# EVALUATION OF ANTIMICROBIAL SUSCEPTIBILITY OF *ESCHERICHIA COLI* AND *SALMONELLA* SPP. ISOLATED FROM CONTAMINATED AREAS OF MAJENGO SLUM IN MERU COUNTY, KENYA

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## DECLARATION

**Student Declaration**

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

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## DEDICATION

To my late Dad, Prof. Thomas Omao Getabu, my mum Martha Morangi Ondieki and siblings, Job Ombuya, Duke Ombuya and Lydia Ombuya.

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## ABBREVIATIONS AND ACRONYMS

| | |
|---|---|
| AGISAR: | Integrated Surveillance of Antimicrobial Resistance |
| AMR: | Antimicrobial resistance |
| API: | Analytical Profile Index |
| ARB: | Antimicrobial resistant bacteria |
| ARG: | Antimicrobial resistant gene |
| ATCC: | American Type Culture Collection |
| CAZ: | Ceftazidime |
| CPR: | Ciprofloxacin |
| CTX: | Cefotaxime |
| CXT: | Cefoxitin |
| EHEC: | Enterohaemorrhagic *E. coli* |
| EIEC: | Enteroinvasive *E. coli* |
| ETEC: | Enterotoxigenic *E. coli* |
| GIT: | Gastrointestinal |
| IMP: | Imipenem |
| MDR: | Multidrug resistant |
| NEMA: | National Environment Management Authority |
| SDG: | Sustainable development goals |
| SMAC: | Sorbital MacConkey |
| SS: | Salmonella Shigella Agar |
| TBX: | Tryptone Bile Glucuronide |
| TSI: | Triple Sugar Iron |
| WASH: | Water, sanitation and hygiene |
| WHO: | World Health Organization |
| WWTP: | Wastewater treatment plant |

## OPERATIONAL DEFINITION OF TERMS

| | |
|---|---|
| American Type Culture Collection: | It's an Organization That Collects and Distributes Reference Organisms for Research |
| Antimicrobial resistant: | Antibiotics lacks the ability to kill disease causing microorganisms |
| Cefotaxime: | Antimicrobial Agent That Is Used to Treat Bacterial Infections |
| Cefoxitin: | Antibiotic That Belongs to Second Generation of Cephamycin |
| Ceftazidime: | Antibiotic That Belongs To Third Generation Of Cephalosporin |
| Ciprofloxacin: | An Antibiotic That Belong To Class Of Fluoroquinolones |
| Contaminated areas: | It's a surface that has been polluted with waste that's harmful to human and animals |
| *E. coli 0157:* | Is a serotype of *E. coli* species commonly known as enterohemorrhagic *E. coli* |
| *Escherichia coli:* | It a bacteria that's is commonly found in intestines as a commensal |
| Evaluation: | Getting insight of subject that is under study in order to get information |
| Facultative anaerobe: | They are microorganisms that are able to grow in the presence or absence of oxygen |
| Gram negative: | They are microorganisms that are unable to hold crystal violet stain. |
| Imipenem: | Is Carbapenem type of drug that is used to treat bacterial infections |
| Intermediate: | Sensitivity of an antibiotic in the midpoint of a cutoff point |
| Macconkey Agar: | A differential media that allows growth of gram negative enteric bacteria |

| | |
|---|---|
| Media: | It Is a Substance That's Used in Growing Bacteria in The Lab |
| Normal flora: | Microorganisms that are found on the skin surface and inside the mucous membrane of human beings and they are harmless |
| Resistance: | Inability for an antibiotic to kill a microorganism |
| Salmonella Shigella Agar: | A Selective Media That Only Allows the Growth Of *Salmonella* and *Shigella* spp |
| *Salmonella* spp*:* | A group of bacteria that resides in the intestines of the human and animals and can cause disease |
| Sanitation environments: | A state of managing and controlling human waste that pose a risk for the outbreak of diseases |
| Sensitivity: | Ability of Given Antibiotic to Inhibit Growth of a Bacteria |
| Sorbital Macconkey: | Differential Media That Differentiates 0157 *E. coli* from other strains of *E. coli.* |
| Strata: | A population is divided into subgroups |
| Susceptibility: | A Laboratory Test Carried out to Check for Drug Response to Bacteria |
| Tryptone Bile X-Glucuronide Agar: | Selective Media Used in The Isolation of *E. coli* |

## ABSTRACT

The aim of this study was to evaluate antimicrobial susceptibility patterns of *Escherichia coli* and *Salmonella* spp. in Majengo slum that have emerged due to poor sanitation implementation. Multidrug resistance of *E. coli* and *Salmonella* has increased in the recent years and this has led to resistance of microorganisms to antimicrobials. The impact of antimicrobial resistance will be high mortality rates being recorded especially among young children in low income areas. Antimicrobial drug resistant is of great concern today since the rate of use and misuse has increased and organisms are changing the genetic make up to survive on the environment. Poor sanitation is one of the cause of this resistance in the globe currently. The main objective was evaluation of antimicrobial resistant strains of *E. coli* and *Salmonella* spp. Isolated from Sanitation Environments of Majengo Slum in Meru County, Kenya. The study employed standard microbiological procedure such as culturing on MacConkey agar, biochemical testing for the confirmation of the organisms' presence using TBX agar and Indole test for *E. coli* and TSI test for *Salmonella* spp and Urea agar and microbial susceptibility profile on Muller Hinton agar using commonly used antibiotics for enteric bacteria. Statistical analysis revealed a significance in comparison between the two strata (P 0.00052). Highest resistance was shown to Cefoxitin 22(52.38%) while the least was Ciprofloxacin 4(9.52%). Ceftazidime showed highest sensitivity 28(66.67%) while Cefoxitin showed least sensitivity 13(30. 95%).Data was entered into Microsoft Excel 2010 and analyzed using SPSS version 26. Data was compared between each study strata and between each sample type using Kruskal-Wallis tests, and between the two drugs using the Wilcoxon Signed Rank tests. This study showed that *E. coli* isolated from Majengo is pathogenic and resistant to antibiotics. Detection of *E.coli* pose a great risk in the spread of resistant strains in human. However, further research should be carried to find out the resistant genes of organism in this study area.

# CHAPTER ONE

# INTRODUCTION

## 1.0 Introduction

This chapter gives an introductory on research study where, it gives understanding of the topic of study. It further clearly states the research questions and the objectives of the study.

## 1.1 Background of antimicrobial susceptibility of *E. coli*

Infectious organisms are the major cause of diseases worldwide currently. A number of newly recognized pathogens and strains are now emerging. These organisms have resulted to high morbidity and mortality globally. Health (2012) found that, emergence of these pathogens include microbial evolution and creation of new environment. This has resulted to drug resistance. Also, improved surveillance and monitoring and greater commitment to sanitation and water management are needed to ensure protection from associated re-emerging infectious diseases.

Multidrug resistance of *E. coli* has been contributed by the emergence of hybrid plasmids that are resistant and virulence (Szmolka & Nagy,2013). According to Pedley and Pond (2003) noted that, 30% bacteria are currently emerging this is because of wastewater, agricultural practices. Water management could act as a barrier to prevent spread of pathogens (Fletcher, 2015). Prevention of pathogens could reduce antibiotic use and misuse and eventually mutation of microorganisms (Kwong *et al*., n.d.).

Multidrug resistance was observed in *E. coli* strains isolated from treated wastewater(Kumar *et al.,* 2020). In humans and food producing animals MDR *E. coli* is on the rise (Sidjabat & Paterson,2015). Multidrug resistance has increased which has led to multidrug resistance of infection causing organisms (Sidjabat & Paterson,2015). Studies have shown that, provision of sanitation facilities is one of the most important interventions to be put in place in order to stop the spread of resistant bacteria (WHO, 2018). In another study on typhoid among young children, Town *et al.*, (2022) concluded that, careful

monitoring of antimicrobial resistance was required to prevent an increase of this infection to the public.

High levels of *E. coli* from rivers can be a source of antimicrobial resistance (Bessa *et al.*, 2014). *E. coli* resistance to antimicrobials has been greatly been contributed by wastewater (Akiba *et al*., 2015).Diarrhea genic *E. coli* has demonstrated a significant resistant to beta lactams antibiotics that are commonly prescribed (Iseghohi *et al*., 2021). In the same study they highlight that, the contributing factors to diarrhea cases are due to poor quality of foods, water, hygiene and sanitation. The main source of contamination on the environment with antimicrobial resistant bacteria is human and animal waste (Haenni *et al*., 2022). Further they noted that, the contamination resulted in the formation of biofilms that supported the bacterial resistance.

While studying on prevalence and antibiotic resistance of *Salmonella* spp (Qamar *et al.*, 2020) .During wastewater treatment Odjadjare & Olaniran, (2015)noted that treatment plants are still reservoirs of antibiotic resistance and virulence of *Salmonella* spp. Antibiotic resistance was exhibited from multiple bacteria population that were isolated from water samples (Mawa *et al.*, 2021). They further concluded that, management of wastewater was key for proper aqua farming. Goldman, Ian and Pabari,(2021) observes that the cost of healthcare will increase as a result of prolonged hospitalization that is caused by reduced efficacy of antibiotics leading to high mortality rates.

Contamination of *E. coli* isolated from livestock, food and water had virulent genes that are pathogenic (Mukami, 2021). Fresh produce from vendors contained multidrug resistance (Baloyi *et al.*,2021). It was further noted that, *E coli* that was isolated was resistant to the antimicrobials and contained resistant genes. Hassan *et al.*,(2022) in a study on meat stands, fruits and vegetables, noted prevalence of (26%) *E. coli*. The health of consumers is at a great risk of drug resistant microorganisms through the trade of street food items (Nur *et al.,* 2021).Unhygienic handling by vendors was the cause of microbial contamination which could cause outbreak of foodborne diseases. In one study, Mshana *et*

*al.*,(2021) noted that, in Africa the degree of resistance ampicilin, tetracycline, trimethoprim/sulfamethoxazole is high. This is due to the presence of genes that are resistant to human, animals and environment. Consequently, they strongly recommended, proper environmental sanitation and personal hygiene to eradicate the pathogenic organism.

While studying on the prevalence of antibiotics resistance of *E. coli* in groundwater, Tahri *et al.*, (2021) noted that, *E. coli* was resistance to all the antibiotics used. Occurrence of *Salmonella* spp and *E. coli* in surface water has resulted to persistence of AR (Cho *et al.*,2020). Wastewater treatment plants have emerged as the main source of antibiotic resistance in the environment leading to contamination of water sources for agriculture and home use (Pazda *et al.,* 2020). A study conducted by Korzeniewska and Harnisz,(2018) found an increase in percentage of bacteria resistant to the new generation antibiotics in effluents. Govender *et al.,(*2021) while studying on antibiotic resistance in wastewater, concluded that, virulence genes contained in wastewater was a great threat to the communities by directly or indirectly exposing them to water.

A study conducted by Kumar *et al*.,(2020) on antibiotic prevalence and resistance in rivers found that, *E. coli* isolation ranged between 10-27 CFU ml respectively. Further they noted that antibiotic resistance was higher on old generation antimicrobials such as tetracycline and sulfamethoxazole. Another study conducted by Liu *et al*., (2021) on antibiotic resistance genes in drinking water, found that, pollution of surface water by antimicrobial resistant gene could affect the health of the human. On the same study they noted that, int11 and eam36 genes were the main ARG found in this water surfaces. Antibiotic residues in wastewater need to be continuously be monitored in order to develop strategies of controlling (Ngigi *et al*., 2019).

A study done by Kumar *et al.*,(2021) showed an increase from 60% to 85% of antidrug resistance from *E. coli*. Also from the same study it was noted that, non-fluoquinolone was more resistant than quinolone antibiotic. Wastewater that finds its way to aquatic ecosystem could act as a reservoir for

ARGs that could get into bacteria hence becoming pathogenic (Devarajan *et al*., 2016). Another study by Papajová *et al*.,(2022), noted that *E. coli* was resistant to cefotaxime by up to 5%. They concluded by highlighting that, wastewater that was treated possessed resistant ARG and MDR hence, a rise in infections.

A study conducted by Mbanga *et al*.,(2021) on resistant of *E. coli* from wastewater showed that, 64.6% showed multidrug resistance. Further they noted that, all samples collected for the study contained *E. coli* which is a clear indication of insufficient treatment of wastewater. Similar results was noted by (Hubeny *et al.*, 2019).Ultraviolet-C disinfection could remove resistant bacteria from being spread but still not effective as resistant traits are seen in the final effluent hence, improvement on wastewater treatment is essential to stop the spread of the bacteria (Silva *et al*., 2018)..

**1.2 Problem Statement**

According to Wuijts *et al.*,(2017) wastewater is one of the sources of antimicrobial resistance. Diseases that were earlier cured with commonly available antibiotics have now become a major cause of mortality especially in informal settlements with poor sanitation. This is due to increase of resistant bacteria and resistant genes that evades the antibiotics. This problem can be best addressed by implementing of WASH to combat resistance of antibiotics. WASH can be used to address the gap that could enable reduction in the use of antibiotics by provision of quality water, on site sanitation and hygiene. Kalule *et al.*,(2019) while looking at antibiotic susceptibility patterns in urban informal settlements to enteric bacteria, it was noted that there was high resistance diarrheal pathogens in stool and water.

Antimicrobial resistance globally, resistance of antibiotics is on the rise (Pulingam *et al*., 2022). A study conducted by Alba *et al.*, (2018) noted that, *beta* lactamase producing *E. coli* was resistant to colistin. In a related study WHO (2018) *E. coli* and *Salmonella* spp expressed mcr-gene of colistin. There is high antimicrobial misuse, insufficient drinking water, drainage and sanitation infrastructure

*(*Nadimpalli *et al*., 2020). In a related study Sulis *et al.*, (2022) noted that, poor hygiene and sanitation contributes to AMR faster. Improved hygiene and management practices on the use of antibiotics will reduce the spread of AMR (LeJeune *et al*., 2022). Monitoring wastewater could be used to predict resistance in clinical levels hence, guide on the use of antibiotics and management of resistance (Mesquita *et al.,* 2021).

Antimicrobial resistance implementation in Africa according to global action plan on antimicrobial resistance is inadequate (Iwu and Patrick, 2021). The was 0% surveillance of antimicrobial use and resistance(Founou *et al.*, 2017) . Slum areas are highly congested and the houses are erected very close to each other (Mutai *et al.*, 2020). This makes it difficult for construction of latrines and provision of clean water hence, there are high incidences of diarrhea leading to increased morbidity (Guillaume *et al.*, 2020). Studies have shown that, slum areas are composed of low income communities who cannot afford latrine construction and clean water (Latif *et al*., 2016). Another study noted that, people living in slum areas struggle for water since they are excluded socially, economically and politically (Subham *et al.*, 2020).

A study by Ampaire *et al*., (2016) while reviewing resistance of antibiotics in East Africa, noted that, ampicillin, gentamycin and ceftriaxone were among the commonly resistant antibiotics. Shared water and un boiled milk were among the risk factors contributing to resistant of *E. coli* (Katale *et al*., 2020). Need microbial use can be minimized by provision of water, sanitation and hygiene infrastructure (Loosli *et al.*, 2021).

### 1.3 Justification

Slum areas are faced with challenges in sanitation facilities caused by overcrowding and extreme poverty. Environmental contamination is in the increase due to inadequate management of human waste from this slum. This has led to increase of infections caused by *E. coli* and *Salmonella* spp and antimicrobial resistance caused by overuse and misuse of antibiotics (Karimi *et al*.,2023). According

to Shodikin *et al.*,(2022) 90% of long beans were contaminated by *E.coli.* Further proposed, provision of clean water, sanitation and good trader hygiene could reduce the contamination. According to Zone and Seyoum, (2022) fecal matter can cause contamination of udder, calf stalls and hands of the milking person. Implementation of SDGs such as provision of clean water, sanitation and inequalities subjected to this community would be of great importance in dealing with AMR (Ercumen *et al.*, n.d.). Provision of affordable housing with well-designed on site sanitation could reduce environmental contamination (Jaiswal, 2019). Outbreak of diseases could cease to occur (Nandi *et al.,* 2017). To achieve this, different stakeholders for instance the government, NGOs, private sectors and the community at large need to be brought on board in addressing the problem (Kobusingye *et al.*,2017). The stakeholders would help in developing policies, providing infrastructure and financial support (Mensah, 2019). This would ensure availability of clean environment to all and affordable housing to low income areas that would be well equipped with sanitation facilities.

## 1.4 Research Questions

What are the common contaminants of Majengo environment, in Meru County, Kenya?

What are the *E. coli* and *Salmonella* spp isolates found in Majengo Meru County susceptible to antimicrobials?

What are the common strains of *E. coli* and *Salmonella* spp that are found in the environments of in Majengo slum, in Meru County, Kenya?

## 1.5 Objectives

### 1.5.1 Main Objective

Evaluation of Antimicrobial Resistant Strains of *E. coli* and *Salmonella* spp Isolated from Sanitation environments of Majengo Slum in Meru County, Kenya

### 1.5.2 Specific objectives

To Isolate *Escherichia coli* and *Salmonella* spp for antimicrobial susceptibility testing from the sanitation environments of Majengo slums in Meru County, Kenya

To determine the antimicrobial susceptibility patterns of stains of *E. coli* and *Salmonella* spp. obtained from the sanitation environments of Majengo Slums, in Meru County, Kenya

To examine presence of 0157 *Escherichia coli* and *Salmonella* spp. strains that are resistant to commonly used antimicrobial drugs resistance from the sanitation environments of Majengo slum, in Meru County, Kenya.

# CHAPTER TWO

# LITERATURE REVIEW

## 2.0 Introduction

This chapter captures and evaluates reviewed relevant literature on antimicrobial resistant in regard to study objectives.

