MAPPING THE DISTRIBUTION PATTERNS OF MULTIPLE-DRUG RESISTANCES GRAM-NEGATIVE BACTERIAL STRAINS RECOVERABLE FROM FOOD AND ENVIRONMENTAL SAMPLES IN KIBERA INFORMAL SETTLEMENTS

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Mapping the Distribution Patterns of Multiple-Drug Resistances Gram-Negative Bacterial Strains Recoverable From Food and Environmental Samples in Kibera Informal Settlements

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DECLARATION

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

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DEDICATION

I dedicate this thesis to my dear parents Mr. Josphat Maina Kiiru and Mrs. Grace Wangui Maina.

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Special thanks to God the Almighty for granting me the strength, good health, and seeing me throughout my study period.

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LIST OF ACRONYMS AND SYMBOLS

µg	Microgram
µl	Micro litre
A.baumannii	*Acinetobacter baumannii*
AMP	Ampicillin
AMR	Antimicrobial resistance
ATCC	American type culture collection
ATM	Aztreonam
bla	β-lactamase gene
$\boldsymbol{bla}_{\textbf{CTX-M}}$	Cefotaxime Munich gene
$\boldsymbol{bla}_{\textbf{OXA}}$	Oxacillin resistance gene
$\boldsymbol{bla}_{\textbf{SHV}}$	Sulfhydryl variant gene
$\boldsymbol{bla}_{\textbf{TEM}}$	Temoneria antimicrobial-resistant gene
C	Chloramphenicol
C.freundii	*Citrobacter freundii*
CAZ	Ceftazidime
CFU/mL	Colony forming units per milliliter
CIP	Ciprofloxacin
CLSI	Clinical and laboratory standard institute

interpretations

CMR	Centre for Microbiology Research
CN	Gentamicin
CO_2	Carbon dioxide
CRO	Ceftriaxone
CTX	Cefotaxime
DNA	Deoxyribonucleic acid
E. coli	*Escherichia coli*
E.agglomerans	*Enterobacter agglomerans*
E.tarda	*Edwardsiella tarda*
EMBA	Eosin Methylene blue medium
ESβLS	Extended-spectrum β-lactamases
FAO	Food and Agriculture Organization of the United Nations
FEP	Cefepime
FOX	Cefoxitin
GPS	Global positioning system
H.alvei	*Hafnia alvei*
HACCP	Hazard analysis and critical control point system
intI	integrase of an integron
IPM	Imipenem

IRT gene	Inhibitor resistance Temoneria gene
ITROMID	Institute of Tropical Medicine and Infectious diseases
JKUAT	Jomo Kenyatta University of Agriculture and Technology
K. pneumonia	*Klebsiella pneumoniae*
KEMRI	Kenya Medical Research Institute
M.morganii	*Morganella morganii*
MDR	Multidrug resistance strains
NA	Nalidixic acid
^{o}C	Degree centigrade
P.aeruginosa	*Pseudomonas aeruginosa*
PCR	Polymerase chain reaction
pH	potential hydrogen
RL	Sulfamethoxazole
S	Streptomycin
SERU	Scientific Ethical Review Unit
SIM	Sulfur indole motility medium
Spp	Species
Sr marcesence	*Serratia marcesence*
TCBS	Thiosulfate-citrate-bile salts-sucrose agar

TSI	Triple sugar iron medium
UV	Ultraviolet light
W	Trimethoprim
WHO	World Health Organisation

DEFINITION OF TERMS

ESβL: ESβL refers to Extended-spectrum β-lactamases enzymes. Bacteria that produce or carry these enzymes are characterized by the hydrolysis capability of penicillin's, first-, second-, third-generation cephalosporins, and aztreonam but not the cephamycins. These enzymes act by deactivating the β-lactam ring of β-lactam antibiotics making them ineffective against ESβL – producing bacteria strains.

MDR-strains: MDR-strains in this context, refer to bacterial strains that are resistant to 3 or more antimicrobial agents of distinct classes. In this study, antimicrobial activity of 16 agents that belong to β-lactams, Aminoglycosides, Monobactam, Cefamycin, Phenicol, Floroquinolone and Quinolone were tested against Gram-negative bacteria isolates.

βFQA phenotype: This refers to bacterial strains with co-resistance to β-lactams specifically the 3rd generation cephalosporin (ceftazidime, ceftriaxone and/or cefotaxime), a fluoroquinolone (ciprofloxacin), a quinolone (nalidixic-acid) and advanced aminoglycosides such as gentamicin and amikacin.

GTG_5 fingerprinting: This low-resolution method detects small tandem repeats in bacteria DNA and generates polymorphic patterns essential in distinguishing bacterial strains. Bacterial strains with more similar tandem repeats tend to be closely related and may infer clonal expansion in a particular ecosystem.

ABSTRACT

In this cross-sectional study conducted in Kibera informal settlements in the west side on Nairobi, ready-to-eat food samples that consisted of kales, cabbage, Managu, rice, ndengu, githeri, beans, meat, omena(a preparation of *Rastrineobola argetea*) and ugali were analyzed to determine the diversity of Gram-negative bacteria and contamination levels. Sewage, sludge and soil samples collected within a 10 meters radius of the sampled food vending points were also collected and analyzed. Analysis of contamination levels detected microbial contamination levels exceeding 10^4 CFU/mL in 106 (38%) of the 281 ready-to-eat foods analyzed. The burden of contamination of these foods ranged from a mean of 4.0×10^4 to 2.3×10^6 CFU/mL, which is above the recommended minimum threshold of 1.0×10^2 CFU/mL considered fit for human consumption. Among these foods, vegetables, especially kales, were the most contaminated foods with a mean of 2.3×106 CFU/mL, followed by meats at 1.0×106 CFU/mL, while omena (a type of tiny fish) had a mean CFU/mL of 4.0×10^4. Statistical analysis revealed a significant difference in contamination levels among foods sold near sewage, toilet, open space vending point layout, and near dumping from those not close to these features (P 0.0001)). Sites that did not have a clean water supply were found to have more contaminated foods exceeding the recommended threshold for human consumption than those with a constant supply of treated municipal water (P: 0.0001, C.I: 5.23 – 5.35, O.R: 5.28). The study recovered seven types of non-fastidious bacteria species in these foods and these included were; *Klebsiella* (29%), *Escherichia coli* (26%), *Enterobacter agglomerans* (22%), *Salmonella spp* (7%), *Proteus mirabilis* (7%), *Citrobacter freundii* (7%) and *Serratia marcesence* (3%). A total of 14 bacteria genera were also recovered from sewage, sludge and soil samples; *E. coli* (21%), *Klebsiella spp* (20%), *Enterobacter spp* (19%), *Citrobacter spp* (8%), *P. aeruginosa* (8%), *Proteus spp* (4%), *Salmonella* (5%), *Shigella* (3%), *M. morganii* (3%), *Edwardisella spp* (2%), *Hafnia spp* (2%), *S. marcesence* (1%), and *A. baumannii* (1%). On average, Ampicillin, trimethoprim and sulfamethoxazole were the most resisted antimicrobial agents in both food and environmental isolates, while Imipenem, cefepime, ciprofloxacin and ceftazidime were the most effective. Class 1 integron (*intI*1) was more prevalent than *intI*2 among the screened isolates. This study's cluster analysis suggested possible microbial contamination of ready-to-eat foods from Kibera's immediate vending environment.

CHAPTER ONE

INTRODUCTION

1.1 Study background

Gram-negative and positive bacteria have been implicated with serious infections across the globe. However, Gram-negative bacteria, particularly those belonging to the family Enterobacteriaceae, have been associated with difficult-to-treat diseases due to the emergence and spread of antimicrobial resistance in this family via horizontal gene transfer (Woerther *et al.* 2011). This spread in antimicrobial resistance has mostly been associated with integron elements that can acquire and express resistance gene cassettes through a promoter region. Integron cassettes encoding resistance to important antimicrobial agents such as β-lactams, aminoglycosides and fluoroquinolones have been documented in clinical and environmental microbial isolates (Blair *et al.* 2015;Kiiru *et al*. 2013).

Various genes encoding resistance to β-lactams, aminoglycosides and fluoroquinolones have been reported, however, Extended-spectrum β-lactamases (ESβL) remain the principal documented resistance determinants in Gram-negative microbial (Lynch III *et al*. 2013). These resistance genes can hydrolyze the β-lactam ring hence making β-lactam antimicrobials ineffective. To date, the bla_{TEM}, bla_{CTX}, $bla_{\text{SHV}}$and bla_{OXA} ESβL-variants that are to hydrolyze 3rd generation cephalosporins remain the most prevalent amongst members of the family Enterobacteriaceae.

To date, many studies across the world have widely documented the prevalence of microbial in clinical settings, their antimicrobial resistance phenotypes and genotypes. However, the role-played by Environment compartments such as sewage, sludge and soil that may serve as refuse and source of infection-causing bacterial strains, especially in an area with poor sanitation infrastructure such as the informal settlements, have been overlooked. In Kenya, like in most sub-Saharan countries, no study has attempted to establish the role of foods, sewage, sludge and fecal contaminated soils as sinks for isolates with MDR-bacteria strains. This is even

more important in informal settlements characterized by poor sanitation, limited access to clean water and over-congestion (Okeke *et al.* 2007). Such data is particularly crucial in outbreaks management and policy, formulations to curb the spread of AMR, and identify hotspots that play a role as flush-points for outbreaks associated with MDR strains.

In Kibera informal settlement in the western region of Nairobi County, Kenya and like in many informal settlements, open raw sewers and uncollected heaps of garbage are vast. In some areas, water supply lines pass through these open sewers and waste, which increases the chances of contamination with Multiple-drug resistance (MDR) strains, some of which may be potential pathogens that can subsequently cause severe infections in both humans and animals (Cantas *et al.* 2013). This unsafe water is often used for cooking and drinking and is, therefore, a health risk to many informal settlements residents.

Ready-to-eat street foods sold by the roadside are trendy in Kibera slums due to their affordability and short waiting-time (Owuor *et al.* 2017). However, due to the poor sanitation infrastructures and limited access to basic amenities like clean water, toilets and sewage facilities, most of these foods are prepared in insufficiently hygienic environments (Mutisya and Yarime 2011). Water shortage that is also a common problem in these settings, makes it hard to exercise proper hygiene during the preparation and serving of these street foods. There is also a lack of sufficient clean water to wash vegetables; therefore, the same water can be used repeatedly, which could also lead to cross-contamination. Food-handlers therefore can cross-contaminate raw or processed foods during handling and/or preparation (Akabanda *et al*. 2017). Washing of the utensils in most of these vending points is usually done in a bucket and in many cases, the water is reused several times for the same purpose. Therefore, there is a great possibility that foods prepared and served in such settings get contaminated and may be a source of food-borne infection pathogens (Havelaar *et al.*,2010 ;Kariuki *et al.* 2017).

According to the literature review done in this study, there is no documentation done so far to document the diversity of enteric bacteria recoverable from ready-to-eat

street foods in the informal settlements in Kenya. The date, known antimicrobial resistance phenotypes and genotypes of bacteria from such settings is also very scarce. In this cross-sectional study, we aimed to determine the diversity of non-fastidious Gram-negative bacteria recoverable from ready-to-eat street-vended foods and environmental samples obtained from close to the sale points of such foods. Such samples included sewage, sludge and soil and were obtained from within a maximum of 10 meters from the vending points. Samples were obtained from an informal settlement (Kibera). Notably, this study is the first attempt in Kenya that attempts to link microbial contaminates in ready-to-eat street foods from those recoverable from surrounding environmental sources through genetic fingerprinting using the $(GTG)_5$ primers. Although this technique is not as sensitive as those based on whole-genome sequencing and analysis, it gives a sneak preview of possible genetic diversity of bacterial population sets, predicting outbreaks and expansion of significant clones. We also determine antimicrobial resistance patterns and β-lactamases carriage among recovered isolates.

1.2 Statement of the problem

The World Health Organization estimates that more than half a million persons across the globe suffer Foodborne infections every year and 420,000 dies as a result. Human food may be contaminated at the farm, mostly when unsafe irrigation water is used, at various points in the supply chain, during processing or due to unhygienic handling (Alegbeleye, 2018;Machado-Moreira, 2019). Foodborne diseases cause massive economic losses with the World Bank estimating a total productivity loss of $95.2 billion and an additional $15 billion in treatment annually just in low and middle-income countries (Jaffee *et al*, 2018). The Africa continent has been the most affected by these Foodborne diseases due to inadequate urban sanitation infrastructures such as sewage drainage, insufficient clean water supply, poor hygiene, and environmental pollution, especially in crowded informal settlements.

Street foods are popular among many Nairobi residents, especially those residing in the informal sectors, however, the health risk of these foods mostly unknown or ignored by most vendors (Kariuki *et al.* 2017). Most of these foods are sold in open

spaces, sometimes next to running sewage and open sewers, mounds of garbage, and exposed to known and unknown infectious pathogens (Rane 2011;Zawack *et al.* 2016). Despite the solemn public health risk pose by this, very little research has been conducted to determine any possible environmental sources of bacterial pathogens that are recoverable from ready-to-eat foods. It is expected that foodborne infections are even higher in informal settlements such as Kibera that are usually characterized by deplorable sanitation infrastructure. To the best of my knowledge, the full scope of bacterial diversity that contaminates ready-to-eat street foods and those recoverable from immediate environmental compartments such as sewage, sludge and soil has not been documented in Kenya. Still, no study in Kenya has attempted to link microbial contaminates in ready-to-eat street foods from those recoverable from the immediate surrounding environment.

With the continued emergence and spread of antimicrobial resistance in bacterial pathogens, it is also essential to evaluate resistance profiles of strains from such environmental effluents and foods to establish possible hotspots for multiple-drug resistance bacterial strains (Smith & Fratamico, 2018). Bacterial strains resistant to multiple antimicrobial agents have become a significant health challenge in treating severe infections worldwide (Andersson *et al.* 2001). Of major concern are bacterial isolates that have co-resistance towards β-lactams, aminoglycosides and fluoroquinolones antimicrobial agents. These three classes of antimicrobial agents have a wide acceptance due to their availability, affordability and effectiveness against most Gram-negative bacteria strains. Therefore, there is a high risk of poor treatment outcomes in patients colonized by MDR-strains resistant to these antimicrobial agents.

1.3 Justification

Microbial food contamination is a significant health problem that has led to severe foodborne infections and chronic diarrhea in many parts of the world (Kirk *et al.* 2015). Foods prepared and served in an unhygienic environment are exposed to many predisposing risk factors that lead to microbial contamination. Of the major health concerns are street foods sold in many informal settlements such as Kibera.

This informal settlement is characterized by open burst sewer and uncollected garbage. Most of these street food vending points are free space and close to these open sewage and garbage sites. These foods are exposed to multiple food contamination vectors such as flies. It is, therefore, very likely that these foods are highly contaminated and pose a serious health risk to many consumers (Rane 2011;Zawack *et al.* 2016).

The gradual increase of food-borne infections has led to heavy antibiotic usage across the globe, leading to the emergence of multi-drug resistant (MDR) strains (Doyle 2015). Food-borne pathogens such as *Salmonella spp* are increasingly becoming resistant to advanced antimicrobial agents such as cephalosporins, aminoglycosides, and fluoroquinolones. These three classes of antimicrobial agents are widely used in Kenya to treat infections, especially in hospital settings. Therefore, co-resistance with β-lactams, aminoglycosides, and fluoroquinolones in strains (here-by referred to as the βFQA phenotype) reduces treatment options leading to possible treatment failure. Antimicrobial resistance, especially among Gram-negative bacteria, may be facilitated by exchanging resistance genes within and across species. This exchange is mediated mainly by horizontal transfer of genes borne on integrons that may in turn be borne on conjugative plasmids. Such plasmids may also harbor other genetic elements and their conjugative transfer ensures that the resistances encoded are transferred *en bloc* to the recipient strain. Although more than nine integron classes have been documented to date, type 1 and 2 have widely been reported in Gram-negative bacteria and heavily implicated in AMR (Gillings *et al*. 2008). Although these elements have been described from the hospital and animal isolates in Kenya, little is known about those from food-borne and environmental isolates *(Kiiru et al*. 2012;Langata *et al*. 2019;Pitout *et al.* 2008). Furthermore, data on food contamination and the diversity of bacteria species recoverable from street foods remain scarce in Kenya. Surveillance of food contamination and resistance profiles of microbial strains is therefore essential for food safety and contamination prevention.

The current study sought to determine the diversity of non-fastidious Gram-negative bacteria and their associated antimicrobial resistance patterns. We also analyzed the carriage of β-lactamases genes and possible clonal relatedness of isolates recovered from different ready-to-eat foods in a slum setting. This study's data strongly support the potential advantages of launching antimicrobials resistance surveillance that could help early detection of foodborne outbreaks and identify possible hotspots where multiple-drug resistance strains may arise and cause outbreaks that could in turn be difficult to treat.

1.4 Research questions

This study sought to address the following research questions.

1. What is the level of the microbial contamination of different food-types sold across Kibera informal settlements and are there particular hotspots associated with high levels of contaminations?
2. What are the possible factors associated with microbial food contamination?
3. What is the diversity of Gram-negative bacteria recoverable from environmental and food samples collected from an informal settlement?
4. What are the common antimicrobials resistance phenotypes among isolates recovered from environmental samples and food sold near contaminated sites?
5. What is the genetic basis of resistance towards β-lactams drugs, the largest antimicrobial class used to manage major infections in developing countries, among isolates recovered from food and environmental samples?
6. How are the clustering patterns between isolates from foods and the environment?

1.5 Research hypothesis

1.5.1 Null Hypothesis

1. There is no microbial contamination in the street sold, ready-to-eat foods sold in Kibera informal settlements.

2. Gram-negative bacteria from food and environmental sample in Kibera informal settlements are not diverse?
3. An immediate environmental where food is sold in Kibera do not contribute to microbial contamination
4. Bacterial isolates recoverable from the foods and environmental samples are not resistant to multiple classes of drugs.
5. Gram-negative isolates recoverable from food and environmental samples in Kibera do not carry β-lactamases?
6. There is no microbial cross-contamination of ready-to-eat street foods sold near sewage and sludge refuse in Kibera.

1.6 Research objectives

1.6.1 General Objective

Mapping the Distribution Patterns of Multiple-Drug Resistances Gram-negative bacterial strains recoverable from Food and Environmental Samples in Kibera informal settlements

1.6.2 Specific Objectives

1. To determine microbial contamination levels of ready-to-eat food samples across Kibera informal settlements
2. To establish possible environmental risk factors leading to microbial contamination of streets sold foods at Kibera informal settlements.
3. To determine the diversity of Gram-negative bacteria isolates recoverable from ready-to-eat foods and environmental samples consisting of sewage, sludge and soil collected in Kibera informal settlements
4. To determine relative antimicrobial resistance abundance is microbial isolates from foods and environmental samples collected from Kibera
5. To assess carriage of β-lactamases in food and environmental bacterial isolates from Kibera
6. To assess the clustering patterns among members of each species isolated from different locations within Kibera informal settlements to infer possible genetic analysis

CHAPTER TWO

LITERATURE REVIEW

2.1 Food Contamination and Challenges in Proper Sanitation

Food contamination is a term used to refer to foods that have been spoiled or colonized by microorganisms or toxic substances, therefore, making it unfit for human consumption *(*Parfitt *et al*., 2010). The World Health Organization (WHO) stated that food contamination is a global challenge that needs to be urgently addressed (Hald *et al.* 2016). Foodborne diseases are a common challenge across the globe and have led to devastating outbreaks in many parts of the world and are therefore perceived as a major public health problem (Quaranta *et al.* 2016). Food can be contaminated in the supply chain due to inadequate cooking or unhygienic handling (Tan *et al*. 2013). Vegetables such as kales are among the most contaminated foods and this has mostly been attributed to urban irrigation using fecal-contaminated water (Mritunjay and Kumar 2017).

Kibera informal, just like many others in Kenya lacks proper sanitation infrastructure. Such factors lead to deteriorated hygiene and poor living conditions (Darkey and Kariuki 2013). Many houses in this informal settlement do not have toilet facilities and therefore many residents rely on few communal facilities built by Nairobi county and other non-governmental organizations. Accessing these facilities often is problematic since most are inconveniently located for the majority of people. Toilet excreta that leak in open sewers is also a common feature in many parts of this slum.

Water shortage is also a common problem for many residents of Kibera informal settlement. Only a few homesteads have access to clean water formally supplies by the Nairobi County water line (Mutisya and Yarime 2011). As a result, most residents have to purchase their water from various sources, such as commercial water points. Access to clean water from sellers is also a challenge due to the distance of watering points and high cost). Previous studies determined that the cost of accessing water for these residents is nearly seven times more than residents living

in the formal settlements (Mutisya & Yarime 2011).Chronic water shortage has, in turn, led to a series of poor hygiene practices among foods vendors leading which raised the chances of food contamination.

The Kibera informal settlement is also heavily contaminated with animal and human feces, among other domestic wastes (Akullian *et al.* 2015). These conditions have been attributed to the lack of a proper drainage system, leading to open sewers (Mutisya & Yarime 2011). The open sewers pose a serious health risk and are a possible source of MDR bacterial strains, especially during the rainy season when the open sewer is overflown. High levels of poverty and poor hygiene have been attributed to illness and disease in Kibera informal settlements (Breiman *et al.* 2011). Most of Kibera residents can hardly afford better health services and most opt for self-medication, which poses a risk of developing MDR-bacteria strains.

2.2 Bacteriological Food Safety of Street Food

Several studies have been done to determine microbial contamination of street foods in many parts of the world. In the East African region, one such cross-sectional study has been conducted in Bahir Dar town in Ethiopia to determine bacteriological food safety and susceptibility of bacteria isolated from street sold white lupin (Kibret and Tadesse 2013). In this study, out of the 40 samples analyzed, a total of 29 (72.5%), *E. coli* 23 (57.5%), *Salmonella spp* and 8 (20%), *Shigella spp* were isolated. Most of the isolates exhibited resistance to tetracycline, co-trimoxazole (sulfamethoxazole-trimethoprim combinations) and erythromycin. Multi-drug resistance was 72.2%. Multi-drug resistance was defined as resistance to at least three classes of antimicrobials. Therefore, this study clearly shows that microbial contamination of foods can be diverse and based on the prevailing AMR profiles, it is also possible that some of the contaminating strains can potentially cause difficult-to-treat infections.

A similar study done in the Gondar region of Ethiopia reported a microbial contamination prevalence of 5.5% in food from animal origin (Ejo *et al*. 2016). In this study conducted in Ethiopia, 12% prevalence was reported for *Salmonella spp* in

raw meats. A single multidrug resistance strain resistant to Amoxicillin and Tetracycline was detected from raw milk. Four multidrug resistance strains with varied resistance profiles to Amoxicillin, Gentamicin, Nitrofurantoin, Nalidixic acid, Sulfamethoxazole and Tetracycline were detected from raw eggs sample. A total of three MDR strains were also detected from raw meat, exhibiting varied resistance to Amoxicillin, Ampicillin, Gentamicin, Nitrofurantoin, Sulfamethoxazole and Tetracycline. Resistance to nearly all drugs used in veterinary medicine and human medicine was detected in the tested *Salmonella ssp.* Therefore, these studies clearly show microbial contamination of foods can be diverse with some resistant strains that may cause it hard to treat infections.

Microbial contamination in ready-to-eat meat foods has been attributed to food safety non-compliance by the food handlers (Bagumire *et al.* 2017). In a study done by Bagumire *et al.*(2017) in Uganda; ready to eat beef and chicken samples collected in highway markets found coliform contamination levels exceeding the recommended thresholds in 68% of analyzed foods. Another study in Windhoek, Namibia, reported a prevalence of 83% Gram-negative bacterial contamination in 96 ready to eat meat samples collected from street vendors (Shiningeni *et al.* 2019). However, 26% of these microbial contaminated foods exceeded food safety levels and, therefore, unfit for human consumption.

The use of polluted water for irrigation has been considered one of the significant causes of vegetable contamination (Ijabadeniyi *et al.* 2011). Inadequate cooking temperatures and long holding time accompanied by improper storage have also been implicated in the microbial contamination of vegetable food (Alimi, 2016). Microbial contamination of cereal foods, meats, and ready-to-eat vegetables has also been linked to the vending points' immediate environmental factors. Most street foods in the informal settlements are sold in open spaces are exposed to insects, rodents, dust, heaps of garbage and open sewage (Alimi, 2016).

2.3 Hygiene and Sanitary Practices of Street Food Vendors in Nairobi

Hygiene and sanitary practices of vendors of street foods in Dandora and Kayole in Nairobi, Kenya, has been accessed and documented in a descriptive study (Muinde, *et al*, 2005). In this study by Muinde, et al. (2005), 80 food vendors were recruited and environmental conditions, utensils washing and food preservation methods were evaluated. From observations made in this study, 85% of vendors prepared and sold food in an open unhygienic environment next to uncollected garbage. A vast majority of vendors making up to 95.5% of these vendors, lacked proper waste disposal facilities and disposed of their rubbish next to vending points, making the surrounding environment dirty and unhygienic. In this study by Muinde *et al.,* 2005, houseflies (*Musca domestica*), were common among most food vending points. This study observed that due to chronic water shortage, most vendors did not wash fresh foods adequately and that the surfaces used for the preparation of foods were not frequently cleaned. Different food types were prepared over the same surface, which could promote cross-contamination of food products. The storage and display of ready-to-eat foods were mostly in open basins and were therefore prone to contamination and spoilage. Most of these foods were not covered hence leaving them exposed to flies and dust. The study observed that most foods were not adequately cooked and ready to eat raw vegetables such as fruit salads were inappropriately stored. However, this study did not determine the level of microbial contaminations and the associated AMR prevalence.

Muinde, *et al* (2005) also observed personal hygiene among the vendors in the study 2005. All the vendors evaluated handled money as they sold food items, a practice that has a high potential to contaminate foods. About 60% of the vendors handled food with bare hands without first washing the hands therefore raising the possibility of food contamination. From the interviews, 32.1% said they consume the foods' leftovers while the remaining said they keep the food to sell the next day.

A cross-sectional study conducted in Nairobi county documented various aspects of microbial quality and safety of raw chicken meats (Odwar *et al.* 2014b). This study sought to determine coliform contamination levels from raw chicken and the

microbial resistance patterns of obtained isolates. Samples were bought from chicken outlets across Nairobi County in both formal and informal settlements based on residential segregation.

The coliform contamination rate was 97% upon analysis of meat and skin samples using the 3M Petri-film coliform count. Microbial contamination by *E. coli* and coliforms was found to be above 100 CFU/mL, which is above the acceptable range set by the Hazard Analysis and Critical Control Point system. From a total of 200 samples, only 80 were within the allowable range. The study also found that from 156 *E. coli* isolates, 42.9% of the isolates were resistant to more than three antimicrobials. However, there was no significant difference in antimicrobial resistance prevalence between the sampled outlets. This study, therefore, shows that the contamination level of food is high and raises a concern regarding the increasing antimicrobial resistance strains in Human food.

However, Gram-negative bacterial isolates' diversity recoverable from ready-to-eat street foods has not yet been studied in Kenya. Therefore, the link between microbial food contamination and the immediate environment remains to be established. This is particularly important because sanitation standards of food preparation area significantly affect food quality and, consequently, consumers' health outcomes.

2.4 Hygiene Standards of Kibera Slums

Kibera slum is situated in Nairobi county's western region and hosts one of the largest low social economic and slum populations in the continent (Ayah *et al.* 2013). This informal settlement lacks essential services such as proper water drainage systems, leading to frequent cases of open or burst sewers near homesteads and food-vending points. There is also a lack of an organized garbage collection system and this potentially results in the accumulation of uncollected garbage that pollutes the environment. This has led to unhealthy and hazardous living conditions for many residents in this informal settlement. This garbage also provides a source of food and shelter for rodents and insects that in turn serve as vectors of foodborne pathogens (Sharma and Mazumdar 2014). Occasionally, garbage from heaps is washed away

during rainy seasons into water channels and rivers. Lastly, access to the few public toilets that are inconveniently located for most residents and mostly unclean is a severe problem in these types of settings (Worrell *et al.* 2016).

