceae. Emerg Infect Dis17: 1791–1798.

Nuneza, K. J. M., Abella, D. M. M., Maratas, L. L., & Nuneza, O. M. (2015). Antibiotic phenotypic profile of fecal Escherichia coli from pediatric patients of Adventist Medical Center, Iligan City, Philippines. *Adv Environ Biol*, *9*, 129–138.

O'Bryan, C. A., Crandall, P. G., & Ricke, S. C. (2017). *Antimicrobial Resistance in Foodborne Pathogens*. *Food and Feed Safety Systems and Analysis*. Elsevier Inc. https://doi.org/10.1016/B978-0-12-811835-1.00006-3

O'Neill, J. (2014). Review on antimicrobial resistance. *Antimicrobial resistance: tackling a crisis for the health and wealth of nations*, *2014*(4).

Odwar, J. A., Kikuvi, G., Kariuki, J. N., & Kariuki, S. (2014). A cross-sectional study on the microbiological quality and safety of raw chicken meats sold in Nairobi, Kenya. *BMC research notes*, *7*(1), 627.

Okumura T, Seto Y, Fuse A: Countermeasures against chemical terrorism in Japan. For Sci Int 2013, 227:2-6 http://dx.doi.org/ 10.1016/j.forsciint.2012.11.008 Epub 2013/02/26. PubMed PMID: 23434376.

Olowe, B., Oluyege, J., Famurewa, O., Ogunniran, A., & Adelegan, O. (2017). Molecular identification of Escherichia coli and new emerging enteropathogen, Escherichia fergusonii, from drinking water sources in Ado-Ekiti, Ekiti State, Nigeria. *J. Microbiol. Res*, *7*, 45-54.

Olsen, I. (2015). Biofilm-specific antibiotic tolerance and resistance. *European Journal of Clinical Microbiology & Infectious Diseases*, *34*(5), 877–886.

Oliva, G., Sahr, T., & Buchrieser, C. (2015). Small RNAs, 5′ UTR elements and RNA-binding proteins in intracellular bacteria: impact on metabolism and virulence. *FEMS Microbiology Reviews*, *39*(3), 331–349.

Onyango, D. M. (2019). Source Tracking and Antibiotic Resistance Patterns of Selected Pathogenic Bacteria Isolated from Street Vended Food in Kisumu County, Kenya. *EC Pharmacology and Toxicology*, *7*, 585-596.

Owusu-Kwarteng, J., Wuni, A., Akabanda, F., Tano-Debrah, K., & Jespersen, L. (2017). Prevalence, virulence factor genes and antibiotic resistance of Bacillus cereus sensu lato isolated from dairy farms and traditional dairy products. *BMC Microbiology*, *17*(1), 65.

Omulo, S., Thumbi, S. M., Njenga, M. K., & Call, D. R. (2015). A review of 40 years of enteric antimicrobial resistance research in Eastern Africa: what can be done better? *Antimicrobial resistance and infection control*, *4*(1), 1.

Okeke, I. N., Kariuki, S., Dinant, G. J., Driessen, C., & Stobberingh, E. E. (2004). Antibiotic resistance of faecal Escherichia coli from healthy volunteers from eight developing countries. *Journal of Antimicrobial Chemotherapy*, *54*(5), 952–955.

Oksanen, J., Kindt, R., Legendre, P., O'Hara, B., Stevens, M. H. H., Oksanen, M. J., & Suggests, M. (2007). The vegan package. *Community Ecology Package*, *10*(631–637), 719.

Oladipo, I. C., & Adejumobi, O. D. (2010). Incidence of antibiotic resistance in some bacterial pathogens from street vended food in Ogbomoso, Nigeria. *Pakistan Journal of Nutrition*, *9*(11), 1061-1068.

Pepperell, C., Kus, J. V., Gardam, M. A., Humar, A., & Burrows, L. L. (2002). Low-virulence Citrobacter species encode resistance to multiple antimicrobials. *Antimicrobial agents and chemotherapy*, *46*(11), 3555-3560.

Peng, Q., Jiang, S., Chen, J., Ma, C., Huo, D., Shao, Y., & Zhang, J. (2018). Unique microbial diversity and metabolic pathway features of fermented vegetables from Hainan, China. *Frontiers in Microbiology*, *9*, 399.

PerezPerez, G. I., Rothenbacher, D., & Brenner, H. (2004). Epidemiology of Helicobacter pylori infection. *Helicobacter*, *9*, 1-6.

Price, M. N., Dehal, P. S., & Arkin, A. P. (2010). FastTree 2–approximately maximum-likelihood trees for large alignments. *PloS One*, *5*(3), e9490.

Pushpakanth, P., Kennedy, Z. J., & Balachandar, D. (2019). Source tracking of Shiga-like toxin-producing Escherichia coli in the fresh vegetable production system of South India. *Annals of Microbiology*, *69*(9), 885–893.