## 2.1 Overview of the sanitation associated illnesses

Rapid urban growth is one of the challenge facing slums in the provision of sustainable sanitation hygiene (Bishoge, 2021). While looking at health risks in our environment in Kampala Uganda Ssemugabo *et al.,* (2021) noted that overcrowding and inadequate access sanitation and hygiene resulted to diseases such as diarrhea. Inadequate access to land in urban poor leads to environmental degradation (Surya *et al.,* n.d.). According to Takyi *et al.,* (2021) slums will develop due migration to the city and this will lead to poor environmental sanitation.

Disease related to poor sanitation can occur due to unavailability of household latrines, poor washing facilities and inadequate drinking water that is clean (Anas, 2020). A study conducted by Zerbo *et al.*, (2021) indicated that 75% of total deaths are due to diarrheal diseases. Further they noted that, the diseases were as a result of poor water sanitation and hygiene services in urban areas. While looking at water and sanitation risk exposure to children under five, Murtaza *et al.,* (2021) noted that 57.5% of children were at risk of infection caused by poor water and sanitation. Outbreak of diarrhea related diseases can be minimized by provision of knowledge and practices of the use of antibiotics. (Mahapatra *et al.,* 2021). In another study Iyer *et al., (*2021) found that, involvement and empowerment can bring about local solutions to global problems such as antibiotic resistance.

## *2.2 E. coli* isolated from slum areas

*E. coli* is bacteria that belongs to enterobacteriaceae, it is gram negative and facultative anaerobe (Mukami, 2021). It is found in the small intestines as a normal flora and change of its habitat in the

body can lead to diseases such as urinary tract infections (monicah cheesbrough, 2006). According to (Harada *et al.,* 2018) 17 out of 18 samples of stored drinking water were contaminated with *E. coli.* While studying on multidrug resistance of *E. coli* in Kibera slum Gitahi *et al.*, (2018) noted that, 17% of the samples had *E. coli.* While studying on contamination of ground water in rural areas, Bindra *et al*., (2021) noted that, 50% of groundwater was contaminated by *E. coli.*

Open drains in slum areas can be a source for the manifestation of disease causing microorganisms(Muriuki *et al*., 2020). Drains can be contaminated by human waste due to poor sanitation facilities(Kwiringira *et al*., 2016). A study conducted by (Ginn *et al.,* (2021), found out 52% of the samples were positive for *E. coli.* Open drains contained 89 % concentration of *E. coli*,(Berendes *et al*., 2020). While accessing fecal contamination in the environmental samples, Amin *et al.*, (2019) found out that open drains had 49% concentration of *E. coli.*

While studying on enteric bacteria of public health in dumpsite soil World *et al*., (2018) recorded 9.9% of *E. coli* in soil samples. In another study, Pickering *et al*., (2018) recorded 94% of *E. coli* isolated from soil. According to Poma *et al.*, (2016), 67% of *E. coli* was recorded while Sobur *et al*., (2019) 100% *E. coli* was isolated from farm soils. Other studies conducted by Loots *et al*., (2021),Holvoet *et al*., n.d.) recorded 28% and 37% of *E. coli* from the soil. A studies conducted by World *et al.*, (2018, Sobur *et al*., (2019) noted that 8.5% and 72% of the soil isolated had *Salmonella* spp. Pornsukarom and Thakur, (2016) recorded 13.22% prevalence of *Salmonella* spp. In other studies conducted by Loots *et al.,* (2021), Johannessen *et al.,* (2015) 0% *Salmonella* spp. was isolated.

Infections such as diarrhea can rise in slums due to poor sanitation(Corburn and Hildebrand, 2015). While studying on the prevalence of enterobacteriaceae isolated from diarrhea samples of children, Samuel *et al.,* (2019) found that 35.2% of *E. coli* was the most organism isolated. In a related study Haque *et al.,* (2003) isolated 9% of *E. coli* from diarrhea samples. Out of forty-two samples analyzed, 70% were positive for *E. coli* (Lindeberg *et al.,* 2018). According to Mubarak, (2020), 15.55% of *E.*

*coli* was isolated from diarrhea samples of children. In another study, Dhale1 *et al.,* (2019) isolated 55% of *E. coli* from cute diarrhea of children under age of 5 years.

### 2.3 Microbiological features of *E. coli* and *Salmonella* spp.

#### 2.3.1 Characteristics of *E. coli*

*E. coli* belongs to enterobactericeae family and Escherichia genus. It is a gram negative and facultative anaerobic bacteria. It is motile with peritrichous flagella. It rod-shape and stains red with a gram stain. It is normally found in the large intestine of mammals (Gebisa, 2019;Gomes *et al.*, 2016). It is non-spore forming bacteria. In the laboratory it is isolated by growing on MacConkey differential medium and incubated to 37ºC for 24 hours. It yields pink colonies after fermenting lactose( *Barcella et al.*, 2016). Biochemically it is oxidase negative, catalase positive and indole positive. Strains of *E. coli* are not pathogenic, but there are serotype that are infectious (Gomes *et al.*, 2016). The organism is found on the environment due to fecal contamination. This contamination cause disease when food and water get contact with the fecal matter.

#### 2.3.2 *E. coli* Enterotoxigenic (ETEC)

This strain produces colonization factors that the bacteria use to attach itself on the walls of intestinal epithelia cells. The virulence factors of this bacteria are through production of enterotoxin. These toxins are either heat labile and heat toxin(Tabaran *et al.,* 2017). These toxins stimulate the membrane in channels hence, causes massive fluid and ion loss in form of diarrhea (Mirhoseini *et al*., 2019). ETEC is known to cause travelers' diarrhea and neonates' diarrhea in developing countries. Thus resulting to high death rates.

#### 2.3.3 *E. coli* Enterohaemorrhagic (EHEC)

This strain is distributed and produces toxins known as shiga-toxins. These toxins attach to the intestinal epithelial cells causing acute bloody diarrhea. This leads to development of hemolytic uremic syndrome (HUS). Infections caused by this strain has been found to affect infants and children. Acute

renal failure has been reported due to this infection (Newell & La Ragione, 2018). *E. coli* 0157:H7 a protype of EHEC has been associated with outbreak in the global leading to cases of hemorrhagic colitis and HUS.

### 2.3.4 *E. coli* Enteroinvasive (EIEC)

Enteroinvasive *E. coli* is third world countries is the leading cause of dysentery. The infection caused by this organism are similar to those caused by *Shigella* species (Cowley *et al.*,2018). It invades and destroys the enterocytes (Cabrera-Sosa & Ochoa 2020). Drinking water is one of the sources of the infection caused by(EIEC) (Swedan & Alrub, 2019). Out breaks that have been reported in united kingdom have been caused by salad (Michelacci *et al.*, 2016).

### 2.3.5 Characteristics of *Salmonella* spp

*Salmonella* spp is a Gram negative. It belongs to enterobacteriaceae family and *Salmonella* genus. It is a non-lactose fermenter. Produces hydrogen sulphide gas which mostly observed as a black precipitate on triple sugar agar. It is non-spore forming facultative organism Rahman *et al.,* (2016). In the laboratory it is isolated by growing on Salmonella Shigella agar selective media at 37ºC for 24 hours. It is oxidase negative and catalase positive Rokeya Ahmed, (2019). Strains that are pathogenic includes *S. typhi*,*S typhimurium*,*S. enteritidis* and *S. paratyphi* (Dougnon *et al.*, 2017).

## 2.4 Antimicrobials of infections caused by *E. coli* and *Salmonella* spp

Antimicrobial therapy is the management that is required in treating infections caused by the two organisms. Bacterial organisms can be killed by antimicrobial and also can be used as a basis to develop antimicrobial resistance. A global overview to drug resistance as shown 40% towards *E. coli* A. White and (Hughes, 2019). Various agents are being used in the treatment of the organism which includes beta-lactams, cephalosporings, fluoroquinolones, tetracycline and aminoglycoside.

### 2.4.1 Beta- lactams

Class of these drugs include penicillins, carbepenems and cephalosporings. The mechanism of action of these drugs is by synthesis of the cell wall hence the bacteria is deactivated. Beta-lactamases microbial enzymes are able to deactivate beta lactam antimicrobials to be infective by hydrolyzing (Pandey & Cascella, 2021). *E. coli* has shown resistance to beta lactams recently. This resistance has developed in the following ways; target sites are altered and production of beta lactamases causing inactivity(Pandey & Cascella, 2021).

### 2.4.2 Fluoroquinolones

This class of antibiotic is classified into four generations. Nalidixic acid belongs to first generation while ciprofloxacin belongs to second generation, levofloxacin belongs to third generation and dilafloxacin belongs to fourth generation. The antibiotic act by inhibition of DNA gyrase hence lowering the activity of microbiological by hindering replication and transcription (Ezelarab *et al.*, 2018). Chromosomal mutation has been found to cause resistance of these agents (Boni *et al.*, 2022).

### 2.4.3 Aminoglycosides

This class of antibiotics includes gentamycin and streptomycin. They act by inhibiting the growth of bacteria by binding on 30S subunit by the bacteria's ribosome. This results to mismatch between codons and anticodons (Saravolatz & Stein, 2020). The resistance of aminoglycoside is brought about enzyme modification and inactivation of small molecules (Reeves *et al.*, 2021).

## 2.5 Pathogenic *E. coli* and *Salmonella* species

*E. coli* is a normal flora in the human intestines. However, *E. coli* has been associated in causing infections in the human body such as diarrhea. The infections result from fecal contamination on

drinking water (Blyton and Gordon, 2017; Riley, 2020). Different strains have been found to causes disease in human health leading to high morbidity and mortalities especially to the young children (Gomes et al., 2016). Kobayashi *et al.,* (2021) noted that, there were some strains that were not pathogenic hence, do not cause disease to human.

Human health is under a threat due to an increase in ground water contamination (White *et al.*, 2021). Inadequate of lack of proper treatment of waste water, contaminated water surfaces leading to high concentration of *E. coli* (Blaak *et al.,* 2014). Pathogens that are caused by extended spectrum beta lactamase organisms are on the rise in the community. Blaak *et al.*, (2014) also observes that these organism are difficult to treat in low income areas. These beta lactamase organisms are resistant to extended spectrum cephalosporins.

*E. coli* and *Salmonella* organisms are on the rise due to human waste, contaminated food and poor hygiene Alburo and Otadoy, (2021) also people continued to defecate in the open leading ton increase in diarrhea cases. Ramlal *et al.,* (2022) observed contamination with *E. coli* posed a great risk of infections. Environmental management can be done through stakeholder partnership (Kumutha *et al.*, 2020). Isolates from pit latrines showed 14% of *E. coli* had multidrug resistance (Beukes *et al.*, 2017). Maloo *et al.,* (2017) while studying on *E. coli* multiple resistance to antimicrobials, it was noted that enter coding genes of enterohaemorrhagic *E. coli* and shiga toxin *E. coli* were the isolates found in their study. A study conducted by Ram and Kumar (2020) showed that 60% resistance of *E. coli* to antimicrobials. Further they noted that discharge of wastewater to rivers was the contributing factor to this contamination. Provision of water, sanitation and hygiene education could provide positive interventions to slums (Chowdhury *et al*., 2021).

### 2.6 Burden of Antimicrobial resistance

A study done by Jeffrey L. Fortman and Aindrila Mukhopadhyay, (2016) noted that, there is rise of resistance of antimicrobials. The antimicrobial activity to microorganism is decreasing because of the

resistance. This has led to exhaustion of available antibiotics. Focus should not only look at control of resistance but also the efficacy of the currently used antimicrobial agents (Paphitou, 2013). The mutation of multidrug resistance of pathogenic bacteria is on the spot by world organization of animal health, food and agriculture organization as a global problem to animals and humans. Programs that can help combat antimicrobial should be implemented (WHO, 2014).

Developing countries are faced with highly infectious organisms but due to cost constraints by most new antimicrobials, disease causing organisms are difficult to control Van Boeckel *et al.,* (2019) . According to Godman *et al.,* (2021) resistance of antimicrobials is a great concern as it impacts on morbidity and mortality and costs. The spread of antimicrobial resistance is facilitated by overcrowding, poor sanitation and misuse of antibiotics (Ayukekbong *et al.*, 2017). The spread of antimicrobial resistance can best be assessed with surveillance (WHO, 2014). Use of Carbapenem in treatment of multidrug resistance bacterial infections is now under siege due to increase and spread of Carbapenem resistant Enterobacteriaceae (Potter *et al.,* 2016).

According to Dadgostar (2019), antimicrobial resistance has been contributed by rapid evolution of bacteria and passing of genes that are resistant. Further highlights that to end antimicrobial resistance is challenging since progress seem not to be there. The use of substandard drugs, excessive use and inappropriate handling of antimicrobials has resulted to multidrug resistance. Santajit and Indrawattana, (2016). A study done by Berry, (2019) on impact of antimicrobial resistance in the global, noted that poverty in third world countries are increasingly faced antimicrobial resistance. This is due to partial prescription, counter dispensing and lack of education on the users on the need to finish diseases. For instance, Kenya inappropriate use of antibiotics is due to inadequate registration and over registration of antibiotics that are not of priority resulting to misuse (Lyus *et al*., 2020). According to Muthuma *et al.,* (2016) abuse and misuse of antimicrobials can be managed if laws are implemented .

In Africa, African center and disease control prioritizes on improvement of surveillance, ensures adherence to clinical treatment this will reduce the emergence of antimicrobial resistance Varma *et al*., (2018). Loosli *et al.* (2021) while addressing how to improve access to quality care and resistance of microbial agents, recommend that cases of substandard drugs should be reported and implementing monitoring at all levels of supply chains.

### 2.7 Transportation of antimicrobial resistance in the environment

The antimicrobial resistance has been steered by human activities such as agriculture, direct release of contaminants to the environment and use of antibiotics in animals Holmes *et al.*, (2016). According to Fouz *et al*. (2020), human and animals are the origin of ARB and ARG on the environment. Growth promoters used in industries for food animals has contributed to the spread of resistance traits of antibiotics (Vidovic &Vidovic, 2020).

Transportation of antimicrobial resistance between man and animals can be explained in two ways. The first one is through food and water (Ma *et al.*, 2021). The other way is by transfer of genes in form of bacteriophages that contain antimicrobial resistance genes. According to Shah *et al*., (2016), it was noted that resistance of *E. coli* was due to transfer of Kanamycin resistance by phages that contain antimicrobial resistance genes. The spread of antimicrobial resistance can be associated with newly resistant gene fixation and emergence as a result of microbiome of human and animals and pathogen to human due resistance gene mobilization and transfer (Bengtsson-Palme *et al.*, 2018). Surface water could involve in dissemination of anti-resistant bacteria since they are contaminated by wastewater from treatment plants (Kraemer *et al*.,2019). *E. coli* isolated from river showed anti-resistant gene and integrons that were contributed by wastewater (Dhawde *et al*., 2018). Bleichenbacher *et al*., (2020) while studying on spread of Enterobacteriaceae that produces Carbepenemase noted that river ecosystem hosts resistance replicating and evolving polluting genes.

## 2.8 Antimicrobial resistance globally

According to Essack (2021), an increase of antibiotic use has been contributed by poor sanitation and hygiene. This has increased the spread of organisms that are drug resistant amongst communities. He concluded is study by suggesting hygiene improvement like handwashing could prevent spread of infections. A study conducted by Samreen *et al.,* (2021) noted that the route entry of resistant microorganism and resistant genes into the environment included wastewater. Further recommended that, provision of clean water, sanitation as remedy to the global problem.

According to Loayza and Graham (2020) on *E. coli* a global antimicrobial resistance crisis, they indicate the organism as a major problem in antimicrobial resistance in the world. Collignon *et al.,* (2018) argues that reducing the consumption of antibiotics use could not stop the spread of resistant strains and gene from contaminating the environment but they suggested that if sanitation is improved, clean water provision is increased and ensuring good governance, then globally antimicrobial resistance will reduce. Thakur and Gray (2019) argue that prevention of antimicrobial resistance requires global cooperation between countries.

According to Antimicrobial Resistance Division *et al.,* (2021) refill that, 46% of countries reported decreases in susceptibility to ceftriaxone , 73.3% to azithromycin and 76.8% to ciprofloxacin which was the highest showing resistance. Alvarez-Uria *et al.,* (2018) in their study concluded that, by 2030 antibiotics of third generation will be ineffective to a number of infections to *E. coli* .A. White & Hughes (2019) highlight that, ways of combating resistance and existing gaps should be prioritized. This is through collaboration and engagement of key partners.

## 2.9 Antimicrobial Resistance in Africa

A study that was conducted in Uganda on antimicrobial resistance in pathogenic aerobic bacteria, showed that 55.56% of *E. coli* was resistant to antibiotics that were tested (Hope *et al.*, 2019). Also study conducted by Hope *et al.,* (2019) showed that 95% of cefazolin and 93% cefotaxime were

resistant to *Salmonella enterica*. A study conducted in northern Tanzania on multidrug resistant of *E. coli* resistance was higher on imepenem 79.8%,cefotaxime (79.7%) and tetracycline (73.7%) (Sonola *et al*., 2021). Fleece *et al.* (2019) noted that *E. coli* resistant to antibiotics was consistent among children and concludes that, interventions like provision of sanitation, clean drinking water and hygiene will reduce these infections.

There was 73.5% of *E. coli* resistance to Ampicilin and 30.2%resistant to *Salmonella typhi* in a study conducted by (Ombelet *et al.,* 2022).Studies conducted on street foods in Burkina faso showed that, six serotypes of *Salmonella* were multidrug resistant (Nikiema *et al*., 2021). Another study conducted on *Salmonella* isolates from chicken in South Africa showed that 81% *Salmonella* showed multidrug resistant to antibiotics (Mokgophi *et al.*, 2021). In the other study done on drivers in resistant in low-income countries. Iskandar *et al*.,(2020) indicate that causes of multidrug resistance includes release of highly contaminated waste effluents.

