# PREVALENCE AND ANTIBIOTIC RESISTANCE PATTERNS OF *PSEUDOMONAS AERUGINOSA*, *KLEBSIELLA PNEUMONIAE* AND *ESCHERICHIA COLI* AMONG HOSPITALIZED PATIENTS AT THIKA LEVEL 5 HOSPITAL

**CECILIA WANJIRU NDUNG'U**

**MASTER OF SCIENCE**

**(Medical Microbiology)**

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# Prevalence and Antibiotic Resistance Patterns of *Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Escherichia coli* among Hospitalized Patients at Thika Level 5 Hospital

**Cecilia Wanjiru Ndung'u**

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## DECLARATION

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

Signature …… Date …….

**Cecilia Wanjiru Ndung'u**

This thesis has been submitted for examination with our approval as University Supervisors

Signature …… Date …….

**Prof. Samuel Kariuki, PhD**
**KEMRI, Kenya**

Signature …… Date …….

**Prof. Anne W. T. Muigai, PhD,**

**JKUAT, Kenya**

## DEDICATION

I dedicate this work to my late mother, Emily Gathoni and my father Daniel Ndung'u, my husband Samuel, my children Julie, Jefferson and Justin for their inspiration and encouragement in the course of this work.

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## ABBREVIATIONS AND ACRONMYS

| | |
|---|---|
| **A/A** | Acid Butt and Acid Slant |
| **aac(3)** | The Gene Encoding AAC(3`) |
| **AAC(3)** | Aminoglycoside Acetyltransferases(3`) |
| **AAC(6`)-Ib** | Aminoglycoside Acetyltransferases(6`)-Ib |
| **aac(6`)-Ib** | The Gene Encoding AAC(6`)-Ib |
| **aac(6`)-Ib-cr** | The Gene Encoding AAC(6`)-Ib-cr |
| **AAC(6`)-Ib-cr** | Aminoglycoside Acetyltransferases (6`) -Ib-Ciprofloxacin Variant |
| **AACs** | Aminoglycoside Acetyltransferases |
| **AADs / ANTs** | Adenylyltransferases or Nucleotidyltransferases |
| **AIDS** | Acquired Immuno-Deficiency Syndrome |
| **AK** | Amikacin |
| **AMC** | Amoxycillin-Clavulanic Acid |
| **AMEs** | Aminoglycoside Modifying |
| **AMP** | Ampicillin |
| **AmpC** | Genes Encoding Extended Spectrum Beta Lactamases |
| **APHs** | Aminoglycoside Phosphoryltransferases |
| **ATCC** | American Type Culture Colony |
| **BSA** | Bovine Serum Albumin |

| | |
|---|---|
| **C** | Chloramphenicol |
| **cAMP** | Cyclic Adenosine Monophosphate |
| **CAZ** | Ceftazidime |
| **CDC** | Center of Disease Control and Prevention |
| **CF** | Cystic Fibrosis |
| **CIP** | Ciprofloxacin |
| **CLED** | Cysteine Lactose Electrolyte Deficiency |
| **CLSI** | Clinical Laboratory Standards Institute |
| **CN** | Gentamycin |
| **CTX** | Cefotaxime |
| **CTX-M** | Genes Encoding Beta Lactamases with Extended Spectra |
| **dATP** | Deoxy Adenine Triple Phosphate |
| **dCTP** | Deoxy Cytosine Triple Phosphate |
| **dGTP** | Deoxy Guanine Triple Phosphate |
| **DNA** | Deoxyribose Nucleic Acid |
| **dNTPs** | Deoxy Nucleotides |
| **dTTP** | Deoxy Thiamine Triple Phosphate |
| ***E.coli*** | *Escherichia coli* |
| **EDTA** | Ethylene Diamine Tetra Acetic Acid |

| | |
|---|---|
| **ERC** | Ethical Review Committee |
| **ESBLs** | Extended-Spectrum β-Lactamases |
| **HCl** | hydrochloric Acid |
| **ICU** | Intensive Care Unit |
| **IMP** | Imipenem |
| **K** | Kanamycin |
| **K/K** | Alkaline Slant and Alkaline Butt |
| **KCl** | Potassium Chloride |
| **KOH** | Potassium Hydroxide |
| **MBLs** | Metallo-Beta Lactamase Producers |
| **$MgCl_2$** | Magnesium Chloride |
| **MIO** | Motility-Indole-Ornithine Media |
| **MOH** | Medical Officer of Health |
| **MR-VP** | Methyl Red and Voges-Proskauer |
| **N, N, N', N'** | Tetra-Methyl-P-Phenylenediaminedihydrochloride |
| **NA** | Nalidixic Acid |
| **NCCLS** | National Committee for Clinical Laboratory Standards |
| **OFL** | Ofloxacin |
| **OXA** | Type Oxacillinases |

| | |
|---|---|
| **PCR** | Polymerase Chain Reaction |
| **PRL** | Piperacillin |
| **SCC** | Scientific Steering Committee |
| **SHV** | Genes encoding beta lactamases (some with extended spectra) |
| **SXT** | Sulfamethoxazole-Trimethoprim |
| **TEM** | Genes Encoding Beta Lactamases (Some with Extended Spectra) |
| **TET** | Tetracycline |
| **TL5H** | Thika Level 5 Hospital |
| **TOB** | Tobramycin |
| **TSI** | Triple Sugar Iron Agar |
| **TZP** | Piperacillin-Tazobactam |
| **UTI** | Urinary Tract Infection |
| **VCR** | Variable Cassette Region |
| **WHO** | World Health Organization |

## ABSTRACT

The rise of antibiotics resistance is a global public health challenge, particularly in the context of hospital-acquired infections. Pathogens such as *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Escherichia coli* play a significant role in nosocomial infections. This study assessed the carriage rates, antibiotic resistance patterns, and the presence of genes conferring resistance of these pathogens to β-lactams, aminoglycosides, and (fluoro) quinolones. Conducted at Thika Level 5 Hospital in Kiambu County, the cross-sectional study spanned five months. Pus swabs from surgical and burn wounds along with aseptically collected urine samples from catheterized inpatients were collected and analyzed. Isolates were identified through biochemical and serological tests following specimen culture. Antibiotic susceptibility testing was performed using the Kirby-Bauer disc diffusion method, with interpretation based on recommendations from the Clinical Laboratory Standards Institute. Genes encoding resistance to the three main classes of antibiotics were detected using Polymerase Chain Reaction. A total of 450 specimens were collected, yielding 42 *E. coli*, 41 *K. pneumoniae*, and 41 *P. aeruginosa* isolates. Importantly, 4.9% of *P. aeruginosa*, 7.1% of *E. coli*, and 14.6% of *K. pneumoniae* isolates demonstrated resistance to carbapenems. Additionally, resistance of isolates to sulfamethoxazole-trimethoprim was *E. coli* (78.6%), *K. pneumoniae* (46.3%), and *P. aeruginosa* (29.3%) isolates being classified as multidrug-resistant. Resistance genes were detected using PCR: aminoglycoside phosphotransferase (*aph (3')III*) ββat 17% in *E. coli* and 7% each for *P. aeruginosa* and *K. pneumoniae.* Prevalence of $bla_{\text{TEM}}$ for *E. coli*, *P. aeruginosa*, and *K. pneumoniae* was 10 %, 20 % and 24% respectively. Integrons in *P. aeruginosa* and *K. pneumoniae* were each at 20%, and at 33% for *E. coli*. In conclusion, the study highlights significant resistance to commonly used antibiotics including last- resort options like carbapenems. Routine antibiotic susceptibility testing and surveillance are crucial for managing wound and urinary tract infections, especially due to MDR.

# CHAPTER ONE

# INTRODUCTION

## 1.1 Background

Antibiotic resistance poses a significant global challenge, impacting the effectiveness and cost of treatment for infectious diseases (Amin, 2019). Antibiotics resistance increases morbidity and mortality by hampering the provision of effective treatment, and makes treatment more costly (Majumder *et al*., 2020). This issue is particularly critical in developing countries, where alternative treatment options may be limited or unaffordable for the majority of the population (Godman *et al*., 2021).

Hospital-acquired infections (HAIs) significantly contribute to the problem of antibiotic resistance, resulting in increased healthcare costs, morbidity, and mortality (Vivas *et al*., 2019). The distribution of pathogens causing HAIs, especially those resistant to antibiotics, varies over time and across different hospitals and locations within hospitals (Nekkab *et al.,* 2017). Several factors contribute to the development of antibiotic resistance in HAI-causing pathogens, including the widespread use of antibiotics in hospitals, the growing number of immune compromised patients, and the increased use of medical devices (Gunardi *et al*., 2021).

*Pseudomonas aeruginosa (P. aeruginosa*), *Klebsiella pneumoniae* (*K. pneumoniae*) and *Escherichia coli (E. coli)* are prominent Gram-negative bacteria implicated in nosocomial infections worldwide (Iseppi *et al*. , 2020).

*P. aeruginosa* is an obligate aerobe, non-fermenting and saprophytic bacteria (Shrestha *et al*., 2019). Patients typically acquire *P. aeruginosa* infections through direct or indirect contact with contaminated surfaces and environment (Amin, 2019). *P. aeruginosa* is an opportunistic pathogens with broad-spectrum antibiotic resistance that has emerged extensively in hospital environments, causing serious infections in immuno-compromised hosts (Breijyeh, Jubeh and Karaman, 2020). *P.aeruginosa* is known to cause sepsis, pneumonia, meningitis and urological infections (CDC, 2018). This species has been reported to have intrinsic resistance to

several antibiotics due to the presence of lipo-polysaccharides in the outer membrane. However, persistent administration of antibiotics has resulted in the emergence of induced multi-drug resistance among strains of *P.aeruginosa* (Lundstedt *et al*., 2020). This combination of resistance factors reduces the number of therapeutic options leading to significant morbidity and mortality (Liu *et al.*, 2018).

*K. pneumoniae* are enteric Gram-negative rods, often capsulated and capable of both aerobic and anaerobic respiration (Dadgostar, 2019). The gut of human and animals is usually the normal habitat of this species, but *Klebsiella* strains are also found in moist inanimate environments, especially water and soil (Moran *et al*., 2019). Like *P. aeruginosa, K. pneumoniae* is mainly an opportunistic pathogen causing hospital-acquired infections and infections in debilitated or immuno-compromised patients (Martin and Bachman 2018). It accounts for up to 10% of all nosocomial bacterial infections (Paczosa and Mecsas, 2016). Usually, these infections are treated with beta-lactam antibiotics. Resistance to these antibiotics is mainly mediated by hydrolytic activity of β-lactamases (Tamma *et al*., 2020).

*E. coli* are facultative anaerobe Gram-negative rods that are motile with some strains possessing capsules while others are non-capsulated (Shnawa, 2021). *E. coli* is of clinical importance due to its cosmopolitan nature and ability to initiate, establish and cause various kinds of infections (Biggel, 2020). It is normally found in the gut of both humans and animals. However, it also colonizes the lower end of urethra and vagina. *E. coli* causes infections including neonatal meningitis, urinary tract infections, diarrhea and septicaemia (Abushaheen *et al*., 2020). *E. coli* is able to survive in abiotic environments and can be found in water, soil and vegetation (Samaddar *et al*., 2021). It is the leading pathogen causing urinary tract infections (Kudinha, 2017). *E. coli* is also among the most common pathogens that cause blood stream infections (Dat *et al.,* 2017), wounds, otitis media and other complications in humans. Resistance to antibiotics in *E. coli* has been reported worldwide (Vila *et al*,. 2016). There is increasing rates of resistance among *E. coli* which is a growing concern in both developed and developing countries (Singh *et al*., 2018). As such routine monitoring of antibiotic resistance provides data for antibiotic therapy and

resistance controls, prescription programs, making policy decisions and assessing the effectiveness, (Malekzadegan *et al*., 2018). It is important to understand the nature of antibiotic resistance among strains encountered among hospitalized patients due to potential exposure to high doses of antibiotics in such settings.

Limited data exist on the resistance profiles of *E. coli, Klebsiella* and *Pseudomonas* strains from hospitalized patients and those from various specimens obtained from patients seeking treatment in various hospitals in Kenya. In this study, isolates obtained from urine, wound and burns specimens from patients at Thika Level 5 hospital which is located in a cosmopolitan setting were investigated. Strains belonging to these three bacterial species were tested for susceptibility to various antibiotics and further screened for various genes conferring resistance to selected important classes of commonly used antibiotics including aminoglycosides, fluoroquinolones, and β-lactam antibiotics (Galani *et al*., 2021).

### 1.2 Problem Statement

*Pseudomonas aeruginosa, Klebsiella pneumoniae* and *E. coli* strains have been implicated in major hospital outbreaks and infections. Due to their ability to act both as harmless commensals and virulent pathogens, these species may pose a great danger to the community, especially to individuals with predisposing conditions (Fodor *et al*., 2020). These bacterial strains can also colonize inert surfaces making the environment serve as a potential source of such strains, especially for the health-care givers and hospitalized patients (Exner *et al*., 2017). The emergence of MDR strains, especially *E. coli* and *Klebsiella pneumoniae* is due to pressure from heavy use of antibiotics in the hospital environment, thus pause a potential for hospitals to become a reservoir of antibiotics resistance facilitating the spread of resistant strains to the community (Anyanwu *et al*., 2020). It is critical to determine if strains from different specimen types and those from patients in different wards carry similar or different resistance profiles, especially to three critical classes of antibiotics:-β-lactams, aminoglycosides and (fluoro) quinolones. These antibiotics are commonly used as single agents or in combination in the management of infections caused by multi-drug of these species (Jung, 2019).

## 1.3 Justification

Several studies on *Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Escherichia coli* isolated in large tertiary hospitals in Kenya have shown a high prevalence of multi-drug resistance. However, there is limited data on the resistant prevalence to other antibiotics among similar strains obtained from county hospitals such as Thika Level 5 hospital, which serves a significantly large cosmopolitan population. In these settings, Infections in wound and in catheterized patients are common, but causative agents are rarely isolated through culture. This proposed study has not been done to date. Also, little work has been done to determine the mechanisms of resistance to the three important classes of antibiotics that is β-lactams, aminoglycosides and (fluoro) quinolones among similar isolates in Kenya. The data obtained from this study will shed light on the prevalence and mechanisms of resistance to these antibiotics and provide policy direction on treatment options for such infections. This data will also serve as a basis for future research studies aimed at expanding knowledge on the molecular and epidemiological aspects of dispersal of multi-drug resistance strains belonging to these species.

## 1.4 Research Questions

i. Are *Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Escherichia coli* important pathogens affecting hospitalized patients at Thika hospital, especially those with bed sores, indwelling catheters and wounds? These conditions are known to predispose patients to colonization with these species
ii. What are the resistance profiles for *Pseudomonas aeruginosa, Klebsiella pneumoniae,* and *Escherichia coli* obtained from such patients?
iii. Which genes are responsible for encoding resistance to important classes of β-lactams, aminoglycosides, and (fluoro) quinolones antibiotics?

### 1.4.1 Hypothesis

**$H_{01}$:** *Pseudomonas aeruginosa, Klebsiella pneumoniae,* and *Escherichia coli* are not common in specimens such as wounds and urine obtained from catheterized patients hospitalized at Thika Level 5 Hospital (TL5H).

**$H_{02}$:** *Pseudomonas aeruginosa*, *Klebsiella pneumoniae,* and *Escherichia coli* strains from specimens obtained from hospitalized patients at TL5H do not exhibit resistance to major classes of antibiotics, especially β-lactams, aminoglycosides, and (fluoro) quinolones.

**$H_{03}$:** *Pseudomonas aeruginosa*, *Klebsiella pneumoniae,* and *Escherichia coli* do not have genes responsible for encoding resistance to β-lactams, aminoglycosides, and (fluoro) quinolones antibiotics.

## 1.5 Objectives

### 1.5.1 Broad Objective

To determine the prevalence and resistance profiles of *Pseudomonas aeruginosa, Klebsiella pneumonia,* to selected antibiotics, including β-lactams, aminoglycosides, and (fluoro) quinolones, isolated from wounds and urine of catheterized patients hospitalized at Thika Level 5 Hospital.

### 1.5.2. Specific Objectives

i. To determine prevalence of carriage of *Pseudomonas aeruginosa*, *Klebsiella pneumoniae,* and *Escherichia coli* in urine from catheterized patients and in wounds samples from patients hospitalized at Thika Level 5 Hospital.
ii. To determine antibiotics resistance patterns of *Pseudomonas aeruginosa*, *Klebsiella pneumoniae,* and *Escherichia coli* obtained from various specimens.
iii. To identify the genes encoding resistance to three classes of commonly used antibiotic agents: β-lactams, aminoglycosides, and (fluoro) quinolones.

## 1.6 Significance of the Study

The research will make a significant contribution to existing knowledge and will provide data on resistance patterns in *Pseudomonas aeruginosa*, *Klebsiella pneumoniae,* and *Escherichia coli.* This data will be crucial for developing effective antibiotics stewardship programs and in informing clinical treatment guidelines. The

findings have potential to influence the public health policy and clinical practices, particularly in regions with high incidences of these infections.

# CHAPTER TWO

# LITERATURE REVIEW

## 2.1 Infections Associated with *Pseudomonas aeruginosa*

Infections caused by *Pseudomonas aeruginosa* (*P. aeruginosa*) include dermatitis, soft tissue infections, bacteremia in immune-compromised individuals such AIDS, neutropenia, hematologic malignancies (Chahal *et al*., 2021) and diabetes mellitus (Sivanmaliappan and Sevanan, 2011). It also causes bone and joint infections (Ribera *et al.*, 2015), gastrointestinal infections (Markou and Apidianakis, 2014), endocarditis in intravenous drug users and meningitis when introduced by lumber puncture (Parr *et al.*, 2017).

*P. aeruginosa* is a leading cause of nosocomial infections among critically ill patients in intensive care unit (Esfahani *et al.,* 2017). It is responsible for respiratory tract infections due to contaminated respirators (Pachori *et al*., 2019), urinary tract infection (Ferreiro *et al*.,2017) and is associated with catheterization, surgery or instrumentation (Medina-Polo *et al*., 2021). It constitutes 12% of all UTIs (Ferreiro *et al.,* 2017) and can invade the bloodstream from the urinary tract, accounting for 40% of *Pseudomonas*-bacteremia infections (Duplessis *et al*., 2018).

*P.aeruginosa* causes infections in burns and wounds giving rise to blue green pus (Spernovasilis *et al*., 2021). It is also responsible for ear infections including external otitis in diabetic patients and in swimmers (Norbury *et al*., 2016), and severe eye infections especially after injury or surgical procedure which can lead to loss of the entire eye (Teweldemedhin *et al*., 2017). *Pseudomonas aeruginosa* contribute to 25% of all hospital acquired gram-negative bacteremia infections and its mortality rate remains over 10% (Peters *et al*., 2019).

*Pseudomonas aeruginosa*, the most important species in genus *Pseudomonas* is an obligate aerobe, non-fermenting, saprophytic, motile gram-negative bacilli and is widespread in nature, particularly in moist environments (Fujitani *et al*., 2017). It occurs as single bacterial cells, in pairs, and sometimes as short chains. It is an

opportunistic pathogen both in human and plants (Malhotra *et al*., 2019). It secretes pigments including pyocyanin, pyoverdin and pyorubin and often exhibits fluorescence (Czerwonka *et al*., 2019).

The disease process associated with *P.aeruginosa* infection begins with alterations of normal host defenses or compromise on tissue integrity due to trauma including disruption of mucous membranes and skin by direct tissue damage, or the use of intravenous or urinary catheters (Petrocheilou *et al*., 2017). Individuals with poor immune responses especially those with neutropenia associated with cancer therapy are susceptible to infection. (Bhat *et al*., 2021). The main stages of infections are; bacterial attachment to and colonization of the mucous membrane or skin, local invasion and disseminated systemic disease (Khatoon *etal*., 2018). Most *Pseudomonas* infections are invasive and toxigenic (Yan *et al*., 2020).The pathogenesis of disease is enhanced by pili, enzymes including proteases, elastases and toxins such as exotoxin A, hemolysin (heat-labile phospholipase C and heat-stable glycolipid) that increases virulence of *P. aeruginosa* (Rocha *et al*., 2019).

### 2.2 Infections Associated with *E. coli*

*E. coli* is a gram negative, facultative anaerobic and non-sporulating bacterium that is commonly found in the lower intestine of warm-blooded animals, and implicated in a number of infections in humans (Heredia and García, 2018) and (Ebbensgaard *et al*., 2018). The most common infections are gastrointestinal and urinary tract infections (Guglietta, 2017). Majority of *E. coli* strains are harmless, forming part of the normal flora of the gut and even benefit their hosts by producing vitamin K2 (Frame *et al*., 2020). They also benefit the host by preventing the establishment of pathogenic bacteria within the intestinal system (De la Fuente, 2021). However, virulent strains of *E. coli* can cause gastroenteritis, urinary tract infections, and neonatal meningitis (Guglietta, 2017). Virulent strains are also responsible for hemolytic-uremic syndrome (HUS), peritonitis, mastitis, septicemia and gram-negative pneumonia (Elbayoumi *et al*., 2018).

### 2.3 Infections Associated with *Klebsiella pneumoniae*

*Klebsiella pneumoniae* causes suppurative infections, bacteremia, and a significant proportion of nosocomial infections (Durdu *et al*., 2016). Most clinical isolates of *K. pneumoniae* possess a well-defined polysaccharide capsule which appears to be a critical virulence factor (Russo *et al*., 2018). Members of the *Klebsiella* genus usually express two types of antigens on their cell surface: a lipopolysaccharide referred to as O antigen and a capsular polysaccharide (K antigen). These antigens contribute to the pathogenicity of *Klebsiella pneumoniae.* Some capsular serotypes are isolated at significantly higher frequency than others (Choi *et al*., 2020)

### 2.4 Role of *P. aeruginosa, Klebsiella pneumoniae* and *E. coli* Strains as Causes of Nosocomial Infections

Strains belonging to these species cause infections among newly admitted patients and thus are important nosocomial pathogens (Liu *et al*., 2020). Nosocomial infections are hospital-acquired infections and that typically manifest three days after a patient is admitted to a hospital or other health care facility (Barranco *et al*., 2021). *E. coli* is the leading pathogen, implicated in 24% of all nosocomial urinary tract infections (UTIs) while *K.pneumoniae* is the fifth leading cause of nosocomial UTIs according to NNIS data from 1909 to 1996 (Yang *et al*., 2021). In the United States, about 5-10% of patients admitted to hospitals develop a nosocomial infection (CDC, 2018). These infections can be prevented by about 25% if healthcare workers take proper precautions when caring for patients (Sikora & Zahra, 2021). Hospital-acquired infections caused by these species may develop from surgical procedures, catheters placed in the urinary tract or blood vessels, or from material from the nose or mouth that is inhaled into the lungs or procedure used to diagnose or treat patient's illness or injury (Musila *et al*., 2021). The Center for Disease Control (CDC) estimates that over two million patients develop hospital-acquired infections in the United States each year and causes death in approximately 90,000 of these patients (Corrado *et al*., 2017). These species are some of the most significant causes of these deaths. Individuals at greater risk include young children, the elderly and immune-

compromised patients. Other risk factors include indwelling catheters, long hospital stays, overuse of antibiotics and invasive procedures (Djordjevic *et al.*, 2016).

