Escherichia coli and *Klebsiella* species as indicators for antimicrobial resistance among isolates recovered from garbage and dumpsites in selected sites in Nairobi County

BY

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DECLARATION

I, Grace Wambui Waturu, hereby declare that the work presented in this thesis is my original work and from my own effort. It has not been submitted by anyone for award of a degree in any University.

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DEDICATION

To my Family

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ACRONYMS AND ABBREVIATIONS

bla	beta-lactamase gene
BHI	Brain Heart infusion broth
CTX-M	CefoTaXimases 'Munich'
CDC	Centers for Disease Control
CMR	Centre for Microbiology Research
DNA	Deoxyribonucleic acid
E. coli	*Escherichia coli*
EAEC	Enteroaggregative *Escherichia coli*
EHEC	Enterohemorrhagic *Escherichia coli*
EPEC	Enteropathogenic *Escherichia coli*
ESBL	Extended or Expanded Spectrum beta-Lactamase
ETEC	Enterotoxigenic *Escherichia coli*
KEMRI	Kenya Medical Research Institute KEMRI
KPC	*Klebsiella Pneumoniae* Carbapenemase
MDROs	Multidrug resistant Organisms
MSW	Municipal Solid Waste
NCCLS	National Committee for Clinical Laboratory Standards
PCR	Polymerase chain reaction
SHV	Sulfhydrl Variable Enzymes
Spp	Species
TEM	Temoneira Enzymes
UoN	University of Nairobi
WHO	World Health Organization

ABSTRACT

Introduction

Dumpsites and garbage collection areas can act as reservoirs of highly resistant bacterial strains. The objective of this study was to determine the potential of garbage collection areas and dumpsites in different parts of Nairobi as possible sources of resistant strains using *E. coli* and *Klebsiella* as indicator species.

Methodology

A total of 126 samples were collected during the sampling period. The samples were then transported to the laboratory in the sterile bottles that they were collected in for analysis. The samples were cultured on MacConkey agar. Gram staining was done on discrete isolates based on colony characteristics. Biochemical tests were performed on colonies from primary cultures for final identification of the isolates. Antimicrobial disc susceptibility tests and pathogenicity tests were also carried out on the indicator isolates.

Results

Highest bacterial burden was recorded from Muthurwa estate dumpsite, with a mean viable count of 8.2 x 10^{10}cfu/gm while the least was from Dandora dumpsite with mean count of 1.1 x 10^{11}cfu/gm. Overall, Gentamicin was the most effective agent on *Klebsiella* and meropenem was the most effective on both *E.coli* and *Klebsiella* strains. The isolates showed high resistance to ampicillin, streptomycin and trimethoprim-sulfamethoxazole. ESBL production had a prevalence rate of 16%. Presence of bla_{TEM}, $bla_{CTX\text{-M}}$, and bla_{SHV} resistance genes was also determined among the indicator strains. From the ten isolates 10% was EnteroToxigenic *Escherichia coli* and 4 EnteroAggregative *Escherichia coli*.

Conclusion

It concluded that, municipal waste dumpsites and garbage collection areas bear heavy burdens of potentially virulent resistant *E.coli* and *Klebsiella* species which may constitute major public health hazards to scavengers and those living near the dumpsite. There is need therefore to educate people on the use of appropriate protective materials. Proper disposal and recycling of these wastes also ought to be mandatory.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Proper structure in management and collection of solid waste materials is critical to health and well-being of urban dwellers (Baker, 2008). Waste generation and its control have taken an important role in our environment. With the doubling of population and changing lifestyle pattern of the inhabitants the quantity of generated municipal waste is increasing in an alarming rate. Most of this waste is subjected to dumping in a specified disposal yard. In Nairobi, like most cities in the developing world, several tons of municipal solid waste and other wastes are disposed incorrectly, clogged drains, creating conducive environment for pests that spread disease and creating a myriad of related health and infrastructural problems. A substantial part of the urban residents in the down-town part of Nairobi, schools and market places have little or no access to solid waste collection services (World Bank, 2002, 2003). This is due to poor land planning which resulted in the creation of informal settlements with narrow streets that are inaccessible that make it difficult for garbage trucks to reach many areas. The result is that a large portion of the population is left without access to solid waste management services making them particularly vulnerable to infections. The diversity of infectious bacteria in these breeding grounds is still not known. *Escherichia coli* and *Klebsiella* species are good indicators as they are widely isolated from both clinical and environmental samples and they easily undergo gene transfer (Nabegu, 2010).

Antimicrobial resistance threatens the effective prevention, control and treatment of an ever-increasing range of infections caused by various pathogens. It is an increasingly serious threat to global public health that requires action across all government sectors and society. New resistance mechanisms emerge and spread globally. In the early 1970s, physicians had to abandon the belief that, administration of a vast array of effective antimicrobial agents clears all bacterial infections. This optimism was shaken by the emergence of resistance to multiple antibiotics among such pathogens as *E. coli*, *Pseudomonas aeruginosa* and a host of others (Lowy, 2003).

Products such as disinfectants, sterilants, and heavy metals used in industries and in household products along with antibiotics, are creating selective pressure in the environment that lead to mutations in microorganisms (Baquero & Negri, 1998). In an environment with multiple presences of these products makes it favorable, in terms of survival, for a bacterium to acquire resistance from the stresses present. If the resistance is carried on plasmids, bacteria with clusters of resistance genes are likely to pass on those genes to other bacteria (Clermont *et al*., 2008).

Enterobacteriaceae are one of the major causes for a significant number of infections and death in the world. The prevalence of antibiotic resistance in this family of bacteria e.g. *Escherichia coli*, *Salmonella*, *Shigella* has risen over the years. One reason for this increase is the dissemination of *Klebsiella pneumonia* carbapenemase (KPC), a class A serine carbapenemase first isolated from *Klebsiella pneumoniae* in 1996 (Gonzalo & Drobniewski 2013).

Resistance in pathogenic organisms poses a distinct clinical challenge. However commensal bacteria may play a crucial role in the spread of antimicrobial resistance within a community by acting as a major reservoir for resistance genes. Exposure of commensals such as *Escherichia coli* to antimicrobials, increases the carriage levels of resistant organisms. *Escherichia coli* was used in this study as an indicator species because they are common in humans and animals, can cause disease, have also been used in other studies to gauge the spread of acquired resistance and that they may serve as markers of the transfer of resistance from animal to human intestinal micro flora (Chopra & Roberts, 2001).

Antimicrobial resistance and related genes are omnipresent, with most of the genes that encode resistance to human pathogens having originated in bacteria from the natural environment (e.g., β-lactamases and fluoroquinolones resistance genes, such as *qnr*). This rapid change and spread of antimicrobial resistance genes has been catalyzed by modern human activities and their influence on the environmental resistome (Rita L *et al*., 2013). This shows the importance of including the role of the environmental vectors, such as bacterial genetic diversity within soil and water, in resistance risk management. We need to take more initiative to decrease the spread of these resistance genes in environmental bacteria to human pathogens, to decrease the spread of resistant bacteria to people and animals through foodstuffs, water and wastes to minimize the levels of antibiotics and antimicrobial bacteria introduced into the environment. By reducing this,

improved management of waste measures must be considered containing antibiotic residues and antimicrobial-resistant microorganisms (Rita L *et al*., 2013). Extended-spectrum β-lactamases (ESBLs), including the AmpC type, are important mechanisms of resistance among *Enterobacteriaeceae*. CTX-M type extended-spectrum β- lactamases, of which there are now over 90 variants, are distributed globally yet appear to vary in regional distribution. AmpC β-lactamases hydrolyze third generation cephalosporins, but are resistant to inhibition by clavulanate or other β-lactamase inhibitors *in vitro*. Fecal carriage and rates of colonization by bacteria harboring these resistance mechanisms have been reported in patients with community-acquired infections and in healthy members of their households. Expression of these ESBLs compromises the efficacy of current antibacterial therapies, potentially increasing the seriousness of hospital- and community-acquired *Escherichia coli* infections (Rita L *et al*., 2013).

The main aim of this study was to determine the potential of garbage collection areas and dumpsites in different parts of Nairobi as possible sources of resistant strains using *Escherichia coli* and *Klebsiella* as indicator species

1.2 Statement of the problem

The Dumping Sites and garbage collection areas are a problem to human populations and may pose a risk of contamination related illnesses. Many people's lives are at risk, especially when a large population already lives in environments with large amounts of garbage. Contamination related illnesses are a challenge to the urban-poor population living in informal settlements and slums face. Recently, there has been reports of emergence and spread of Multi-Drug Resistant (MDR) strains that are resistant to important classes of antimicrobials such as ampicillin and aminoglycosides. Treatment of these diseases has been a challenged especially among indicator species. These antimicrobials are important "chemotherapeutic replacer agents" that are recommended for alternative treatment of infections caused by ESBL-producers that have now become widespread in patients across all ages. The differences in resistances, the prevalence of ESBL colonization and associated *bla* genes in dumpsites and garbage collection areas is still not known. It is not known what resistance phenotypes are observed among *Escherichia coli* and *Klebsiella* as indicator species. It is also not known what danger such sites pose to residents and

waste handlers. There is therefore a need to study the kind of bacteria recovered from such sites, with a view of identifying solutions that would minimize contamination related illnesses.

1.3 Justification

Dumpsites are largely reported as breeding grounds for most microbes associated with diseases. With the increasing incidences of Multi Drug Resistant Organisms (MDROs) among indicator strains found in the dumpsites and garbage collection areas, chances of them encroaching into the general population is high. The data generated in this study on resistance profiles among isolates recovered from dumpsites and garbage collection areas, will aid in better management of contamination related illnesses associated with such strains. The data will also find use among the county government of Nairobi seeking to make proper disposal and recycling practices. This data also sheds light on the contamination levels of various dump sites and garbage collection areas in Nairobi with *Escherichia coli* and *Klebsiella* as indicator species, the genetic basis of resistance among isolates obtained in these sites and the kind of *Escherichia coli* pathotypes recoverable from these sites. Until this study, only a few related studies had reported such information from environmental samples.

1.4 Research questions

- What are the contamination levels of various dump sites and garbage collection areas in Nairobi using *Escherichia coli* and *Klebsiella* as indicator species?
- To what antimicrobials are the *Escherichia coli* and *Klebsiella* species from these dump sites and garbage collection areas resistant to?
- What are the antimicrobial resistant genes present among isolates obtained from these dump sites and garbage collection areas?
- What kinds of *Escherichia coli* pathotypes are recoverable from these dump sites and garbage collection areas?

1.5 Objectives

1.5.1 General objectives

To determine the potential of garbage collection areas and dumpsites in different parts of Nairobi as possible sources of resistant strains using *Escherichia coli* and *Klebsiella* as indicator species

1.5.2 Specific objectives

- To determine the contamination levels of various dump sites and garbage collection areas in Nairobi using *Escherichia coli* and *Klebsiella* as indicator species.
- To investigate the levels of resistance to various antimicrobials encountered in garbage collection areas and dumpsites using *Escherichia coli* and *Klebsiella* as indicator species.
- To determine the presence of antimicrobial resistant genes among isolates obtained from these dump sites and garbage collection areas.
- To determine diversity of *Escherichia coli* pathotypes recoverable from these dump sites and garbage collection areas.

1.6 Significance of the study and anticipated output

The data generated from the study was used to advise the County government on the true state of contamination in the dumpsites and garbage collection areas as well as alert Ministry of health (MoPHs). The waste disposal practices are also poor leading to the increase in the contamination cases and diversity of the antimicrobial resistant bacteria. Therefore the study will advise the county management, staff and the general public on the purpose of the study and the benefits of the results in order to improve dumping practices. The data from this work also provided a platform for assessing the level of MDR contamination in the environment and prevalence of *Escherichia coli* pathotypes.

CHAPTER TWO

2.0 LITERATURE REVIEW

Municipal solid waste (MSW) is defined as non-air and sewage emissions created within and disposed of by a municipality, including household garbage, commercial refuse, construction and demolition debris, dead animals, and abandoned vehicles (Cointreau, 1982). The majority of substances composing municipal solid waste include paper, vegetable matter, plastics, metals, textiles, rubber, and glass. Municipal solid waste disposal is an enormous concern in developing countries across the world, as poverty, population growth, and high urbanization rates combine with ineffectual and under-funded governments to prevent efficient management of wastes (Walling & Wilhelm, 2004). The barriers to effective MSW management are not simply lack of policy, but lack of infrastructure, education, social awareness of problems and solutions, and lack of institutions promoting sustainable actions. (Barrett, Lee, & McPeak, 2005) recommended that the "conservation community" needs to invest in research to work out institutional design questions, and in building and linking conservation institutions.

Some investigation have been made on the operations of the state agency responsible for waste management in the Kano metropolis Nigeria and report showed that a significant portion of the population, about 80%, does not have access to waste collection services, only 20% of the waste generated is actually collected and vast majority of users of the service 92% consider the service as very poor (Nabegu, 2010). Composition of municipal solid waste provides a description of the constituents of the waste and it differs widely from place to place. most of the differences is due to organic content which is much higher in the low income areas than the high income, while the paper and plastic content is much higher in high income areas than low income areas (Zhang *et al*., 2012). This reflects the difference in consumption pattern, cultural and educational differences. In high-end estates disposable material and packaged food are commonly used in higher quantities; this results to having higher calorific value wastes, lower specific density and lower moisture content. In the case of lower income areas, the usage of fresh vegetables to packaged food is much higher. This results in a waste composition that has high moisture content, high specific weight and low calorific value (Hakami & Seif, 2015). The 'blind technology transfer' of machinery like the garbage trucks and the separate liter bins from

developed countries to developing countries and its subsequent failure has brought attention to the need for appropriate technology to suit the conditions in developing countries (Beukering & Sehker, 1999).

The recovery of the usable items like clothes bags, glass bottles, and metal containers occurs at the household level by the itinerant collectors who generally pay a nominal amount for the material or provide a useful material in exchange. This is found at the middle and lower income levels of the populace of which there is a vast majority. This helps in reducing the quantity of refuse generated but prompts gross negligence on the part of the generators inducing littering and unmonitored disposal. At the level of the waste collection systems, the rag pickers and the scavengers would litter the garbage around the bins or enclosures causing nuisance which in most cases is neglected by the collection system for transport (Mcintyre *et al*., 2013).