According to Tahri *et al.*,(2021), study on antimicrobial resistance of *E. coli* isolated from ground water, it was found that, 68.75% of *E. coli* strains showed multidrug resistance. Further they noted 12.50% of the *E. coli* strains were resistance to at least 7 antimicrobial agents, 10.42% were resistant to at least 10 agents. On the same study they highlighted that, amoxicillin and Ceftzidime showed highest resistance hence, putting human life to danger of infections.

Effluents from wastewater treatment plant showed multidrug resistant to twelve diarrheagenic *E. coli (*Mbanga, Amoako, *et al.*, 2021). A study on multidrug resistant phylogroups of *E. coli*, revealed that 98% of the antimicrobials were resistant to sulphamethoxazole- and penicillin (Titilawo *et al.*, 2021). A study conducted by Ngene *et al.*, (2021), on wastewater and soil, it was noted organisms showed 100% resistant to Ceftazidime while Augmentin and Ampicilin were 95% resistant. They concluded their study by recommending that, management of wastewater properly and discouraging improper use of antibiotics.

## 2.10 Antimicrobial Resistance in Kenya

In Kenya, Langata *et al.,* (2019) noted that, *E. coli* showed antimicrobial resistance to all antibiotics except gentamycin and ciprofloxacin. Highest resistance was found in amoxicillin at 54%. Also 50% of salmonella were resistant to amoxicillin antibiotic. According to Ngai *et al.,* (2021) on *Salmonella* and *E coli* on poultry it was observed that ampicillin had the highest resistant (41%) for *Salmonella* spp. and (62%) for *E. coli* isolates. Further highlights that contamination of poultry feeds will results to contamination of the bacteria across the community.

According to Nadimpalli *et al.,* (2020), on urban informal settlement hotspots for antimicrobial resistant it was highlighted that water and sanitation infrastructure improvement will disrupt environmental antimicrobial resistance. Study done by Felipe *et al.,* (2018) on antimicrobial resistance obtained from waste water,72% of Amoxicillin recorded highest resistance. In another study conducted by Omulo *et al.,* (2021), it was concluded that, sanitation, hygiene, and disease transmission will minimize the increase of resistant bacteria in slum settlement. Also Gitahi *et al.,*(2018) demonstrated that 55% of *E. coli* was resistant to tetracycline antibiotics.

Ampicillin was more resistant to gram negative organisms. However, imipenem and ciprofloxacin antibiotics showed highest sensitivity. Also it was noted that resistant was dominant in slum kibera area than middle income areas of Juja (Maina *et al.,* 2019). In another study on multidrug resistant of food and environmental samples they noted that, most resistant agents were Ampicillin, trimethoprim and sulfamethoxazole isolated from food and environmental and drugs that showed sensitivity included Imipenem, cefepime, ciprofloxacin and ceftazidime.

## 2.11 Call for response on antimicrobial resistance in the globe

Countries should invest highly on order to contain antimicrobial resistance. Global economy will be under threat if antimicrobial resistance is not addressed (World Bank, 2017). In developing countries, antimicrobial resistance studies are neglected and they are lacking the resources that will help in

addressing the risk (Founou *et al.,* 2017). Establishment of laboratories for antimicrobial testing minimizing use of antimicrobial agents in production of food will help in addressing the antimicrobial resistance (Agisar, 2015).

According to Bürgmann *et al.,* (2018), less attention is given to antimicrobial resistance to animals than human and 73% of the antibiotics are used in animals. Global commitment towards the increase of antimicrobial resistance should be based on sustainable development goals plans (Jasovský *et al.,* 2016). Alternative methods have been introduced in addressing antimicrobial resistance. The use of vaccines instead of antibiotics is being adapted (Fortman & Mukhopadhyay, 2016).

### 2.12 Emergence of enterohemorrhagic *E. coli* in slum environment

Enterohemorrhagic *E. coli* is a strain of *E. coli* that causes diarrhea in human. The bacteria cause HUS in those infected. It produces a shiga toxin that cause acute diarrhea and kidney failure (Mukami, 2021). A study conducted by Zhou *et al.,* (2021) 0.31% of 0157 *E. coli* in patients with acute diarrhea. While determining Shiga toxins in sheep, Ghaderi *et al.,* (2022) noted that, three out of forty two isolates had *E. coli* 0157 strain. In a related study, Heydari *et al.,* (2020) found five out of seventy eight samples contained *E. coli* 0157. They concluded that, *E. coli* 0157 is the potential source of human infection and it is caused by contamination of fecal matter.

According to Fesseha *et al.,* (2022) *E. coli* 0157 was identified from 46% from the samples analyzed. The further found out that poor unhygienic conditions were the reason for the presence of bacteria. In another study, Ghali-mohammed and Ayoade, (2023)noted that 2.3% of *E. coli* 0157 were found in raw milk. In a similar study Mumma and Baker, (2022),Disassa *et al*., (2017) noted that, unpacked milk was the most contaminated where *E. coli* 0157 was the pathogen present.

While studying on the occurrence of *E. coli* virulence genes in feces of wild birds, Bertelloni *et al.,* (2019) noted that 8.26% of positive samples possessed eaeA genes that belongs to *E. coli* 0157. In

another study, Engidaw Abebe, Getachew Gugsa, Meselu Ahmed and Shimelis Abegaz, (2022) noted 6.5% of *E. coli* 0157 from foods that originated from bovine. They also indicate that resistant antimicrobial was observed from all the isolates of *E. coli* 0157.

Reuse of wastewater from wastewater treatment plant was found to have 5% of indicator organism(Garre *et al.*, 2022). In another study Bolukaoto *et al.,* (2019) recorded 2.35%. While studying on the prevalence of *E. coli* 0157 on irrigation water and agricultural soil, Iwu *et al.,* (2021) noted 28% of *E. coli* 0157 from both irrigation water and agricultural soil. In a related study,Enabulele & Uraih, (2009) noted that *E. coli* 0157 was present in fresh meat 6.94%. Further they highlight that, slaughter houses had poor sanitary environment hence, concluded that sanitary practices were minimal.

# CHAPTER THREE

# RESEARCH METHODOLOGY

## 3.0 Introduction

This section presents the study methodology as guided by the objectives. It covers the study area, the study design and population, sampling procedure, sample size determination, analysis, data interpretation and ethical considerations.

## 3.1 Study area

The study was conducted in Majengo area, an informal settlement in Meru County within the Eastern region of Kenya. As with other slum areas, Majengo has congested households with poor sanitation and limited access to safe toilet facilities. It is located at Imenti North sub-County and Ntima west ward. Intima west has a population of 33,265 (Kenya National Bureau of Statistics, 2019).River Kathita passes along the slum. The contamination of this river poses a great risk to the large population downstream. Outbreak of diseases due to infectious organisms could occur. Poverty is high in this area, and residents often consume street food usually prepared in unhygienic standards (Kimathi 2018). Solid and liquid waste is mostly dumped into clogged drains proximal to the households (Tanni *et al*., 2015).

## 3.2 Study design and population

The study was cross-sectional in design. Environmental samples were collected from water collection points, soils near latrines, and open drains in Majengo area. Samples that were collected included water, soils, and swabs of open drains. The study did not involve households or interactions with residents of Majengo area.

## 3.3 Sampling procedure

To identify sites for sample collection, the study utilized a stratified sampling frame. First, Majengo area was stratified using a major road as point A and B. In each point, areas of sampling were randomly selected based on availability of water collection points, soils near latrines, and open drains. The

number of sampled areas or type of sample in each point was dependent on accessibility and spatial/temporal distribution of the sample types. The sampled areas were marked using google map to pin sample collection points. Figure **3.1** shows sampled areas in Majengo slum. The area was divided into two strata **A** and **B** as shown on the map.


Figure 3. 1 Sampled areas in Majengo slum

### 3.4 Sample size determination

To determine the required sample size, the following formula was used at a 95% confidence interval.(Cochran, 1977)

$n = \frac{z^2 \times p(p-1)}{e^2}$ Where;

n= sample size

$Z^2$= Test power for level of confidence (95%)

p= expected proportion (0.2) a prevalence (Pourhoseingholi *et al.*, 2013)

e= precision

$$n = \frac{1.96 \times 1.96 \times 0.2(1-0.2)}{0.1 \times 0.1}$$

Sample size = 61 samples

### 3.5 Isolation methods

#### 3.5.1 Water sample collection

Water sampling was conducted using sterile 500ml bottle, stickers and marker pen for labelling for proper collection.

#### 3.5.2 River water collection

The cap of the bottle was aseptically removed, then the mouth of the bottle was laid in water facing upstream. The bottle was immersed 30cm deep in water and was allowed to fill. It was labelled and tightly capped (monicah cheesbrough, 2006,p.148).

#### 3.5.3 Tap water

To start with tap splash nozzle was removed. The tap then was turned on and water allowed to run for 1 minute before collection. Before sample collection undertaken, the tap was sterilized using a flamed wet alcohol cotton swab. Water was allowed to run and the sample bottle was held close the tap to fill it. After the bottle was filled it was tightly capped (monicah cheesbrough, 2006,p.148)

#### 3.5.4 Water transportation

Water samples were packed in a cooler box with ice bags (Chauhan *et al.*, 2017). The ice bags ensured there is low temperatures so that the organisms didn't die before analysis in the laboratory. The samples were transported immediately after collection.

#### 3.5.5 Soil sample collection and transportation to the laboratory for analysis

Soil collection required sterile polythene bags, a sterile spoon, a pair of gloves and marker pens. Samples were collected near a toilet. Soil samples were collected 10cm deep with a sterile spoon(John maina, 2020) and placed into a sterile polythene bag and tightly sealed. The soil samples were transported in a cooler box with an ice pack (Ramírez et al., 2017).

### 3.5.6 Drain swab sample collection transportation to the laboratory for analysis

Drain collection required sterile gloves, transport media and sterile dry cotton swabs.

In swab collection, the cover was first untwisted to remove swab. Sample collection was by moving the swab in a clockwise circular motion(Lemarié *et al.*, 2022). The swab then was returned aseptically into the vial containing transport media. It was labelled and packed in cooler box containing an ice pack for transportation to the laboratory for analysis.

Sample analysis begun the same day of collection and the analysis was done at Meru University of Science and Technology's Biological Sciences Laboratory.

### 3.5.6 Media preparation prior to the inoculation

Requirement for media preparation included: Macconkey agar (oxoid CM007), Macconkey broth (oxoid CM 0505), Triple sugar iron agar (European pharmacopoeia), Muller Hinton (CMO337 Oxoid), Salmonella Shigella Agar (Techno Pharmchem), Trypton water (CM 0087Oxoid), TBX agar, Urea Agar Autoclave, autoclave tape, petri dishes, heater,1000ml capped bottle, capped test tubes, measuring cylinder, weighing balance and bijou bottles. Five types of media were prepared as described below:

### 3.5.6.1 MacConkey agar

It's a differential media that's used to grow organisms that are able to ferment lactose and those not able to. The organisms that are lactose fermenter are observed by the presence of pink colonies.

The media was first 52grams were weighed using a weighing balance. The media was then suspended in 1000ml bottle containing 1000ml of distilled water. The mixture was allowed to boil in order for the media to dissolve. Once the ingredients were dissolved, autoclaving was done at 121ºC for 15 minutes. Once sterilization was complete the media was transferred to a water bath at 50ºC.

### 3.5.6.2 Salmonella Shigella Agar

Salmonella Shigella agar is the selective media that's used to isolate *Salmonella* and *Shigella* species. Growth of Salmonella is characterized by growth of black colonies.

60 grams of the media was weighed first. The required amount was suspended in a conical flask containing 1000ml of distilled water. The medium was then mixed well to dissolve completely. It was then boiled with agitation frequently. After boiling for one minute, the media was transferred to 50ºC water in a water bath for 10 minutes and allowed to cool. The media was then dispensed to petri dishes and allowed to solidify.

### 3.5.6.3 Urea Agar

Urea is the media that's used in microbiology to differentiate between non lactose fermenters *Salmonella* and *Proteus* species.

24 grams of agar media were weighed and suspended in a conical flask containing 950ml of distilled water. The media was mixed well and the solution subdivided into 5mls bijou bottle. The media was then sterilized at 115ºC for15minutes. The media was allowed to cool to 50ºC for 10 minutes. 50ml of 40% urea were added and mixed well.

### 3.5.6.4 Triple Sugar Agar

It's a differential media that is used to test for organisms that have the ability to utilize and ferment glucose and lactose to produce acid and gas. This media was used to identify sulfur reducers such as *Salmonella* species since they have the ability to produce a black precipitate. It was also used to identify *E. coli* since it's able to ferment lactose to produce acid and gas as indicated by the formation of yellow color and clear zone due to gas formation.

64.42g were weighed and suspended in 1000ml distilled water in a conical flask. The media was then heated to boil to ensure complete dissolving. 10ml was then distributed in capped test tubes. The media was then autoclaved at 115ºC for 20 minutes. After sterilization the tube were held diagonally to form a slope.

### 3.5.6.5 Cary Blair Transport Media

It's the media that's used to transport samples from the site of collection to the laboratory. Enteric pathogens may die if not transported in the transport media.

12.6 grams of the media were weighed then the media was suspended in 991ml of distilled water and mixed well. The medium was heated in order to dissolve the solute completely. It was allowed to cool to 50ºC after which 9ml of 1% anhydrous calcium chloride was added. The media was then sub divided into 7ml of capped bijou bottles. The media was finally steamed at 100ºC for 15 minutes and refrigerated thereafter.

#### 3.5.6.6 Dispensing of media on petri dishes

Sterile plates were laid on a flat surface in a biosafety cabinet. The media was mixed gently by rotating the bottle in which it was held. The neck of the bottle was flamed to keep it sterile. 25ml of media was dispensed into the plates and allowed to solidify.

#### 3.5.6.7 Test for sterility of the prepared media

Media was incubated overnight at 37ºC for 24 hours to check for sterility. Presence of turbidity on fluid media or growth on solid media with microbial indicated contamination of the media hence, not suitable to be used to carry out the inoculation.

#### 3.5.6.8 Inoculation Technique for culturing of organisms

The inoculation required; a wire loop, a flame, pair of gloves, a straight wire. The working bench was decontaminated. Gloves were worn to prevent contamination from the infectious organisms. A wire loop was passed through a burning flame and allowed to cool. A sample was picked using the wire loop. First the main inoculum was made at the edge of the plate. The wire loop was sterilized again and allowed to cool. Then the sample was spread out from the main inoculum to ensure single colony growth.

#### 3.5.6.9 Quality control of the cultures

Control organism used was ATCC *E. coli*. A plate was inoculated with control organism using the previously described procedure. The plate was incubated for 24hours at 37ºC. The result was compared to the growth of the isolated organism.

### 3.5.6.10 Soil samples preparation prior to the inoculation

A sample of soil weighing 1g was dissolved in sterile distilled water in a 250ml conical flask (Amoafo *et al.*, 2022). A serial dilution was performed up to $10^5$for *E. coli* all dilutions were cultured into MacConkey agar and incubated at 37ºC for 24 hours using microbiological standards of culturing. On the other hand, dilutions to detect *Salmonella* spp. were cultured in Salmonella Shigella agar and incubated at 37ºC for 24 hours. After this duration colonies of target organisms were sub cultured in Tryptone Bile Glucuronide (TBX) to detect *E. coli* while *Salmonella* spp in Salmonella Shigella agar in order to obtain pure colonies that were inoculated in triple sugar agar. Confirmation for *Salmonella* spp and *E. coli* was done on indole test for *E. coli* and Triple sugar for *Salmonella* spp and urease test was used for confirmation of *Salmonella* species.

### 3.5.6.11 Drain swabs

In the laboratory, drain swabs samples were cultured both in MacConkey agar to isolate *E. coli* and Salmonella Shigella Agar to isolate *Salmonella* spp. Both media were incubated at 37ºC for 24 hours. After 24 hours' growth of target organisms in MacConkey agar were sub cultured in Tryptone Bile Glucuronide (TBX) to detect *E. coli*. Growth of green colonies was an indicator for the presence of *E. coli*. In Salmonella Shigella agar growth of black colonies was an indication for *Salmonella* growth.

During isolation of the target microorganisms on the culture media, other microorganisms were isolated on MacConkey and Salmonella Shigella agar. However, these microorganisms were not of significant to the study since only *E. coli* and *Salmonella* spp hence, further study can be done to identify these microorganisms.

### 3.5.6.12 Water analysis using most probable number method

Water analysis for most probable number required sterile bottles with MacConkey broth, Durham tubes for gas collection.

Each bottle with sample was appropriately labelled. The water was mixed thoroughly by inverting the bottle many times. The cap of the bottle was removed and the mouth of the bottle flamed. The water sample were then inoculated (Yeboah *et al*., 2022). After inoculation the bottles were incubated at 44 ºC for 24hours the samples were examined for color change and gas formation. Table 3.1 shows water analysis set for untreated and treated water samples.

Table 3. 1 Water analysis set for untreated and treated water samples

| Sample Type | No. of Bottles | Ml of Broth | Strength of Broth |
|---|---|---|---|
| Untreated Water | 1 | 50 | Double |
| | 5 | 10 | Double |
| | 5 | 5 | Single |
| Treated Water | | | |
| | 1 | 50 | Double |
| | 5 | 10 | Double |

Positive samples were identified by formation of yellow color and the positive samples sub cultured in TBX for18-24hrs at 37ºC to detect for *E. coli* an indicator for water contamination (Chukwu *et al*., 2022).

