

**PREVALENCE OF BACTERIAL CONTAMINANTS AND THEIR  
ANTIBIOTIC SUSCEPTIBILITY PROFILES AT KITUI COUNTY REFERRAL  
HOSPITAL, KENYA**

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## DECLARATION AND APPROVAL

This is my original work and has not been presented for a degree in any other university or for any other award.

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## DEDICATION

To all Medical Laboratory Scientists and family, I am grateful. I may not have enough to give you, but I dedicate the fruits of this work to you.



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## ABSTRACT

The study determines the prevalence of bacterial contaminants, major sources and their antibiotics susceptibility profiles in Kitui County Referral hospital, Kenya. Bacterial contaminants are the major sources of Nosocomial infections which causes hospital acquired infections among health care workers, patients and visitors in the health facilities. Nosocomial infections are acquired during provision of health care services within the health facility set up. The buildings provide the space used to provide hospital care while the equipment entails the tools and machines used to run the operations of the hospital. Thus, environment in Surgical and medical wards have a huge impact on the health safety of the patients. This study was done by collection of 195 swabs samples in the patient care and treatment environment which involved floors, beds, drugs trolleys, infusion stands, sinks, door handles, chairs, tables and bedside lockers of medical and surgical wards. The study was done through culturing of specimen in MacConkey, Sheep blood agar and chocolate blood agar media. The identification was done by gram staining technique and biochemical tests; Citrate utilization test, Catalase, Coagulase, Indole, Methyl Red, Voges Proskauer and Oxidase test. A total of 177 bacteria isolates contaminants were identified from both surgical and medical wards. The study found that the primary types of bacteria in hospital setting being *Staphylococci aureus*, *Escherichia coli*, *Klebsiella oxytoca*, and *Pseudomonas aeruginosa*. *S. aureus* had the highest prevalence at 43%, *Escherichia coli* 30%, *Klebsiella* 14% and *Pseudomonas aeruginosa* was the least 13%. Proportion of contaminants on sources were; lockers 28%, bed 15 %, sink 16%, floor 9%, table 7.5 %, chair 7%, drug trolley 6%, infusion stand 5.5%, and doors 6%. The study found that hospital surfaces, including doors, tables, lockers, chairs, sinks, trolleys, and beds were the primary source of contaminants. *S. aureus* was distributed mainly on doors, while lockers, beds, sinks, and drug trolleys were contaminated with all bacteria isolates. Similarly, floors were contaminated with *S. aureus*, *P. aeruginosa*, and *E. coli*. Chairs and infusion stands were contaminated with *S. aureus* and *E. coli*. Tables had *S. aureus*, *E. coli*, and *K. oxytoca* contaminants. *S. aureus*, *P. aeruginosa*, *E. Coli*, and *K. oxytoca* were the predominant bacteria isolate from surgical and medical ward surfaces and equipment. Of all the four isolates, *S. aureus* was the most prevalent in medical and surgical wards (51%). Antimicrobial susceptibility test showed that all the isolated bacteria were sensitive to Meropenem. *P. aeruginosa* showed high sensitivity to Meropenem (100%) but averaged 33.3 % against Piperacillin Tazobactam, Ampicillin -clavulanic acid, and Ciprofloxacin. *E. coli* was only susceptible to Tazobactam, Ciprofloxacin, and Ceftazidime (20%), Augmentin (40%), and Meropenem (80%). Similarly, *K. oxytoca* was (100%) susceptible to Meropenem and ranked second with the most sensitivity to drugs tested. Vancomycin, Oxacillin, Penicillin, and Levofloxacin had no activity on bacteria isolates. Similarly, *S. aureus* showed sensitivity to most of the drugs tested. The study recommends inclusion of infection prevention and control measures to enhance safety against Nosocomial infections. Further, molecular techniques are recommended for further studies.

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## LIST OF ABBREVIATIONS AND ACRONYMS

AMR:	Antimicrobial Resistance
AST:	Antimicrobial Susceptibility Testing
ATCC:	American Type culture collection
BSIs:	Blood Stream Infections
CAUTIS:	Catheter-Associated Urinary Tract Infections
CDC:	Centers for Disease Control and Prevention
CLABSIs:	Central-Line-Associated Bloodstream Infections.
CLSI:	Clinical Laboratory Standard Institute
CRE:	Carbapenem Resistant Enterobacteriaceae
DNA:	Deoxyribonucleic Acid
ERC:	Ethical Review Committee
HAI:	Health Acquired Infections
HAP:	Hospital Acquired Pneumonia
IPC:	Infection Prevention and Control
KEMRI:	Kenya Medical Research Institute
MHA:	Muller Hinton Agar
MRSA:	<i>Methicillin Resistant Staphylococci aureus</i>
MSSA:	<i>Methicillin Susceptible Staphylococci aureus</i>
NACOSTI:	National Commission for Science, Technology and Innovation
NBU:	New Born Unit
NHSN:	National Healthcare Safety Network
NIS:	Nosocomial Infections
OECD:	Organization for Economic Co-Operation and Development
PBP:	Penicillin Binding Proteins

QC:	Quality Control
SOP:	Standard Operating Procedures
SPSS:	Statistical Package of Social Science
SSIs:	Surgical Site Infection
UTI:	Urinary Tract infection
VAEs:	Ventilator –Associated Events
VAP:	Ventilator Associated Pneumonia
VRE:	Vancomycin Resistant Enterococci



## CHAPTER ONE

### INTRODUCTION

#### 1.0 Background

“Nosocomial” term covers any disease acquired by patients during their hospital stay ((Khan *et al.*, 2021). Recently, the infections caused by prolonged hospital stay have become common and have been termed as healthcare associated infections, characterized by their high-risk factors towards health problems, leading to death. These infections are rampant in developing countries-accounting to 75% of the burden infections (World Health Organization, 2015). Hospital staff, visitors to the hospital facility, and other healthcare workers may also acquire some infections in the course of their activities. These infections may also be in the list of nosocomial infections.