Climatic factors play another crucial role in the municipal solid waste management as Nairobi experiences long wet season, and heat and humidity causes the municipal solid waste to be of higher moisture content thus increasing the weight of the refuse. In addition, high humidity with heat causes the organic portion of the waste to decompose quickly that causes problems in handling and disposal, which directly affects the environmental health of the waste workers and the inhabitants in the surroundings (Visvanathan & Trankler, 2001)

Identification of what waste is composed of is therefore important in identifying a way of treatment, taking essential health precautions and space needed for the treatment facilities. Despite this recognition, there has been no study on the analysis of potential microbes found in municipal, school and market place wastes in Nairobi. This study will therefore attempt to fill this gap by providing data on the microbial composition, and sources of these wastes in different parts of the city for the purpose of understanding the type of waste generated, the total microbial load and the presence of *E. coli* and *Klebsiella* species.

2.1 Waste management in Nairobi County

Nairobi does not have a comprehensive waste separation and disposal system and garbage and litter is common within neighborhoods. Such garbage poses a greater risk if near schools, market place and may be hospitals. A study on Dandora dumpsite showed that it is home to all kinds of wastes. The report further reveals that agricultural wastes such as fungicides and herbicides and

hospital waste including packaging materials, and containers, used syringes and other sharp, biological waste and pharmaceuticals are all dumped at the site. The dumpsite has a number of youth and women co- operatives which sort and recycle some of this waste. These cartels hire them to sort out and recycle wastes from industries and residential estates in Nairobi. Due to hard economic times which has spawned a culture of survival, these people earn between Ksh 50-150 (USD 0.75-2.3) a day. They work under harsh conditions without any protective clothing. Their employers do not cover them when they get sick. Getting sick here is common and this is manifested in the high death rate of those working at the dumpsite (Unep, 2007).

These sites pose a serious health hazard to those working in and around the area. Since burning is widely used to reduce the amount of waste, the site is a major source of dioxins, furans, lead and cadmium, elements which have been scientifically proved to be toxic to both humans and the environment. The health hazards associated with the Dandora dumpsite according to the UNEP report includes skin disorders, respiratory abnormalities, abdominal and intestinal problems central nervous system and blood disorders. Diseases such as malaria, chicken pox, lung cancer, septic wounds and genital abnormalities are more prevalent among the people living around the dumpsite. No work has been done to assess the potential of garbage in Nairobi as sources of pathogens and especially those that are Multi-Drug-Resistant (MDR). No one knows if such strains are distributed based on neighborhood from which the garbage comes from.

2.2 Carriage of bacteria in dumpsites

Dump sites and garbage collection areas carry a wide range of bacteria that have both positive and negative effects on the environment and the people living and working on them. Previous studies have shown the type of bacteria and fungi and their frequency of isolation from the waste dump sites in Eagle Island, Southern Nigeria. The bacteria isolated from the dump site included, *Arthrobacter* species (4.7%), *Bacillus* species(15.2%), *E. coli* (12.1%), *Klebsiella* species(9.6%), *Micrococcus* species (2.5%), *Proteus* species (10.2%), *Pseudomonas* species (5.4%), *Serratia* species (2.5%), *Staphylococcus* species (21%), *Streptococcus* species (16.8%). The order of their decreasing frequency of isolation was *Staphylococcus-Streptococcus-Bacillus-Micrococcus* and *Serratia* species (Okpoitari, 2013).

Another study on ambient microbial pollution was conducted in Mandur dumping site India. The mean number of *staphylococcus aureus* in the air near the dumping site was 3×10^4 CFU/m^3 and *Enterococcus* spp. was found to be much lower at 2.1×10^3 CFU/m^3. The viridians group of *Streptococci* was found to be 1.1×10, while *Aeromonas* and *Escherichia coli* were 23% and 10% respectively (Velsivasakthivel & Nandini, 2014). These results show that contamination of a dump site results to contamination of the air around the dumpsite thus putting a higher number of people at risk of contamination related illnesses. From these studies *Escherichia coli* and *Klebsiella* are among the bacteria isolated with a high prevalence.

A previous study in Benin City isolated eleven Bacteria genera from topsoil's and leacheates (F. E. Oviasogie & Agbonlahor, 2013). They were in the following order of predominance: *Bacillus* spp. (18.20%) *Staphylococcus* spp. (13.93%), *Escherichia coli* (12.72%), *Proteus* spp. (12.12%), *Streptococcus* spp. (12.12%), *Klebsiella* spp. (9.70%), *Pseudomonas* spp. (7.90%), *Citrobacter* spp. (5.45%), *Bacteroides* spp. (3.03%), *Clostridium* spp. (2.42%), *Serratia* spp. (2.42%).

2.3 Common bacteria isolated from dump sites

Most dumpsites that have been studied have had some common bacteria that have been isolated from them. *Escherichia coli* and *Klebsiella* species have always been isolated in most if not all of the dumpsites and garbage collection areas studied.

A previous study that studied the recovery of bacterial flora of different types from hospital waste found out that the different color-coded bags containing plastics and sharps were mostly sterile after hypochlorite treatment, or had no pathogenic bacteria at 0 hours of generation, another had *Acinetobacter* species isolated at 24 hours of generation and *Escherichia coli* and *Acinetobacter* species at 48 hours of generation. The cultures of two bags were found sterile even after 48 hours of generation. The samples at 0 hrs of generation for all the infectious waste bags were sterile. At 24 hours, *E coli, Staphylococcus* species and *Acinetobacter* species were isolated in some samples. All the samples had two or more types of pathogenic microorganisms at the end of 48 hours. The general waste contained many types of organisms, such as *E. coli, Klebsiella* species and *Staphylococcus* species, even in samples at 0 hours of generation. All the samples at the end of 24 hours and 48 hours had multiple types of organisms (Saini, Das, Kapil,

Nagarajan, & Sarma, 2004). This shows that *Escherichia coli* can be found in areas where it is thought to be sterile and can survive almost everywhere.

In another study in a private university in Nigeria found out that some of the common bacteria found on the dump sites were: *Bacillus, Klebsiella, Pseudomonas, Serratia, Staphylococcus, Enterobacter, Enterococcus, Aeromonas* and *Streptococcus,* respectively (Olukanni, Akinyinka, Ede, Akinwumi, & Ajanaku, 2014).

In another study on the antibiogram status of bacterial isolates from air around dumpsite of Ekiti state destitute centre at Ilokun, Ado-Ekiti, Nigeria found the bacterial distribution in the air to be, 37% of *Escherichia coli*, 19% of *Klebsiella* spp. 13% of *Pseudomonas* spp, 15% of *Serratia* spp. 8% of *Staphylococcus* spp. 7% of *Enterococcus* spp. and only 1% of *Salmonella* spp. (A. Odeyemi, 2012).

E. coli and *Klebsiella* among other bacteria have therefore shown their ability to grow even in areas thought to be sterile and therefore there was need to carry out our study since there was evidence of hospital wastes in the dumpsites.

2.4 *E. coli* pathogenicity

Gut of warm-blooded animals including human are normal inhabitants of *E. coli* and most *Escherichia coli* species in the gut are non-pathogenic commensals (Zorcolo & Casula, 2006). Certain strains may carry a combination of virulence genes which enable them to cause intestinal infections such as diarrhea or haemolytic colitis, or to cause extra-intestinal infections such as neonatal meningitis, nosocomial septicaemia, haemolytic uremic syndrome, urinary tract and surgical site infections (Zorcolo & Casula, 2006). Pathogenic *Escherichia coli* species can be classified into intestinal (IPEC) and extra intestinal (ExPEC) on the basis of their virulence factors and clinical symptoms. IPEC can be further classified into enterotoxigenic (ETEC), enteropathogenic (EPEC), enterohemorrhagic (EHEC), enteroinvasive (EIEC), and enteroaggregative (EaggEC) *E. coli* and diffusely adherent *E. coli* (DAEC) (Weintraub, 2007).

It has to be noted that the possession of a single or multiple virulence genes does not necessarily indicate that a strain is pathogenic unless that strain has the appropriate combination of virulence genes to cause disease in a specific host. Fecal matters from domestic, wild animals and humans may contain high numbers of *Escherichia coli* species harboring one or more virulence genes

(Odagiri *et al*., 2016). This may result in the antimicrobial resistance in dump sites since most of the garbage have fecal contamination.

2.5 Antimicrobial resistant profiles

Antimicrobial resistance is a growing problem that threatens modern healthcare globally. Resistance has traditionally been viewed as a clinical problem, but recently non-clinical environments have been highlighted as an important factor in the dissemination of antimicrobial resistance genes. Horizontal gene transfer events are likely to be common in aquatic environments; integrons in particular are well suited for mediating environmental dissemination of antimicrobial resistant genes. A growing body of evidence suggests that antimicrobial resistant genes are ubiquitous in natural environments. Particularly, elevated levels of antimicrobial resistant genes and integrons in aquatic environments are correlated to proximity to anthropogenic activities. Bacteria have been sowing resistance against various commonly used antibiotics. This is as a result of various reasons that have been studied to show how various bacteria that did not have resistance acquire their resistance. Molecular resistance mechanisms between clinical pathogens and the common soil bacterium *Streptomyces* were first shown to be similar in 1973. Since then, numerous parallels have been identified between soil microorganisms and clinically important strains, and the abundance of pathogens that can survive in soil results in a potent mixture that can give rise to the emergence of antibiotic resistance in the clinical setting. In recent years, metagenomic approaches have been implemented to characterize the diversity and prevalence of resistance in soil bacteria (Velsivasakthivel & Nandini, 2014).

Airborne MDR bacteria were isolated from municipal solid waste dumping site of Bangalore Karnataka, India using Anderson single stage air sampler. The *staphylococcus aureus* and *Streptococcus* species, developed resistant towards antibiotics like (Amoxicillin, Ampicillin, Ciproflaxin, Rifampin). Similarly, Amoxicillin and Ampicillin resistant *Enterococcus* species and *Streptococcus* species, were isolated inside dumping site which was found to be 24 and 10 isolates (Velsivasakthivel & Nandini, 2014).

Antibiotic resistant developing agents like alcohol, alkene, and steroid based material present in the garbage may induce the drug resistant capacity in gram negative bacteria. In other hand,

organic and inorganic toxic substance in waste materials, are consumed by the bacteria used as nutrients and developed the resistant character. Similar findings found out that percentage of antibiotic resistant bacteria number is more near dumping sites and decreased tendency to distance (Gibbs *et al*., 2006).

Ineffectiveness of antibiotics is as a result of selective pressure brought about by increased use and misuse of antibiotics (Frost, 2010). β-lactams, fluoroquinolones and aminoglycosides remain active against a significant proportion of *E. coli* strains causing nosocomial infections in Kenya (Brooks *et al*., 2006; Talbert, Mwaniki, Mwarumba, Newton, & Berkley, 2010). However, reports of multidrug resistant *Escherichia coli* clone ST131 are changing the existing strategies for chemotherapy. Some of the strains belonging to ST131 clone carry plasmid-borne *aac(6')-lb-cr*, and $bla_{CTX\text{-}M\text{-}15}$ (Clermont *et al*., 2008). The $bla_{CTX\text{-}M\text{-}15}$ genes confer resistance to third generation cephalosporins, while *aa(6')-lb-cr* confer low-level ciprofloxacin resistance and aminoglycosides resistance. For such strains, carbapenems remain the only plausible alternative against strains, antimicrobials that are more expensive and toxic (Rawat and Nair, 2010). It is therefore important to determine the diversity of genes carried by bacteria form the garbage areas and dump sites so as to control the rapid spread of antimicrobial resistance from the environment to human.

A previous study that determined the prevalence and antibiotic resistance phenotype of enteric bacteria from Arusha municipal dumpsite, found a total of 219 enteric bacteria from 75 genera. *Escherichia* spp. and *Shigella* spp. (12%), *Bacillus* spp. (11%) and *Proteiniclasticum* (4%) were the predominant genera. Most of the *Escherichia* spp., *Shigella* spp. and *Bacillus* were from fresh droppings of pigs continuously scavenging on the dumpsite, while *Proteiniclasticum* spp. was from biomedical waste. Some isolates from fresh droppings of pigs continuously scavenging on the dumpsite had 99% sequence similarity to pathogenic *Escherichia furgosonii*, *Shigellasonnei*, *Enterococcus faecium* and *Escherichia coli O154:H4*. Over 50% of the isolates were resistant to Penicillin G, Ceftazidime and Nalidixic Acid. Ciprofloxacin and Gentamicin were the most effective antibiotics with 81% and 79% susceptible isolates, respectively. Of all the isolates, 56% (45/80) were multidrug resistant. *Escherichia* spp. and *Bacillus* spp. (12 isolates each) constituted a large group of multidrug resistant bacteria (Samson Mwaikono, Maina, & Gwakisa, 2015).

2.6 Acquisition of antibiotic resistance by plasmids

Bacteria can acquire resistance to antibiotics by mutating existing genes (vertical evolution), or by acquiring new genes from other strains or species (horizontal gene transfer). A plasmid is a small, circular, double-stranded DNA molecule that is distinct from a cell's chromosomal DNA. Plasmids naturally exist in bacterial cells, and they also occur in some eukaryotes. Often, the genes carried in plasmids provide bacteria with genetic advantages, such as antibiotic resistance. Resistance genes encoded on plasmids are often located within genetic elements transposons. These elements include the transposase function that enables the transposon to recombine into bacterial plasmids (Cointreau, 1982). A resistant gene that is present in a bacteria can be transferred to another non resistant bacteria through the plasmids located in a transposon or integron (O'Brien, 2002)

Plasmids encode for genes that augment the fitness of their hosts, e.g. genes for antibiotic resistance, heavy metal resistance, virulence, fermentation of unusual carbon source, or UV light resistance. They can also block the entry of bacterial phage. Plasmids produce allelopathic substances such as bacteriocins- secreted toxins that can kill other strains of bacteria. Conjugative plasmids can use their capacity for infectious transfer to infect higher fitness bacterial variants. Therefore plasmid can be maintained by selective sweeps that have to occur frequently enough so that the acquisition of the bacterial genes by the chromosome does not occur before the next selective sweep (Samson Mwaikono *et al*., 2015).