### 3.5.6.13 Biochemical identification of *E. coli* using Tryptone water

Isolates were subjected to biochemical identification using Indole test for *E. coli*. The test organism was inoculated in a bijou bottle containing 3ml Tryptone water and incubated at 37ºC for 48 hours. Test for indole was done by adding Kovac's reagent where a formation of a red ring indicated presence of *E. coli* within 10minutes.

### 3.5.6.14 Determination of *E. coli* 0157 using API (Analytical Profile Index) test kit

The test required API agglutination kit the package box as shown in **figure 4.12**, *E. coli* pure colony, preparation sample vials, pipette and a sterile swab.

The test tube reagent was allowed thaw a room temperature. One drop of extraction one was transferred into sample vial. Using a sterile swab, a single colony of the isolate was picked and mixed with the extraction reagent one in the vial. Two drops of extraction 2 and 3 were added respectively. One drop of the mixture pipetted and transferred into a reaction plate. One drop of agglutination latex was added and the plate was swirled to check for agglutination. A positive control was included to ensure validity of the results.

#### 3.5.6.15 Biochemical identification of salmonella species using TSI test

The top of the colony was touched using a straight wire loop. The organism was inoculated by first stabbing the butt at the center from top to bottom. Streaking of the slant was then done. The cap of the bottle was loosened and incubated at 37ºC for 24 hours.

#### 3.5.6.16 Biochemical confirmation of the positive tubes from TSI for presence of *Salmonella spp* using Urease test

The test organism was inoculated into 3ml of sterile urea broth. The bottle was incubated at 37ºC for 4 hours. After this the tubes were observed for color change.

#### 3.5.6.17 Gram staining technique

Gram stain is a technique used in differentiation of Gram positive and gram negative bacteria. The technique is important since it helps in selecting the correct antibiotic during microbial sensitivity. A smear was prepared from the colonies to confirm if it was gram negative rods using gram staining procedure. The procedure required gram stain reagents, slides, oil immersion and a microscope.

Colonies of test organism were emulsified on a glass slide using a wire loop. A drop of normal saline was added to make a smear. The slide was allowed to air dry. The slides were passed through a flame to fix them. The smear was covered with crystal violet solution for 30secs. Then the smear was rapidly

washed off to remove the excess stain. The smear was covered with Gram's iodine for 30 seconds and washed off using tap water. The smear was decolorized by covering with acetone for a few seconds and washed off. The smear was then covered with a counter stain neutral red for 30 seconds. The smear was allowed to dry before being examined at power x100 on a microscope. Presence of gram negative rods confirmed the organism.

### 3.5.7 Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was done using Muller Hinton Agar. Before inoculation of the organism to the Muller Hinton, single colonies were used to make 0.5ml McFarland standard (Ghabban *et al*., 2022; Zelalem *et a*l., 2022). The standard was used to compare the colony suspension prepared in 1ml vial tube that was prepared by picking single colonies and suspending in a vial tube containing normal saline. Once the suspension was similar to the McFarland standard sterile swab was used to pick the sample for inoculation. This inoculum was spread evenly on Muller Hinton Agar plate using sterile cotton swab by inserting the swab in the prepared suspension and antimicrobial susceptibility discs inserted using Kirby Disc method as explained by Clinical Laboratory Standards Institute (CLSI) and Monicah cheesbrough, (2006) respectively. The discs were well spaced to prevent them from overlapping. They were pressed on the media to ensure complete contact. They were incubated for 24 hours at 37ºC. The Clinical standards, were used to determine the zones of inhibition and enable classification of isolates of *E. coli* as either sensitive, intermediate, or resistant Zhao *et al*., (2022) also (Kahlmeter *et al*., 2019). ATCC *E. coli* was used as a control organism during the study. The microbial agents used were as follows in their corresponding concentrations; Imepenem (10µg); Ceftazidime (30µg); Cefotaxime (30µg); Cefoxitin (30µg) and Ciprofloxacin (5µg). Most antibiotics were resistance to all generation of antibiotic used with highest being ampicillin a first generation (Maina *et al.*, 2019). Figure 3.2 shows laboratory work flow for the beginning to the end.

**Laboratory work flow for the beginning to the end**

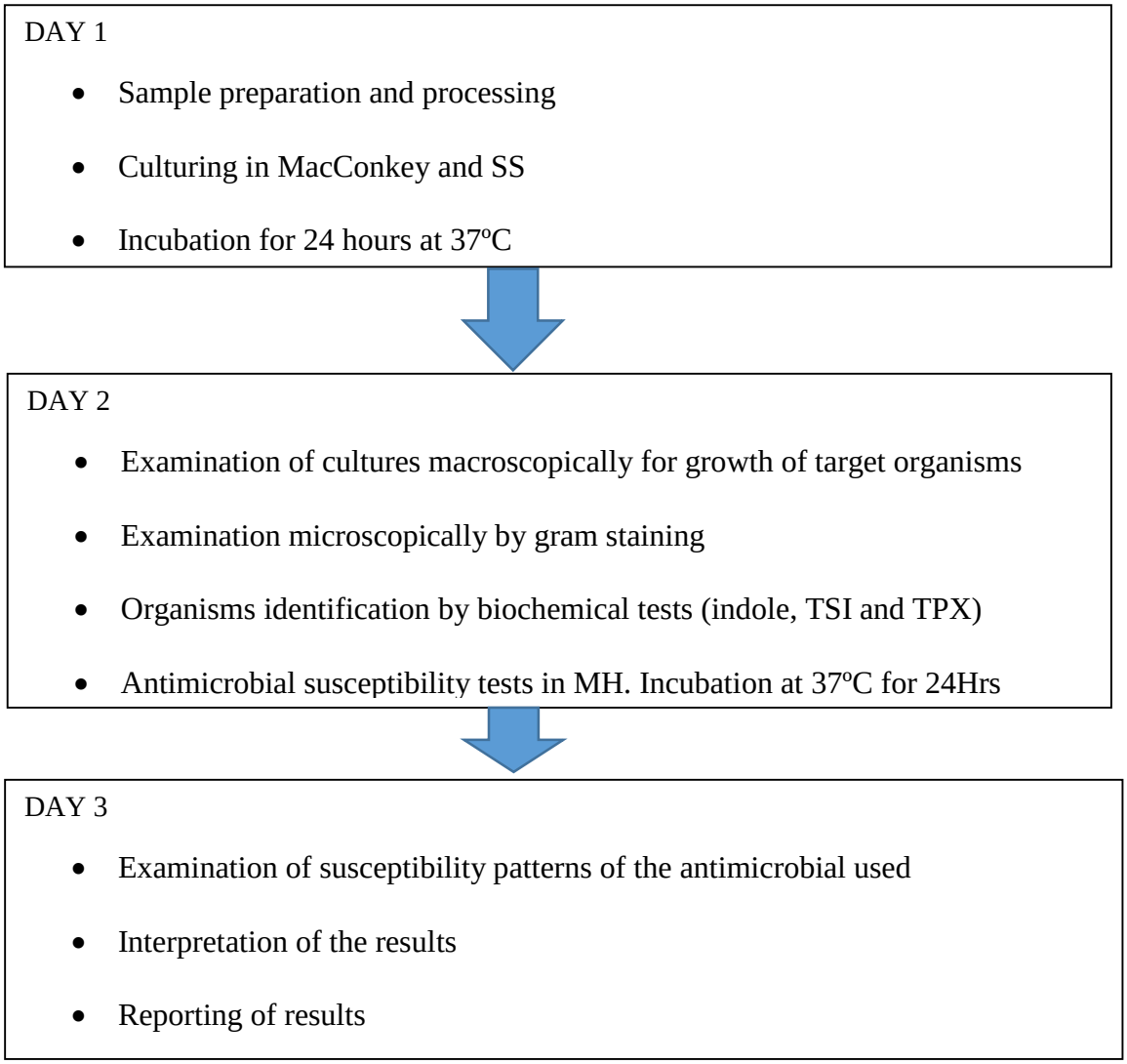



Figure 3. 2 Laboratory work flow for the beginning to the end

### 3.6 Data analysis

Laboratory data was entered into Microsoft Excel 2010 and analyzed using SPSS version 26.0. Descriptive analysis was done using Microsoft excel. The analysis included, frequencies and graphs. Data was compared between each study strata and between each sample type using Kruskal-Wallis tests, between the two drugs using the Wilcoxon Signed Rank test and Findings were presented as figures tables and graphs.

### 3.7 Ethical considerations

Clearance to conduct the study was obtained from the County public health authorities local authorities also and from Meru University of Science and Technology MIRERC (Meru University Institutional Research and Ethics Review Committee). Also permission to conduct the study was also sought from National Environment Management Authority (NEMA) that carries surveys on matters concerning the environment. The residents of Majengo were accorded utmost respect and confidentiality during the study period. The organisms isolated were deactivated before release to the environment.

# CHAPTER FOUR

# RESULTS

## 4.0 Introduction

This chapter presents the outcome of the cross sectional study that was conducted in Majengo slum informal settlement in Meru County. The first section gives identification, isolation, and susceptibility testing. Section 4.12 provides statistical comparison in sensitivity, intermediate and resistance of the drugs using Kruskal-Wallis and Wallis and Wilcoxon Sign Rank test.

## 4.1 Colonies obtained from Tryptone Bile X- Glucuronide and Salmonella Shigella Agar

A total of twenty-two (36.1%) drain swabs thirty-six (59.0%) soils and three (4.9%) samples water was analyzed as described in the previous procedure. Out of this 42(69%) samples had *E. coli* as confirmed by TBX agar while 19(0%) *Salmonella* spp. Figure 4.1 a and b shows green colonies of *E. coli* isolated from TBX agar and suspected black colonies of *Salmonella* spp from Salmonella Shigella Agar.

Figure 4. 1 Left shows green colonies of *E. coli* (b) right suspected black colonies of *Salmonella* spp

## 4.2 Isolates from water samples from both strata

A total of three samples were analyzed as described in the methodology. All the 3(100%) samples were contaminated by *E. coli*. Figure 4.2 shows change of MacConkey broth from purple color to yellow indicating presence of *E. coli*.


Figure 4. 2 Change of MacConkey broth from purple color to yellow indicating presence of *E. coli.*

**4.3 Biochemical identification of *E. coli.***

Kovac reagent was added to tryptone water with inoculated *E. coli is* explained in chapter three. Formation of a red ring at the interface indicated presence of *E. coli*. Figure 4.3 red ring formed after addition of Kovac reagent as explained previously.

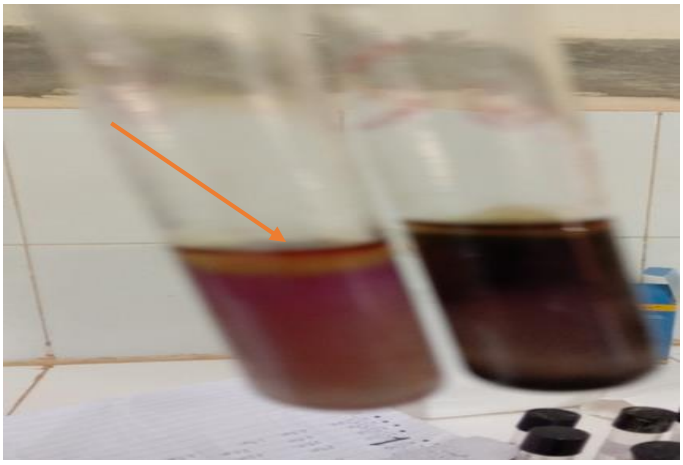


Figure 4. 3 Red ring formed after addition of Kovac reagent

**4.4 Triple Sugar Iron Agar after 24 hours of incubation**

Test tubes that showed red slant, blackening due to gas production and yellow butt, were further tested by urease to confirm if they were *salmonella* or *Proteus*. Figure 4.4 shows TSI tubes with red slant, gas production and yellow slant.

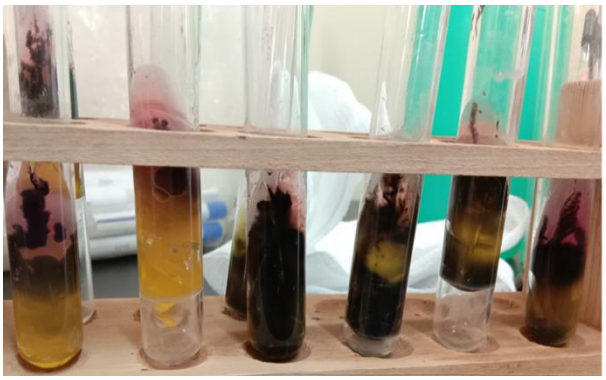


Figure 4. 4 TSI tubes with red slant, gas production and yellow slant

### 4.5 Urease test for identification of *Salmonella* spp

Positive TSI test tubes that had a red slant and yellow butt with black precipitate were confirmed if they were *Salmonella* spp using Urease test method as explained in chapter three. Figure 4.5 shows Urease positive for Proteus. Hence, the test confirmed *Salmonella* negative from the samples collected during the study since salmonella is urease negative (Al-Hadidi *et al.*, 2022).

Figure 4. 5 Urease positive

### 4.6 Identification of the isolates using Gram stain technique

The isolates of *E. coli* colonies were differentiated using Gram staining if they are Gram positive or Gram negative before sensitivity. Figure 4.6 below shows gram negative red rods of *E. coli.* The cell wall of Gram negative is thin hence the stain easily leaks out of the nucleus. (Mahon *et al*., 2022,P. 9)

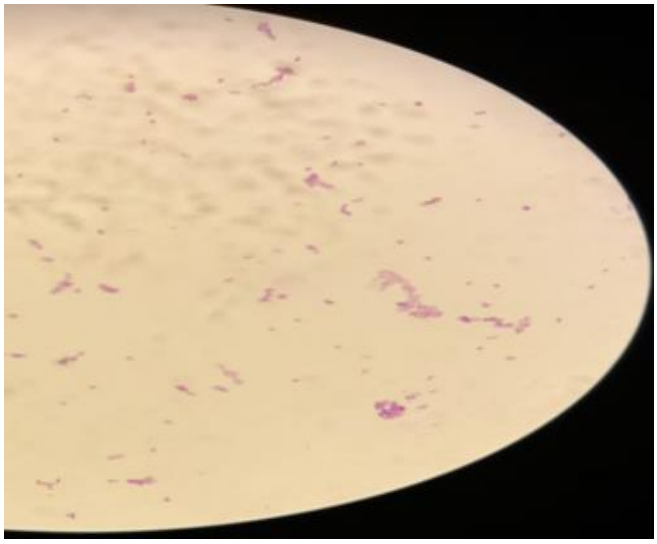


Figure 4. 6 Gram negative rods of *E. coli*

### 4.7 Isolates showing zone of inhibition and control organism ATCC

All the fort two isolates that were confirmed to be *E. coli* were subjected to sensitivity test. Table 4.1 quality control ranges of ATCC obtained from the study. Figure 4.7 shows sensitivity disc showing both the inoculated organism and the control organism a clear zone of inhibition. Table 4.2 shows the ranges of the five drugs according to clinical laboratory standards institute guidelines (CLSI). The interpreted result for the plate labeled QC was compared by the QC range in the table shown below. A result that was with the lower and upper limit confirmed that QC was correct.

Table 4. 1 Quality control ranges of ATCC obtained from the study

| **ATCC *E coli* on agents used** | **QC Range** | **Test Ranges** |
|---|---|---|
| Ceftazidin | 25-32 | 26 |
| Cefoxitin | 23-29 | 25 |
| Cefotaxime | 29-35 | 30 |
| Ciprofloxacin | 30-40 | 34 |
| Imipenem | 26-32 | 27 |

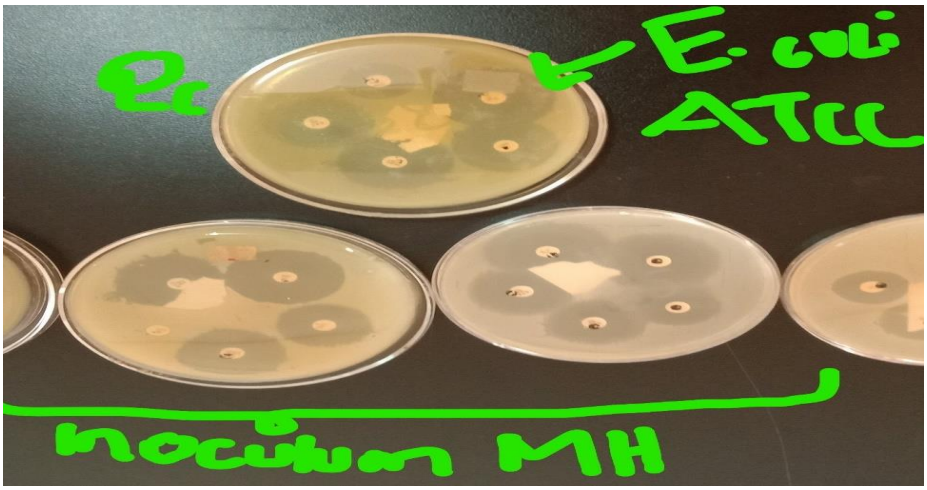



Figure 4. 7 Sensitivity discs and quality control organism showing zone of inhibition

Table 4. 2 Quality control ranges of antimicrobials used in the study

| Drug | Sensitive | Intermediate | Resistant |
|---|---|---|---|
| Cefotaxime | >26 | 23-25 | <22 |
| Cefoxitin | >18 | 15-17 | <14 |
| Ceftazidime | >21 | 18-20 | <17 |
| Imipenem | >23 | 20-22 | <19 |
| Ciprofloxacin | >21 | 16-20 | <15 |

For a drug to be referred as sensitive, intermediate and sensitive a standard for the five drugs was used as show in the table above.