The main risk factors for hospital-acquired urinary tract infections (UTIs) are host and device related, with the presence of urinary catheter being the most important factor, rather than pathogen related (Guglietta, 2017). This is because the catheter provides some opportunity for bacteria to enter the bladder along external or internal surfaces of the catheter, and for development of a biofilm that can protect bacteria from antibiotics and host defenses, thus facilitating adhesion to mucosal surfaces. The catheter may also impair adequate antibacterial polymorphonuclear leukocyte function. Another reason is if the catheter drainage is not optimal, residual urine volumes in the bladder may occur (Surgers *et al*., 2019).

Within the hospital, these strains find numerous reservoirs which include disinfectants, respiratory equipment, food, sinks, taps, toilets, showers and mops (Exner *et al*., 2017). They may also be constantly reintroduced into the hospital environment on materials and food from outside the hospital, by visitors and by patients transferred from other health facilities (Liao *et al*., 2019). Spread occurs from patient to patient through the hands of hospital personnel, by direct patient contact with contaminated reservoirs, and by the ingestion of contaminated foods and water (Barranco *et al*., 2021). Some of these strains are found on the skin of some healthy persons especially the health-care givers and have been isolated from the throat of people in the community (Narayanasamy *et al*., 2019). In a previous study, gastrointestinal carriage rates of *P. aeruginosa* in hospitalized patients increased from 4% to 20% within 72 hours of admission (Cohen *et al*., 2017).

### 2.5 Control of Spread of *Pseudomonas aeruginosa, Klebsiella pneumoniae* and *E. coli* in Hospital Settings

Studies show that these species cause nosocomial infections in various countries; both developed and developing. For instance, the prevalence of *P. aeruginosa* in sputum, urine, wounds, ear swabs, pus and aspirate, and catheter tip swabs obtained from hospital settings in different regions were, 30 % in Pakistan (Cohen *et al*., 2017), 20.3 % in India and 10.5 % in Zaria-Jamaica (ALshaiki & Toweir, 2017).

Since these strains thrive in moist environments, controlling their spread requires special attention to water baths, showers, sinks, hot tubs and all other wet areas that serve as reservoirs in a hospital setting (Salm *et al*., 2016). The spread of these strains, especially *P. aeruginosa,* can be best controlled by observing proper isolation procedures, aseptic techniques, and careful cleaning, sterilization and monitoring of respirators, catheters, and other instruments (Amoureux *et al.*, 2017). High risk procedures such as urinary catheterization should only be performed when necessary, and catheters should be implanted for the shortest possible period (Adesanya *et al*., 2020). Healthcare workers and visitors can reduce the spread of nosocomial strains to hospitalized patients by frequently washing their hands with antiseptics (Ataee *et al*., 2017). Since heavy use of antibiotics may positively select for multidrug strains of any of *Klebsiella, E. coli* or *Pseudomonas* strains, antibiotics should be used in a prudent manner, such as ceftazidime, carbapenems, tazobactam piperacillin, fluoroquinolones, or aminoglycosides (Özgenç,2016). Monitoring drug resistance can be achieved by implementing active surveillance of resistant strains and reporting unique resistance profiles to the hospital epidemiologist (Bertoia, 2016).

### 2.6 Treatment Options for *Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Escherichia coli* Infections

Common agents used for treatment of infections caused by *P.aeruginosa, K. pneumoniae and E. coli* strains include β-lactam antibiotics, aminoglycosides and (fluoro) quinolones (Kanj *et al*., 2022). In most cases, combined therapy comprising β-lactam antibiotics and a (fluoro) quinolone or an aminoglycoside is prescribed for serious infections resulting from strains belonging to any of these three genera. Cephalosporins alone (as a monotherapy) or as a β-lactam/ β-lactamase inhibitor such as amoxicillin-clavulanic acid combinations may be effective against *E. coli* infections but rarely effective against multi-drug resistant *Pseudomonas* or *Klebsiella* strains (Ferrer-Espada *et al*., 2020). Combination antibiotic therapy is used to increase the antibacterial spectrum and also to prevent development of resistance (Abushaheen *et al*., 2020). In clinically significant *P. aeruginosa* infections, a penicillin antibiotic such as ticarcillin, mezlocillin, and piperacillin is used in combination with an aminoglycoside especially gentamycin, tobramycin or amikacin

(Ai *et al*., 2022). Generally, *Pseudomonas* strains that exhibit resistance to multiple classes of antipseudomonal antibiotics are described as multi-drug resistant. Similarly, strains of *K. pneumoniae* that are resistant to multiple antibiotics, including the newer cephalosporins have emerged and the infections caused by them are frequently epidemic in nature and have complicated chemotherapy significantly (Shaikh *et al*., 2015). The choice of therapy depends on clinical factors and on the laboratory antibiotic susceptibility profile reports. Since the susceptibility profiles of a certain species may differ from one region to another, there is need for routine susceptibility profiling to inform treatment options (Abdollahi *et al*., 2016). It is important to note that some *Pseudomonas* strains exhibiting resistance to a significant set of antibiotics may remain susceptible to piperacillin, imipenem, ciprofloxacin, and tobramycin (Pang *et al*., 2019), highlighting the importance of antibiotic resistance surveillance.

Other drugs that could be used for serious infections include aztreonam, imipenem and newer quinolones such as ciprofloxacin, newer cephalosporins including ceftazidime and cefoperazone (Pachori *et al*., 2019). Although many strains are susceptible to gentamycin, tobramycin, colistin, and fluoroquinolones, resistant strains have developed, especially in *P. aeruginosa* (Halfon *et al*., 2019).

### 2.7 Antibiotic Resistance among *P. aeruginosa, K. pneumoniae* and *E. coli*

Antibiotic resistance is a global issue of growing concern and represents a significant public health problem worldwide. It affects both the developed and developing countries (Ayukekbong, Ntemgwa and Atabe, 2017). *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *E. coli* are crucial species known to cause severe infections, particularly in individuals with predisposing factors such as compromised immunity, compromised skin barrier, or in the very young and elderly populations (Liu *et al.*, 2020). These species have a remarkable ability to develop resistance to multiple antibiotics through similar or related mechanisms. Key resistance mechanisms observed in these strains include decreased intracellular drug concentration, drug inactivation, target modification, or target bypass (Munita & Arias, 2016).

*P. aeruginosa*, in particular, demonstrates a substantial capacity for developing or acquiring new antibiotic resistance mechanisms. This can be attributed to its large genome size and its presence in aquatic habitats, which serve as reservoirs for bacteria carrying other resistance genes (Munita & Arias, 2016). *P. aeruginosa* produces extracellular slime enabling it to escape host defenses and resist the antibiotic action (Carette *et al*., 2020).

Multi-drug resistant *P. aeruginosa, K. pneumoniae* and *E. coli* pose serious therapeutic challenges for the treatment of both community-acquired and hospital-acquired infections (Gandra *et al*., 2019). Selecting the appropriate antibiotic for treatment is crucial to optimize clinical outcomes. However, due to the ability of these organisms to rapidly develop resistance to multiple classes of antibacterial agents, even during the course of an infection, this task becomes challenging. Infections caused by drug-resistant *P. aeruginosa*, *K. pneumoniae,* and *E. coli* are associated with increased morbidity, mortality, the need for surgical intervention, prolonged hospital stays, and elevated overall treatment costs (Peters *et al*., 2019).

Monitoring and controlling the spread of multi-drug resistant nosocomial strains is essential, as these strains can quickly disseminate and present a challenge in managing epidemics with high mortality rates (Ramsamy *et al.* , 2018). Routine susceptibility testing is crucial for guiding individual patient treatment and for surveillance of antibiotic resistance (CDC, 2017). However, indiscriminate and inappropriate use of antibiotics in both community and hospital settings has contributed to increased antibiotic resistance leading to difficulties in disease treatment (Ezeuko *et al*., 2021). Limited antibiotics are available for treatment of multi-drug resistant *Pseudomonas, Klebsiella* and *E. coli* strains particularly in hospital settings (Apondi *et al*., 2016).These antibiotics include; β-lactam antibiotics, aminoglycosides and (fluoro)quinolones. However, the emergence of strains exhibiting resistance to combinations of these vital antibiotics poses serious challenges in infection management in many countries (Jaggi *et al*., 2019).

A recent study identified *P. aeruginosa* obtained from patients in intensive care units (ICUs) in a tertiary hospital in Kenya that produce metallo-β-lactamase (MBL)

enzymes, which confer resistance to carbapenems (Mukaya *et al*., 2018), the most potent β-lactam antibiotics currently used in clinical practice. Additionally, the relatively new MBL, NDM-1first described in India and Pakistan in 2008 has also been reported among *K. pneumoniae* isolates obtained from patients in Kenya (van Duin & Doi, 2017). Strains from other African countries have also been reported to be highly multidrug resistant, but a combination of resistances observed in the *Klebsiella* strains obtained in Kenya is rarely reported in Africa (Maina *et al*., 2019).

### 2.8 Resistance to β-lactam Antibiotics among *Pseudomonas aeruginosa, Klebsiella pneumonia* and *E.coli* Strains

Beta-lactam (β-lactam) antibiotics are an important arsenal of antibiotics used to treat infections arising from infections by members of *Pseudomonas aeruginosa, Klebsiella pneumoniae* and *E. coli* (Zango *et al*., 2019). All β-lactam antibiotics contain a core of a β-lactam ring. The β-lactam ring is a cyclic amide with a four-membered hetero-atomic structure made up of three carbon atoms and one nitrogen atom (Hosseyni & Jarrahpour, 2018). This is the basic ring structure found in the primary group of these antibiotics known as penicillins. This is the basic ring structure found in the primary group of these antibiotics known as penicillins. Depending with the complexity of other rings or chemical structures that may be added to this core ring, β-lactam antibiotics can be grouped broadly into penicillins, cephalosporins, monobactams, β-lactamase inhibitors, cephamycins, and carbapenems (Lima *et al*., 2020). Resistance to β-lactam antibiotics among these species may be mediated by efflux mechanisms and porin deficiency in which case, the antibiotic does not gain access to the cell or the cell wall of the bacteria (Zango *et al*., 2019). However, in most cases, this resistance is mediated by production of hydrolytic enzymes known as β-lactamases. These enzymes, such as TEM- or SHV-, β-lactamases can have a narrow range of substrates such as simple penicillins while extended-spectrum β-lactamases (ESBLs) hydrolyze advanced classes of antibiotics such as cephalosporins and cephamycins 7-alpha-methoxy-cephalosporins) such as cefoxitin (Sawa *et al*., 2020). AmpC-β-lactamases such as CMY-1 and CMY -2 are clinically important because they confer resistance to narrow-, expanded-, and broad-spectrum cephalosporins, β-lactam-/β-lactamase inhibitor combinations (such as

amoxicillin-clavulanic acid and piperacillin-tazobactam), cephamycins and aztreonam. Plasmid-mediated AmpC β-lactamases represent a new threat since they confer resistance to these antibiotics and because they have a huge potential for horizontal transmission to susceptible strains (Bajaj, Singh, & Virdi, 2016). These bacteria can also resist cephamycins and even carbapenems which are currently the most potent β-lactam due to loss of outer membrane porins (Sugawara *et al*., 2016).

Hydrolytic enzyme-based resistance to carbapenems such as meropenem remains the greatest challenge in clinical settings (Lisa *et al*., 2017). Resistance to these agents may arise due to decreased outer membrane permeability, increased expression of efflux pumps, alteration of penicillin binding proteins and due to production of carbapenem-hydrolyzing enzymes (Santajit & Indrawattana, 2016).The most important carbapenemases include the metallo-β-lactamases (MBLs). MBLs require divalent cations as cofactors for enzyme activity. Metal ion chelators such as EDTA therefore inhibit these enzymes. MBLs efficiently hydrolyze all β-lactam antibiotics, except aztreonam (Yu *et al*., 2019). The most important MBLs include the VIM and IMP families ,SPM, GIM-1 SIM-1 , AIM-1 KHM-1, and NDM-1 (Bonomo., 2017). The prevalence of metallo-betalactamase producing isolates of carbapenem resistant *P. aeruginosa* has been reported worldwide (Acharya *et al*., 2017). In Turkey, the prevalence is 10%, and it can reach up to 36% in Southeast Asia (Hong *et al*., 2015). Studies done at Aga Khan University Hospital Kenya had all of the carbapenem resistant *P. aeruginosa* isolates being metallo-beta lactamase (MBL) producers (Maina *et al*., 2017).

Among *Enterobacteriaceae* (such as *E. coli* and *Klebsiella pneumoniae*), resistance to β-lactam antibiotics is mainly conferred by bla$_{TEM}$, bla$_{SHV}$ and bla$_{CTX-M}$ (Cornista *et al*., 2019). However, production of chromosome-encoded β-lactamases renders most *Pseudomonas* strains naturally resistant to ampicillin and cephalothin (ur Rahman *et al*., 2018). This intrinsic resistance to β-lactam antibiotics and other non β-lactam antibiotics lowers therapeutic options leading to significant morbidity and mortality arising from infection with *Pseudomonas* species (Galani *et al*., 2021). In recent years however, plasmid-borne MBLs have been reported in *Klebsiella pneumoniae* and *E. coli*, suggesting that these strains could serve as vehicles for transmission of

genes conferring resistance to carbapenems. Although some studies show that β-lactamase-mediated resistance is becoming a major challenge in large tertiary hospitals in Kenya, little has been done in Kenya to investigate occurrence of resistance of this important class of antibiotics among isolates obtained from patients seeking outpatient and inpatient treatment in smaller hospital (sub county hospitals) such as Thika that serves cosmopolitan communities comparable to those served by referral and tertiary hospitals such as Kenyatta National Hospital, Aga Khan or Nairobi hospital.

### 2.9 Resistance to Aminoglycosides among *Pseudomonas aeruginosa, Klebsiella pneumoniae* and *E. coli* Strains

Aminoglycosides are considered among those commonly prescribed broad spectrum antibiotics in treating infectious diseases caused by gram negative bacteria (Tamma *et al*., 2020). Among these species, especially in *Pseudomonas aeruginosa*, resistance to aminoglycosides can be due to decreased membrane permeability (Garneau-Tsodikova & Labby, 2016), modification of 16S RNA or ribosomal modifying proteins, or through the enzymatic modification of aminoglycoside (Shete *et al*., 2017). Impermeability resistance may confer resistance to all classes of aminoglycosides even when the hosts lack any aminoglycoside modifying enzyme (Godinho *et al*., 2019). Impermeability resistance mechanism is often the most common aminoglycoside resistance particularly in cystic fibrosis isolates caused by *Pseudomonas aeruginosa* (López-Causapé *et al*., 2018).

Active aminoglycoside efflux is a rare aminoglycoside resistance mechanism that is mediated by MexXY proteins operating simultaneously with OprM (Abdi *et al*., 2020), and some other outer membrane proteins which include OpmB, OpmG and OpmI thus forming a three component active efflux systems (Saint Auguste, 2017).

Although resistance to aminoglycosides associated with efflux pump and membrane impermeabilities are important, aminoglycoside-modifying enzymes (AMEs) remain the most formidable defense against these antibiotics (Garneau-Tsodikova & Labby 2016). AMEs act by attaching a phosphate, adenyl or acetyl radical to the antibiotic molecule, thus decreasing the binding affinity of the modified antibiotics to the target

in the bacterial cell at 30S ribosomal subunit. AMEs may be borne on chromosomes or plasmids and are divided into aminoglycoside phosphoryltransferases (APHs), adenylyltransferases or nucleotidyltransferases (AADs or ANTs) and acetyltransferases (AACs). Subclasses of these enzymes confer resistance to specific groups of antibiotics (Kayastha *et al*., 2020). For instance, AAC (6')-I for resistance to tobramycin, netilmycin and amikacin and ANT (2')-I for resistance to gentamycin and tobramycin while AAC (6')-II mediates resistance to gentamycin, tobramycin and netilmycin (Fernández-Martínez *et al*., 2015). AAC (3)-I mediates resistance to gentamycin while AAC (3)-II mediates resistance to gentamycin, tobramycin and netilmycin. Some species such as *P. aeruginosa*, can carry multiple enzymes such as two to five modifying enzymes (López-Causapé *et al*., 2018) and they consequently exhibit broad-spectrum aminoglycoside resistance (Munita & Arias, 2016).

Another emerging resistance mechanism against aminoglycosides (target modification), is the 16S ribosomal RNA (rRNA) methylation which is a plasmid mediated resistance mechanism (Gokmen *et al*., 2016). This was reported in *P aeruginosa* in 2003 in Japan. The 16S rRNAmethylases were found to confer extra ordinary high levels of resistance to clinically useful aminoglycosides, such as amikacin, tobramycin, and gentamycin (Yu *et al*., 2019). Information on this newly recognized resistance mechanism has grown rapidly since 2003 and there is documentation on identification of new enzymes and their spread to different species in various regions of the world (Garneau-Tsodikova & Labby, 2016). The resistance genes in *P.aeruginosa* are *rmtA and rmtD. RmtA* gene is structurally associated with a genetic element that resembles a mercury-resistance transposon Tn5041 on a transferable plasmid (Salimizand *et al*., 2018). RmtD was found to be produced by a *P. aeruginosa* clinical strain from Brazil, which also produced metallo-beta-lactamase SPM-1(Nascimento *et al*., 2016).

### 2.10 Resistance Mechanisms to (Fluoro) Quinolones among *Pseudomonas aeruginosa, Klebsiella pneumoniae* and *E. coli* Strains

Nalidixic acid is a basic synthetic antibiotic possessing a naphthyridone core that was first availed for clinical use in 1962 (Zhang *et al*., 2018). Nalidixic acid was

successful in treating infections caused by various bacteria, including *E. coli* (Odonkor and Addo, 2018). Although many countries have discontinued the use of these agents, quinolones are still employed for the treatment of UTIs and other infections caused by β-lactamase-producing *Enterobacteriaceae* strains in resource-poor settings (Sfeir *et al*., 2019). There is some concern however to note that the prevalence of ESBL-producers with concomitant resistance to quinolones has been on the rise among *Enterobacteriaceae* strains (Dirar *et al*., 2020). In *Enterobacteriaceae*, quinolone resistance is typically caused by alterations in the target enzymes, namely DNA gyrase and/or topoisomerase IV. Resistance may also be due to reduced accumulation of the antibiotics in the cells through active extrusion mediated by multi-drug efflux (Correia *et al*., 2017). Resistance to quinolones is also mediated by plasmid-encoded Qnr enzymes, particularly in *K. pneumoniae* and *E. coli* (Majlesi *et al*., 2018).

Ciprofloxacin is a potent synthetic fluoroquinolone that contains a quinolone ring at the core. This antibiotic was introduced in 1980s as a response to rising resistance towards quinolones. Resistance to (fluoro) quinolones is mainly due to point mutation in chromosomal genes (Al-Muhanna *et al*., 2018). Recently, the plasmid-encoded gene aac (6')-lb-cr, which encodes a variant enzyme conferring cross-resistance to fluoroquinolones and gentamicin, has gained significant attention (Pragasam *et al*., 2020). This is due to its transferability and cross-resistance to more than one class of antibiotics (Munita & Arias, 2016). Isolates bearing the aac (6′)-Ib-cr gene therefore, are resistant to both fluoroquinolones and aminoglycosides. The *aac* (6′)-Ib-cr has been shown to be plasmid-borne and is derived through a point mutation (Trp102 Arg and Asp179Tyr) on aac (6')-lb (aacA4) that confers resistance to tobramycin, amikacin, and kanamycin**.** The *aac (6')-lb-cr* occurs as an integron cassette (Costello *et al*., 2019). This gene has been reported in *E. coli* and, in *P. aeruginosa* there is only a single report of its existence. It would therefore be important to determine occurrence of this important gene in *Pseudomonas, Klebsiella* and *E. coli* strains obtained from similar settings in Kenya.

## 2.11 Role of Integrons in the Dispersal of Genes Encoding Resistance to Aminoglycosides, Fluoroquinolones and β-Lactam Antibiotics

Integrons are genetic elements with a unique capacity to capture and express drug resistance genes in bacteria. There are nine classes of integrons described currently (Engelstädter *et al*., 2016). Among the integrons implicated in antibiotic resistance are integron class 1, 2 and 3 (An *et al.*, 2018). All integrons contain a 5'-end. The majority of integrons contain a 5' Conserved Sequence (5'-CS) that carries a recombinase gene, intI, encoding an integrase (Ríos *et al*., 2018). Integrons are named depending on the identity of the integrase they carry. For instance, class 1, 2 and 3 integrons carry *intI*1, *intI*2 and *intI*3 respectively. Most integrons also contain a 3'-CS (Tohya *et al*., 2019). There is a section between the 5'-CS and 3'-terminal known as the Variable Cassette Region (VCR), where gene cassettes are added sequentially. To date, more than 130 different antibiotic resistance gene cassettes have been described. Majority of these cassettes are found within the class 1 integron (An *et al*., 2018).

Cassettes encoding resistance to β-lactam antibiotics include the $bla_{OXA-1}$, $bla_{SHV}$ *and* $bla_{VIM}$ *cassettes* (Ríos *et al*., 2018). Integron cassettes conferring resistance to aminoglycosides include the aac*4, aad-A* and *aad-B* cassettes among others (Obayiuwana and Ibekwe, 2020). The *aac (6') lb-cr* conferring resistance to gentamicin and fluoroquinolones is also found as a class 1 integron cassette (Zamanlou *et al*., 2018). Such cassettes are found in different bacteria genera, including *Pseudomonas, Klebsiella* and *E. coli* strains (Zamanlou *et al*., 2018).

Some surveillance on antibiotics resistance of HAIs has been carried out in referral hospitals in Kenya.

The prevalence of hospital-acquired infections in Kenya, on average is 4.4 %, according to a study conducted in three public healthcare facilities (Ndegwa L, 2015).

These include a study at Mama Lucy hospital on bacteria isolates from ward, operating room and from post- operative wound infections among patients attending

this facility. Antibiotics susceptibility test results showed that the most resistant bacteria causing surgical site infection were *E. coli* and *K.pneumoniae* which are Gram negative bacilli. They had a resistance of 74 % and 81.3 % respectively. On average, all the isolates had a resistance rate of 49.4 % (Auna *et al*., 2021). In a study carried out in Kenyatta National Hospital (KNH) by analyzing data collected retrospectively from inpatients in medical wards, 88 % of the isolates were MDR while 26 % were extensively drug resistant (XDR) (Wangai F K, 2017).