This sharing of genes between bacteria by horizontal gene transfer, occurs by many different mechanisms namely; conjugation transduction, transformation. In bacterial conjugation the prototypical conjugative plasmid is the F-plasmid, or F-factor. The F-plasmid is an episome (a plasmid that can integrate itself into the bacterial chromosome by homologous recombination). The plasmid carries its own origin of replication, the *oriV*, and an origin of transfer, or *oriT*. In a bacterium there can only be one copy of the F-plasmid, which it is either free or integrated, and bacteria that possess a copy are called *F-positive* or *F-plus* (denoted F^+). The cells that lack F plasmids are called *F-negative* or *F-minus* (F^-) whose function as recipient cells. F-plasmid carries a *tra* and *trb* locus, which together are about 33 kb long and consist of about 40 genes. The *tra* locus includes the *pilin* gene and regulatory genes, which together form pili on the cell

surface. The locus also includes the genes for the proteins that attach themselves to the surface of F^- bacteria and initiate conjugation. Though there is some debate on the exact mechanism of conjugation it seems that the pili are not the structures through which DNA exchange occurs. Several proteins coded for in the *tra* or *trb* locus seem to open a channel between the bacteria and it is thought that the traD enzyme, located at the base of the pilus, initiates membrane fusion. When conjugation is initiated by a signal the relaxase enzyme creates a nick in one of the strands of the conjugative plasmid at the *ori T*. The enzymes may work alone or in a complex of over a dozen proteins known collectively as a relaxosome. In the F-plasmid system the relaxase enzyme is called TraI and the relaxosome consists of TraI, TraY, TraM and the integrated host factor IHF. The nicked strand, or *T-strand*, is then unwound from the unbroken strand and transferred to the recipient cell in a 5'-terminus to 3'-terminus direction. The remaining strand is replicated either independent of conjugative action (vegetative replication beginning at the *oriV*) or in concert with conjugation (conjugative replication similar to the rolling circle replication of lambda phage). Conjugative replication may require a second nick before successful transfer can occur (Lujan, Guogas, Ragonese, Matson, & Redinbo, 2007)

Since integration of the F-plasmid into the *Escherichia coli* chromosome is a rare spontaneous occurrence, and since the numerous genes promoting DNA transfer are in the plasmid genome rather than in the bacterial genome, it has been argued that conjugative bacterial gene transfer, as it occurs in the *E. coli* Hfr system, is not an evolutionary adaptation of the bacterial host, nor is it likely ancestral to eukaryotic sex (Lin & Scott, 2012).

Mobile genetic elements, including phages, plasmids and transposons mediate this transfer, and in some circumstances the presence of low levels of the antibiotic in the environment is the key signal that promotes gene transfer, perhaps ensuring that the whole microbial community is protected from the antibiotic (Nicolaudius, Elements, & Genet, 2012). A high proportion of drug resistance in bacteria is known to be associated with the acquisition of plasmid DNA, but the selective pressures that favor the maintenance of resistance are not fully defined (Gillespie, 2001).

Escherichia coli is naturally sensitive to ampicillin and amoxicillin. Acquired resistance conferred by a plasmid-encoded TEM-1 ß-lactamase was first described in 1965, and this has spread so extensively throughout the world that 40-60% of both hospital and community strains

are now resistant. Other plasmid-mediated ß-lactamases are sometimes seen in *Escherichia coli* (Acar & Goldstein, 1997). The plasmid, IncP (ß) which confers gentamicin resistance was identified in enterobacteria isolated from sewage. Bacteria become resistant to antimicrobials with ease and this has been of concern to clinicians, public health officials, and researchers. The use of antimicrobial agents in both human and veterinary medicine exerts a strong selective pressure inducing resistance to antimicrobial agents among bacteria (Moro, Beran, Griffith, & Hoffman, 2000). In generally, high-resistance bacteria are isolated from environments contaminated with antimicrobial agents, e.g., hospitals, farms, fish, sewage effluents, and waste water (Leistevuo *et al*., 1996). However, resistant bacteria have also been isolated from apparently non-selective environments. Many attempts have been made to show that plasmid transfer between bacteria occurs in a variety of natural habitats, e.g., waste water, sewage, sea water, river water, lake water, river epilithon, sediments, soil, gastrointestinal tracts, and grow in gradish plants and aqueous saw dust suspensions. However, most of these studies focused on disease causing microorganism and environmental bacteria belonging to the same bacterial family or group and derived from the same ecological niche. Conjugation and transfer of resistance plasmids (R plasmids) between distantly related bacteria have been described, but most of these experiments have been performed in laboratories under standardized conditions (Frost, 2010)

2.7 Extended Spectrum Beta Lactamases (ESBLs)

β-lactamases are enzymes that are produced by some bacteria and contribute to their resistance to beta-lactam antibiotics. The lactamase enzyme breaks the β-lactam ring open, deactivating the molecule's antibacterial properties (Philippon, Labia, & Jacoby, 1989).

Enterobacteriacae with extended-spectrum β-lactamase (ESBL) have increased in frequency in both clinical and environmental samples during the last decades, and are now considered as an escalating challenge for global health care. This increase is not due to clonal expansion of single genotypes, as there is considerable variation in ESBL genotype distribution between different geographical regions. The rapid spread of ESBLs has largely consisted of different CTX-M types, but the mechanisms behind its greater penetration into *E*scherichia *coli* populations compared to other ESBL classes is largely unknown (Liu *et al*., 2015).

ESBLs are often plasmid mediated and most of the enzymes are members of TEM and SHV families that have been described in many countries. The TEM was first reported in *E. coli* isolated from a patient named Temoniera. The name of the other beta-lactamase, SHV, is due to sulf-hydryl variable active site. Detection of TEM and SHV genes by molecular methods in ESBL producing bacteria and their pattern of antimicrobial resistance can provide useful information about the epidemiology and risk factors associated with certain infections (Ghafourian *et al*., 2011).

OXA beta-lactamases were long recognized as a less common but also plasmid-mediated beta-lactamase variety that could hydrolyze oxacillin and related anti-staphylococcal penicillins. These beta-lactamases differ from the TEM and SHV enzymes in that they belong to molecular class D and functional group 2d. The OXA-type beta-lactamases confer resistance to ampicillin and cephalothin and are characterized by their high hydrolytic activity against oxacillin and cloxacillin and the fact that they are poorly inhibited by clavulanic acid. Amino acid substitutions in OXA enzymes can also give the ESBL phenotype. While most ESBLs have been found in *Escherichia coli*, *K. pneumoniae*, and other Enterobacteriaceae, the OXA-type ESBLs have been found mainly in *P. aeruginosa* (Liu *et al*., 2015).

A previous study conducted in 2009 covering 8 of the 10 Canadian provinces found an average ESBL prevalence of 4.3% in *E. coli* isolated from patients attending hospitals (clinics, emergency rooms, medical and surgical wards, and intensive care units). In this Canadian material the $bla_{\text{CTX-M-15}}$ genotype is most common, and ESBLs was predominantly detected in at least 7 wide-spread *E*scherichia *coli* sequence types, including ST131 and members of the ST10 clonal complex (Zhanel *et al*., 2010).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study site

Dumpsites near schools, residential areas and the municipal general waste dumping sites in Nairobi area were selected for the study and sampled.

3.2 Qualitative survey of the dumpsites and garbage collection areas

In order to verify the most accessed area of these dump sites and garbage collection areas by the street families and other people relying on dumpsites for a living, a qualitative survey of the dump site and garbage areas was conducted. The purpose of the survey was to determine the most appropriate area to sample for the main study. This was done by visiting of the dumpsites and garbage areas before the start of the study and surveying the areas and identifying potential barriers and sample collection areas to our study (**Appendix 8**).

3.3 Sample collection and sample size

Sample collection was randomly carried out in different days in seventeen different points. A total of 126 samples were collected during the sampling period. At each sampling station, the sub-surface soil, mixed solids, leaking water, stagnant water, swabs and food samples were collected from one squire foot area into sterile sampling bottles and appropriately labeled. Six samples were collected from each site. The samples were then transported to the laboratory for analysis.

3.4 Microbiological analysis

3.4.1 Determination of CFU

One gram (1g) of each solid samples and 1mL of the liquid samples and swabs were suspended in 10mL normal saline. Serial dilutions of 10 fold, 5 fold and 1 fold dilutions were prepared from the 10mL suspension and transferred onto duplicate molten Plate Count Agar (PCA) mixed and allowed to cool at room temperature. This was then incubated at 37^0C for 24 hours. Colonies were determined from duplicate plates and the average counts recorded as mean viable bacteria (colony forming units [CFUs]) of the sample. The low and high CFU's were reached by dividing

the dumpsites and garbage collection areas into two, those that had CFU'S above 5.0 were considered to be high and those below 5.0 considered to be low.

3.4.2 Isolation and identification of *E. coli* and *Klebsiella* for analysis

A loop full (1µl) of the mixture incubated in buffered peptone water was then transferred onto MacConkey agar plates and incubated at 37^0c for 24 hours for isolation of *E. coli* and *Klebsiella* species. The plates were then examined for growth and presumptive identification of *E. coli* and *Klebsiella* species (pink non-mucoid for *E. coli* and pink mucoid for *Klebsiella*).

3.4.3 Biochemical identification of suspect isolates

Biochemical and Gram stain tests were performed on colonies from primary cultures for final identification of the isolates. The Biochemical tests done included, IMVIC (Cheesebrough, 2006). Results obtained from cultural and biochemical reactions were used for phenotypic characterization of the bacterial isolates.

3.5 Antimicrobial susceptibility testing

The antimicrobial susceptibility testing was done on 286 isolates on Mueller-Hinton agar plates (Oxoid). In the first plate the following antibiotics were placed: Ampicillin (AMP, 10µg), Cefpodoxime (CPD, 10µg), Ceftazidime (CAZ, 30µg), Cefoxitin (FOX, 30ug) Cefepime (FEP, 30µg). Then Amoxicillin-Clavulanic acid (AMC, 10/100µg ratio) disk was placed at the centre of the plate. In the second plate the following antibiotics were used; Ciprofloxacin (CIP, 10µg), Tetracycline (TE, 30ug), Trimethoprim Sulfamethoxazole (SXT, 30µg), Gentamicin (GEN, 10µg), Chloramphenicol (C, 30µg), Streptomycin (S, 25µg), Nalidixic acid (NA, 10ug), and meropenem (MRP, 10ug). The plates were then incubated at 37°C for 18–24 hours. These antibiotics were chosen on the basis of their use in the management of enteric bacterial infections and their suitability for presumptive identification of ESBL producers. The inoculums for susceptibility testing were compared against the McFarland 0.5 turbidity standards with *E.coli* ATCC 25922 strain being used as the control standard for quality assurance of media and the antimicrobial discs. The interpretation of results was according to Clinical and Laboratory Standards Institute guidelines (CLSI, 2013).

3.6 Confirmation of ESBLs using disc diffusion test

Phenotypic Extended Spectrum β-Lactamases (ESBL) detection was performed on the 286 isolates using disc synergy test following the CLSI 2013 guidelines. Discs containing cephalosporin (ceftazidime, cefepime) were applied next to a disc with amoxicillin + clavulanic acid and incubated over night at 37^{o}C. Positive results were indicated when the inhibition zones around any of the cephalosporin discs were augmented in the direction of the disc containing amoxicillin + clavulanic acid. Isolates that showed exhibiting resistance to at least one or more-third generation cephalosporins with or without concomitant susceptibility to amoxicillin/clavulanic were picked as potential ESBL producers.

3.7 DNA Extraction from Bacteria

In the extraction of DNA, the ten randomly selected isolates were purified on nutrient agar plates (Oxoid, U.K) and pellets were harvested aseptically. DNA extraction was done using the boiling method in sterile distilled water. A pea-sized inoculum of pure colonies of the isolates was added to a tube containing 1ml of sterile distilled water and lysis done by incubation at 95^{O}C for 10 min. The lysates were centrifuged on a table-top centrifuge for 10 min at 14000 rpm. The supernatant was used as the template in PCR analysis.

3.8 Detection of *bla$_{TEM}$*, *bla$_{SHV}$*, *bla$_{CTX-M}$*, genes by PCR

Twenty randomly selected isolates that demonstrated the ESBL-phenotypes (exhibiting resistance to at least one or more-third generation cephalosporins with or without concomitant susceptibility to amoxicillin/ clavulanic) were screened for *bla$_{TEM}$*, *bla$_{SHV}$*, and *bla$_{CTX-M}$* genes. These genes are frequently implicated in the ESBL phenotype among *Enterobacteriaceae*. The presence of the β-lactamase genes was screened via PCR using primers listed in the table below **(Table 3.2)**. The PCR procedure used for the detection of these genes was the one previously described by Kiiru *et al.* (2012). Briefly, the gene of interest was amplified in a total reaction volume of 25µl containing 10pmol each of primer, 20mM of each dNTPs, 10mM Tris-HCl (pH 8.8), 25mM $MgCl_2$, and 1.25U Taq DNA Polymerase (ThermoFischer Scientific, Rockford, IL, USA). At least 2-5µL of template DNA was added to 23 µL of master mixture. The reaction mixture was placed in MJ-mini Bio-Rad thermal cycler (Bio-Rad, USA). The PCR amplification cycle was performed with cycling conditions consisting of an initial denaturation step at 95^{O}C

for 5 min, followed by 35 cycles of 94^{O}C for 30 seconds, annealing temperatures were selected based on the gene of interest for 1 min (55^{O}C for bla_{TEM} or 50^{O}C for bla_{SHV} and 60^{O}C for bla_{CTX-M} and an extension temperature of 72^{O}C for 1min 30 sec. The final extension step was set at 72^{O}C for 10min. The presence and sizes of amplicons were determined on 1.2 % agarose gels (Invitrogen Life Technologies, Paisley, UK), using Gene Ruler 100 bp DNA Ladder Plus (Fermentas Sweden, Helsingborg, Sweden) as a size marker (Hansen *et al.,* 2012). After electrophoresis DNA fragments was visualized by Bio-Rad Gel documentation system (Bio-Rad, USA).

Table 3. 1 Consensus primer table

Target Gene	Primer name	5'-3'sequence	Size (bp)	Accession no.	References
*bla*TEM	TEM	F-ATGAGTATTCAACAT TTCCG R- CCAATGCTTAATCAGTGAGG	717	EF125012 -related	Oviasigie,2010
*bla*SHV	SHV	F- TTCGCCTGTGTATTATCTCCCTG R- TTAGCGTTGCCAGTGTCG	471	AF148850 -related	Oviasigie,2010
*bla*CTX-M	MA1 MA2	ATGTGCAGACCAGTAAGTATGGC TGGGTAARTAGTACCAGAACAGCGG	593	Y10278- related	Oviasigie,2010

3.9 Screening for *E.coli* pathogenic strains

In order to determine the pathotypes of our *E. coli* isolates on ten selected isolates, Screening of five categories of diarrheagenic *E. coli* including; enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC), enterohemorrhagic *E. coli* (EHEC), and enteroaggregative *E. coli* (EAggEC) was done from the *E. coli* strains isolated.