### 4.8 *E. coli* isolated from all the samples collected

A total of sixty samples were analyzed *E. coli* organism was isolated from forty-two (69%) as evidenced from MacConkey Agar after growth. The *E. coli* was isolated from soil twenty-three, drains sixteen and water three. However, there were no *Salmonella* spp nineteen (0%) isolated from this study since all the isolates were negative for *Salmonella* organism as evidenced from urease test done for the nineteen samples that produced hydrogen sulphide gas. All colonies of *E. coli* that grew on MacConkey agar were sub cultured into Sorbitol MacConkey to identify the growth of *E. coli* 0157 strain. They

were incubated for 24hrs at 37 ºC. Growth of colorless colonies showed the presence of 0157 *E. coli.* Table 4.3 A and B shows isolates of *E. coli* both strata. B samples with no isolates of *Salmonella* spp from all samples.

Table 4. 3 *E. coli* isolated from soil, drain swabs and water from both strata.

**Strata 1**

| **Sample ID** | **Source** | **Organism Isolated** | **Total No.** |
|---|---|---|---|
| A2,A4,A9,A10,A14,A15,A16,A18,A21 | Soil | *E. coli* | 9 |
| A3,A5,A6,A8,A13,A17,A19,A20,A22,A23 | Drain Swabs | *E. coli* | 10 |
| A1,A12 | Water | *E. coli* | 2 |
| **Total** | | | **21** |

**Strata 2**

| **Sample Id** | **Source** | **Organism Isolated** | **Total No.** |
|---|---|---|---|
| B1,B2,B5,B6,B9,B10,B13,B17,B18,B19,B20,B21,B28,29 | Soil | *E. Coli* | 14 |
| B12,B4,B7,B8,B15,B16 | Drain Swabs | *E. Coli* | 6 |
| A31 | Water | *E. Coli* | 1 |
| **Total** | | | **21** |

Table 4. 4 (B) samples with no isolates of *Salmonella* spp from all samples

| **Isolate No** | **Strata1** | **Source** | **Culture** | **Total** |
|---|---|---|---|---|
| A7,A24,A25,A26,A29,A30 | strata1 | Soil | no growth | 6 |
| A11,A27,A28 | strata1 | Drain swab | no growth | 3 |
| **Total** | | | | **9** |

| Isolate no | strata2 | source | culture | Total |
|---|---|---|---|---|
| B11,B14,B23,B24,B25,B27,B31 | strata2 | soil | no growth | 7 |
| B3,B12,B22 | strata2 | Drain swab | no growth | 3 |
| Total | | | | 10 |

### 4.9 Antimicrobial Susceptibility Testing

#### 4.9.1 Antimicrobial susceptibility from both strata

The sampled areas were divided into two groups called strata and they were identified as A and B.

All the samples that grew *E. coli* were subjected to antimicrobial sensitivity using Kirby Disc diffusion method as described in chapter 3. A total of 42 isolates were tested for antimicrobial susceptibility in both strata. Out of these antimicrobials, 5(50.48%) were susceptible,5 (22.86%) were intermediate and 5(26.67%) were resistant. Out of the five antimicrobial agents used, Ceftazidime 28(66.67%) showed the highest sensitivity followed by Ciprofloxacin 26 (61.90%) and Imepenem 25(59.52%) respectively. Cefotaxime and Cefoxitin showed least sensitivity at 14(33.33%) and 13(30.95%) respectively. In intermediate Imepenem and Ciprofloxacin were the highest with 12(28.57%) followed by Cefotaxime 10(23.81%). The least intermediate was observed in Ceftazidime and Cefoxitin both at 7(16.67%). Highest resistance was observed in Cefoxitin 22(52.38%), followed by Cefotaxime at 18(42.86%). Ciprofloxacin, Imepenem and Ceftazidime had the lowest resistance 4(9.52%),5 (11.91%) and 7(16.67%) respectively. Figure 4.8 A bar plot of the percentages of different drugs in response to *E. coli.*


Key: IMP-imipenem; CAZ-ceftazidime;CXT-cefoxitin;CTX-cefotaxime;CIP-ciprofloxacin

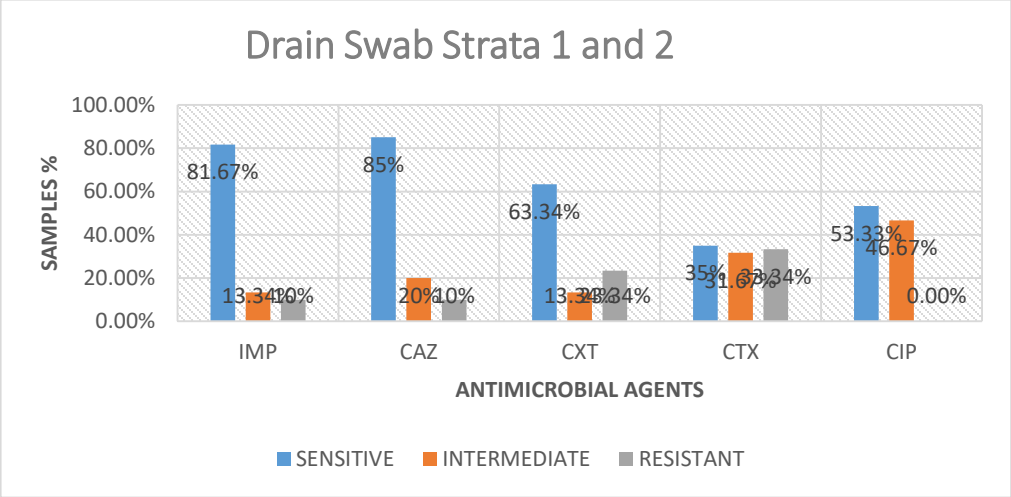
Figure 4. 8 Percentages of different drugs under different classes of response to *E. coli.*

**4.9.2 Antimicrobial susceptibility from drain swabs**

All the samples that grew *E. coli* were subjected to antimicrobial sensitivity using Kirby Disc diffusion method. Five agents were used to do sensitivity test for all the samples. A clear zone was measured in millimeters and the figure compared with the stand chart hence a drug was recorded as either resistant, sensitive or intermediate. A total of 16 isolates from drain swabs were tested for antimicrobial susceptibility. Out of these antimicrobials 5 (61.25%) were susceptible to the microbial, 5(23.75%) were intermediate and 5 (15%) were resistant. The highest resistance was shown to be Cefotaxime 6 (33.34%), followed by Cefoxitin at 4 (23.34%) while Imipenem and Ceftazidime were least resistant at 2 (10%) respectively. Ciprofloxacin was 0% resistant to the isolates tested. Table 4.5 below shows antimicrobial susceptibility patterns from drain swabs in both strata. Out of the five drugs Ceftazidime showed highest susceptibility at 13(85%) while Imipenem followed at 13(81.67%) respectively. Ciprofloxacin was the highest in intermediate at 8(46.67%) while Imipenem and Cefoxitin were the least intermediate at 2(13.34%) respectively. Figure 4.9 shows comparison in % between sensitivity, intermediate and resistance.

Table 4. 5 Antimicrobial susceptibility Frequencies from drain swabs in both strata.

| Antimicrobial | Sensitive | Intermediate | Resistant |
|---|---|---|---|
| Imipenem | 13(81.67%) | 2(13.34%) | 1(10%) |
| Ceftazidime | 13(85%) | 2(20%) | 1(10%) |
| Cefoxitin | 10(63.34%) | 2(13.34%) | 4(23.34%) |
| Cefotaxime | 5(35%) | 5(31.67%) | 6(33.34%) |
| Ciprofloxacin | 8(53.33%) | 8(46.67%) | 0(0.00%) |



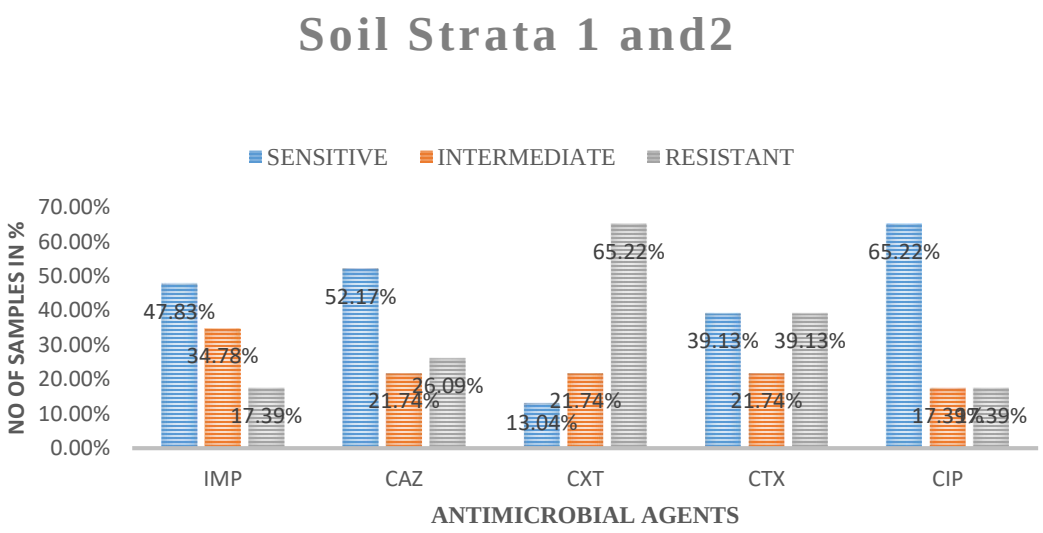

Key: IMP-imipenem; CAZ-ceftazidime;CXT-cefoxitin;CTX-cefotaxime;CIP-ciprofloxacin

Figure 4. 9 Comparison in % between sensitivity, intermediate level and resistance

### 4.9.3 Antimicrobial susceptibility from soil isolates from both strata

A total of 23 samples of soil were tested for antimicrobial sensitivity of which Imepenem resistant were 4 (17.39%),8 (34.78%) intermediate while 11 (48.83%) susceptible. In Ceftazidime resistant were 6 (26.09%), intermediate 5 (21.74%), susceptibility12 (52.17%), Cefoxitin resistant were15 (65.22%), intermediate 5 (21.71%), Susceptible 3 (13.04%), Cefotaxime resistant were 9(9.13%), intermediate 5 (21.74%), susceptible 9 (39.13%) and Ciprofloxacin resistant were 4 (17.39%), intermediate 4 (17.39%), susceptible 15 (65. 22%).Table 4.6 shows *E. coli* antimicrobials susceptibility drug patterns from soil samples in both strata. In both strata ciprofloxacin showed highest susceptibility at 65.22%, followed by Ceftazidime 52.17% while Cefoxitin showed least susceptibility. Imepenem showed

highest intermediate of all the drugs. Cefoxitin showed highest resistance. Figure 4.10 shows comparison in susceptibility patterns of the samples.




Key: IMP-imipenem; CAZ-ceftazidime;CXT-cefoxitin;CTX-cefotaxime;CIP-ciprofloxacin

Figure 4.10 Comparison in susceptibility patterns of the samples

In the first strata, Imepenem at 9 (77.78%) showed highest susceptibility, followed by Ciprofloxacin at 6(66.67%) and Cefoxitin was the least sensitive at 22.22% respectively. Ceftazidime showed highest intermediate at 33.33% while Cefoxitin and Cefotaxime were least intermediate. Cefoxitin showed highest resistance while Imepenem showed no resistant. In strata two Ciprofloxacin 64.29% showed highest susceptibility, followed by Ceftazidime 50%. Cefoxitin showed highest resistance at 64.29%, followed by Cefotaxime at 42.86%. Imepenem showed highest intermediate of all the drugs.

Table 4. 6 *E. coli* antimicrobials susceptibility drug patterns from soil samples in both strata

| **Strata 1** | **Sensitive** | **Intermediate** | **Resistant** | **Total** | **Sensitive** | **Intermediate** | **Resistant** |
|---|---|---|---|---|---|---|---|
| Imipenem | 7 | 2 | 0 | 9 | 77.78% | 22.22% | 0.00% |
| Ceftazidime | 5 | 3 | 1 | 9 | 55.56% | 33.33% | 11.11% |
| Cefoxitin | 2 | 1 | 6 | 9 | 22.22% | 11.11% | 66.67% |
| Cefotaxime | 5 | 1 | 3 | 9 | 55.56% | 11.11% | 33.33% |
| Ciprofloxacin | 6 | 2 | 1 | 9 | 66.67% | 22.22% | 11.11% |

| **Strata 2** | **Sensitive** | **Intermediate** | **Resistant** | **Total** | **Sensitive** | **Intermediate** | **Resistant** |
|---|---|---|---|---|---|---|---|
| Imipenem | 4 | 6 | 4 | 14 | 28.57% | 42.86% | 28.57% |

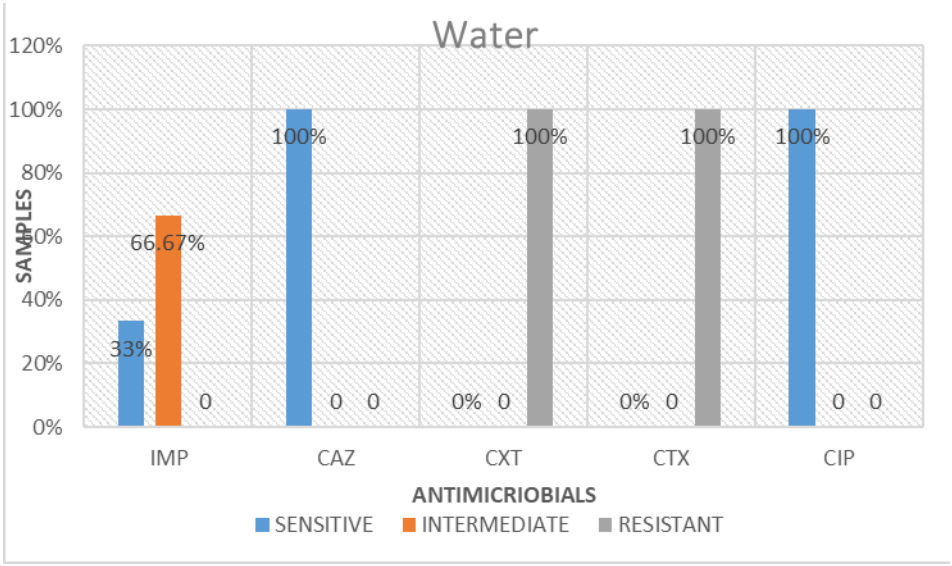
| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Ceftazidime | 7 | 2 | 5 | 14 | 50.00% | 14.29% | 35.71% |
| Cefoxitin | 1 | 4 | 9 | 14 | 7.14% | 28.57% | 64.29% |
| Cefotaxime | 4 | 4 | 6 | 14 | 28.57% | 28.57% | 42.86% |
| Ciprofloxacin | 9 | 2 | 3 | 14 | 64.29% | 14.29% | 21.43% |

### 4.9.4 Antimicrobial susceptibility for water samples

Out of the three water samples analyzed it was observed that, Ceftazidime and Ciprofloxacin showed 100% sensitivity while Imipenem showed least sensitive figure 4.11 shows the comparisons in % between the drugs. Highest resistance was observed on Cefoxitin and Cefotaxime at 100% respectively. Imipenem was the most intermediate at 66.67%. Table 4.7: shows *E. coli* antimicrobials susceptibility drug patterns from water samples from strata 1 and 2.

Table 4. 7 *E. coli* antimicrobials susceptibility of water samples

| Water | Sensitive | Intermediate | Resistant | Total | Sensitive | Intermediate | Resistant |
|---|---|---|---|---|---|---|---|
| Imipenem | 1 | 2 | 0 | 3 | 33% | 66.67% | 0% |
| Ceftazidime | 3 | 0 | 0 | 3 | 100% | 0% | 0% |
| Cefoxitin | 0 | 0 | 3 | 3 | 0% | 0% | 100% |
| Cefotaxime | 0 | 0 | 3 | 3 | 0% | 0% | 100% |
| Ciprofloxacin | 3 | 0 | 0 | 3 | 100% | 0% | 0 |




Key: IMP-imipenem; CAZ-ceftazidime;CXT-cefoxitin;CTX-cefotaxime;CIP-ciprofloxacin

Figure 4. 11 Comparisons in % between the drugs

### 4.10 Agglutination Latex Test for 0157 *E. coli* using 0157 AD kit

Out of fort two (76.19%) samples ten (23.81%) samples that had colorless colonies were tested for *E. coli* 0157 in both strata as shown in table 4.8**.** All the 10 samples tested positive for *E. coli* 0157. Figure 4.12 a API latex and b below shows agglutination of the colonies using 0157 latex antisera and Figure 4.13 shows colorless colonies of non-lactose fermenter of 0157 *E. coli.* The colorless colonies were non lactose fermenters while those that did not for colorless colonies were pink in color since they fermented lactose thirty-two (52.38%).

Table 4. 8 Identification and results for *E. coli* 0157 using API

| **Sample ID** | **Api *E. Coli* 0157 Latex Test Result** |
|---|---|
| A1,A6,A10,A13,A19 | Postive |
| B1,B7,B13,B16,B31 | Postive |


Figure 4. 12 API latex and b shows agglutination of the colonies using 0157 latex antisera

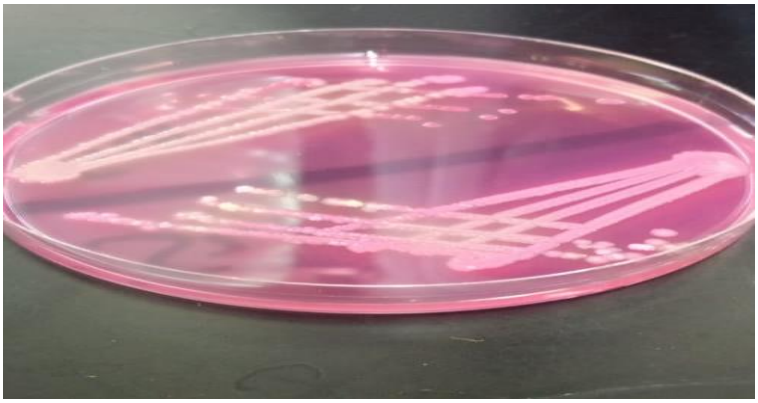


Figure 4. 13 Non-lactose fermenting colonies

### 4.11 Statistical comparison of resistance of the drugs using Kruskal-Wallis Test and Wilcoxon Signed-Rank Test.

The resistance from this study was statistically significant since the p value=0.0005 and the statistic =19.87. The p-value is less than 0.05, and so we reject the null hypothesis. This means that the resistance is not the same across the different drugs. To know which drug is more resistant, Wilcoxon Signed-Rank Test was used. This study found that Cefoxitin and Cefotaxime are statistically significant with a p-value 0.0156 which means they are have highest resistance among the rest of the drugs. Table 4.9 shows p-values between compared drugs.