According to the Centre for Disease Control and Prevention National Nosocomial Infection Surveillance System notes that 38% of all hospital-acquired infections across the globe occur in surgical sites (Center for Disease Control and Prevention, 2019a). The surveillance ranks hospital-acquired infections within the surgical wards at the top, alongside pneumonia at acute patient care units in the United States of America. Bacteria are unicellular prokaryotic micro-organisms and have no nucleus. Many bacteria have a peptidoglycan cell wall and divides asexually by binary fission. In addition, they have flagella for locomotion. Bacteria come in various forms, which include Bacillus, Coccus, Spirilla (Goyal *et al.*, 2019).

Gupta, (2021) categorizes bacteria into Gram-positive or Gram-negative using the gram staining technique. Gaseous requirements are categorized into aerobic and anaerobic as well as facultative anaerobes (Bhat *et al.*, 2021a). Gupta notes that bacteria could be autotrophs or heterotrophs depending on their energy acquisition mode (Tantray *et al.*,

2022). Chemoautotrophs are autotrophs that produce their own food by chemical processes or sunshine. Heterotrophs obtain energy by ingesting living things whereas saprophytes are bacteria that obtain energy from disintegrating matter.

While defining and classifying nosocomial infections, it is important to consider the situations in which the infections are acquired. Nosocomial infections are Healthcare Associated Infections (Gupta, 2021). They are acquired during delivery of health care services, in contrast to contaminations present or raising at service delivery. According to Seventer & Hochberg (2017), nosocomial infections are acquired through mechanical vectors such as cockroaches, ants, and flies. In addition, none compliant or inadequate knowledge of standard precautions by health care staff working towards infection control result in infections. Nosocomial infections are the foremost reasons for indisposition and death among the hospitalized patients (Kaminsk *et al.*, 2022). They occur within two days of hospital admission, 72 hours of discharge or within 30 days of an operation. Their effect is felt by 1 in 10 patients admitted.

However, when infections were present at the time of admission and their complications increase during hospital stay. Such infections will not be considered nosocomial. Moreover, infections that are acquired trans-placentally as a result of diseases like rubella, syphilis, or toxoplasmosis. They appear two days after birth are and not classified as nosocomial. The infections are typically instigated by viral, bacterial or fungal pathogens (Sikora & Zahra, 2022). In this study, the prevalence of bacterial contaminants and their antibiotic profiles in Kitui County Referral Hospital was determined through culturing of specimen and identification via microscopy and biochemical tests.

Thus, the combined causes of nosocomial infections are the hospital environment. The environment is made up of the building, the equipment and the people working or who

come in touch with the hospital. According to Dramowski *et al.*, (2017), the buildings provide the space used to provide hospital care while the equipment entails the tools . The machines are used to run the operations of the hospital within the building or space. The people could be patients, healthcare workers or visitors. Thus, patient environment has a huge impact on the health safety of the patient , healthcare workers and visitors (Fraser *et al.*, 2021). Infections occur when pathogens attack susceptible patient host. In the contemporary healthcare services, these infections are associated with invasive procedures and surgery, devices within the healthcare facility, and the surrounding environment. The etiology of Nosocomial infections depends on the source of infection, the type of infection, and the pathogen causing it (Khan *et al.*, 2021;). Such pathogens may be bacterial, viral, or fungal.

Hospital acquired infections are a threat to the patient safety and have been rated as the most common adverse threat in health care facilities (Levine *et al.*, 2020). They contribute to high morbidity, mortality and financial burden to the patients and their families. The effect is also felt by the healthcare system through increased congestion of patients, and overstretched resources (Wang *et al.*, 2021).

They lay a huge burden to the health systems as their costs spread from physical, psychological, and economic burdens. The consequences of having nosocomial infections include depression, pain, anxiety, morbidity, and mortality among patients (Filip *et al.*, 2022). Moreover, the burden on patients extends to financial costs as patients have to incur expenditures in seeking medication. This burden aspect is demonstrated by a study on six European nations: Britain, Netherlands, France Germany, and Spain where hospital-acquired infections have been associated with higher hospitalization (Badia *et*

*al.*, 2017). Nosocomial infections cause increased indulgence of antibiotics, leading to antibiotic resistance in hospital-acquired infections (Bhat *et al.*, 2020).