This sewage in Kibera informal settlements is often contaminated by domestic waste and uncollected garbage, a potential source of microbial pathogens that may lead to disease outbreaks in this informal settlement (Mutisya, 2011). Infections and outbreaks caused by pathogens resistant to multiple antimicrobial agents may further complicate the treatment process. Of major concern are the bacterial strains that have co-resistance to readily available and affordable antimicrobial agents that includes the β-lactam class, Fluoroquinolones, quinolones and aminoglycosides, denoted as the BFQA-strains. However, organisms producing the extended-spectrum β-lactamases (ESBL) are more prevalent since these resistance enzymes are readily acquired through horizontal transfer between and across bacterial species (Bhattacharjee *et al.* 2010).

2.5 Extended-spectrum β-lactamases

β- lactam antibiotics are among the most used and successful drugs in treating bacterial infections for a long time (Coleman 2011). These antimicrobial agents are widely accepted in medical settings due to their excellent safety profiles, broad antimicrobial potency, availability of orally bioavailable formulations, and low cost (Hughes *et al.* 2013). However, their effectiveness in treating bacterial infections is on the decline due to the emergence and spreading of resistant strains that have led to their ineffectiveness against many bacterial infections (Allen *et al.,* 2010). This widespread use in clinical settings has led to persistent exposure of bacterial strains β-lactam antibiotics. The consequence of this constant exposure has led to induced resistance and mutation of β-lactamases in susceptible bacteria. This effect of antimicrobial resistance has even developed in newer β-lactam antibiotics (Tang *et al.* 2014).

Resistance to β-lactams has mostly been attributed to the production and spread of the extended-spectrum β-lactamases (ESBLS) enzymes. These enzymes are

characterized by the hydrolysis capability of penicillins, first-, second-, and third-generation cephalosporins, and aztreonam but not the cephamycins (Fernandes *et al.* 2013). The ESBLs enzymes are plasmid-borne rather than chromosomal-associated (Schultsz and Geerlings 2012). This, therefore, means that these enzymes can easily spread from resistant bacteria to susceptible ones leading to the emergence of resistant strains. Among the various variants of ESBLs, bla_{TEM}, $bla_{\text{CTX-M}}$ types are of immense clinical and epidemiologic importance. This is because these enzymes can spread fast than the other ESBL variants and are associated with mobile sequences such as IS*Ecp*1 (Shaikh., *et al*, 2015). The $bla_{\text{CXT-M}}$ variant has been the most abundant ESBL variant in multiple species (Shaikh., *et al,* 2015; Jouini A., *et al,* 2010)

Gram-negative microbial, especially those who belong to the family Enterobacteriaceae, have been mainly associated with difficult to treat infections due to the widespread and acquisition of antimicrobial resistance (Lynch III *et al,* 2013). The mechanisms of resistance in these organisms have been mainly via production and spread of ESBL enzymes harbored in integrons borne in mobile plasmids (Kiiru *et al.* 2013). These enzymes are rapidly evolving with increasing ability to hydrolyze third-generation cephalosporins and aztreonam (Rawat *et al.*, 2010). The ESBL enzymes, combined with other resistance mechanisms, have led to a strong indication of microbial recovery and the ability to adapt to their environment.

Foods such as Chicken meat among food types have been proposed to be a source of ESBLs that eventually colonize and infect humans (Compos *et al,* 2014). However, to the best of our knowledge, no research has so far attempted to link bacterial strains recoverable sewage and sludge systems to microbial contamination of street foods. However, several studies have been done in various settings worldwide to check the prevalence and occurrence of ESBLs from food. A survey conducted in Zigzag, Egypt, documented the distribution of ESBL-producing species among the various members of the family Enterobacteriaceae from chicken meat from different retail points (Abdallah *et al.,* 2015). From 106 isolates obtained, 69 (65.09%) were ESBL producers. The distribution of ESBL-producing species among the various members

of the family Enterobacteriaceae was found to be 44 (63.77%) for *Klebsiella pneumonia and* 10(14.49%) for *E. coli*, 13(18.84%). The bla_{TEM} variant was detected in 61 (57.55%) of the isolates, while 49 (46.23%) of the isolates contained CTX-M genes. The occurrence of bla_{SHV} was found to be 25 (23.58%). Therefore, this research's findings raise serious concerns on food safety and a reservoir for antimicrobial-resistant bacterial strains.

Studies on antimicrobial-resistant Gram-negative strains in clinical settings in Kenya have also been conducted. A study conducted by Kiiru *et al.* (2012) determined β-lactamase phenotypes and carriage of *bla* genes in *E. coli* during the past 18yrs (1992- 2010). Blood, stool and urine samples were obtained from hospitalized and non-hospitalized patients seeking treatment in Kenyan hospitals (Kiiru et al. 2012). Narrow spectrum β-lactamases were the dominant phenotype (30%) of the 912, 8% IRT, 27% ESBL, 4% CMT and 10% ampC-like. Extended Spectrum β-lactamase (ESBL) phenotype were detected in 247 (27%) isolates which carried $bla_{CTX\text{-}M\text{-}14}$ (29%), $bla_{CTX\text{-}M\text{-}15}$ (24%), $bla_{CTX\text{-}M\text{-}9}$ (2%), $bla_{CTX\text{-}M\text{-}8}$ (4%), $bla_{CTX\text{-}M\text{-}3}$ (11%), $bla_{CTX\text{-}M\text{-}1}$ (6%), $bla_{SHV\text{-}5}$ (3%), $bla_{SHV\text{-}12}$ (5%), and $bla_{TEM\text{-}52}$ (16%). However, the occurrence of these genes among isolates from environmental sources is still unknown.

Extended-spectrum β-lactamases are classified into several groups according to their amino acid sequence homology. The four most prevalent types in Gram-negative bacteria have been discussed hereunder;

2.5.1 Tem- β-Lactamases

The bla_{TEM} enzymes are derived from narrow-spectrum β-lactamases $bla_{TEM\text{-}1}$ and $bla_{TEM\text{-}2}$ and are mainly borne in the chromosome (Bush *et al.* 2010). However, these can also be plasmid-borne. TEM enzymes are the most prevalent ESBL type identified in Gram-negative bacteria (Bush, 2010). Over 219 TEM types have been documented so far; however, not all are ESBL phenotype (http://www.lahey.org/studies/temtable.asp). The $bla_{TEM\text{-}1}$ can hydrolyze the β-lactam

ring of penicillins and the first generation Cephalosporins and is incapable of hydrolyzing advanced Cephalosporin.

2.5.2 SHV- β-Lactamases

The SHV β-lactamases result from the SVH-1 enzyme mutation resulting in hydrolysis capabilities of cefotaxime and ceftazidime to some extent (Liakopoulos *et al.* .2016). These enzymes mostly spread from resistant organisms to another through IS26 mobilization. SHV-enzymes are the second most ESBL variants found in Gram-negative bacteria.

More than 185 variants of SHV enzymes have been identified and most of which possess extended-spectrum activity against the newer broad-spectrum Cephalosporins (http://www.lahey.org/studies). SHV enzymes are classified in the molecular class A of β-lactamases and confer resistance to broad-spectrum penicillins such as Ampicillin and ticarcillin.

Extended-spectrum SHV β-lactamases confer a wide spectrum of resistance to β-lactams that includes new generation cephalosporins and monobactams. The SHV type resistance gene is normally encoded by multiple resistant plasmids that are self-transmissible and highly mobile.

2.5.3 CTX-M β-lactamases

More than 100 $bla_{\text{CTX-M}}$ have been sequenced and documented (Bonnet, 2004). The $bla_{\text{CTX-M}}$ type enzymes are encoded by transferable plasmids and are acquired by the horizontal gene transfer from one bacteria to another through conjugative plasmid or transposon (Nhu, 2010). These enzymes are capable of hydrolyzing ceftriaxone, cefotaxime, and aztreonam. However, these enzymes preferentially hydrolyze cefotaxime. The β-lactamase inhibitor tazobactam better inhibits the blaCTX-M than by sulbactam and clavulanate (Shakil *at al*., 2010).

The CTX-M β-lactamases can be divide into five groups based on their amino acid sequences (CTX-M group 1, 2, 8, 9, and 25) (Bonnet, 2004 96 /id).

2.5.4 OXA β-lactamases

The OXA β-lactamases are characterized by hydrolysis rates of cloxacillin and oxacillin greater than 50% (Bush, 2010). The OXA β-lactamases are the less prevalent ESBL type. These enzymes are plasmid-mediated and are capable of hydrolyzing amoxicillin-clavulanic acid, oxacillin and anti-staphylococcal penicillins (Walker *et al.* 2015). These enzymes are more prevalent in *E. coli* and *K. pneumoniae* among Gram-negative bacteria. These two bacterial species are a good indicator of fecal contamination of food and resistance to antimicrobials.

2.6 Bacterial Resistance towards Antimicrobial Agents

Aminoglycosides and fluoroquinolones are broad-spectrum antibiotics and are highly potent for treating life-threatening infections (Piekarska *et al.* 2016). However, the widespread use of these antibiotics has led to a huge resistance reaching up to 50% among Gram negative bacterial species, especially among *Salmonella* and *Klebsiella* species (Strahilevitz, *et al.*, 2007). The problem of aminoglycoside and fluoroquinolone resistance and dissemination heightened with the discovery of plasmid-mediated quinolone resistance (PMQR) genes which can be easily spread across bacterial species (Schultsz and Geerlings 2012). The low level of resistance to fluoroquinolones and aminoglycosides has been attributed to aac(*6′*)-Ib-cr gene, a variant of aminoglycoside acetyltransferase. This aac(*6′*)-Ib-cr gene harbors two base-pair substitutions which have been attributed to acetylate capabilities of ciprofloxacin and norfloxacin hence reducing their effectiveness (Gonsalves 2011). The resistance level varies in various microorganisms and amongst individual strains depending on the amount of enzyme produced, its catalytic efficiency, and the type of antimicrobial. This plasmid-mediated gene is embedded within a gene cassette within an integron. This, therefore, suggests that *aac(6′)*-Ib-cr resistance gene is mobile and is widely distributed among plasmids. The aac(*6′*)-Ib-cr gene variant has been reported as the first enzyme conferring resistance to two structurally different antibiotic families such as aminoglycosides, β-lactams and fluoroquinolones (Ramirez *et al.* 2013). There is therefore a high possibility that the majority of the βFQA-bacterial strains carry this resistance gene.

Fluoroquinolones which are the often preferred treatment option for diarrhea and other gastrointestinal infections are resisted due to point mutations in the quinolone-resistance-determining region; *gryA*, and *parC* gene (Kawai *et al.* 2015). Fluoroquinolones such as ciprofloxacin and norfloxacin may also be resistance through the efflux pump mechanism mediated by the QepA gene. Resistance to fluoroquinolones also involves mutations in target genes (gyrA, gyrB, parC, and parE), aminoglycoside modifying enzymes, or to drug efflux systems (MexAB-OprM, MexCD-OprJ, MexAB-OprM, MexXY-OprM, OqxAB and Qep). Resistance to aminoglycosides, for example, gentamicin and amikacin, involves the MexXY-

OprM efflux pump as well as the aminoglycoside modifying enzymes (AMEs) (Aghazadeh *et al.* 2014). Resistance to aminoglycosides has also developed from chromosomal mutations from ribosomal RNA methylases (RMT), leading to alteration of ribosomal binding sites. Various plasmids mediated RMT have been detected from the Gram-negative bacteria, which include ArmA, RmtA, RmtB, RmtC, RmtD and NpmA. The plasmid-mediated aac(6')-Ib-cr gene has been detected in Garm-negative bacteria though at a low level. Antimicrobial agents such as chloramphenicol are resisted through the hydrolysis action of chloramphenicol-acetyl-transferases (Kapoor *et al*.2017).

Carbapenems such as Imipenem are resisted in Gram-negative bacteria through combined mechanisms such as target inaccessibility, stable derepression of AmpC β-lactamase, overexpression of efflux systems and production of Metallo β-lactamases (MBLs). In Kenya, various studies have documented carriage of bla_{VIM} and bla_{NDM} carbapenemases in Gram-negative bacteria such as *P.aeruginos*a isolates from clinical isolates consisting of blood, urine, tracheal aspirates and pus samples (Kilivwa *et al.*2018; Pitout *et al*. 2008).

2.7 The GTG_5 Fingerprinting Technique

Prokaryotic organisms have conserved inter spread repetitive tendons in their genome. The GTG_5 is a method of repetitive extragenic palindromic PCR that

amplifies the GTG_5 repetitive segments present in the bacterial genome (Kathleen *et al.* 2014). This molecular tool is useful in the differentiation of bacterial strains of the same species. The 5'-GTGTGTGGTGGTGGTG-3' is used as the oligonucleotide primer that binds these repetitive segments in the polymerase chain reaction. This binding of the oligonucleotide primer enables the amplification of DNA fragments of varied sizes detected as multiple bands on a gel electrophoresis image. Bionumeric software such as gelcompar is used to normalize formed bands against the molecular ladder and identify and analyze the banding patterns (Dombek, 2000). Similarity coefficient of the normalized bands using the person correlation or Jaccard methods and eventually generating a dendrogram for the analyzed prokaryotic isolates. Closely related bacteria tend to cluster together with a high similarity matrix of more than 80%.

2.8 Integrons

Integron is gene capture system in the form of resistance gene cassettes. Cassettes carried by integron usually encode multiple resistance mechanisms such as resistance to β-lactams (bla_{TEM}, $bla_{\text{CTX-M}}$, bla_{SHV}, bla_{OXA}), Aminoglycosides (*aacA4*allele) and other antimicrobial agents (Jeong *et al.* 2009). Integrons are linked to plasmids and transposons; therefore, they can move between and across bacterial species. Integron has three essential core elements: The *intI* gene, which encodes an integrase (*IntI*) required for site-specific recombination; *attI*, which is recognized by integrase; and integron, which is associated promoter (Pc) and is needed for transcription and expression of gene cassettes within the integron (Gillings *et al.* 2014). Gene cassettes are genetic elements that encode antibiotic resistance genes and consist of a specific-site recombination recognized by integrase called *attC* [or 59-base elements. The class 1 and 2 integron remains the most common integron found in Gram-negative bacteria such as *Enterobacter* spp, *K. pneumoniae, E. coli* and *Proteus* spp, *P. aeruginosa* and *Acinetobacter* baumannii (Deng, 2015).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

This research study was conducted in Kibera, an informal settlement located on the South West side of Nairobi city. This informal settlement is approximately 2.38 km^2, with a population of close to a million residents. Kibera is the largest informal settlement in Kenya, with a vast majority of people living below the poverty line (Desgroppes and Taupin 2011). This informal settlement has poor sanitation characterized by poor sewage drainage, uncollected garbage in dump sites and chronic water shortages (Worrell *et al.* 2016). Most foods sold in this slum are prepared and served close to open sewers, potentially putting consumers at risk of foodborne infections.

3.2 Study Design

A cross-sectional study design was used in this research study was used to collect ready-to-eat foods and environmental samples that consisted of soil, sewage and sludge from the various parts of Kibera informal settlement. The Microreact® software was used to generate a Kibera settlement map indicating ready-to-eat foods sampling points

3.3 Sample size

Since the prevalence of multiple-drug resistance Gram-negative from street food and the environment has not been previously documented in the region, a prevalence of 50% recommended based on Fisher's *et al.* (2005) formula was adopted. Assuming a standard error [Z] from the mean of 1.96 and an absolute precision [D] of 5%, the sample size [N] was calculated as follows using the fisher's sample size calculation method;

$$N = \frac{Z^2 x P[1-P]}{d^2} = \frac{1.96^2 x\ 0.5\ x\ 0.5}{0.0025}\ \ 384\ minimum\ samples$$

Purposeful sampling was used to collect a minimum of 400 samples, slightly higher than the recommended 384 samples to collect at least 200 environmental and food samples, respectively.

3.4 Recruitment of Target Population

This study-targeted individual who sell ready-to-eat foods in the streets of Kibera informal settlements. Ethical clearance before the study commence was obtained from the scientific ethical review unit, Kenya medical research institute (SERU, KEMRI), approval number KEMRI/SERU/CMR/P00055/3514 **(Appendix 4).** The engagement process involved a brief on purpose and potential outcomes of this research to seek vendors' consent to allow the researcher to collect ready to eat foods samples they sell.

3.4.1 Inclusion Criteria

1. Only ready-to-consume street foods that consisted of kales, cabbage, managu, meat, omena, rice, ndegu, beans, githeri and ugali were collected in this study
2. Environmental samples consisting of sewage, sludge and soil were only collected with 10 meters of vending points sampled.

3.4.2 Exclusion Criteria

1. Vending points whose vendors did not consent to be included were excluded from the study.

3.5 Sampling criteria

This study segmented Kibera informal settlement into four large blocks based on its geography and purposeful sampling of up to 100 sampling points (Figure 3.1) to obtain a minimum of 200 food and environmental samples. A convenient sampling strategy of ready-to-eat foods was applied and selecting sampling points approximately 50 meters apart. Specimen sampling was distributed across all villages of these informal settlements to ensure proper representation. At each of the

sampling sites, all ready-to-eat food types available were collected with no exceptions.

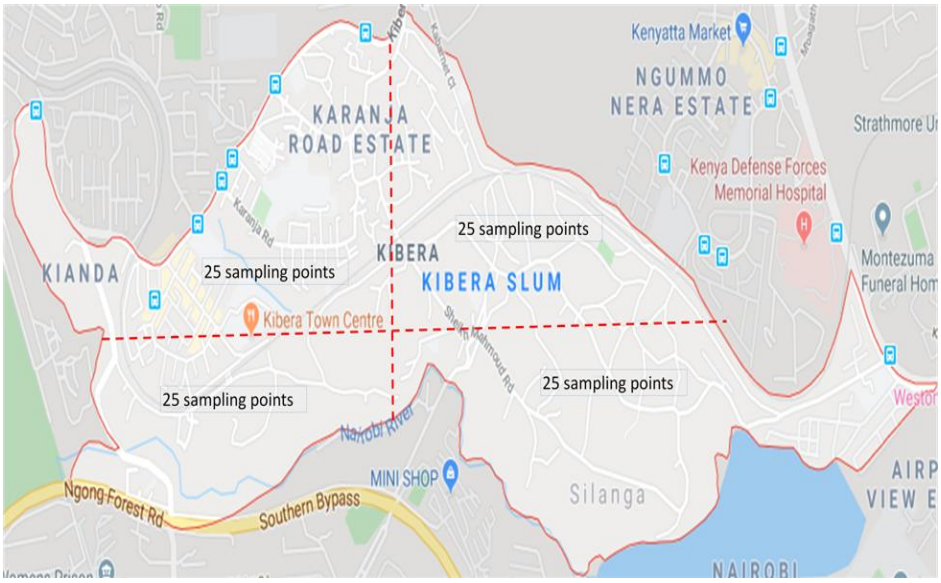


Figure 3.1: A map of Kibera showing the sampling blocks

Key: The entire region of Kibera informal settlements was split into four major sampling blocks for ease of sampling and ensured diversity. In each of the four sampling blocks, approximately 25 food vending points not less than 50 meters apart were sampled.

The study's ready-to-eat foods and environmental samples included soil, sludge and sewage near the food vending points. The GPS coordinates of sampled food vending points were taken to generate a map showing the various MDR occurrence hotspots, Figure 3.2. Details on vending point proximity to open sewage, dumpsites, toilet facility, water supply, and layout features were captured on Epi-info software and a sample collection form. Environmental samples consisting of soil, sewage and sludge samples where available were collected within 10 meters radius of the sampled food vending point.

3.6 Sample Collection

Ready-to-eat-foods were mixed by Stirring before sample collection to ensure homogeneity. These ready to eat foods consisted of beans, githeri, ugali, rice, kales,

ndengu, omena, meat, cabbage and managu. A sterile spoon was used to collect approximately 25 grams of samples that were then transferred in a zip lock bag while 25mL Semi-solid foods were put in a sterile universal bottle. Labeling on the sample container indicating the food sample type, unique code for the particular vending point and village of sampling was also ensured.

A sewage sample was collecting by holding a universal bottle at the base and plunging it below the surface. The bottle was then turned slowly upward to collect an approximately 25ml sample. Scooping with a sterile spoon, collected sludge and soil samples about 25g of sample and transferred to a screw-cap container. All samples collected were transported to the laboratory in a cool box within 6 hours to the molecular biology laboratory at the Center for Microbiology Research, KEMRI.

3.7 Laboratory Processing Collected Samples

a) Assessment of Food Microbial Contamination

Microbial food contamination level was assessed using the Enterobacteriaceae specific 3M Petri film commercial plates. Approximately 1g of beans, githeri, ugali, rice, kales, ndengu, omena, meat, cabbage and managu was put in a stomacher bag and 9 ml of normal saline added to dilute the sample. The sample was then homogenized for 1 min using a stomacher machine. An aliquot of a 1ml-homogenized sample was inoculated on a 3M Petri-film plate and incubation did aerobically for 24 - 48hrs. After 24 hours of incubation, colonies that grew on 3M plates with a typical butterfly appearance characteristic of Enterobaaacteriaceae were enumerated. **Figure 3.1**, a spot map, shows the various Kibera slums' locations where various food samples were collected. Calculation of contamination levels was done using the following formulae;

CFU/mL = colonies counted x 0.1ml of 10^{-6} dilution in 1mL of the original sample

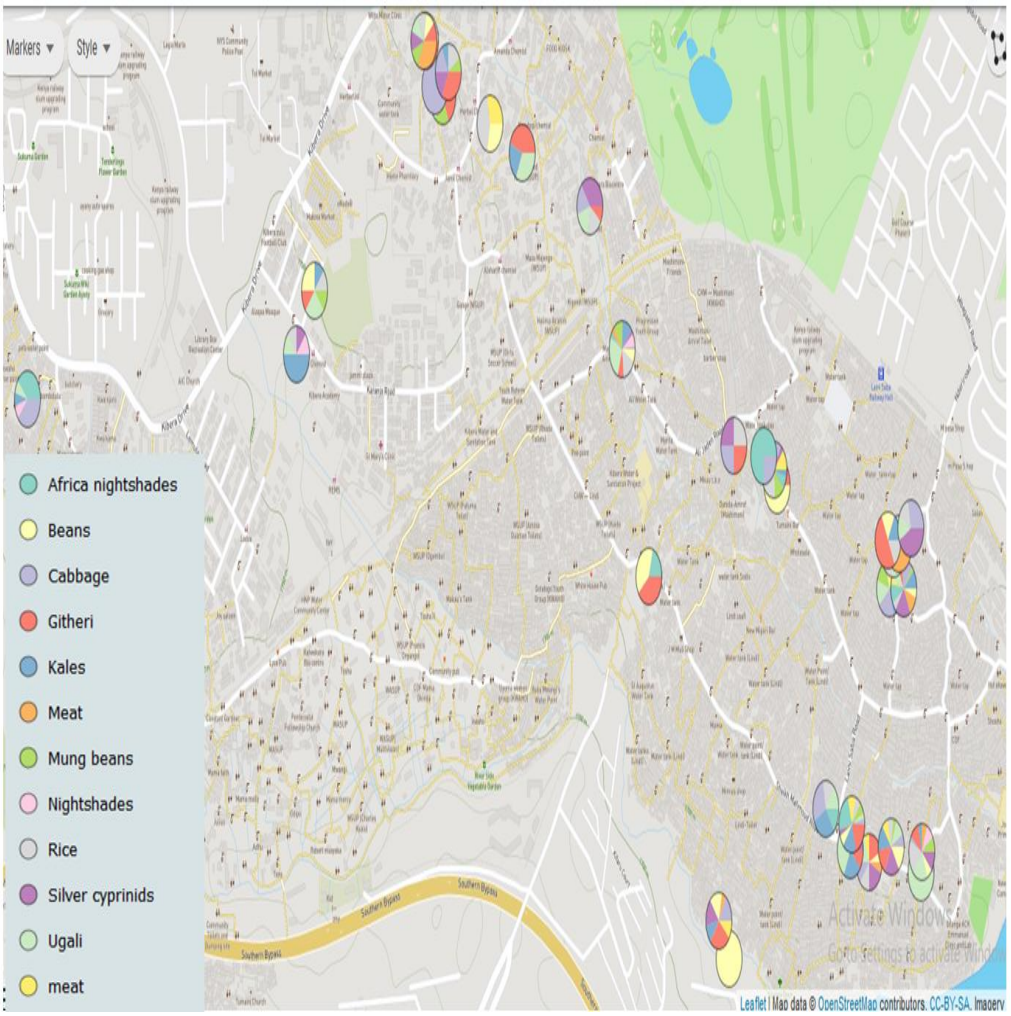


Figure 3. 2: Distribution of ready-to-eat food and environmental sampling points in Kibera

Key: The map shows sampling points of ready-to-eat foods collected across Kibera informal settlements. The sampled sites were distributed across different villages in the slum and notably, all were along access roads. The map shows that most of these vending points are concentrated at populated sections of the informal settlements where consumer availability is high. This in turn constrains available resources leading to frequent water shortages and accumulation of garbage near vending points (within 10 meters radius). These vending points are also in very close proximity to open sewage

b) Enrichment and Bacterial Isolation From Food and Environmental Samples

Approximately 1g of each food and the environmental sample was added in 9ml of buffered peptone water and shaken (in Grant Incubator Shaker) for 1 hour to resuscitate any injured cells. Plating was then directly done onto MacConkey and Eosin Methylene Blue Agar (Oxoid, UK) and incubation done at 37°C for 24hrs. Non-duplicates isolates of each colony type were then subjected to Gram stain and biochemical tests for species identification, **Appendix 2 and 3**.

3.8 Identification of Bacteria Isolates

Pure isolates were subjected to Gram stain and a series of biochemical tests such that includes TSI, Urease, SIM and Simmon's citrate test for organism identification;

3.8.1 Gram Stain

A Gram stain reaction was used in this study to identify Gram-negative bacterial cells (Mahasneh and Bdour 2006). A loop full of the test organism was emulsified in normal saline on a glass slide using a sterile wire loop. The smear was fixed by passing the slide over a flame 3-4 times and cooling in air. Primary stain crystal violet capable of being retained by Gram-positive bacteria was flooded on the slide for 1 minute and then gently washed off with running water. The slide was then flooded with logos iodine which acts as mordant for 1 minute and then washed off gently with running water. Decolonization was done using 95% ethanol for 30 seconds and then washed off with running water. Lastly, a counterstain safranin capable of staining Gram-negative cells pink was flooded on the slide and then washed off after a minute. The cells were then observed on a microscope using a x100 magnifying lens (oil emulsion).

3.8.2 Sulfur Indole Motility Media (SIM)

This is a differential medium that tests an organism's ability to reduce sulfur and produce indole and motility through the agar (Lehman 2014). Sulfur is reduced to

H_2S through catabolism of the amino acid cysteine by the enzyme cysteine desulfurase or by reducing thiosulfate in anaerobic respiration. Bacteria that have the enzyme tryptophanase are capable of converting the amino acid tryptophan to indole. Indole reacts with added Kovac's reagent to form rosindole dye, which is red.

3.8.3 Triple Sugar Iron Test (TSI)

Triple sugar iron differential medium was used to test organisms' ability to ferment glucose and lactose to produce acid and gas. This medium allowed the identification of sulfur reducers such as *Salmonella* and *Proteus* species, which was indicated as a black precipitate in the batt of the TSI medium. The TSI media was also used to separate lactose fermenting members of the family Enterobacteriaceae such as *E. coli, Enterobacter* and *Klebsiella* species from other members that do not ferment lactose, like *Shigella dysenteriae*. Organisms capable of fermenting lactose sugar within 18-24 hours of incubation produced acidic byproducts that consequently turned phenol red indicator in the medium to yellow. Organisms that cannot use lactose as a source of energy use the amino acids in the media. The deamination of these amino acids created ammonium, which turned the medium to alkaline. The alkaline pH causes the phenol red indicator to turn red (Lehman 2014).