# CHAPTER THREE

# MATERIALS AND METHODS

## 3.1 Study Area

This study was carried out at Thika Level 5 Hospital in Kiambu County. Pus swabs from surgical and burn wounds were collected from patients hospitalized in various wards at Thika Level 5 Hospital (TL5H).This was done between September 2011 and January 2012. Urine samples were also collected from catheterized patients. TL5H serves as a referral hospital in Kiambu County with a bed capacity of 250 and seven wards. It serves an average of 450 to 500 patients daily.

## 3.2 Study Population

Urine samples were obtained from patients admitted to medical and surgical wards. All patients with indwelling catheters were included in this study. Bed sore specimens were also taken from all patients with wounds, regardless of the ward. More specimens were obtained from wounds and burns, especially from patients admitted to the burns unit. Specimens were only obtained from patients who consented to participate in this study. Both adults and children were included in this study. For children, consent was sought from their parents or guardians. All study participants had been admitted to the ward for at least 48 hours. Patient information including age, date of admission, and diagnosis was obtained from patient records.

### 3.2.1 Inclusion Criteria

Eligible subjects were all in-patients with wounds, burn wounds, with or without indwelling catheters. All participants had been hospitalized for at least 48 hours. Only patients who gave consent to participate were eligible for inclusion in the study.

### 3.2.2 Exclusion Criteria

These were in-patients who had been admitted for less than 48 hours and those without wounds or bed sores. Patients in the renal unit without indwelling catheters

were also excluded. All patients who did not consent to participate, regardless of meeting other inclusion criteria were not legible to participate.

### 3.3 Study Design

This was a cross-sectional study. Patients were sampled only once and more than one sample type was taken from patients with a combination of wounds, burns and indwelling catheters.

### 3.4 Sample Size Determination

The Sample size was determined using the formula of (Shen and Fisher, 1999). The assumed prevalence of *Pseudomonas, Klebsiella* and *E. coli* is 20% (Jroundi *et al*., 2007). Therefore, this was taken as the least prevalence and used for sample calculation for all the three species:

$$N=Z^2 P (1-P) /D^2 = 1.96^2 X\ 0.2(0.8)/0.05^2 =246$$

Where N = Minimum sample size

Z=1.96 (Standard Error)

P =Expected prevalence of *P. aeruginosa* (0.2)

D=0.05 (inverse of 95% allowable error)

Therefore, a minimum of 246 patients was included in the study. From these patients, urine, wounds, and burns specimen were collected.

### 3.5 Specimen Collection

Sterile cotton swabs were used to aseptically collect specimens from fresh burn sites and surgical wounds. The swab was gently rolled on the surface area of the wound for about five seconds. The cotton swab was then inserted directly into sterile bottle containing Stuart transport media. The handle of the swab was snapped off, leaving cotton tip in the transport media. The bottle was sealed and labeled with the patient's

study number, ward, and date. Urine specimens were obtained from catheter port. The sampling port was first disinfected with 70 % alcohol and allowed to dry. Sterile syringe tip was inserted into the sampling port and 10 ml of urine aspirated and syringe disconnected. The urine sample was put in sterile universal bottle and capped to avoid leakage and contamination. The sampling port was disinfected again with alcohol swab to prevent contamination. These specimens were also labeled with the study number, ward, and date. All specimens were delivered immediately to the microbiology department in the laboratory for processing.

### 3.6 Laboratory Procedures

#### 3.6.1 Processing of Urine and Swabs Specimens

Bacterial isolates from swabs collected from wounds and catheter urine were processed according to standard operating procedures. Swab specimens were inoculated onto blood agar media to observe colony morphology, hemolysis, and pigmentation. They were also inoculated onto MacConkey media to determine lactose and non-lactose fermentation. Smears were then prepared, and a Gram stain performed. The culture plates were incubated aerobically at 37 °C for 18-24 hours. The following morning, the plates were observed for colonial morphology, pigmentation on blood agar and for hemolysis and non- lactose fermentation to identify *P. aeruginosa*. The characteristic grape-like odor and large, flat colonies were noted. For *K. pneumoniae* and *E. coli*, lactose fermentation was observed. Large mucoid colonies were also observed. Urine specimens were first inoculated on blood agar and then on CLED media. The plates were incubated at 37 °C for 18-24 hours. Colonial morphology was observed and the pale color of the colonies indicated they are non-lactose fermenters. Colonies suggestive of *P. aeruginosa, K. pneumoniae and E. coli* were subjected to biochemical tests.

#### 3.6.2 Biochemical Tests for *P. aeruginosa, K. pneumoniae* and *E. coli*

Identification was done by conducting a series of biochemical tests, including Gram staining, tests for oxidase, methyl red, Voges-Proskauer reactions, indole, citrate,

catalase, urea hydrolysis, lactose fermentation, nitrate reduction, and sugar fermentation, were conducted (Farid and Larsen, 1980).

#### 3.6.2.1 Oxidase Test

Impregnated oxidase test strips from Oxoid were used. A sample of the bacterial colony from a fresh growth was rubbed onto filter paper impregnated with the oxidase reagent (N, N, N', N'-tetra-methyl-p-phenylenediaminedihydrochloride) using a wooden applicator stick (Kalawat *et al*., 2012). A blue/purple color appearing within ten seconds indicated an oxidase-positive result.

#### 3.6.2.2 Triple Sugar Iron Agar (TSI)

A well-isolated colony was touched at the top with a straight inoculating needle. Inoculation on Triple Sugar Iron Agar (TSI) was performed by first stabbing through the center of the medium to the bottom of the tube and then streaking the surface of the agar slant. The cap was loosely placed, and tubes incubated at 35-37 °C in ambient air for 18-24 hours (Upgade *et al*., 2012). An alkaline slant and alkaline (K/K), acid butt and acid slant (A/A), hydrogen sulfide and gas production were observed.

#### 3.6.2.3 Motility Test in *P. aeruginosa, K. pneumoniae* and *E. coli*

The motility of the above organisms was tested using motility-indole-ornithine media (MIO). A freshly isolated colony was touched at the top with a straight inoculating needle and then inoculated to the bottom of the tube containing MIO medium. The tubes were incubated at 35-37°C for 18-24 hours and observed for motility. Motility was indicated by generalized turbidity or growth extending from the line of inoculation (Kalawat *et al*., 2012). Indole test was performed by adding a drop of Kovac's reagent on MIO media. Immediate color change to pink indicated Indole positive while Indole negative was indicated by no color change.

### 3.6.2.4 Citrate Utilization Test for *P. aeruginosa, K. pneumoniae* and *E. coli*

Citrate utilization test was performed by picking a single colony from a fresh culture plate using a straight wire. Inoculation was done onto the Simon's Citrate agar by singly stabbing the butt and streaking the slant. The caps were loosely placed, and the tubes then incubated aerobically at $37^0$C overnight. Presence of blue color indicated citrate utilization. Persistent green color indicated negative for citrate utilization (Yusuf *et al*., 2012).

### 3.6.2.5 Methyl Red and Voges-Proskauer (MR-VP)

Methyl Red and Voges-Proskauer (MR-VP) test is very useful in separating members of the family *Enterobacteriaceae* and some *Streptococcus species*. Some bacteria can be distinguished on the basis of their production of acetoin, which is a neutral end product, after incubation in buffered peptone-glucose media. Addition of alpha-naphthol and KOH solutions resulted in a pink-red color within a few minutes. The tube was lightly inoculated with a single colony from an 18-24-hour culture. The cap was slightly loosened, and the tubes incubated at 35°-37°C for 48 hours.

After incubation, a sterile pipette was used to remove two aliquots of one ml each and placed into two small tubes labeled M-R and V-P, respectively. Five drops of methyl red were added to the tube labeled MR, and results read immediately taking care not to mix the tube.

Voges-Proskauer test was performed by adding two drops of Voges-Proskauer A (alpha-naphthol). The tube was gently mixed well to aerate the sample since oxygen was needed to complete the reaction. Then, three drops of Voges-Proskauer B (40 % Potassium hydroxide) were added to the tube. The results were read within five to thirty minutes.

Positive Methyl red test was indicated by formation of a red color at the surface, while a negative test was indicated by a yellow color at the surface.

A positive Voges-Proskauer test was indicated by formation of a thin pink-red color developing at the surface of the broth within five to thirty minutes.

### 3.6.2.6 Acetamide Broth medium for the Confirmation Test of *P. aeruginosa*

Acetamide is a liquid medium used as the sole source of carbon, with its utilization by many bacteria indicated deamination. This results in color change from orange-red to purple-red. A sterile straight wire was used to touch the top of fresh colony (18-24 hours old) and inoculated in tube containing acetamide, by stubbing the butt then streaking the slant. The tubes were incubated at 37°C for 48 hours. An intense purple-red indicated positive results (Washington, 2012).

### 3.6.2.7. Reduction of Nitrates to Nitrites Test in *P. aeruginosa, E. coli* and *K. pneumoniae*

Bacteria in the *Enterobacteriaceae* family, as well as *Pseudomonas* reduce nitrates to nitrites. A fresh colony was inoculated by stabbing the medium in the tube. The tubes were then incubated at 35°C-37°C for 8, 12, and 24 hours. About five drops of Solution A followed by five drops of Solution B were added to the tubes. Formation of a red color in 1-2 minutes indicated reduction of nitrates to nitrites hence positive test. If no color appeared, a pinch of zinc powder was added to the tube which was free of nitrates and nitrites. The tubes were observed for red color formation or if the culture remained colorless.

Interpretation of the results: if no color appeared, it indicated that the organism reduced the nitrate present in the culture medium to nitrite, possibly carrying the reaction to the gaseous nitrogen, indicating a positive test for nitrate reduction.

If there was no reduction of nitrate present in the culture medium by the microorganism, the zinc reduced the nitrate to nitrite and formed a red color upon reacting with the Griess reagent, indicating a negative test for nitrate reduction). The test organism was negative hence absence of nitrates (Washington, 2012).

## 3.7 Growth of *P aeruginosa* at 42°C

Isolates suggestive of *P. aeruginosa* were inoculated on culture plates and incubated at 42 °C. Plates were observed the following day for growth. *P. aeruginosa* grew at this temperature (LaBauve and Wargo, 2012).

### 3.8 Stocking of *Pseudomonas aeruginosa, K. pneumoniae,* and *E. coli* Isolates

Stocking was done after confirmation of identity of the isolates. The organisms were cultured on Mueller-Hinton plates and incubated at $37^0$C overnight. About two to three colonies were emulsified in tryptic soy broth with 15% glycerol in cryotubes (Ndegwa, 2014) and were stored at -20 $^0$C for molecular analysis and at -80 $^O$C for further studies.

### 3.9 Antibiotic Susceptibility Testing

Antibiotic susceptibility testing was done using Kirby-Bauer Disc diffusion method. At least four to five isolated colonies of the same morphologic type were selected from an overnight agar plate of a pure culture and emulsified in 10 ml sterile normal saline (0.85% sodium chloride) in sterile glass tubes. The organisms were diluted using sterile normal saline to obtain a turbidity equivalent to the 0.5 McFarland standards. Within 15 minutes of diluting the organisms, a sterile cotton swab was dipped into the standardized solution, lifted slightly out of the suspension and the swab firmly rotated several times against the upper inside wall of the tube to express excess fluid. The entire surface of Mueller–Hinton agar plates was swabbed, turning the plate and the swab at 90 degrees between swabbing so as to obtain a confluent growth. The lid was left ajar for two minutes for the surface moisture to be absorbed. The disks were applied aseptically using a dispenser and pressed down with sterile forceps to make contact with the surface. All antibiotics discs used were obtained from Oxoid. Susceptibility to commonly used antibiotics was performed using the Kirby-Bauer method (Hudzicki, 2009). The panel used for *Pseudomonas aeruginosa* strains included aminoglycosides antibiotics such as tobramycin (10µg), gentamicin (10µg), and amikacin (30µg). B-lactam antibiotics included piperacillin (100µg) for penicillins, ceftazidime (30µg) for third generation cephalosporins, and imipenem (10µg), a carbapenem. Aztreonam (30µg), a monobactam commonly used against *P. aeruginosa* was also tested. The β-lactamase inhibitor included in this panel was piperacillin/ tazobactam (100/10 µg). Two fluoroquinolones, ciprofloxacin (5µg), ofloxacin (5 µg) were also included.

Antibiotics used to test susceptibilities for *K. pneumoniae* and *E. coli* included the following aminoglycosides: gentamicin (10µg), amikacin (30µg), and kanamycin (30µg). β-lactams included piperacillin (100µg), amoxicillin/clavulanic acid (20/10 µg), piperacillin/tazobactam (100/10 µg), cefotaxime(30µg), ceftazidime (30µg) and imipenem (10µg). Fluoro-quinolones included nalidixic acid (30µg), ciprofloxacin (5µg) and ofloxacin (5 µg). Other antibiotics tested were trimethoprim/ sulfamethoxazole (25µg) and tetracycline (30µg).

Quality control organisms used to test for disc potency and media quality were ATCC *P. aeruginosa* 27853 and ATCC *E. coli* 25922. The plates were incubated at 37$^{o}$C for 18-24 hours. Susceptibility results were interpreted according to the Clinical Laboratory Standards Institute 2011 guidelines (Polsfuss *et al*., 2012).

### 3.10 PCR Tests

#### 3.10.1 DNA Extraction for PCR Reactions

Target isolates were obtained from the stock and sub-cultured on MacConkey media at 37°C overnight to obtain fresh colonies. A fresh bacterial colony was picked and suspended in 1000 µL of sterile double-distilled water. DNA used as a template in PCR reactions was obtained by boiling the suspension at 95$^{o}$C for 5 minutes. The supernatant was stored at -20$^{o}$ C until further use. Subsequent PCR amplifications were carried out in a final volume of 25 µl with 5-10 µl of template DNA and 1 µl of 10 mM concentration of both forward and reverse primers. Appropriate positive control strains were used depending on the test gene while sterile distilled water served as negative control (Crăciunaş e*t al*., 2010) .

#### 3.10.2 Detection of Selected Resistance Genes

A sterile Eppendorf tube was labeled “master-mix” with a permanent marker. The PCRs were done using the PureTaq Ready-to-Go PCR beads according to manufacturer’s instructions (GE Healthcare, Buckinghamshire, UK). These PCR beads comprise of puReTaq DNA Polymerase, stabilizers, BSA, dATP, dCTP, dGTP, dTTP, and reaction buffer. When a bead is reconstituted to a 25 µl final

volume, the concentration of each dNTP is 200µM in 10mM Tris-HCl, (pH 9.0 at room temperature), 50 mM KCl and 1.5 mM $MgCl_2$.

Master-mix was prepared by first pipetting 25µl of sterile distilled PCR water per sample. Respective primers were then added by pipetting 1 µl forward primer and 1 µl reverse primer per sample. The master-mix was then gently vortexed. A volume of 28 µl of the prepared master-mix was then dispensed into labeled eppendorf tubes containing PCR beads. Subsequently, 6 µl DNA from respective samples was added into these tubes, which were then capped with PCR lids. The contents were gently mixed and placed on the thermo-cycler (Peltier DYAD Company, UK) which had been pre-set.

The concentration of each reagent was calculated depending on the number of the samples being analyzed. The PCR steps included initiation denaturation at $95^0$C for 5 minutes, followed by denaturation at $94^0$C, annealing and extension at 72°C between 1-2 minutes and final elongation at 72°C for 10 minutes. The number of cycles was 35 for all primers used, but varied in the annealing temperature and incubation period depending on the genes being detected.

The thermocycler was programmed according to the reaction conditions specific to the primers and genes being detected. For aminoglycoside modifying enzymes the following genes were tested for *aac-(6')-lb*, *aac-(3)* and *aph-(3)-III*. For fluoroquinolones resistance, only the plasmid-borne *aac(6')lb-cr* gene was screened for, since this gene is of clinical and epidemiologic importance due to its potential for horizontal acquisition and encoding cross resistance to fluoroquinolones and gentamicin. The following genes encoding for β-lactamases of critical which are importance to *Pseudomonas aeruginosa, K. pneumoniae* and *E. coli* were screened for $bla_{TEM}$, $bla_{SHV}$ and $bla_{OXA}$.

**Table 3.1: Primers used in Detection of Resistant Genes in *P. aeruginosa, K. pneumoniae* and *E. coli***

| Primer Target gene | Name | Primer Sequence | Anealing temp(°C) | approximate size | Accession |
|---|---|---|---|---|---|
| blaTEM | TEM-F | GCGGAACCCCTATTTG | 50 | 964 | EF125012-related |
| | TEM-R | TCTAAAGTATATATGAGTAAACTTGGTCTGAC | | | |
| blaSHV | SHV-F | TTCGCCTGTGTATTATCTCCCTG | 50 | 854 | |
| | SHV-R | TTAGCGTTGCCAGTGYTCG | | | AF148850-related |
| blaCTX-M | CTX-M- F | ATGTGCAGYACCAGTAARGTKATGGC | 60 | 593 | Y10278-related |
| | CTX-M -R | TGGGTRAARTARGTSACCAGAAYCAGCGG | | | |
| blaOXA-1 | OXA-1F | ATGAAAAACACAATACATATCAACTTCGC | 62 | 820 | JO2967-related |
| | OXA-1R | GTGTGTTTAGAATGGTGATCGCATT | | | |
| intI1 | INT-1U | GTTCGGTCAAGGTTCTG | 50 | 923 | U12338 |
| | INT-1D | GCCAACTTTCAGCACATG | | | |
| intI2 | INT-2U | ATGTCTAACAGTCCATTTT | 50 | 450 | AJ001816.1 |
| | INT-2D | AAATCTTTAACCCGCAAAC | | | |
| intI3 | IntI3-F | GCAGGGTGTGGACGAATACG | 57 | 760 | AY219651 |
| | IntI3-R | ACAGACCGAGAAGGCTTATG | | | |
| Class 1 VCR | in-F | GGCATACAAGCAGCAAGC | 52 | | U12338 |
| | in-B | AAGCAGACTTGACCTGAT | | Variable | |
| Aph(3')III | APH (3´) I-F | 5'-AACGTCTTGCTCGAGGCCGCG-3' | 52 | 780 | Oka *et al.*, (1981) |
| | APH(3´)I I-B | 5'-GGCAAGATCCTGGTATCGGTCTGCG-3' | | | |
| aac(6′)-Ib-cr | aac(6′)-Ib-cr-F | TTGCGATGCTCTATGAGTGGCTA | 55 | 482 | AAL93141.1 |
| | aac(6′)-Ib-cr-R | CTCGAATGCCTGGCGTGTTT | | | |

(Robledo *et al*., 2010)

### 3.10.3 Determination of the Size of the Variable Cassette Region of Integrons

Integrons play a critical role in the dispersal of resistance determinants in *Pseudomonas, Klebsiella* and *E. coli* strains. Screening for the prevalence of integron class 1, 2 and 3 was performed and the sizes of variable cassette regions (VCRs) of the detected integrons, were determined.

The detection of class 1 integron, the 3'-CS and amplification of the integron VCR region were conducted as described by (Deng *et al*., 2017) while detection of class 3 was done according to (Falbo *et al*., 1999). Amplification of class-specific integrase (*intI*1, 2 or 3) was done using a combination of primers that target the integrase gene, *intI* located at the 5'-CS. Amplification of the VCR region was done using a combination of primers targeting the 5'-CS and the 3'-CS of each integron type. PCRs were done as described in section 3.16.2 while the list of primers and the annealing temperatures are indicated in table 1.

### 3.11 Analysis of PCR Products

All PCR products for resistance genes were analyzed by electrophoresis in 1.5 % agarose gels while large amplifications from integron VCRs were analysed in 1.0% agarose gels. Tris-Boric EDTA buffer was used for casting gels and running the electrophoresis. Small PCR products such as those of resistance genes were subjected to electrophoresis at 100V for 2 hours while larger products such as the integron VCRs were ran at 70-80 V for up to 3 hours to ensure proper resolution of the bands. To ensure that the PCR products sank into the wells and in order to monitor the progress of electrophoresis, PCR products were mixed with 3 µl of bromothymol blue dye before loading into the wells of the electrophoresis gels. Appropriate markers were used for estimating the PCR product sizes. All gels were stained with ethidium bromide, visualized under UV light and the image recorded with a gel documentation system (Bio-Rad Laboratories, Hercules, Ca, USA).

### 3.12 Data Management

Data on patients' code number, gender, age, ward, type of specimen, organism isolated and antibiotic susceptibility results were recorded and entered in MS Excel. The Data were organized and presented in tables, bar graphs and charts. Data analysis was performed using MS Excel and STATA version 13.1 tool respectively. P values of $< 0.05$ were considered to be statistically significant.

### 3.13 Ethical Consideration

Ethical clearance for the study was obtained from the Kenya Medical Research Institute (KEMRI) Scientific Steering Committee and Ethical Review Committee (ERC No. 2081). Approval was also obtained from Medical Superintendent of Thika Level 5 Hospital and informed consent from the patients or their guardians.

There were minimum risks to the patients as only urine and swabs from open wounds were collected both of which are non-invasive procedures. However, the patients were informed that collection of specimens from septic wounds would cause mild irritation and discomfort.

Only patients' and laboratory numbers were used, ensuring the identity of the patients and results were treated with utmost confidentiality. Patients were identified by coding and the results were kept in a lockable cabinet and saved in a password-protected computer accessible only to the investigator and clinicians.

### 3.14 Benefits of the Study

The information generated from this study benefited patients because they were treated according to the individual antibiotic susceptibility results. Patients' contacts were used to inform them of the results in case they were discharged before results were completed. Clinicians benefited because the resistance profiles of circulating pathogens causing nosocomial infections informed them of treatment options. The results were released to the clinicians and then recorded in the patient's file. This information also guided on the need to upgrade prescription policy in patient management, assessment and control of *P. aeruginosa, K. pneumoniae* and *E. coli* spread and colonization in the hospital.

# CHAPTER FOUR

# RESULTS

## 4.1 Introduction

A total of 450 patients participated in the study. In total, 450 specimens were collected from surgical and non- surgical specimens (burns, bedsores and urine). The distribution of bacterial isolates was as follows: 41 for both *P. aeruginosa*, 41 for *K.pneumoniae* and 42 for *E.coli*, resulting in a prevalence of 9.1% (41/450) for both *P. aeruginosa*, 41 for *K.pneumoniae* and 9.3% for (42/450) *E.coli*.

**Table 4.1: Distribution of Bacterial Isolates in Inpatients from Various Sources of Specimens at Thika L5H**

| **Bacterial isolates** | **Frequency (n=450** | **%** |
|---|---|---|
| *P. aeruginosa* | 41 | 9.1 |
| *E. coli* | 42 | 9.3 |
| *K. pneumoniae* | 41 | 9.1 |

## 4.2 Demographic Characteristics of Inpatients at Thika L5H

Distribution of patients by age group was 74 % for those aged 0- 59 years and 26% for those aged 60 years and above. Regarding gender, males constituted 49% and females 51%.