Patient environment is however different for intensive unit, multi-bed facility, and acute care unit's care. The patient environment for Intensive Care Unit refers to the bed space and items included in the bed space. Patient environment in acute care involves equipment, medical services, bathroom, personal items, telecommunication equipment, and furniture inside the curtain (Rosenthal *et al.*, 2022). The patient environment is invested with pathogens that are shed by the patients. The pathogens can remain active for many days, lying or attaching to air, water and surfaces (Bullen's *et al.*, 2022). The pathogens number depends on the number of people within the hospital facility, activities on run, moisture and available equipment that can support the life of microbes (Rosenthal *et al.*, 2022). The rate of removal of microbes in the air and the orientation of surfaces also determines the number and types of microorganisms (Marin *et al.*, 2022; Rosenthal *et al.*, 2022; Odoyo *et al.*, 2021; Dramowski *et al.*, 2017).

There are different types of nosocomial infections sites as classified by the National HealthCare Safety Network with Centre for Disease Control(Weiner *et al.*, 2020).. Some of the most common sites include gastroenteritis, urinary tract infections, surgical and soft tissue infections, respiratory infections and meningitis. Nosocomial infections are caused by various microbes within a healthcare setting. Bacteria are the most common causes of Hospital acquired infections, accounting for over ninety percent infections (Elseiver *et al.*, 2021). Protozoa, fungi, viruses and mycobacteria, on the other hand, are also causes of Nosocomial infections but to a lesser extent compared to bacteria.

The prevalence of high bacteria contaminants in the hospital environment has become a menace to the health system (Gola *et al.*, 2021). The threat is attributed to poor

decontamination and sterilization, poor disinfection and inefficient antimicrobial management strategies. The threat has also become a menace because of continued hospital visits, high rates of antibiotic resistance and inadequate healthcare safety information (Ling *et al.*, 2015). The Center for disease Control and Prevention asserts that approximately 3.8 million people in European countries are infected with Hospital acquired infections (Odoyo *et al.*, 2021). Pneumonia has been the most prevalent case alongside urinary tract infections, surgical site infections, bloodstream infections, and gastrointestinal infections. With the emergence of multidrug resistant organisms, hospital acquired infections control has become complicated.

The risks of hospital acquired infections in Asian region have been estimated to be more than in developed countries by 2-20 times. Over a quarter of the hospitalized patients have acquired Hospital acquired infections in the Asian-Pacific region (Rosenthal *et al.*, 2016). The hospital acquired infections incidence density in Asia is 20 cases per 1000 Intensive care unit-days in Southeast Asia (Ling *et al.*, 2015). In Africa, hospital acquired infections among the inpatients are frequent with a prevalence rate of 3%-15%. According to Najed *et al.*, 2011), the Gram-negative bacteria are the most widespread in surgical site infections and ventilator-associated pneumonia, however, disproportionate in sub-Saharan region as it is estimated to have a range of 2.5%-14.8%. HAIs rates in Kenya are 4.4 per 100 admissions of patients (Ndegwa, 2015). They are linked to hospital environment and high transmission rates through contact (Odoyo *et al.*, 2021). Most common Hospital acquired infections from surface contamination in Kenyan hospitals are caused by *Clostridium difficile*, *Acinetobacter baumannii*, oxacillin-resistant *Staphylococcus aureus*, Carbapenem-Resistant *Enterobacteriaceae* and vancomycin-resistant enterococci. (Wahome *et al.*, 2021)

The bacteria lie on hospital environment: on sinks or water dispensing points, couches, clothes and medical instruments. As a result of high prevalence rates, Kenya has resorted to Infection prevention and control protocols that appropriate cleaning of the environment and monitoring of bacterial contaminants (Odoyo et al., 2021). The World Health Organization posits that for every 100 hospitalized patients, in developed and in developing countries contract least one hospital acquired infections (Mnyambwa et al., 2021). Some of the bacteria species which have been reported to cause NIs include: *Staphylococci aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter species*, *Citrobacter*, *Enterococcus* and *Pseudomonas aeruginosa* (Khan et al., 2021; Alera & Merga, 2018).

Transmission of Nosocomial infections can be through direct or by indirect contact (Kaminsk et al., 2022). Direct contact occurs when someone comes into contact with an infected individual while indirect contact occurs when an individual touches an infected object or surface. In health facilities most of Nosocomial infections outbreaks are associated fomites that have been contaminated by pathogens (Kaminsk et al., 2022). In this study, the major sources of bacteria causing nosocomial infections in Kitui County Referral Hospital were determined by the researcher. Determination of the major sources of the bacteria contributed to effective management of nosocomial infections. For example, cleaning and decontamination protocols was analyzed and adopted as per the findings.

Antibiotics are antimicrobial substances produced by living organisms and are used to treat and prevent bacterial infections (Kourkouta et al., 2018). Antibiotics are classified into two classes according to the mode of action (Stokes et al., 2019). Bacteriostatic antimicrobials when given in their usual dosage prevent active multiplication while

bactericidal kill bacteria. Antimicrobials are further categorized as broad and narrow spectrum (Melander & Zurauski, 2018). Broad antimicrobials have activity against wide range of gram positive and gram-negative bacteria while narrow spectrum antimicrobials inhibit or kill limited species of microbes. The antibiotics act by inhibiting production of nucleic acid, protein, cell wall formation and damaging bacterial cell wall membrane (Fraser *et al.*, 2021).