3.8.4 Simmon's Citrate Agar

This medium was used to the test organisms' ability to use citrate as a sole carbon source (Lehman 2014). Organisms capable of utilizing citrate as a carbon source hydrolyzed citrate enzyme into oxaloacetic acid and acetic acid. The oxaloacetic acid was then hydrolyzed into pyruvic acid and CO_2. CO_2 produced reacted with components of the medium to produce an alkaline compound. The alkaline then turned the pH indicator bromthymol blue from green to blue, which indicates a positive test.

3.8.5 Urease Test

Urease differential medium was used to test the ability of test organisms to produce urease. The urease produced hydrolyzes urea to ammonia and carbon dioxide. Urease

medium contains pH buffers, urea and minute nutrients and phenol red, which is a pH indicator. In an acid environment, phenol red changes to yellow and fuchsia in the alkaline environment and the medium turns red (Hemraj *et al*. 2013).

3.9 Antimicrobial Susceptibility Testing

Susceptibility to commonly used antimicrobials agents against Gram-negative bacteria was determined using the disc on agar diffusion method using 13 antimicrobial agents as described in the Clinical Laboratory Standards Institute (CLSI 2017), **Table 1**. Isolates resistant to Ampicillin and any or all of the 3rd generation cephalosporin were presumed as producers of extended-spectrum β-lactamases. Isolates that had combined resistances to β-lactams, fluoroquinolones (ciprofloxacin), and Quinolones such as nalidixic acid and Aminoglycosides such as gentamicin were denoted as βFQA-strains. MDR-strains are strains that were resistant to ≥3 antimicrobial classes. Emulsifying pure bacterial colonies made a 0.5 turbidity McFarland standard in 3mL sterile normal saline. A sterile cotton swab was immersed in the O.5 McFarland standard and spread on Mueller Hinton agar plate to ensure a confluent growth. Antimicrobial discs were placed using a disc dispenser and then incubation was done at 35°C for 12 – 18 hours. The zone of inhibition was measured in millimeters across the diameter. *Escherichia coli* ATCC 25922 was used as a quality control of antimicrobial discs potency and media quality. The interpretation of inhibition zones was made using the CLSI standards (CLSI, 2017).

Table 3. 1: Antimicrobial agents tested against Gram-negative isolates in this study

Antimicrobial agents	Acronym	Disc potency	Manufacturer
Penicillin			
Ampicillin	AMP	10µg	Oxoid™
3rd Generaion cephalosporin			
Ceftazidime	CAZ	30µg	Oxoid™
Ceftriaxone	CRO	30µg	Oxoid™
Cefotaxime	CTX	30µg	Oxoid™
4th Generaion cephalosporin			
Cefepime	FEP	5µg	Oxoid™
Monobactam			
Aztreonam	ATM	30µg	Oxoid™
Floroquinolone			
Ciprofloxacin	CIP	5µg	Oxoid™
Quinolone			
Nalidixic acid	NA	30µg	Oxoid™
Aminoglycosides			
Gentamicin	CN	10µg	Oxoid™
Streptomycin	S	10µg	Oxoid™
Phenicol			
Chloramphenicol	C	30µg	Oxoid™
Sulfomides			
Sulfamethoxozole	RL	300µg	Oxoid™
Trimethoprim	W	5µg	Oxoid™
Cephamycin			
Cefoxitin	FOX	30µg	Oxoid™
Inhibitor (Agumentin)			
Amoxicillin-clavulanic acid	AMC	110µg	Oxoid™
Carbapenem			
Imipenem	IPM	10µg	Oxoid™

Key: Table 3.1 shows the 13 antimicrobial agents belong to different classes tested for antibacterial activities against recovered Gram-negative bacteria.

3.10 Molecular Analysis of Multiple Drug Resistant Isolates

3.10.1 DNA Extraction

The extraction of DNA for the PCR template and fingerprint analysis was done using the boiling method. Briefly, a loop full of the pure isolate was emulsified in 1ml of distilled water in an Eppendorf tube. The tube content was then boiled at 95°c for 10 minutes using a heating block. Centrifuging at 140000 Xg for 5 minutes did separation of DNA. Using a micropipette, 500µl of the supernatant containing extracted DNA was then transferred in another sterile Eppendorf tube.

3.10.2 Screen For β-Lactamase Genes And Carriage of Class 1 And 2 Integron

Potential producers of extended-spectrum β-lactamases based on disc diffusion were screened for carriage of *bla*$_{TEM}$, *bla*$_{CTX-M}$, *bla*$_{SHV}$ and *bla*$_{OXA,}$ **Table 3.2**. These are the most common reported β-lactamases genes in the family Enterobacteriaceae (Fernandes *et al.* 2013; Manoharan *et al*. 2011). Potential producers of extended-spectrum β-lactamases based on disc diffusion (resistant to all or either ceftazidime, ceftriaxone and cefotaxime) were all subjected to PCR screening to affirm. In addition to this, bacterial strains resistant to multiple antimicrobial agents were screened for carriage of class 1 and 2 integrase.

PCR amplification was done using published primers as summarized in **Table 3.**2 (Hasman, 2005;Hasman, 2005;Mohapatra, 2007;Taherikalani, 2011;Yu, 2014)**.** Amplification of resistance genes was done under the following conditions; initial denaturation at 95 °C for 2 min, annealing at 50-62 °C for 1 minute (depending on the target gene and the primer), extension at 72 °C for 1 min for 30 cycles and a single final extension step at 72 °C for 15 minutes. Amplified PCR products were run in 1.2% agarose gel and bands visualization using the UV gel max machine. Analysis of the gel images was done by checking the molecular weight of formed bands against a 1kb molecular ladder.

3.10.3 Establishing Genetic Relatedness in Recovered Bacteria Strains

To establish possible clonal spread and genetic relatedness, the $(GTG)_5$-PCR fingerprint method was conducted as previously described (Mohapatra *et al*. 2007). PCR amplification using the $(GTG)_5$ primer was performed under the following amplification conditions; denaturation step at 95°C for 7 minutes followed by 30 cycles of 1 minute at 94°C, 1 minute at 40°C and 8 minutes at 60°C. The final elongation was done for 16 minutes at 65°C. PCR products were then separated on a 1.5 % agarose gel electrophoresis in 1x TAE buffer at 80V for 1 hour and banding patterns visualized under UV light. Fingerprint analysis and generation of the dendrogram tree was done using the bio numeric software version 6.6. Banding patterns were analyzed using Gelcompar®2 software versions 6.6 with the cluster analysis was done using the dice method using the unweighted paired group method with arithmetic mean. The interpretation of the generated dendrogram was done by checking the clustering patterns. Isolates clustered together with a similarity matrix of ≥ 80% were considered closely related to those that were < 80% were deemed distantly related. Metadata on resistance phenotypes and genotypes were also added to the dendrogram to also aid in analysis. Isolates that had similar resistance phenotypes and genotypes were considered closely related and possibly clonal strains.

Table 3. 2: PCR amplification primers used in this study

Target gene	Primer name	Primer sequence	Annealing TºC	Product size	Reference
bla TEM	TEM-F	5′-GCGGAACCCCTATTTG-3′	50	964 bp	(Hasman, *et al* , 2005)
	TEM-R	5′-TCTAAAGTATATATGAGTAAACTTGGTC			
bla CTX-M	CTX-M-F	5′-ATGTGCAGYACCAGTAARGTKATGGC-3	60	593 bp	(Hasman *et al* ., 2005)
	CTX-M-R	5′-TGGGTRAARTARGTSACCAGAAYCAGCC			
bla SHV	SHV-F	5'- TTCGCCTGTGTATTATCTCCCTG- 3'	50	854 bp	(Hasman *et al* ., 2005)
	SHV-R	5'- TTAGCGTTGCCAGTGYTCG- 3'			
bla $_{OXA}$	OXA-IF	5'-ATGAAAAACACAATACATATCAACTTCG	62	820 bp	(Hasman *et al* ., 2005)
	0XA-1R	5'- GTGTGTTTAGAATGGTGATCGCATT-3'			
IntI 1	intM1_D	5'-GAAAGGTCTGGTCATACATG-3'	50	500bp	Yu *et al.* , 2014
	intM1_U	5'-ACGAGCGCAAGGTTTCGGT-3'			
IntI 2	INT2-L	5'-CACGGATATGCGACAAAAAGGT-3'	50	789	Taherikalani *et al.* , 2011
	INT2-R	5'-GTAGCAAACGAGTGACGAAATG-3'			
(GTG)5	(GTG)5	5'-GTGGTGGTGGTGGTG- 3'	40	variable	Mohapatra et al, 2007

Key: Table 3.2 shows the screened β-lactamases and integrase screened in selected Gram-negative isolates from food and environmental samples. A low resolution fingerprinting method referred to as the $(GTG)_5$ was used to assess bacterial species' possible relatedness. The $GTG_{5,}$ which is based on the detection of repetitive tendons on the bacterial genome, did not have a specific band size and formed multiple variable bands based on the number of amplified oligonucleotide segments.

3.11 Data Management and Analysis

Epi-info version 7 was used to capturing field and lab data. Manual backup of field and laboratory data was also done on an A4 hardcopy book. The association between independent (condition) and dependent (outcome) variables were accessed using Fischer's chi-square to determine test P-value, odds ratio and confidential interval. The relationship between microbial food contamination and proximity to open sewage, uncollected garbage, handling money while serving, wearing personal

protective equipment and layout of the vending point was also tested using chi-square.

3.14 Dissemination of Study Findings

Data generated from this study was published in a free access journal of advances in Microbiology (D01: 10.4236/aim.2019.96031 Jun.18, 2019), **Appendix 8**. Information was also disseminated through presentation in training and seminars, including the FAO training workshop held in KEMRI, Nairobi July 2018. Data on the diversity of environmental microbial isolates and their antimicrobial sensitivity profiles was also presented during the 3rd National antimicrobial resistance conference in Kampala, Uganda, in November 2018.

CHAPTER FOUR

RESULTS

4.1 Microbial Food Contamination

4.1.1 Microbial Food Contamination Analysis Based on Food Type

Ready-to-eat street food samples consisting of cereal, vegetables and meats **(Figure 4.1)** were analyzed to determine the microbial contamination load. Enumeration detected microbial contamination levels exceeding 2.0×10^4 CFU/mL in 106 (38%) of the 281 samples of ready-to-consume food. The Mean CFU for all foods was 4.9×10^5 CFU/mL. More than 50% of the food samples had a CFU/mL count of between 3.7×10^5 to 2.1×10^6 CFU/mL. These values are beyond the $\leq 10^2$ CFU/mL minimum thresholds for fit human food contamination based on (HACCP standards set by the Food and Agriculture Organization (FAO) of the United Nations. Analysis done noted that kales were the most contaminated foods with a Mean of 2.3×10^6 CFU/mL, followed by meats at 1.0×10^6CFU/mL. At the same time, omena (a preparation of *Rastrineobola argetea*) was the least contaminated food with a mean CFU/mL of 4.0×10^4 , **Table 4.1**.

Table 4. 1: Food microbial load in colony-forming unit per milliliter (CFU/mL)

Food category	Food type	n	Enterobacteriaceae food contamination (CFU/mL)		
			Mean	CFU_{50}	CFU_{90}
Vegetables					
	Kales	31	2.3×10^6	2.3×10^6	5.0×10^6
	Managu	25	1.0×10^5	1.3×10^5	3.7×10^6
	Cabbage	25	1.3×10^5	1.1×10^5	1.7×10^5
Meat					
	Beaf	41	1.0×10^6	1.0×10^6	4.7×10^6
	Omena	17	4.0×10^4	3.0×10^4	1.2×10^5
Cereals					
	Beans	32	3.4×10^5	3.0×10^5	6.6×10^5
	Rice	28	3.1×10^5	2.1×10^5	3.0×10^6
	Githeri	36	2.0×10^5	1.1×10^5	3.0×10^6
	Ndengu	24	7.0×10^4	7.2×10^4	1.5×10^5
Others					
	Ugali	22	2.3×10^5	2.4×10^5	4.0×10^5

Key: The level of Enterobacteriaceae food contamination was determined using the 3M® Petri-film plate. Bacteria growth enumeration after 24 hours was determined and expressed in CFU/mL (colony-forming unit per milliliter). Microbial contamination levels of these foods were classified according to guidelines stated by of the Analysis and critical control point system (HACCP) by the Food and Agriculture Organization (FAO). A microbial contamination level of $\leq 10^2$ CFU/ml is considered acceptable for human consumption, while food with a 103 CFU/mL value and beyond is deemed to be acceptable for human consumption. Githeri is a staple food in Kenya consisting of a mixture of boiled beans and while ugali is a stiff porridge prepared by mixing cornflour with boiling water before simmering. Silver cyprinids(*Rastrineobola argetea*) are a type of tiny fish commonly known as *omena* in Kenya. *Vigna radiate*, which is popularly known by its local name Ndengu in Kenya, is a legume type of lentils while Managu (*Solanum villosum*) is a bitter type of vegetable of the nightshade family. The CFU_{50} and CFU_{90} show the 50^{th} and 90^{th} percentilr score values for each food type.

4.1.2 Microbial Food Contamination In Relation To Vending Point Surrounding Features

This study showed that foods sold close to sewage and dumping sites were the most contaminated with a mean CFU/mL of 5.5×10^5 and $5.1 \times 10^{5,}$ respectively, in **Table 4.2.** Statistical analysis revealed a significant difference in contamination levels among foods sold near open sewage, toilets, free space vending point layout and near

dumping from those that were not close to these features (**P**< 0.0001). Sites that did not have constant clean water supply were found to have more contaminated foods exceeding the recommended threshold considered fit human consumption than those with a continuous water supply (**P**< 0.0001, **C.I** 5.23 – 5.35, **O.R** 5.28). **Plate 4.1** shows an image of clean Water supply lines laid on burst sewers in Kibera slums. Lack of proper personal protective equipment (PPE) and handling money significantly contributed to microbial food contamination (**P**< 0.0001, **C.I** 0.027 – 0.0.028, **O.R** 0.028).

Table 4. 2: Food contamination levels in relation to various risk factors

Proximity of the foos vending point		n	Enterobacteriaceae food contamination			Chi-square test (×2)			
			Mean	CFU50	CFU90	P value	Odds ratio (O.R)	95% confidence interval (C.I)	
								Lower limit	Upper limit
Sewage	**near**	**67**	5.5×10^5	7.9×10^5	2.1×10^6	< 0.0001*	0.1183	0.1162	0.1204
	not near	**33**	1.4×10^4	1.7×10^5	2.7×10^5				
Toilet	**near**	**28**	4.1×10^4	9.4×10^4	2.0×10^4	< 0.0001*	1.0278	1.0142	1.0415
	not near	**72**	1.3×10^5	2.9×10^5	3.9×10^6				
Dumpising site	**near**	**59**	5.1×10^5	1.2×10^5	2.0×10^6	< 0.0001*	0.0235	0.0232	0.0238
	not near	**41**	4.0×10^4	4.0×10^5	6.8×10^5				
Water supply	**near**	**47**	5.9×10^4	1.1×10^5	1.9×10^5	< 0.0001*	5.2825	5.2196	5.3461
	not near	**53**	3.4×10^5	1.2×10^5	2.0×10^6				
Personal protective	**worn**	**9**	2.3×10^4	1.1×10^5	1.8×10^5	< 0.0001*	12.8063	12.6062	13.0097
	not warn	**91**	2.7×10^5	6.8×10^5	1.6×10^6				
Handling	**Yes**	**78**	2.4×10^5	3.1×10^5	4.0×10^5	< 0.0001*	0.0283	0.0278	0.0288

Key: CFU/mL: Colony-forming units per milliliter. Hygiene features in and around the food-vending point were capture and the level food microbial determined in relation to proximity features recorded. CFU_{50}: is the median CFU/ml value, CFU_{90} is the 90^{th} percentile, while CFU/mL mode is the most common CFU/ml value in the respective food vending point proximity category. * denotes a statistically significant P-value. Association between microbial contamination means and proximity to the various environmental risk factors calculated using Fischer's chi-square test. In this table, the acronym 'n' is a count of the food vending points that were either near or not near the analyzed environmental features.

Figure 4.1: Clean water pipes laid in open sewage and highly polluted environment in Kibera informal settlements

Key: This image taken in Kibera informal settlements shows clean water supply pipes meant for domestic usage laid on untreated sewage and uncollected garbage.

4.2 Prevalence of Gram-negative Bacterial Isolates

4.2.1 Diversity of Bacterial Isolates In Environmental Samples From Kibera

This study recovered 209 non-fastidious Gram-negativeisolates from sewage, 268 from sludge and 56 from the soil, **Table 4.3**. These non- fastidious isolates felt under 14 bacteria genera. The isolates included known indicator organisms for AMR trends in human, animal and the environment [*Escherichia* (21%) and *Klebsiella* (20%)] and known Enteropathogens [*Salmonella spp* (5%) and *Shigella spp* (3%)]. The other category of isolates recovered included bacterial trains associated with high intrinsic resistance to antimicrobials [*Acinetobacter baumannii* (1%), *Pseudomonas aeruginosa* (8%), *Morganella morganii* (3%), *Serratia marcesence* (1%)] and other bacteria genera that are not significantly associated with diseases but are known opportunistic infection pathogens [*Citrobacter spp* (8%), *Enterobacter spp* (19%), *Edwardisella spp* (2%), *Proteus spp* (4%), *Yersinia spp* (4%) and *Hafnia spp* (2%)](Figure 4.1).

Table 4. 3: Diversity of Gram-negative microbial recovered from environmental

Sample type	Total isolates (n)	*Citrobacter spp*	*Enterobacter spp*	*E.tarda*	*E.coli*	*H.alvei*	*Klebsiella spp*	*M.morgannii*	*P.aeruginosa*	*A.baumannii*	*Proteus spp*	*Salmonella spp*	*Shigella spp*	*Yersinia spp*	*S.marcesence*
Sewage	209	7	24	10	61	10	26	5	13	5	9	18	11	10	0
Sludge	268	34	67	0	31	0	65	10	25	0	12	9	5	10	0
Soil	56	3	12	0	19	0	13	0	3	0	1	0	0	0	5

Key: spp: species**, n:** total number***, E. tarda:*** *Edwardisella tarda****, E. coli:*** *Escherichia coli****, P. aeruginosa:*** *Pseudomonas aeruginosa****, S. marcesence:*** *Serratia marcesence.*

4.2.2 Diversity of Gram-Negative Bacteria Isolates From Ready-To-Eat Foods

This study recoverd 405 Gram-negative bacterial isolates from the 281 ready-to-eat food samples collected, **Table 4.4**. These isolates fit into seven (7) enteric genera that namely *Klebsiella ssp* (29%), *Escherichia coli* (26%), *Enterobacter agglomerans* (22%), *Salmonella spp* (7%), *Proteus mirabilis (*7%), *Citrobacter freundii* (7%) and *Serratia marcesence* (3%), **Table 4.4**. From the 405 microbial isolates, 24% were from kales, 19% from meat and 16% from Managu, **Table 4.4**. *Escherichia coli* colonies' growth on MacConkay agar medium recovered from foods specimen in Kibera slums was as shown on **Plate 4.2**.

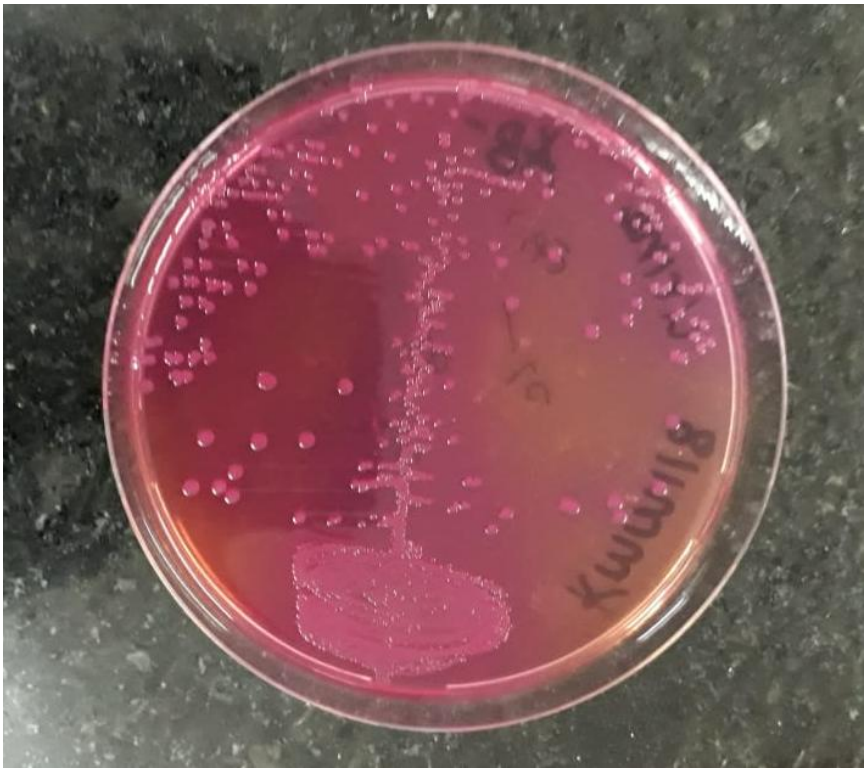


Plate 4.1: Lactose fermenters *Escherichia coli* in MacConkey agar plate

Key: The image shows monoculture rounded pinkish lactose fermenters *Escherichia coli* isolates from Ugali specimens obtained from a street food vending point in Kibera. Isolation of this enteric microbial in a ready-to-eat food sample is highly indicative of fecal contamination that may lead to diarrhea and other gastrointestinal infections.

Table 4.4: Diversity of bacterial isolates collected in various ready-to-eat food samples

Food type	Samples analyzed	*Citrobacter spp*	*Enterobacter spp*	*E.coli*	*Klebsiella spp*	*Proteus spp*	*Salmonella spp*	*S.marcesence*
Kales	31	9	22	27	21	8	10	2
Meat	41	3	11	20	28	4	8	4
Beans	32	2	9	6	8	2	1	2
Rice	28	1	5	7	4	0	0	0
Ugali	22	0	3	5	8	0	0	0
Githeri	36	7	6	15	7	5	0	2
Cabbage	25	1	3	5	4	2	0	0
Managu	25	4	13	11	24	5	6	1
Ndengu	24	1	10	5	7	2	0	1
Omena	17	0	7	4	5	0	2	0
	281	28	89	105	116	28	27	12

Key: This table shows the count of various bacterial species isolated from the ten analyzed food types. The acronym spp used in this table means species.

4.3 Antimicrobial Resistance Patterns of Gram-Negative Isolates

4.3.1 Overall Antimicrobial Resistance in Bacteria Isolates From Ready-To-Eat Foods

The relative antimicrobial resistance abundance among microbial isolates from the 283 foods samples collected from Kibera was such that out of the 405 Gram-negative isolates, the prevalence of multiple-drug resistance (MDR) strains was 93(23%), that of extended-spectrum β-lactamases (ESβL)-producing strains was 17(4%). The prevalence of those with combined resistance to β-lactams, Fluoroquinolones, Quinolones and Aminoglycosides (βFQA-phenotype) was 10 (2%). The βFQA phenotype was prevalent in *Klebsiella spp* (4%) followed by *E. coli* (3%) and was absent in *Citrobacter, Proteus, Salmonella* and *Serratia* genera.
Out of the seven microbial genera recovered from foods in this study, *Klebsiella spp* were the most resistant to any set of antimicrobial agents tested, with the highest values been recorded towards ampicillin (AMP, 41%), sulfamethoxazole (RL, 29%), and trimethoprim (TRIM 32%), **Table 4.5.** These species also recorded the highest resistance towards advanced generations of cephalosporins (CAZ, 4% and FEP, 3%), relatively advanced classes of aminoglycoside (CN, 6%) and quinolones NA (14) (fluoroquinolone; CIP, 4%). However, none of the *C. freundii, Salmonella spp* and *Sr. marcesence* isolates was resistant towards cephalosporins, gentamicin or ciprofloxacin, indicating that these genera are relatively susceptible to locally available drugs and that the BFQA phenotype is still not prevalent among these genera. Resistances similar to those recorded in *K. Pneumoniae* were observed in *E. coli* isolates (CAZ 2%, FEP 2%, CN 5%, NA 10%, and CIP 3%). All the 405 isolates recovered in this study were susceptible to imipenem (IPM, 100%), while 98% were susceptible to cefepime, a fourth-generation cephalosporin.

From the ten food types collected and analyzed in this study, Kales followed by meat isolates were the most resistant overall to tested antimicrobial agents, while those from Rice, Ndengu and ugali were the least resistant, **Table 4.6**. The analysis also showed that isolates from kale samples were also the most resistant to advanced antimicrobial agents; AMC 13%, CN 7%, CIP 6%, FEP 4% and CAZ 4%, **Table 4.6.**

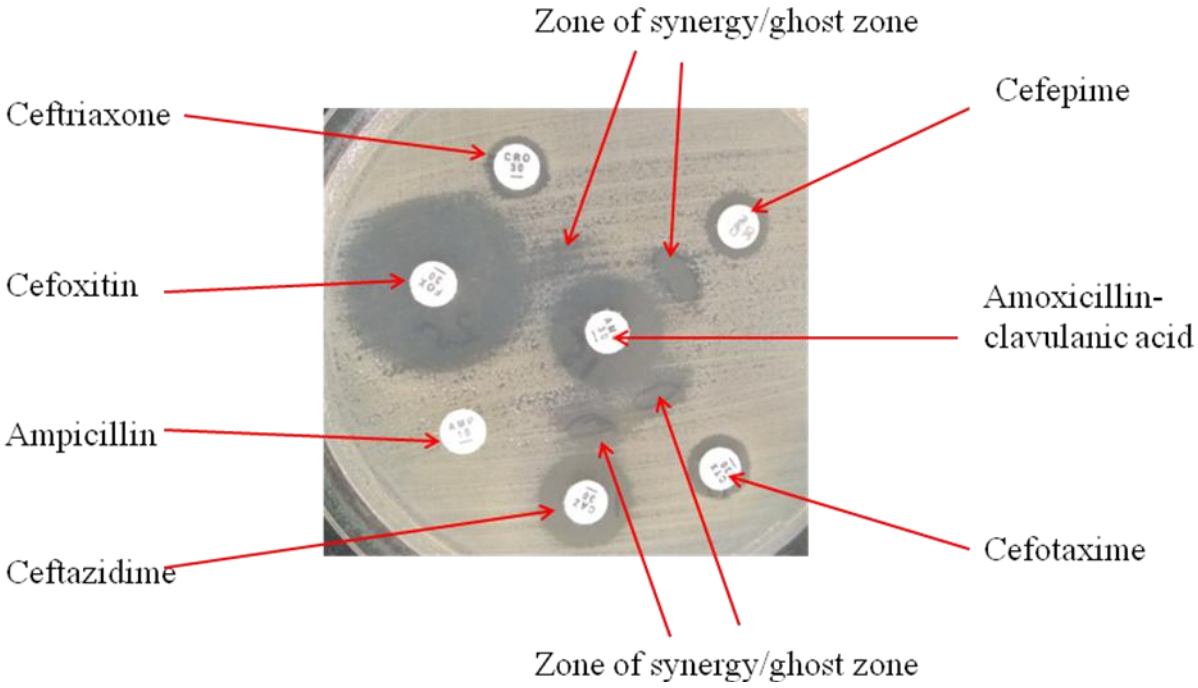


Plate 4.2: This plate shows an *Escherichia coli* producing extended-spectrum β-lactamase

Key: ***Escherichia coli* isolate in** plate 4.2 shows an extended-spectrum β-lactamases producing phenotype characterized by resistance towards penicillin (Ampicillin) and third-generation cephalosporins (CAZ, CRO and CTX). The isolate was also resistant to more advanced fourth-generation cephalosporin (cefepime). There were synergistic reactions indicated by the ghost zones between cephalosporins antimicrobial agents and amoxicillin-clavulanic acid bacterial inhibitor.