**Table 4.2: Distribution of Patients in Terms of Age- Group and Sex at Thika L5H**

| Age- group (years) | Frequency (n=450) | % |
|---|---|---|
| 0-59 | 335 | 74 |
| ≥60 | 115 | 26 |
| Sex | Frequency (n=450) | % |
| Male | 221 | 49 |
| Female | 229 | 51 |

The composition of specimens according to the source was as follows: urine 26.9%, surgical 26.7%, burns 24.7% and non-surgical pus swabs 19.1% and bedsores 2.7%.

**Table 4.3: Distribution of Specimen from Various Sources in Inpatients at Thika L5H**

| Source of specimen | Frequency (n=450) | % |
|---|---|---|
| Urine | 121 | 26.9 |
| Surgical pus swab | 120 | 26.7 |
| Burns pus swabs | 111 | 24.7 |
| Bedsores swabs | 12 | 2.7 |
| Other non-surgical pus swabs | 86 | 19.1 |

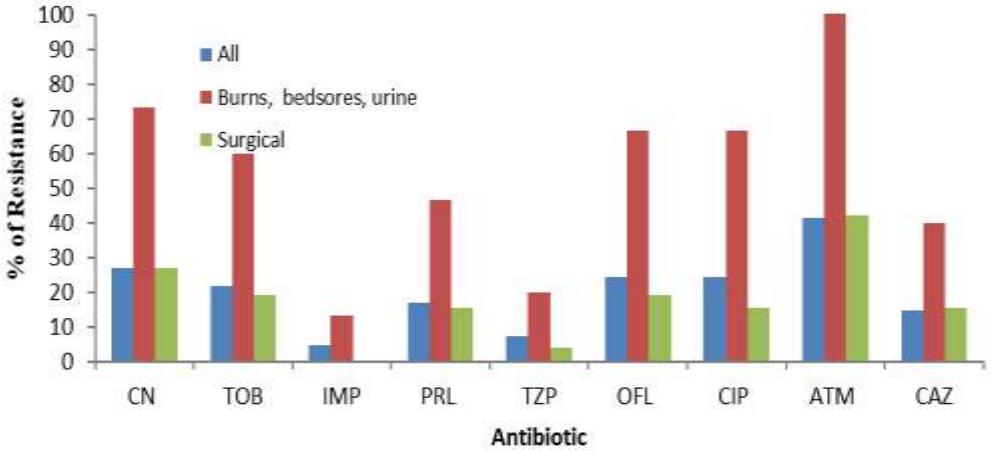
Antibiotic susceptibility Testing (AST) data was obtained by using various classes of antibiotics including aminoglycosides, beta-lactams and fluoroquinolones. Resistance was considered in relation to age, gender, source of clinical specimen and combination of antibiotics and presented in figures and tables. In general, *Pseudomonas* strains were resistant to fewer antibiotics than *E. coli* (P= 0.002) and *Klebsiella* strains (P= 0.0025). *P. aeruginosa* isolates from non-surgical sites were

more resistant to multiple antibiotics than those from surgical sites (P= 0.0110). Strains resistant to imipenem were only obtained from non-surgical specimen. All *P. aeruginosa* isolates from non-surgical sites were resistant to aztreonam, a recommended anti-pseudomonas drug. Overall, distribution of MDR was *E. coli* 78.6%, *K. pneumoniae* 46.3% and *P. aeruginosa* 29.3%

PCR amplification tests were carried out to determine resistance genes in the various isolates from different specimens. All *Pseudomonas* isolates from urine samples did not carry any of the genes tested and only 1 (33.3%) *aph (3') III* was obtained from bedsore specimens. Carriage of β-lactamases was poor with a prevalence of 17% ($bla_{CTX-M}$) and 2% ($bla_{SHV}$, $bla_{OXA-1}$, $bla_{TEM}$) most being isolated from surgical sites. It was noted that *Escherichia coli* isolates from urine had all the genes that were tested compared to *Pseudomonas* strains. Among β-lactamases tested in bedsores and burns, only $bla_{CTX-M}$ at a prevalence of 22 % (2) and 11% (1). Carriage of β-lactamases in *Klebsiella* isolates ranged between 24% and 27%. Most of the genes detected were from urine isolates which carried *acc (6')-1-cr, aph (3')III* and *ant(4')-IIb* with a prevalence of 100%, and 12.5 % from surgical sites. Prevalence of class I integrons was 33 % in *E. coli* and 20% each in *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* strains.

### 4.3 Resistance to Antibiotics among *Pseudomonas aeruginosa* Isolates

Resistance of *P. aeruginosa* strains from non- surgical specimens was highest in aztreonam at 100% and least to imipenem at 13.3 %. Other antibiotics resistance was shown in gentamycin and tobramycin 73% and 60% respectively, ciprofloxacin and ofloxacin 66.7% each, piperacillin 46.7%, piperacillin-tazobactam 20% while ceftazidime was 40% from non- surgical specimens. In isolates from surgical sites, all were susceptible to imipenem. The pattern of resistance for surgical site isolates to other antibiotics was aztreonam 42.3 %, gentamicin 26.9 %, tobramycin and ofloxacin 19.2 %, ciprofloxacin, ceftazidime and piperacillin 15.4 % and piperacillin-tazobactam 3.5 %. Amikacin had zero (0 %) resistance to both surgical and non-surgical specimens.



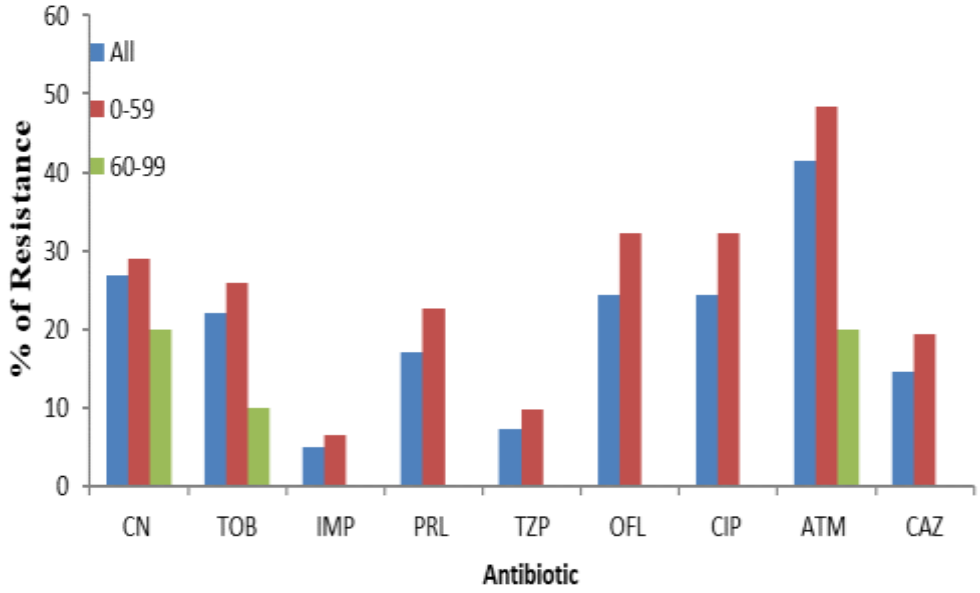

**Key:** CN- Gentamicin, TOB- Tobramycin, IMP- Imipenem. PRL- Piperacillin, TZP- Piperacillin-Tazobactam, OFL- Ofloxacin, CIP- Ciprofloxacin, ATM- Aztreonam, CAZ- Ceftazidime

**Figure 4.1: Analysis of Resistance to Multiple Antibiotics among *Pseudomonas aeruginosa* Isolates from Surgical and Non-Surgical Specimens.**

Isolates from non-surgical sites (burns, bedsores and urine) were more likely to be resistant to multiple antibiotics than those from surgical sites (P= 0.0110). Isolates resistant to imipenem were only obtained from non-surgical specimen. All isolates from non-surgical sites were resistant to aztreonam. Regardless of the source, imipenem was the most effective antibiotic followed by piperacillin-tazobactam, and ceftazidime. Although ciprofloxacin was also effective against 80.5% of all isolates, only 70% of strains from non-surgical sites were susceptible to this antibiotic.

### 4.4 Resistance Patterns of *P. aeruginosa* in Two Different Age Groups

In this study, regardless of the age group, aztreonam had the highest resistance at 41.5 %, followed by gentamicin at 26.8 %, ofloxacin and ciprofloxacin 24.4 % each, tobramycin at 22 %, piperacillin at 17.1 %, ceftazidime at 14.6 % and piperacillin-tazobactam at 7.3 %. Imipenem showed the least resistance at 4.9 %.



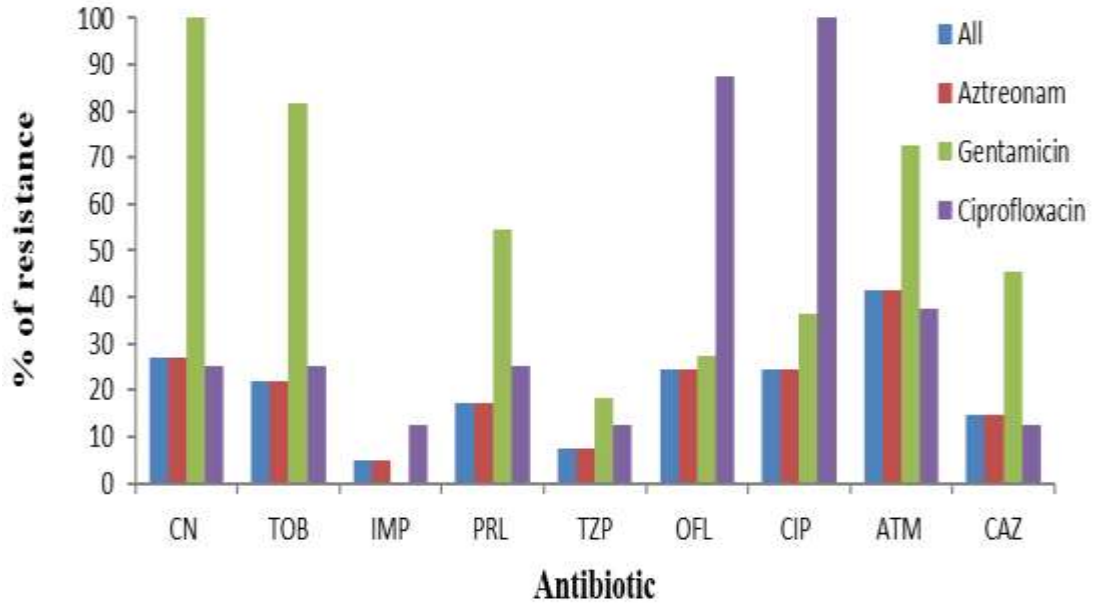

**Key:** CN- Gentamicin, TOB- Tobramycin, IMP- Imipenem. PRL- Piperacillin, TZP- Piperacillin-Tazobactam, OFL- Ofloxacin, CIP- Ciprofloxacin, ATM- Aztreonam, CAZ- Ceftazidime

**Figure 4.2: Analysis of Resistance to Multiple Antibiotics among *P. aeruginosa* Isolates in Different Age Groups.**

A comparison of resistance patterns of *P. aeruginosa* strains obtained from patients of different age groups showed that isolates obtained from patients below 60 years of age were likely to be resistant to multiple antibiotics than those obtained from sixty (60) years and above.

### 4.5 Co-Resistance of *P. aeruginosa* to Multiple Antibiotics

Resistance patterns for co- resistance to multiple antibiotics were as follows: gentamicin 100 %, tobramycin 81.8 %, piperacillin 54.5 %, piperacillin-tazobactam18.2 %, ofloxacin and ciprofloxacin 27.3 %, aztreonam 72.7 % and ceftazidime 45.5 %. All isolates were susceptible to imipenem.




**Key:** CN- Gentamicin, TOB- Tobramycin, IMP- Imipenem. PRL- Piperacillin, TZP- Piperacillin-Tazobactam, OFL- Ofloxacin, CIP- Ciprofloxacin, ATM- Aztreonam, CAZ- Ceftazidime

**Figure 4.3: Co-Resistance of *P. aeruginosa* to Multiple Antibiotics**

**Analysis for Co-Resistance to Multiple Antibiotics among Isolates Resistant to Aztreonam, Gentamicin or Ciprofloxacin**

Analysis for distribution of *P. aeruginosa* in the various wards and their resistance phenotypes

**Table 4.4: Number (%) of *Pseudomonas* Isolates from Different Hospital Wards with Specific Resistance Phenotypes.**

| **Resistance phenotype combinations** | | **Number(%) of isolates with this phenotype (N=41)** | **Number (%) of isolates with a given phenotype per hospital ward** | | |
|---|---|---|---|---|---|
| | | | **Surgical (n=12)** | **Renal (n=15)** | **Burns (n=14)** |
| Aminoglycosides | | | | | |
| Resistant to:- TOB, K, CN, AK | Susceptible to:-None | 11 (27) | 3 (25) | 6 (40) | 2 (14) |
| TOB, K | Susceptible to:-CN and AK | 15 (37) | 6 (50) | 3 (20) | 6 (43) |
| β-lactams | | | | | |
| Resistant to:-none | Susceptible to:- all | 5 (12) | 2 (16) | 0 | 3 (21) |
| IMP | PIP, ATM and CAZ | 20 (49) | 7 (58) | 2 (13) | 11 (19) |
| PIP and AMP | ATM and CAZ | 16 (39) | 5 (42) | 6 (40) | 5 (36) |
| PIP, ATM, CAZ | IMP | 4 (10) | 1 (8) | 2 (13) | 1 (7) |
| β-lactamase inhibitors | | | | | |
| Resistant to:-None | Susceptible to:-AMC and TZP | 18 (44) | 5 (42) | 6 (40) | 7 (50) |
| AMC | TZP | 12 (29) | 2 (17) | 7 (47) | 3 (21 |
| TZP | AMC | 22 (54) | 8 (67) | 5 (33) | 9 (34) |
| (Fluoro)quinolones | | | | | |
| Resistant to:- CIP | Susceptible to:-OFL | 12 (29) | 3 (25) | 7 (47) | 2 (14) |
| OFL | CIP | 15 (37) | 7 (58) | 2 (13) | 6 (43) |
| CIP and OFL | None | 5 (12) | 1 (8) | 3 (20) | 1 (7) |

**Key:** TOB-Tobramycin, K- Kanamycin, CN- Gentamicin, AK- Amikacin, PIP- Piperacillin, AMP- Ampicillin, AZT- Aztreonam, CTX- Cefotaxime, CAZ- Ceftazidime, AMC- Amoxycillin-clavulanic acid, TZP- Piperacillin-Tazobactam, IMP- Imipenem, CIP- Ciprofloxacin, OFL- Ofloxacin

It was found that isolates from non-surgical sites were more likely to be resistance to multiple antibiotics than those from surgical sites (P=0.0110).Thus, there was significant difference in resistance prevalence for the different bacterial isolates.

This study revealed that 40% of *Pseudomonas aeruginosa* strains from urine were not susceptible to all the aminoglycosides tested, which included tobramycin, kanamycin gentamicin and amikacin. However, amikacin and gentamycin was effective against 30% of the isolates tested.

All *P. aeruginosa* isolates from urine were resistant to at least one β-lactam compared to 16% and 21% of strains from surgical and burns wounds respectively. A high proportion of strains from surgical specimens were resistant to penicillins (piperacillin and ampicillin) but were susceptible to advanced classes of β-lactam such as aztreonam (monobactam), cefotaxime and ceftazidime (third generation cephalosporins). A total of four *P. aeruginosa* isolates that were resistant to all classes of β-lactams including imipenem were identified. These imipenem resistant strains were however susceptible to fluoroquinolones and aminoglycosides, in particular amikacin and/or gentamicin. Resistance to Amoxycillin-clavulanic (AMC) with concomitant susceptibility to TZP was observed in 44% of all *P. aeruginosa* isolates tested. This study also revealed that 54% of all isolates were resistant to at least one β-lactam but were susceptible to TZP. Also twelve isolates that were resistant to AMC but remained susceptible to cephalosporins such as ceftazidime and aztreonam were identified.

**Table 4.5: Distribution of *P. aeruginosa* Strains Positive for Selected Resistance Markers**

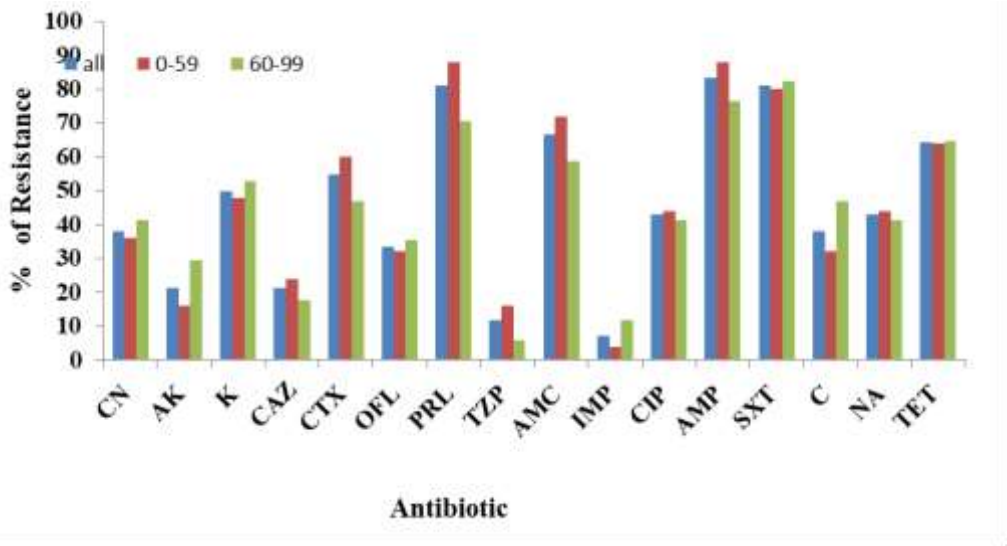
| | | | Distribution of positive isolates | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Specimen distribution | | | | Gender | |
| **Resistance mechanism** | **Gene** | **Prevalence N=41** | **Renal** | **bed sores** | **surgery** | **burns** | **Male** | **Female** |
| Ciprofloxacin/ gentamicin cross resistance | *aac(6')-lb-cr* | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Aminoglycoside phosphotransferase | *aph(3')III* | 3 (7%) | 0 | 1 (33%) | 2 (67%) | 0 | 0 | 3 (100%) |
| '-aminoglycoside nucleotidyltransferase | *ant(4')-IIb* | 1 (2%) | 0 | 0 | 1 (100%) | 0 | 1 (100%) | 0 |
| β-lactamases | $bla_{CTX-M}$ | 7 (17%) | 0 | 0 | 2 (29%) | 5 (71%) | 5 (71%) | 2 (29%) |
| β-lactamases | $bla_{SHV}$ | 1 (2%) | 0 | 0 | 1 (100) | 0 | 1 (100) | 0 |
| β-lactamases | $bla_{OXA-1}$ | 1 (2%) | 0 | 0 | 1 (100%) | 0 | 1 (100% ) | 0 |
| β-lactamases | $bla_{TEM}$ | 1 (2%) | 0 | 0 | 1 (100%) | 0 | 1 (100%) | 0 |
| Integron | *IntI*1 | 8 (20%) | 0 | 0 | 4 (50%) | 4 (50%) | 7 (88%) | 1 (12%) |

Key; *aac(6')-lb-cr*- gene encoding AAC(6`)-Ib, *aph(3')III*- gene encoding *Aph(3')III*, *ant(4')-IIb*- genes encoding *ANT(4')-IIb*,$bla_{CTX-M}$,$bla_{SHV}$,$bla_{OXA-1}$, $bla_{TEM}$-Genes encoding β-lactamases with extended spectra, *IntI*1- Integron

Compared to *E. coli* and *K. pne umoniae*, *P. aeruginosa* isolates had a low prevalence of all the genes tested. Unlike the *E. coli* isolates, most *P. aeruginosa* isolates from urine samples did not carry any of the genes tested. While the aph (3') III and ant (4′)-IIb genes were detected in three and one isolates, respectively. These isolates did not detect the aac (6')-lb gene encoding an aminoglycoside- modifying enzyme. The $bla_{CTX-M}$ gene was also not detected in bed sores isolates, and carriage of other β-lactamases in this collection was also poor. None of the *P. aeruginosa* isolates obtained from urine tested positive for these genes. Instead, these genes were detected among isolates obtained from surgical wounds. This was in sharp contrast with *E. coli* strains from urine samples that tested positive for the genes tested.

### 4.6 Resistance Profile of *E. coli* to Various Classes of Antibiotics in Relation to Age Groups

Resistance of isolates to the antibiotics for all the age groups showed that the higest resistance with ampicillin at 83%, trimethoprim-sulfamethoxazole and piperacillin at 81% each, amoxicillin-clavulanic acid at 66.7%, tetracycline 64%, cefotaxime 54% and kanamycin 50%. Resistance profile for others was nalidixic acid and ciprofloxacin 43% each, gentamicin and chloramphenicol at 38%, ofloxacin at 33%, and amikacin at 21%. The least resistant were imipenem at 7.1 %, piperacillin-tazobactam at 12 %, ceftazidime, and amikacin at 21 % each.




**Key:** CN=Gentamicin, AK=Amikacin, K=Kanamycin, CAZ= Ceftazidime, CTX=Cefotaxime, OFL=Ofloxacin, PRL=Piperacillin, TZP=Piperacillin/Tazobactam, AMC=Amoxycillin Clavulanic, IMP=Imipenem, CIP=Ciprofloxacin, AMP=Ampicillin, SXT=Sulfamethoxazole-trimethoprim, C=Chloramphenicol, NA=Nalidixic acid, TET=Tetracycline

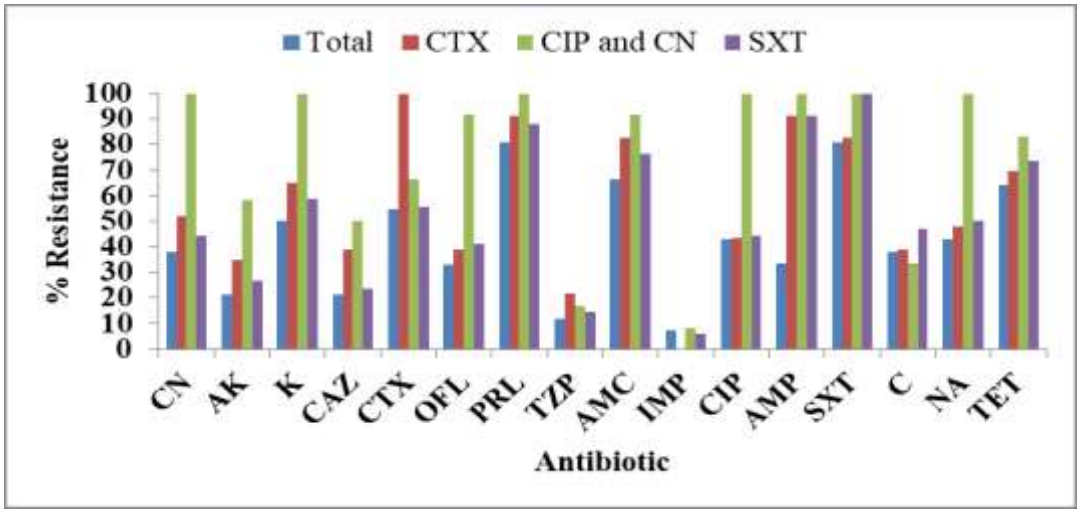
**Figure 4.4: Resistance Profile of *E. coli* to Various Classes of Antibiotics in Relation to Age Groups**

Resistance profiles of *E. coli* showed that the majority of strains were resistant to penicillins, such as ampicillin and piperacillin. Resistance was equally high for tetracycline, sulfamethoxazole-trimethoprim, and kanamycin. Based on the proportion of resistant strains, the most effective antibiotics were ceftazidime, piperacillin-tazobactam, and imipenem. Among aminoglycosides, amikacin, gentamicin, and kanamycin were the most effective, respectively.