Antimicrobial resistance is the process in which microbes develop the ability to defend themselves against drugs that are meant to kill them; thus, continuing to grow despite being exposed to drugs (Centre for Disease Control, 2019). The impact of hospital acquired infections is compounded by antimicrobial resistance which causes long stay in the hospital, patient-care complications and deaths (Dramowski *et al.*, 2017; Nkengasong *et al.*, 2017). According to Organization for Economic Co-operation and Development (OECD) (2017), one antimicrobial resistant infection could cost the European nations approximately EUR 8,500 to 34,000 compared to non-resistant infections. In Asia, Hospital acquired infections have had significant impact in health management (Ling *et al.*, 2015). They frequently compromise the safety of the patients, lead to increased stay in the hospital and cause antibiotic resistance.

The major challenges in the use of Antibiotics are failure against bacterial infections (Peter & Pauline, 2020). Bacteria become resistance to antibiotics by genetic mutation, alteration of permeability of their cell membrane or by production of enzymes that destroy or inactivate antimicrobials (Fraser *et al.*, 2021). In addition, resistance occurs due to overutilization or underutilization of drugs, extensive use, and misuse. Moreover, there is lack of proper adherence to drug management and use where healthcare workers do not follow the treatment guidelines keenly (Sserwadda *et al.*, 2018). Following the absence

of strict guidelines on usage and treatment against infections, the healthcare entities have resorted to use of broad spectrum of antibiotics which has resulted into increase in antibiotic resistance (Pittet *et al.*, 1999).

The resistance has therefore become a global health threat (World Health Organization, 2019). The Center for Disease Control and Prevention shows that there has been an increase in hospital acquired infections since 2020 compared to the previous years in the United States (Peter & Paulin, 2020). The Center for Disease Control and Prevention associates this rise with the Covid-19 pandemic (Rosenthal *et al.*, 2022). According to National Healthcare Safety Network (NHSN) data, central-line-associated bloodstream infections rates are on the rise which is the same for the Catheter-associated urinary tract infections and ventilator –associated events (VAEs) (Bulens *et al.*, 2022). *Methicillin-resistant Staphylococcus aureus* (MRSA) also recorded a substantial data. In this study, antimicrobial susceptibility profiles of bacteria isolated in Kitui County Referral Hospital was determined to give insight of drugs efficacy. Health practitioners may use it in prescribing effective antibiotics against the nosocomial infections.

## **1.2 Statement of the problem**

Hospital acquired infections are still a global problem. They affect approximately 100,000 people, more than HIV/AIDs, cancer, and road accidents. To worsen the situations, Hospital acquired infections are more prevalent in developing countries, accounting for 75% of all infections. Data from Center for Disease Control and Prevention shows that antimicrobial resistance is on the rise due to frequent use of antibiotics, and lack of adequate information on susceptibility patterns. Apart from mortality, bacterial contamination is linked with increased health care costs including high financial burden to patients, families, and the government through the healthcare system. Moreover, it increases the workload and congestion at the healthcare facilities.

Further, bacterial contamination and rising Hospital acquired infections have become a core driver in elevating antimicrobial resistance. The war against antimicrobial resistance is tedious and costly as it is associated with disease burdens and frequent misuse of antibiotics. Understanding the bacterial contamination patterns, distribution and susceptibility patterns will help in addressing the rise of hospital acquired infections in healthcare facilities, and the associated problems. Globally, the problem with Hospital acquired infections continues to persist. Key information concerning bacterial distribution, sources associated with the bacteria in hospital set up, and prevalence is scanty. For instance, there is no adequate surveillance and data on antimicrobial resistance, bacterial distribution, and susceptibility patterns in Kenya and globally. Consequently, there is need to develop an antibiogram that will direct on handling bacterial infections, antibiotic use, and resistance patterns. There is need to investigate the sources and distribution of bacterial contaminants in hospital facilities. Moreover, a study on the prevalence and susceptibility patterns on drugs to curb the mortality rates from nosocomial infections.

### **1.3 Study Justification**

Bacterial contamination in health settings has been playing a huge role in elevating hospital-acquired infections across the world. The elevated contamination is also associated with antimicrobial resistance. The management of common Hospital acquired infections depends on data that is available on etiology. Kenya and most of the Sub-Saharan African countries do not have adequate information and diagnostics of bacterial contamination and hospital acquired infections. The findings of this study will provide baseline data and information to aid in handling, managing and preventing nosocomial infections in hospitals.

The Ministry of Health may use the findings of this research to establish bacterial contamination prevention protocols for care givers, healthcare professionals, and the general hospital staff. The Ministry of Health may rely on the susceptibility patterns to control the issuance and distribution of antibiotics by healthcare professionals to patients.

The types of bacterial contaminants and their susceptibility to antibiotics are lacking in most health facilities including Kitui County Referral Hospital. These bacterial contaminants are the major sources of nosocomial infections in health care workers and visitors. This study determines the prevalence of bacterial contaminants, major sources and their antibiotics susceptibility profiles in the hospital environment. Knowledge generated from this study can be adopted by the relevant stakeholders (Ministry of Health, health based Non-Governmental Organizations) in effective management of nosocomial infections. It will be achieved through sterilization of the identified major sources of infections within the health facility. In addition, information on the susceptibility of the identified bacteria to antibiotics may guide health practitioners in prescribing effective antibiotics against the nosocomial infections.