Table 4. 5: Relative abundance (%) of isolates antimicrobial-resistant from ready-to-eat foods

Gram-negative bacteria	n	Antimicrobial resistance profiles of Gram negative microbial (%)															
		AMP	CTX	CAZ	CRO	FEP	ATM	FOX	AMC	CN	S	CIP	NA	IPM	RL	W	C
All	405	133(33)	17(4)	7(2)	17(4)	7(2)	21(5)	46(11)	39(10)	15(4)	40(10)	10(2)	36(9)	0(0)	95(23)	110(27)	38(9)
C.freundii	27	7(26)	0(0)	0(0)	0(0)	0(0)	0(0)	1(4)	1(4)	0(0)	1(4)	0(0)	1(4)	0(0)	5(19)	6(22)	1(4)
E.agglomerans	88	24(27)	3(3)	0(0)	3(3)	1(1)	4(5)	9(10)	7(8)	2(2)	7(8)	2(2)	7(8)	0(0)	20(23)	22(25)	5(6)
E.coli	104	34(33)	5(5)	2(2)	5(5)	2(2)	6(6)	14(13)	11(11)	5(5)	12(12)	3(3)	10(10)	0(0)	25(24)	29(28)	10(10)
Klebsiella spp	116	47(41)	8(7)	5(4)	7(6)	4(3)	9(8)	17(15)	15(13)	7(6)	15(13)	5(4)	14(12)	0(0)	34(29)	37(32)	15(13)
P.mirabilis	30	9(30)	1(3)	0(0)	1(3)	0(0)	1(3)	2(7)	2(7)	1(3)	2(7)	0(0)	2(7)	0(0)	5(17)	7(23)	2(7)
S.Typhis	28	9(32)	0(0)	0(0)	1(4)	0(0)	1(4)	2(7)	2(7)	0(0)	2(7)	0(0)	1(4)	0(0)	4(14)	7(25)	2(7)
Sr.marcesence	12	3(25)	0(0)	0(0)	0(0)	0(0)	0(0)	1(8)	1(8)	0(0)	1(8)	0(0)	1(8)	0(0)	2(17)	2(17)	3(11)

Key: A maximum of three isolates per colony type from a given primary plate were subjected to susceptibility testing against some of the most commonly used antimicrobial agents. The Disc diffusion method was used in this sensitivity testing using inoculums of 0.5 McFarland standards.

Table 4. 6: Relative abundance in antimicrobial resistance among isolates recovered from the various foods types from Kibera informal settlements

Food type	*n*	AMP	CTX	CAZ	CRO	FEP	ATM	FOX	AMC	CN	S	CIP	NA	IPM	RL	W
Kales	99	55(56)	7(7)	4(4)	7(7)	4(4)	9(9)	17(17)	13(13)	7(7)	13(13)	6(6)	11(11)	0(0)	32(32)	45(45)
Meat	78	32(41)	4(5)	2(3)	4(5)	2(3)	4(5)	9(12)	7(9)	1(1)	9(12)	3(4)	6(8)	0(0)	14(18)	23(29)
Beans	30	10(33)	1(3)	0(0)	1(3)	0(0)	1(3)	3(10)	3(10)	1(3)	3(10)	0(0)	5(17)	0(0)	11(37)	5(17)
Rice	17	4(24)	0(0)	0(0)	0(0)	0(0)	1(6)	2(12)	2(12)	0(0)	1(6)	0(0)	2(12)	0(0)	5(29)	3(18)
Ugali	16	5(31)	0(0)	0(0)	0(0)	0(0)	0(0)	2(13)	1(6)	0(0)	1(6)	0(0)	1(6)	0(0)	4(25)	5(31)
Githeri	42	10(24)	2(5)	0(0)	2(5)	0(0)	2(5)	5(12)	6(14)	2(5)	5(12)	0(0)	4(10)	0(0)	7(17)	7(17)
Cabbage	15	4(27)	0(0)	0(0)	0(0)	0(0)	0(0)	2(13)	1(7)	0(0)	1(7)	0(0)	1(7)	0(0)	3(20)	4(27)
Managu	64	4(27)	3(5)	1(2)	3(5)	1(2)	3(5)	4(6)	4(6)	2(3)	4(6)	1(2)	4(6)	0(0)	9(14)	9(14)
Ndengu	26	4(15)	0(0)	0(0)	0(0)	0(0)	1(4)	1(4)	1(4)	0(0)	2(8)	0(0)	1(4)	0(0)	5(19)	5(19)
Omena	18	5(28)	0(0)	0(0)	0(0)	0(0)	0(0)	1(6)	1(6)	0(0)	1(6)	0(0)	1(6)	0(0)	5(28)	4(22)
All	405	133(33)	17(4)	7(2)	17(4)	7(2)	21(5)	46(11)	39(10)	13(3)	40(10)	10(2)	36(9)	0(0)	95(23)	110(27)

Key: The table shows the antimicrobial resistance abundance of various isolates recovered from the ten food sample types collected in this study. The acorn 'n' used in this table indicates the count of bacterial isolates recovered from each of the ten food types collected and analyzed in this study.

4.3.2 Antimicrobial Resistance Patterns of Isolates Recovered From Environmental Samples

This study reported 14 bacteria among which *P. aeruginosa* isolates were the most resistant to tested antimicrobial agents, **Table 4.7**. The prevalence of isolates with the BFQA phenotype was also highest among *P. aeruginosa* isolates (13%). However, none of the *Yersinia* species and *Edwardisella tarda* exhibited this resistance, **Table 4.7**. Of the 41 *P. aeruginosa* isolates recovered from the informal environmental samples, two isolates from sewage were resistant to Imipenem. Resistance prevalence for *Klebsiella* strains towards any class of antimicrobials was not significantly higher than that of *E. coli* strains (P: 0.36, C.I: 1.88, O.R:1.22). There was no difference in the resistance prevalence between two known enteropathogens recovered in this study, *Shigella Salmonella* (P: 1.25, C.1:4.33, O.R:1.25). Isolates from slum sludge were overall more resistant to any antimicrobial agents tested, **Table 4.8**. High resistances were recorded towards ampicillin (AMP 54%), trimethoprim (W 50%) and sulfamethoxazole (RL 47%) among sludge isolates recovered from the slum, **Figure 4.2.**

A hotspot map was developed to show the distribution pattern of hotspots of multiple-drug resistance bacteria strains recovered in this study, **Figure 4.3**. Most of these hotspots were concentrated in sections of the slum (Laini Saba and Soweto) characterized by poor sewage drainage and garbage heaps.

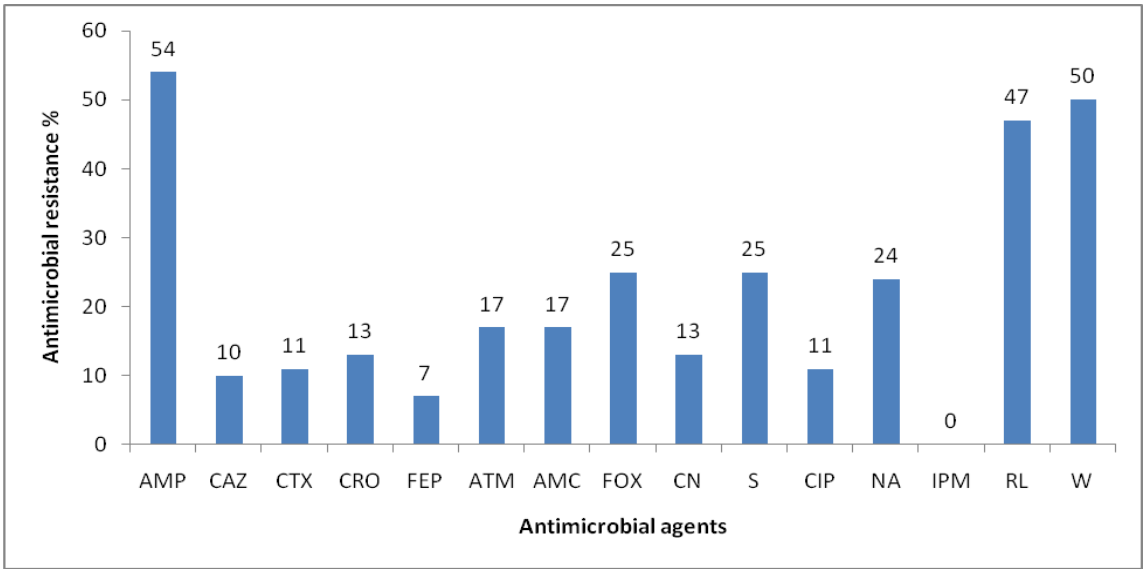


Figure 4. 2: Antimicrobial resistances (%) environmental Gram-negative isolates from Kibera informal settlements

Table 4.7: Antimicrobial resistance (%) in microbial isolates from environmental samples

Study site	n	AMP	CAZ	CTX	CRO	FEP	ATM	FOX	AMC	CN	S	CIP	NA	IPM	RL	W
E.coli	111	58	9	12	13	7	14	29	15	14	29	9	15	0	52	55
K.pneumoniae	104	60	12	14	14	10	17	32	17	15	33	12	32	0	55	58
Salmonella ssp	27	44	7	11	11	4	11	19	15	15	22	4	33	0	48	44
Shigellaspp	16	50	6	13	13	0	13	19	13	13	19	6	25	0	44	50
A.baumannii	5	40	20	20	20	0	20	40	20	20	40	20	40	0	40	40
P.aeruginosa	41	49	15	20	22	12	37	48	37	20	37	15	48	5	44	48
M.morganii	15	53	13	13	20	7	20	27	20	13	27	8	33	0	53	47
S.marcesence	5	40	0	20	20	0	20	20	20	0	20	0	20	0	40	20
Citrobacter spp	44	43	9	11	11	7	11	18	14	9	18	5	16	0	60	40
Enterobacter spp	103	48	10	12	13	5	14	19	13	11	13	5	13	0	50	50
E.tarda	10	30	0	0	10	0	10	20	10	0	10	0	20	0	20	10
Proteusspp	22	41	10	10	14	5	14	23	23	10	27	5	23	0	45	41
H.alvei	10	40	0	10	10	0	10	10	20	0	20	0	20	0	50	40
Yersiniaspp	20	25	0	5	5	0	5	10	5	5	15	0	15	0	10	15

Key: Table 4.5 shows the percentage of antimicrobial-resistant patterns of Gram-negative bacteria isolates recovered from sewage, sludge and soil samples collected in informal (Kibera).

Table 4. 8: Relative abundance (%) of isolates resistance from environmental samples

Sample type	n	AMP	CAZ	CTX	CRO	FEP	ATM	AMC	FOX	CN	S	CIP	NA	IPM	RL	W
Sewage	209	111(53)	19(9)	23 (11)	27 (13)	15 (7)	31 (15)	36 (17)	50 (24)	25 (12)	48 (23)	21 (10)	44 (21)	2 (1)	82 (39)	90 (43)
Sludge	268	153 (57)	29(11)	32(12)	36 (14)	19 (7)	51 (19)	51 (19)	75 (28)	36 (14)	72 (27)	32(12)	70 (26)	0 (0)	147(55)	150 (56)
Soil	56	22 (40)	4(7)	5(9)	6 (10)	3(6)	6(10)	6 (10)	8(15)	6(10)	12 (22)	4 (7)	12 (22)	0 (0)	22 (40)	25(44)

Key: AMP: Ampicillin, **CTX**: Cefotaxime, **CRO**: Ceftriaxone, **FEP**: Cefepime, **ATM**: Aztreonam, **FOX**: Cefoxitin, **AMC:** Amoxicillin-clavulanic acid, **CN**: Gentamicin, **S**: Streptomycin, **CIP**: Ciprofloxacin, **NA**: Nalidixic acid, **IPM**: Imipenem, **C**: Chloramphenicol, **RL**: Sulfamethoxazole, **W**: Trimethoprim, **n**=total number bacteria isolates. The acronym ‘n’ used in this table is the number of isolates analyzed from each sample type.

4.3.3 Comparison of Antimicrobial Resistance Profiles Among Isolates From Environmental And Food Samples

Bacterial isolates from environmental samples were more resistant to any test antimicrobial agents than those recovered from food samples. Isolates from the 2 sample types were however more resistance towards AMP (54% vs 33%), W (50% vs 27%) and (RL (47% vs 23%). There was also a significant difference in resistance towards advanced antimicrobial agents between food and environmental isolates [(FEP: P=.0.002, C.1 1.49 – 6.67, O.R 3.15)(CIP: P<0.0001, C.I 2.64 – 11.42, O.R 5.49)]. Imipenem and ciprofloxacin were the most effective antimicrobial agents against isolates from both food and environmental samples.

4.4 PCR Screening for *BLA* and *INT*I Genes in Gram-negative Bacteria Recovered from Food and Environmental Samples

This study selected 104 and 93 Gram-negative isolates from envivronmental and foods samples respectively to screen carriage of β-lactamases and integron. The isolates selected were resistant to 3rd generation cephalosporins and were screened for carriage of bla_{TEM}, bla_{CTX-M}, bla_{SHV}, bla_{OXA} and class 1 and 2 integron which has widely been reported in Gram-negative bacteria. Among environmental and food isolates, bla_{TEM} was the most prevalent ESBL type (**FD**-55%, **EN**-45%), while bla_{SHV} was the least detected (**FD**-8%, **EN**-19%), **Figure 4.3**. All isolates that carried these genes were resistant to ampicillin and at least one 3rd generation cephalosporin (ceftriaxone, cefotaxime and/or ceftazidime). Co-carriage of bla_{TEM}, bla_{CTX-M}, bla_{OXA} and *intI*1 was prevalent in *P aeruginosa* (13%). Class 1 integron (*intI*1) was more prevalent [**FD**-23%, **EN**-17%)] than *intI*2 **[FD**-3%, **EN**-0%] among these MDR-strains. Distribution of *bla* genes prevalent in *K. pneumoniae* and *E.coli* respectively was as follows; bla_{TEM} (76%, 63%) bla_{CTX-M} (12%, 15%) bla_{OXA} (45%, 41%), **Table 4.9**. **Figures 4.4 and 4.5** show the distribution of *bla* genes and integrase detected in food and environmental isolates obtained across Kibera.

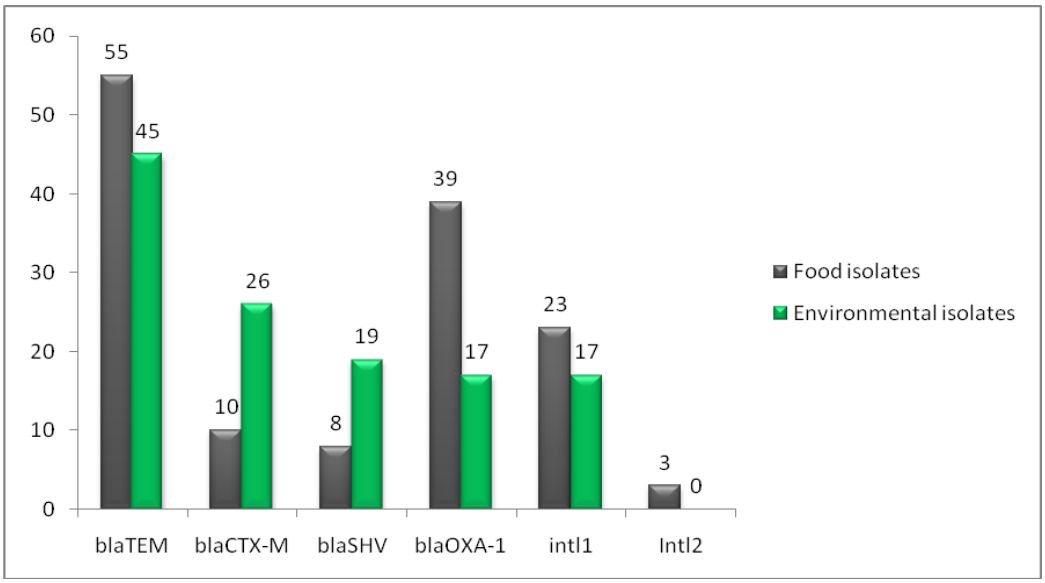


Figure 4.3: Carriage of β-lactamases and integron (%) in Gram-negative isolates from food and environmental samples from Kibera

Key: A total of 104 and 93 isolates from environmental and food samples were analyzed for carriage of β-lactamases and integron carriage. The acronym bla_{TEM} means Temoneria β-lactamases, $bla_{CTX-M:}$ cefotaxime Munich β-lactamases, bla_{OXA-1}: oxacillin β-lactamases type one variant, bla_{SHV}: Sulfahydryl β-lactamases and *intI*1 and 2: integrase of class 1 and 2 integron.

Table 4. 9: Relative abundance of isolates with Carriage of *bla* genes and integron class 1

Microbial isolates	Screened isolates		Carriage of β-lactamases genes and integron in Gram negative microbial											
			TEM		CTX-M		SHV		OXA-1		int1		Int2	
	FD	EN	FD	EN	FD	EN	FD	EN	FD	EN	FD	EN	FD	EN
C.freundii	5	10	1(20)	3(30)	0(0)	1(10)	0(0)	0(0)	1(20)	1(10)	0(0)	0(0)	0(0)	0(0)
E. agglomerans	14	20	5(36)	3(30)	1(7)	2(10)	0(0)	2(10)	7(50)	0(0)	3(21)	0(0)	0(0)	0(0)
E. coli	27	20	17(63)	12(60)	4(15)	10(50)	2(7)	3(15)	11(41)	4(20)	7(26)	4(20)	1(4)	0(0)
Klebsiella ssp	33	20	25(76)	12(60)	4(12)	4(20)	5(15)	10(50)	15(45)	6(30)	10(30)	5(25)	2(6)	0(0)
P. mirabilis	5	9	2(40)	5(56)	0(0)	2(22)	0(0)	1(11)	1(20)	1(11)	1(20)	1(11)	0(0)	0(0)
Salmonella spp	6	3	1(17)	1(33)	0(0)	1(33)	0(0)	0(0)	1(17)	0(0)	0(0)	0(0)	0(0)	0(0)
Sr .marcesence	3	1	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
P.aeruginosa	0	15	0(0)	10(67)	0(0)	7(47)	0(0)	4(27)	0(0)	6(40)	0(0)	7(47)	0(0)	0(0)
A.baumannii	0	2	0(0)	1(50)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1(50)	0(0)	0(0)
M.morgannii	0	2	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
E.tarda	0	1	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
H.alvei	0	1	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Shigella spp	0	2	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)

Key: Table 4.7 shows the number and percentage in brackets of isolates positive for the carriage of β-lactamases and class 1 and 2 integrases. The acronyms FD and EN refer to food and environmental samples collected and analyzed in this study. None of the target β-lactamases and integrase was detected in Morganella *morgannii, Edwardisella tarda, Hafnia alvei* and *Shigella* species.

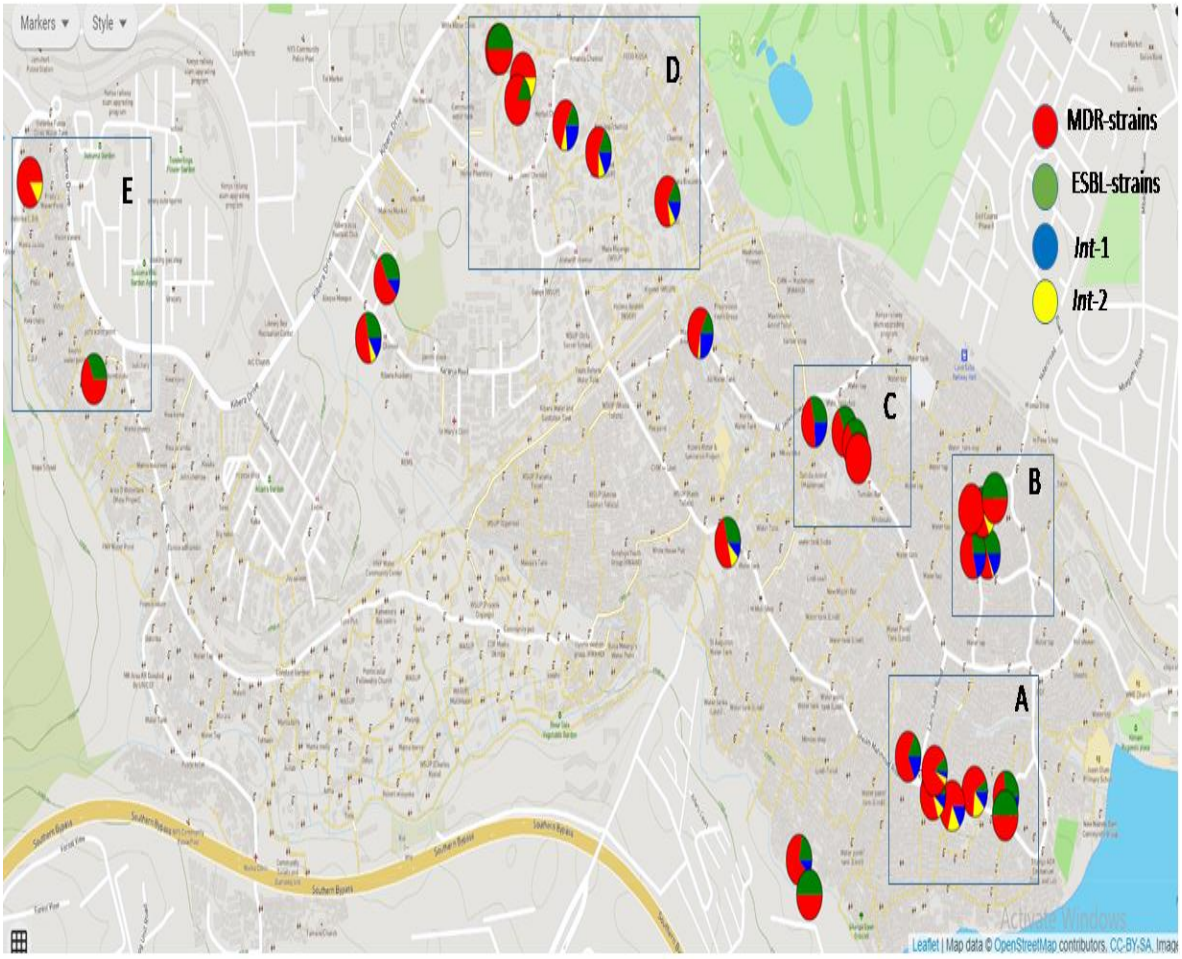


Figure 4. 4: Distribution MDR, ESBLs-strains and carriage of integron in microbial isolates from food

Key: MDR strains were recovered from foods isolates across all sampled vending points. However, the distribution of extended-spectrum β-lactamases (ESBL) and multiple-drug resistance (MDR) bacterial strains were more concentrated in most populated areas of Kibera [(A,B – Silanga)(C – Laini saba)]. Integron class 1 (*intI*1) was distributed nearly across to sampling sites, but class 2 integron (*intI*2) was more common in Silanga (A) and Makina (D) villages.

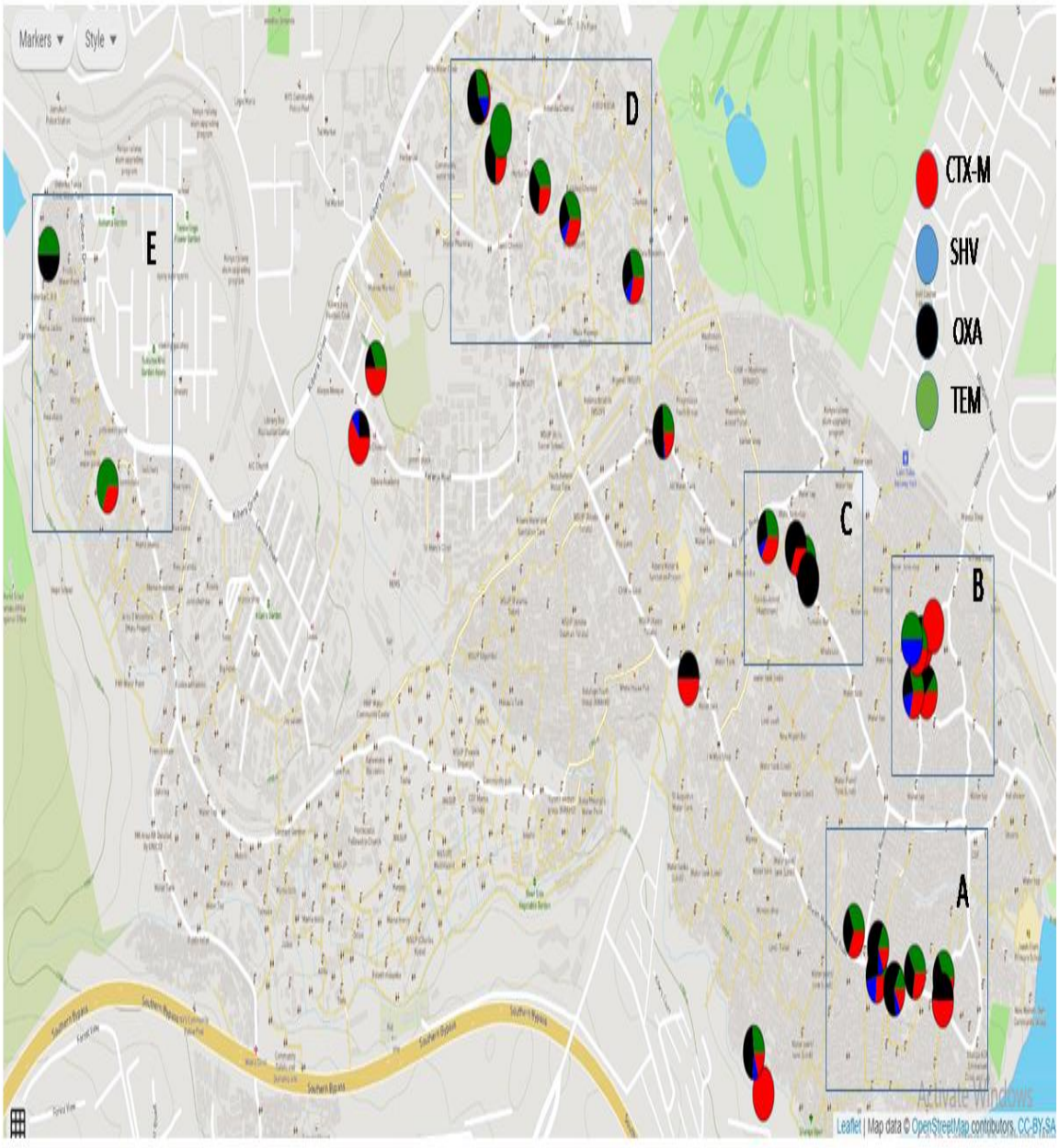


Figure 4. 5: Distribution pattern of *bla* gene microbial isolates from foods in Kibera.

Key: TEM was the most common β-lactamase gene detected in isolates recovered from all sites. Distribution of CTX-M and SHV associated with resistance to ceftriaxone and ceftazidime was more common Silanga (A, B) and Makina (D) villages. The map also shows that OXA-1 related to amoxicillin-clavulanic acid (AMC) resistance was common across most sampled sites.