Table 4. 9 p-values between compared drugs

| p-value | Ceftazidime | Cefoxitin | Cefotaxime | Ciprofloxacin |
|---|---|---|---|---|
| **Imipenem** | 0.1088 | 0.0156 | 0.0156 | 0.7150 |
| **Ceftazidime** | | 0.0156 | 0.0156 | 0.0679 |
| **Cefoxitin** | | | 0.2249 | 0.0156 |
| **Cefotaxime** | | | | 0.0156 |

Imipenem Ceftazidime and Ciprofloxacin are not significant to this study p>0.05. This p-value correlates to that was found in a study conducted by John maina, (2020) who recorded a p-value (0.36) towards *E. coli.*

Statistical analysis summary using plot box. Figure 4.14 A box plot showing summary of means, standard errors.

A box plot showing summary of descriptive statistics

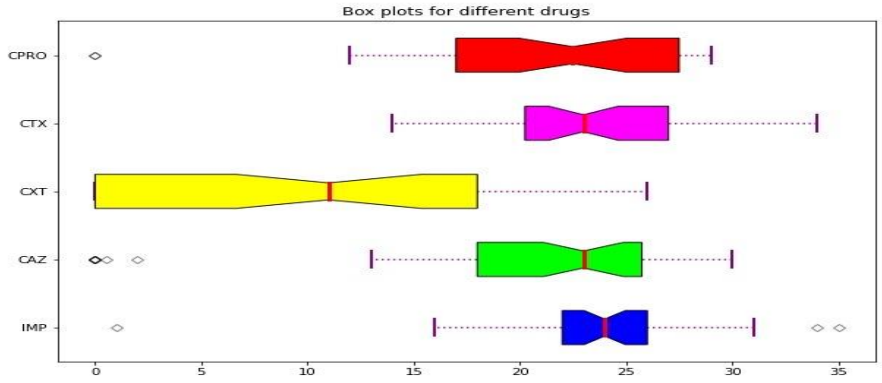



Figure 4. 14 A box plot showing summary of descriptive statistics

Table 4. 10 Means and standard errors

| **Drug** | **Mean ± SE** |
|---|---|
| **Imipenem** | 23.809524 ± 5.460448[a] |
| **Ceftazidime** | 19.440476 ± 9.395252[a] |
| **Cefoxitin** | 9.333333 ± 9.030211[b] |
| **Cefotaxime** | 23.452381 ± 4.695007[b] |
| **Ciprofloxacin** | 21.547619 ± 7.012888[a] |

Those with the same letter are statistically the same.

### 4.12 Statistical comparison of sensitivity of the drugs using Kruskal-Wallis Test and Wilcoxon Signed-Rank Test.

Sensitivity from this study was significant to the study since the p value=0.01 is between the strata. This means that the sensitivity is not the same across the different drugs. To know which drug is more sensitive, Wilcoxon Signed-Rank Test was performed. It was observed that, Ciprofloxacin, Ceftazidime and Imipenem were the most sensitive drug with a p-value >0.05. Table 4.11 shows the p-values of the antimicrobials in the study.

Table 4. 11 p-values of the antimicrobials

| p-value | Ceftazidime | Cefoxitin | Cefotaxime | Ciprofloxcin |
|---|---|---|---|---|
| **Imipenem** | 0.4630 | 0.0156 | 0.0277 | 0.9375 |
| **Ceftazidime** | | 0.0156 | 0.0260 | 0.3454 |
| **Cefoxitin** | | | 0.9165 | 0.1158 |
| **Cefotaxime** | | | | 0.0156 |

# CHAPTER FIVE

# DISCUSSION

## 5.0 Introduction

This chapter gives a discussion of the results based on the specific objectives of the study. Section explains how presence of *E. coli* on the environment of Majengo is an indication of contamination. Further, it explains resistance of the antimicrobials used during the study to isolated organism. The last section explains the impact of Enterohemorrhagic *E. coli* to human living in this informal settlement.

## 5.1 Isolation of *E. coli* in informal settlements of Majengo slum

The current study recorded a significant number of *E. coli* bacteria from forty-two samples analyzed 69%. This finding is lower than that recorded by Dewi *et al*., (2022) who isolated 100% *E. coli* from pond water. This result is an indication that the environment of Majengo slum in Meru County is highly contaminated. This poses a risk to human health to the people living in this area that will result to outbreak of diseases.

The current study recorded 72.7% of *E. coli* from drain swabs this finding is much lower than (Berendes *et al*., 2020) who recorded 89% of *E. coli* for open drains. Other studies conducted by Amin *et al*., (2019), Ginn *et al*., (2021) recorded 49% and 52% of *E. coli.* Their finding is lower from the findings recorded from the current study. The current study and precious studies hence agree that open drains are highly contaminated with waste water released from homes and open defecation.

While studying on *E. coli* isolated from community toilet wastewater and stored drinking water, (Harada *et al*., 2018) noted that 94% of stored drinking water had *E. coli*. In other studies, (Bindra et al., 2021, Gitahi *et al*., 2018) recorded 50% and 17% *E. coli* in ground water. The current study recorded 100% isolation of *E. coli* from three water samples analyzed. This contamination from current and previous studies could be due to unsafe disposal of human waste that contaminates water.

### 5.2 Antimicrobial resistance from water samples.

The current study showed 100% resistance to water samples analyzed during the study. This resistance is a likely indication that there is high contamination of waste water, that is in return used for home use by the residents**.** However, this study didn't do a study from the source to find out whether contamination starts from the source downstream. Further studies should be conducted to find out if there is contamination. The findings from the current study was higher by 20% from a related study conducted by Kumar *et al*., (2021) on antidrug resistance on Indian rivers. Mukami (2021) while investigating resistance from water found that Ceftazidime and Ciprofloxacin had almost the same resistance 1.7% and 1.8% respectively. These findings are higher than the current study which recorded 0% resistance on the drugs. Praveenkumarreddy *et al*.,(2020) while investigating the occurrence of antimicrobial of *E. coli* in wastewater plants found 60% resistance towards Ciprofloxacin, this value was higher than the current study which found 0% resistance.

### 5.3 Antimicrobial resistance patterns of *E. coli* from the study area

This study reported a range of multidrug resistance to *E. coli* from all the samples analyzed. Therefore, there is high chance that *E. coli* isolated from this study area emerges from the surrounding poor unhygienic conditions and inadequate sanitation facilities from the residents. This findings agrees with a study on contamination of street food in Burkina faso that, enteric organisms emerge from cross contamination (Nikiema *et al.*, 2021). Same observation were recorded by Tahri *et al.*, (2021). A study conducted on antimicrobial profile in Juja and Kibera found that, Cefotaxime, Ceftazidime and Ciprofloxacin recorded a greater than 30% of antimicrobial resistance (Maina et *al.*, 2019*).* This result is much lower than the one reported in the current study that recorded 25% increase. Fleece *et al*., (2019) observed that this increase in resistance was due to use of antibiotics in the treatment of diseases associated with poor unhygienic conditions. This study found the highest resistance on soil where it was noted that, Cefoxitin was at 65.22%, an indication that soil was highly contaminated in Majengo

slum. These findings correlates with study on urban informal settlement on antimicrobial resistance on the environment which noted that less attention was being given on contamination and posed a great risk to antimicrobial resistance (Iskandar *et al*., 2020). Samreen *et al.*, (2021) while studying at environmental resistance, he highlights that soil is a hotspot carrier of resistant genes.

The current study recorded 34% antimicrobial resistance towards Cefotaxime, imepenem 10% and zero resistance to Ciprofloxacin which agree with Divyashree *et al*., (2020) in a similar study that recorded 0% resistance to Ciprofloxacin 1.68% to Imipenem and 8.94% Cefotaxime respectively. However, both studies recorded 0% to Ciprofloxacin. Sonola *et al*., (2021) while looking at multidrug resistance to *E. coli* noted that Cefotaxime showed 79.7% resistance to *E. coli.* In a related study by Hope *et al*., (2019) recorded 93% resistance to *E. coli*. The current study recorded a lower figure of 42.86% antimicrobial resistance to Cefotaxime.

The current study showes the least resistance Ceftazidime (16.67%) which is much lower than that resistance obtained from (Ngene *et al*., 2021) at100%.

### 5.4 Global partnership in curbing antimicrobial resistance

The current study noted that slum need to be supported in order to manage waste. This will in turn ensure proper management of waste generated from informal settlement. This studies agrees with a study conducted by Oppong *et al.*, (2015) while studying on slums and health globally noted that, slum neglect in developing countries is a huge disaster since, it may increase multidrug resistance to microorganism that causes infections. This current study indirectly highlights need for partnership in order for slum areas to get water, sanitation and hygiene. This observation correlate to that of Gupta and Guin, (2015) who proposes need for non-governmental organizations to lead in provision of amenities in urban poor areas. In another study, Adane *et al*., (2017) suggests creation of preventive programs that mainly focuses on improving cleanliness of sanitation facilities. Also Khan *et al.*, (2017)

while investigating emerging antimicrobial resistance, suggests need for improving health, hygiene and sanitation at the community may reduce spread of infectious diseases.

### 5.5 Isolation of enterohemorrhagic *E. coli* on the Majengo environment.

Enterohemorrhagic *E. coli* is a strain of *E. coli* that release a toxin known as shiga toxin that causes acute diarrhea and kidney failure (Mukami, 2021). A study conducted by (Ghaderi *et al*., 2022) while studying on shiga toxin in sheep recorded 7.4% of *E. coli* 0157 present. In a related study (Heydari *et al*., 2020) noted 6.4% of *E. coli* 0157 was present in five samples out of seventy eight analyzed. The current study also isolated *E. coli* 0157 from ten out of forty-two samples analyzed.

In a study conducted by Fesseha *et al*., (2022) 46% of *E. coli* 0157 was recorded and unhygienic conditions were the reason for the availability of the bacteria. This result is higher than the current study which recorded 23.8%. In another study, (Ghali-mohammed and Ayoade, 2023) noted 2.3% of *E. coli* 0157 from raw milk. This result is lower than the one recorded by the current study. Unpacked milk was the most contaminated (Mumma and Baker, 2022,Disassa *et al*., 2017). Maina *et al*., (2019) highlights that contamination of the environment is the cause of emergence of strains that are infectious and resistant to antimicrobials. This study concurs with the current study which noted contamination of the environment which could lead to emergence of strains that are highly infectious.

### 5.6 Limitations for the study

Several shortcomings were experienced during this study that can form a basis for more robust studies in the future.

The study was only able to screen for microbial sensitivity without looking at resistance genes from the isolated organism.

The study only focused on isolation of organism without looking on the sanitation facilities in the study area.

The study was unable to isolate *Salmonella* spp. from this area hence more studies on alternative sample collection and analysis is needed.

# CHAPTER SIX

# CONCLUSION AND RECOMMENDATION AND PUBLICATION

## 6.0 Introduction

This chapter give a conclusion of the study and recommendation based on the findings from the study.

## 6.1 Study conclusion

The study arrived at the following conclusions after findings and Analysis.

High contamination noted strongly suggest sanitation standards are poor in this area which is a significant risk to human health. Resistance noted from this study result to increase of infections that may be a challenge to treat leading to high mortality rates. Isolation of *E. coli* strain 0157 is a strong indication of high risk of diarrhea to young children hence, high death rate. Salmonella species susceptibility was not recorded since the organism was not isolated during analysis.

## 6.2 Study recommendation

The researcher came up with the following recommendation based on the primary data collected:

Proper sanitation and hygiene awareness practices should be provided through education to the residents of this area. Water supply and proper standards for sanitation and WASH programs to control the contamination noted hence, reduce outbreak of diseases caused by microorganisms. Improvement of the environmental contamination by human waste could reduce the risk of contamination that originates from the surrounding. The County government of Meru should take the initiative and improve sanitation infrastructure in slum which include better sewerage drainage systems. Molecular methods should be in future be used to look into more details the resistance genotype of the isolate from the study.

### 6.3 Publication

A journal article was prepared and submitted to F1000 research journal. The article was on " Evaluation of antimicrobial susceptibility of *Escherichia coli* isolated from contaminated areas of Majengo slum in Meru County, Kenya ". The article was referenced as Ombuya J, Kennedy G, Kagendo D, Naomi M: Evaluation of antimicrobial susceptibility of escherichia coli isolated from contaminated areas of majengo slum in Meru County, Kenya. This publication is provided as appendix IV.

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## APPENDICES

### Appendix I: Consent

**Meru University of Science and Technology: Participant Information Sheet and Consent Form**

**Lay Title:** Evaluation of Antimicrobial Susceptibility of *E. coli* and *Salmonella* Spp Isolated from Majengo Slum in Meru County, Kenya

**Investigators and Institutional Affiliations**

| **Name** | **Institutional Affiliation** |
|---|---|
| OMBUYA JARED | MERU UNIVERSITY OFTECHNOLOGY |

**Who is carrying out this study?**

This study is being carried out by OMBUYA JARED. JARED is a master's student taking a study in MSC sanitation

**What is this study about?**

The study is about evaluation of antimicrobial susceptibility patterns of *E. coli* and *Salmonella spp* that will be isolated from poor environments of Majengo slum Meru County.

**What will it involve for me?**

The study will not involve human subject during data collection.

**Who has allowed this research to take place?**

Clearance to conduct the study will be obtained from the County public health authorities and local authorities also from Meru University of science and technology **MIRERC**. Also will seek permission to conduct the study from **NEMA**.

I have followed the study SOP and it will not involve human subject participation. I have understood the nature and the purpose of the study.

**Designee/investigator's signature:** …….. **Date** ……..

**Appendix II: Log Frame**

| **Project Description** | | **Objectively Verifiable indicators of achievement** | **Source and means of verification** | **Assumptions** |
|---|---|---|---|---|
| **Goal** | To determine the antimicrobial resistant level in slum areas | Resistant of *E.coli* and *Salmonella spp* | Pre-assessment data collected | Environmental pollution contribute to the public health risk of faecal contamination. |
| **Purpose** | Implementation and overall public health policies to prevent environmental contamination | Decreased diarrheal diseases in slum areas | Policy review<br><br>County hospital reports. | Policy change inhibits faecal contamination.<br><br>Correct use of latrines improves health |
| **Output** | 1. Descriptive statistics analysis of concentration of *E. coli* for each sample analyzed.<br>2. Exposure assessment profiles. | Source of exposure is determined | Statistical charts and histogram | Increase in fecal contamination results to contamination |
| **Activities** | 1. Preliminary assessment.<br>2. Environmental data collection.<br>3.Laboratory sample analysis;<br>-Multiple tube method of water analysis<br>-Plate count method | -determining risk of exposure pathways presence of *E. coli* in analyzed samples<br>- Observable (30-300) colonies on the agar media | Laboratory analysis | Growth of organism on the culture plate<br><br>Gas production in water samples |

## Appendix III: Ethical clearance

MERU UNIVERSITY INSTITUTIONAL RESEARCH & ETHICS REVIEW COMMITTEE
(MIRERC)

TO: Ombuya Jared Nyang'au
Through: Dean, School of Engineering and Architecture
FROM: Chairman MIRERC
REF: MU/1/39/28 Vol.2 (31)
SUBJECT: MIRERC clearance and approval of Research
DATE: 17th February 2022

I hereby forward Ethical clearance and approval of your research proposal*: Evaluation of Antimicrobial Susceptibility of Escherichhiacoli and Salmonella SPP Isolated from Majengo Slum in Meru County, Kenya* for implementation: Note that the implementation of the project should strictly adhere to and follow expected attributes of Justice, Respect, Beneficence and Non-maleficence to the study subjects.
The committee expects to be informed on the progress of the project from time to time and any amendments that may be instituted or incorporated into the proposal during its implementation to be pointed out.

The committee also expects this research project implementer(s) will not at any time risk the study subjects/data in terms of unfair disclosure of information that may come to their knowledge by way of this project or subject the study subjects/data to any bias or consequences whatsoever if or not a study subject withdraws from the project or access to data is denied.

The committee and study subjects will expect to be considered favorably for any benefits that arise from this study. The university would be grateful to act as repository for the data that your project will generate.

The MIRERC committee therefore accords the clearance and approval for this project to be implemented by the investigator(s) during the period specified by the project.