Again, the findings of this study are fundamental for healthcare providers because provision of data on epidemiology of bacterial isolates, their distribution and antibiotic sensitivity will be a useful guide to direct the expected bacterial infection controls. The general public may rely on the findings of this research to learn on self-safety and preventive measures with respect to taking antibiotics. The findings may also be important to the general public as they shed light on the risks associated with hospital acquired infections. risky areas and sites regarding possible contamination, and infection prevention protocols to observe.

Finally, this research may play an integral part in adding to the body of knowledge or literature on bacterial contamination, types and distribution. Other researchers may borrow from the findings of this study as they advance on their scholarly work. The research provides broad information on causes, types, and distribution of bacterial contaminants. It also gives empirical data on bacterial contaminants, their distribution and types. This information may be useful as a reference point to scholars and researchers in microbiology and healthcare field.

#### **1.4 Research questions**

Three research questions were used to guide the researcher. They include:

- i. What is the prevalence of bacteria contaminants in Kitui County Referral Hospital?
- ii. What are the major sources of bacteria causing nosocomial infections in Kitui County Referral Hospital?
- iii. What are the susceptibility profiles of bacteria isolated in Kitui County Referral Hospital?

#### **1.5 General objective**

To determine the prevalence of bacterial contaminants and their antibiotics susceptibility profiles at Kitui County Referral Hospital, Kenya

##### **1.5.1 Specific objectives**

The specific objectives for this study included:

- i. To investigate prevalence of bacteria contaminants in Kitui County Referral Hospital.

- ii. To examine the major sources of bacterial contaminants in Kitui County Referral Hospital.
- iii. To determine antibiotic susceptibility profiles of bacteria in Kitui County Referral Hospital.

### **1.6 Limitations**

Due to limited financial and time resources, the study was only conducted in one hospital

### **1.7 Significance of the study**

Nosocomial infections are acquired by patients from the hospital environment during care and treatment. The study will assist in identifying various prevalence of bacteria contaminants in the patient environment and subsequently design and implement infection prevention and control measures antimicrobials to suppress or kill the contaminants isolated in the facility.

### **1.8 Basic assumptions**

The researcher assumed that data and knowledge generated from Kitui County Referral Hospital was a representative of all health care facilities in Kenya.

## 1.9 Operational Definitions of Key Terms

**Antibiotic:** Antimicrobials that inhibit growth or suppress growth of bacteria.

**Antimicrobial susceptibility:** This is a measure of susceptible, intermediate or resistance to growth of microbes

**Antimicrobial:** They are substances produced by living microorganisms or synthetically produced to kill or suppress microbes' growth

**Bacterial contamination:** This occurs when microbes enter and multiply in surfaces or patients.

**Bacterial distribution:** This is spread of bacteria in various surroundings over time.

**Fomites:** These are items or surfaces which may contain microbes and hence cause spread of microbes.

**Inanimate objects:** These are non-living things in the environment.

**Nosocomial infections:** These are infections a patient acquires from the hospital during care and treatment.

**Patient care surrounding:** This is the hospital environment which comes into contact with health care providers, patients and visitors.

## CHAPTER TWO

### LITERATURE REVIEW

#### 2.0 Introduction

This chapter discusses various studies or literatures done by other scientists and correlates the findings with the previous studies in the country and global.

#### 2.1. Nosocomial Infections and their Prevalence

Nosocomial infections are as old as the hospitals. They happen while receiving hospital care and are not present while being admitted (Sikora & Zahra, 2022). Environmental surfaces within the hospital facilities tend to act as bacteria's reservoirs and end up being acute sources of Nosocomial infections (Hanna *et al.*, 2021). The contamination from environmental surfaces is therefore referred to as environmental contamination. It occurs where a health care worker contaminates their gloves, or hands by coming into contact with contaminated surfaces (Chang *et al.*, 2022). It also occurs if patients come into direct contact with such surfaces causing transfer of bacteria from the surfaces to the patients through the hands of the health worker or through direct transfer (Chang *et al.*, 2022). The chief causes of bacterial isolates are the hands, which are frequently in contact with high touched surfaces (Gupta, 2021).

Nosocomial infections are also known as Hospital Acquired Infections. They occur due to adverse reactions to toxins, or infectious agents within two days or more after a patient has been admitted Artika and Ma'roef (2017) classified infections by health workers and other staffs, or visitors to the hospital environment as nosocomial infections. The urinary tract infection associated with catheter has been ranked as the leading nosocomial infections, while Surgical site infections take the second position, with bloodstream infections being third (Artika & Ma'roef, 2017). Blood stream infections are commonly linked to the use of intravascular devices (Artika & Ma'roef, 2017). They are followed

by pneumonia infections, which are linked with ventilation problems. Transmission is influenced by the inoculum size and virulence, transmission route, micro-environment and patient status of immunity (Seventer & Hochberg, 2017).

Nosocomial infections manifest themselves during hospitalization or after the patients are discharged. Studies conducted by Sikora and Zahra *et al* reveal that Nosocomial infections pathogens are prevalently endogenous while others occur through exogenous flora such as cross contamination, contaminated hospital tools and environment. According to Suleyman *et al.*, 2018), bacteria, fungi and parasites are linked to Nosocomial infections . The leading bacteria causing Nosocomial infections include; *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumonia*, *Staphylococci aureus*, and *Proteus species*. The World Health Organizations reports that Hospital acquired infections are part of the global pandemics sabotaging the economy. They affect approximately 2 million people across the globe (Fatima *et al.*, 2017). Hospital infections lead to a high mortality rate, prolonged hospital stays, disabilities, and substantial global economic losses (Fatima *et al.*, 2017).