Raele, D. A., Galante, D., Pugliese, N., La Salandra, G., Lomuto, M., & Cafiero, M. A. (2018). First report of Coxiella burnetii and Borrelia burgdorferi sensu lato in poultry red mites, Dermanyssus gallinae (Mesostigmata, Acari), related to urban outbreaks of dermatitis in Italy. *New Microbes and New Infections*, *23*, 103–109.

Rahman, B. A., Wasfy, M. O., Maksoud, M. A., Hanna, N., Dueger, E., & House, B. (2014). Multi-drug resistance and reduced susceptibility to ciprofloxacin among Salmonella enterica serovar Typhi isolates from the Middle East and Central Asia. *New Microbes and New Infections*, *2*(4), 88–92.

Rantsiou, K., Kathariou, S., Winkler, A., Skandamis, P., Saint-Cyr, M. J., Rouzeau-Szynalski, K., & Amézquita, A. (2018). Next generation microbiological risk assessment: opportunities of whole genome sequencing (WGS) for foodborne pathogen surveillance, source tracking and risk assessment. *International Journal of Food Microbiology*, *287*, 3-9.

Ramnarain, J., Yoon, J., & Runnegar, N. (2019). Staphylococcus pasteuri infective endocarditis: A case report. *IDCases*, *18*, e00656.

Rasmussen, L. H., Kjeldgaard, J., Christensen, J. P., & Ingmer, H. (2013). Multilocus sequence typing and biocide tolerance of Arcobacter butzleri from Danish broiler carcasses. *BMC Research Notes*, *6*(1), 322.

Ravin, N. V., Mardanov, A. V., & Skryabin, K. G. (2015). Metagenomics as a tool for the investigation of uncultured microorganisms. *Russian Journal of Genetics*, *51*(5), 431-439.

Reiner, K. (2010). Catalase test protocol. *American Society For Microbiology, ASMMicrobeLibrary*.

Richards, C. L., Broadaway, S. C., Eggers, M. J., Doyle, J., Pyle, B. H., Camper, A. K., & Ford, T. E. (2018). Detection of pathogenic and non-pathogenic bacteria in drinking water and associated biofilms on the crow reservation, Montana, USA. *Microbial Ecology*, *76*(1), 52–63.

Rodriguez, C., Taminiau, B., Van Broeck, J., Delmée, M., & Daube, G. (2016). Clostridium difficile in food and animals: a comprehensive review. In *Advances in Microbiology, Infectious Diseases and Public health* (pp. 65–92). Springer.

Rodriguez-Lazaro, D., Cook, N., Ruggeri, F. M., Sellwood, J., Nasser, A., Nascimento, M. S. J., & Bosch, A. (2012). Virus hazards from food, water and other contaminated environments. *FEMS microbiology Reviews*, *36*(4), 786-814.

Salines, M., Andraud, M., & Rose, N. (2017). From the epidemiology of hepatitis E virus (HEV) within the swine reservoir to public health risk mitigation strategies: a comprehensive review. *Veterinary Research*, *48*(1), 31.

Sambrook, J., & Russell, D. W. (2001a). Molecular cloning: A laboratory manual, the third edition. Cold spring harbor laboratory press, cold spring harbor, New York.

Sambrook, J., & Russell, D. W. (2001b). Molecular clonning: a laboratory manual. In *Molecular clonning: a laboratory manual*.

Sambrook, J., Fritsch, E. F., Maniatis, T., & Sambrook-Fritsch-Maniatis (1989). *Molecular Cloning: A Laboratory Manual;[prepared for Use in the CSH Courses on the Molecular Cloning of Eukaryotic Genes]*. Cold Spring Harbor Laboratory Press.

Schoder, D., Strauß, A., Szakmary-Brändle, K., Stessl, B., Schlager, S., & Wagner, M. (2015). Prevalence of major foodborne pathogens in food confiscated from air passenger luggage. *International Journal of Food Microbiology, 209*, 3-12.

Sicuia, O.-A., Grosu, I., Constantinescu, F., Voaides, C., & Cornea, C. P. (2015). Enzymatic and genetic variability in Bacillus spp. strains with plant beneficial qualities. *AgroLife Scient. J*, *4*, 124–131.

Sifuna, A. W., & Onyango, D. M. (2018). Source Attribution of Salmonella and Escherichia coli Contaminating Lake Victoria fish in Kenya. *African Journal of Tropical Hydrobiology and Fisheries*, *16*(1), 39-47.

Sharma, A. K., Sharma, V., & Saxena, J. (2016). Enhancement of extracellular lipase production by strain improvement of fungus Aspergillus niger LPF-5. *Int J Sci Res Environ Sci*, *4*, 0145-0152.

Schnappinger, D., & Hillen, W. (1996). Tetracyclines: antibiotic action, uptake, and resistance mechanisms. *Archives of microbiology*, *165*(6), 359-369.

Standards, P., & Testing, A. S. (2018). *M100 Performance Standards for Antimicrobial*.