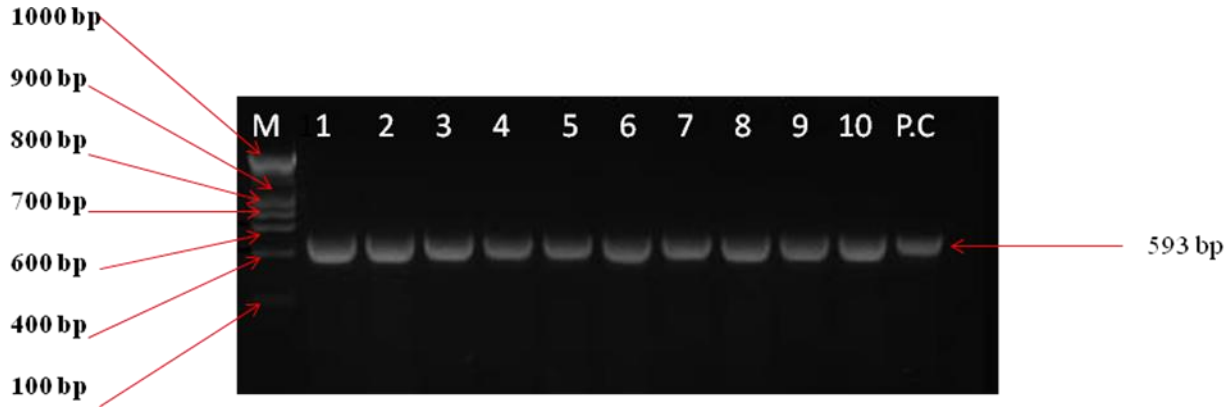


Plate 4.3: *bla*$_{CTX-M}$ gel electrophoresis image

Key: Plate 4.3 shows a gel image of 10 isolates positive for *bla*$_{CTX-M}$ carriage. The first lane with multiple bands is a 1kb molecular ladder (M) and positive control (P.C).

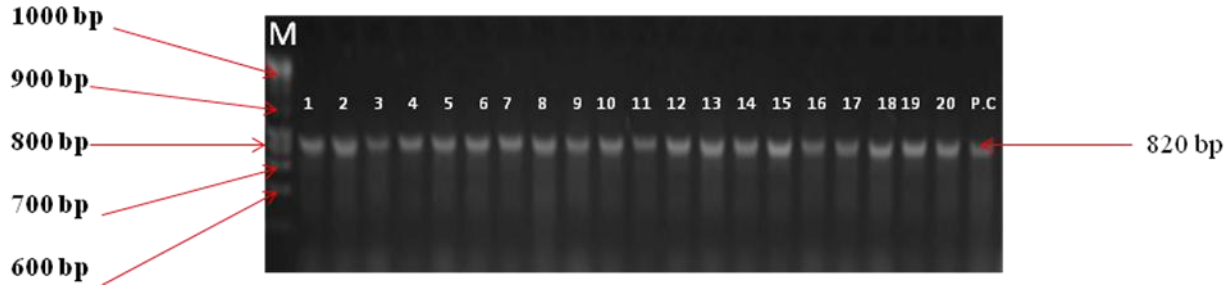


Plate 4. 4: *bla*$_{OXA}$ gel electrophoresis image

Key: This gel image shows ten isolates positive for *bla*$_{OXA}$ carriage. The first lane with multiple bands is a 1kb molecular ladder. The acronym PC used on this image refers to the positive control.

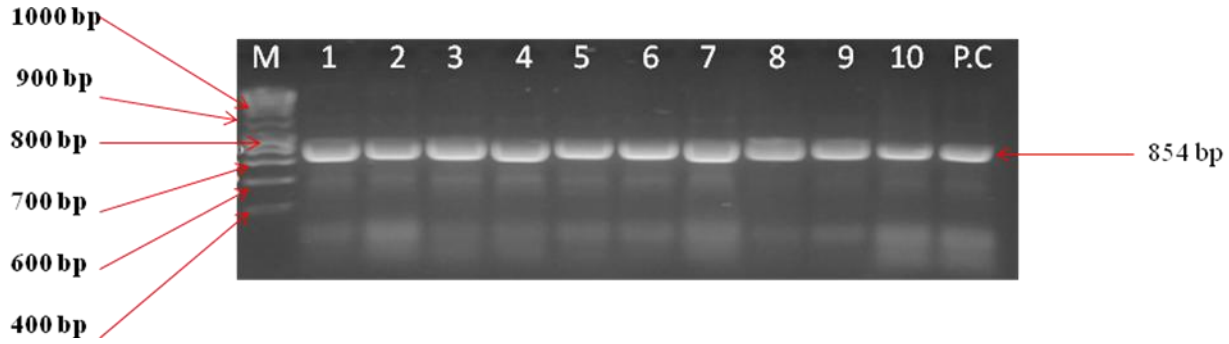


Plate 4. 5: *bla*$_{SHV}$ gel electrophoresis image

Key: This gel image shows isolates positive for *bla*$_{SHV}$ carriage. The first lane with multiple bands is a 1kb molecular ladder. The acronym M and P.C used on this image refer to the molecular ladder and positive control.

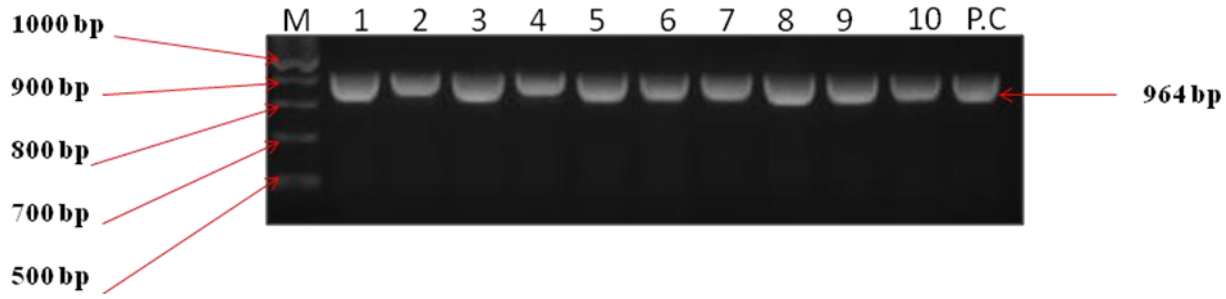


Plate 4. 6: *bla*$_{TEM}$ gel electrophoresis image

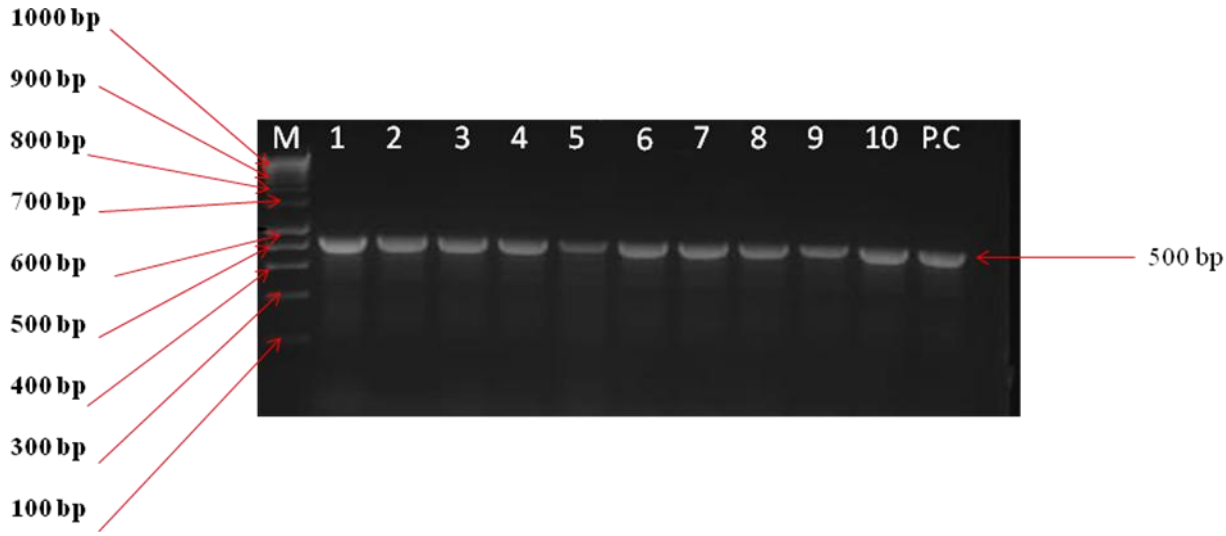


Plate 4.7: Class 1 integron (*intl*1) gel electrophoresis image

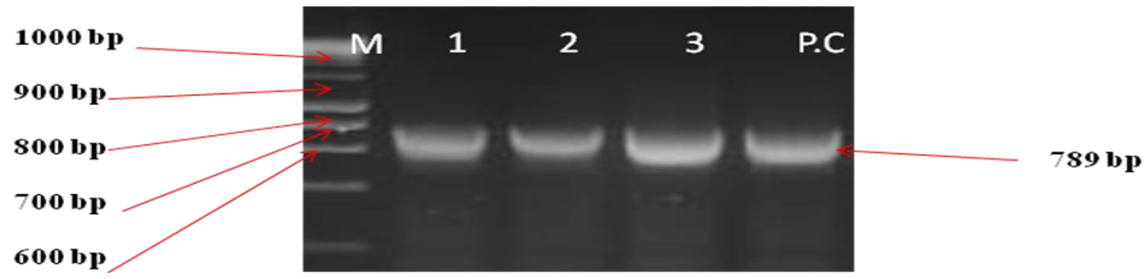


Plate 4.8: Class 2 integron (*intl*2) gel electrophoresis image

4.5 Cluster Analysis

4.5.1 Cluster Analysis Of Bacteria Species Isolated In Environmental Samples From Kibera

The cluster analysis revealed significant genetic similarity among *E. coli, K. pneumoniae, E. agglomerans* and *P. mirabili*s *strains*. Still, less relatedness was noted among strains belonging to *Citrobacter freundii,* **Figure 4.7-4.11**. Further analysis showed possible clonal expansion of *E.agglomerans* (**Figure 4.7, cluster 2**) and *Klebsiella pneumonia* (**Figure 4.8, cluster 2**) within the environmental ecosystems. Such strains had identical resistance phenotypes and shared more than 80% genetic similarity. Other strains such as *Salmonella Typhi* showed a similarity of above 70% in resistance patterns, **Figure 4.9.** Based on the banding patterns, this significant difference further indicates that different clones may have acquired similar sets of resistance determinants independently. These results call for high-resolution analysis using whole-genome analysis strategies in future studies.

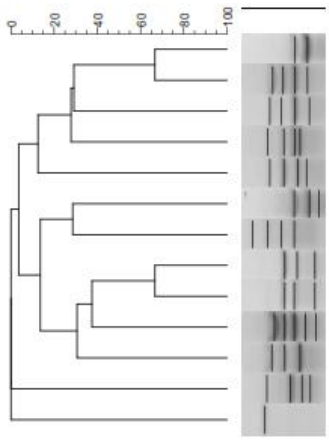
4.5.2 Cluster Analysis of Selected Bacteria Species Isolated In Ready-To-Eat Food Samples From Kibera.

A similarity matrix of less than 40% was noted in *E. coli* isolates obtained from different foods types across Kibera slums, **Figure 4.11**. Further analysis found no major clones for *E. coli* due to low percentage similarities and variation in antimicrobial resistance. Isolates in *E. coli* phylogeny were mostly resistant to ampicillin, trimethoprim and sulfamethoxazole in addition to other varying antimicrobial agents, indicating low genetic similarity (**Figure 4.11**). However, closer relatedness of 80% was found in a single cluster with two K. *pneumoniae* isolates obtained from Omena (Silver cyprinid) and beans obtained from different villages of Kibera informal settlements, **Figure 4.12.** The variations in antimicrobial resistance between these two isolates, however, suggest an independent evolution in antimicrobial resistance.

4.5.3 Cluster Analysis of Selected Bacteria Species From Ready-To-Eat Food And Environmental Samples From Kibera.

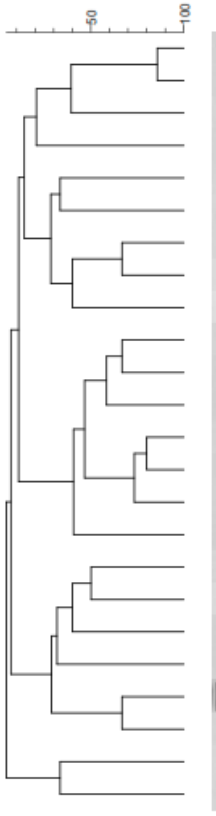
Selected *E. coli* and *K. pneumoniae* isolated from ready-to-eat foods and environmental samples were analyzed for genetic similarities to assessing possible cross-contamination**.** Several clusters with more than 60% similarities were noted among *E.coli* and *K.pneumoniae* isolates from food and environmental samples, **Figure 4.13 and 4.14.** However, most isolates

from food types sold in different villages of Kibera informal settlements had different resistance phenotypes that suggest minimum genetic relatedness. Phylogeny analysis of *K. pneumoniae*, however, revealed 2 isolates from beans and sewage respectively also exhibited identical resistance phenotypes, which strongly suggest cross-contamination. Other clusters in this phylogeny had similarities of less than 70% with varying antimicrobial resistances among isolates.



084	S.Typhi	Kibera	Soil	F/S	N/D
119	S.Typhi	Kibera	Sewage	AMP,S,RL,,W	N/D
601	S.Typhi	Kibera	Sewage	AMP,FOX,AMC,C,RL,W	TEM,INT-1,VCR
122	S.Typhi	Kibera	Sewage	AMP,CTX,CAX,CRO,FEP,ATM	N/D
2485	S.Typhi	Kibera	Sludge	AMP,FOX,AMC,CN,S,NA,RL,W	CTX-M,OXA,TEM,INT-1,VCR
2477	S.Typhi	Kibera	Sewage	Susce' to IPM,FEP&CIP only	OXA,CTX-M,TEM,INT-1,VCR
2437	S.Typhi	Kibera	Sewage	AMP,AMC,C	TEM,INT-1,VCR
2461	S.Typhi	Kibera	Sludge	AMP,S,NA,RL,W	N/D
2488	S.Typhi	Kibera	Sewage	Susce to CAZ,FEP,CIP &IPM only	CTX-M,OXA,INT-1,TEM,VCR
566	S.Typhi	Kibera	Sludge	AMP,NA,RL,W	N/D
2439	S.Typhi	Kibera	Sewage	AMP,S,CIP,NA,C,RL,W	OXA
2442	S.Typhi	Kibera	Sludge	AMP,FOX,AMC,NA,RL,W	N/D
2435	S.Typhi	Kibera	Sludge	AMP,ATM,RL,W	N/D

Figure 4.6: Cluster analysis of recover *Salmonella* Typhi from sewage, soil and sludge



11	E.agglomerans	Kibera	Soil	AMP,FOX,AMC	N/D
19	E.agglomerans	Kibera	Sludge	AMP,ATM,NA	N/D
20	E.agglomerans	Kibera	Sewage	AMP,S,RL,W	N/D
10	E.agglomerans	Kibera	Slugde	AMP,CTX,AMC,S,RL,W	TEM
5	E.agglomerans	Kibera	Sludge	AMP,CAZ,CTX,CRO,FEP,S,NA,RL,W	TEM,CTX-M
21	E.agglomerans	Kibera	Sewage	RL,W	N/D
3	E.agglomerans	Kibera	Sludge	AMP	N/D
23	E.agglomerans	Kibera	Sludge	AMP,FOX	N/D
24	E.agglomerans	Kibera	Sludge	AMP,AMC,S,NA,RL,W	N/D
9	E.agglomerans	Kibera	Sewage	RL,W	N/D
18	E.agglomerans	Kibera	Sludge	AMP,CTX,FOX,AMC,S,	TEM
14	E.agglomerans	Kibera	Sludge	AMP,FOX,AMC,S,NA,RL,W	TEM
7	E.agglomerans	Kibera	Sewage	AMP,RL,W	N/D
12	E.agglomerans	Kibera	Sludge	AMP,RL,W	N/D
17	E.agglomerans	Kibera	Sludge	AMP,AMC,RL,W	N/D
13	E.agglomerans	Kibera	Sludge	AMP,CTX,CAZ,CRO,FEP,NA,C	TEM,CTX-M
8	E.agglomerans	Kibera	Sludge	AMP,CAZ,ATM,AMC,NA,RL,W	TEM,CTX-M
22	E.agglomerans	Kibera	Sludge	AMP	N/D
16	E.agglomerans	Kibera	Sewage	AMP,FOX,AMC	N/D
15	E.agglomerans	Kibera	Sludge	AMP,CRO,ATM,AMC,S,C,RL,W	N/D
1	E.agglomerans	Kibera	Sludge	AMP,AMC,S,NA	N/D
6	E.agglomerans	Kibera	Sewage	AMP,ATM,FOX,AMC,W	TEM
2	E.agglomerans	Kibera	Sludge	AMP,FOX,S,RL,W	N/D
4	E.agglomerans	Kibera	Sludge	AMP,FOX,AMC	N/D

Figure 4.7: Cluster analysis of recover *Enterobacter agglomerans* from sewage, soil and sludge

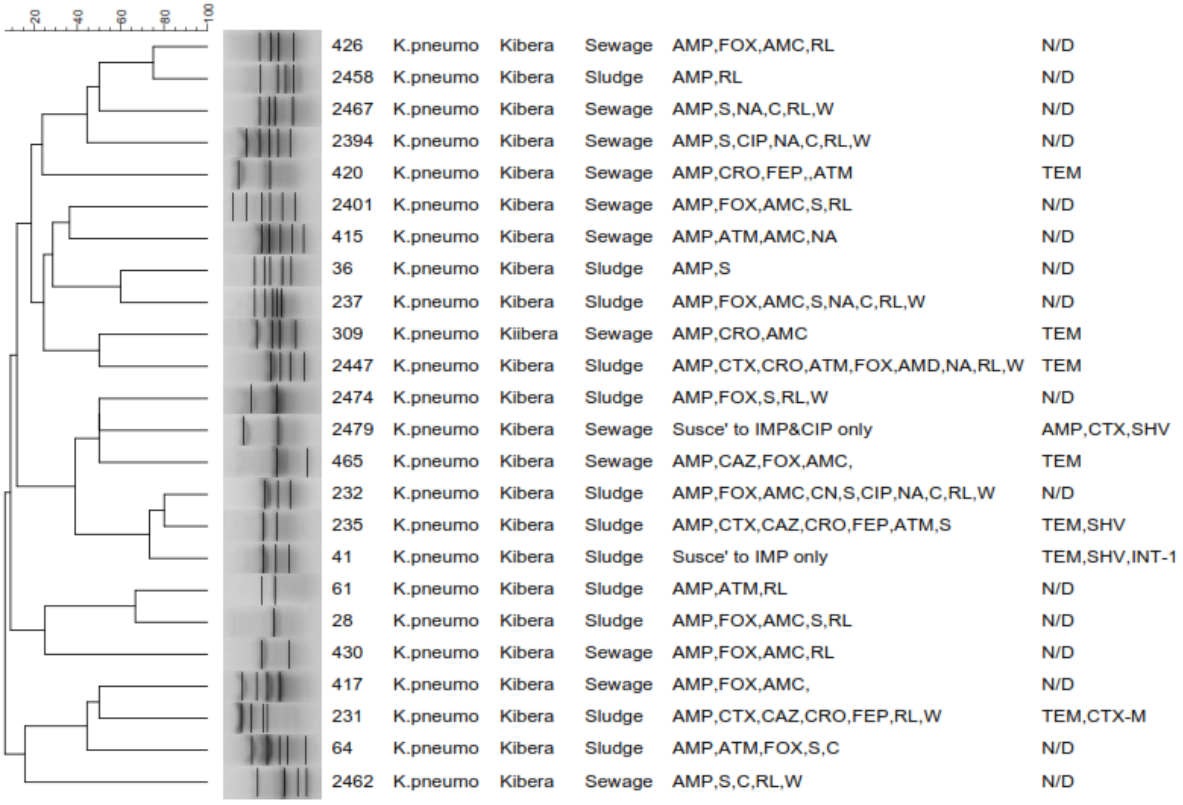


Figure 4.8: Cluster analysis of recover *Klebsiella pneumoniae* from sewage and sludge

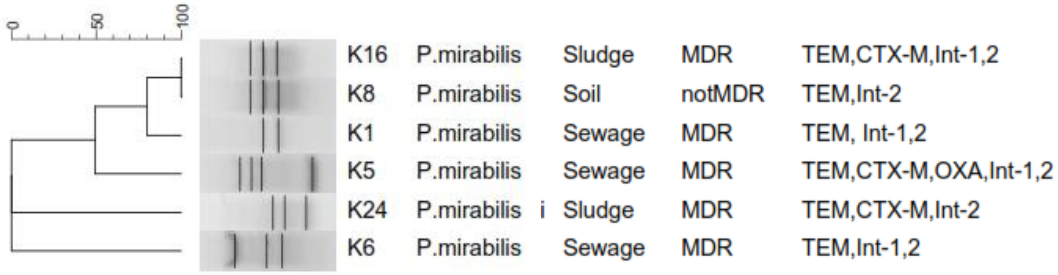


Figure 4.9: Cluster analysis of recover *Proteus mirabilis* from sewage, sludge and soil

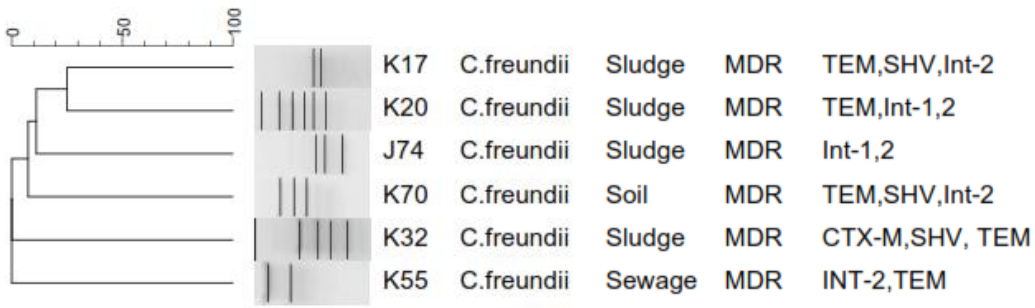


Figure 4.10: Cluster analysis of recover *Citrobacter freundii* from slum soil, sewage and sludge samples

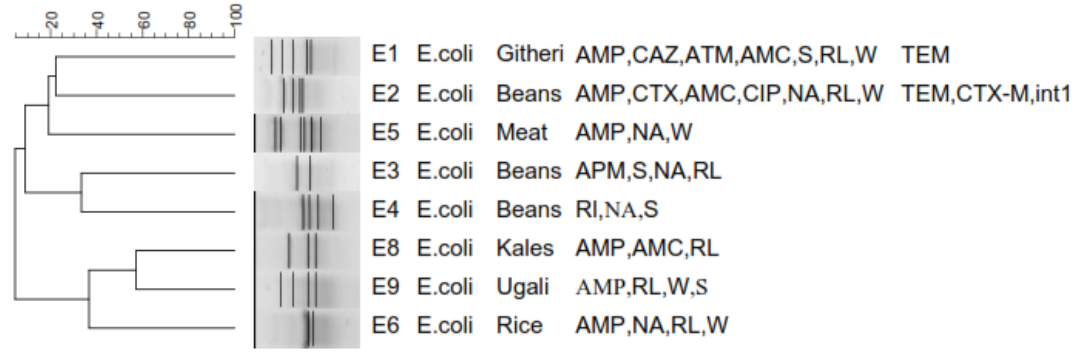


Figure 4.11: Phylogeny analysis of *Escherichia coli* isolates from ready-to-consume foods

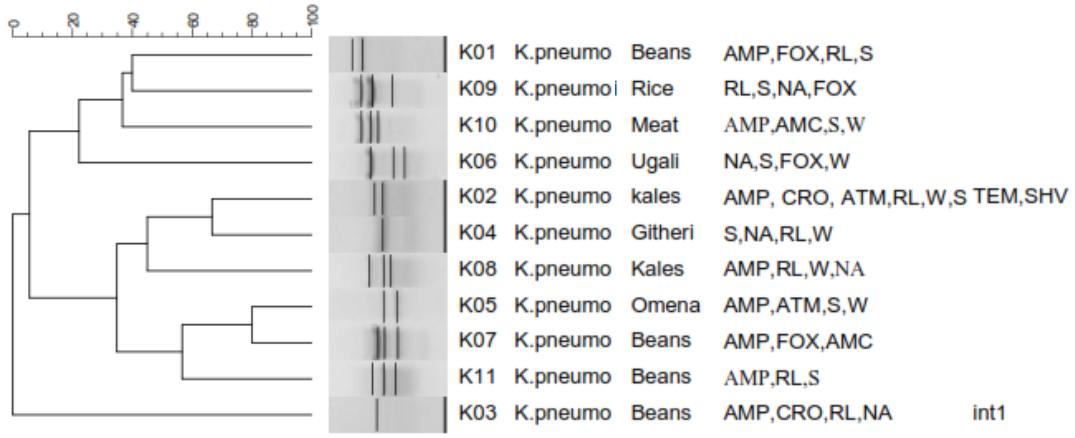


Figure 4.12: Phylogeny analysis of *Klebsiella pneumoniae* isolates from ready-to-consume foods

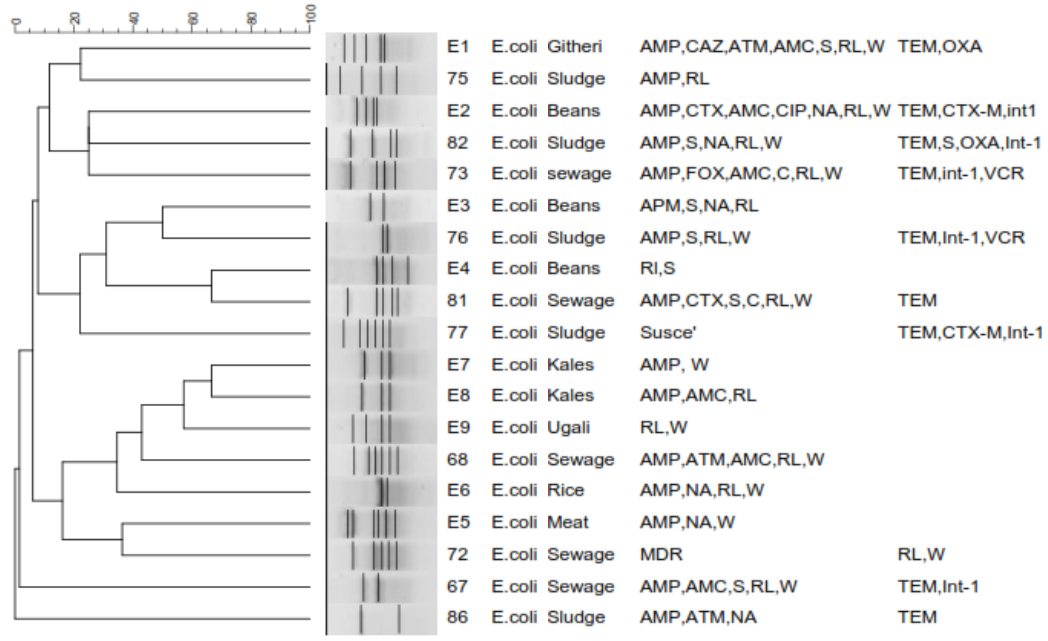


Figure 4.13: Cluster analysis of *Escherichia coli* isolates from ready-to-consume foods and environmental samples from Kibera slums.