WHO reports that 15% prevalence has been discovered among patients who have been hospitalized (Hassan *et al.*, 2017). The global pervasiveness rate of Nosocomial infections was 14.0%, from 4.6% to 27.3% in the surveyed medical facilities (Maria *et al.*, 2016). Additionally, the infections result in 5-15% of hospitalization of the affected people (Wakefield *et al.*, 1992); Magill *et al.*, 2018). The Nosocomial infections prevalence varies between countries in which the prevalence rate in the United States of America is 3.2% for patients in hospitals as per a 2015 study. The factors leading to this prevalence are associated with critical care locations, 57.5% in wards, and 6.1% in special care centers. Prevalence of Healthcare Associated Infections in emerging countries and

developed countries varied from 5.7% to 19.1% (World Health Organization, 2011) and prevalence rates of 7.7 and 9.0% respectively.

Also, according to Sikora & Zahra (2022), responsible disease-causing microorganisms originate from vast sources and are represented by different types of nosocomial infections. Based on the Centre for Disease Control and Prevention (CDC), the infections are broadly classified into four. They include; central line-associated bloodstream infections (CLABSI), catheter associated urinary tract infections (CAUTI), surgical site infections SSI and ventilator-associated pneumonia (VAP). (Suleyman *et al.*, 2018) also ascertained that other types of nosocomial infections include non-ventilator associated hospital-acquired pneumonia, other primary bloodstream infections not associated with central catheter use, Urinary tract infections is not associated with catheter use and gastrointestinal infections including (*Clostridium difficile*) (Suleyman *et al.*, (2018). They also maintained that they may also be grouped by specifically affected body systems. For example, infections of the ears, nose and throat, and the lower respiratory tract like bronchiolitis, tracheitis and lung abscess without pneumonia. Others include skin and soft tissue infections, reproductive tract, central nervous system, cardiovascular and bone and joint infections.

A study conducted in 2016 by Maria *et al.*, showed that major nosocomial infection in the acute hospital setting is pneumonia, gastrointestinal infections, surgical site infections, blood stream infections and urinary tract infections respectively. Major pathogens that cause nosocomial infections include bacteria, fungi and viruses. They also further argued that patient population, healthcare setting and facility location define the prevalence of infections caused by specific micro-organisms. Overall, bacteria are the leading causative agents of nosocomial infections followed by fungi then viruses.

According to the Center for Disease Control National Nosocomial Infection Surveillance System notes that 38% of all hospital-acquired infections across the globe occur in surgical sites (Center for Disease Control and Prevention, 2019a). The surveillance ranks hospital-acquired infections within the surgical wards at the top, alongside pneumonia at acute patient care units in the United States of America. Moreover, the surgical sites' nosocomial infections were among the leading nosocomial infections in Europe and Australia (Badia *et al.*, 2017). Globally, nosocomial infections depict glaring variations. For instance, surgical site infections vary considerably from 2.6% to 5.8%. The United States of America is leading in feeling the impact of nosocomial infections from surgical wards (Loyola University Health System., 2017). According to the report, surgical site infections eat up to over \$10 billion every year Moreover, has been found *Clostridium difficile* is the most prevalent pathogen and it accounts for 15% of the reported infections. Bhat *et al.*, (2021) notes that other prevalent pathogens include gram-negative bacteria namely *Klebsiella pneumoniae* and *Klebsiella oxytoca*, *Escherichia coli*, *Proteus mirabilis*, *Enterobacter*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Burkholderia cepacian*. *Acinetobacter baumannii* has inherently multi-drug resistant properties. The Gram positive bacteria include *Staphylococcus*, *Streptococcus*, *Corynebacterium*, *Clostridium*, *Listeria* and *Bacillus* (Seventer & Hochberg, 2017).

Alternatively, the United Kingdom reports a high incidence of nosocomial infections. In England alone, for instance, the Public Health of England reported a rapid increase in wound infections in both surgical and medical ward units in 2018. The Surgical site infection incidence was 8.7% between 2013 and 2018, involving minor bowel surgeries. A surveillance report for seven years (2003-2010) by Health Protection Scotland (HPS) showed that surgical site infections were as high as 88% of discharged (Center for Disease Control and Prevention, 2019b) patients after cesarean section.

Moreover, the dominance of hospital acquired infections and antimicrobial application in the European Union for 28 countries included 1,209 patients from intensive care facilities (Cassini *et al.*, 2019). The exact prevalence surveys show 117,138 persons to have acquired Hospital acquired infections from facilities intended for prolonged stay or long-term hospital stay in 23 European countries. by the European Centre for Disease Prevention and Control (ECDC) on the costs associated with six key healthcare-associated infections namely: healthcare-associated pneumonia, urinary tract infection, surgical site infection, *Clostridium difficile* infection, neonatal sepsis, and primary bloodstream infection) in Europe revealed that Hospital acquired infections in Europe had a higher burden than that of other 31 combined infectious diseases.