A comparison of resistance profiles of isolates obtained from two age groups, , those between 0 to 59 years and those above 60 years, showed no significant differences in resistance to combinations of antibiotics between the two groups (P: 0.9115).

### 4.7 Comparison of Resistance Patterns among Strains Showing Resistance to a Cephalosporin, Combination Resistance to Ciprofloxacin and Gentamicin and those Resistant to Sulfamethoxazole- Trimethoprim

Resistance patterns of isolates resistant to a combination of ciprofloxacin and gentamicin were 100% for gentamicin, kanamycin, piperacillin, ampicillin, ciprofloxacin, nalidixic acid, and sulfamethoxazole-trimethoprim. Resistance to ofloxacin was 91%, tetracycline 83%, amoxicillin-clavulanic acid 76%, cefotaxime 66%, amikacin 58%, ceftazidime 50%, and chloramphenicol 33%. Imipenem showed the least resistance at 8.3%.




**Key:** CN=Gentamicin, AK=Amikacin, K=Kanamycin, CAZ= Ceftazidime, CTX=Cefotaxime, OFL=Ofloxacin, PRL=Piperacillin, TZP=Piperacillin/Tazobactam, AMC=Amoxycillin Clavulanic, IMP=Imipenem, CIP=Ciprofloxacin, AMP=Ampicillin, SXT=Sulfamethoxazole-trimethoprim, C=Chloramphenicol, NA=Nalidixic acid, TET=Tetracycline

**Figure 4.5: Comparison of Resistance Patterns of *E. coli* among Strains Showing Resistance to a Cephalosporin, Combination Resistance to Ciprofloxacin and Gentamicin, and those Resistant to Sulfamethoxazole-Trimethoprim.**

Resistance patterns were compared among isolates showing resistance to at least a cephalosporin such as cefotaxime (CTX), and those with combined resistance to ciprofloxacin and gentamicin and those resistant to sulfamethoxazole-trimethoprim. Those with combined resistance to ciprofloxacin and gentamycin were resistant to more combinations of antibiotics than those susceptible to these antibiotics (P=0.0066).

**Table 4.6: Percentage (%) Number of *E. coli* Isolates with Specific Resistance Phenotypes from Different Hospital Wards**

| **Resistance phenotype combinations** | **Number(%) of isolates with this phenotype (N=42)** | | **Number (%) of isolates with a given phenotype per hospital ward** | | |
|---|---|---|---|---|---|
| | | | **Surgical (n=12)** | **Renal (n=16)** | **Burns (n=14)** |
| **Aminoglycosides** | | | | | |
| Resistant to:- | Susceptible to:- | | | | |
| K, CN, AK | None | 16 (38%) | 5 (42%) | 8 (42%) | 3 (21%) |
| K | CN, AK | 19 (45%) | 6 (50%) | 9 (64%) | 4 (29%) |
| β-lactams | | | | | |
| Resistant to:- | Susceptible to:- | | | | |
| None | PIP, CTX, CAZ | 9 (21 %) | 4 (33%) | 2 (14%) | 3 (21%) |
| PIP and AMP | CTX, CAZ | 7 (17%) | 4 (33%) | 3 (21%) | 0 |
| PIP, CTX, CAZ | IMP | 9 (21%) | 2 (17%) | 5 (36%) | 2 (14%) |
| CTX, CAZ or IMP | | 3 (7%) | 0 | 3 (21%) | 0 |
| β-lactamase inhibitors | | | | | |
| Resistant to:- | Susceptible to:- | | | | |
| AMC | TZP | 21 (50%) | 5 (42%) | 12 (86%) | 4 (29%) |
| TZP | AMC | 16 (38%) | 6 (50%) | 8 (57%) | 2 (14%) |
| Any other β-lactams | TZP | 14 (33%) | 3 (25) | 10 (71%) | 1 (7%) |
| (Fluoro)quinolones | | | | | |
| Resistant to:- | Susceptible to:- | | | | |
| None | NA, CIP or OFL | 9 (21%) | 2 (17%) | 6 (43%) | 1 (7%) |
| NA | CIP, OFL | 13 (31%) | 3 (25%) | 8 (57%) | 2 (14%) |
| NA, CIP, or OFL | none | 9 (21%) | 2 (17%) | 5 (35%) | 2 (14%) |
| Others | | | | | |
| Resistant to:- | Susceptible to:- | | | | |
| SXT | Any other antibiotic | 12 (29%) | 3 (25%) | 7 (50%) | 2 (14%) |
| Any other antibiotic | SXT | 5 (12%) | 3 (25%) | 0 | 2 (14%) |
| SXT, TET | no other antibiotic | 9 (21%) | 3 (25%) | 5 (35%) | 1 (7%) |

**Key:** CN=Gentamicin, AK=Amikacin, K=Kanamycin, CAZ= Ceftazidime, CTX=Cefotaxime, OFL=Ofloxacin, PRL=Piperacillin, TZP=Piperacillin/Tazobactam, AMC=Amoxycillin Clavulanic, IMP=Imipenem, CIP=Ciprofloxacin, AMP=Ampicillin, SXT=Sulfamethoxazole-Trimethoprim, C=Chloramphenicol, NA=Nalidixic acid, TET=Tetracycline

A total of 42 *E. coli* isolates were investigated for resistance to different combinations of antibiotics. Among the 12 and 16 isolates, from surgical and renal specimens, respectively at least 42% were resistant to all aminoglycosides. This phenotype was less common (21%) among the 14 isolates from burns wounds and bed sores. The majority of isolates from surgical (50%) and those from renal (64%) were resistant to most aminoglycosides but remained susceptible to gentamicin and amikacin. However, this resistance pattern to aminoglycosides was only observed in 29% of isolates from burn wounds and bed sores.

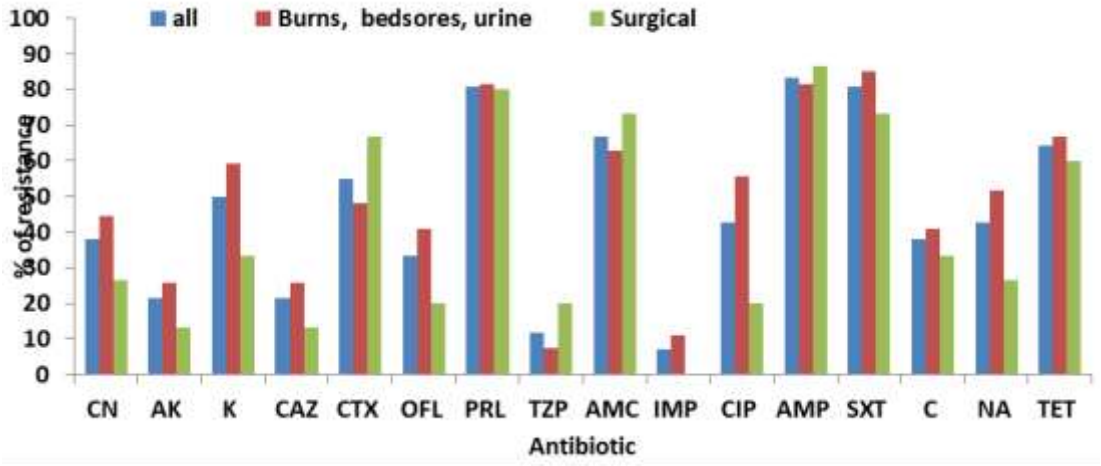
Of the 42 *E. coli* isolates, 9 (21.4%) exhibited combined resistance to penicillins, monobactams, and cephalosporins but were susceptible to imipenem. Five of these isolates were obtained from urine specimens, two from burns wounds, and others from surgical specimens. However, only 3 *E. coli* isolates (all from urine specimens) were resistant to all classes of β-lactams, including imipenem. At least half of the isolates from surgical (50%) and those from urine (57%) specimens were resistant to various cephalosporins and amoxicillin-clavulanic, while only 14% of isolates from wounds exhibited such phenotypes.

Fluoroquinolones are frequently used as alternative antibiotics for treating infections caused by β-lactams -resistant *E. coli* isolates. It was established that nine isolates resistant to β-lactams were susceptible to fluoroquinolones, with majority of these isolates (67%) being from urine samples. Another thirteen isolates that are resistant to nalidixic acid but susceptible to other fluoroquinolones were also identified, with (62%) of these also from urine specimens. Most isolates resistant to chloramphenicol, sulfamethoxazole-trimethoprim, and tetracycline (55%) were also resistant to cephalosporins, while only (35%) of the isolates were resistant to fluoroquinolones.

### 4.8 Resistance Patterns of *E. coli* from Different Sources of Specimen

Resistance of isolates to antibiotics in *E. coli* from non-surgical sites (burns, bedsores, urine) highest for sulfamethoxazole-trimethoprim at 85%, ampicillin, and piperacillin at 81.5%, tetracycline at 67%, amoxicillin-clavulanic acid at 62.9%, kanamycin at 59%, ciprofloxacin at 55.6% and nalidixic acid at 51.8%. Other

resistance patterns were cefotaxime at 48%, gentamicin at 44.4%, ofloxacin, chloramphenicol at 40.7% each, and ceftazidime and amikacin at 25.9% each. The least resistant was observed for imipenem and piperacillin-tazobactam at 11.1% and 7.4%, respectively. All isolates from surgical sites were susceptible to imipenem. Other antibiotics with low resistance were amikacin and ceftazidime at 13.3%, ofloxacin, ciprofloxacin, and piperacillin-tazobactam at 20%, gentamicin, and nalidixic acid at 27% and kanamycin and chloramphenicol at 33%. The highest resistance was noted in ampicillin at 87%, piperacillin at 80%, sulfamethoxazole-trimethoprim and amoxicillin-clavulanic acid at 73%, cefotaxime at 66.7% and tetracycline at 60%.




**Key:** CN=Gentamicin, AK=Amikacin, K=Kanamycin, CAZ= Ceftazidime, CTX=Cefotaxime, OFL=Ofloxacin, PRL=Piperacillin, TZP=Piperacillin/Tazobactam, AMC=Amoxycillin Clavulanic, IMP=Imipenem, CIP=Ciprofloxacin, AMP=Ampicillin, SXT=Sulfamethoxazole-Trimethoprim, C=Chloramphenicol, NA=Nalidixic acid, TET=Tetracycline

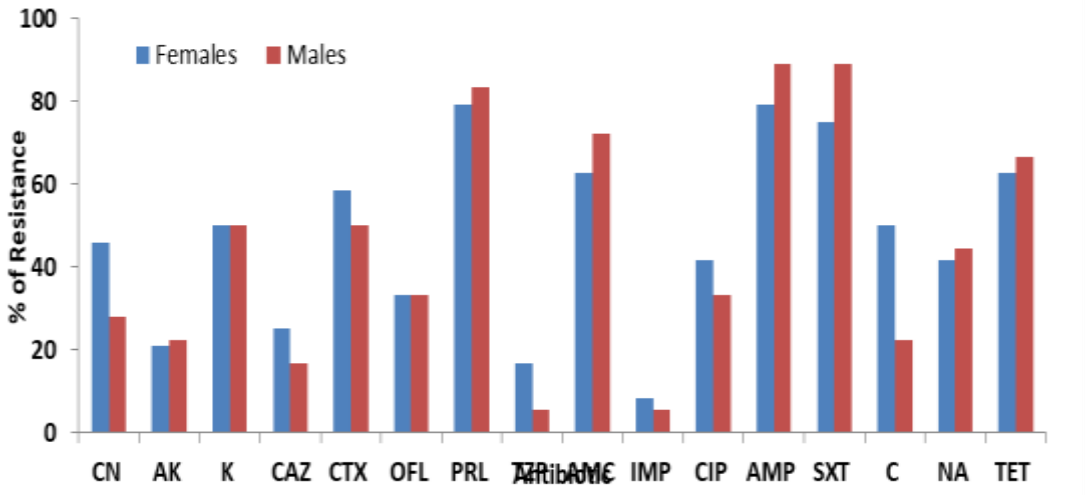
**Figure 4.6: Resistance to Multiple Antibiotics Patterns of *E. coli* from Different Sources of Specimen**

Comparison of resistance patterns between isolates from surgical sites and those from burns, bedsores and urine combined. Showed that isolates from non-surgical sites were slightly more resistant than those from surgical specimen (P= 0.0261).

### 4.9 Comparison of Resistance Patterns of *E. coli* in Relation to Gender

Among male isolates, resistance to antibiotics was highest for ampicillin and sulfamethoxazole-trimethoprim at 88.8%, piperacillin at 83%, amoxicillin-clavulanic acid at 72%, tetracycline 67%, nalidixic acid 44.4% ofloxacin, and ciprofloxacin were 33.3%, gentamicin 27.7%, amikacin, and chloramphenicol were at 22.2%. The most effective antibiotics were imipenem and piperacillin-tazobactam at 5.6%, and ceftazidime at 16.6%. For female isolates was highest for ampicillin and piperacillin at 79%, sulfamethoxazole at 75%, amoxicillin-clavulanic acid and tetracycline at 62.5%, cefotaxime at 58%, chloramphenicol at 50%, gentamycin at 45.8%, nalidixic acid and ciprofloxacin at 41.6%, ofloxacin at 33.3%, ceftazidime at 25%, amikacin at 20.8%. The most effective were imipenem and piperacillin-tazobactam at 8.3% and 16.7%, respectively. Cefotaxime showed a resistance of 50% in both genders.

Comparing resistance patterns between *E. coli* strains from males and females revealed no significant differences (P=0.8575)




**Key:** CN=Gentamicin, AK=Amikacin, K=Kanamycin, CAZ= Ceftazidime, CTX=Cefotaxime, OFL=Ofloxacin, PRL=Piperacillin, TZP=Piperacillin/Tazobactam, AMC=Amoxycillin Clavulanic, IMP=Imipenem, CIP=Ciprofloxacin, AMP=Ampicillin, SXT=Sulfamethoxazole-Trimethoprim, C=Chloramphenicol, NA=Nalidixic acid, TET=Tetracycline

**Figure 4.7: Comparison of Multiple Antibiotics Resistance Patterns of *E. coli* in Relation to Gender**

Analysis showing distribution of *Escherichia coli* isolates with carriage of various resistance markers was done and presented in terms of specimen source and gender

**Table 4.7: Distribution of *E. coli* Isolates Carrying Various Resistance Markers**

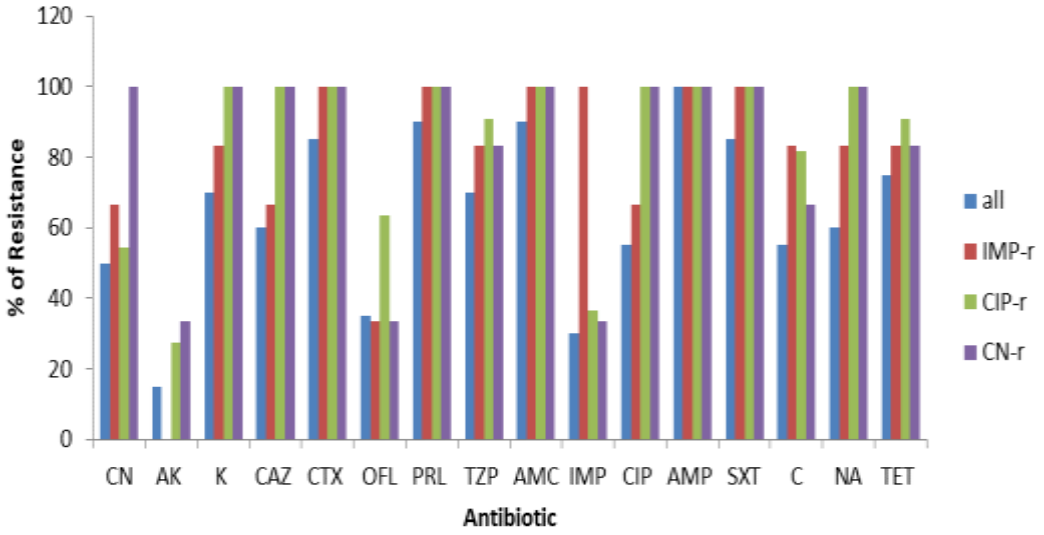
| | | | Specimen sources | | | | Gender | |
|---|---|---|---|---|---|---|---|---|
| **Resistance mechanism** | **Gene** | **Prevalence N=41** | **urine** | **bed sores** | **Surgery** | **burns** | **male** | **female** |
| ciprofloxacin/ gentamicin cross resistance | *aac(6')-lb-cr* | 14 (33%) | 8 (57%) | 2 (14%) | 4 (29%) | 0 | 4 (28%) | 10 (72%) |
| aminoglycoside phosphotransferase | *aph(3')III* | 7 (17%) | 3 (43%) | 2 (29%) | 1 (14%) | 1 (14%) | 3 (43%) | 4 (57%) |
| β-lactamases | $bla_{CTX-M}$ | 9 (21%) | 4 (44%) | 2 (22%) | 1 (11%) | 1 (11%) | 3 (33%) | 6 (66) |
| β-lactamases | $bla_{TEM}$ | 4 (10%) | 3 (75%) | 0 | 1 (25%) | 0 | 1 (25%) | 3 (75%) |
| β-lactamases | $bla_{SHV}$ | 4 (10%) | 3 (75%) | 0 | 1 (25%) | 0 | 1 (25%) | 3 (75%) |
| β-lactamases | $bla_{OXA-1}$ | 4 (10%) | 3 (75%) | 0 | 1 (25%) | 0 | 1 (25%) | 3 (75%) |
| Integron | *IntI*1 | 14 (33%) | 7 (50%) | 3 (21%) | 3 (21%) | 1 (7%) | 7 (50%) | 7 (50%) |

Key; *aac(6')-lb*- gene encoding AAC(6`)-Ib, *aph(3')III*- gene encoding *Aph(3')III*, *ant(4')-IIb*-genes encoding *ANT(4')-IIb*,$bla_{CTX-M}$,$bla_{SHV}$,$bla_{OXA-1}$, $bla_{TEM}$.Genes encoding β-lactamases with extended spectra, *IntI*1- Integron

The prevalence of selected genes among *E. coli* isolates is shown. The integrons occurred at a prevalence of 33%. Integrons were detected in isolates obtained from all specimen types. 50% of the integrons were detected among isolates obtained from the urine. These elements were equally distributed among isolates obtained from male and female patients. Urine and surgical specimens tested positive for all the genes tested, but isolates from bedsore wounds and wounds from burns did not carry the majority of β-lactamases. In general, isolates recovered from female patients, especially those from suspected UTI cases, were more likely to test positive for the genes tested than those from male patients (P= 0.010). Thus there was a significant difference in the genes prevalence among patient isolates from different wards.

### 4.10 Resistance of *Klebsiella pneumoniae* to Various Antibiotics

Resistance patterns in *K. pneumoniae* to a combination of imipenem, ciprofloxacin, or gentamicin was 100% for kanamycin, ceftazidime, cefotaxime, piperacillin, amoxycillin-clavulanic acid, ciprofloxacin, ampicillin, sulfamethoxazole-trimethoprim, and nalidixic acid. Other resistance patterns were amikacin at 27.3%, ofloxacin at 63.6%, piperacillin-tazobactam at 83.3%, imipenem at 36.4%, chloramphenicol at 81.8% and tetracycline at 90.9 %.




**Key:** CN=Gentamicin, AK=Amikacin, K=Kanamycin, CAZ= Ceftazidime, CTX=Cefotaxime, OFL=Ofloxacin, PRL=Piperacillin, TZP=Piperacillin/ Tazobactam, AMC=Amoxycillin Clavulanic, IMP=Imipenem, CIP=Ciprofloxacin, AMP=Ampicillin, SXT=Sulfamethoxazole-Trimethoprim, C=Chloramphenicol, NA=Nalidixic acid, TET=Tetracycline

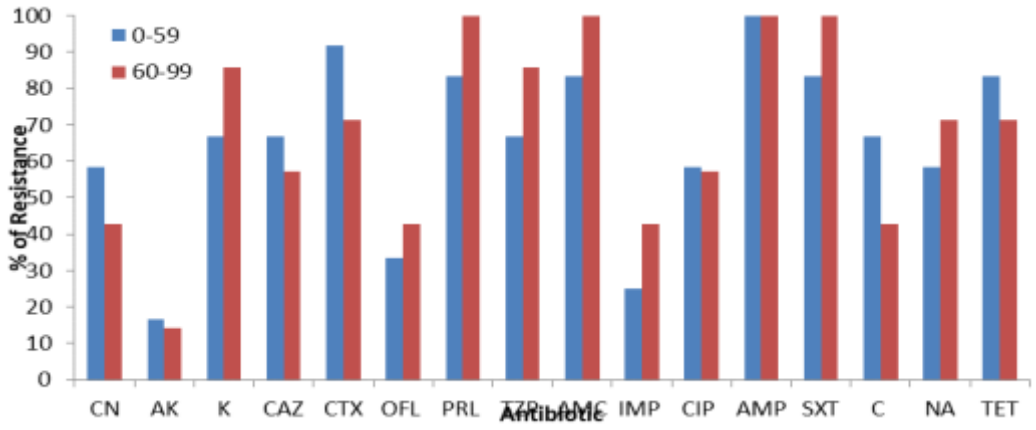
**Figure 4.8: Resistance of *Klebsiella pneumoniae* to Various Antibiotics**

**Analysis for Co-Resistance to Multiple Antibiotics among Isolates Resistant to Imipenem, Ciprofloxacin or Gentamicin.**

*Klebsiella* isolates were analyzed for resistance to various antibiotics. A higher proportion of these isolates were resistant to more combinations of antibiotics than *E. coli* isolates obtained from similar specimens. Isolates resistant to imipenem, ciprofloxacin, or gentamicin were resistant to more combinations of antibiotics than those susceptible to these combinations (P= 0.004).