Screening for these isolates was done using the multiplex PCR primers listed in **Table 3.3**. Briefly, each multiplex PCR assay was performed in 50 µl of reaction mixture containing 1 mm deoxynucleoside triphosphate mix, 10 pmol of each primer, 1.5 mm MgCl2, 1x reaction buffer (10 mm Tris-HCl, 50 mm KCl), 0.25U of Taq DNA polymerase, and 3 µl of DNA as the template. In order to prevent non-specific amplification a hot-start technique (where the reaction mix is heated for 94°C for 5 min before Taq polymerase is added) was applied. The samples were then subjected to 35 cycles of amplification each consisting of 1.5 min at 94°C, 1.5 min at 55^{o}C, 1.5 min at 64°C, and 1.5 min at 72°C. A final extension step was carried out for 5min at 72°C before the PCR products were separated by electrophoresis in 1.5% agarose gels and stained with ethidium bromide visualized through UV transmission, and photographed.

Table 3. 2 PCR Primers used in this study

Target gene	Description	Sequence (5' to 3')	Amplicon size (bp)	References
*Agg*R	EAEC, EAggEC	GTATACACAAAAGAAGGAAGC ACAGAATCGTCAGCATCAGC	254	Adekanle, 2014
*eae*A	STEC, EPEC	AAACAGGTGAAACTGTTGCC CTCTGCAGATTAACCTCTGC	917	Adekanle, 2014
STp	ETEC	TCTGTATTATCTTTCCCCTC ATAACATCCAGCACAGGC	186	Adekanle, 2014
STh	ETEC	CCCTCAGGATGCTAAACCAG TTAATAGCACCCGGTACAAGC	186	Adekanle, 2014
LT	ETEC	AGCAGGTTTCCCACCGGATCAC CA GTGCTCAGATTCTGGGTCTC	218	Adekanle, 2014
*Stx*2	STEC, EHEC	TTCGGTATCCTATTCCCG TCTCTGGTCATTGTATTA	474	Adekanle, 2014

3.10 Ethical Considerations

No ethical consideration was required in this study since there were no human samples involved. However permission was sort from the waste workers, those living on or adjacent to the dump site or garbage collection area and the municipal council of Nairobi. They all gave their agreement before commencement of the project.

CHAPTER FOUR

4. 0 RESULTS

4.1 Qualitative Survey of the Dumpsites and garbage collection areas.

A total of 17 dump sites (permanent dumping area) and garbage collection areas (temporary dumping area where garbage is dumped awaiting collection) were sampled in different parts of Nairobi area. 30 samples were from Dandora dumpsite since the dumpsite is very large. In the 17 dumpsites and garbage collection areas, 12 had evidence of fecal contamination (human and animal feces). There was evidence of recycling of vegetables and reselling at a cheaper price in all major dumpsites near market. In all the dumpsites and garbage collection sites, there was stagnant water regardless the season. Most of this water was seepage from these sites. In most of the dumpsites there was evidence of ongoing human activities such as people working as dumpsite attendants, recycling waste foods and other recyclable items such as bottles and metals. It was not unusual to find children playing near or in these garbage collection sites and dump sites.

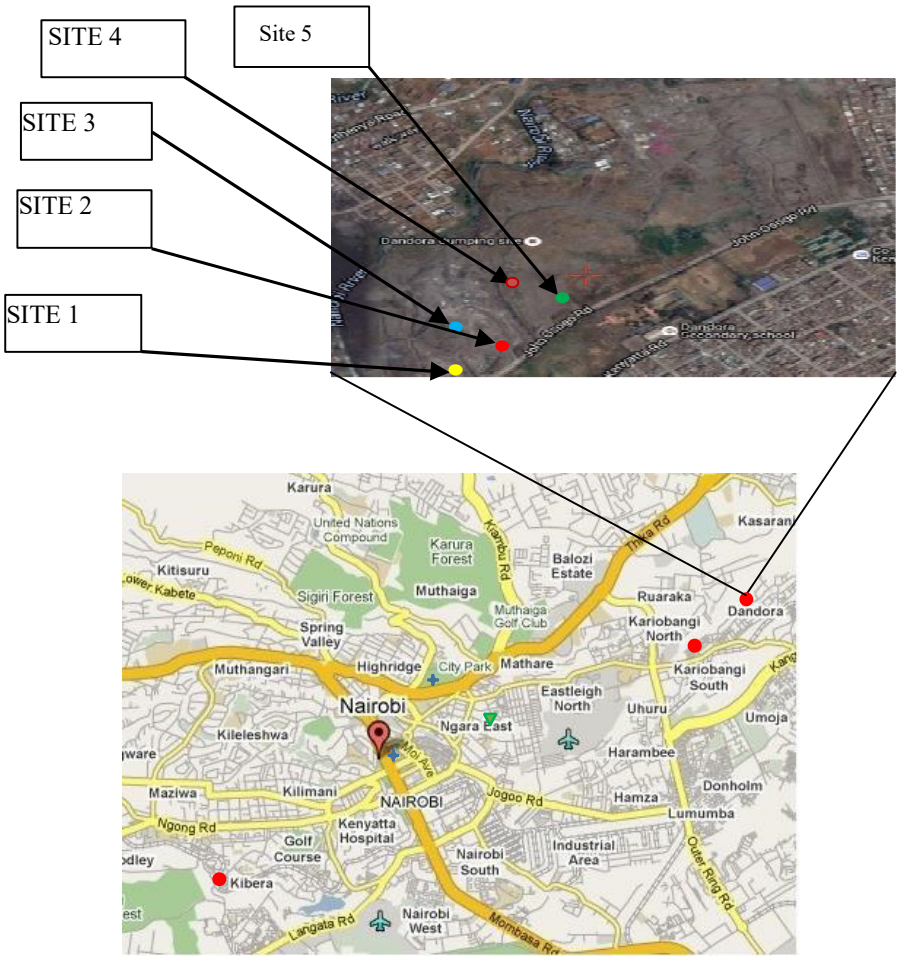


FIGURE 4. 1 Aerial view of the areas sampled in different parts of Nairobi County

KEY
- -Dumpsites and garbage Collection areas near residential places
- -Dumpsites and garbage Collection areas in Market areas
- -Dumpsites and garbage Collection areas near Schools

The different dump sites and garbage collection areas sampled were in market places (**D**) near residential places (**A** and **C**) and schools (**B**), **Plate 4.1**. The presence of garbage trucks coming in and out of the dumpsites was observed in most of the dumpsites.

PLATE 4. 1 Qualitative survey of the dumpsites and garbage collection areas

Key: A- A garbage truck ferrying garbage into the dumpsite that is adjacent to residential houses. **B**- An uncollected garbage collection area adjacent to a school **C**- Children playing adjacent to a dump site. **D**- A dump site inside a market in Nairobi area.

4.2 Contamination levels of the dumpsites and garbage collection areas

High *Enterobacteriaceae* CFUs is an indicator of possible fecal contamination. The lowest CFU from any given sampling point was 1.1 x 10^{11} that was recorded in Dandora dumpsite. The site with the highest CFU/unit volume value was Muthurwa Estate Dumpsite that recorded 8.2 x 10^{10}. Other sites with high CFU counts were Umama garbage collection area (Komarock), Kawangware Market Dumpsite, Kenyatta staff quarter garbage collection area, Kweria garbage collection area, City market garbage collection area, Central police garbage collection area, Kibera Dumpsite, and Kenyatta Market Dumpsite while Seven of the dumpsites and garbage collection areas (Ayany Dumpsite, Dandora Dumpsite, Ngara market garbage collection area, Muthurwa Market garbage collection area, Masai Market dumpsite, Mareba garbage collection area (Kibera), District Commissioner garbage collection area (Kibera) recorded CFUs below $2.0x10^{10}$. With most of the dumpsites and garbage collection areas having human activity, this poses great danger to contamination and infection also increases the possibility of spread these bacteria from the environment to people and animals, **Table 4.1**.

Table 4. 1 Average microbial load of the samples from dumpsite and garbage collection areas and their characteristics

Dumpsite/garbage collection area	Area in Nairobi	Location	Average CFUs	Category of CFU	Dumpsite/garbage collection area characteristics
Muthurwa Estate Dumpsite	East	Residential	8.2×10^{10}	High	Fecal contamination, Domestic/Rodents
Umama garbage collection area (Komarock)	North Western	Residential	7.9×10^{10}	High	Seepage, Fecal contamination,
Kawangware Market Dumpsite	East	Market	7.7×10^{10}	High	Fecal contamination, Domestic/Rodents
Kenyatta staff quarter garbage collection area	South	Market	7.3×10^{10}	High	Seepage, Fecal contamination, Domestic/Rodents, Human activity
Kweria garbage collection area	Central	Residential	7.2×10^{10}	High	Fecal contamination, Domestic/Rodents, Human activity
City market garbage collection area	Central	Market	6.7×10^{10}	High	seepage, Human activity
Central police garbage collection area	West	Residetial	6.1×10^{10}	High	Seepage
Kibera Dumpsite	South	Residential	5.0×10^{10}	High	Seepage, Fecal contamination, Domestic/Rodents, Human activity
Kenyatta Market Dumpsite	South	Residential	5.0×10^{10}	High	Seepage, Fecal contamination, Domestic/Rodents, Human activity
City park Market Dumpsite	South Western	Market	2.0×10^{10}	High	Seepage, Domestic/Rodents, Human activity
Muthurwa Market garbage collection area	East	Residential	1.8×10^{11}	Low	Fecal contamination, Human activity
Mareba garbage collection area (Kibera)	South Eastern	School	1.5×10^{11}	Low	Fecal contamination, Domestic/Rodents, Human activity
Ayany Dumpsite	South Eastern	Residential	1.5×10^{11}	Low	Seepage, Human activity
Ngara market garbage collection area	West	Residential	1.4×10^{11}	Low	Fecal contamination, Domestic/Rodents

Masai Market dumpsite	West	Market	1.2×10^{11}	Low	Human activity
District Commissioner garbage collection area (Kibera)	South	Residential	1.2×10^{11}	Low	Seepage, Fecal contamination, Human activity
Dandora Dumpsite	North Western	Residential	1.1×10^{11}	Low	Seepage, Fecal contamination, Domestic/Rodents, Human activity

Key: Seepage- leakage of water into the ground in and around the dumpsite or garbage collection area, **Fecal contamination-** presences of feces on the dumpsite or garbage collection area, **Domestic/Rodents-** Presence of livestock (e.g. goats) or rodents (e.g. rats) on the dumpsite and garbage collection area, **Human activity-** presence of humans on or adjacent to the dumpsite and garbage collection area.

4.3 Antimicrobial susceptibility profile of *E. coli* and *Klebsiella* species

Resistance to all antimicrobials for *E. coli* and *Klebsiella* was above 5% except for ciprofloxacin (3.3% *E.coli*, and 2.4% *Klebsiella*), meropenem (1.7% *E.coli* and 1.8% *Klebsiella*) and gentamicin (3.3% *E.coli* and 0% *Klebsiella*). In general, there was no difference in resistance to most antimicrobials between *E. coli* and *Klebsiella* since the Chi- square P values were above 0.05 **(Table 4.2).**

Table 4. 2 Distribution of antibiotic resistance of *E. coli* and *Klebsiella* among the antibiotics used.

Antimicrobial	*E. coli* (%)	*Klebsiella* (%)	P value
Ceftazidime (CAZ)			
Resistance	6 (5.0)	14 (8.5)	0.237
Intermediate	0	3 (1.8)	0.999
Susceptible	115 (95.0)	148 (89.7)	
Cefpodoxime (CPD)			
Resistance	24 (19.8)	44 (26.7)	0.404
Intermediate	23 (19.0)	15 (9.1)	0.031
Susceptible	74 (61.2)	106 (64.2)	
Cefoxitin (FOX)			
Resistance	15 (12.4)	20 (12.1)	0.918
Intermediate	2 (1.7)	1 (0.6)	0.408
Susceptible	104 (86.0)	144 (87.3)	

Cefepime (FEP)			
Resistance	16 (13.2)	30 (18.2)	0.145
Intermediate	39 (32.2)	61 (37.0)	0.211
Susceptible	66 (54.5)	74 (44.8)	
Ciprofloxacin (CIP)			
Resistance	4 (3.3)	4 (2.4)	0.664
Intermediate	2 (1.7)	4 (2.4)	0.662
Susceptible	115 (95.0)	157 (95.2)	
Amoxicilin clavulanic acid (AMC)			
Resistance	15 (12.4)	19 (11.5)	0.710
Intermediate	18 (14.9)	18 (10.9)	0.299
Susceptible	88 (72.7)	128 (77.6)	
Tetracyclin (T)			
Resistance	33 (27.3)	41 (24.8)	0.756
Intermediate	6 (5.0)	13 (7.9)	0.361
Susceptible	82 (67.8)	111 (67.3)	
Meropenem (MRP)			
Resistance	2 (1.7)	3 (1.8)	0.744
Intermediate	19 (15.7)	51 (30.9)	0.003
Susceptible	100 (82.6)	111 (67.3)	

Streptomycin (S)			
Resistance	55 (45.5)	75 (45.5)	0.929
Intermediate	59 (48.8)	80 (48.5)	0.920
Susceptible	7 (5.8)	10 (6.1)	
Nalidixic acid (NA)			
Resistance	11 (9.1)	14 (8.5)	0.883
Intermediate	0	2 (1.2)	0.999
Susceptible	110 (90.9)	149 (90.3)	
Cloramphenical (C)			
Resistance	7 (5.8)	13 (7.9)	0.483
Intermediate	2 (1.7)	4 (2.4)	0.636
Susceptible	112 (92.6)	148 (89.7)	
Gentamicin (GEN)			
Resistance	4 (3.3)	0	0.999
Intermediate	3 (2.5)	8 (4.8)	0.337
Susceptible	114 (94.2)	157 (95.2)	
Trimethoprim sulfamethoxazole (SXT)	35 (28.9)	48 (29.1)	0.809
Resistance	6 (5.0)	0	0.999
Intermediate	80 (66.1)	117 (70.9)	
Susceptible			

Ampicilin (AMP)			
Resistance	51 (42.1)	97 (58.8)	0.009
Intermediate	16 (13.2)	16 (9.7)	0.925
Susceptible	54 (44.6)	52 (31.5)	

Key: **AMP**-ampicillin (10µg), **CPD**-cefpodoxime (10µg), **CAZ**-ceftazidime (30µg), **FOX**-cefoxitin (30µg), **FEP**-cefepime (30µg), **CIP**-ciprofloxacin (10µg), **AMC**-amoxicillin clavulanic acid (10µg), **TE**-tetracycline (30µg), **MRP**-meropenem (10µg), **S**-streptomycin (10µg), **NA**-nalidixic acid (10µg), **C**-cloramphenical (10µg), **GEN**-gentamicin (10µg), **SXT**-trimethoprim sulfamethoxazole (30µg)

4.4 Antimicrobial resistance in dumpsites and garbage collection areas with high and low Colony Forming Units

In general, resistances prevalence was similar for *E. coli* and *Klebsiella* obtained from samples with high CFUs to those obtained from samples with low CFUs. In the dumpsites and garbage collection areas that had high CFUs, such as Muthurwa estate dumpsite, Central police garbage collection area, City market garbage collection area, City park market dumpsite **Figure 4.2A**, there were high resistance prevalence's of above 25% to streptomycin, ampicillin, tetracycline and trimethoprim sulfamethoxazole for isolates belonging to both species. In contrast there was low resistance to meropenem, gentamicin, and ciprofloxacin (≤5%) in both species. The study also found that 42% of *E. coli* were resistant to ampicillin compared to 59% of *Klebsiella* isolates found in this study. Resistance to gentamicin among isolates from sites recording high CFUs was only observed for *E. coli* (6%) while resistance to meropenem from the same population of isolates, was observed in *Klebsiella* (2.8%).