Similarly, studies show that at least one patient out of 20 who were hospitalized ended up acquiring a Nosocomial infection. Greece, for instance, recorded a 9.1% prevalence rate of Hospital acquired infections where Surgical sites infections, urinary tract infections, blood stream infections were common. Saka *et al* (2016) identify *Staphylococcus species*, Gram-positive and Gram-negative rods as the most common isolates in medical and surgical wards. A meta-analysis of different literature on the cost of NIS Southeast Asia demonstrated a prevalence of 21.6% (Bhat *et al.*, 2021b). In the South Asian countries, Indonesia reported a prevalence rate of 30.4%, the highest compared to the lowest in Singapore, which recorded an 8.4% Hospital acquired infections prevalence rate. Researchers in India found that Hospital acquired infections in surgical wards have a pooled prevalence rate of 21.9% and an incidence rate of 12.72%.

In Sub-Saharan Africa (SSA), prevalence rates for nosocomial infections have been reported to be between 1.6-28.7%. In Botswana, the prevalence rate is 13.4% and 16.9%

in Ethiopia. This elevated prevalence in Sub-Saharan Africa is attributable to poor infection control measures and insufficient prevention capacity (Sartorius et al., 2024). Moreover, hand hygiene, insufficient knowledge, poor training, inadequate resources, and increased workloads have been associated with a high prevalence rate of healthcare-acquired infections in Sub-Saharan Africa. Other researchers have reported an 4.6% rate of SSI infections and a pooled prevalence of 2.5-30.9% in South Africa and Africa, respectively (Nair et al., 2018)

Also, in developing countries including Asia and Sub-Saharan Africa, 16% of hospitalized patients acquire hospital acquired infections. According to World Health Organization (WHO) (2011), Hospital acquired infections are responsible for 75% neonatal deaths in Sub-Saharan Africa and South East Asia. Studies therefore show that both gram positive and gram-negative bacteria cause Nosocomial infections (Fraser et al., 2021). In other studies, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* have been reported as the predominant bacteria isolates in surgical areas (Călina et al., 2017); Patel et al., 2019). In Morocco by Chaoui et al. (2019) posit that Enterobacteria (31.6%) is the primary contaminant in surgical sites. However, in Nigeria, Olowo-Okere and Babandina's (2018) argue that *Staphylococcus aureus* was the primary contaminant in surgical sites with 42%. In Kenya, there is limited data and research on healthcare-associated infections. However, a study of Kenyatta National Hospital's nosocomial incidence rate revealed that HAIs are rapid at 38% incidence rate. A 2012 surveillance in Kenyan hospitals in 2010-2012 showed that nosocomial infections among pediatrics were 6%, but none were confirmed (Ratemo, 2014).

### **2.3 Burden of Nosocomial Infections**

Nosocomial infections have a substantial burden in the society. Its cost spread from physical, psychological, and economic burdens (Badia *et al.*, 2017). The consequences of having nosocomial infections include depression, pain, anxiety, morbidity, and mortality among patients. Moreover, the burden on patients extends to financial costs as patients have to incur expenditures in seeking medication. This may sink them into debt. A study on six European nations: Britain, Netherlands, France Germany, and Spain found that hospital-acquired infections were associated with higher hospitalization costs due to elevated need for research, treatment, and hospitalization (Badia *et al.*, 2017). Nosocomial infections cause increased indulgence of antibiotics, leading to antibiotic resistance in hospital-acquired infections (Bhat *et al.*, 2021b; Gelaw *et al.*, 2014; Wang *et al.*, 2020).

#### **2.4 Sources of Bacteria for Nosocomial Infections**

According to Chester *et al.*, (2023) key sources of bacteria include surfaces, table tops, door handles, and hands of healthcare staff, among others, act as catchments for contaminants in the hospital setting. (Chester *et al.*, 2023) assert that most Hospital acquired infections are acquired from contact with hospital surfaces or interaction with contaminated equipment. Chester *et al.*'s research shows that the ward surfaces have the highest number of isolates at 58.7% (n=27).

In the hospital set-up, access to the surrounding environment may pose as a risk of bacterial contamination. Table tops can be contaminated by patients, healthcare workers and family members during a patient's hospital stay (Magill *et al.*, 2018). Patients can be sharing tables in cases where the hospital set-up has limited resources and this can lead to contamination of the table tops hence transmission of pathogens from one patient to another (Magill *et al.*, 2018). Also, healthcare workers can cause contamination when the table tops come into contamination with already used patient equipment like intravenous

infusion sets, branulas, used needles, swabs and strappings among others (Magill *et al.*, 2018).

The disease-causing micro-organisms tend to colonize and multiply on the surface. When a patient comes into contact with the pathogens, then the disease transmission cycle ensues and the patient acquires another disease while in the hospital set-up. However, in this case, the disease to be acquired will depend on the organism that the patient acquires. The same principle applies to other objects that pose as sources of bacteria contaminants (Magill *et al.*, 2018). The employees, hospital facility surfaces, instruments and devices are prone to colonization with different microbial contaminants (Goyal *et al.*, 2019). Bacteria are known to survive on devices, instruments and tools such as dustcoats, computer devices, communication devices, furniture, clothes, stethoscopes, and elevator buttons among others. This makes patients, healthcare staff, and the environment form the main sources of contaminants in healthcare facilities (Artika & Ma'roef, 2017). The healthcare facilities are a major reservoir for potential microorganisms. Due to their repeated interaction with patients or through infected inanimate objects (Ling & Mandriaga, 2015). The hospital facilities could contain gram positive and gram-negative bacteria which are able to survive for many days on inanimate surfaces of the hospital facility. Some of the bacteria that are table on dry hospital environment include methicillin resistant *Staphylococcus aureus* (MRSA), *Pseudomonas spp.*, *Acinetobacter spp.* and *Vancomycin Resistant Enterococci* (VRE) (Wang *et al.*, 2020).