### 4.11 Resistance Patterns of *K. pneumoniae* Obtained from Different Age Groups

Resistance to multiple antibiotics in the 0-59 age-group was highest in ampicillin 100%, cefotaxime 92%, and piperacillin, amoxicillin-clavulanic acid, sulfamethoxazole-trimethoprim, and tetracycline were (83.3%). Resistance rates for anamycin, ceftazidime, piperacillin-tazobactam and chloramphenicol 66.7%, ciprofloxacin, gentamicin and nalidixic acid were 58.3% and ofloxacin was 33.3%. The least resistant was for amikacin (16.7%), and imipenem, (25%). In the age group 60-99 years, resistance was 100% for piperacillin, amoxycillin-clavulanic acid, and sulfamethoxazole-trimethoprim; kanamycin and piperacillin-tazobactam was 85.7%, cefotaxime; nalidixic acid, and tetracycline was 71.4%; ceftazidime, and ciprofloxacin was 57.1%, while gentamicin, ofloxacin, imipenem, and chloramphenicol were 42.9%. The most effective antibiotic was amikacin, with a resistance of 14.3%.



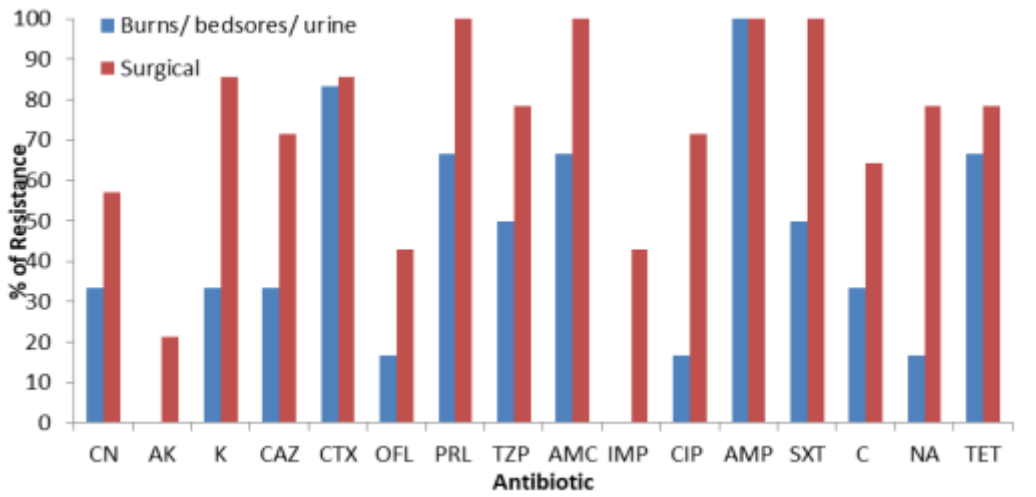

**Key:** CN=Gentamicin, AK=Amikacin, K=Kanamycin, CAZ= Ceftazidime, CTX=Cefotaxime, OFL=Ofloxacin, PRL=Piperacillin, TZP=Piperacillin/Tazobactam, AMC=Amoxycillin Clavulanic, IMP=Imipenem, CIP=Ciprofloxacin, AMP=Ampicillin, SXT=Sulfamethoxazole-Trimethoprim, C=Chloramphenicol, NA=Nalidixic acid, TET=Tetracycline

**Figure 4.9: Resistance Patterns to Multiple Antibiotics of *K. pneumoniae* Obtained from Different Age Groups.**

Analysis of the findings of resistance patterns of *K. pneumoniae* obtained from different age groups showed that there were no significant differences between those obtained from all patients below 59 years and those above the age of 60 (P= 0.8482).

### 4.12 Comparison of *K. pneumoniae Antibiotics* Resistance from Surgical and Non-Surgical Sources

This study showed that there was 100% resistance to ampicillin in isolates from surgical and non- surgical sources, while all isolates from non-surgical sites were susceptible to imipenem and amikacin. Other resistance patterns for isolates from non-surgical sources were cefotaxime at 83.3%, piperacillin, amoxicillin-clavulanic acid, and tetracycline at 66.7%, piperacillin-tazobactam and sulfamethoxazole-trimethoprim 50% and gentamicin, kanamycin, ceftazidime, and chloramphenicol at 33.3%. Ofloxacin, ciprofloxacin and nalidixic acid showed 16.7% resistance. Further resistance in isolates from surgical sources was 100 % also in amoxicillin-clavulanic acid, ampicillin, and sulfamethoxazole-trimethoprim. Kanamycin and cefotaxime were at 85.7%, piperacillin-tazobactam, nalidixic acid, and tetracycline were at 78.6%, ciprofloxacin, and ceftazidime were at 71.4%, chloramphenicol at 64.3%, gentamicin at 57.1%, ofloxacin and imipenem was 42.9%. The least resistance in these isolates was at 21.4% in amikacin.




Key: CN=Gentamicin, AK=Amikacin, K=Kanamycin, CAZ=Ceftazidime, CTX=Cefotaxime, OFL=Ofloxacin, PRL=Piperacillin, TZP=Piperacillin/Tazobactam, AMC=Amoxycillin-Clavulanic, IMP=Imipenem, CIP=Ciprofloxacin, AMP=Ampicillin, SXT=Sulfamethoxazole-Trimethoprim, C=Chloramphenicol, NA=Nalidixic acid, TET=Tetracycline

**Figure 4.10: Comparison of *K. pneumoniae* Antibiotics Resistance from Surgical and Non-Surgical Sources.**

**Analysis for Resistance to Multiple Antibiotics to Isolates from Various Specimen Types.**

While data from *E. coli* isolates showed that isolates from non-surgical sources were more resistant than those from surgical sites, data from *K. pneumoniae* showed that isolates from surgical sites were found to be more resistant to combinations of antibiotics than those from non-surgical sites (P= 0.0025).

**Table 4.8: Percentage (%) Number of *K. pneumoniae* Isolates with Specific Resistance Phenotypes from Different Hospital Wards**

| **Resistance phenotype combinations** | | **Number(%) of isolates with this phenotype (N=41)** | **Number (%) of strains with a given phenotype per hospital ward** | | |
|---|---|---|---|---|---|
| | | | **Surgical (n=12)** | **Renal (n=15)** | **Burns (n=14)** |
| Aminoglycosides | | | | | |
| Resistant to:- | Susceptible to:- | | | | |
| K, GN, AK | None | 21 (51) | 7 (58) | 11 (73) | 3 (21) |
| K | GN, AK | 14 (34) | 3 (25) | 8 (53) | 3 (21) |
| β-lactams | | | | | |
| Resistant to:- | Susceptible to:- | | | | |
| None | PIP, CTX, CAZ | 18 (44) | 9 (75) | 6 (40) | 3 (22) |
| PIP and AMP | CTX, CAZ | 25 (61) | 10 (83) | 9 (60) | 6 (43) |
| PIP, CTX, CAZ | IMI | 17 (42) | 5 (42) | 9 (60) | 3 (22) |
| CTX, CAZ or IMI | None | 6 (15) | 2 (16) | 4 (27) | 0 |
| β-lactamase inhibitors | | | | | |
| Resistant to:- | Susceptible to:- | | | | |
| AMC | TZP | 27 (66) | 10 (83) | 12 (80) | 5 (36) |
| TZP | AMC | 21 (51) | 8 (67) | 12 (80) | 1 (7) |
| None | TZP and AMC | 16 (39) | 4 (33) | 8 (53) | 4 (29) |
| (Fluoro)quinolones | | | | | |
| Resistant to:- | Susceptible to:- | | | | |
| None | NA, CIP or OFL | 21 (51) | 5 (42) | 10 (67) | 6 (43) |
| NA | CIP, OFL | 25 (61) | 6 (50) | 14 (93) | 5 (36) |
| NA, CIP, or OFL | None | 11 (27) | 5 (42) | 6 (40) | 0 |
| Others | | | | | |
| Resistant to:- | Susceptible to:- | | | | |
| TRIM/SUL | any other antibiotics | 12 (29) | 4 (33) | 5 (33) | 3 (22) |
| Any other antibiotics | TRIM/SUL | 5 (12) | 1 (8) | 2 (13) | 2 (14) |
| TRIM/SUL, TET | None | 14 (34) | 3 (25) | 7 (47) | 4 (29) |

**Key:** CN=Gentamicin, AK=Amikacin, K=Kanamycin, CAZ= Ceftazidime, CTX=Cefotaxime, OFL=Ofloxacin, PRL=Piperacillin, TZP=Piperacillin/Tazobactam, AMC=Amoxycillin Clavulanic, IMP=Imipenem, CIP=Ciprofloxacin, AMP=Ampicillin, SXT=Sulfamethoxazole-Trimethoprim, C=Chloramphenicol, NA=Nalidixic acid, TET=Tetracycline

The resistance profiles among *K. pneumoniae* strains were very close to those observed in *E. coli.* Resistance to aminoglycosides was relatively high; in this case, 21(51%) of the 41 isolates were resistant to all aminoglycosides, including kanamycin, gentamicin, and amikacin. Close to 58% of isolates from surgical specimens and 73% of those from urine exhibited this phenotype. This phenotype was, however, much rare among isolates from burns and bed sores, of which only 21% exhibited resistance to these combinations. As with E. *coli* strains, many *K. pneumoniae* strains were susceptible to amikacin and gentamicin. In this regard, 25%, 53%, and 21% of isolates from surgical, urine, and burns/bedsores exhibited such phenotypes towards these aminoglycosides.

Similar to *E. coli*, many *K. pneumoniae* strains were resistant to penicillins but susceptible to other β-lactams. Of the 41 isolates, 17 (42%) were resistant to all other β-lactams but were susceptible to carbapenems. These 17 isolates were from surgical specimens (5), urine (9), and burns (3). There were six isolates of *K. pneumoniae* resistant to imipenem but were susceptible to fluoroquinolones and to at least one class of aminoglycosides (especially amikacin and or gentamicin). Of the six carbapenem-resistant, two of these isolates were from surgical, and four were from the urine; none were from burns or bed sore wounds.

As in *E. coli*, some *K. pneumoniae* strains were resistant to the amoxicillin-clavulanic acid combination but were susceptible to piperacillin-tazobactam (TZP). Twenty seven of the 41 (66%) *K. pneumoniae* isolates exhibited this phenotype. A high proportion (83% and 80%) of isolates from surgical and urine specimens respectively exhibited this phenotype. In contrast, only a small proportion of 36% of isolates from burns and bed sore wounds exhibited this phenotype. Another 21 (51%) isolates were resistant to amoxicillin-clavulanic acid (AMC) but susceptible to at least one or more third-generation cephalosporins. This study also shows that nearly 51% of *K. pneumoniae* strains resistant to cephalosporins and aminoglycosides remained susceptible to fluoroquinolones or nalidixic acid. Ciprofloxacin and ofloxacin were more effective than nalidixic acid; in this case, 61% of strains resistant to nalidixic acid were susceptible to one or both of these fluoroquinolones.

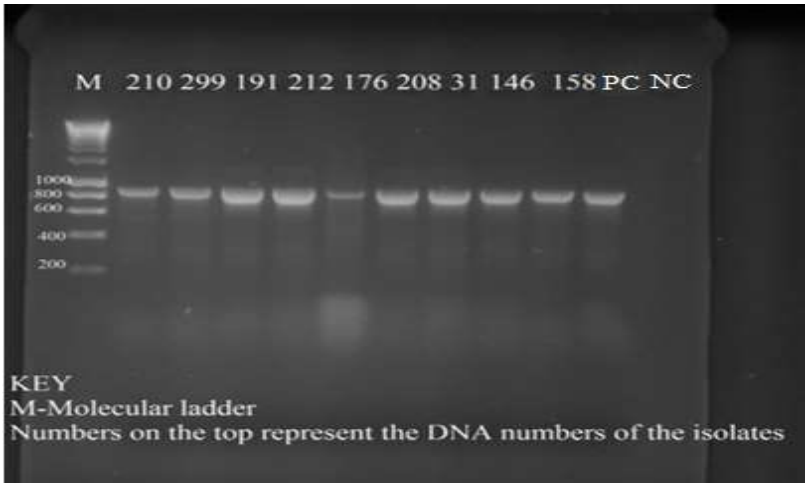
Also, eleven isolates of *K. pneumoniae* were resistant to fluoroquinolones but were susceptible to cephalosporins and imipenem.

**Table 4.9: Distribution of *Klebsiella pneumoniae* Isolates Positive for Selected Resistance Markers. Analysis for Resistance Genes in Relation to Specimen Type and Gender**

| | | | Specimen distribution | | | | Gender | |
|---|---|---|---|---|---|---|---|---|
| **Resistance mechanism** | **Gene** | **Prevalence n=41** | **Urine** | **bed sores** | **surgery** | **burns** | **Male** | **Female** |
| Ciprofloxacin/gentamicin cross resistance | *aac(6')-Ib-cr* | 3 (7%) | 3 (100%)) | 0 | 0 | 0 | 0 | 3 (100%)) |
| Aminoglycoside phosphotransferase | *aph(3')III* | 3 (7%)) | 3 (100%)) | 0 | 0 | 0 | 0 | 3 (100%)) |
| 4'-aminoglycoside nucleotidyltransferase | *ant(4')-IIb* | 1 (2%)) | 1 (100%)) | 0 | 0 | 0 | 0 | 1 (100%)) |
| β-lactamases | $bla_{CTX-M}$ | 11 (27%)) | 10 (91%)) | 1 (9%)) | 0 | 0 | 2 (18%)) | 9 (81%)) |
| β-lactamases | $bla_{SHV}$ | 10 (24%)) | 8 (80%)) | 1 (10%)) | 0 | 1 (10%)) | 2 (20%)) | 8 (80%)) |
| β-lactamases | $bla_{OXA-1}$ | 10 (24%)) | 8 (80%)) | 1 (10%)) | 0 | 1 (10%)) | 2 (20%)) | 8 (80%)) |
| β-lactamases | $bla_{TEM}$ | 10 (24%)) | 8 (80%)) | 1 (10%)) | 0 | 1 (10%)) | 2 (20%)) | 8 (80%)) |
| Integron 1 | *IntI*1 | 8 (20%)) | 6 (75%)) | 1 (12.5%) | 1 (12.5%)) | 0 | 2 (25%)) | 6 (75%)) |

In comparison with *E. coli* and *P. aeruginosa* isolates, a significant proportion of *K. pneumoniae isolates* were positive for all genes tested in this study.

Carriage of β-lactamases was prevalence in 24% and 27% of isolates. Integrons were detected in 20% of these isolates. Most genes were detected among isolates recovered from urine samples, whereas these were rare among isolates from surgical wounds. In general, isolates recovered from female samples were more likely to test positive for any gene tested than those from male patients (P=0.029).




Lanes: M: Molecular Marker, PC: Positive Control, NC: Negative Control, Samples: 210, 299,191, 212, 176, 208, 31, 146, 158

**Figure 4.11: PCR Amplification of TEM β-Lactamases among *E. coli, P. aeruginosa* and *K. pneumoniae* Isolates.**

Analysis for prevalence of $bla_{TEM}$ was 10% in *E. coli, 20 % in P. aeruginosa,* and 24% *K. pneumoniae.*

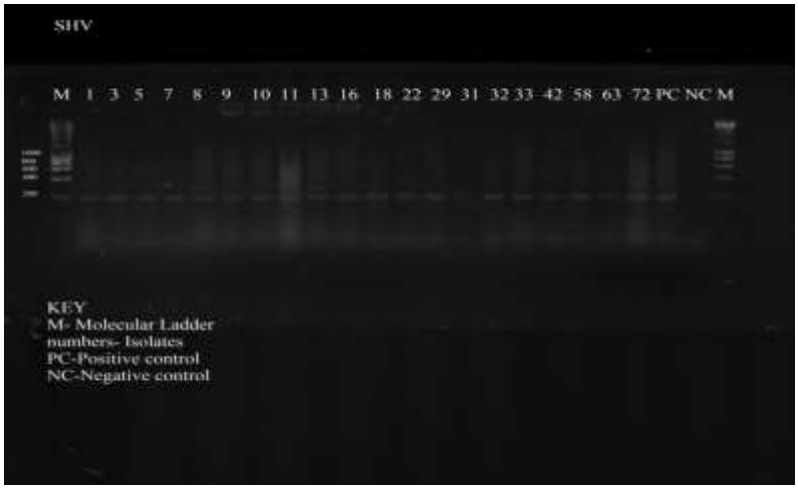
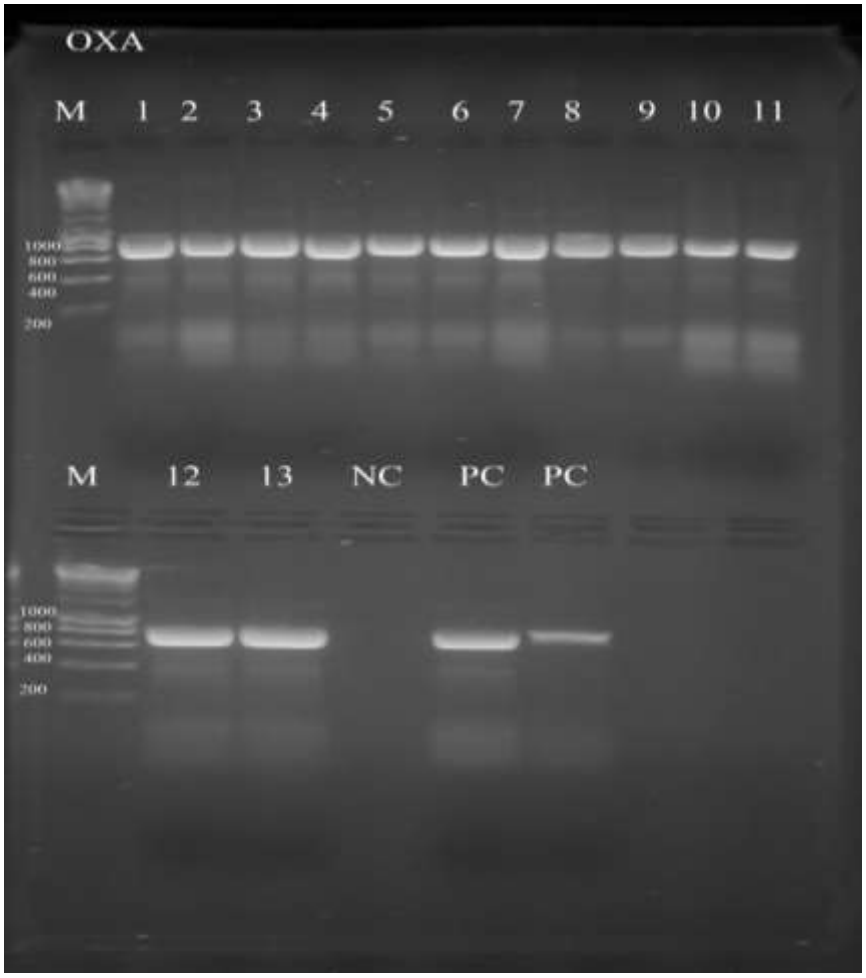



**Figure 4.12: PCR Amplification of SHV β-Lactamases among *E. coli, P. aeruginosa* and *K. pneumoniae***

Analysis for prevalence of $bla_{SHV}$ was 10% in *E. coli, 2 %* in *P. aeruginosa,* and 24% *K. pneumoniae*




Lanes: M: Molecular Marker, PC: Positive Control, NC: Negative Control, Samples: 1-13

**Figure 4.13: PCR Amplification of OXA β-Lactamases among. *E. coli, Pseudomonas aeruginosa* (*Ps*) and *Klebsiella pneumoniae* (*Kleb*) Isolates.**

Analysis for prevalence of $bla_{OXA}$ was 10% in *E. coli, 2 % in P. aeruginosa,* and 24% *K. pneumoniae* isolates

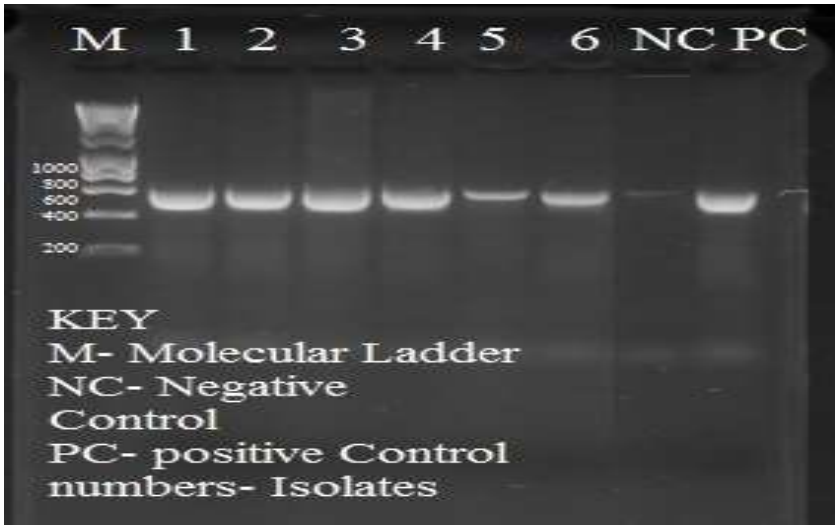



**Figure 4.14: PCR Amplification of CTX-M β-Lactamases among *E. coli* and *Klebsiella pneumoniae***

Analysis for prevalence of CTX-M β-lactamases was 21 % in *E. coli*, 17% in *P. aeruginosa*, and 27% in *K .pneumoniae* isolates

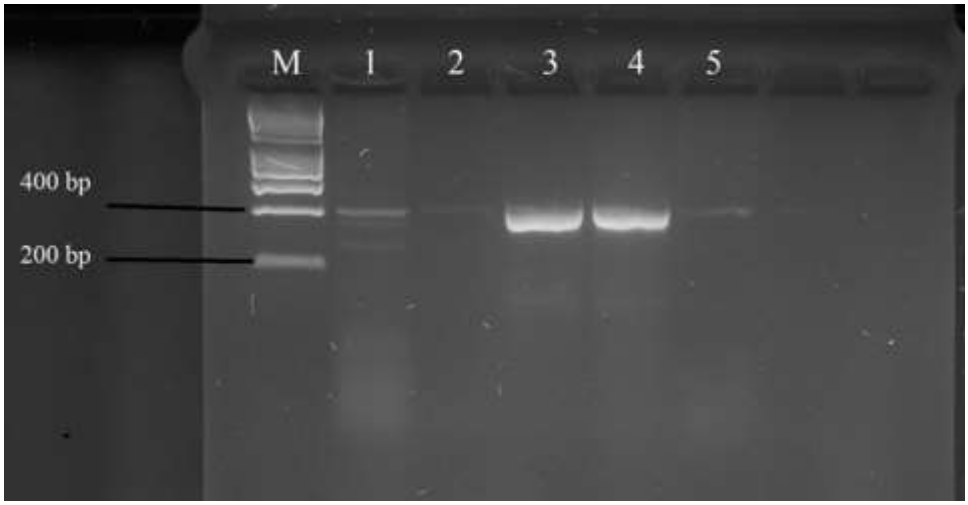



**Figure 4.15: PCR Amplification of aac (6')-lb-cr among *E. coli* and *K. pneumoniae* Isolates.**

Lanes: Molecular marker, 1, 3 and 5 are test samples while 2=negative controls and 4=positive control Analysis for prevalence of aac (6')-1b-cr β-lactamases was 33 % in *E. coli* and 7% in *K .pneumoniae* isolates