Sowah, R. A., Habteselassie, M. Y., Radcliffe, D. E., Bauske, E., & Risse, M. (2017). Isolating the impact of septic systems on fecal pollution in streams of suburban watersheds in Georgia, United States. *Water research*, *108*, 330-338.

Sulman, S., & Rehman, A. (2013). Isolation and characterization of cellulose-degrading Candida tropicalis W2 from environmental samples. *Pak J Zool*, *45*, 809–816.

Tang, K.-Y., Wang, Z.-W., Wan, Q.-H., & Fang, S.-G. (2019). Metagenomics reveals seasonal functional adaptation of the gut microbiome to host feeding and fasting in the Chinese alligator. *Frontiers in Microbiology*, *10*, 2409.

Taylor, A. J., Paris, D. H., & Newton, P. N. (2015). A systematic review of mortality from untreated scrub typhus (Orientia tsutsugamushi). *PLoS Negl Trop Dis*, *9*(8), e0003971.

Too, J. K., Kipkemboi Sang, W., Ng'ang'a, Z., & Ngayo, M. O. (2016). Fecal contamination of drinking water in Kericho District, Western Kenya: role of source and household water handling and hygiene practices. *Journal of water and health*, *14*(4), 662-671.

Torres, C., Leandro, D. O., Jamile, P., & Pereira, Q. (2016). Screening of Bacteria for Protease Production and Feather Degradation. *Waste and Biomass Valorization*. https://doi.org/10.1007/s12649-015-9464-2.

Torres, M. J., Petroselli, G., Daz, M., Erra-Balsells, R., & Audisio, M. C. (2015). Bacillus subtilis subsp. subtilis CBMDC3f with antimicrobial activity against Gram-positive foodborne pathogenic bacteria: UV-MALDI-TOF MS analysis of its bioactive compounds. *World Journal of Microbiology and Biotechnology*, *31*(6), 929–940.

Team, C. (2012). nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-104R Development Core Team (2011) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. *URL Http://www. R-Project. Org*.

Van den Bergh, B., Michiels, J. E., Wenseleers, T., Windels, E. M., Boer, P. Vanden, Kestemont, D., Fauvart, M. (2016). Frequency of antibiotic application drives rapid evolutionary adaptation of Escherichia coli persistence. *Nature Microbiology*, *1*(5), 16020

Vestergaard, G., Schulz, S., Schöler, A., & Schloter, M. (2017). Making big data smart—how to use metagenomics to understand soil quality. Springer.

Watkins, R. R., Smith, T. C., & Bonomo, R. A. (2016). On the path to untreatable infections: colistin use in agriculture and the end of 'last resort'antibiotics.

Wang, Q., Holmes, N., Martinez, E., Howard, P., Hill-Cawthorne, G., & Sintchenko, V. (2015). It is not all about single nucleotide polymorphisms: comparison of mobile genetic elements and deletions in Listeria monocytogenes genomes links cases of hospital-acquired listeriosis to the environmental source. *Journal of Clinical Microbiology*, *53*(11), 3492–3500.

Walsh, F., & Duffy, B. (2013). The culturable soil antibiotic resistome: a community of multi-drug resistant bacteria. *PloS one*, *8*(6), e65567.

WHO, 2015. 10 Facts on Food Safety.Available online at. http://www.who.int/features/ factfiles/food_safety/facts/en/index.html (Accessed on April 12, 2016).

WHO (World Health Organization). (2014). Antimicrobial resistance: global 304 report

WHO/AFRO (World Health Organization Regional Office for Africa), 2013. Integrated Disease Surveillance and Response AFENET Pre-Conference Workshop. Addis Ababa, Ethiopia, November 17, 2013.

Xie, Z., Dong, Z., Zhu, P., Zhang, L., Chen, X., & Dong, C. (2017). Antibiotic Susceptibility and Molecular Characterization of <i>Escherichia coli O157</i> Isolates from Urinary Tract Infections. *Urologia Internationalis*, *443003*(183). https://doi.org/10.1159/000479090

Yu, K., & Zhang, T. (2012). Metagenomic and metatranscriptomic analysis of microbial community structure and gene expression of activated sludge. *PloS One*, *7*(5), e38183.

Zhang, X. X., Zhang, T., & Fang, H. H. (2009). Antibiotic resistance genes in water environment. *Applied microbiology and biotechnology*, *82*(3), 397-414.

Zhao, Y., Chen, C., Gu, H. J., Zhang, J., & Sun, L. (2019). Characterization of the genome feature and toxic capacity of a Bacillus wiedmannii isolate from the hydrothermal field in Okinawa Trough. *Frontiers in cellular and infection microbiology*, *9*, 370.