Thank you.
Yours Sincerely,

Prof. Eric Muchiri Ph. D
Chairman, MIRERC
Cc: Director, RDE

M.U.S.T IS ISO 9001:2015 CERTIFIED

## Appendix IV: Publication



F1000Research

RESEARCH ARTICLE

# REVISED Evaluation of antimicrobial susceptibility of *Escherichia coli* isolated from contaminated areas of Majengo slum in Meru County, Kenya [version 3; peer review: 1 approved]

Jared Ombuya [1], Kennedy Gachoka[2], Kagendo Dorothy[3], Naomi Mutea[4]

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## Abstract

**Background:** Antimicrobial drug resistance is of great concern today. Infections by the antimicrobial resistant strains of *Escherichia coli*, including enteropathogenic as well as enterotoxigenic strains have been reported as a major cause of deaths, especially among young children in low- and middle-income countries. This has been augmented by antimicrobial misuse, over the counter availability and poor sanitation especially in low income areas.
This study aimed at characterizing antimicrobial resistant strains of *Escherichia coli* isolated from sanitation environments of the Majengo slum in Meru County, Kenya
**Methods**: A cross-sectional study was conducted on 61 samples from soil, water and drains swabs. These were tested against five antimicrobial drugs by the Kirby disk diffusion method.
**Results:** A total of 42 (69%) of the samples had *Escherichia coli.* These recorded antimicrobial drug susceptibility as follows: Out of the five antimicrobial agents used, ceftazidime 28 (66.67%) showed the highest sensitivity followed by ciprofloxacin 26 (61.90%) and imepenem 25 (59.52%) respectively. cefotaxime and cefoxitin showed least sensitivity at 14 (33.33%) and 13 (30.95%) respectively. In intermediate imepenem and ciprofloxacin were the highest with 12 (28.57%) followed by cefotaxime 10 (23.81%). The least intermediate was observed in ceftazidime and cefoxitin both at 7 (16.67%). The highest resistance was observed in cefoxitin 22 (52.38%), followed by cefotaxime at 18 (42.86%). Ciprofloxacin, imepenem and ceftazidime had the lowest resistance 4 (9.52%), 5 (11.91%) and 7 (16.67%) respectively. The p-value <0.05 was considered significant to the study.

**Open Peer Review**

**Approval Status** ✔

1

**version 3**

(revision) view

12 Apr 2023

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**version 2**

(revision) view

26 Jan 2023

**version 1**

04 Oct 2022

1. **Eman S. Ibrahim**, National Research Centre, Dokki, Egypt

Any reports and responses or comments on the article can be found at the end of the article.

**Conclusions:** This study showed that *Escherichia coli* isolated from Majengo is pathogenic and resistant to antibiotics. Detection of *Escherichia coli* poses a great risk in the spread of resistant strains in human. Proper sanitation and hygiene awareness practices should be provided through education to the residents of this area.

**Keywords**

E. coli, Susceptibility Testing, Antimicrobial Resistance, Multidrug Resistance, Ciprofloxacin, Ceftazidime, Cefotaxime, Imepenem, Cefoxitin

**Corresponding author:** Jared Ombuya (jashnyash@gmail.com)

**Author roles: Ombuya J**: Conceptualization, Formal Analysis, Investigation, Methodology, Writing – Original Draft Preparation, Writing – Review & Editing; **Gachoka K**: Supervision; **Dorothy K**: Conceptualization, Methodology, Validation; **Mutea N**: Supervision

**Competing interests:** No competing interests were disclosed.

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SED Amendments from Version 2

Changes were made to the conclusion as per proposed by peer reviewer.

Any further responses from the reviewers can be found at the end of the article

## Introduction

Infectious organisms are currently the major cause of diseases worldwide. A number of newly recognized pathogens and strains are now emerging. These organisms have resulted in high morbidity and mortality globally.[1] The emergence of these pathogens has been attributed to microbial evolution, high mutations as the organisms try surviving in different environments as well as creation of new environments. This microbial evolution and high mutations has resulted to drug resistance, especially due to deposition of drug residues released in different environments, and more especially in areas with poor drainages and poor sanitation systems. As a result, there has evolved a need for surveillance and monitoring systems with emphasis on sanitation and water management. This will help curve the spread of emerging and re-emerging infectious diseases.

Human population growth currently has faced great challenges in accessing proper quality and quantity water resources. This has led to an increase in the number of water borne infectious diseases recently.[2] An estimated 30% of the bacterial population has been reported to be emerging because of wastewater, agricultural practices, and poor sanitation systems. Water management could act as a barrier to prevent the spread of pathogens. Prevention of pathogens could reduce environmental contamination, antibiotic misuse and eventually mutation of microorganisms.

In different environmental set ups, studies have indicated increased multidrug resistance strains which has led to increased mortality and morbidity resulting from exposure to infection causing organisms. Improved sanitation facilities is one of the most important interventions needed in order to stop the spread of resistant bacteria.[3] A study on typhoid among young children,[4] stressed the need for careful monitoring of antimicrobial resistance in a view to prevent increase of resistance strains and resultant infections to vulnerable communities.

Diarrheagenic E. coli has demonstrated a significant resistance to beta lactams antibiotics that are commonly prescribed,[5] with contributing factors to diarrhea cases attributed to poor quality of foods, poor water systems, lack of proper hygiene often due to lack of water and poor sanitation. Studies have shown that human and animal waste are the main sources of contamination on the environment that hosts strains that are antimicrobial resistance.[6] The study indicated that contamination resulted in formation of biofilms that supported bacterial resistance.

## Methods

### Ethical considerations

Approval to carry out the study was done by Meru University of Science and Technology MIRERC (Meru University Institutional Research and Ethics Review Committee). Approval Ref NO: MU/1/39/28 VOL.2(31). Date 17th February 2022.

### Study area

The study was conducted in the Majengo area, an informal settlement in Meru County within the Eastern region of Kenya. As with other slum areas, Majengo has congested households with poor sanitation and limited access to safe toilet facilities. It is located at Imenti North sub-county and Ntima west ward. River Kathita passes along the slum.

### Study design and population

The study employed a cross-sectional study design with an aspect of laboratory analysis. Environmental samples were collected from water collection points, soils near latrines, and open drains in the Majengo area. Samples that were collected included water, soils, and swabs of open drains. The samples were then transported to the laboratory for analysis. The study did not involve households or interactions with residents of the Majengo area.

### Sampling procedure

To identify sites for sample collection, the study utilized a stratified sampling scheme, where the Majengo area was stratified by its constituent villages using available maps. In each village, areas of sampling were randomly selected based on availability of the water collection points, soils near latrines, and open drains. The number of sampled areas or type of

samples in each village was dependent on accessibility and spatial/temporal distribution of the sample types. The sampled areas were marked using Google map to pin sample collection points. Figure 1 shows sampled areas in the Majengo slum. The area was divided into two strata A and B as shown on the map.

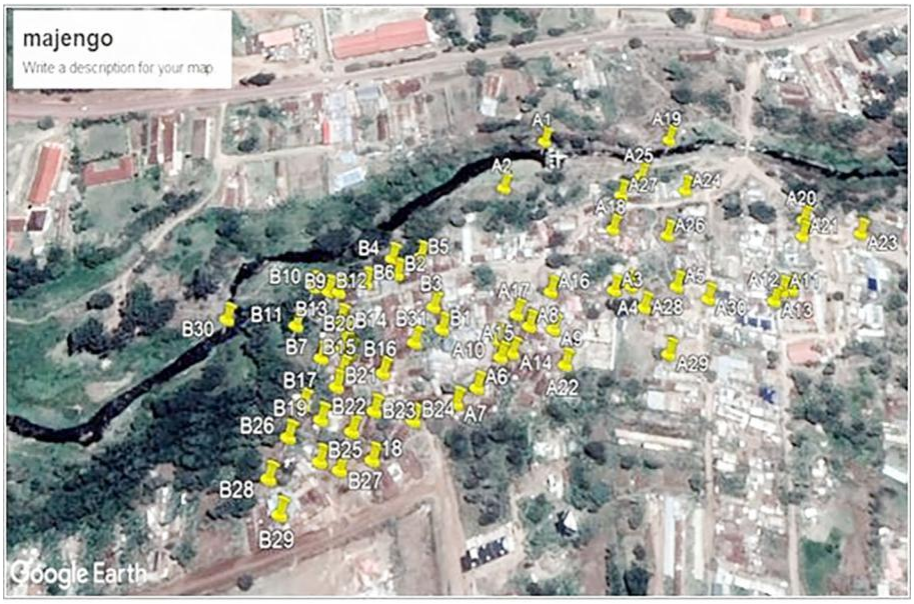


Figure 1. Shows a map of the sampled areas in the Majengo slum. Maps data ©2022: Google, ©2022 CNES/ Airbus, Maxar Technologies.

## Sample size determination

To determine the required sample size, the following formula was used at a 95% confidence interval.[7]

$$n = \frac{1.96 \times 1.96 \times 0.2(1-0.2)}{0.1 \times 0.1}$$

Sample size = 61 samples

## Sample processing

A 500 ml river water sample was collected into 500 ml sterile bottles by laying the bottle in water facing upstream. Tap water was collected into sterile 500 ml bottles. To do this, the individual tap was sterilized using a flamed alcohol cotton swab, a little water was allowed to flow, before getting collected into the sterile sample bottle. Approximately 10 g of soil samples were collected near toilets and dumping areas using a sterile spoon. In the swab collection, the cover was first untwisted to remove the swab and the sample collection was done by moving the swab in a clockwise circular motion. The swab was then returned aseptically into the vial containing transport media. It was labelled and packed in a cooler box containing an ice pack for transportation to the laboratory for analysis.

## Samples preparation prior to the inoculation

Soil sample

A sample of soil weighing 1g was dissolved in sterile distilled water in a 250 ml conical flask.[8] A serial dilution was performed up to $10^5$ for E. coli all dilutions were cultured into MacConkey agar (oxoid CM007) and incubated (Biobase) China at 37°C for 24 hours using microbiological standards of culturing. After this duration pink colonies of target organisms were sub cultured in Tryptone Bile Glucuronide (TBX) CM 0964 (Techno Pharmchem, India) to detect E. coli. Confirmation of E. coli was done on indole test.

Drain swabs

In the laboratory, drain swabs samples were cultured in MacConkey agar (Oxoid CM007) to isolate E. coli. The media was incubated at 37°C for 24 hours. After 24 hours' growth pink colonies of E. coli in MacConkey agar (Oxoid CM007) were sub cultured in TBX to detect E. coli. Growth of green colonies was an indicator for the presence of E. coli.

### Water analysis using most probable number method

The most probable number method was used to analyze water samples using MacConkey broth (Oxoid CM0505CM 0505) UK. Each bottle with sample was appropriately labelled. The water was mixed thoroughly by inverting the bottle several times. The cap of the bottle was removed and the mouth of the bottle flamed. The water samples were then inoculated by arranging the bottles in an incubator independently inside the incubator grill.[9] After inoculation the bottles were incubated at 44°C for 24 hours and the samples were examined by observing the color change and gas formation. Table 1 shows water analysis set for untreated and treated water samples.

### Microbiological identification of E. coli from the samples

In the laboratory, samples were cultured in MacConkey agar to isolate E. coli. The cultures were incubated at 37°C for 24 hours. After 24 hours' growth of target organisms in MacConkey agar were sub cultured in TBX to detect E. coli. Growth of green colonies was an indicator for the presence of E. coli.

### Biochemical identification of E. coli using Tryptone water

Isolates were subjected to biochemical identification using the indole test for E. coli. The test organism was inoculated in a bijou bottle containing 3 ml Tryptone water (Oxoid CM0087) UK and incubated at 37°C for 48 hours. The test for indole was done by adding one drop of Kovac's reagent (Himedia) and the formation of a red ring within 10 minutes indicated presence of E. coli.

### Gram staining technique

Gram staining is a technique used in differentiation of gram-positive and gram-negative bacteria. The technique is important since it helps in selecting the correct antibiotic during microbial sensitivity. A smear was prepared from the colonies to confirm if it was gram-negative rods using gram staining procedure. The procedure required gram stain reagents, slides, oil immersion and a microscope.

Colonies of the test organism were emulsified on a glass slide using a wire loop. A drop of normal saline was added to make a smear. The slide was allowed to air dry. The slides were passed through a flame to fix them. The smear was covered with crystal violet solution for 30 seconds. Then the smear was rapidly washed off to remove the excess stain. The smear was covered with Gram's Iodine (Pro-Lab Diagnostics Netherland) for 30 seconds and washed off using tap water. The smear was decolorized by covering with acetone for a few seconds and washed off. The smear was then covered with a counter stain neutral red for 30 seconds. The smear was allowed to dry before being examined at power 100 on a microscope (Olympus CX22). Presence of gram-negative rods confirmed the organism.

### Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was done using Mueller-Hinton Agar. Before inoculation of the organism to the Mueller-Hinton (CMO337 Oxoid) UK, single colonies were used to make 0.5 ml McFarland standard.[10,11] Colony suspension was first prepared by picking single colonies and suspending them in a vial containing 1ml normal saline. The suspension was compared with McFarland's standard in order to obtain the required concentration of the isolate. Using a sterile cotton swab, the isolate was picked and spread evenly on Mueller-Hinton agar. Once all the Mueller-Hinton agar was completely inoculated by suspension, antimicrobial discs were inserted on the swabbed Mueller-Hinton agar.[12]

Table 1. Water analysis set for untreated and treated water samples.

| Sample type | No. of bottles | Ml of broth | Strength of broth |
|---|---|---|---|
| Untreated water | 1 | 50 | Double |
| | 5 | 10 | Double |
| | 5 | 5 | Single |
| Treated water | 1 | 50 | Double |
| | 5 | 10 | Double |

The discs were well spaced to prevent them from overlapping. They were pressed on the media to ensure complete contact. They were incubated for 24 hours at 37°C. After 24 hours of incubation, the susceptibility and resistance was determined by measuring the diameter of the zone of inhibition using Vernier Calipers. The measurement was recorded in millimeters. This enabled classification of isolates of E. coli as either sensitive, intermediate, or resistant.[11,13] ATCC 25922 E. coli was used as a control organism during the study. The microbial agents used were as follows in their corresponding concentrations; imepenem (10 µg); ceftazidime (30 µg); cefotaxime (30 µg); cefoxitin (30 µg) and ciprofloxacin (5 µg).

### Data analysis

Laboratory data was entered into Microsoft Excel v 2010 and analyzed using SPSS version 26.0. Descriptive analysis was done using Microsoft Excel. The analysis included, frequencies and graphs. Data was compared between each study strata and between each sample type using Kruskal-Wallis tests, between the two drugs using the Wilcoxon Signed Rank test and findings were presented as figures tables and graphs. A p-value of 0.05 or less was considered significant between comparison in susceptibility and resistance between the strata A and B.

## Results

### Antimicrobial susceptibility from both strata

A total of 42 isolates were tested for antimicrobial susceptibility in both strata. Out of these antimicrobials, 5 (50.48%) were susceptible, 5 (22.86%) were intermediate and 5 (26.67%) were resistant. Out of the five antimicrobial agents used, ceftazidime 28 (66.67%) showed the highest sensitivity followed by ciprofloxacin 26 (61.90%) and imepenem 25 (59.52%) respectively. cefotaxime and cefoxitin showed least sensitivity at 14 (33.33%) and 13 (30.95%) respectively. In intermediate imepenem and ciprofloxacin were the highest with 12 (28.57%) followed by cefotaxime 10 (23.81%). The least intermediate was observed in ceftazidime and cefoxitin both at 7 (16.67%). The highest resistance was observed in cefoxitin 22 (52.38%), followed by cefotaxime at 18 (42.86%). ciprofloxacin, imepenem and ceftazidime had the lowest resistance 4 (9.52%), 5 (11.91%) and 7 (16.67%) respectively. Figure 2 shows a bar plot of the percentages of susceptibility patterns of different drugs under classes of response to E. coli.

### Antimicrobial susceptibility from drain swabs

A total of 16 isolates from drain swabs were tested for antimicrobial susceptibility. Out of these antimicrobials, 5 (61.25%) were susceptible to the microbial, 5 (23.75%) were intermediate and 5 (15%) were resistant. The highest resistance was shown to be cefotaxime 6 (33.34%), followed by cefoxitin at 4 (23.34%), while imipenem and ceftazidime were the least resistant at 2 (10%) respectively. ciprofloxacin was 0% resistant to the isolates tested. Table 2 shows antimicrobial susceptibility patterns from drain swabs in both strata. Out of the five drugs ceftazidime showed the highest susceptibility at 13 (85%) while imipenem followed at 13 (81.67%) respectively. ciprofloxacin was the highest in intermediate at 8 (46.67%) while imipenem and cefoxitin were the least intermediate at 2 (13.34%). Figure 3 shows antimicrobial susceptibility pattern from drain swabs.

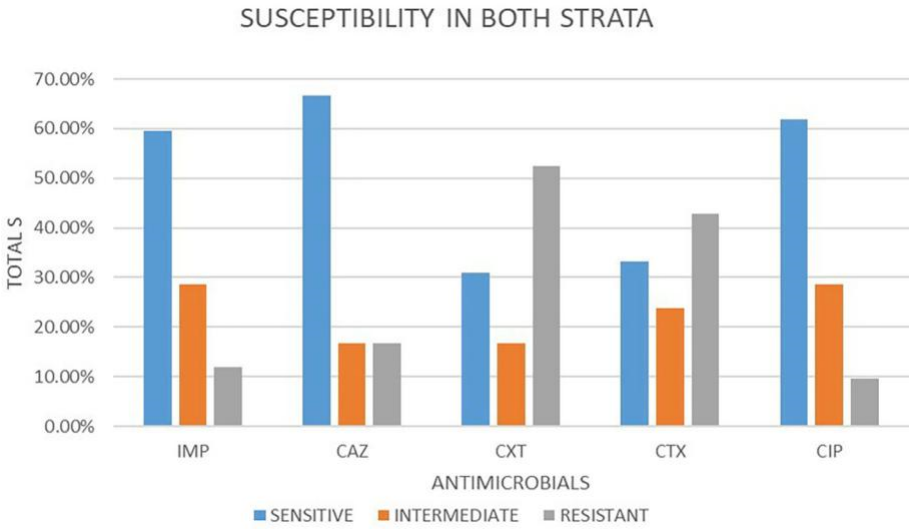

Figure 2. Shows a bar plot of the percentages of susceptibility patterns of different drugs under classes of response to E. coli in both strata. IMP-imipenem; CAZ-ceftazidime; CXT-cefoxitin; CTX-cefotaxime; CIP-ciprofloxacin.