There was high resistance (> 20%) to streptomycin, ampicillin, tetracycline, trimethoprim sulfamethoxazole and cefpodoxime for both species in the dumpsites and garbage collection areas that had low CFUs such as the Ngara market garbage collection area, Ayany dumpsite, Dandora dumpsite, Muthurwa market garbage collection area **Figure 4.2B.** In such sites, there was low resistance of *E. coli* and *Klebsiella* to meropenem, gentamicin, and ciprofloxacin (< 5%). The only *E. coli* strains found to be resistant to gentamicin were from the sites with low CFUs.

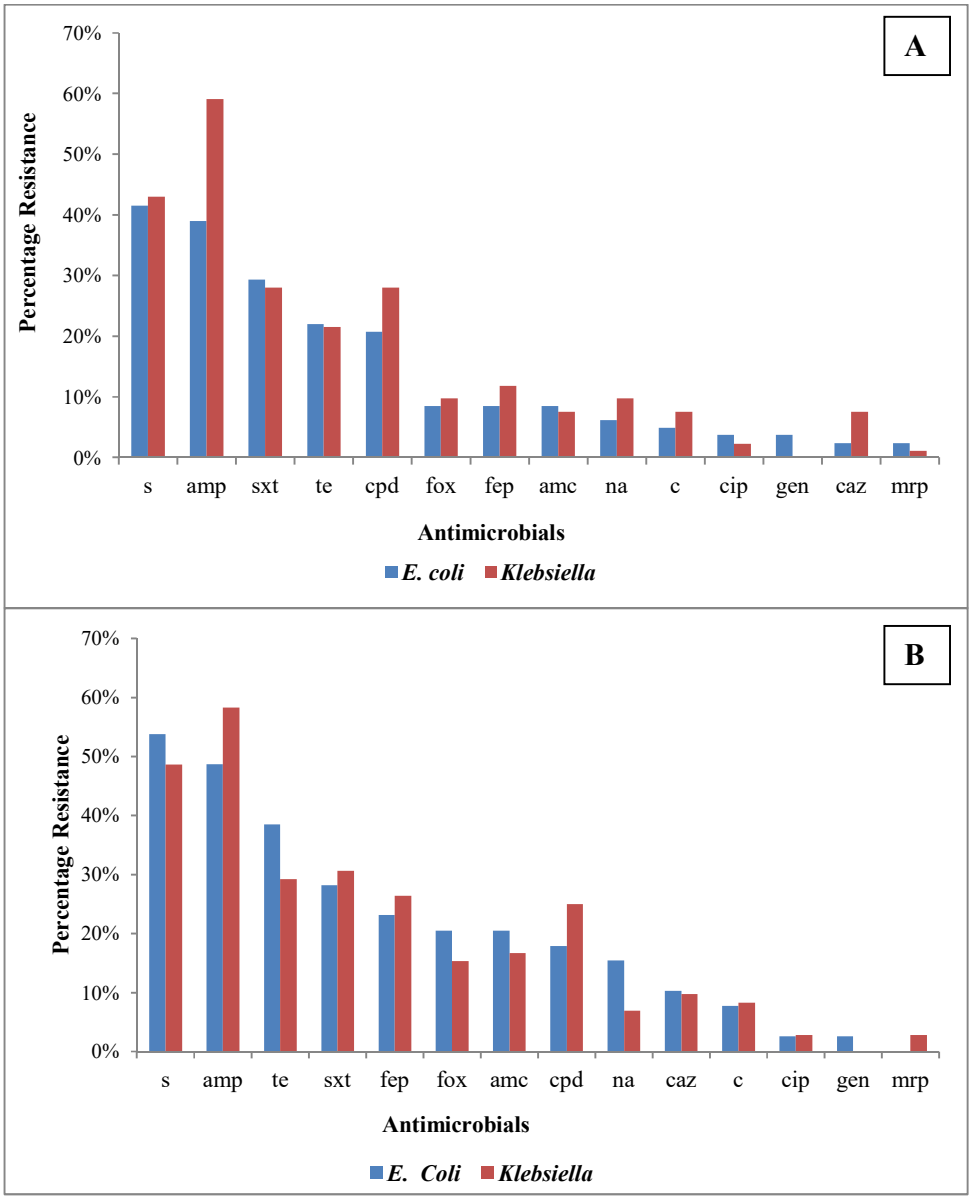


Figure 4. 2 A-antimicrobial resistance in dumpsites and garbage collection areas with high Colony Forming Units, B,-antimicrobial resistance in dumpsites and garbage collection areas with low Colony Forming Units

Key: **AMP**-ampicillin (10µg), **CPD**-cefpodoxime (10µg), **CAZ**-ceftazidime (30µg), **FOX**-cefoxitin (30µg), **FEP**-cefepime (30µg), **CIP**-ciprofloxacin (10µg), **AMC**-amoxicillin clavulanic acid (10µg), **TE**-tetracycline (30µg), **MRP**-meropenem (10µg), **S**-streptomycin (10µg), **NA**-nalidixic acid (10µg), **C**-cloramphenical (10µg), **GEN**-gentamicin (10µg), **SXT**-trimethoprim sulfamethoxazole (30µg)

4.5 Antimicrobial resistance profiles

There were no isolates that were fully susceptible in 7(41%) of the seventeen dumpsites and garbage collection areas sampled. Most (61%) of the isolates were resistant to 1-3 antimicrobials. Another 23% of isolates were resistant to more than 3 antimicrobials and were thus multidrug resistant (MDROs) **Table 4.3**. In the Umama garbage collection area (Komarock) 5(71%) out of the 7 isolates recovered, were MDROs but no MDROs strain was recovered from the Kawangware Market Dumpsite. The sites with the highest prevalence of MDROs strains included Muthurwa Estate Dumpsite (66%) and Umama garbage collection area (Komarock) (71%) that both had high CFUs **Table 4.1**.

Table 4. 3 Distribution of isolates with different resistance profiles across dump sites

		Number of antimicrobials to which *E. coli* and *Klebsiella* are resistant (%)		
Dumpsites/garbage areas	**No. of isolates**	**Fully susceptible**	**1-3 antimicrobials**	**> 3 antimicrobials (MDROs)**
Ayany Dumpsite	19	2 (10.5)	10 (52.6)	7 (36.8)
Central police garbage collection area	18	1 (5.6)	12 (66.7)	5 (27.8)
City market garbage collection area	22	3 (13.6)	12 (54.5)	7 (31.8)
City park Market Dumpsite	29	5 (17.2)	20 (69.0)	4 (13.8)
Dandora Dumpsite	33	1 (3.0)	27 (81.8)	5 (15.2)
District Commissioner's garbage collection area (Kibera)	9	0	7 (77.8)	2 (22.2)
Kenyatta Market Dumpsite	15	3 (20.0)	10 (66.7)	2 (13.3)
Kenyatta staff quarter garbage collection area	24	10 (41.7)	13 (54.2)	1 (4.2)

Kibera Dumpsite	15	0	12 (80.0)	3 (20.0)
Kweria garbage collection area	24	3 (12.5)	17 (70.8)	4 (16.7)
Mareba garbage collection area (Kibera)	7	0	4 (57.1)	3 (42.9)
Masai Market dumpsite	14	0	11 (78.6)	3 (21.4)
Muthurwa Estate Dumpsite	12	0	4 (33.3)	8 (66.7)
Muthurwa Market garbage collection area	17	1 (5.9)	10 (58.8)	6 (35.3)
Ngara market garbage collection area	17	3 (17.6)	12 (70.6)	2 (11.8)
Kawangware Market Dumpsite	4	0	4 (100.0)	0
Umama garbage collection area (Komarock)	7	0	2 (28.6)	5 (71.4)

Key: The fully susceptible are those that did not show any resistance while resistance is showing resistance to 1-3 antimicrobials and MDROs are those that show resistance of more than 3 antimicrobials.

4.6 Frequency distribution of the zones sizes for the antimicrobials tested

The study also tested the resistant, intermediate and susceptible ranges of some of the antimicrobials used in this study. This was so as to understand how resistant, intermediate or susceptible an organism was by looking at where the frequencies clustered.

4.6.1 Distribution of inhibition zone sizes for cephalosporins

In this study, susceptibility profiles for ceftazidime, cefpodoxime, cefoxitin and cefepime Cephalosporins were determined. They are used in the treatment of a wide range of bacterial infections such as ear infections, pneumonia and meningitis. As indicated in **Table 4.2**, only a small proportion of *E. coli* (5%) and an equally small proportion of *Klebsiella* (9%) were resistant to ceftazidime. Majority of isolates belonging to the two species clustered within the susceptible range of 24mm and 30mm of ceftazidime **Figure 4.4A** thus implies that the proportion of resistant strains to this antimicrobial is likely to increase.

The resistance to cefpodoxime for *E. coli* was 20% and that of *Klebsiella* was 27% **Table 4.2**. Majority of *E. coli* (60%) and 51% of *Klebsiella* clustered between 22mm and 30mm and while (12%) of *Klebsiella* and (8%) of *E.coli* clustered at the extreme resistant range of 6mm for cefpodoxime **Figure 4.4B.** Resistance to this antimicrobial was low for both species with only *E. coli* 12.4%, and *Klebsiella* 12.1% exhibiting resistance to this agent **Table 4.2** and the zones of inhibition on the susceptible range clustered between 22mm and 28mm **Figure 4.4C**. It is possible that a higher proportion of strains belonging to this species may become resistant considering that 75% *E. coli*, and 79% *Klebsiella* clustered within the mid susceptible range of between 22mm-28mm.

Only a small proportion of *E. coli* (3%) and *Klebsiella* (9%) were resistant to the fourth generation cephalosporin, cefepime **Table 4.2**. Majority of the isolates exhibited susceptibility zones between greater than 26mm, **Figure 4.4D** indicating that this antimicrobial would be a good choice for treatment of infections caused by similar strains.

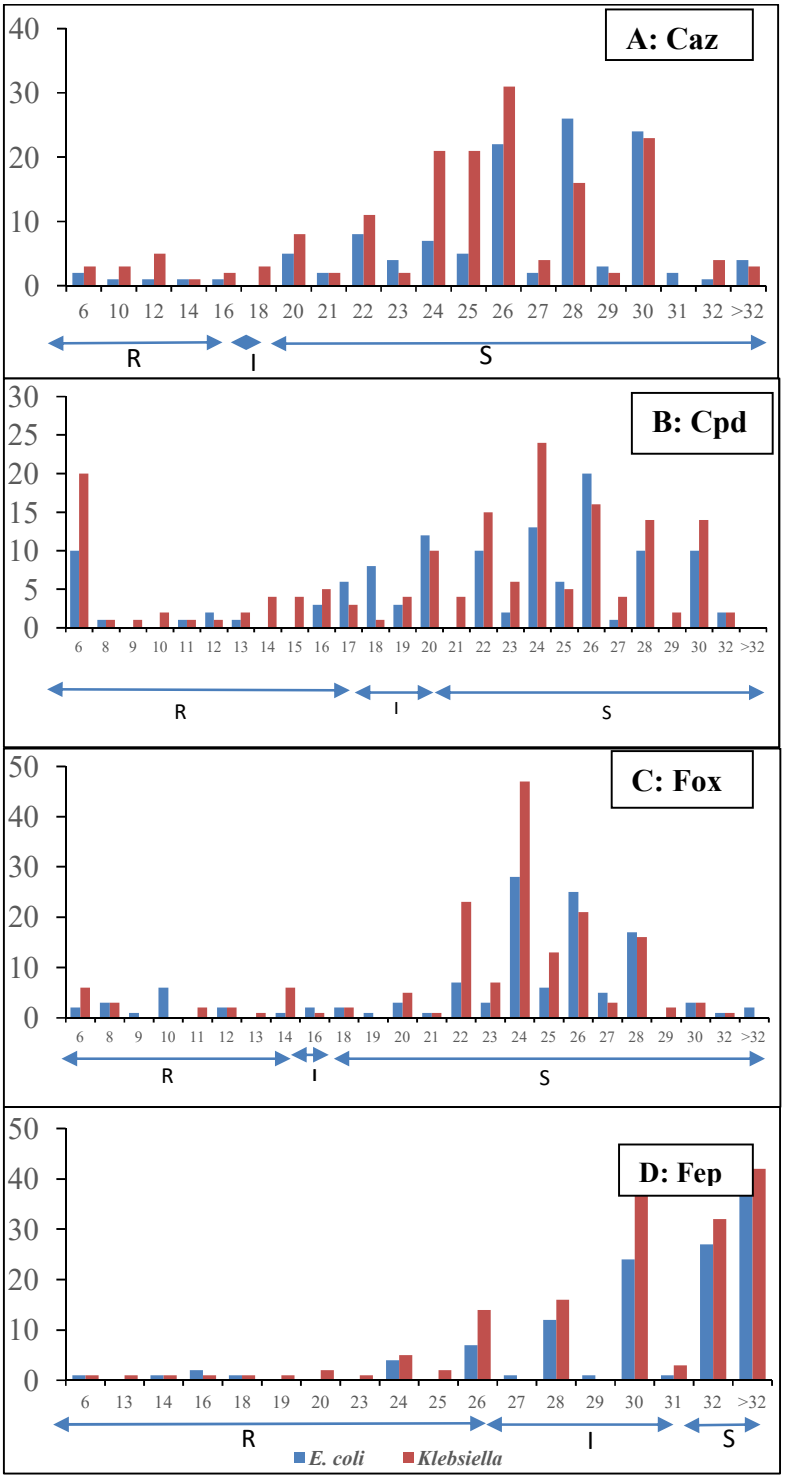


Figure 4. 3 Zones of inhibition for cephalosporins

Key: R – Resistance, **I** – Intermediate & **S** – Susceptible, A- zones of inhibition for cefpodoxime, **B**- zones of inhibition for cefoxitin, **C**- zones of inhibition for ceftazidime, **D**- zones of inhibition for cefepime

4.6.2 Distribution of inhibition zone sizes for Meropenem

Meropenem would be a good drug for the treatment of the isolates recovered from our study since it recorded a low resistance of 2% to both *E. coli* and *Klebsiella* **Table 4.2**. From the figure below it is evident that most of the isolates recovered clustered between the zone sizes of 22mm->32mm **Figure 4.4**. However some of the isolates in the susceptible range could become resistant since they were weak in their susceptibility.