Zhao, X., Lin, C., Wang, J., & Oh, D. (2014). Advances in rapid detection methods for foodborne pathogens. *J. Microbiol. Biotechnol*, *24*(3), 297–312. https://doi.org/10.4014/jmb.1310.10013

Zaulet, M., Dumitrache, R., Tanasuica, R., Nichita, C., Kevorkian, S. E. M., & Buburuzan, L. (2016). Prevalence of some foodborne pathogens in meat products in Romania. *Romanian Biotechnological Letters*, *21*(5), 11949-11958.

https://www.who.int/news-room/fact-sheets/detail/food-safety:year-2020

https://reliefweb.int/report/kenya/fear-cholera-outbreak-three-hospitalised-embu

APPENDICES

Appendix 1: Morphological characterization of bacterial isolates from fast foods and the surrounding environment that produced extracellular enzymes

Isolate	Location	Sample	Lac	Suc	Glu	Dea	Cata	Citrate	H2S	Cracks	Cell shape	Gram	*Probable Identity*
KS7	Kangaru	Soil	-	-	+	-	++	+	-	+	Rod	-	*Providencia spp.,*
KF27	Kangaru	Fish	-	-	-	-	+++	-	-	-	Rod	-	*Shigella spp.,*
KRM34	Kangaru	Roosted meat	+	+	+	-	+++	-	-	-	Rod	-	*E.coli*
KFR38	Kangaru	Fruit	-	-	-	-	+	-	-	-	Rod	+	*Bacillus pacificus*
KFR42	Kangaru	Fruit	-	-	-		+++	+	-	-	Rod	+	*Bacillus amyloliquefaciens*
KAF45	Kangaru	Meat pie	+	+	+	-	+++	+	-	-	Rod	-	*Pseudomonas spp.,*
KFR52	Kangaru	Fruit	-	-	+	-	+++	+	-	-	Rod	-	*Bacillus megaterium*
KSM63	Kangaru	Samosa	+	+	+	-	-	-	-	-	Rod	-	*Shigella sonnei*
KS65	Kangaru	Soil	-	-	+	-	+++	+	-	-	Rod	+	*Paraclostridium benzoelyticum*
KC67	Kangaru	Chips	-	-	+	-	++	-	-	-	Rod	-	*Pseudomonas aeruginosa*
KVS68	Kangaru	Vegetable salad	-	-	+	-	+++	-	-	-	Rod	-	*Enterobacter spp.,*
KC75	Kangaru	Vegetable salad	+	+	+	-	-	-	-	-	Rod	-	*Bacillus wiedmannii*
TVS81	Kangaru	Vegetable salad	-	-	-	-	+++	-	-	-	Rod	-	*Bacillus thuringiensis*
KVS82	Kangaru	Vegetable salad	-	-	-	-	+++	-	-	-	Rod	-	*Serratia marcescens*
KVS85	Kangaru	Vegetable salad	-	-	-	-	+++	-	-	-	Rod	-	*Serratia marcescens*
KAF95	Kangaru	Meat pie	+	+	+	-	++	+	-	-	Rod	-	*Serratia marcescens*
KF101	Kangaru	Fish	-	-	-	-	+	+	-	-	Rod	+	*Bacillus velezensis*
KS104	Kangaru	Soil	+	+	+	-	+++	+	-	+	Rod	+	*Virgibacillus phasianinus*
KS109	Kangaru	Soil	+	+	+	-	++	-	-	+	Rod	+	*Lysinibacillus parviboronicapiens*
KS116	Kangaru	Soil	-	-	+	+	++	+	-	-	Rod	-	*Providencia spp.,*
KVS120	Kangaru	Vegetable salad	-	-	-	-	+++	-	-	-	Rod	+	*Bacillus subtilis*
KC122	Kangaru	Chips	-	-	+	-	+++	-	-	-	Rod	-	*Pseudomonas spp.,*
KFR131	Kangaru	Fruit	-	-	+	-	+	-	-	-	Rod	-	*Pseudomonas spp.,*
KVS147	Kangaru	Vegetable salad	+	+	+	-	++	+	-	-	Coccus	+	*Staphylococcus aureus*
KF148	Kangaru	Fish	+	+	+	-	+++	-	-	-	Rod	-	*Shigella spp.,*
TF152	Town	Fish	+	+	+	-	+++	+	-	-	Rod	-	*E. coli*