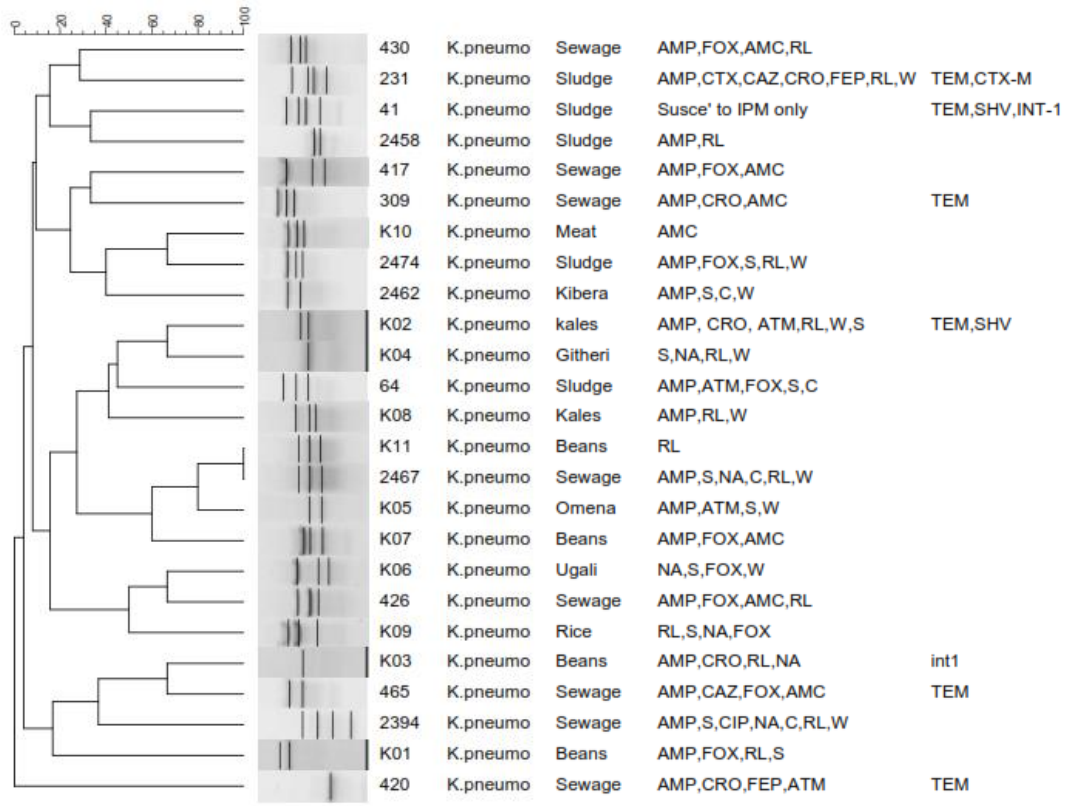


Figure 4.14: Cluster analysis of *Klebsiella pneumoniae* isolates from ready-to-consume foods and environmental samples from Kibera slums.

CHAPTER FIVE

DISCUSSION

5.1 Microbial Food Contamination of Ready-To-Eat Foods From Kibera Informal Settlements

The current study recorded significant microbial contamination of greater than 10^4 CFU/mL in 38% of ready-to-eat foods sold on the streets of Kibera informal settlements in Kenya. These results are an indication that most street food sold in this slum is unfit for human consumption and health risk to such consumers. This is the first attempt to link contamination level of food-types and the sanitation conditions of the vending points in Kenya. This is the first report on major resistance phenotypes and genotypes encountered among isolates recovered from foods sold in Kenya's slum setting.

The mean of between 4.0×10^4 CFU/mL and 2.3×10^6 CFU/mL recorded in the current study for foods sold in these settings are close to 6.7×10^5 to1.7×10^6 CFU/mL published on fish foods in Ethiopia (Eromo *et al.* 2016a). The highest contamination was recorded in beans among the cereal-type foods with a mean of 3.4×10^5CFU/mL, which is lower than 8.1×10^3 CFU/mL reported in maize/beans (Githeri) in Mukuru slum in Kenya (Muoki *et al.* 2008). This study showed that vegetables such as kales recorded the highest levels of contamination (2.3×10^6 CFU/mL) and these findings correlate with a similar study in South Africa that showed prevalent contamination among vegetable foods (6.8 $\log_{10}$ CFU/g^{-}) (Nyenje *et al*. 2012). Vegetable contamination has widely been attributed to unsafe urban irrigation using sewage water heavily contaminated with human excreta (Qadir *et al.* 2010). This study, therefore, confirms that most street foods sold in this slum are highly contaminated. Therefore, the increase in cases of diarrhea and other gastrointestinal infections may be as result from the contaminating enteropathogens (Muoki *et al.* 2008; Nyokabi *et al.* 2018). Some entero-pathogens such as *Salmonella spp* have a low infection dose and isolation from these foods pose a risk of diarrhea and gastrointestinal infections by the consumers.

5.2 Environmental Risk Factors for Microbial Contamination of Street Foods

Inadequate cooking of foods is a significant source of food-borne infections, however, contamination from fecal and environmental sources is frequently implicated heavily in such diseases (Odwar *et al.* 2014). The vending points map generated in this study showed that street food-vending points were concentrated in areas with a broad customer reach, which consequently constrain the resources available. As a result, waste emanating from food processing is disposed of very close to the vending points, which may serve as breeding sites for rodents, flies and other vectors that disseminate food-associated pathogens (Rane, 2011). This data strongly suggests that lack of proper sanitation facilities such as adequate sewage drainage systems, sufficient toilets and clean water significantly contributes to the contamination of slum foods. These findings correlate with those from a previous study that noted that ready-to-consume foods sold near unhygienic environments such as dumping sites and burst sewers are more contaminated, which therefore calls for better sanitation and hygiene standards (Kariuki *et al.* 2017; Kariuki *et al.* 2018).

5.3 Microbial Diversity of Food Contaminants and Environmental Compompartments

The current study recorded a diverse group of non-fastidious Gram-negative bacteria isolates in environmental samples from the slum area. The majority of genera isolated are those known to reside in human and animal gut such as *Escherichia, Klebsiella* and some are known Enteropathogens such as *Salmonella* and *Shigella* species. Although clinical reports of *Salmonella* and *Shigella* species are on declines, environment colonization may be a source of typhoid and *Shigella* pathogens. Human fecal waste and untreated domestic waste could likely be seeding the environment with bacteria of enteric origin and some of these may have clinical roots. This study underpins the role of environmental contamination as a driving factor for the emergence of outbreaks, some of which are caused by MDR strains. Also isolated were multiple drug-resistant *P. aeruginosa* and *A. baumannii;* These organisms have previously been associated with a wide range of difficult-to-treat infections (Bonomo and Szabo 2006). This study's bacteria diversity is comparatively broader compared to that reported in a related study conducted in 2013 and analyzed the microbial content and resistance profiles of isolates obtained from the sewage-contaminated Nairobi river (Musyoki *et al.* 2013). Therefore, there is a clear indication that ecosystem contamination with domestic wastes in a gradual increase in contamination of the environment.

This study recovered a wide range of Gram-negative bacterial isolates implicated in severe and difficult to treat infections such as *Salmonella ssp, Pseudomonas aeruginosa* species and *Acinetobacter baumannii*. Among food samples, kales and meat had the most diverse bacterial contaminants, with *E.coli* and *Klebsiella spp* been the most dominant species. Considering that diarrhea is a significant cause of morbidity and mortality in developing countries and that *Shigella, E.coli* and *Salmonella* genera are documented as the major etiological agents' slums such as Kibera, the fact that this study recovered strain belonging to these pathogens strongly implicates contamination of food to the transmission of these pathogens (Samuel *et al*. 2019).

5.4 Antimicrobial Resistance Patterns of Gram-Negative Bacteria Isolates from Sewage, Sludges and Soil Isolates from Kibera

The current study reported a diverse range of MDR bacteria belonging to species such as *Salmonella spp, Shigella spp, E. coli* and *Klebsiella spp* from sewage, sludge and soil in Kibera near these food vending points (Maina *et al.* 2019). Therefore, there is an excellent possibility that most of the enteric isolates reported in street foods emanate from surrounding unhygienic surroundings. Although virulence genes among such isolates were not screened for, consumption of foods contaminated with *Salmonella spp* may potentially cause gastrointestinal infections and death (Linscott ,2011). *Salmonella* species in this study were recovered without the usual 24 hours enrichment in modified Rappaport Vassiliadis protocols and therefore, it is possible that the occurrence of this pathogen in our food specimen could be higher than what is reported.

A recent study conducted in Kibera that sought to characterize *E coli, Salmonella* and *Shigella* species in water, soil, vegetables and meat reported a resistance prevalence of 56.8% towards ampicillin, 29.6% for trimethoprim-sulfamethoxazole, 13.6% for streptomycin, 4.9% for nalidixic acid and 2.5% for gentamicin (Christabel *et al.* 2018). This previous study's values were generally lower than what we reported in this study by a factor of greater than 10%. Although the reasons behind this apparent rise in resistance prevalence are not clear, (Kummerer 2009) has reported that more than 80% of antibiotics consumed by humans and animals are excreted through urine and feces. Therefore, many active residues may end up in the sewage, sludge, and other environmental compartments, thereby presenting a strong selection pressure that favors MDR strains' proliferation. Such agents in the environment can consequently provide an intense selective pressure that preferentially allows MDR clones to

spread (Sarmah *et al.* 2006). Although this study did not assess the presence of antimicrobial agents' residues in the environment, these finds strongly underpin the need for such a study.

This study shows an increase in antimicrobial resistance in environmental compartments than a previous study conducted in Kakamega town in Kenya (Malaho *et al.* 2018). The former research reported *E. coli* from recovered from dumpsites, sludge and wastewater. This study reported a 3% increase in resistance towards amoxicillin-clavulanic acid, gentamicin and ciprofloxacin in *Citrobacter species, Enterobacter spp.* and *Klebsiella spp* compared to the survey by Malaho *et al.* (2018). This indicates that the more populated urban environment is highly contaminated with resistant bacterial strains, possibly due to the high pollution rate in these settings.

5.5 Antimicrobial Resistance Patterns of Gram-Negative Bacteria Isolates from Ready-To-Eat Foods from Kibera Informal Settlements

Besides high resistance among isolates from environmental sources, this study also showed higher resistance values to antimicrobials among isolates obtained from foods. Most food isolates were resistant to ampicillin (AMP), trimethoprim (W) and sulfamethoxazole (RL) and this finding are in line with those published previously showing the resistance of 34% towards AMP and of 49.4% towards sulfamethoxazole-trimethoprim (SXT) among *E.coli* recovered from raw chicken in Kenya (Odwar *et al*. 2014). Compared to the finds of Odwar *et al* (2014) study, the current study recorded higher resistances towards amoxicillin-clavulanic acid (AMC 11% vs. 2.6%), ceftazidime (CAZ 2% vs. 0%), and gentamicin (CN 5% vs 0.6%). However, a similar study in Ethiopia has documented higher resistances of up to 80% for ampicillin, 14.3% for ceftriaxone and 9.5% for gentamicin in *E coli* from fish (Eromo *et al.* 2016). The variation in resistance patterns strongly indicates microbial isolates in these regions are evolving and developing antimicrobial resistance independently, which is likely to reflect the pattern of antimicrobial usage and AMR burden but these assumptions remain to be elucidated. These results collectively suggest that the resistant strains may be emanating from the immediate surroundings to contaminate foods consumed and sold in the informal settlements. Resistances of up to 2% towards ceftazidime, ciprofloxacin and 9% against aztreonam have been documented in *Klebsiella* species from stool samples in Kenya (Taitt *et al.* 2017). These resistances are similar to those recorded in food isolates in our study, suggesting a high possibility that such isolates may also have a clinical origin. In the

current study bacterial isolates from kales were the most resistant to tested antimicrobial agents, possibly due to contamination from sewage-contaminated water.

5.6 Carriage Of β-Lactamases and Integron in Gram-Negative Isolates from Food and Environmental Samples from Kibera Informal Settlements

Carriage of extended-spectrum β-lactamases (ESβL) was prevalent in environmental isolates compared to those recovered from food (12% vs. 4%). The distribution of ESβL-producing strains was more concentrated in highly populated villages in the Kibera, possibly due to microbial contamination emanating from highly contaminated surroundings. Distribution of integron classes 1 and 2 was also prevalent in the same region. Therefore, we suspect that these elements could be spreading associated resistances and the exchange of plasmids carrying such elements in the sewage. Although this study did not screen for plasmids implicated in these resistances, *bla*$_{\text{CTX-M}}$, *bla*$_{\text{SHV,}}$ *bla*$_{\text{OXA}}$ and *bla*$_{\text{TEM}}$ have been reported in plasmids such as *inc*F, *inc*FIB, L/M, *inc*F, *inc*F11 and *inc*H12 in clinical *E. coli* isolates in Kenya (Kiiru *et al.* 2011). The resistances observed in this study strongly suggest the involvement of integron cassettes encoding resistance to aminoglycosides such as gentamicin, trimethoprim and β-lactams ceftriaxone and cefotaxime. Past studies in Kenya have reported the involvement of integrons carrying sets of resistance cassette arrays such as the *dfrA*1-*aadA*1, *aadA*1, *drfA*17-*aadA*1 in class 1 integron that were associated with a broad range of antimicrobial resistances such as aminoglycosides sulfamethoxazole-trimethoprim in the enteric belonging to the Enterobacteriaceae family (Kiiru *et al*. 2013). The prevalence of class 1integron was higher than that of class 2 integrons and this finding is correlated with that of another study in Iran (Tajbakhsh *et al.* 2015). Integron class 1 is mostly associated with mobile plasmids easily shared among Gram-negative bacteria hence more prevalent (Gillings *et al*. 2008). Although the current study did not determine the detected integrons' content, the *drfA*1-*sat*2-*aad*A1, *sat*2, *sat*1-aadA1cassettes in class 2 integron have previously been associated with resistance towards sulfonamides, diaminopyrimidines, streptothricin and β-lactams (Wu *et al.* 2012). Most isolates that carried class 2 integrase were also positive *intI*1 carriage. It is there likely that most isolates that were resistant to trimethoprim and sulfamethoxazole had these integrons.

5.7 Genetic Relatedness of Bacterial Species from Food and Environmental Isolates from Kibera Informal Settlements

Most environmental and food isolates had less than 70% similarity in resistance phenotypes and genotypes, suggesting little evidence of the clonal expansion among *Citrobacter* and *Salmonella* species. However, tight clustering of ≥ 70 similarity matrix among some few *E. coli, K. pneumoniae, E. agglomerans* and *P. mirabili*s strains isolates also had similar resistance phenotypes. These findings suggest that MDR, BFQA and/or ESBL phenotypes may be spreading through horizontal gene transfer mechanisms rather than through the expansion of major clones. Some isolates from foods were significantly close to those from environmental sources, phenotypically and genetically, further suggesting that food may be contaminated from environmental sources, most likely water. The methods used in this study for the clonal association are not highly sensitive such as current methods that utilize whole genome sequencing (WGS) strategies. Therefore, it is possible to use next-generation approach in future to gain insights into the transmission pathways of the isolate from the environment to the food and, eventually, to patients. Such methods will also reveal the mechanism of emergence, exchange and dispersal of critical resistance determinants such as ESBLs, and those encoding resistance to fluoroquinolones and advanced classes of aminoglycosides such as amikacin and gentamicin.

5.8 Study Limitations

This study experienced several shortcomings that can be a basis for the formulation of more robust studies in the future;

1. The study was only able to screen for a few β-lactamases genes. This study could also not determine the bases of resistance to other classes of antimicrobial agents such as aminoglycosides and fluoroquinolones.
2. The study was also unable to determine the content of detected *int*1and whether they were borne in mobile genetic elements such as plasmids.
3. Low resolution $(GTG)_5$ fingerprint method was used to establish bacteria phylogeny and genetic relatedness. Futures studies should apply high-resolution methods such as whole-genome sequencing and SNP typing, shedding more light on resistance gene content and bacteria evolution in the environmental compartments.

CHAPTER SIX

STUDY CONCLUSION AND RECOMMENDATIONS

6.1 Study Conclusion

Findings and analysis from this study arrived at the following vital conclusions;

1. The high level of food contamination noted strongly suggests that most ready-to-eat foods sold in the streets surrounding poor sanitation standards are unfit for human consumption and a significant health risk.
2. These findings suggest a need for clean water supply and proper sanition stards and hygiene (WASH programs) to control contamination and reduce foodborne illnesses.
3. Recovery of enteric pathogens such as *P.aeruginosa, Salmonella* and *Shigella spp* is a strong indication of high risks of dispersal and contamination of foods by sewage and sludge site of food preparation and vending.
4. Some MDR-strains recovered in this study may cause foodborne infections that may be challenging to treat with β-lactams, high-end aminoglycosides, and fluoroquinolones ciprofloxacin.

6.2 Study Recommendations

Foods served and prepared in unhygienic environments pose a risk of emanating and spreading severe foodborne infections. To close or minimize loopholes that may lead to foods outbreaks, a public health official and relevant government bodies should address the following issues;

1) Provide awareness education to food vendors on proper sanitation and hygiene practices
2) Improving the environment where foods are prepared to reduce the risk of contaminations that may emanate from such surroundings.
3) The government should deliberate efforts to improve sanitation infrastructure in these informal settlements that should include better sewage drainage systems and effective garbage management.

4) Most vendors and residents of Kibera informal settlements constant clean water supply; this essential resource key in all aspects of life should therefore be made available to ensure food is prepared in a hygienic way as possible.
5) Similar future studies should use high-resolution methods such as the whole genome sequencing (WGS) to shed more insight into resistance genotype and phenotypes of isolates recoverable from environment ecosystems.

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APPENDsICES

Appendix I: Research Consent

Research title

Mapping the distribution patterns of Multi-drug resistance strains of selected Enterobacteriaceae species from food and environmental samples in Kibera, Nairobi.

Research investigators

Principal investigator:

John Ndemi Maina

Affiliation: Jomo Kenyatta University of Agriculture and Technology (JKUAT).

Attribute: Medical Microbiology M.Sc. student

Co-investigators

1. Dr. John Kiiru
   Affiliation: Kenya Medical research institute (KEMRI)
   Attribute: Senior researcher at KEMRI.
2. Prof. Anne W T Muigai
   Affiliation: Jomo Kenyatta University of Agriculture and Technology (JKUAT).
   Attribute: Professor of genetics at JKUAT.

What is Jomo Kenyatta University of Agriculture and Technology (JKUAT)?

Jomo Kenyatta University of Agriculture and Technology (JKUAT) is a public university situated in Juja, Kiambu County in Kenya. This university was started in 1981 as a middle-level college offering certificate and diploma courses. Today the university offers courses in agriculture, architecture, engineering, technology and biology.

What is Kenya Medical research institute (KEMRI)?

Kenya Medical research institute is a medical research institution in Kenya, established in 1979. This institution was established as a national body for carrying out health research. This institution has 14 centers, among them the Center for microbiology research (CMR).

What is the purpose of this research?

You are being invited to take part in this study. We aim to determine the presence of bacteria that can cause illness in food and drinking water. The purpose of collecting the environmental samples that include sewage, soil and sludge is to detect the source of such bacteria that can find their way into our food through unhygienic practices.

Therefore, we request your consent to participate in this study and allow us to do the following; however, it is within your rights to decline to participate in this study.

1. To allow this study to obtain a small sample of food and drinking water from your food vending point.
2. To allow this study to a sample of sewage, soil and sludge near your food vending point.
3. To allow this study to ship the specimen to our laboratory for analysis. We also assure you that the analysis results will be treated with confidentiality and no unauthorized person will have access.
4. To allow this study to use the collected samples in this study and any other related research in the future that may generate data that have potential benefits in improving human health or improved strategies for treatment
5. To allow this study to ship the isolates or the DNA obtained from your sample to other laboratories in Kenya and abroad if need be for experiments that are not available or are not easy to perform locally.

I would also like to disclose to you the following;

a) That some questions I will ask may be uncomfortable. Still, care will be taken to ensure that the data collected will also be confidential and will only be accessible to relevant authorities. The data will also be encrypted to ensure your privacy.

b) This study intends to do tests on the food and water samples to determine whether the sample has microbial contamination.
c) This study intends to test the environmental samples that I will collect to determine whether the recoverable bacteria are similar to those isolated from the food and water samples.
d) I intend to take the GPS coordinates of your food sampling point.
e) That there are no monetary gains for participating in this study.
f) That there are no penalties for declining participation in this study.
g) That you can withdraw from this study at any time.
h) That you may decline to answer any of my questions or any question that you may find inappropriate.

Your part in the research

To investigate the presence of bacterial food and water contamination, I ask you to consent on your behalf to participate in this research project.

You are also requested to fill a questionnaire that I will read and explain to you in either English or Swahili if you cannot do that.

What will happen during and after the study?

The samples and data collected will determine the microbial contamination of foods and the occurrence of disease-causing germs. The bacterial isolates obtained from the sample collected will determine antimicrobial resistance and screen for resistance determinants.

If need be, the shipment of isolates or the DNA obtained from the isolates will be shipped to other laboratories in Kenya and abroad for experiments that are not available or are not easy to perform locally, such as the whole-genome sequencing. The samples shipped overseas will be destroyed immediately after the stated analysis has been completed.

After analyzing the data obtained, a map will be generated to show the distribution of multiple drug resistance hotspots.

What if I change my mind about helping with this research?

If you agree to participate in this study and later change your mind, you are free to withdraw at any time. You will not be discriminated against in any way in the future due to your decision to withdraw or to decline to participate.

Who will read or hear about information collected from me?

The information collected from those who help with this research will be stored using codes so that each individual cannot be recognized. Coded information will be held on computers protected by passwords known to the research team only.

Do you have any questions that you would like me to answer now? If you would like to know more details about the research or have any issues about your rights that need to be discussed in the future you can contact any of the following people with priority being the SERU contact.

1. **The Secretary, KEMRI/SERU. P.O Box 54840-00200, Nairobi Tel. 2722541 Ext. 3307.**
2. **John N Maina- 0715132829**
3. **Dr. John Kiiru, CMR- KEMRI - 0721-805285**

I have read the above information and have had the opportunity to ask questions and all of my questions have been answered satisfactorily. I consent to participate in the study as has been explained and as I have understood it.

Signature …….

Date …….

Name of principal investigator……

Signature……

Date…….

Thumb print impression for those who can't sign

Appendix II: Ridhaa ya utafiti
Kichwa cha utafiti

Kuweka mtawanyiko wa vimelea visivyo sikia dawa vinavyo patikana kwenye chakula na mazingira kwenye ramani.

Wachunguzi Wa utafiti

Mtafiti mkuu:

John Ndemi Maina

Uhusiano: Chuo kikuu cha ukulima na tecnologia cha Jomo Kenyatta (JKUAT)

Sifa: Mwanafuzi wa somo la sayansi ya uzamili wa viini

Wahusika wengine:

1. Dr. John Kiiru
   Uhusioano: Shirika la utafiti wa matitabu la Kenya (KEMRI)
   Sifa: mtafiti mkuu katika Shirika la utafiti wa matitabu la Kenya (KEMRI).
2. Prof. Anne W T Muigai
   Uhusiano: Chuo kikuu cha ukulima na tecnologia cha Jomo Kenyatta (JKUAT)
   Sifa: Profesa wa somo la genetics katika Chuo kikuu cha ukulima na tecnologia cha Jomo Kenyatta

Je ni nini maana ya Chuo kikuu cha ukulima na tecnologia cha Jomo Kenyatta (JKUAT)?

Chuo kikuu cha jomo Kenyatta ni chuo cha kitaifa kinachopatikana mjini Juja katika jumbo la kiambu. Chuo hiki kilianzishwa mnamo mwaka 1981 kama chuo cha elimu ya juu ya cheti na stashahada. lakini kwani hivi sasa chuo hiki kinatoa shahada mbalimbali katika masomo ya ukulima, ujenzi, inginia, teknolojia na biologia.

Je nini maana ya shirika la utafiti wa matitabu la Kenya (KEMRI)

KEMRI ni shirika la utafiti wa kimatibabu lililo anzishwa mnamo mwaka 1979. Shirika hili na madhumuni ya kuwa shirika kuu la kitaifa linalo husika na utafiti wa matitabu Kenya. Shirika hili la KEMRI lina vituo kumi na vinne mojawepo ikiwemo kituo cha utafiti wa viini (CMR) ambacho kinahusika na utafiti wa viini vinavyo sababisha magonjwa.

Ni ipi azima ya utafiti huu?

Mwanzo ni kukualika kuhusika katika utufafiti huu. Madhumuni ya utafiti huu ni kutafuta vimelea vinavyo sababisha magonjwa kutokana na vyakula na maji tunayo kunywa. Utafiti huu pia utahusisha ukusanyaji wa sampuli kwenye mazingira kama vile maji machafu na tope kubaini chanzo cha vimelea vinavyo patikana kwenye vyakula na maji ya kunywa kwa njia ya kutozingatia usafi. Ili kuwezeshesha kubaini njia ama mbinu zinazohusika katika kusababisha mchafuko wa vyakula na maji, tutaukuuliza maswali kadhaa baada ya kuchukua sampuli ya vyakula na maji ya kunywa.

Tunakuomba kutoa kibali chako kuhusika katika utafiti huu na kuturuhusu kufanya yafuatao; Lakini pia nikujulishe unahaki ya kukataa kuhusika katika utafiti huu.

1. Uturuhusu kukusanya sehemu ngogo ya sampuli ya vyakula na maji ya kunywa katika kibanda chako.
2. Pia tunaomba kibali chako uturuhusu kuchukua sampuli ya maji chafu, mchanga na tope karibu na kibanda chako.
3. Uturuhusu pia tupeleka sampuli tulizo kusanya katika maabara yetu. Pia tunatoa hakikisho letu kuwa matokeo ya utafiti huu yatakuwa ya siri na kwamba hakuna mtu asiyekuwa na kibali ataweza kupata matokio haya.
4. Tunaomba kibali chako ilikutumia sampuli tutakazo chukua katika utafiti huu kama njia ama mbinu ya kuhifadhi na kuboresha afya
5. Tunaomba kibali chako pia kutuma sampuli katika maabara mengine hapa kenya ama ulaya iwapo maktaba yetu hayatakua na umezo wa kufanya utafiti ulio bora zaidi.hii ni Iwapo tu kutakua na hoja.

Pia ningetaka kukujulisha maelezo yafuatayo;

a) Baadhi ya maswali tutakayouliza yanaaibu lakini hakikisho letu ni kuwa majibu utakayotupatia itakuwa siri na hakuna yeyote asiyekuwa na kibali atayapata.

b) Nina madhumuni ya kufanya utafiti nikitumia sampuli ya vyakula na maji kubaini idadi ya vimelea.
c) Ni madhumuni yangu pia kufanya utafiti katika sampuli ya mazingira ili kubaini kama vimelea kitakavyo patikana ni sawa na vitavyopatikana katika sampuli ya vyakula na maji.
d) Ningetaka kukujulisha ya kwamba nitatumia simu yangu ya rununu kuchukua kodi speshieli za kubaini kibanda chako kwenye ramani ya dunia.
e) Lamuhimu pia ni kukujulisha kwamba hakuna manufaa yeyote ya kifedha utakayopata kutokana na kuhusika kwako katika utafiti huu.
f) Pia hakuna adhabu yeyote itatokana na uamuzi wako wa kutojihusisha na utafiti huu.
g) Unahaki pia ya kujiondoa katika utafiti huu ata baada ya kutoa idhini ya kuhusika.
h) Ningekata kukujulisha pia unahaki ya kutojibu swali ama maswali yeyote utakayohisi hayajakufurahisha.

Jukumu lako katika utafiti huu

Ili kuwezesha kubaini uwezekano wa vimelea katika vyakula na maji, ningependekeza utoa idhini kuhusika katika utafiti huu.

Ni ombi langu pia utatusaidia katika utafiti huu kwa kujibu maswali kadhaa tutakayo uliza katika lugha unayoielewa vizuri.

Ni yapi yatakayotendeka katika na baada ya utafiti huu?

Matokeo ya utafiti huu yatahusika katika kutoa taarifa inayoelezea mbinu na njia za kutambua vimelea vinavyohusika katika uchafuzi wa maji na chakula katika mazingira na katika vibanda vya chakula.Aina ya vimelea vitakavyopatikana kusababisha uchafuzi wa maji na chakula vitatumika katika ubainishaji wa dawa bora za kutumia katika maraadhi.

Kunauwezekano kuwepo na mahitaji ya kusafirisha sampuli tulizo kusanya kupelekwa kwenye maabara mengine hapa nchini kenya n ahata ughaibuni kwa utafiti Zaidi kuhusiana na mpangilia wa dna

Baada ya utafiti kuhitimishwa ramani itakayoonyesha utapakaaji wa vimelea vya uchafuzi wa chakula na maji katika sehemu tofauti ambako utafiti huu ulifanyika

Itakuwaje nikigeuza nia yangu kuhusu kujihusisha na utafiti huu

Ukikubali kujihusisha na utafiti huu halafu ugeuze nia utakuwa na uhuru wa kutoka wakati wowote Hautotelekezwa ama kulaumiwa kwa sababu ya maamuzi yako na wala hutokatazwa kujihusishwa tena katika utafiti huu.