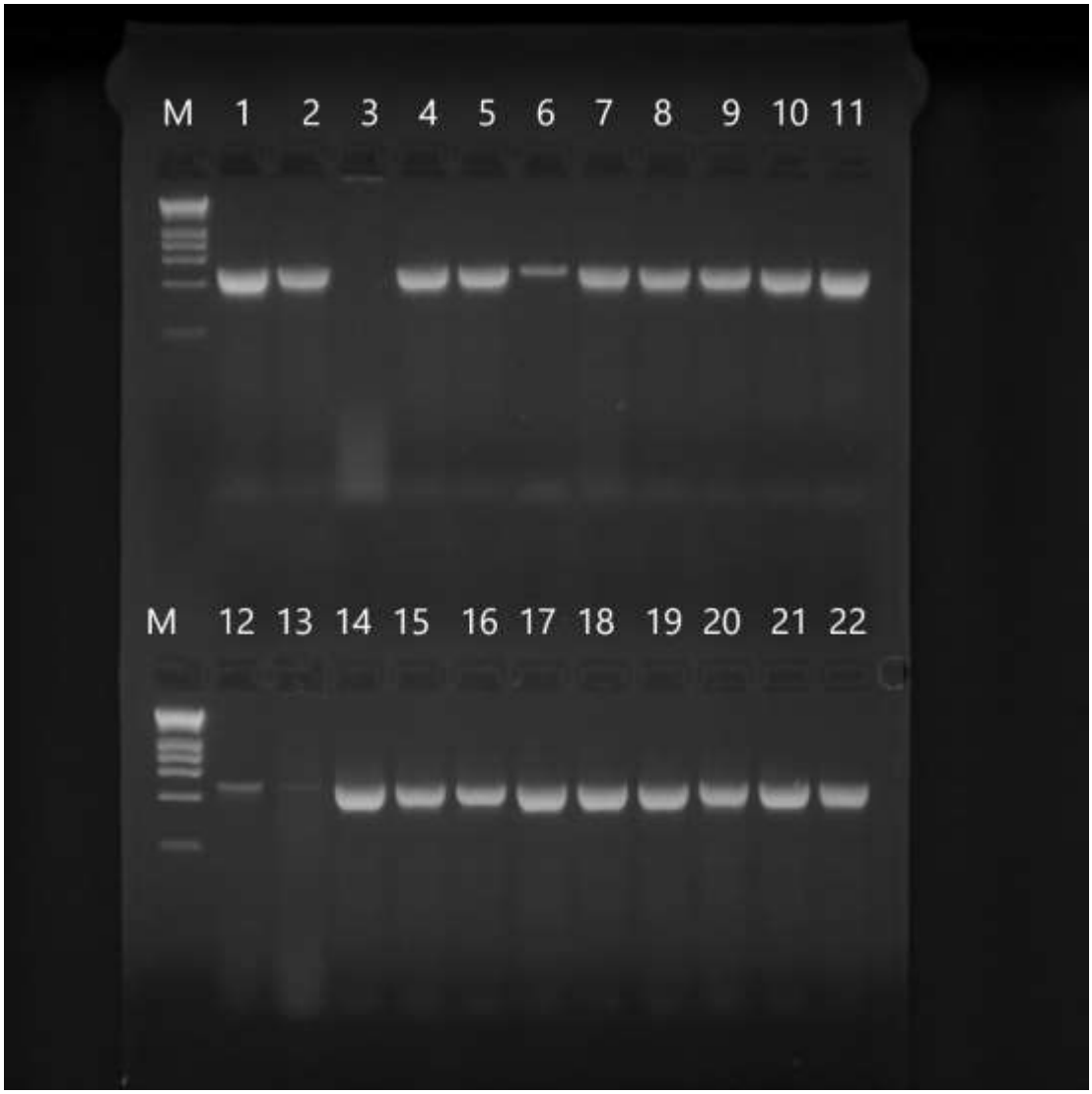



**Figure 4.16: PCR Amplification Showing aph (3) iii Gene among *E. coli, Pseudomonas aeruginosa* (*Ps*) and *Klebsiella pneumoniae* (*Kleb*) Isolates.**

Lanes: M: Molecular marker, 1, 2, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 are all test samples whereas 3= Negative Control and 21 and 22= Positive Control

Analysis for prevalence of aph (3') iii gene was 17 % in *E. coli* and 7% in both *P. aeruginosa* and *K .pneumoniae* isolates


**Figure 4.17: PCR Amplification Showing Class I Integron Gene among *E. coli, Pseudomonas aeruginosa* (*Ps*) and *Klebsiella pneumoniae* Isolates (*Kleb*)**

Analysis for prevalence of class 1integron gene was 33 % in *E. coli* and 20 % in both *P. aeruginosa* and *K .pneumoniae* isolates

# CHAPTER FIVE

# DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

## 5.1 Discussion

The results of the antibiotic resistance pattern are alarming, as the predominant bacterial isolates in Kenya showed high resistance to commonly available antibiotics. Most isolates from all specimen types were found to be multi-drug resistant (MDR) (Table 4.4, Table 4.6 and Table 4.8). Similar results have been published before (Dou *et al*., 2017), indicating a consistent problem. The study revealed that isolates from burns and surgical wounds were the second most resistant after those from urinary tracts. *P. aeruginosa* isolates were rare compared to *E. coli* and *Klebsiella pneumoniae* isolates.

Although most isolates were susceptible to carbapenems, some *E. coli*, *Klebsiella*, and *Pseudomonas* isolates resistant to imipenem were identified. Carbapenems are still considered drugs of choice against the isolates investigated, as reported in a related study (Gajdács *et al*., 2019). The study revealed that a significant proportion of isolates from wounds and burns are highly multi-drug resistant. Burn patients are especially susceptible to infections due to the disruption of the skin's mechanical integrity and immune suppression (Salman *et al*., 2020). The protein-rich, avascular environment in burns provides a favorable niche for microbial colonization and proliferation. The use of topical microbial applications containing heavy metals like silver, neomycin, and tetracycline in these patients may promote the emergence of highly resistant strains. Such practices may promote the emergence of highly resistant strains.

Interestingly, the frequency of *P. aeruginosa* (9.1%) was lower than in previous reports, where it is often responsible for most invasive burn wound infections (Wardhana *et al*. , 2017). The proliferation of highly resistant isolates, as observed in burns and wounds, poses a challenge in finding effective treatment options (Abdi *et al*., 2020).

In the case of urinary tract isolates *E. coli* and *K. pneumoniae* isolates were more resistant to antibiotics compared to *K. pneumoniae,* and *P. aeruginosa* isolates. Regardless of the species, isolates from non-surgical sites, especially from urine, were slightly more resistant than those from surgical specimens (P= 0.0261). Similarly, high resistance among urine isolates has been reported in previous studies. For instance, resistance rates among isolates of *E. coli* isolated from women with UTI average 30% for both sulphonamides and ampicillin, varying from 17% to 54% in different countries (Morrill *et al*., 2017). Sulfamethoxazole-trimethoprim resistance ranges from 11% in Scandinavian countries to 34% in Spain and Portugal, while fluoroquinolone resistance can reach 20% in southern Europe (Dalhoff, 2012). In Spain, where antibiotics can be used without restrictions, reduced susceptibility to sulfamethoxazole-trimethoprim (SXT) and fluoroquinolones have been reported to be as high as 26% and 16% respectively among *E. coli* isolates from UTI patients (Campos *et al*., 2018). However, the isolates obtained from urine in this study were more susceptible to common antibiotics used to treat UTIs compared to reports from other countries (Zivanovic *et al*., 2017).

Most isolates from urinary tracts were multi-drug resistant (MDR), and a significant proportion of these were resistant to sulfamethoxazole-trimethoprim (SXT). These isolates were also resistant to other antibiotics, limiting the choice of alternative antibiotics. These results indicated similar sensitivity patterns among the uropathogenic observed in Kolkata, India. International clinical practice guidelines recommend sulfamethoxazole-trimethoprim and ampicillin as the first drug of choice for UTI treatment and empiric treatment for uncomplicated urinary tract infections (Mullakary *et al*., 2021). In contrast, as revealed in the present study, SXT cannot be the treatment of choice among most of these isolates. A recent report also indicated that SXT and ampicillin were not suitable drugs of choice for treating UTIs (Jodal, 2021). However, another study showed that amikacin and nitrofurantoin are the most effective treatments for UTIs in China. Since inappropriate use of antibiotics may lead to complications and treatment failure, this study suggests that there is a need to have an idea of the resistance phenotypes of isolates from urine before commencing treatment. It is, therefore, important to conduct culture and susceptibility testing on

all bacterial isolates to determine susceptibility patterns to avert the development of resistant mutants.

As with *E. coli* and *K. pneumoniae* isolates, most isolates resistant to co-trimoxazole were also resistant to other antibiotics, especially β-lactams. The second and third-generation cephalosporins were effective against most isolates investigated in this study regardless of the bacterial isolates. This study also reveals that some *E. coli, K. pneumoniae,* and *P. aeruginosa* isolates were resistant to at least one β-lactam. The present study also showed considerable resistance to cefotaxime and ceftazidime among *E. coli, K. pneumoniae,* and *P. aeruginosa* isolates. Other isolates were resistant to amoxicillin-clavulanic acid (AMC) but remained susceptible to ceftazidime (CAZ) and piperacillin-tazobactam (TZP) combinations. These results, therefore, show that TZP and CAZ are more potent antibiotics against isolates belonging to the three species.

In recent years, a number of authors have reported the emergence of the CTX-M Extended Spectrum Beta-Lactamases (ESBLs) worldwide (Quiñones *et al*., 2020). The emergence of ESBL-producing strains among *Enterobacteriaceae* (*E. coli, K. pneumoniae)* is of special concern. This is because these otherwise commensals strains are capable of causing serious infections and can exhibit high MDR phenotypes (Corrado *et al*., 2017). There is, therefore, a need to check the spread of ESBL-producing strains such as those reported in this study.

The ESBL phenotype was more prevalent among *E. coli* from urine, while none of *the P. aeruginosa* isolates exhibited a true ESBL phenotype (Table 4.5 and Table 4.7). Carriage of β-lactamases was in the prevalence of between 24% and 27%. This study showed that $bla_{CTX\text{-}M}$, $bla_{SHV}$, $bla_{OXA\text{-}1}$, and $bla_{TEM}$ were particularly associated with resistance to advanced classes of β-lactams. The gene $bla_{CTX\text{-}M}$ was particularly implicated in the resistance to advanced cephalosporins such as ceftazidime. In contrast, resistance to AMC was common among isolates carrying a combination of *blaSHV* gene, especially $bla_{CTX\text{-}M}$ *and* $bla_{OXA\text{-}1}$.

Compared with *E. coli* and *Pseudomonas isolates*, most *Klebsiella* isolates were positive for all genes tested in this study (Table 4.9). Most genes were detected

among isolates recovered from urine samples but rare among isolates from surgical wounds. In general, isolates recovered from female samples were more likely to test positive for any gene tested than those from male patients (P: 0.029). These results further show that urine samples are likely to yield highly resistant isolates compared to other samples. This is probably due to the high usage of different classes of antibiotics to treat complicated and uncomplicated UTIs.

In another study, it was reported that the majority of patients with UTI are those under the age of 50 years, and the predominant bacterium is likely to be *E. coli* (Lewis and Gilbert, 2020). These reports are in agreement with the findings of this study. This has been attributed to the fact that otherwise healthy non-pregnant women with normal genitor-urinary tract may suffer from acute uncomplicated UTI (uUTI) more frequently than males, hence more likely to harbor MDR strains (Dubbs *et al*., 2019). Other studies have found significant geographical variations in resistance among urine isolates from females (Gunduz *et al*., 2018). The most important driving factor of resistance is the overuse of antibiotics (Dadgostar, 2019). Frequent episodes of UTIs may lead to an increase in patient morbidity, costs of reassessment and re-treatment, and the use of broader spectrum antibiotics. Females who suffer frequent episodes of complicated or uncomplicated UTI may have a higher risk of colonization with MDR strains. Although past studies reported that MDR UTI isolates are common among post-menopausal women owing to the loss of estrogen and consequent changes in vaginal flora (Shaheen *et al*., 2019), this data did not reveal the difference between resistances among isolates from women of different ages. This study also found that, in general, isolates recovered from female patients, especially those from suspected UTI cases, were more likely to test positive for resistance genes tested than those from male patients (P= 0.010). Based on data from this study, women may represent an important reservoir for MDR *E. coli*, *K. pneumoniae*, and *P. aeruginosa* strains.

Comparing *E. coli* and *Klebsiella pneumoniae*, *Pseudomonas* isolates had a low prevalence of all the genes tested. This is possible because antibiotic resistance among *P. aeruginosa* isolates is mediated by intrinsic mechanisms such as drug efflux pumps rather than horizontally acquired genes.

The study did not observe significant differences in resistance patterns between isolates obtained from patients below 59 years of age and those above 60 years (P:0.8482). This contrasts studies that have reported that age influences and impacts resistance rates, especially in urinary tract infections (Medina and Castillo- Pino, 2019). From the current study findings, we may infer a pattern of misuse of antibiotics across all ages. This study found that bacterial isolates with combined resistance to ciprofloxacin and gentamicin were more likely to exhibit resistance to more combinations of antibiotics than those susceptible to these antibiotics (P= 0.0066). This may be due to the high use of fluoroquinolones since it is considered the antibiotic group of choice, especially in treating UTIs. Since most ciprofloxacin is given without laboratory investigations, misuse of these antibiotics to treat various infections drives the resistance trends observed among *E. coli, Klebsiella pneumoniae,* and *P. aeruginosa* investigated in the current study. Similar reports have been made in countries like Germany (Dadgostar, 2019). Several studies have shown that physicians' prescribing habits drive antibiotic resistance (Machowska and Stålsby, 2019). Although prescription habits have not been properly studied in Kenya, it was found that 37% of physicians prescribe SXT, closely followed by fluoroquinolones (32%), and the average duration of antibiotic therapy was 8.6 days in the United States (Townsend *et al*., 2018). It was revealed that fluoroquinolones were Israel's most frequently prescribed drugs (25.57%) (Gomila *et al*., 2018). It has also been reported that resistance against fluoroquinolones is strongly associated with many prescriptions for this group of antibiotics (Dadgostar, 2019). Inappropriate use of cheaper fluoroquinolones such as ciprofloxacin may necessitate the use of more effective but more expensive alternatives such as moxifloxacin and levofloxacin as has been reported in other countries (Machowska and Stålsby, 2019). The study reveals the presence of integrons in MDR isolates of *E. coli, Klebsiella* and *Pseudomonas*. These elements were more common among isolates from urine and those resistant to sulfamethoxazole-trimethoprim. Similar reports have been made in Malaysia (Sabbagh *et al*., 2021). Previous studies showed a strong association between integrons and antibiotics resistance, both MDR and single-drug resistance, particularly to SXT, which agreed with this study. In these studies, class 1 integrons were found in about 70% of SXT-resistant isolates. The studies that

examined class 2 integrons in Korea, Southern India and Sweden found them in 5 to 15% of clinical isolates, also highly correlated with SXT resistance (Ayukekbong *et al*., 2017). Most studies, including this one, indicate that integrons of either class 1 or 2 are very rare in SXT susceptible isolates. It has been previously reported that class 1 and 2 integrons are common in *E. coli* and much rare in *Pseudomonas isolates*. The carriage of integrons encoding resistance to SXT may favor the selection of MDR strains, the majority of which are also resistant to β-lactams, fluoroquinolones, and aminoglycoside.

### 5.2 Conclusions

1. The prevalence of *P. aeruginosa, K. pneumoniae* and *E. coli* at Thika Level 5 Hospital was 9.1 % for both *P. aeruginosa* and *K. pneumonia*, and *E. coli* was 9.3 %.

2. Distribution of multi- drug resistance was *P. aeruginosa* at 29.3 %, *K. pneumoniae* at 46.3 % and *E. coli* at 78.6 %. The study highlights significant resistance to commonly used antibiotics including last- resort options like carbapenems.

3. The most prevalent resistance genes were *aac(6')-1br-cr* and *intl1* from *E. coli* both at 33 %. Specimens were urine at 57 % and 50 % *aac(6')-1br-cr* and *intl1* for respectively.

### 5.3 Recommendations

Based on the findings from this study, the recommendations are as follows:

1. There is a need for routine culture for identification of these Gram negative strains in clinical settings and susceptibility testing and for surveillance of antibiotics resistance
2. Hospital should have antibiotic susceptibility profiling for guiding individual patient treatment and implement active AMR surveillance and reporting unique resistance profiles to hospital epidemiologist. There is need to review the existing empiric treatments based on current susceptibility data to the various bacterial isolates.

3. There is need for continuous surveillance on resistance genes circulating within the clinical settings in the hospital. This will enable the policy makers and epidemiologists make informed decisions in the proper management of patients

### 5.4 Application of these Findings

These findings have the following applications:

1. The data generated in this study was shared with clinicians, aiding in timely diagnosis and effective treatment of patients in a more cost-effective manner.
2. This data could find important application in developing effective antibiotics steward programs and informing clinical treatment guidelines and in policy formulation concerning use of antibiotics and curbing development of drug resistance.
3. The findings from this study provide information on resistance genes circulating within the study population and hence inform on the best drug of choice for treatment. It also has potential to influence the public health policy and clinical practices especially in regions with high incidences of these infections.

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# APPENDICES

## Appendix I: Biochemical Tests

### Oxidase Test

Oxidase test is used to determine if an organism possesses the cytochrome oxidase enzyme and is used to differentiate pseudomonads from related species. It is also used for the differentiation of *Neisseria, Moraxella, Campylobacter* and *Pasteurella* species which are oxidase-positive (Isenberg *et al*., 2004). The cytochrome system is usually only present in aerobic organisms which are capable of utilizing oxygen as the final hydrogen receptor. The test reagent*, N, N, N', N'-tetra-methyl-p-phenylenediaminedihydrochloride* acts as an artificial electron acceptor for the enzyme oxidase and the oxidized reagent forms the colored indophenol blue compound. The end product of this metabolism is impregnated oxidase test strips (MacFaddin *et al.,* 2000)

Positive control: *Pseudomonas aeruginosa* NCTC 10662

Negative control: *Escherichia coli* NCTC 10418

### Procedure and Results using impregnated oxidase test strip Method

A sample of the bacterial colony from a fresh culture growth was rubbed onto filter paper impregnated with the oxidase reagent (N, N, N', N'-tetra-methyl-p-phenylenediaminedihydrochloride) using a wooden applicator stick. A blue/purple color within ten seconds was oxidase positive indicating oxidase production while absence of blue color was interpreted as negative result (Kalawat *et al*., 2012).

### Triple sugar Iron agar (TSI)

This test is used to determine whether a gram-negative rod utilizes glucose and lactose or sucrose during fermentation and forms hydrogen sulfide for differentiation of Gram negative enteric bacteria (Upgade *et al*., 2012). Triple sugar Iron agar (TSI) contains three sugars thus 1% lactose, 1% sucrose and 0.1% dextrose. Phenol red is

used as acidification indicator in addition to thiosulfate while ferrous sulfate is indicator for the detection of the ability to form hydrogen sulfide. A well-isolated colony from a differential plate was touched at the top with a straight inoculating needle. Inoculation on triple sugar iron agar (TSI) was performed by first streaking the slope and stabbing the butt through the center of the medium to the bottom of the tube. The cap was loosely placed and tubes incubated at 35-37 °C in ambient air for 18-24 hours. Organisms fermenting dextrose together with lactose and /or sucrose change the color of both the slope and butt to yellow by the formation of acids. Organisms that ferment dextrose but not lactose, the slope becomes red in color because of the weak acid formation and heavy reversion of reaction due to growth under aerobic condition.

Tubes showing original color of the medium indicate non-fermentation of dextrose, lactose and sucrose. Fermentation of these carbohydrates with gas is observed by the formation of cracks in the butt of the medium, and the formation of hydrogen sulfide by its blackening.

**Motility Test**

Motility test is used to determine if an organism is motile hence possess flagella or non-motile. Generally bacilli organisms are motile although a few cocci are non-motile.

**Semi Solid Agar -Motility-Indole-Ornithine (MIO)**

Test organisms were inoculated at the center of glass tube containing MIO media by stabbing with a straight wire. The tubes were incubated at 35°C-37°C for 18-24 hours. Motility was indicated by generalized turbidity or growth extending from the line of inoculation while negative result was indicated by organisms remaining at the line of inoculation (Kalawat *et al*., 2012).

## Appendix II: Polymerase Chain Reaction (PCR)

### Principle

Polymerase chain reaction is an in vitro technique for exponentially amplifying DNA, via enzymatic replication without using a living organism. PCR allows the in vitro amplification of specific target DNA sequences by a factor of $10^6$ and is an extremely sensitive technique. It is based on an enzymatic reaction involving the use of synthetic oligonucleotides flanking the target nucleic sequence of interest. This oligonucleotides act as primers for the thermal stable Taq polymerase.

### Materials

Template DNA, forward and reverse primers, DNA Polymerase (5u/µl) , 25mM $MgCl_2$, nuclease-free water, dNTP mix, 10mM of each dNTP, thermal cycler and micro-pipettes (10,100,1000µl).

### PCR Procedure

Master-mix was prepared by mixing template DNA, primers, dNTP's and Taq polymerase, buffer, distilled sterile water and Mg2+ in a single tube. The concentration of each item was calculated depending on the number of the samples to be analyzed. Master-mix was prepared by mixing template DNA, primers, dNTP's and Taq polymerase, buffer, distilled sterile water and Mg2+ in a single tube. The PCR steps were an initiation denaturation (95°C, 5 minutes, denaturation (94°C, annealing (depending with the gene and the primers), extension at 72°C between 1-2 minutes and final elongation (72°C for 10 minutes). The number of cycles was 35 for all the primers used, but varied in the annealing temperature and incubation period depending on the genes being detected.

Micro-centrifuge tubes were marked with a permanent marker. The required volume of the sample was pipetted into the tube and the recommended volume of the master mix added into the tube. The lowered temperature was to allow the primers to anneal to the single-stranded DNA. Elongation step was when the DNA polymerase was to synthesize new DNA strands. The PCR will consist of 35 cycles of denaturation,

annealing and elongation. Final elongation took ten minutes at 72 $^{o}$C and final hold was at 4-15 $^{o}$C though 15 $^{o}$C is recommended to avoid accumulation of moisture in the heating block which would interfere with the results. On completion of PCR cycles, the tubes were removed and labeling confirmed. The amplified DNA was separated according to sizes by electrophoresis procedure using agarose gel.