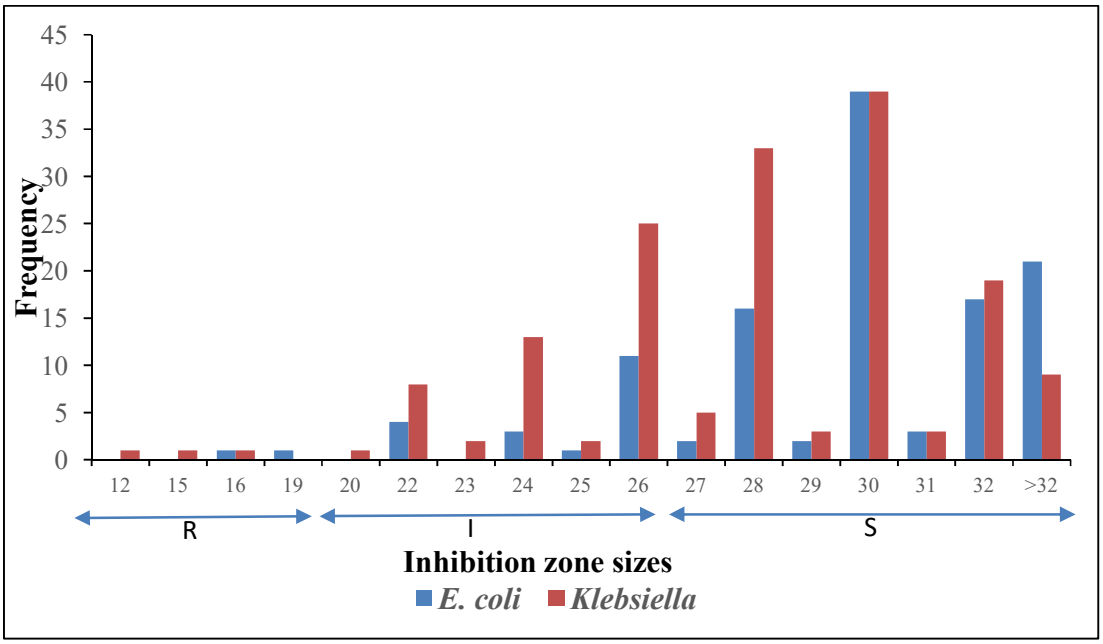


Figure 4. 4 Zones of inhibition for meropenem

Key: R – Resistance, **I** – Intermediate &**S** – Susceptible Expand this Legend**.**

4.7 ESBL producing strains

Out of the 286 samples tested, there was low prevalence's of ESBL producers (16%) across the dumpsites and garbage collection areas **(Table 4.4)**. There was no presence of ESBL producers in Kawangware market dumpsite and the Umama garbage collection areas. Both sites had a high CFU of 7.7 x 10^{10} and 7.9 x 10^{10} respectively. Kibera dumpsite that had CFU of 5.0 x 10^{10} had the highest percentage (33%) of ESBL producers.

Table 4. 4 Occurrence of ESBLs and Non- ESBLs producers

Dumpsite/Garbage collection area	n	ESBL Producers (%)	Non ESBL producers (%)
Ayany Dumpsite	19	3(16%)	16(84%)
Central police garbage collection area	18	3(17%)	15(83%)
City market garbage collection area	22	4(18%)	18(82%)
City park Market Dumpsite	29	5(17%)	24(83%)
Dandora Dumpsite	33	6(18%)	27(82%)
District Commissioner's garbage collection area (Kibera)	9	2(22%)	7(78%)
Kenyatta Market Dumpsite	15	3(20%)	12(80%)
Kenyatta staff quarter garbage collection area	24	5(21%)	19(79%)
Kibera Dumpsite	15	5(33%)	10(67%)
Kweria garbage collection area	24	4(17%)	20(83%)
Mareba garbage collection area (Kibera)	7	1(14%)	6(86%)
Masai Market dumpsite	14	1(7%)	13(93%)
Muthurwa Estate Dumpsite	12	2(17%)	10(83%)
Muthurwa Market garbage collection area	17	2(12%)	15(88%)
Ngara market garbage collection area	17	2(12%)	15(88%)
Kawangware Market Dumpsite	4	0	4(100%)
Umama garbage collection area (Komarock)	7	0	7(100%)

4.8 Detection of *blaTEM, blaSHV, blaCTX-M,* genes, across the dumpsites and garbage collection areas

In comparisons of the different genes tested, three genes were identified. *blaTEM* had the highest occurrence with three being found among *E.coli* and two among *Klebsiella*. *blaSHV,* was only found among *Klebsiella,* among the isolates tested and *blaCTX-M in E.coli.*

Table 4. 5 Distribution of *blaTEM, blaSHV, blaCTX-M,* genes, across the dumpsites and garbage collection areas

Gene	*E.coli* (n=10)	*Klebsiella* (n=10)
blaTEM,	3	2
blaSHV,	0	1
blaCTX-M	1	0

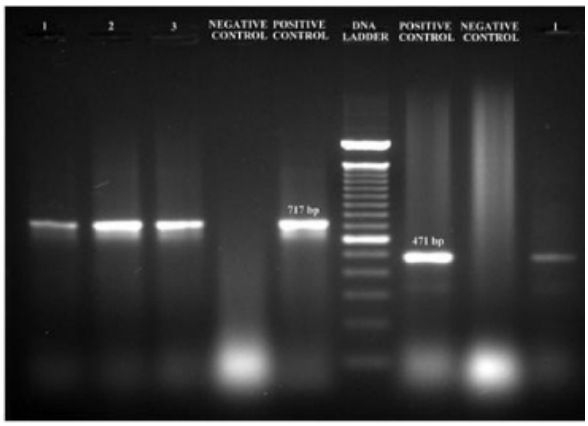


Figure 4. 5. PCR amplified fragments blaTEM (on left of the ladder); Positive control in-house control (*E. coli*; Resistant to Aztreonam, Cefoxitin, ampicillin, Cefotaxime, Ceftazidime, Phenotype confirmed), Negative control - *E. coli* ATCC 25922, and blaSHV (on right of the ladder); Positive control - K. pneumonia ATCC 700603, Negative control - *E. coli* ATCC 2592.

4.9 Resistant phenotypes and associated genes

In this study the most antimicrobial profile associated antibiotic resistance gene was bla_{TEM} which was isolated in three of the antimicrobial profiles and the least was bla_{SHV} and $bla_{CTX\text{-}M.}$

Table 4. 6 Presence of different phenotypes and the genes they carried.

Profiles	Number tested (n)	bla_{TEM}	bla_{SHV}	$bla_{CTX\text{-}M}$
Amp, Cip, TE, C, NA,SXT, Cpd, Fox, Caz	10	0	0	1(10)
S, Na, Te, Amp, Amc, Caz, Cpd	10	2(10)	0	0
Cip, S, Na, Te, Amc, Amp	10	0	1(10)	0
S, Na, Fep, Caz	10	1(10)	0	0
Cip, Na, Caz, SXT, Amc, Cpd, Fep, S, Te, Amp	10	2(10)	0	0
CIP, Te, NA, S, SXT, Amp, Fep, Caz, Cpd	10	0	0	0

Key**:** AMP**-ampicillin (10µg),** CPD**-cefpodoxime (10µg),** CAZ**-ceftazidime (30µg),** FOX**-cefoxitin (30µg),** FEP**-cefepime (30µg),** CIP**-ciprofloxacin (10µg),** AMC**-amoxicillin clavulanic acid (10µg),** TE**-tetracycline (30µg),** S**-streptomycin (10µg),** NA**-nalidixic acid (10µg),** C**-chloramphenical (10µg),** SXT**-trimethoprim sulfamethoxazole (30µg)**

4.10 *E. coli* Pathotypes

A total of ten samples from each dumpsite randomly selected were tested for the *E. coli* pathotypes. In the samples selected from the dumpsites and garbage areas, EAEC pathotype revealed a predominance rate of 20% in Masaai market dumpsite and Kweria garbage collection area **Table 4.7** and both had presence of human activity. Most of the pathotypes were not present in the samples tested in our study. However the most common gene was *AggR* associated with EAEC pathotype and the least common was *Stx*2 associated with EHEC pathotype. Most of the dumpsites and garbage collection areas that had no pathotype were found in market areas and one from residential area.

Table 4. 7 Details of *E.coli* pathotypes recovered from dumpsites and garbage collection areas with their target gene amplification.

Dumpsites	**Site CFU value**	**(n)**	**ETEC** *(LT)*	**EHEC** *(Stx2)*	**EAEC** *(AggR)*
Central police garbage collection area	6.1×10^{10}	10	0	0	0
Umama garbage collection area	7.9×10^{10}	10	1(10%)	0	0
Masai market dumpsite	1.2×10^{11}	10	0	0	2(20)
Kweria garbage collection area	7.2×10^{10}	10	0	0	2(20)
City market garbage collection area	6.7×10^{10}	10	0	0	0
Kenyatta market dumpsite	5.0×10^{10}	10	0	0	0

Key: ETEC- enterotoxigenic *E. coli* (producing heat-labile enterotoxin (*LT*), **EHEC-** enterohemorragic *E. coli* (producing a shiga-like toxin *Stx2*), **EAEC-e**nteroaggregative *E. coli* (with a transcriptional regulator *AggR*)

CHAPTER FIVE

5.0 DISCUSSION

Based on the qualitative survey this study showed that there was evidence of human contact with garbage and dumpsites surveyed. Some of these sites had high CFUs indicating a high possibility of contamination with fecal material. It is therefore possible that these interactions pose a serious danger to the public who work and sell their salvaged merchandise from such sites. Such merchandise may include fruits and vegetables. This may result in the spread of pathogens to the unsuspecting public. These results are in agreement with a study done in Nigeria that found that waste scavenging poses a great threat to the public. In addition, such dumpsites allow the growth of many pathogenic bacteria including those that may be MDR (Wachukwu, Mbata, & Nyenke, 2010). Human and animal scavengers were invariably at the site at all times.

This study's results show that the mean colony counts obtained from the dumpsite and garbage collection areas in residential areas and market places close to the dumpsite were relatively high. Among the dumpsites and garbage collection areas that had high CFUs, 70% were found in residential areas and 20% in market areas. In the dumpsites and garbage collection areas that had low CFUs, 57% were in residential areas and 14% in market areas. These high CFUs were probably as a result of presence of fecal contamination and human activity in the dumpsites and garbage collection areas. The results obtained in this study correlates with that of Odeyemi, 2012 that showed that the mean total bacterial counts obtained from the dumpsite and in residential area are relatively higher than those obtained at neighboring streams or samples collected at least 50m away from dumpsites. This shows how fast resistant bacteria from the environment can gain entry into the human body through contamination from these dumpsites and garbage collection areas, thus creating high health concerns in the public health sector. It is also worthy of note that the heaviest bacteria burden in this study was found at Muthurwa estate dumpsite and that the least was at Dandora dumpsite. This may not be too surprising since Muthurwa estate dumpsite had fecal contamination and a high number of trespassers thus bringing and taking a high number of the bacteria with them. Dandora dumpsite was the only dumpsite with a structured management system. This is the largest dumpsite in East and Central Africa, where rubbish/garbage from different parts of the city is dumped. This potentially offers a chance of

transfer of pathogens from such sites to human residential sites since Dandora dumpsite is closely located to an ever busy residential area.

This present study also determined antibiotic resistance profile of *E. coli* and *Klebsiella* from the sampled sites. A high proportion of *E. coli* and *Klebsiella* strains were resistant to ampicillin (42%, 59%), streptomycin (46%, 46%) and trimethoprim/sulfamethoxazole (29%, 29%) respectively. While these values may be lower than those reported from clinical studies, these results suggest that resistance to antimicrobials is rising and this may be due to either the intrinsic resistance of many microorganisms to antibiotics or acquired resistance of the organisms enabled by the transfer of resistance of drug resistance plasmids (Njoroge 2015). A high level of resistance has been found with members of the family *Enterobacteriaceae* which are increasingly becoming MDR. The origin of this resistance can probably be traced to the fecal constituent of the wastes or dump produced by people or animals that have been treated indiscriminately with various antibiotics and also to antibiotics production naturally by soil microorganisms (Njoroge 2015). The resistance prevalence of the two species was almost similar in all the antimicrobials used in this study (P: >0.05). This indicates that the action of the antimicrobial to the two species works in an almost similar way thus the close resistance prevalence's.

There was low resistance to cephalosporins, ceftazidime (*E. coli* 5%, *Klebsiella* 9%), cefoxitin (12%), cefepime (*E. coli* 13%, *Klebsiella* 19%) and cefpodoxime (*E. coli* 20%, *Klebsiella* 27%) in this study. Cephalosporins are of great importance in the public health sector and as first line therapy for a wide variety of infections, hence its continuous relevance and usage (Tenover, 2006). Most bacteria of clinical importance have become resistant to the antimicrobials found in this group, thus posing great danger to the sector. A high proportion of the cephalosporins used in our study clustered at the mid susceptible ranges of their zone distributions and decreased towards the extreme susceptible ranges with the exception of cefepime that increased towards the extreme susceptible range (26mm- >32mm). Based on the susceptibility patterns observed among our isolates towards cephalosporins, these antimicrobials are likely to remain potent for a long time against bacteria of environmental origin. These observations are in constract to those made on isolates from clinical backgrounds (Kiiru, Kariuki, Goddeeris, & Butaye, 2012) that suggest the apparent rise of ESBLs. A study in Daegu, Korea reported that the rate of third generation cephalosporin resistance among *E. coli* and *K. pneumoniae* causing community-onset

bacteremia over a 7-year period from 2003 to 2009 is on the rise. They concluded this rise in resistance is largely attributable to the spread of CTX-M-type ESBLs in the community especially among *Escherichia coli* (Tamang *et al*., 2014).