Watakao husika katika ujumbe nitakao utoa

ujumbe unaokusanywa na wanaohusishwa katika utafiti huu utahifadhiwa kwenye kodi spesheli ilikuwezesha kutogunduliwa kwa wahusika. Kodi hizo spesheli zitafungiwa kwenye kompyuta na watu wasiohusika hawatoweza kufikia ujumbe huu kwa hali yoyote.

je unaswali lolote ungelipenda nikujibu? Kama ngependa kujua Zaidi kuhusu utafiti ama labda Zaidi kuhusu haki unazopendekeza zizingatiwe kwasasa ama hapo badae unaweza kuwasiliana na wahusika ambao ni SERU contact

1. **The secretary, KEMRI/SERU.P.O BOX 54840-00200, Nairobi Tel.2722541 Ext.3307**
2. **Mr. John N Maina-0715132829**
3. **Dr. john Kiiru,CMR-KEMRI-0721-805285**

Nimeusoma ujumbe huu na nikapewa nafasi ya kuuliza maswali na nikajibiwa hadi nikaridhika

Nimeridhia kuhusika katika utafiti huu kama jinsi nilivyoelezwa nikaelewa.

Sahihi ……

Tarehe ……..

Jina la mtafiti mkuu ……..

Sahihi ……..

Tarehe ……..

Thumb print impression for those who can’t sign

Appendix III: Sample collection form

Mapping the distribution patterns of multidrug resistance strains of selected Enterobacteriaceae *spp* from food and environmental samples in Kibera, Nairobi.

Sampling location

Sampling date

Sample code number

GPS coordinates

Sample type

Food ☐ Drinking water ☐ Water from pools ☐

Soil ☐ Sludge ☐

Food type

Observation features during sampling

1. Sanitary features around the food vending point;

i.	Near a toilet	Yes	☐	No	☐
ii.	Near a dumping site	Yes	☐	No	☐
iii.	Near open or burst sewer	Yes	☐	No	☐
iv.	Near council water supply	Yes	☐	No	☐

v. Other features

2. The layout of the food vending point; Open ☐ Closed ☐

3. Food type sold at the sampled vending point;

4. Does the vendor wear PPE such as aprons, gloves and headscarves when handling food?

   Yes ☐ No ☐

5. Does the vendor handle money while serving or preparing food?

   Yes ☐ No ☐

Enrichment medium

BPW ☐ APW ☐ MSRV ☐

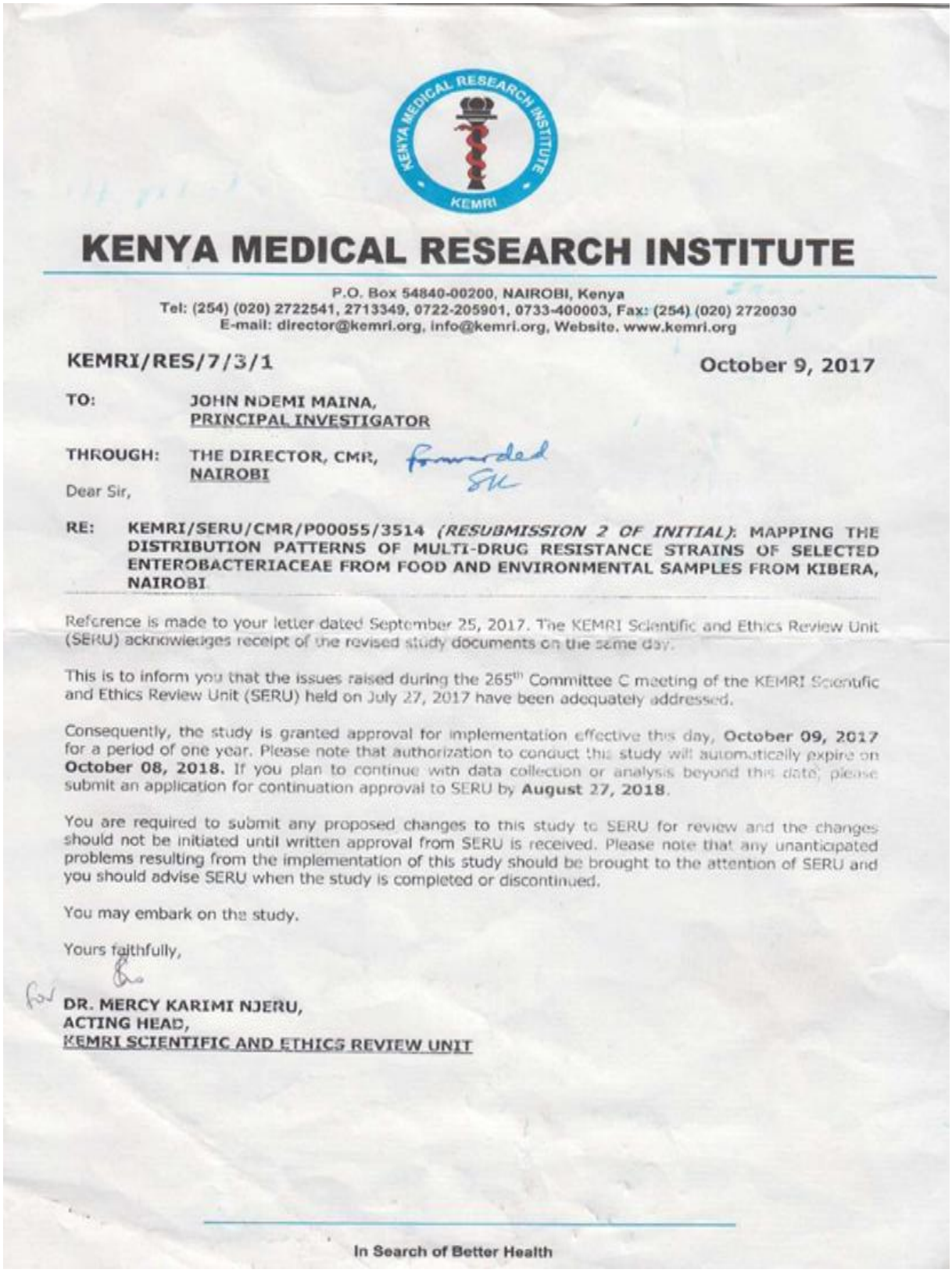
Isolation medium

EMBA ☐ MacC ☐ TCBS ☐ XLD ☐

Isolation medium	Colony morphology	Presumed organism	No. of isolates stocked	Gram stain	Organism (after biotyping)
Eosin methylene blue agar (EMBA)					

MacConkey salt agar (MacC)					
Thiosulfate citrate bile salt sucrose agar (TCBS)					
Xylose lysine deoxycholate agar (XLD)					

Appendix IV: SERU, KEMRI study approval letter



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KEMRI/RES/7/3/1 **October 9, 2017**

TO: **JOHN NDEMI MAINA,**
PRINCIPAL INVESTIGATOR

THROUGH: **THE DIRECTOR, CMR,**
NAIROBI

Dear Sir,

RE: **KEMRI/SERU/CMR/P00055/3514** ***(RESUBMISSION 2 OF INITIAL)*****: MAPPING THE DISTRIBUTION PATTERNS OF MULTI-DRUG RESISTANCE STRAINS OF SELECTED ENTEROBACTERIACEAE FROM FOOD AND ENVIRONMENTAL SAMPLES FROM KIBERA, NAIROBI**

Reference is made to your letter dated September 25, 2017. The KEMRI Scientific and Ethics Review Unit (SERU) acknowledges receipt of the revised study documents on the same day.

This is to inform you that the issues raised during the 265th Committee C meeting of the KEMRI Scientific and Ethics Review Unit (SERU) held on July 27, 2017 have been adequately addressed.

Consequently, the study is granted approval for implementation effective this day, **October 09, 2017** for a period of one year. Please note that authorization to conduct this study will automatically expire on **October 08, 2018.** If you plan to continue with data collection or analysis beyond this date, please submit an application for continuation approval to SERU by **August 27, 2018**.

You are required to submit any proposed changes to this study to SERU for review and the changes should not be initiated until written approval from SERU is received. Please note that any unanticipated problems resulting from the implementation of this study should be brought to the attention of SERU and you should advise SERU when the study is completed or discontinued.

You may embark on the study.

Yours faithfully,

DR. MERCY KARIMI NJERU,
ACTING HEAD,
KEMRI SCIENTIFIC AND ETHICS REVIEW UNIT

In Search of Better Health

Appendix V: Published manuscript

Scientific Research Publishing

Advances inMicrobiology,2019,9, 507-524
http://www.scirp.org/journal/aimI
SSN Online: 2165-3410
ISSN Print: 2165-3402

Antimicrobial Profiles of Selected Gram-Negative Bacteria Recoverable from Sewage and Sludge from Juja and Kibera Informal Settlements of the Larger Nairobi Metropolis

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Abstract

Africahasexperiencedrapidurban migration inthepasttwodecades.New informalsettlementscontinue toemergeandexpandbutthesanitation provisionoffacilitieshasnotimprovedatthesamepaceandthisposesaserious healthconcerntothepublicespeciallytheurbanpoor.Opensewagesystems and sludge-clogged drainage systems as well as soil contaminated with industrial anddomesticwastesarepossiblesourcesofgermsthat probably causeclinicalinfectionsandepidemics.Inthiscross-sectionalstudy,we recordeddiversegeneraofGram-negativenon-fastidious bacteriathatincluded; Escherichiacoli(23%),Klebsiellaspp(21%),Enterobacterspp(19%),Citrobacterspp(10%),Pseudomonasaeruginosa (8%),Proteusspp(7%),Salmonella(3%),Yersiniaspp(3%),Shigellaspp(2%),Morganellamorganii(2%), Edwardisellaspp(1%),Hafniaspp(1%),Serratiamarcesence(0.5%),and Acinetobacterbaumannii(0.5%).Mostoftheseisolateswereresistanttoampicillinwhile imipenemandciprofloxacinwere themosteffective antimicrobi- al agents.Resistancecombination towardsampicillin,trimethoprim,sulfamethoxazoleand streptomycin wasalsonoted inrecoveredisolates(16%).An overallhighantimicrobial resistancewasrecordedamongisolatesfromslum ascompared tothoserecoveredfromJuja,amiddle-classsettlementlocated attheedge of Nairobimetropolis.

isolates(13%)butnoneoftheYersiniaspeciesandEdwardisellatardaexhibitedthisresistance.Carriageof bla_{TEM}(52%)wasmostprevalentinallbacteriaspeciesfollowedbybla_{CTX-M}(20%),bla_{SHV}(18%)whilebla_{OXA}(17%)was theleastcommon. Thephylogenyanalysisrevealedsignificantgeneticsimilarityamongstrainsbelongingto*E.coli*,*K.pneumoniae*,*E.agglomerans*and *P.mirabilis*strainsbutlessrelatednesswasnotedamongstrainsbelongingto *C. freundii*.Furtheranalysisshowedpossibleclonalexpansionof*E. agglomerans*and*K.pneumoniae*withintheenvironmentalecosystems.

Keywords

AntimicrobialResistance,Multiple-DrugResistance(MDR),Extended Spectrumβ-Lactamases(ESBL),Enterobacteriaceae,SewageandSludge

1.Introduction

Theemergence and spread ofantimicrobial resistance havebecome aglobal concern andaffectourabilitytocombatsevereinfections.Itisestimated that nearly1Mdeathsannuallyoccuracrosstheglobeas aresultof treatment failure duetoantimicrobial resistance[1].Notably,inresource-poor countries, especiallythoseinSub-SaharaAfrica,cases ofmorbidityandmortalityduetotreatmentfailureareontherise.Theincreaseininfectionscausedby Gram-negative bacteriahasalso ledtoasurgeinusage andover-relianceonantimicrobialsfor humanandanimalinfectiontreatmentandprophylaxis.

Asaresultofover-useandmisuseofantibioticsinhumanandanimalhealth, domesticwasteislikelytobehighlycontaminated withfecalmaterialswhichin turnhavebacteriathatareresistanttomultipleantimicrobialsduetotheirpossibleclinicalorigin[2].Previousstudieshavedocumented sludgeandsewageeffluentsasmajorsourcesofantimicrobial resistantbacteriastrainsintheenvironmentalcompartment[3][4].

AntimicrobialresistanceinGram-negativebacteriaisontherisewhichispar- tially attributed totheease ofspreadandacquisitionofresistancegenesespeciallyamongstthemembersofthefamilyEnterobacteriaceae.Horizontal transferoftheseresistancegeneshaslargely beenattributed toplasmid-borne integronswhichareable toassembleresistancegenesincassettes[5].Multiple-drug resistance(MDR)strainsinsewage,sludgeorsoilcaneasily getintowaterbo- diessuchas riverandmunicipalwatersupplylinesandendupasclinicalstrains responsible forvariousinfections inhumans and animals.Thisisevenmore plausibleininformal settlements wherewatersupplylinesoftenpassthrough opensewersandwheremanyillegalwaterconnectionshappen.Insuchsettings, mostofstreetfood-vending points areoftensituated nexttoopensewerand handhygieneishardlyobservedfurtherincreasingriskofacquisitionandspread ofMDR-strains. Therefore, foodssoldin such environments cancausefood borneinfectionsandoutbreaks.Someantimicrobial agentssuchastetracycline

andquinolonehavealongshelf-lifeinenvironmental compartmentswhileotherssuchas sulfonamidesarewatersolubleandtheirpersistencemaycreateselectionpressurethatleadstoemergenceofresistantclones[6][7].Contaminationandlong-term exposureofsoiltoantimicrobial residualsduetolivestock rearingandagriculturalpracticesmaythereforelead toresistancebuild-upand positivelyselectforhighlyresistantstrains[8].

In Kenya,likein most sub-Saharan countries, the role played bysewage, sludgeandfecalcontaminated soilsassourcesforisolateswithMDRstrainshas notbeeninvestigated.Thisis evenmoreimportant ininformalsettlementscharacterizedbypoorsanitation,limitedaccesstocleanwaterandover-congestion [9].Suchdatais particularlyimportant becauseitmayfindimportant applica- tions inoutbreaks management and inpolicyformulations tocurbspread of AMRandforidentification ofhotspotsthatplayaroleasflush-points foroutbreaksassociatedwithMDRstrains.

Inthiscross-sectional study,wedetermined thediversityofnon-fastidious Gram-negativebacteriaandtheirassociated antimicrobialresistancepatternsto commonantimicrobialsandthephylogenicrelatednessof strainsfromdifferent sites.ThecurrentstudywasconductedinKiberainformalsettlementinNairobi whichisthesecondlargestsluminAfricacontinent andinJujatown,amid- dle-classsettlementattheedgeofNairobimetropolis.

2. Materials and Methodology

2.1.StudyDesignandSampleCollection

Aconvenientrandom samplingdesignwas usedinthiscross-sectionalstudyto obtain soil,sludgeandsewagesamplesfromsitesnearfoodvendingpoint in KiberainformalsettlementsandJuja town,Figure1andFigure2.A purposeful 100sampleseachofsewage,sludgeandsoilwereobtainedacross13villagesof

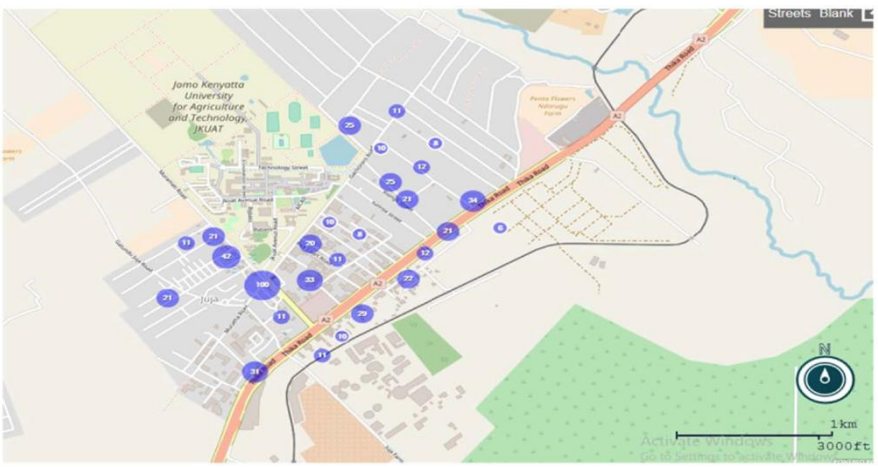

Figure1.Distribution pattern ofmultipledrugsresistanceGramnegativebacteriaisolatesfromJuja metropolis.Thespotmapshowthedistribution patternofEnterobacteriaceaeisolatesthatwereresistanttomorethan3antimicrobialagentsbelongingtodiffer- entclasses.

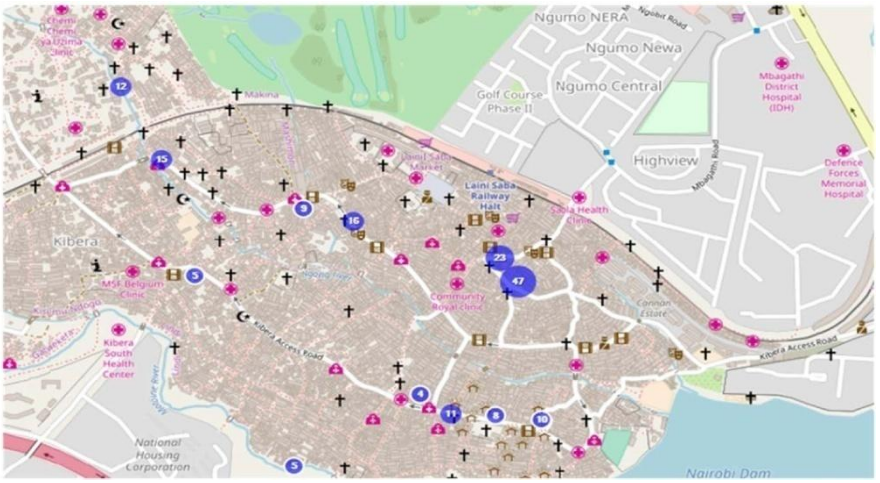

Figure2.Distribution pattern ofmultipledrugsresistanceGramnegativebacteriaisolatesfromKiberaslums.Thespotmapshowsthedistribution pattern ofEnterobacteriaceaeisolatesthatwereresistanttomorethan3antimicrobialagentsbelongingtodiffer- entclasses.

KiberaslumbetweenJulytoDecember2017.Duringthatperiod,asimilarsetof sampleswerealsoobtained inJujatown.Approximately 30mlofsewageand sludgesamplewasobtainedby holdinguniversalbottleatthebaseandplunging itbelowthesurfaceof flowingsewer.Approximately30gofsoilsamplewasalso collectednearthepointofsewageandsludgecollectionsite.Thesampleswere thentransported tolaboratorywithin2hoursforprocessingandculture.

2.2.SampleProcessing

Inorder toisolatesnon-fastidious Gram-negative bacteriaespeciallythosebelongingtofamilyEnterobacteriaceae, approximately 1mLofsewageorsludge samplewas inoculatedin9mlofbufferedpeptonewaterandalkalinepeptone water,shakenfor1hrinordertoresuscitateanyinjuredcellsbeforeplatingdi- rectlyonto oxoid™MacConkeyand EosinMethyleneBlueAgar(EMBA)and bloodagar(BA) plates.Fromeachplate,threedistinctcoloniesofsimilarmorphologywerepurifiedonEMBAandontryptone soyagar(TSA).Pureisolates wereidentifiedthroughGram-staining andaseriesof biochemicalteststhatincluded;triplesugariron, lysine-indole motility, citrate utilization, methylred vogesproskauertestandureasetestasdescribedinthepast[10].

2.3.AntimicrobialSusceptibilityTesting

Antimicrobial susceptibility profileswereperformed using the diskdiffusion methodonMuellerHinton medium.Theantimicrobialpanelusedconsistedof ampicillin(AMP,10µg),amoxicillin/clavulanicacid(AMC,20/10µg),cefoxitin (FOX,30µg), ceftriaxone(CRO,30µg), cefotaxime(CTX,30µg),ceftazidime (CAZ,30µg), cefepime(FEP,30µg),aztreonam (ATM,30µg),ciprofloxacin (CIP,5µg),nalidixicacid(NA,30µg),gentamicin(CN,10µg),streptomycin(S, 10µg),imipenem (IPM,10µg),sulfamethoxazole(RL,200µg)andtrimetho-

prim(W,5.2µg).E. coli ATCC25922referencestrainswasusedforensuring media qualityand discpotency. Interpretationofantimicrobial susceptibility zoneswasdoneusingtheCLSI2017guidelines.Chi-squaretestwasusedforstatisticanalysiswhereavalueof0.05orlesswasconsideredasindication ofsignificantdifferencebetweentestvariables.

2.4.PCRDetectionof*β*-LactamaseGenes

Extraction ofDNAwasdoneusingtheboilingmethod. Thisprocessentailed emulsifyingbacterialcoloniesin1000µlmolecular gradewater.Celllyses was thendonebyboilingthepreparation at95˚Conathermalblockfor10-15min. Separation wasthedonethrough centrifugation at14,000rpm for5minutes. Thesupernatant containingDNAwasthenstoredat−20˚C.Detectionofgenes thatarefrequentlyassociatedwithresistancetoimportant classes ofß-lactams suchasbla_{TEM},bla_{CTX-M},bla_{SHV},bla_{OXA}andclass 1integronwasdoneusingpublishedprimers,Table1[11][12][13]. ThefinalvolumeineachPCRtubewas25 µlwhichincluded10µlof Qiagenmastermix,1µlbutane,2ulDNA,10µlPCR waterand2µlforwardsandreverseprimer. AmplifiedPCRproducts wereseparatedin1.5%gelandbandingpatternsvisualizedunderUVgelimager.

2.5.FingerPrintingofRecoveredBacterialIsolates

Phylogeneticrelatednesswasdetermined usingthe$(GTG)_5$-PCRmethod using publishedstrategiesandprimersindicatedinTable1.(13)PCRamplificationof target DNA sequence wasdone at 40˚C annealing temperature. Amplified productswereseparatedbyrunningin1%agarosegelforIhour.Visualizationof bandingpatterns wasdoneusingaGelmax®UVimager.Bandingpatterns were analyzedusingbionumerics Gelcompar®2softwareversion6.6withthecluster

Table1.PCRamplificationprimersused.

Targetgene	Primername	Primersequence	AnnealingT(˚C)	Productsize	Reference
blaTEM	TEM-F	5'-GCGGAACCCCTATTTG-3'	50	964bp	
		5'-TCTAAAGTATATATGAGTAAACTTGGTCTGAC-3'			12
blaCTX-M	CTX-M-F	5'-ATGTGCAGYACCAGTAARGTKATGGC-3'	60	593bp	12
	CTX-M-R	5'-TGGGTRAARTARGTSACCAGAAYCAGCGG-3'			
blaSHV	SHV-F	5'-TTCGCCTGTGTATTATCTCCCTG-3'	50	854bp	12
	SHV-R	5'-TTAGCGTTGCCAGTGYTCG-3'			
blaOXA	OXA-IF	5'-ATGAAAAACACAATACATATCAACTTCGC-3'	62	820bp	12
	0XA-1R	5'-GTGTGTTTAGAATGGTGATCGCATT-3'			
Int1	intM1_D	5'-GAAAGGTCTGGTCATACATG-3'	50	500bp	11
	intM1_U	5'-ACGAGCGCAAGGTTTCGGT-3''			
(GTG)5	(GTG)5	5'-GTGGTGGTGGTGGTG-3'	50	variable	13

Key:bla: β-lactamasegene,bp: molecularweightinbasepair.

analysisdoneusingthedicemethod basedonUPGMAarithmetic mean.Randomlyselectedbacteriaspeciesfromsixmostprevalentgenerawereanalyzedfor geneticrelatedness.Acorrelation of≥80%amongbacterialspecieswasconsideredasstrongevidenceof geneticrelatednessamongisolatesaspreviouslydescribed[14].

2.6.EthicalApproval

Ethicalapprovalpriortothestudycommencewasobtainedfromscientificethicalreviewunit, Kenyamedical research institute (SERU,KEMRI),approval numberKEMRI/SERU/CMR/P00055/3514.

3.Results

3.1.BacterialIsolates

Atotal of348Gram-negative isolateswereobtained from sewage,364from sludgeand211 fromsoil,Table2.Atotalof14non-fastidious bacteriagenera wereidentifiedfromthesesamplesobtained fromtheslum(Kibera)andfrom middleclasssettings(Juja).Isolatesobtainedinthisstudywerecategorizedin4 groups;indicator organisms forAMRtrends inhuman, animal andtheenvi- ronment [Escherichia(23%)andKlebsiella(21%)],generaofknownEnteropa- thogens species[Salmonella(3%)and Shigella(2%)],genera associated with high intrinsic antmicrobial resistances [A.baumannii (0.5%),P.aeruginosa (8%),M.morganii(2%),Sr.marcesence(0.5%)]andothergenerathatarenot significantlyassociatedwithdiseases butsignificantasopportunistic infection pathogenssuchas [Citrobacterspp(10%),Enterobacterspp(19%),Edwardisella spp(1%),Proteusspp(7%),Yersinia spp(3%)andHafniaspp(1%)].

Table2.Bacterialdiversityfromsewageandsludgesamples.

	Gramnegative bacteriadiversity inenvironmentalsamples													
	Citrobacterspp	Enterobacterspp	E.tarda	E.coli	H.alvei	Klebsiellaspp	M.morgannii	P.aeruginosa	A.baumannii	Proteusspp	Salmonellaspp	Shigellaspp	Yersiniaspp	S.marcesence
Sewage														
Jujan=139	17	28	0	38	0	31	0	10	0	10	0	0	5	0
Kibera n=209	10	31	10	43	10	33	5	13	5	14	15	10	10	0
Sludge														
Jujan=153	22	33	0	39	0	35	0	12	0	12	0	0	0	0
Kibera n=211	25	42	0	32	0	41	10	18	0	15	12	6	10	0
Soil														
Jujan=75	9	12	0	25	0	22	0	7	0	0	0	0	0	0
Kibera n=136	10	30	0	36	0	30	0	10	0	15	0	0	0	5

Lctn: Location, spp: species,n: total number, E.tarda:Edwardisellatarda,E.coli:Escherichiacoli,P.aeruginosa:Pseudomonasaeruginosa,S. marcesence:Serratiamarcesence.

3.2.SpatialDifferenceinAntimicrobialResistanceProfiles

Isolatesfromslumsludgewereoverly moreresistanttoanytestedantimicrobial agents.Highresistanceprevalencewasrecordedtowardsampicillin(AMP57%), trimethoprim(W56%)andsulfamethoxazole(RL55%) amongsludgeisolates recoveredfromtheslum,Table3.SoilisolatesfromJujamiddleclasssetting were theleastresistancetoanytestedantimicrobialagent.Anoverallhighanti-microbialresistancewasrecorded inisolatesfromslumascompared tothose recoveredfromJuja,a townlocatedattheedgeofNairobimetropolis.Resistance towardscephalosporins wasrelativelyhigheramongslumisolateswithhighest resistance valuesrecorded against ceftriaxone (CRO 14%),cefotaxime (CTX 12%) and ceftazidime (CAZ 11%) respectively. Further analysis showed significantdifferenceincephalosporin resistancee.g. againstceftazidimeresis-tancebetweenthe2studysites[OR:0.31, CI0.59,P:0.00002)].Isolatesrecov- ered from the slumsarea(Kibera)weremore resistant tociprofloxacin than thosefromobtained fromthemiddle-classareawhereasignificantdifference was alsonoted(OR:2.13,CI:3.69,P:0.006).Slumisolatesweremoreresistance toaminoglycosides,withno significantdifferencebeenrecorded [gentamicin (OR1.26,CI1.93,P:0.28)].Mostisolateswereresistanttoampicillinwhileim-ipenemandciprofloxacinwerethemosteffective antimicrobialagents.