## Appendix III: Gel Electrophoresis

### Agarose gel electrophoresis

Agarose gel electrophoresis is an easy technique used to separate DNA fragments according to their sizes and then visualizing them. It is a basic diagnostic procedure used in molecular biological laboratories.

### Principle of Electrophoresis

Electrophoresis is based on the fact that DNA is negatively charged at neutral pH due to its phosphate backbone thus, when an electrical potential is placed on the DNA it will migrate towards the positive electrode. The migration rate of DNA is slowed by making the DNA migrate through an agarose gel which forms a porous lattice in the buffer solution, thus the DNA must slip through the holes in the lattice in order to move toward the positive pole thereby slowing them down. Larger molecules are slowed down more than smaller molecules, since the smaller molecules can fit through the holes easier. This results in a mixture of large and small fragments of DNA that have been separated by size.

### Electrophoresis- Agarose Gel Preparation

Tris/Borate/EDTA (TBE) buffer solution was used to dissolve agarose which was heated to 90°C in a microwave to melt the agarose. Ethidium bromide was cautiously added into the gel and was the stain. The melted gel was poured into the casting plate, and comb placed in position and gel allowed to cool for at least 20 minutes. Masking tape was removed from the ends of the casting plate.

### Loading the gel

Gel comb was removed and using a micropipette, each sample was carefully added to the well to ensure that the sample sank to the bottom of the well. A sample of known molecular weight was used as a positive control while sterile double distilled water was used as a negative control. A DNA marker ladder was added to the right hand lane.

**Reading the results**

Power to electrophoretic tank was then switched off, gel removed and placed on the dual intensity ultra violet transilluminator (UVP) to visualize the DNA bands. Polaroid Gelcam EP H7 camera was used to photograph the bands. These results were documented.

## Appendix IV: Scientific Review Approval

KENYA MEDICAL RESEARCH INSTITUTE

P.O. Box 54840-00200, NAIROBI, Kenya
Tel (254) (020) 2722541, 2713349, 0722-205901, 0733-400003; Fax: (254) (020) 2720030
E-mail: director@kemri.org info@kemri.org Website:www.kemri.org

ESACIPAC/SSC/9576 18th July, 2011

Cecillia W. Ndung'u

Thro'
Director, CMR forwarded : 19/7/2011
NAIROBI

REF: SSC: No.2081 (Revised) –Prevalence and antibiotic resistance patterns of *pseudomonas aeruginosa* among hospitalized patients at Thika District Hospital

I am pleased to inform you that the above mentioned proposal, in which you are the PI, was discussed by the KEMRI Scientific Steering Committee (SSC), during its 180th meeting held on 5th July, 2011 and has since been approved for implementation by the SSC.

**Kindly submit 4 copies of the revised protocol to SSC for onward transmission to ERC office.**

We advise that work on this project can only start when ERC approval is received.

Sammy Njenga, PhD
SECRETARY, SSC

In Search of Better Health

## Appendix V: Ethical Review Approval

# KENYA MEDICAL RESEARCH INSTITUTE

P.O. Box 54840-00200, NAIROBI, Kenya
Tel (254) (020) 2722541, 2713349, 0722-205901, 0733-400003; Fax: (254) (020) 2720030
E-mail: director@kemri.org info@kemri.org Website:www.kemri.org

**KEMRI/RES/7/3/1** **August 16, 2011**

**TO:** **Ms. CECILIA WANJIRU NDUNGU (PRINCIPAL INVESTIGATOR)**

**THROUGH :** **DR. S. KARIUKI, DIRECTOR, CMR NAIROBI**

Dear Madam,

**RE:** **SSC PROTOCOL No. 2081 – REVISED (*RE-SUBMISSION*): PREVALENCE AND ANTIBIOTIC RESISTANCE PATTERNS OF *PSEUDOMONAS AERUGINOSA* AMONG HOSPITALIZED PATIENTS AT THIKA DISTRICT HOSPITAL**

We acknowledge receipt of:

a. The Study Protocol – version dated 15 August 2011;
b. The Informed Consent Documents - English and Kiswahili versions.

The Committee is satisfied that the issues raised at the initial review have been adequately addressed and grants approval for implementation of the proposed study effective this **16th day of August 2011**. Please note that authorization to conduct this study will automatically expire on **August 14, 2012**. If you plan to continue with data collection or analysis beyond this date, please submit an application for continuing approval to the ERC Secretariat by **July 3, 2012**.

Any unanticipated problems resulting from the implementation of this protocol should be brought to the attention of the ERC. You are also required to submit any proposed changes to this protocol to the ERC to initiation and advise the ERC when the study is completed or discontinued.

You may embark on the study.

Sincerely,

**Christine Wasunna,**
**FOR: SECRETARY,**
**KEMRI/NATIONAL ETHICS REVIEW COMMITTEE**

## Appendix VI: Publication

*East African Medical Journal Vol. 91 No. 6 June 2014*

PREVALENCE AND ANTIBIOTIC RESISTANCE PATTERNS OF *ESCHERICHIA COLI* AMONG HOSPITALISEDPATIENTS AT THIKA DISTRICT HOSPITAL

C. Ndung'u, HND, BSc, A. W. T. Muigai, BEd, MSc, PhD, Professor, Jomo Kenyatta University of Agriculture and Technology, P. O. Box 62000-00202, Nairobi and S. Kariuki, BVM, MSc, PhD, Professor, Centre for Microbiology Research, Kenya Medical Research Institute, P. O. Box 54840-00200, Nairobi, Kenya.

Request for reprints to: C. Ndung'u, Jomo Kenyatta University of Agriculture and Technology, P. O. Box 62000-00202,Nairobi, Kenya

# PREVALENCE AND ANTIBIOTIC RESISTANCE PATTERNS OF *ESCHERICHIA COLI* AMONG HOSPITALISED PATIENTS AT THIKA DISTRICT HOSPITAL

C. NDUNG'U, A. W. T. MUIGAI and S. KARIUKI

## ABSTRACT

***Background*: Emerging resistance to antibiotics increases morbidity and mortality by hampering the provision of effective chemotherapy, and makes treatment more costly. The emergence of resistance to antibiotics is a global public health problem, especially in pathogens causing nosocomial infections.**

*Objectives:* To determine the carriage of *E. coli* from wounds and urine in catheterised inpatients at Thika District Hospital (TDH) and to determine antibiotics resistance patterns to β-lactams, aminoglycosides and (fluoro) quinolones.

***Design*: A cross-sectional study.**

*Setting*: Thika District Hospital among hospitalised patients.

*Subjects*: A total of 450 specimens were collected and forty two (42) *Escherichia coli* isolated. Pus swabs were collected from wounds and urine was collected aseptically from the inpatients with catheters. *Escherichia coli* were identified by culture methods and biochemical tests. Antibiotic susceptibility testing was performed by Kirby-Bauer disc diffusion method and interpreted according to Clinical Laboratory Standards Institute recommendations.

*Results*: Susceptibility results in aminoglycosides were, resistance for amikacin, gentamicin and kanamycin was 20%, 39% and 51% respectively. Resistance in penicillin was ampicillin 85% and piperacillin 83%. Resistance for sulfamethoxazole was 83%, tetracycline 66 %, nalidixic acid 44 % and chloramphenicol 39%. In amoxicillin/clavulanic acid, resistance was 68%. Cephalosporins' resistance was ceftazidime 22 %, cefotaxime 56 %. Resistance for imipenem and tazobactam was 7% and 12 % respectively.

*Conclusion*: Due to observations on resistance to antibiotics commonly used in Thika District Hospital, this shows that there is need to revise antibiotics policyin this region in the treatment of *E. coli* infections.

## INTRODUCTION

*Escherichia coli* (*E.coli*) are facultative anaerobe Gram-negative rods, are motile, and some posses' capsules while others are non-capsulated (1). It is normally found in the gut of both man and animals. However it also colonises the lower end of urethra and vagina. *Escherichia coli* cause urinary tract infections, neonatal meningitis, diarrhoea and septicaemia (1). It is the leading pathogen causing urinary tract infections (2). *Escherichia coli* is also among the most common pathogens that cause blood stream infections (3), wounds, otitis media and other complications in humans (4).

Resistance to antibiotics in *E. coli* has been reported worldwide (5). There is increasing rates of resistance among *E. coli* which is a growing concern in both developed and developing countries (6). Treatment options for *E. coli* strains include β-lactam antibiotics, aminoglycosides and (fluoro)- quinolones. In most cases combined therapy comprising, a β-lactam antibiotic and a (fluoro) quinolone or an aminoglycoside is prescribed for serious infections. Cephalosporins alone as a monotherapy or as a β-lactam/ β-lactamase inhibitor such as amoxicillin-clavulanic acid combinationsmay be effective against *E. coli* infections.

## MATERIALS AND METHODS

*Study site*: The study was conducted at Thika District Hospital, Kiambu County, Kenya.

*Study population*: The study population included both adults and children who gave consent to participate in this study. For children, consent was given by their parents or guardians. All study participants had been admitted in the ward for at least 48 hours. They were all in-patients with wounds, burns wounds and those with indwelling urine catheters. Information sheets explaining the purpose of the study and the procedures involved were given to the patients/guardians. Patients/guardians who agreed to participate in the study were given the consent form to sign.

*Design of the study*: A cross-sectional study design was used.

*Sampling*: Sterile cotton swabs were used to aseptically collect specimens from fresh burn sites and surgical wounds. The swab was rolled gently on the surface area of the wound for about five seconds. The cotton swab was inserted directly into sterile bottle containing Stuart transport media. The handle of the swab was snapped off, with cotton tip remaining in the transport media. The bottle was sealed and then labeled with the patient's study number, ward and date. Urine specimens were collected in sterile universal bottles from patients with catheters. The specimens were labeled with the study number, ward and date.

These specimens were delivered immediately to the microbiology department in the laboratory for processing.

*Laboratory procedures*: Processing of urine and pus swabs specimens. Swabs collected from wounds and urine from catheterised patients was processed according to standard operating procedures. Swab specimens were first inoculated on blood agar media and then in MacConkey media for lactose fermentation determination. Smears were then prepared and a Gram stain performed. Culture plates were incubated aerobically at 37 °C for 18-24 hours. The identity of the isolates was confirmed bystandard laboratory methods which included colony morphology, lactose fermentation, gram staining, oxidase, triple sugar iron, motility, and indole and citrate utilization.

*Antibiotic Susceptibility Testing*: Antibiotic susceptibility test was done using Kirby-Bauer Discdiffusion method. At least four to five isolated coloniesof the same morphologic type was selected from an overnight agar plate of a pure culture and emulsified in 10 ml sterile normal saline (0.85% sodium chloride) in sterile glass tubes. Susceptibilities were tested to antibiotics commonly used for treatment of infections caused by *E. coli* including aminoglycosides thus gentamycin (10µg), amikacin (30µg), and kanamycin (30µg). Beta-lactams included piperacillin (100µg), amoxicillin/clavulanic acid (20/10 µg), piperacillin/tazobactam (100/10 µg), cefotaxime (30µg), ceftazidime (30µg) and imipenem (10µg). Fluoro-quinolones included nalidixic acid (30µg), ciprofloxacin (5µg) and ofloxacin (5 µg). Other antibiotics were trimethoprim/ sulfamethoxazole (25µg) and tetracycline (30µg) (all from Oxoid, Basingstoke,UK).

Quality control organisms used to test for discpotency and media quality were ATCC E. coli 25922. The plates were incubated at 37oC for 18-24 hours. Susceptibility results were interpreted according to the Clinical Laboratory Standards Institute (CLSI) 2011.

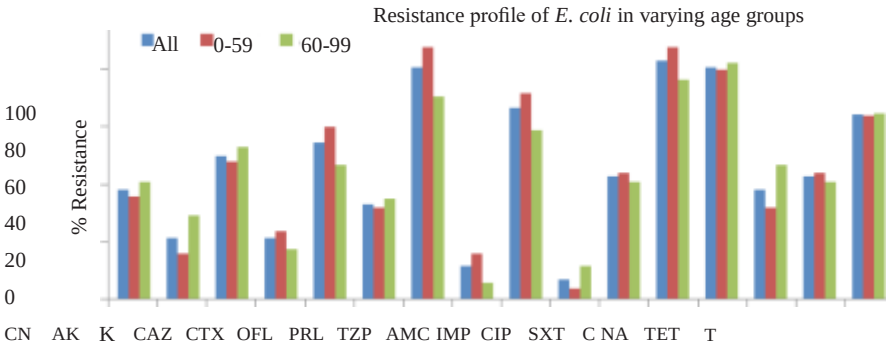
ntry and analysis were d using Ms excel and .ool respectively. Categorical data were tested using chi-square test. P-values of <

0.05 were considered statistically significant. Ethical clearance for the study was obtained from Kenya Medical Research Institute (KEMRI) Scientific Steering Committee and Ethical Review Committee (ERC No. 2081). Approval was also obtained from Medical superintendent, Thika District Hospital and informed consent from the patients or their guardians.

## RESULTS

### Figure 1

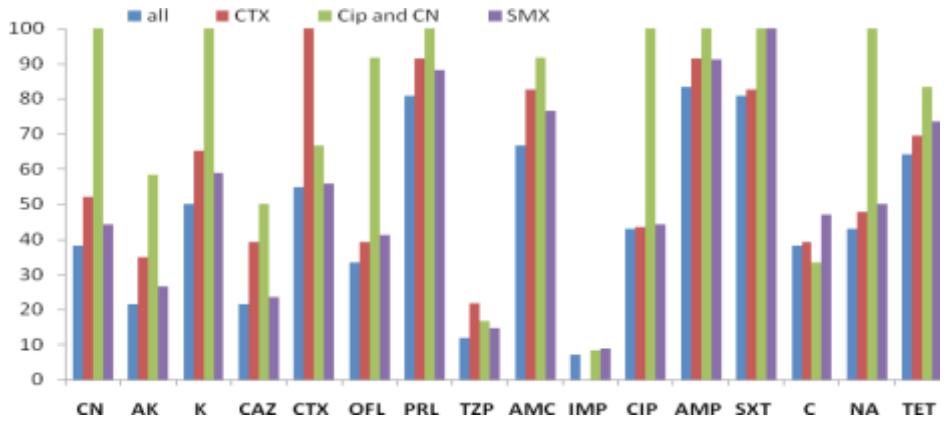
Resistance profile of *E. coli* to various classes of antibiotics in relation to two age groups




A comparison of the resistance profiles of strains obtained from two age groups was done, that is, those between 0- 59 years and 60 years and above. It was noted that generally, there were no significant differences in resistance to combinations of antibiotics between the two groups (P: 0.9115).

### Figure 2

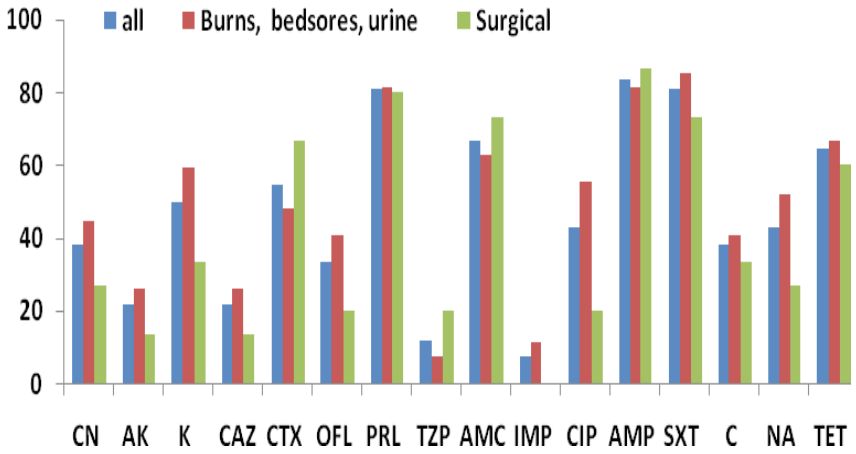
Comparison of resistance patterns of *E. coli* among a cephalosporin, combined resistance to gentamicin and ciprofloxacin, and sulfamethoxazole




Resistance patterns were compared among strains showing resistance to at least a cephalosporin such as cefotaxime and those with combined resistance to ciprofloxacin, gentamicin and those resistant to sulfamethoxazole. Those with combined resistance to ciprofloxacin and gentamicin were resistant to more combinations of antibiotics than those susceptible to these antibiotics (P: 0.0066).

**Figure 3**

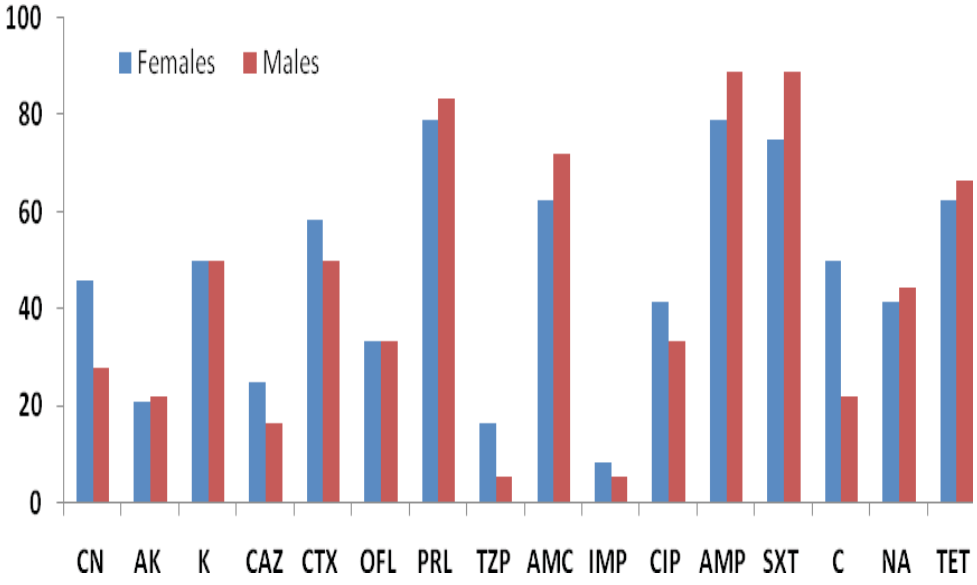
Resistance patterns of *E. coli* from different sources of specimen




Investigations were done on whether there were significant differences in resistance patterns of strains depending on the source of specimen. A comparison of resistance of strains obtained from surgical sites and those from burns, bedsores and urine was done and it was found that isolates from non-surgical siteswere slightly more resistant than those from surgical specimens ( P: 0.0261).

**Figure 4**

Comparison of resistance patterns of *E. coli* between males and females




Analysis to compare resistance patterns between *E. coli* strains obtained from males and

females revealed no differences between the two populations (P: 0.8575).

## DISCUSSION

*Escherichia coli* have widely been associated with various clinical hospital acquired infections (7). The results of antibiotics resistance pattern give serious cause for concern because the predominant bacterial isolates were highly resistant to the commonly available antibiotic agents in Kenya. Majority of strains from all specimen types were found to be multi-drug resistant (MDR). Similar results have been published before (8). These findings show that in general, isolates from burns and surgical wounds are the second most resistant strains after those from urinary tracts.

Although majority of strains were susceptible to carbapenems, some *E. coli* strains that were resistant to imipenem were identified. Carbapenems however remain the drugs of choice against the strains investigated as reported in a related study (9).

This study reveals that a significant proportion of isolates from wounds and burns are highly multidrug resistance. The burn patients are particularly predisposed to different infections which are linked to impaired resistance from disruption of the skin's mechanical integrity and generalised immune suppression (10). In these patients, the skin barrier isreplaced by a protein rich, avascular environment that provides a favorable niche for microbial colonisationand proliferation (11). It is also common for patients with wounds and burns to use topical microbial applications especially those containing heavy metals such as silver and those with neomycin and tetracycline. Such practices may promote emergence of highly resistant strains.

This study shows that *E. coli* isolates from urine were significantly more resistant than those from wounds that is burns and surgical sites. Isolates from non-surgical sites especially from urine, were slightly more resistant than those from surgical specimen (P: 0.0261). Similar high resistances among urine strains have been reported in related studies for instance, resistance rates among strains of *E. coli* isolated from women with urinary tract infection (UTI) averages 30% for both sulphonamides and ampicillin, varying from 17% to 54% in different countries (12). Trimethoprim resistance ranges from 11% in Scandinavian countries to 34% in Spain and Portugal. Most Scandinavian countries record low resistance to fluoroquinolones but resistance to fluoroquinolones may reach 20% in southern Europe(12).

In Spain where antibiotics can be used without restrictions, reduced susceptibility of

*E. coli* strains isolated from patients with UTI to sulfamethoxazole-trimethoprim (SXT-TRIM) combinations has been reported to be as high as 26% and 16% to fluoroquinolones (13).

This current study shows that isolates obtained from urine were fairly susceptible to common antibiotics used for the treatment of UTIs compared to those reported in other countries. These results differ from the expectation given the fact that the sample population was hospitalised patients who are generally reported to have isolates that are highly resistant (14). Majority of isolates from urinary tracts were MDR and a significant proportion of these were resistant to sulfamethoxazole-trimethoprim (SXT-TRIM). These strains were also resistant to other classes of antibiotics further limiting the choice of alternative antibiotics. These results indicated similar sensitivity patterns amongst the uropathogens as those observed in Kolkata, India. The World Health Organization (WHO) guidelines recommend sulfamethoxazole-trimethoprim and ampicillin as the first choice for the UTI treatment

(15) and empiric treatment is recommended for treatment of uncomplicated urinary tract infection (uUTI) (16). However, as was revealed in the present study, SXT-TRIM cannot serve as treatment of choice among majority of these isolates.

A recent report also indicated that SXT-TRIM and ampicillin were not suitable drugs of choice for treating UTIs (17). However, another study showed that amikacin and nitrofurantoin are the most effective treatments for UTIs in China (18). Since inappropriate use of antibiotics may lead to complications and treatment failure, this study suggest that there is a need to have an idea of the resistance phenotypes of isolates from urine before commencing treatment. It is therefore important to conduct culture and susceptibility testing for serious infections.

In conclusions, this study shows that isolates from urine and wounds (bedsore, burns and surgical wounds) are significantly resistant to common antibiotics available for use in Kenya. The *E. coli* strains from urine are particularly resistant. A few isolates are resistant to carbapenems indicating that treatment of such strains would present a great challenge in clinical settings. This study shows that SMX-TRIM is no longer effective against most *E. coli* isolates and that a significant proportion of these strains are MDR. It has also been shown that women may present a significant reservoir for MDR strains. There is therefore need to check antibiotic use patterns in the management of burns and surgical wound, UTIs and bedsore. There is also need to promote culture and susceptibility testing for isolates implicated in serious infections.

## ACKNOWLEDGEMENTS

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