Meropenem would be a good antimicrobial for the treatment of the infections that may arise from strains recovered from this study sites. Only 2% to both *E. coli* and *Klebsiella* exhibited resistance to this antimicrobial. From the measurement of the zones of inhibition, it is evident that most of the isolates recovered from this study were found on the zone ranges of 26mm and >32mm with a small proportion on the resistant range. This however is not promising enough since a significant proportion (52%) of the isolates found on the intermediate and lower susceptible range (20mm to 28mm) has a potential to become fully resistant hence increasing the prevalence of strains no longer susceptible to this antimicrobial. Carbapenemase producing strains, which are primarily found in the medical field, have increasingly been found in the environment, thus posing potential risks to public health (Yinka *et al*., 2014). Related studies have also recorded low resistances of 5% to meropenem, among isolates obtained from water samples collected from ten rivers in Osun State, South-western Nigeria, (Yinka *et al*., 2014). This indicates that meropenem is effective to *E. coli* and *Klebsiella* strains found in the environment.

Multi Drug Resistance is the resistance of three or more antimicrobials belonging to different classes of antimicrobial compounds (Njoroge 2015). The highest multi drug resistance was found in Umama garbage collection area (71%), Muthurwa estate dumpsite (67%) and Mareba garbage collection area (43%). The lowest multidrug resistance was found in Kenyatta staff quarter garbage collection area (4%) and Ngara market garbage collection area (12%). This difference may be due to the difference in diversity of dumpsites and garbage content and possible difference in the amount of human and animal fecal contaminants in different sites. In this study, we repeat environmental isolates exhibiting combined resistance of cephalosporins and other useful classes of antimicrobials such as fluoroquinolones and aminoglycosides. Such resistances were more common in both dumpsites and garbage collection areas that were in market and residential areas. Some sites recorded an MDR prevalence of more than 50% indicating that resistance to antimicrobials is on the rise and thus may lead to an increase in resistance related complications. Presence of high MDR phenotypes among environmental samples is an indication

that resistant clinical samples are gaining entry into these sites. The possible factors driving the emergence of MDR phenotype could be poor use of combined therapy.

In this study, the prevalence of ESBLs was 16 % among both *E. coli* and *Klebsiella* strains. The ESBL phenotype is largely attributed to resistance to third generation cephalosporins mediated by extended-spectrum β -lactamases belonging to bla_{TEM}, bla_{SHV} and bla_{CTX-M}. The current study revealed that bla_{CTX-M} was the most common ß-lactamase among ESBL-producers and the second most common ESBL gene was bla_{shv}. The prevalence of β-lactamases was 8% for bla_{TEM}, 2% for bla_{CTX-M} and 2% for bla_{shv}. The dumpsites and garbage collection areas that had the highest ESBL counts were City market garbage collection area, Kweria garbage collection area, Masai market dumpsite, and Central police garbage collection area, that were found in market and residential areas. The closeness of these dumpsites and garbage collection areas to areas with high human activity could be responsible for these high resistances. A previous study in Benin reported the prevalence of these genes from samples collect from Market Garden Products and Irrigation Water at 67.50% for bla_{TEM}, 10% for *bla*SHV and 22.5% for bla_{CTX-M} (Wassiyath *et al*., 2015). These results are higher than those reported in this current study but indicate that the presence of ESBL producers is on the rise in the environment and leading to greater use of carbapenems and thus may result to possible rise in the resistance. Any emergence of carbapenem resistance is therefore a serious concern and, with evidence of its scatter in the dumpsites and garbage collection areas, there is a clear need for a nationwide survey to determine the prevalence's of these bacteria. The presence of clinical bacterial strains in the dumpsites and garbage collection areas could also be a possible cause of the increase of the isolates in the environment. The co-production of ESBLs with inhibitor-resistant β-lactamases such as OXA-1 renders these strains resistant to commonly used β-lactamase inhibitors like clavulanic acid.

In this study, *E. coli* belonging to ETEC and EAEC pathotypes were recovered. The most prevalent pathotype was EAEC and this was recovered from Kweria garbage collection area and Masai market dumpsite sites and both had presence of human activity. Some of these strains, especially those belong to EAEC pathotype were also MDR. Taken together these results suggest that there is possibility of proliferation of clinical strains in the environments which could in turn bring rise to the pathotypes and increased deaths. Previous studies have also isolated similar pathotypes from the environment (Wassiyath *et al*., 2015, Miyuki *et al*., 2009). From the results

in this study, resistance was only recorded in ETEC pathotype with a resistance rate of 100%**.** The sample was collected from Dandora dumpsite which poses a serious risk since there were men, women and children working on the site. The presence of resistance of these pathotype indicated the possibility of having patients with severe diarrhea problems and the increase of death cases in those that come into contact with the dumpsite and garbage collection areas or recycled products from them. This study showed that *E. coli* and *Klebsiella* are good indicators of antimicrobial resistance as they were isolated in all the sampled areas. Some of our isolates are potential pathogens that have been incriminated with various human diseases. The presence of some of these organisms on the dumpsite and garbage areas is alarming as those working and living on the dumpsites could serve as reservoirs for potential contamination of other close contacts. The presence of these isolates in leacheates at the dumpsites is also a major public health threat as leacheates may seep into nearby surface or underground potable waters. Besides health care settings, the environment is likely to have a role in the dissemination of ESBL-producing bacteria and may serve as an exposure route to humans. Previously recreational waters were identified as a potential exposure source of ESBL-producing *E.* coli. Even though (outside the clinical setting) *E. coli* is generally considered a relatively harmless inhabitant of the human (and animal) gut, major public health risks may be associated with the spread of ESBL-producing commensal bacteria (Liu *et al*., 2015). Firstly, upon colonization ESBL-producing commensal bacteria may disseminate and transfer ESBL-encoding genes to intestinal pathogens through horizontal gene transfer. Secondly, while relatively harmless for healthy individuals, these opportunistic bacteria may cause disease in more vulnerable individuals, such as hospitalized individuals, the elderly or newborns. Thirdly, exposure to ESBL-producing pathogenic *E. coli* variants may directly result in hard-to-treat infection, also in healthy individuals. The public health impact of exposure to ESBL-producing *E. coli* (and other AMR commensal bacteria) is determined by the sum of these individual risks (Liu *et al*., 2015). The results from this current study are in line with those of a study in South Korea that found presence of ESBLs in the environment but the prevalence was however higher than those recorded in the current study. The study from South Korea reported 60% of ESBL-producing *E. coli* isolated from a river to be potentially pathogenic, which was markedly higher than the current findings (Jang J *et al*., 2013).

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

1. Poor waste disposal and recycling practices are still rampant across the dumpsites and garbage collection areas.
2. Isolates from dumpsites and garbage collection areas were resistant to a high proportion of the antimicrobials. *Klebsiella* species were more resistant to the antimicrobials than *E. coli* species. This shows that clinical bacterial strains are gaining access into the environment thus the high resistance prevalence.
3. From the study, there was high occurrence of *Klebsiella* and *E. coli* across the dumpsites and garbage collection areas indicating environmental contamination. Most of the dumpsites and garbage collection areas with high CFUs were found in residential areas indicating that contamination of the dumpsites and garbage collection areas maybe on the rise due to human interference.
4. The prevalence of MDR isolates was higher in the garbage collection areas than in dumpsites. These findings concluded that human activities during collection of the garbage can play a role in the contamination of the dumpsites with potential clinical isolates which can help spread antimicrobial resistance to the environmental strains.
5. The most effective antimicrobial was gentamicin to *E. coli* and meropenem to both strains. The most non- effective antimicrobial was ampicillin and streptomycin indicating that the future of the antimicrobials is at risk with the changing bacterial pressure.
6. Proportion of ESBL producing *E. coli* was low indicating that isolates from the dumpsites and garbage collection areas do not produce as much extended spectrum β lactamases as reported by other studies.
7. The growing resistance of *E. coli* and *Klebsiella* species may be highly due to improper use of the antimicrobials and resistant gene acquisition.

6.2 Recommendations

- Regular treatment of the dumpsites should be carried out in order to reduce the growth of potentially pathogenic organisms especially our indicator species.

- Hazardous wastes from garbage collection areas should be sorted and decontaminated before being disposed in the designated disposal yard.
- Proper use of the antimicrobials should be encouraged and emphasized to reduce the increase of antimicrobial resistance.
- Proper training of safe practices when handling garbage to those collecting the garbage should be carried out in order to reduce the spread of MDROs.
- Encouragement of waste management practices of waste reduction, waste re-use and recycling.
- The County government should come up with ways to relocate the dumpsites and garbage collection areas to areas where there is no human activity.
- Further studies on the effectiveness of the antimicrobials should be done to determine their future of the antimicrobials with the changing bacterial pressures.
- Government should control the settlement patterns of individuals and communities and ensure that residential are far removed from dumpsites.
- Public health organizations and other relevant bodies should embark on public awareness and enlightenment campaigns to enlighten individuals on the hazards of indiscriminate waste disposal.

6.3 Study limitations

1. All potential sites were not sampled since some of the dumpsites had scavangers that controlled the areas where you could collect samples.

2. This current study was not able to cover some gaps e.g. sequencing and populations structures of these strains and therefore would recommend further studies to build on our data

3. Some of the tests run in this study did not have controls due to unavoidable circumstances'.

7. 0 REFERENCES

A. T, Odeyemi. (2012). Antibiogram Status of Bacterial Isolates from Air Around Dumpsite of Ekiti State Destitute Centre at Ilokun, Ado-Ekiti, Nigeria. *Journal of Microbiology Research*, *2*(2), 12–18. http://doi.org/10.5923/j.microbiology.20120202.03

Acar, J. F., & Goldstein, F. W. (1997). Trends in bacterial resistance to fluoroquinolones. *Clinical Infectious Diseases : An Official Publication of the Infectious Diseases Society of America*, *24 Suppl 1*, S67–S73. http://doi.org/10.1093/clinids/24.Supplement_1.S67

Baker, J. L. (2008). Urban Poverty : A Global View. *The World Bank Group*, v-27. http://doi.org/http://www.worldbank.org/urban/

Baquero, F., & Negri, M. (1998). Antibiotic-Selective Environments, *27*(Suppl 1), 5–11.

Barrett, C. B., Lee, D. R., & McPeak, J. G. (2005). Institutional arrangements for rural poverty reduction and resource conservation. *World Development*, *33*(2 SPEC. ISS.), 193–197. http://doi.org/10.1016/j.worlddev.2004.07.008

Beukering, P. V, & Sehker, M. (1999). Analysing Urban Solid waste in Developing Countries: a perspective on Bangalore, India. *Working Paper No. 24. Corpwatch*, *http*(24), //wwwcorpwatchorg17/7/03.

Brooks, J. T., Ochieng, J. B., Kumar, L., Okoth, G., Shapiro, R. L., Wells, J. G., … Slutsker, L. (2006). Surveillance for Bacterial Diarrhea and Antimicrobial Resistance in Rural Western Kenya , 1997 – 2003. *Clinical Infectious Diseases*, *43*((15 August)), 393–401.

Clermont, O., Lavollay, M., Vimont, S., Deschamps, C., Forestier, C., Branger, C., … Curie-paris, P. M. (2008). The CTX-M-15-producing Escherichia coli diffusing clone belongs to a highly virulent B2 phylogenetic subgroup, (March), 1024–1028. http://doi.org/10.1093/jac/dkn084

CLSI, editor (2013), Clinical and Laboratory Standards Insitute. Performance Standards for Antimicrobial Susceptebility Testing: Twenty-third Informational Supplement M100-S23. CLSI, Wayne, PA, USA.

Chopra I, Roberts M. Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance. Microbiol Mol Biol Rev. 2001;65:232–260. [PMC free article] [PubMed]

Cointreau, S. J. (1982). Environmental Management of Urban Solid Wastes in Developing Countries, *4*(4), 795–806.

Frost, L. S. and G. K. (2010). Regulation of bacterial conjugation: balancing opportunity with adversity. *Future Microbiology*, *5*(7), 1057–1071. http://doi.org/10.2217/FMB.10.70

Ghafourian, S., Bin Sekawi, Z., Sadeghifard, N., Mohebi, R., Kumari Neela, V., Maleki, A., … Ranjbar, R. (2011). The Prevalence of ESBLs Producing Klebsiella pneumoniae Isolates in Some Major Hospitals, Iran. *The Open Microbiology Journal*, *5*(2005), 91–5. http://doi.org/10.2174/1874285801105010091

Gibbs, S. G., Green, C. F., Tarwater, P. M., Mota, L. C., Mena, K. D., & Scarpino, P. V. (2006). Isolation of antibiotic-resistant bacteria from the air plume downwind of a swine confined or concentrated animal feeding operation. *Environmental Health Perspectives*, *114*(7), 1032–1037. http://doi.org/10.1289/ehp.8910

Gillespie, S. (2001). Antibiotic Resistance, Methods and Protocols. *Methods in Molecuar Medicine*, *1*(November). http://doi.org/10.1017/CBO9781107415324.004

Gonzalo, X., & Drobniewski, F. (2013). Is there a place for ??-lactams in the treatment of multidrug-resistant/extensively drug-resistant tuberculosis? Synergy between meropenem and amoxicillin/clavulanate. *Journal of Antimicrobial Chemotherapy*, *68*(2), 366–369. http://doi.org/10.1093/jac/dks395

Hakami, B. A., & Seif, E. S. A. (2015). Household Solid Waste Composition and Management in Jeddah City , Saudi Arabia : A planning model, *4*(1), 1–10.

Hansen, D. S., Schumacher, H., Hansen, F., Stegger, M., Hertz, F. boëtius, Schønning, K., … FrimodtmØller, N. (The D. S. G. (2012). Extended-spectrum β -lactamase (ESBL) in Danish clinical isolates of Escherichia coli and Klebsiella pneumoniae: Prevalence, β -lactamase distribution, phylogroups, and co-resistance. *Scandinavian Journal of Infectious Diseases*, (44), 174–181. http://doi.org/10.3109/00365548.2011.632642 Scand J Infect Dis Downloaded from informahealthcare.com by HINARI on 04/17/12 For personal use only.

Jang J[1], Suh YS, Di DY, Unno T, Sadowsky MJ, Hur HG (2013) Pathogenic Escherichia coli strains producing extended-spectrum β-lactamases in the Yeongsan River basin of South Korea. Environ Sci Technol. 2013 Jan 15;47(2):1128-36. doi: 10.1021/es303577u. Epub 2013 Jan 2.

Kiiru, J., Kariuki, S., Goddeeris, B. M., & Butaye, P. (2012). Analysis of beta-lactamase phenotypes and carriage of selected beta-lactamase genes among Escherichia coli strains obtained from Kenyan patients during an 18-year period. *BMC Microbiol*, *12*, 155. http://doi.org/10.1186/1471-2180-12-155

Leistevuo, T., Toivonen, P., Österblad, M., Kuistila, M., Kahra, A., Lehtonen, A., & Huovinen, P. (1996). Problem of antimicrobial resistance of fecal aerobic gram-negative bacilli in the elderly. *Antimicrobial Agents and Chemotherapy*, *40*(10), 2399–2403.