3.3.AntimicrobialResistancePatternsBasedonSpecies

Amongthe14bacteriagenerareportedinthisstudy,P.aeruginosaisolateswere themostresistantandonaverage wereresistanttoatleast9ofthetestedanti-microbialagents,Table4.Thisspeciesalsohadthehighestprevalenceofisolates (13%)withacombinedresistanceto3rd generationcephalosporins (ceftriaxone 22%, cefotaxime20%, andceftazidime15%),advancedclassesofaminoglyco-sides(gentamicin20%)andotherwisepotentclassesofquinolones(ciprofloxacin

Table3.Antimicrobialresistancepatternsofallbacterialisolatesrecovered fromJujaandKibera.

		Antimicrobialresistance%														
	n	AMP	CAZ	CTX	CRO	FEP	ATM	AMC	FOX	CN	S	CIP	NA	IPM	RL	W
Sewage																
Juja	139	50	8	10	11	5	14	11	22	10	19	5	18	0	42	39
Kibera	209	53	9	11	13	7	15	17	24	12	23	10	21	1	39	43
Sludge																
Juja	153	55	10	11	12	6	15	18	26	12	20	6	23	0	46	48
Kibera	211	57	11	12	14	7	19	19	28	14	27	12	26	0	55	56
Soil																
Juja	75	33	5	8	9	4	13	15	16	6	20	3	13	0	33	35
Kibera	136	40	7	9	10	6	10	15	15	10	22	7	22	0	40	44

AMP: Ampicillin,CTX:Cefotaxime,CRO:Ceftriaxone,FEP:Cefepime,ATM: Aztreonam,FOX:Cefoxitin,AMC:Amoxicillin-clavulanicacid,CN:Genta-micin,S:Streptomycin,CIP:Ciprofloxacin,NA:Nalidixicacid, IPM:Imipenem,RL:Sulfamethoxazole,W: Trimethoprim, n=totalnumberbacteriaisolates.

Table4.Antimicrobialresistanceprofiles ofsewage,sludgeandsoilsamples.

	Antimicrobialresistance(%)inGramnegativebacteria																
	Studysite	n	AMP	CAZ	CTX	CRO	FEP	ATM	FOX	AMC	CN	S	CIP	NA	IPM	RL	W
E.coli																	
	Juja	102	50	8	10	12	5	13	24	13	10	15	5	13	0	46	48
	Slum	111	58	9	12	13	7	14	29	15	14	29	9	15	0	52	55
K.pneumoniae																	
	Juja	88	53	9	11	14	7	15	26	14	13	26	6	24	0	49	50
	Slum	104	60	12	14	14	10	17	32	17	15	33	12	32	0	55	58
Salmonellassp																	
	Juja	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Slum	27	44	7	11	11	4	11	19	15	15	22	4	33	0	48	44
Shigellaspp																	
	Juja	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Slum	16	50	6	13	13	0	13	19	13	13	19	6	25	0	44	50
A.baumannii																	
	Juja	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Slum	5	40	20	20	20	0	20	40	20	20	40	20	40	0	40	40
P.aeruginosa																	
	Juja	29	52	14	17	21	10	31	41	31	17	41	10	45	0	62	45
	Slum	41	49	15	20	22	12	37	48	37	20	37	15	48	5	44	48
M.morganii																	
	Juja	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Slum	15	53	13	13	20	7	20	27	20	13	27	8	33	0	53	47
S.marcesence																	
	Juja	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Slum	5	40	0	20	20	0	20	20	20	0	20	0	20	0	40	20
Citrobacterspp																	
	Juja	49	43	6	8	10	2	12	16	12	8	12	2	16	0	0	0
	Slum	44	43	9	11	11	7	11	18	14	9	18	5	16	0	60	40
Enterobacterspp																	
	Juja	73	51	7	10	11	4	11	14	11	7	15	4	14	0	48	51
	Slum	103	48	10	12	13	5	14	19	13	11	13	5	13	0	50	50
E.tarda																	
	Slum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Juja	10	30	0	0	10	0	10	20	10	0	10	0	20	0	20	10
Proteusspp																	
	Slum	22	41	10	10	14	5	14	23	23	10	27	5	23	0	45	41
	Juja	44	55	9	11	14	7	16	25	25	14	30	9	30	0	52	50
H.alvei																	
	Juja	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Slum	10	40	0	10	10	0	10	10	20	0	20	0	20	0	50	40
Yersiniaspp																	
	Juja	5	20	0	0	0	0	0	20	0	0	20	0	20	0	20	0
	slum	20	25	0	5	5	0	5	10	5	5	15	0	15	0	10	15

15%)WealsorecoveredtwoP.aeruginosaisolatesfromslumsewagethatwere resistanttoimipenem.However,noneoftheYersiniaspeciesandEdwardisella tarda exhibitedtheseresistance.TheresistanceprevalenceforKlebsiellastrains towardsanyclassofantimicrobials wasnotsignificantlyhigherthanthatofE. colistrains(P:0.36,CI:1.88,OR:1.22).AmongknownEnteropathogens, there wasnodifferenceinresistanceprofilesbetweenShigellaandSalmonellastrains (P: 1.25,C1:4.33,OR:1.25).

3.4.PCRDetectionof*β*-LactamasesGeneandIntegronin BacterialIsolates

Atotalof106selectedisolatesthatwereresistantto3rd generationcephalosporinswerescreenedforcarriageofbla_{TEM},bla_{CTX-M},bla_{SHV},bla_{OXA}andclass1integronwhichhaswidelybeenreportedinGram-negativebacteria.Carriageofbla_{TEM} (52%)wasmost prevalent ESBL typefollowedbybla_{CTX-M} (20%),bla_{SHV} (18%)whilebla_{OXA}(17%)wastheleastcommon, Table5.Allisolatesthatcarriedthesegeneswereresistanttoampicillinandatleastone3rd generation cephalosporin (ceftriaxone,cefotaximeand/or ceftazidime).Co-carriageofbla_{TEM}, bla_{CTX-M},bla_{OXA}andint1wasprevalentinP.aeruginosa(13%)while$bla_{SHV}$was commoninKlebsiellaspecies.

3.5.FingerprintAnalysis

Thephylogenyanalysis revealedsignificantgeneticsimilarityamongstrainsbelongingtoEscherichiacoli,Klebsiellapneumoniae,Enterobacteragglomerans

Table5.β-lactamasesandintegrondetectedincephalosporinresistancebacteria.

Organism	Total screened	%b-lactamaseandintegronclass1 inGrambacteriaisolates.				
		blaTEM	blaCTX-M	blaSHV	blaOXA	int1
Citrobacterspp	n=10	30	10	0	10	0
E.coli	n=20	60	50	15	20	20
Proteusspp	n=9	56	22	11	11	11
P.aeruginosa	n=15	67	47	27	40	47
Salmonellaspp	n=3	33	33	0	0	0
Klebsiellaspp	n=20	60	20	50	30	25
Enterobacterspp	n=20	30	10	10	0	0
Shigellaspp	n=2	0	0	0	0	0
Acinetobacterbaumannii	n=2	50	0	0	0	50
Morganellamorgannii	n=2	0	0	0	0	0
Serratiamarcesence	n=1	0	0	0	0	0
Edwardisellatarda	n=1	0	0	0	0	0
Hafniaalvei	n=1	0	0	0	0	0

andProteusmirabilis strainsbutlessrelatednesswasnotedamongstrainsbelongingtoCitrobacterfreundii,Figures3-7.Further analysisshowedpossible clonalexpansionwithintheenvironmental ecosystemsofE.agglomeransFigure 4,cluster2),Klebsiellapneumoniae(Figure5,cluster2)andE.coli(Figure8, cluster1,3).Suchstrainshadidenticalresistancephenotypesandsharedmore than 80%geneticsimilarity.Other strainssuchasSalmonellaTyphishoweda similarityofabove70%intermsofresistancepatternsbutdifferedsignificantly

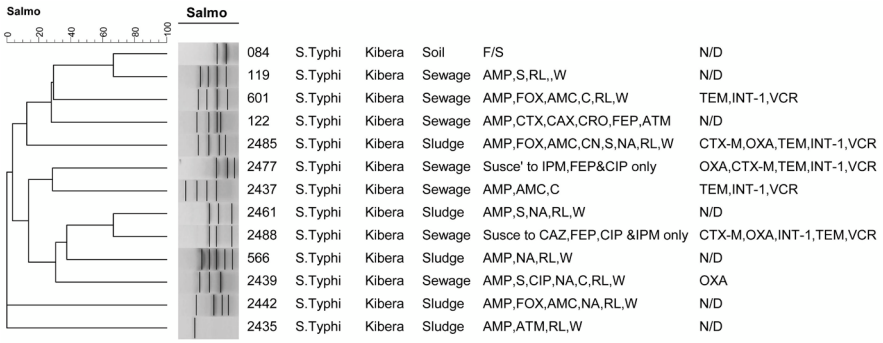

Figure3.ClusteranalysisofrecoverSalmonellatyphifromsewage,soilandsludge.The figureshowphylogenyanalysisofSalmonellaTyphiisolatesfromsewagesludgeandsoil samplefromKiberaslums.Descriptionofvariousacronymsonthisfigureisasfollows;S. Typhi:SalmonellaTyphi,TEM:Temoneria β-lactamase,CTX-M:CefotaximeMunich β-lactamase,SHV:Sulhydrylvariantβ-lactamase,Int-1:Integron,VCR:Variablecassette region,N/D:noneofthescreenedgeneweredetected.

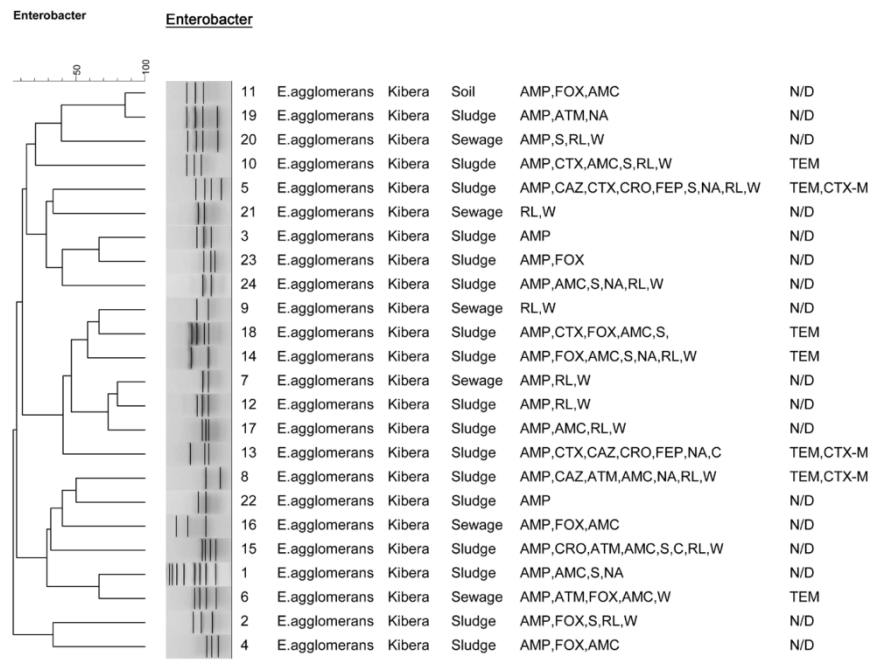

Figure4.ClusteranalysisofrecoverEnterobacter agglomeransfromsewage, soiland sludge.ThefigureshowphylogenyanalysisofEnterobacteragglomeransisolatesfrom sewagesludgeandsoilsamplefromKiberaslums.Description ofvariousacronymson thisfigureisasfollows;E.agglomerans:Enterobacteragglomerans,N/D:none ofthe screenedgeneweredetected.

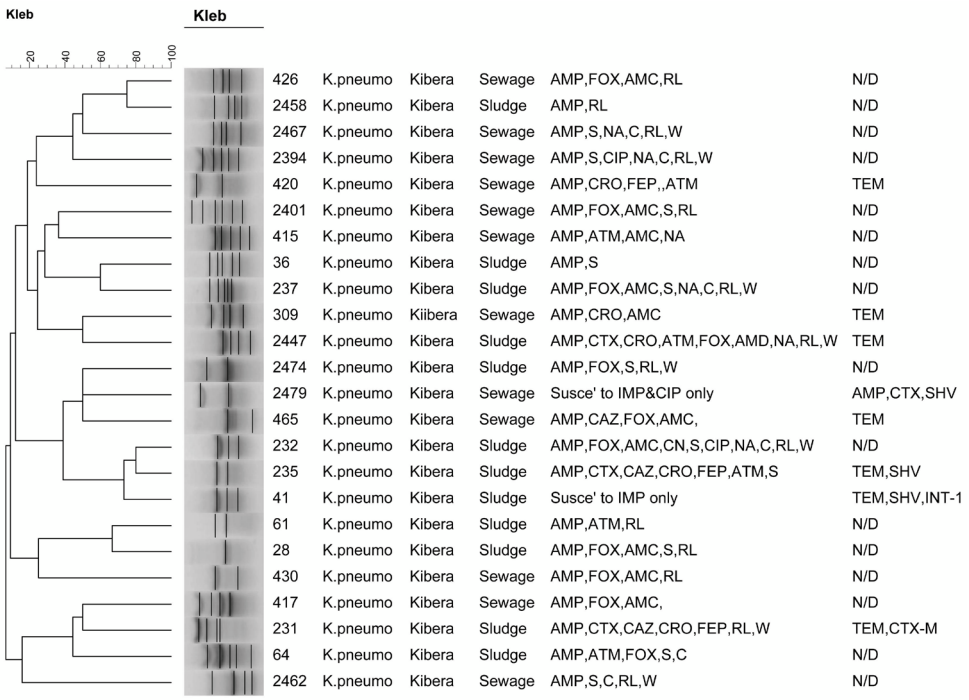

Figure5.ClusteranalysisofrecoverKlebsiellapneumoniaefromsewage andsludge.Thefigure showphylogenyanalysisofKlebsiellapneumoniaeisolatesfromsewage sludgeandsoilsample fromKiberaslums.Descriptionofvariousacronymsonthisfigureis asfollows;Kpneumo:Kleb-siellapneumoniae,Int-1:Integron,N/D:noneofthetargetedgenswasdetected.

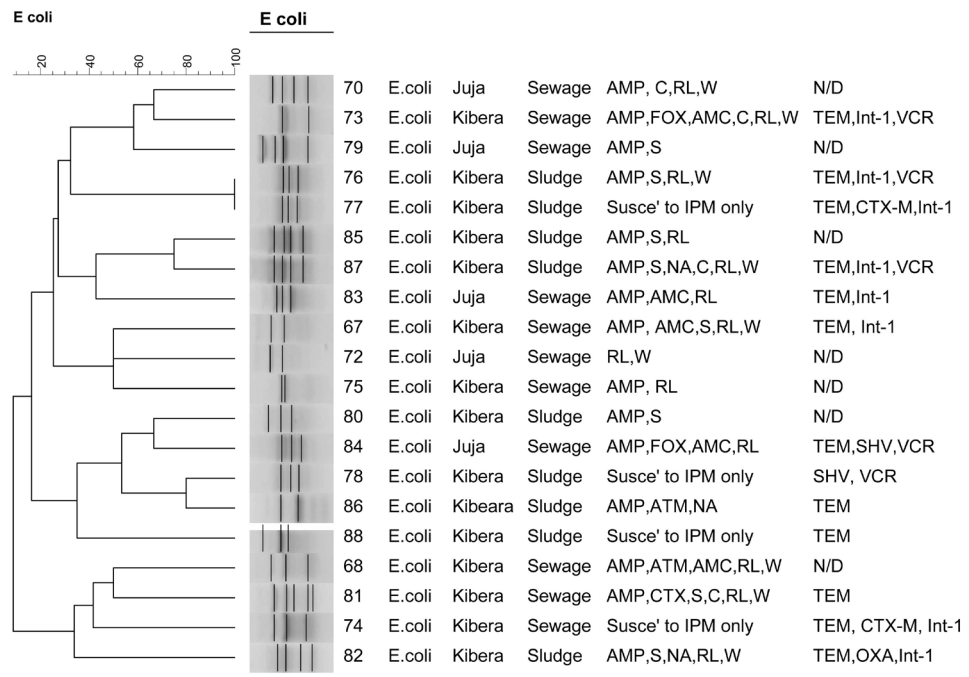

Figure6.ClusteranalysisofrecoverEscherichiacolifromsewageandsludge.Thefigureshow phylogenyanalysisofEscherichiacoliisolatesfromsewagesludgeandsoilsamplefromKibera slumandJujatown.Description ofvariousacronymsononthisfigureisasfollows;E.coli:Es-cherichiacoli,Int-1:Integron,VCR:Variablecassetteregion,So:Soil,Se:sewage,Sl:Sludge,N/D: noneofthetargetedgenswasdetected.

Figure7.ClusteranalysisofrecoverProteusmirabilisfromsoil,sludgeandsewagesamples.Thefigureshowphylogenyanalysis ofProteusmirabilisisolatesfromsewagesludge andsoil samplefromKiberaslums.Descriptionofvariousacronymsonthisfigureis as follows;P.mirabilis:Proteusmirabilis,MDR:Multipledrugresistance,TEM:Temoneria β-lactamase, CTX-M: Cefotaxime Munich β-lactamase, SHV: Sulhydryl variant β-lactamase,Int:Integron,VCR:Variablecassetteregion.

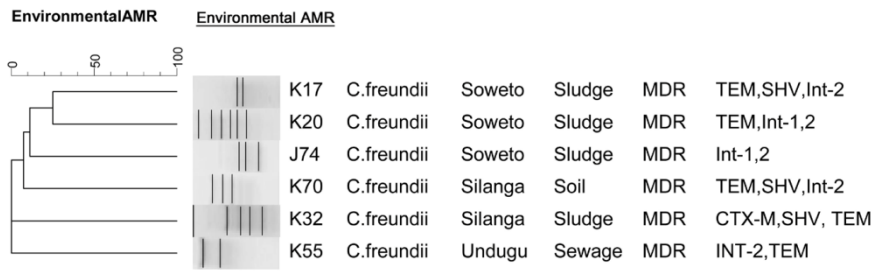

Figure8.ClusteranalysisofrecoverCitrobacterfreundiifromsewage,soilandsludge. ThefigureshowphylogenyanalysisofCitrobacter freundiiisolatesfromsewagesludge andsoil samplefromKiberaslums.Descriptionofvariousacronymsonthisfigureis as follows;C. freundii:Citrobacterfreundii,Int:Integron,N/D:noneofthetargetedgenes weredetected.

basedonthebandingpatternsfurtherindicatingthatdifferentclones mayhave acquiredsimilarsetsof resistancedeterminants independent (Figure3).Tight clusteringofE.colistrainsfromslum(Kibera)andthemiddleclasssettlement (Jujatown)was alsonoted;theseisolateshoweverhaddifferentresistancephe- notypes suggesting independent evolution (Figure 8,cluster 1).Apparently, sampletypeand study sitewasnot akeydeterminant in isolatesclustering. Theseresultscall forapplicationofhighresolutiontypingofsuchstrainsusing wholegenomeanalysis strategiesinfuturestudiesinordertoidentifythemost stableclones that could significantlyberelated to emergence and spread of AMR.

4. Discussion

Itisestimatedthatbytheyear2050theurbanpopulationwilldouble.However, withpoor urban planning especiallyindevelopingcountries, highunemploymentrateandpoverty,informalsettlementsare increasinglybecomingrampant [15].Denselypopulatedneighborhoods withpoorsanitationinfrastructure pose aserioushealthrisk[16].Previousindependent observations havenoted that rawandpartiallyprocessedexcretaleaksfromdomestictoiletsinKiberaslums andfindtheirwayintheopensewer[17].Inordertoreducechancesofrelease

ofentericbacteriaandantimicrobial residuesintotheenvironment, thereisa needforprovisionofimprovedbasic sanitationsuchas cleantoiletsandproper drainagesysteminslumsandurbancenters.

Inthecurrentstudywerecordedadiverse groupofnon-fastidiousGram-negative bacteriaisolates inenvironmental samplesfromslumandmiddleclasssetting. Majorityofgeneraisolatedarethoseknowntoresideinhumanandanimalgut suchasEscherichia,Klebsiellaand someareknown Enteropathogens suchas Salmonellaand Shigellaspecies.Although clinicalreports ofSalmonellaand Shigellaspecies isondeclines,environment colonizationstillremainapossible sourceoftyphoid,andShigellosispathogens. Itislikelythathuman fecaland untreateddomesticwaste couldbeseedingbacteriaofentericoriginintotheen- ironmen andsomeofthesemayhaveclinicaloriginsbasedontheirresistance profiles.Our study underpins the role ofenvironmental contaminationasa drivingfactorforemergenceofoutbreaks someofwhicharecausedbyMDR strains.WealsoisolatedMDRP.aeruginosaandA.baumannii;theseorganisms havepreviouslybeenassociatedwithwiderangeofdifficult-to-treat infections [18].The bacteria diversity recorded in this study iscomparatively broader compared tothosereported inarelatedstudyconducted in2013andanalyzed themicrobialcontent andresistanceprofilesofisolatesobtained fromthese- wage-contaminated Nairobiriver[19].Thereis thereforeaclearindicationthat ecosystemcontaminationwithdomesticwastes isonagradualincreaseincon-taminationoftheenvironment.

Highantimicrobialresistancestowardsampicillin(57%),trimethoprim(56%), cefoxitin(28%),sulfamethoxazole(27%)andnalidixicacid(26%)wasrecorded inisolatesfromslum.Thesefindingsstronglysuggest thatbacteriafromtheen-vironment compartments are ableaccumulate many antimicrobial resistance determinants. Theoccurrenceofsuchstrainswithresistancetoa combinationof antimicrobials raisesconcerns becauseifsuchstrains areimplicated ininfec-tions,availabletreatmentoptionswouldbehighly limited.

Ourdatashowahigherprevalenceof resistancetoantimicrobialsamongiso-latesrecoveredfromslumenvironment compartments compared toaprevious studyconducted inthesameslum,Kiberain2012 [20].Theformerstudythat soughttocharacterizeE.coli,SalmonellaandShigellaspeciesinwater,soil,veg- etables andmeatreportedandreporteda resistanceprevalenceof56.8%towards ampicillinagainst57%reportedhere,13.6%forstreptomycinagainst27%,4.9% fornalidixicacidagainst26%and2.5%forgentamicinagainst14%recordedin ourstudy.Thus,thevalues fromthispreviousstudyweregenerallylowerthan whatwereportedinthepresentstudybyafactorofgreaterthan10%.Although the reasons behind this apparent rise in resistance prevalence is not clear, previousstudyhavereported that morethan 80%ofantibiotics consumed by humansandanimalsareexcretedthroughurineandfeces[21].Therefore,asig-nificantamount ofactiveresiduesmayendupinthesewage,sludgeandother environment compartments therebypresentinga strongselectionpressurethat favorproliferation ofMDRstrains.Contaminationofsoilwithentericbacteria

andantimicrobial compounds comesfromdomesticandmunicipal wasteand alsofromsewage sludgesystems[22].Significantamounts ofantimicrobial residuesbelongingtomorethan6antimicrobial classes havepreviouslybeenreportedinsewageinAsiaandEuropeandsomeclassesofantimicrobialssuchas tetracyclineandchloramphenicol canpersistinsoil,sewageandsludgefordecades[23].Thepresence ofsuchagentsintheenvironment canconsequently provide astrong selectivepressure that preferentially allowsMDRclonesto spread[24].Althoughourstudydidnotassesspresenceofantimicrobialagents residuesintheenvironment, ourfindsstronglyunderpins theneedtosucha study.

Higher levelsofantimicrobial resistances than reported in our study with greater than 30%towards ceftazidime, cefotaxime,ceftriaxone, ciprofloxacin, aztreonam and gentamicin in A.baumannii, E.coli,P.aeruginosaand K. pneumoniae fromblood,trachealaspirates,wound,pusandurinesampleshave beendocumented inKenya [25][26].Ourstudyhowevershowanincreasein antimicrobial resistance in environmental compartmentscompared to apre- viousstudyconducted inKakamegatowninKenya[27].Theformerstudyre- ported E.colifromrecoveredfromdumpsites, sludgeandwastewater.Wereportedmore3%increaseinresistancetowardsamoxicillin-clavulanicacid,gen- tamicin and ciprofloxacininCitrobacterspecies,Enterobacterspp.and Klebsiellaspp.comparedtoformerstudy.Althoughthethreatofantimicrobialresistanceismore profound inclinicalsettings,our findingsshowenvironmental bacteriastrainsare increasinglybecomingresistantandthereforeshouldnotbe overlooked.

Carriageofbla_{TEM}(52%)wasmostprevalentinallbacteriaspeciesfollowedby bla_{CTX-M}(20%),bla_{SHV}(18%) whilebla_{OXA}(17%) wastheleastcommonblagene. Co-carriageofbla_{TEM},bla_{CTX-M},bla_{OXA} andint1wasprevalent inP.aeruginosa (13%)whilebla_{SHV} wascommon in Klebsiella species.Previous studies have shownthat most bacteriathat carryblagenesalsocarryintegrons whichare mostlyharbored inmobilegeneticelementssuchplasmidsthereforesuggesting spreadandacquisitioninbacteriacommunity[28].

Mostofourisolateshadlessthan70%similaritywithdistinctresistancephenotypesandgenotypessuggestingvery littleevidenceofclonalexpansioninthe environment. Tight clustering of≥70similarity matrix among some fewEscherichiacoli,Klebsiellapneumoniae,Enterobacteragglomerans and Proteus mirabilisstrainsisolatehoweversuggestsindependent acquisition ofasimilar setofresistancedeterminants amongisolateswithdifferentprofiles.Although wewerenotabletodeterminethecauseofabovementioned resistancefeatures, suchphenomenon haspreviouslybeenassociatedwithcarriageofgeneticelementssuchasplasmids[29].

5. Conclusion

Thehighdiversityofmultiple-drugs resistanceinsewageand sludgesamples observedinthisstudyposeaseriouspublichealthhazardinemanation ofseri-

ous infection. Unregulated discharge ofdomestic wastehighlypolluted with fecal materialonopensewerisagreatrisktomanyresidentsofover-polluted Kiberainformal settlements. Thereistherefore adireneedforproper sewage managementtoreduceoreliminetheserioushealthriskposedbyopensewer.

6.Study Limitations

Ourstudyexperiencedanumberofshortcomingsthatcanbeabasis for formulationofstrongerstudiesinfutures.

1)Thebroadrangeofbacteriagenerareported inthisstudymakesfocused discussionchallenging.

2)Wewereonlyabletoscreenforafewβ-lactamasesgenes.Ourstudywas alsonotabletodeterminethebasesofresistancetootherclassesofantimicrobial agentssuchasaminoglycosidesandfluoroquinolones.

3)Wewereunabletodeterminethecontentofdetectedint1andwhetherthey wereborninmobilegeneticelementssuchasplasmids.

4)Lowresolution $(GTG)^5$ fingerprint method wasusedtoestablishbacteria phylogenyandgeneticrelatedness.Futuresstudiesshouldapply highresolution methods suchaswholegenome sequencing and SNPtyping whichcanshed morelightresistancegenecontent andbacteriaevolutionintheenvironmental compartments.

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Author Contributions

JohnMainadesignedandwrotetheresearchprotocolforthisstudyinaddition toconductinglabworkandthemanuscript writing.HelenOnyangoassistedin labworkandstatisticalanalysis. JohnKiiruassistedindesigningthestudyand writingthemanuscript. Allauthors readandapprovedfinalmanuscript. Anne MuigaiandPerpetualNdung'uprovidedassist inprotocolcorrectionand amendments instudymethodologies.JoelMukaya,SusanWambui,TerryJudah, JoyceKinyua,JoystellaMuriuki,LynneChesenge,LydiaKisoo,RebeccaThuku, BonifaceWachira,VincentBettandThomasGachukiofferedlaboratory tech-nicalsupport.

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Conflict ofInterest

Theauthors declarenoconflictsofinterest regarding thepublication ofthis paper.

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