Isolate	Location	Sample	Lac	Suc	Glu	Dea	Cata	Citrate	H2S	Cracks	Cell shape	Gram	Probable identity
KAF159	Kangaru	Meat pie	-	+	+	-	++	-	-	-	Rod	-	*Salmonella spp.,*
KS160	Kangaru	Soil	+	+	+	-	++	-	-	-	Long rod	-	*Citrobacter spp.,*
KF169	Kangaru	Fish	-	-	+	+	++	+	-	-	Rod	-	*Proteus spp.,*
KAF188	Kangaru	Meat pie	-	-	+	-	+++	+	-	-	Short rod	-	*Aerobacter aerogenes*
KFR200	Kangaru	Fruit	-	-	-	-	++	-	-	-	Short rod	-	*Enterobacter spp.,*
KFR204	Kangaru	Fruit	+	+	+	-	+++	-	-	-	Rod	-	*Pseudomonas spp.,*
KVS214	Kangaru	Vegetable salad	+	+	+	-	+++	-	-	-	Coccus	+	*S. pneumoniae*
KFR217	Kangaru	Fruit	+	+	+	-	+++	-	-	-	Rod	-	*Klebsiella spp.,*
KFR222	Kangaru	Fruit	-	+	+	-	++	+	-	-	Rod	+	*Bacillus subtilis*
KC231	Kangaru	Chips	-	-	-	-	+	-	-	-	Coccus	+	*S. pneumoniae*
KFR245	Kangaru	Fruit	-	-	-	-	+++	-	-	-	Coccus	+	*P. aeruginosa*
KF246	Kangaru	Fish	-	-	-	-	-	-	-	-	Coccus	+	*S. pneumoniae*
KVS249	Kangaru	Vegetable salad	-	-	-	-	+++	+	-	-	short rod	+	*Pseudomonas spp.,*
KAF253	Kangaru	Meat pie	-	-	+	-	++	-	+	-	Rod	-	*Salmonella spp*
KS260	Kangaru	Soil	+	+	+	-	++	+	-	+	Rod	-	*E. coli*
KS267	Kangaru	Soil	+	+	+	-	++	-	-	+	Rod	+	*Bacillus weidmannii*
KVS271	Kangaru	Vegetable salad	-	-	-	-	+	-	-	-	Rod	-	*Pseudomonas spp.,*
KFR285	Kangaru	Fruit	-	-	-	-	+++	-	-	-	Rod	-	*Hafnia spp.,*
KFR286	Kangaru	Fruit	-	-	-	-	+++	-	-	-	Rod	-	*Pseudomonas spp.,*
KFR301	Kangaru	Fruit	-	+	+	-	++	-	+	-	Rod	-	*Salmonella spp.,*
KAF321	Kangaru	Fish	+	+	+	-	+++	+	-	-	Coccus	+	*Streptococcus pneumoniae*
TFR329	Kangaru	Meat pie	-	-	-	-	+	-	-	-	Rod	+	*Bacillus proteolyticus*
KVS330	Town	Fruit	+	+	+	-	-	-	-	-	Rod	-	*E. coli*
KAF337	Kangaru	Vegetable salad	-	-	-	-	+++	-	-	-	Long rod	-	*Citrobacter freundii*
KRM349	Kangaru	Meat pie	-	+	+	-	-	+	+	-	Rod	-	*Salmonella spp.,*
KS376	Kangaru	Roasted meat	+	+	+	-	+++	+	-	+	Rod	-	*Pseudomonas spp.,*
TS378	Kangaru	Soil	+	+	+	-	++	-	-	-	Rod	-	*E.coli*
TS380	Town	Soil	+	+	+	-	+++	+	+	+	Rod	-	*Salmonella spp.,*
KF381	Town	Soil	-	+	+	-	++	-	-	-	Rod	-	*Pseudomonas spp.,*
KF389	Kangaru	Fish	-	-	+	-	+++	-	-	-	Coccus	-	*Micrococcus spp.,*
KF391	Kangaru	Fish	+	+	+	+	+++	+	-	-	Rod	-	*Morganella spp.,*

Isolate	Location	Sample	Lac	Suc	Glu	Dea	Cata	Citrate	H2S	Cracks	Cell shape	Gram	Probable identity
KF393	Kangaru	Fish	+	+	+	-	++	-	-	-	Rod	-	*Serratia marcescens*
KF395	Kangaru	Fish	-	-	-	-	+++	-	-	-	Rod	-	*Yersinia spp.,*
KF399	Kangaru	Fish	-	-	+	-	+	-	-	-	Rod	-	*Pseudomonas aeruginosa*
TS427	Kangaru	Fish	+	+	+	-	+++	+	-	-	Rod	-	*Salmonella enterica*
KC430	Town	Soil	+	+	+	-	+++	+	-	-	Rod	+	*Pseudomonas spp.,*
KRM435	Kangaru	Chips	+	+	+	-	+++	-	-	-	Rod	-	*Pseudomonas spp.,*
KF463	Kangaru	Roasted meat	+	+	+	-	++	-	-	-	Rod	+	*Bacillus parathrancis*
TS572	Kangaru	Fish	-	-	+	-	++	+	-	-	Rod	-	*Bacillus subtillis*
KS606	Town	Soil	+	+	+	-	+	-	-	+	Rod	+	*Bacillus anthracis*
TS621	Town	Soil	+	+	+	+	-	-	-	-	Rod	+	*Bacillus cereus*
KF317	Kangaru	Soil	+	+	+	-	++	+	-	-	Coccus	+	*Enterococcus feacalis*
KS585	Kangaru	Soil	+	+	+	-	-	-	-	-	Rod	+	*Bacillus toyonensis*
TS472	Town	Soil	+	+	-	-	+++	-	-	-	Rod	-	*Proteus spp.,*