Lin, G. G., & Scott, J. G. (2012). NIH Public Access, *100*(2), 130–134. http://doi.org/10.1016/j.pestbp.2011.02.012.Investigations

Liu, H., Wang, Y., Wang, G., Xing, Q., Shao, L., Dong, X., … Ma, L. (2015). The prevalence of Escherichia coli strains with extended spectrum beta-lactamases isolated in China. *Frontiers in Microbiology*, *6*(APR), 1–5. http://doi.org/10.3389/fmicb.2015.00335

Lowy, F. (2003). Antimicrobial resistance: the example of *Staphylococcus aureus*. *Journal of Clinical Investigation*, *111*(9), 1265–1273. http://doi.org/10.1172/JCI200318535.In

Lujan, S. A., Guogas, L. M., Ragonese, H., Matson, S. W., & Redinbo, M. R. (2007). Disrupting antibiotic resistance propagation by inhibiting the conjugative DNA relaxase. *Proceedings of the National Academy of Sciences of the United States of America*, *104*(30), 12282–7. http://doi.org/10.1073/pnas.0702760104

Mcintyre, S., Badawi, N., Goldsmith, S., Hines, M., Karlsson, P., Novak, I., … Love, S. (2013). Report 2013.

Moro, M. H., Beran, G. W., Griffith, R. W., & Hoffman, L. J. (2000). Effects of heat stress on the antimicrobial drug resistance of Escherichia coli of the intestinal flora of swine, 836–844.

Nabegu, A. B. (2010). An Analysis of Municipal Solid Waste in Kano Metropolis, Nigeria. *J Hum Ecol*, *31*(2), 111–119.

Njoroge S (2015). Phenotypic and Genotypic Resistance Patterns among faecal E. Coli Isolated from severely Malnourished and Non-Malnourished children attending Mbagathi District Hospital, Nairobi.

Nicolaudius, A., Elements, A. P., & Genet, C. (2012). Genomics of Chloroplasts and Mitochondria. *Genomics of Chloroplasts and Mitochondria*, 502. http://doi.org/10.1007/978-94-007-2920-9

O'Brien, T. F. (2002). Emergence, Spread, and Environmental Effect of Antimicrobial Resistance: How Use of an Antimicrobial Anywhere Can Increase Resistance to Any Antimicrobial Anywhere Else. *Clinical Infectious Diseases*, *34*(s3), S78–S84. http://doi.org/10.1086/340244

Odagiri, M., Schriewer, A., Daniels, M. E., Wuertz, S., Smith, W. A., Clasen, T., … Jenkins, M. W. (2016). Human fecal and pathogen exposure pathways in rural Indian villages and the effect of increased latrine coverage. *Water Research*, *100*, 232–244. http://doi.org/10.1016/j.watres.2016.05.015

Okpoitari, S. P. (2013). Effect of Municipal Solid Waste on Bacteria Population in Soil, *2*(12), 65–68. http://doi.org/10.5901/ajis.2013.v2n12p65

Olukanni, D. O., Akinyinka, O. O., Ede, A. N., Akinwumi, I. I., & Ajanaku, K. O. (2014). Appraisal of Municipal Solid Waste Management , Its Effect and Resource Potential in A Semi-Urban City : a Case Study, *2014*. http://doi.org/10.5171/2014.705695

Oviasogie, F. E., & Agbonlahor, D. E. (2013). The burden, antibiogram and pathogenicity of bacteria found in municipal waste dumpsites and on waste site workers in Benin city. *Journal of Medicine and Biomedical Research*, *12*(2), 115–130.

Oviasogie, F. E., Ajuzie, C. U., & Ighodaro, U. G. (2010). Bacterial Analysis of Soil From Waste Dumpsite. *Archives of Applied Science Research*, *2*(5), 161–167.

Philippon, A., Labia, R., & Jacoby, G. (1989). MINIREVIEW Extended-Spectrum j-Lactamases. *Antimicrobial Agents and Chemotherapy*, *33*(8), 1131–1136. http://doi.org/10.1128/AAC.33.8.1131.Updated

Rawat, D., & Nair, D. (2010). Extended-spectrum β-lactamases in Gram Negative Bacteria. *Journal of Global Infectious Diseases*, *2*(3), 263–274.

Saini, S., Das, B. K., Kapil, A., Nagarajan, S. S., & Sarma, R. K. (2004). The study of bacterial flora of different types in hospital waste: Evaluation of waste treatment at AIIMS Hospital, New Delhi. *Southeast Asian Journal of Tropical Medicine and Public Health*, *35*(4), 986–989.

Samson Mwaikono, K., Maina, S., & Gwakisa, P. (2015). Prevalence and Antimicrobial Resistance Phenotype of Enteric Bacteria from a Municipal Dumpsite. *Journal of Applied & Environmental Microbiology*, *3*(3), 82–94. http://doi.org/10.12691/jaem-3-3-4.

Talbert, A. W. a, Mwaniki, M., Mwarumba, S., Newton, C. R. J. C., & Berkley, J. a. (2010). Invasive bacterial infections in neonates and young infants born outside hospital admitted to a rural hospital in Kenya. *The Pediatric Infectious Disease Journal*, *29*(10), 945–949. http://doi.org/10.1097/INF.0b013e3181dfca8c

Tamang, M. D., Gurung, M., Kang, M. S., Nam, H. M., Moon, D. C., Jang, G. C., … Lim, S. K. (2014). Characterization of plasmids encoding CTX-M ??-lactamase and their addiction systems in Escherichia coli isolates from animals. *Veterinary Microbiology*, *174*(3–4), 456–462. http://doi.org/10.1016/j.vetmic.2014.10.004

Tenover, F. C. (2006). Mechanisms of antimicrobial resistance in bacteria. *Am J Infect Control*, *34*(5 Suppl 1), S3-10-73. http://doi.org/10.1016/j.ajic.2006.05.219

Unep. (2007). Environmental Pollution and Impact.

Velsivasakthivel, S., & Nandini, N. (2014). Airborne Multiple Drug Resistant Bacteria Isolated from Concentrated Municipal Solid Waste Dumping Site of Bangalore , Karnataka , India, *3*(10), 43–46.

Visvanathan, C., & Trankler, J. (2001). Municipal Solid Waste Management in Asia: A Comparative Analysis, 1–14. Retrieved from http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.572.6735&rep=rep1&type=pdf

Wachukwu, C. K., Mbata, C. A., & Nyenke, C. U. (2010). The health profile and impact assessment of waste scavengers (Rag Pickers) in Port Harcourt, Nigeria. *Journal of Applied Sciences*.

Walling, E., & Wilhelm, E. (2004). Antibiotic resistance: Not just for people anymore. Journal of Young Investigators,3: 1-4.

Wambugu, P., Habtu, M., Impwi, P., Matiru, V. and Kiiru, J. (2015) Antimicrobial Susceptibility Profilesamong *Escherichia coli* Strains Isolated from Athi River Water in Machakos County, Kenya. *Advances in Microbiology*, 5,711-719. http://dx.doi.org/10.4236/aim.2015.510074

Weintraub, A. (2007). Enteroaggregative Escherichia coli: Epidemiology, virulence and detection. *Journal of Medical Microbiology*, *56*(1), 4–8. http://doi.org/10.1099/jmm.0.46930-0

World Bank, (2002). Urban poverty, The International Bank for Reconstruction and Development/The World Bank

World Bank. (2003). Thailand Environmental Monitor2003. A joint publication of the Pollution Control Department, Royal Thai Government. The World Bank, US Asia Environmental Partnership.

Yinka Titilawo & Timothy Sibanda & Larry Obi & Anthony Okoh (2014) Multiple antibiotic resistance indexing of Escherichia coli to identify high-risk sources of faecal contamination of water

Zhanel, G. G., DeCorby, M., Adam, H., Mulvey, M. R., McCracken, M., Lagacé-Wiens, P., … Hoban, D. J. (2010). Prevalence of antimicrobial-resistant pathogens in Canadian hospitals: Results of the Canadian Ward Surveillance study (CANWARD 2008). *Antimicrobial Agents and Chemotherapy*, *54*(11), 4684–4693. http://doi.org/10.1128/AAC.00469-10

Zhang, Y., Yue, D., Liu, J., Lu, P., Wang, Y., Liu, J., & Nie, Y. (2012). Release of non-methane organic compounds during simulated landfilling of aerobically pretreated municipal solid waste. *Journal of Environmental Management*, *101*, 54–58. http://doi.org/10.1016/j.jenvman.2011.10.018

Zorcolo, L., & Casula, G. (2006). Urinary and sexual involvement in IBD. *Inflammatory Bowel Disease and Familial Adenomatous Polyposis: Clinical Management and Patients' Quality of Life*, 141–155. http://doi.org/10.1007/88-470-0434-9_15

8.0 APPENDICES

Appendix 1: Potassium hydroxide test

- Place 2-3 drops of KOH on a slide.
- Stain the Inoculum of the test organism with KOH.
- Use a wire loop to touch the mixture and lift to observe for the presence or absence of mucus.
- Presence of mucus means the test organism is Gram negative and absence of mucus means the organism is Gram positive (20).
- Palmer KL, Kos VN, Gilmore MS. Horizontal gene transfer and the genomics of enterococcal antibiotic resistance. *Curr. Opin. Microbiol.* 13, 632–639 (2010).

Appendix 2: Catalase test

- Take 2-3 ml of hydrogen peroxide in a test tube
- Take a colony of test organism with sterile wooden or glass rod and immerse it into hydrogen peroxide solution.
- Observe for generation of bubbles. This indicates oxygen production.

Appendix 3: Indole test

- Inoculate the tryptophan broth with broth culture or emulsify isolated colony of the test organism in tryptophan broth.
- Incubate at 37°C for 24-28 hours in ambient air.
- Add 0.5 ml of Kovac's reagent to the broth culture.
- Positive: Pink colored rink after addition of reagent
- Negative: No color change even after the addition of reagent.

Appendix 4: Triple sugar iron agar test

- With a sterilized straight inoculation needle touch the top of a well-isolated colony
- Inoculate TSI Agar by first stabbing through the center of the medium to the bottom of the tube and then streaking the surface of the agar slant.
- Leave the cap on loosely and incubate the tube at 35 in ambient air for 18 to 24 hours.

Appendix 5: Kirbybauer susceptibility testing

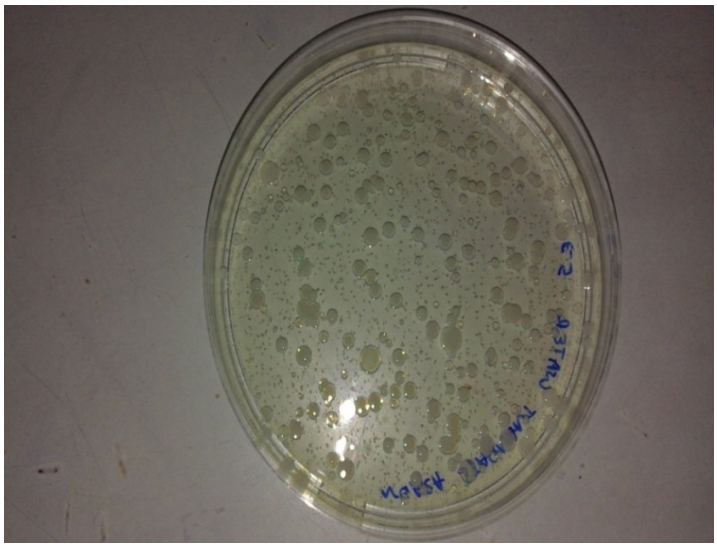
- Prepare the inoculum from the primary culture plate by touching with a loop the tops of each of 3 – 5 colonies, of similar appearance, of the organism to be tested and transfer this growth to a tube of saline.
- If the inoculum has to be made from a pure culture, suspend a loopful of the confluent growth similarly.
- Compare the tube with the 0.5 McFarland turbidity standard and adjust the density of the test suspension to that of the standard by adding more bacteria or more sterile saline.
- Inoculate the plates by dipping a sterile swab into the inoculum.
- Remove excess inoculum by pressing and rotating the swab firmly against the side of the tube above the level of the liquid.
- Streak the swab all over the surface of the medium three times, rotating the plate through an angle of 60 ° after each application.
- Pass the swab round the edge of the agar surface.
- Leave the inoculum to dry for a few minutes at room temperature with the lid closed.
- Place the antibiotic discs on the inoculated plates using a pair of sterile forceps.
- A maximum of seven discs are placed on a 9-10 cm plate.
- Each disc is gently pressed down to ensure even contact with the medium.
- Incubate at 35 °C within 30 minutes of preparation.
- After overnight incubation, the diameter of each zone (including the diameter of the disc) is measured and recorded in mm.

Appendix 6: DNA extraction

- Set a heating block to a temperature of 95^{o}C
- Identify pure colonies for DNA isolation. Label each culture in series…1, 2, 3, 4, etc and record in the laboratory book the identity of each isolates (*e.g.* 1=*E. coli* 1235, 2=*Shigella* 2345, etc.).
- Add at least 1ml of molecular grade water to 2 ml appendorf tubes
- Mark each tube with a number corresponding to the isolate to be analyzed
- Using a sterile swab, scrap a pea-sized amount of inoculum from a culture and transfer the inoculum to the corresponding tube
- Place the tubes in the heating block and leave to heat for a maximum of 12min
- Switch the heat block off
- Wait for 3 minutes before removing the tubes. This is important!! High pressure may develop in the hot tubes and the lids may pop-open when the tubes are shaken. The hot liquid can seriously burn your hands and face.

Appendix 7: Total colony forming units per sample

There was bacterial growth in all the samples collected. This plate shows the colony count of stagnant water sample collected in one of the dump sites in Nairobi area.



Appendix 8 Dump site details form

DATE……

NAME……

GPS……..
GEOGRAPHICALLOCATION……

- URBAN……..
- RESIDENTIAL…
- SCHOOL…….
- MARKET…….
- INSTITUTION…

SEASON: RAINY …….. DRY…….

TYPE OF GARBAGE/DUMP SITE: PERMANENT……TEMPORARY……..

Is there seepage in the soil in the dumpsite/ garbage area? YES……. NO…….

Is there leakage from the dumpsite/garbage area? YES…….. NO……..

Is there human activity on the dump site/ garbage area? YES…… NO……

What kind of human activity takes place on the dump site/ garbage area? .....

Are there domestic animals/ rodents in the dumpsite/ garbage area? YES…….. NO……

Is there evidence of human defecation on the dumpsite/ garbage area? YES…….... NO…….

Is there proper maintenance of the dumpsite/ garbage area? YES……. NO…….

RESIDENTIAL COPLAINTS

1. ……
2. ……
3. ……