Table 2. Antimicrobial susceptibility frequencies from drain swabs in both strata.

| Antimicrobial | Sensitive | Intermediate | Resistant |
|---|---|---|---|
| Imipenem | 13(81.67%) | 2(13.34%) | 1(10%) |
| Ceftazidime | 13(85%) | 2(20%) | 1(10%) |
| Cefoxitin | 10(63.34%) | 2(13.34%) | 4(23.34%) |
| Cefotaxime | 5(35%) | 5(31.67%) | 6(33.34%) |
| Ciprofloxacin | 8(53.33%) | 8(46.67%) | 0(0.00%) |

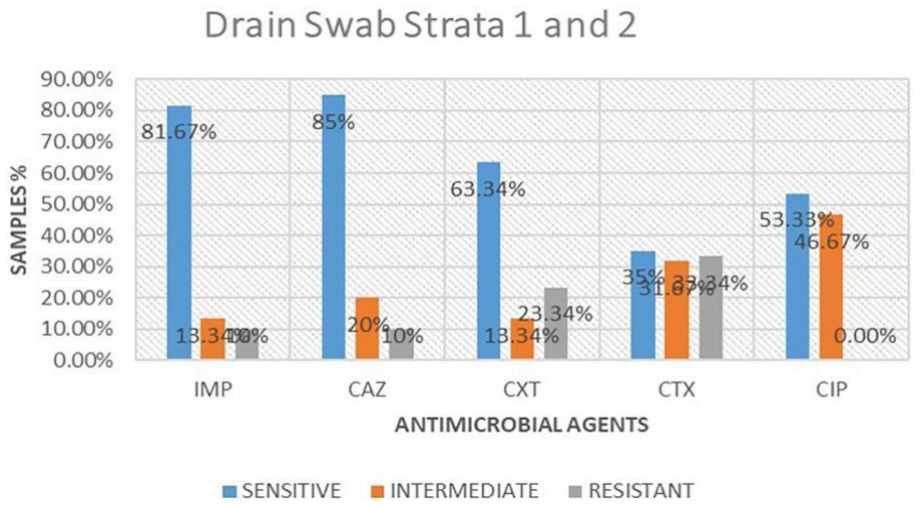


Figure 3. Shows antimicrobial susceptibility pattern from drain swabs in both the strata. IMP-imipenem; CAZ-ceftazidime; CXT-cefoxitin; CTX-cefotaxime; CIP-ciprofloxacin.

## Antimicrobial susceptibility from soil isolates from both strata

A total of 23 samples of soil were tested for antimicrobial sensitivity using diffusion disc method of the five antimicrobials of which imepenem resistant were 4 (17.39%), 8 (34.78%) intermediate while 11 (48.83%) susceptible. Out of the five antimicrobials used in this study in ceftazidime resistant were 6 (26.09%), intermediate 5 (21.74%), susceptibility 12 (52.17%), cefoxitin resistant were 15 (65.22%), intermediate 5 (21.71%), susceptible 3 (13.04%), cefotaxime resistant were 9 (9.13%), intermediate 5 (21.74%), susceptible 9 (39.13%) and ciprofloxacin resistant were 4 (17.39%), intermediate 4 (17.39%), susceptible 15 (65. 22%). Table 3 shows E. coli antimicrobials susceptibility drug patterns from soil samples in both strata. In both strata ciprofloxacin showed the highest susceptibility at 65.22%, followed by ceftazidime at 52.17%, while cefoxitin showed the least susceptibility. Imepenem showed the highest intermediate of all the drugs. Cefoxitin showed the highest resistance. Figure 4 shows the comparison in susceptibility patterns of the samples.

In the first strata, imepenem at 9 (77.78%) showed the highest susceptibility, followed by ciprofloxacin at 6 (66.67%) and cefoxitin was the least sensitive at 22.22%. Ceftazidime showed the highest intermediate at 33.33% while cefoxitin and cefotaxime were least intermediate. Cefoxitin showed the highest resistance while imepenem showed no resistant. In strata two ciprofloxacin 64.29% showed the highest susceptibility, followed by ceftazidime 50%. Cefoxitin showed highest resistance at 64.29%, followed by cefotaxime at 42.86%. Imepenem showed the highest intermediate of all the drugs.

## Antimicrobial susceptibility for water samples

Out of the three water samples analyzed it was observed that, ceftazidime and Ciprofloxacin showed 100% sensitivity while imipenem showed the least sensitivity Figure 5 shows the comparisons in % between the drugs. The highest resistance was observed in both cefoxitin and cefotaxime at 100%. Imipenem was the most intermediate at 66.67%. Table 4 shows E. coli antimicrobials susceptibility drug patterns from water samples from strata 1 and 2.

Table 3. E. coli antimicrobials susceptibility drug patterns from soil samples in both strata.

| | Sensitive | Intermediate | Resistant | Total | Sensitive | Intermediate | Resistant |
|---|---|---|---|---|---|---|---|
| Strata 1 | | | | | | | |
| Imipenem | 7 | 2 | 0 | 9 | 77.78% | 22.22% | 0.00% |
| Ceftazidime | 5 | 3 | 1 | 9 | 55.56% | 33.33% | 11.11% |
| Ceroxitin | 2 | 1 | 6 | 9 | 22.22% | 11.11% | 66.67% |
| Cefotaxime | 5 | 1 | 3 | 9 | 55.56% | 11.11% | 33.33% |
| Ciprofloxacin | 6 | 2 | 1 | 9 | 66.67% | 22.22% | 11.11% |
| Strata 2 | | | | | | | |
| Imipenem | 4 | 6 | 4 | 14 | 28.57% | 42.86% | 28.57% |
| Ceftazidime | 7 | 2 | 5 | 14 | 50.00% | 14.29% | 35.71% |
| Ceroxitin | 1 | 4 | 9 | 14 | 7.14% | 28.57% | 64.29% |
| Cefotaxime | 4 | 4 | 6 | 14 | 28.57% | 28.57% | 42.86% |
| Ciprofloxacin | 9 | 2 | 3 | 14 | 64.29% | 14.29% | 21.43% |

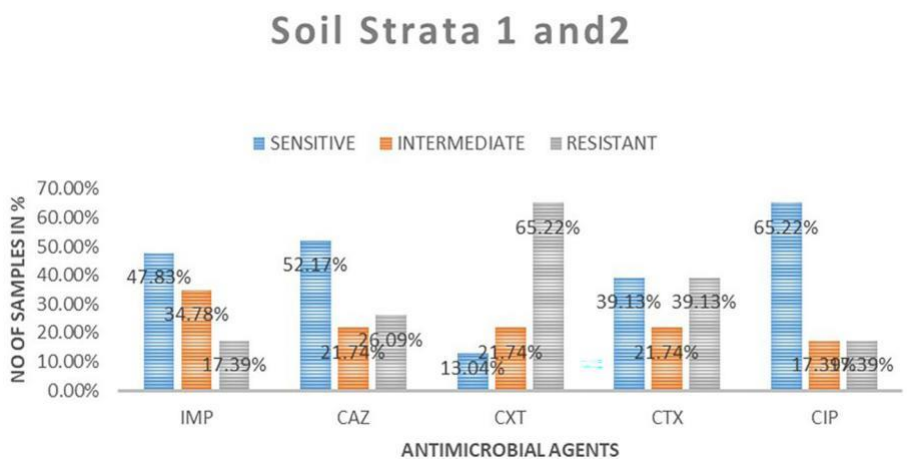


Figure 4. Shows the comparison in susceptibility patterns of the soil samples in both strata. IMP-imipenem; CAZ-ceftazidime; CXT-cefoxitin; CTX-cefotaxime; CIP-ciprofloxacin.

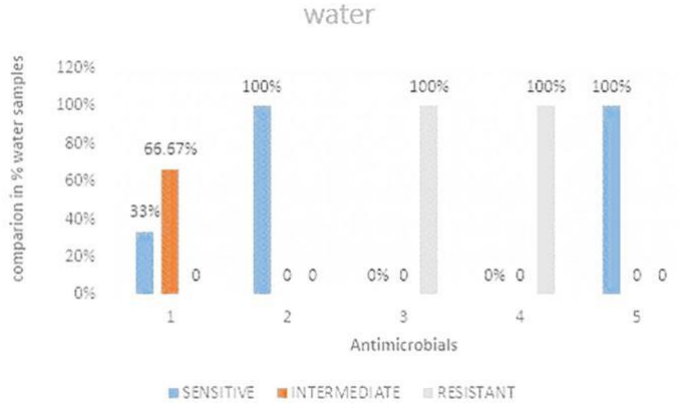

Figure 5. Shows the comparisons in percentages between the drugs for water samples. IMP-imipenem; CAZ-ceftazidime; CXT-cefoxitin; CTX-cefotaxime; CIP-ciprofloxacin.

Table 4. E. coli antimicrobials susceptibility of water samples.

| Water | Sensitive | Intermediate | Resistant | Total | Sensitive | Intermediate | Resistant |
|---|---|---|---|---|---|---|---|
| Imipenem | 1 | 2 | 0 | 3 | 33% | 66.67% | 0% |
| Ceftazidime | 3 | 0 | 0 | 3 | 100% | 0% | 0% |
| Cefoxitin | 0 | 0 | 3 | 3 | 0% | 0% | 100% |
| Cefotaxime | 0 | 0 | 3 | 3 | 0% | 0% | 100% |
| Ciprofloxacin | 3 | 0 | 0 | 3 | 100% | 0% | 0 |

Table 5. p-values between compared drugs.

| p-value | Ceftazidime | Cefoxitin | Cefotaxime | Ciprofloxacin |
|---|---|---|---|---|
| Imipenem | 0.1088 | 0.0156 | 0.0156 | 0.7150 |
| Ceftazidime | | 0.0156 | 0.0156 | 0.0679 |
| Cefoxitin | | | 0.2249 | 0.0156 |
| Cefotaxime | | | | 0.0156 |

Table 6. Means and standard errors.

| Drug | Mean | SE | |
|---|---|---|---|
| Imipenem | 23.809524 | 5.460448 | [a] |
| Ceftazidime | 19.440476 | 9.395252 | [a] |
| Cefoxitin | 9.333333 | 9.030211 | [b] |
| Cefotaxime | 23.452381 | 4.695007 | [b] |
| Ciprofloxacin | 21.547619 | 7.012888 | [a] |

Means within a column followed by the same letter are not statistically different at p>0.05 as to resistance.

### Statistical comparison of resistance of the drugs using Kruskal-Wallis test and Wilcoxon Signed-Rank test

The resistance from this study was statistically significant since the p value=0.0005275354994284686 and the statistic= 19.87938408896494. The p-value is less than 0.05, and so we reject the null hypothesis. This means that the resistance is not the same across the different drugs. To know which drug is more resistant, the Wilcoxon Signed-Rank test was used. This study found that cefoxitin and cefotaxime are statistically significant with a p-value 0.0156 which means they have highest resistance among the rest of the drugs. Table 5 shows p-values between compared drugs.

Imipenem, ceftazidime and ciprofloxacin are not significant to this study since the p value is greater than p>0.05. This p-value correlates to what was found in a study conducted by Ref. 14 who recorded a p-value of 0.36 towards E. coli.

Statistical analysis summary using plot box. Figure 6 shows a box plot showing summary of descriptive statistics of the means and standard errors of the antimicrobials.

### Statistical comparison of sensitivity of the drugs using Kruskal-Wallis test and Wilcoxon Signed-Rank test

Sensitivity from this study was significant to the study since the p value=0.01171770477419787 between the strata. This means that the sensitivity was not the same across the different drugs. To know which drug is more sensitive, the Wilcoxon Signed-Rank test was performed. It was observed that, ciprofloxacin, ceftazidime and imipenem were the most sensitive drug with a p-value>0.05.

Table 7 shows the p-values of the antimicrobials in the study.

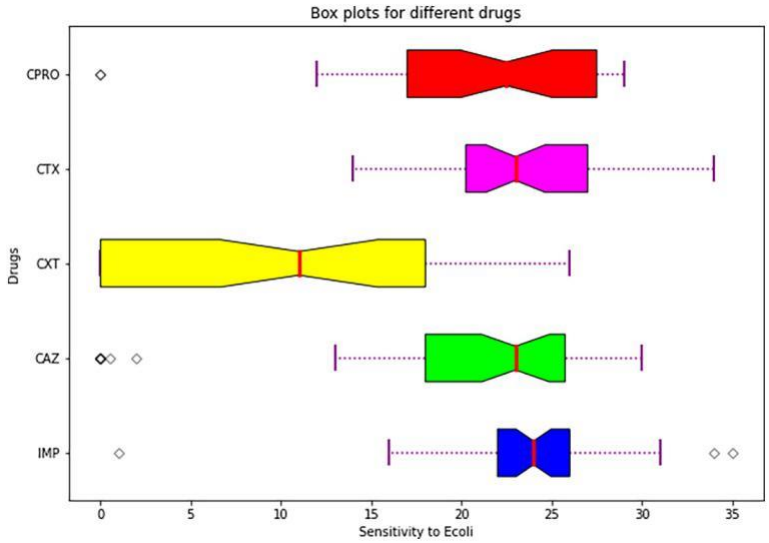


Figure 6. Shows a box plot showing a summary of descriptive statistics of the means and standard errors of the individual drugs used.

Table 7. p-values of the antimicrobials.

| p-value | Ceftazidime | Cefoxitin | Cefotaxime | Ciprofloxcin |
|---|---|---|---|---|
| Imipenem | 0.4630 | 0.0156 | 0.0277 | 0.9375 |
| Ceftazidime | | 0.0156 | 0.0260 | 0.3454 |
| Cefoxitin | | | 0.9165 | 0.1158 |
| Cefotaxime | | | | 0.0156 |

## Discussion

### Antimicrobial resistance patterns of E. coli from the study area

This study reported a range of multidrug resistance to E. coli from all the samples analyzed. Therefore, there is high chance that E. coli isolated from this study area emerges from the surrounding poor unhygienic conditions and inadequate sanitation facilities from the residents. These findings agree with a study on contamination of street food in Burkina Faso that enteric organisms emerge from cross contamination.[15] A study conducted on antimicrobial profile in Juja and Kibera found cefotaxime, ceftazidime and ciprofloxacin recorded a greater than 30% of antimicrobial resistance[16] this result is much lower than the one reported in the current study that recorded 25% increase. A study conducted by Ref. 17 observed that this increase in resistance is due to use in antibiotics in the treatment of diseases associated with poor unhygienic conditions. This study found the highest resistance on soil where it was it was noted that cefoxitin 65.22% an indication that soil was contaminated in this area. These findings correlate with study on urban informal settlement on antimicrobial resistance on the environment. It was noted from this study that less attention is being given on this contamination and pose a great risk to antimicrobial resistance.[18] Another study noted that soil is a hotspot carrier of resistant genes.[19]

The current study recorded 34% towards cefotaxime, imepenem10% and zero resistance to Ciprofloxacin. In a similar study,[20] recorded 0% resistance to Ciprofloxacin and 1.68% to imipenem and 8.94% cefotaxime. This finding on cefotaxime and imepenem is much lower than the ones from the current study. However, both studies recorded 0% to ciprofloxacin. According to Ref. 21 multidrug resistance to E. coli was noted on cefotaxime at 79.7% resistance. A related study[22] recorded 93% resistance to E. coli. The current study recorded a lower figure of 42.86% resistance to cefotaxime.

The current study showed the least resistance towards ceftazidime (16.67%) which is much lower that the resistance obtained from Ref. 23 100%.

## Antimicrobial resistance from water samples

The current study showed 100% resistance to water samples analyzed during the study. This resistance is a likely indication that there is high contamination of wastewater that is in return used for home use by the residents. The current

study only focused on river water samples passing through the study area and not water samples from the source area. This calls for a study in order to determine if there is contamination. The findings from the current study was higher by 20% from a related study conducted by Ref. 24 on antidrug resistance on Indian rivers. A study done by Ref. 25 while investigating resistance from water found that ceftazidime and ciprofloxacin had almost the same resistance; 1.7% and 1.8% respectively. These findings are higher than the current study which recorded 0% resistance on the drugs. According to Ref. 26, E. coli in wastewater plants was 60% resistant towards ciprofloxacin, which was higher than the current study at 0% resistance.

## Conclusion

Antimicrobial resistance noted from this study will result in an increase of infections caused by treatment failure hence, high mortality rates. Hence call for future mitigation measures to curb the rising antimicrobial resistance in informal settlement.

## Recommendations

Proper sanitation and hygiene awareness practices should be provided through education to the residents of this area. In the future, molecular methods should be used to look in more detail at the resistance genotype of the isolate from the study.

## Author's contribution

JO: Developed the concept, wrote the project proposal, collected the research data, analyzed the data, and wrote the thesis.

KG: Corrected the concept, provided necessary guidance, and corrections at the proposal writing, data analysis, and thesis writing.

NM: Corrected the concept, provided necessary guidance, and corrections at the proposal writing, data analysis, and thesis writing

## Data availability

### Underlying data

Figshare: EVALUATION OF ANTIMICROBIAL SUSCEPTIBILITY OF ESCHERICHIA COLI ISOLATED FROM CONTAMINATED AREAS OF MAJENGO SLUM IN MERU COUNTY, KENYA. https://doi.org/10.6084/m9.figshare.20325345.v1.[27]

This project contains the following underlying data:

- my data.xlsx (The data from this study represents data using measures of central tendency and the findings in percentages and bar graphs and statistical analysis involved using Kruskal Wallis and Wilcoxon from SPSS software.)



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