Key; lac (lactose), Suc (Sucrose), Glu (Glucose), Dea (Deamination)

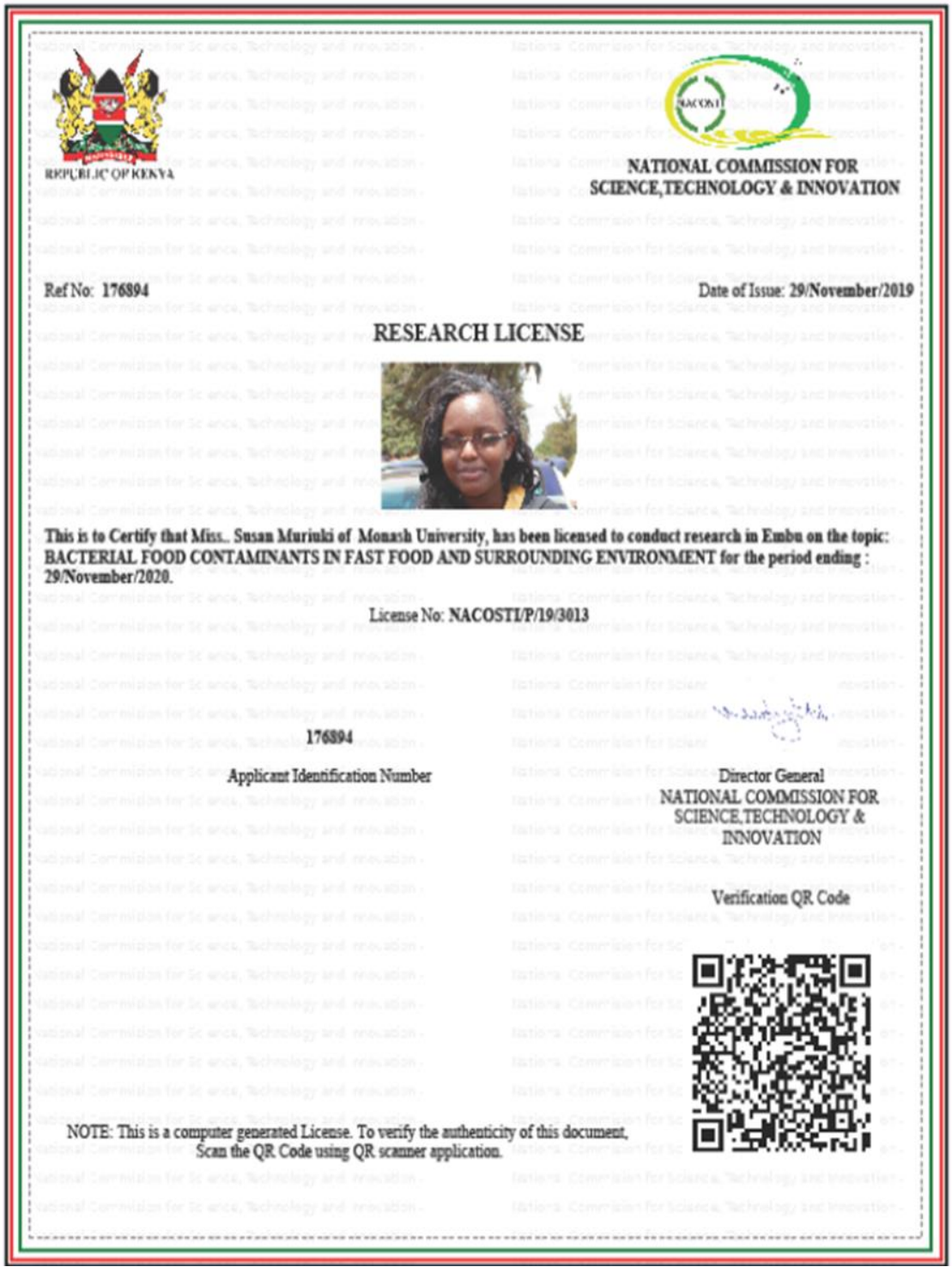
Appendix 2: Morphological and biochemical characterization of antibiotic-resistant bacterial isolates isolated from Embu town and neighboring Kangaru market in 2018

Isolate			**Colony Characterization**						**Extracellular Enzyme detected**			
	Location	**Sample**	**Color**	**Shape**	**Elevation**	**Margin**	**Cell shape**	**Gram**	**Protease**	Amylase	Cellulase	Esterase
KS7	Kangaru	Soil	Brown	Circular	Raised	Curled	Coccus	+	+	-	+	+
KF27	Kangaru	Fish	Colorless	Circular	Raised	Curled	streptococcus	+	+	-	-	+
KRM34	Kangaru	Roosted meat	Pink	Irregular	Raised	Curled	Long rod	-	+	-	-	-
KFR38	Kangaru	Fruit	Colorless	Circular	Raised	Entire	streptococcus	+	+	-	-	-
KFR42	Kangaru	Fruit	light pink	Irregular		Entire	streptococcus	-	-	-	-	-
KAF45	Kangaru	Meat pie	cream white	Irregular	Flat	Entire	filamentous	-	+	-	-	-
KFR52	Kangaru	Fruit	Pink	Circular	Raised	Entire	diplococcus	-	+	-	+	+
KSM63	Kangaru	Samosa	Orange	Irregular	Flat	Curled	Coccus	-	+	-	+	-
KS65	Kangaru	Soil	Yellow	Irregulars	Raised	Curled	diplococcus	-	+	-	+	+
KC67	Kangaru	Chips	Yellow	Irregular	Flat	Undulate	Bacillus	+	-	-	-	-
KVS68	Kangaru	Vegetable salad	Colorless	Irregular	Raised	Undulate	diplococcus	+	+	-	-	-
KC75	Kangaru	Vegetable salad	cream white	Irregular	Raised	Entire	streptococcus	-	+	+	+	-
TVS81	Kangaru	Vegetable salad	Colorless	Circular	Raised	Entire	diplococcus	+	+	-	-	-
KVS82	Kangaru	Vegetable salad	Brown	Irregular	Flat	Curled	diplococcus	-	+	+	-	-
KVS85	Kangaru	Vegetable salad	Colorless	Irregular	Flat	Curled	diplococcus	+	+	-	+	-
KAF95	Kangaru	Meat pie	Pink	Circular	convex	Curled	streptococcus	-	+	+	-	+
KF101	Kangaru	Fish	cream white	Circular	Raised	Entire	Coccus	+	+	+	-	-
KS104	Kangaru	Soil	Black	Irregular	Flat	Curled	Short rod	+	+	+	-	+
KS109	Kangaru	Soil	cream yellow	Irregular	Raised	Curled	Coccus	-	+	+	+	-
KS116	Kangaru	Soil	cream yellow	Circular	Raised	entire	Coccus	-	+	+	+	+
KVS120	Kangaru	Vegetable salad	Colorless	Irregular	Flat	curled	streptococcus	-	-	-	-	+
KC122	Kangaru	Chips	Orange	Irregular	Flat	curled	streptococcus	-	+	-	-	-

Isolate	**Location**	**sample**	**Color**	**Shape**	**Elevation**	**Margin**	**Cell shape**	**Gram**	**Protease**	**Amylase**	Cellulase	Esterase
KFR131	Kangaru	Fruit	Yellow	Circular	Raised	entire	diplococcus	-	+	+	+	+
KVS147	Kangaru	Vegetable salad	cream yellow	Irregular	convex	entire	streptococcus	+	+	+	+	+
KF148	Kangaru	Fish	light purple	Circular	Raised	filiform	Short rod	-	+	+	+	+
TF152	Town	Fish	Purple	Circular	convex	curled	Coccus	+	+	-	-	-
KAF159	Kangaru	Meat pie	Pink	Circular	Raised	entire	Coccus	+	+	-	-	+
KS160	Kangaru	Soil	Purple	Irregular	Flat	curled	Coccus	+	+	-	-	-
KF169	Kangaru	Fish	cream white	Circular	Flat	entire	Coccus	-	+	-	-	+
KAF188	Kangaru	Meat pie	Pink	Irregular	Raised	lobate	Short rod	-	+	-	-	+
KFR200	Kangaru	Fruit	mucoid purple	Irregular	convex	entire	Short rod	-	+	+	+	-
KFR204	Kangaru	Fruit	cream white	Circular	Umbonate	entire	Long rod	-	+	-	-	-
KVS214	Kangaru	Vegetable salad	cream yellow	Circular	Flat	curled	Coccus	+	+	-	-	-
KFR217	Kangaru	Fruit	Pink	Circular	Flat	curled	Short rods	+	+	-	-	-
KFR222	Kangaru	Fruit	Pink	Irregular	Flat	entire	streptococcus	+	+	-	-	+
KC231	Kangaru	Chips	cream white	circular	Raised	entire	streptococcus	+	+	-	-	-
KFR245	Kangaru	Fruit	cream white	Irregular	Flat	undulate	Coccus	+	+	-	+	+
KF246	Kangaru	Fish	Yellow	Circular	Raised	entire	Coccus	+	+	+	-	+
KVS249	Kangaru	Vegetable salad	cream white	Irregular	convex	entire	short rod	+	+	+	+	+
KAF253	Kangaru	Meat pie	cream white	Irregular	Flat	undulate	Coccus	-	+	+	-	+
KS260	Kangaru	Soil	Pink	Irregular	Flat	entire	diplococcus	+	+	-	-	+
KS267	Kangaru	Soil	cream yellow	Circular	Raised	entire	Coccus	+	+	+	-	+
KVS271	Kangaru	Vegetable salad	cream white	Circular	Flat	curled	Bacillus	+	+	+	-	-
KFR285	Kangaru	Fruit	Brown	Circular	Flat	entire	Short rod	+	+	+	-	-
KFR286	Kangaru	Fruit	Yellow	Circular	Raised	curled	streptococcus	-	+	-	-	+
KFR301	Kangaru	Fruit	White	Circular	Flat	curled	Coccus	-	+	-	-	+
KF317	Kangaru	Fish	Purple	Circular	Raised	entire	Long rod	+	+	+	+	-
KAF321	Kangaru	Meat pie	cream white	Circular	Flat	entire	Coccus	-	+	+	+	-

Isolate	**Location**	**Sample**	**Color**	**Shape**	**Elevation**	**Margin**	**Cell shape**	**Gram**	**Protease**	**Amylase**	Cellulase	Esterase
TFR329	Town	Fruit	Orange	Circular	Raised	undulate	Coccus	+	+	+	-	-
KVS330	Kangaru	Vegetable salad	cream white	Circular	Crateriform	entire	diplo-bacillus	+	+	-	-	-
KAF337	Kangaru	Meat pie	Brown	Circular	Raised	curled	Short rods	-	+	-	+	-
KRM349	Kangaru	Roasted meat	cream white	Irregular	Folate	filiform	Bacillus	+	+	-	+	-
KS376	Kangaru	Soil	cream white	Circular	convex	entire	diplococcus	-	+	+	+	+
TS378	Town	Soil	metallic sheen	Circular	Crateriform	entire	diplobacillus	+	+	-	+	+
TS380	Town	Soil	cream white	Circular	convex	entire	Bacillus	-	+	-	-	-
KF381	Kangaru	Fish	Yellow	Circular	Raised	entire	Bacillus	-	+	+	+	-
KF389	Kangaru	Fish	light pink	Circular	Flat	curled	streptococcus	-	+	-	+	-
KF391	Kangaru	Fish	cream yellow	Circular	Flat	entire	Coccus	-	+	-	-	-
KF393	Kangaru	Fish	light pink	Circular	convex	entire	diplococcus	+	+	-	+	+
KF395	Kangaru	Fish	light brown	Circular	Raised	entire	Short rod	-	+	-	-	-
KF399	Kangaru	Fish	cream white	Circular	Flat	entire	diplococcus	-	+	+	+	+
TS427	Town	Soil	White	Circular	Flat	curled	diplococcus	-	-	-	-	-
KC430	Kangaru	Chips	cram white	Circular	Flat	curled	diplococcus	+	+	-	-	+
KRM435	Kangaru	Roasted meat	Colorless	Circular	Flat	entire	Diplococcus	-	+	-	-	-
KF463	Kangaru	Fish	cream white	Circular	Raised	entire	Short rod	-	-	-	-	+.
TS472	Town	Soil	cream white	Irregular	Flat	filiform	Streptococcus	-	+	+	-	-
TS572	Town	Soil	cream white	Oval	Raised	entire	Bacillus	+	+	-	-	-
KS585	Kangaru	Soil	light pink	Irregular	Raised	entire	Streptococcus	+	+	-	-	-
KS606	Kangaru	Soil	Black	Circular	Flat	entire	Streptococcus	-	+	+	+	-
TS621	Town	Soil	light purple	Circular	convex	entire	Streptococcus	-	+	+	-	-

Appendix 3: National Commission for science, Technology & Innovation (NACOSTI) approval



REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: 176894

Date of Issue: 29/November/2019

RESEARCH LICENSE

This is to Certify that Miss.. Susan Muriuki of Monash University, has been licensed to conduct research in Embu on the topic: BACTERIAL FOOD CONTAMINANTS IN FAST FOOD AND SURROUNDING ENVIRONMENT for the period ending : 29/November/2020.

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Appendix 4: Public Health Ministry of Embu County approval

Received
23/7/2018

EMBU COUNTY GOVERNMENT

OFFICE OF COUNTY DIRECTOR OF HEALTH

Mobile: +254 771 204 003/+254 707 192 924 Tel: 254 68 30686/30656
Address: P. O. Box 36 – 60100 Embu Town House Email: info@embu.go.ke Web:www.embu.go.ke

Our Ref No: A.9/VOL.III/22 Date: 6TH JUNE, 2018

THE MEDICAL OFFICER OF HEALTH

MANYATTA SUB-COUNTY

(ATTN: SUB-COUNTY PUBLIC HEALTH OFFICER)

RE: MSC PROJECT, MURIUKI SUSAN WAIRIMU REG B132/1148/2017

The above named is a MSc student at University of Embu pursuing MSc in Applied Microbiology. She is interested in carrying out research on microbial food contaminants. As such she is interested in assessing food premises within Manyatta sub-county. Kindly assist her to achieve her objective.

DR STEPHEN KANIARU

COUNTY DIRECTOR OF HEALTH

EMBU COUNTY

CC

-COH

CEC