Sources of bacteria are either exogenous from the environment or endogenous from the patient (Wang *et al.*, 2020). The endogenous flora of the patient could also be a source of microbes. Nosocomial infections can also originate from patient food in healthcare facilities, medical devices, and equipment (Wang *et al.*, 2020). Microbes could be passed

through direct contact (*Escherichia coli* and *Staphylococci*) and droplets from infections surfaces or people. Indirect contact is a frequent way illness is spread in hospitals. The healthcare workers can spread microorganisms through their hands. Infected patients disseminate microorganisms in the hospital surfaces through expectorate drops, fluids from the body or infected wounds, blood and excrements. Clothes could also be a source of pathogens from infected patients (Tantray *et al.*, 2022). Visitors to the hospital and health care providers could also be carriers when colonized by pathogens. It is common for asymptomatic carriers to be sources of bacteria such as *Neisseria meningitidis*, *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Corynebacterium diphtheria* (Goyal *et al.*, 2019). Water distribution points and aerosols released from water cooling machines could also be a source of pathogens (Feng *et al.*, 2019).

Bacterial contamination can occur when pharmaceuticals are undergoing distribution, or when food and beverages are served to the patients. Moreover, poor handling of hospital waste could be a contamination source. Water from the tap could also contaminate the medical equipment (Wang *et al.*, 2020). Alternatively, Bhatta *et al.*, (2021) argues that the high distribution of bacteria on surfaces and equipment observed may be due to overcrowded wards, high bed occupancy for surgical areas, patients admitted with different clinical conditions from other health facilities, and lack of compliance to infection control practices (ICP). Bhatta *et al.* assert that patients, visitors, health professionals, and workers could contaminate the hospital environment and equipment, thus leading to microbial colonization. The sources of contamination in a hospital setting may include medical devices, medical equipment such as infusion stands, health care personnel hands, and contaminated surfaces such as floors, doors, and tables.

### **2.4.1 Medical Devices and Equipment**

Medical equipment plays a vital role in patient care, but it can also become a breeding ground for bacteria if not properly cleaned and disinfected. Studies have shown that a wide range of equipment, from stethoscopes and blood pressure cuffs to ventilators and ultrasound machines, can be contaminated with bacteria, including multidrug-resistant (MDR) pathogens (Călina *et al.*, 2017).

The contamination can occur in several ways. Patients themselves can shed bacteria onto equipment during procedures or examinations. Additionally, healthcare workers' hands can inadvertently transfer bacteria from one patient to another through contaminated equipment. Furthermore, some bacteria can survive on dry surfaces for extended periods, further increasing the risk of transmission (Wang *et al.*, 2020).

The consequences of contaminated medical equipment can be severe. HAIs are a significant burden on healthcare systems, leading to increased morbidity, mortality, and healthcare costs (Wang *et al.*, 2021). Bacteria on medical equipment can contribute to the spread of these infections, particularly in vulnerable patient populations with weakened immune systems.

### **2.4.2 Contaminated Surfaces**

Beyond medical equipment, hospital surfaces are another major source of bacterial contamination. Frequently touched surfaces like bed rails, doorknobs, light switches, and keyboards can harbor a variety of bacteria (Cardo *et al.*, 2018). Similar to equipment, these surfaces can become contaminated through patient shedding or contact with contaminated hands. The risk associated with contaminated surfaces is particularly concerning in areas with high patient turnover, such as waiting rooms and patient care areas. Bacteria can persist on surfaces for extended periods, creating a potential source of transmission for patients and healthcare workers alike. For instance, a study by Goyal *et*

*al.* (2019) found that *Clostridium difficile* (C. Diff), a bacterium known for causing severe diarrhea, could survive on surfaces for weeks. This highlights the importance of thorough and regular cleaning and disinfection of all hospital surfaces. It may even be transmitted from lockers and doors.

### **2.4.3 Hands of Healthcare Personnel**

Healthcare workers' hands are often the final link in the chain of bacterial transmission in hospitals. During routine patient care, healthcare workers frequently come into contact with patients, their bodily fluids, and contaminated surfaces. This constant contact can readily transfer bacteria onto their hands, making them a potential vector for spreading infections (World Health Organization, 2015). Research indicates that even with proper hand hygiene practices, some level of bacterial contamination can remain on healthcare workers' hands (Boyce & Pittet, 2002). This underscores the critical role of hand hygiene in preventing HAIs. The World Health Organization (WHO) recommends a five-moment hand hygiene strategy, which outlines specific times when healthcare workers should clean or disinfect their hands (World Health Organization, 2015). Following these guidelines is essential for minimizing the risk of bacterial transmission via hands.

### **2.5 Types of Pathogens Causing Hospital acquired infections**

The main pathogens associated with hospital stay infections have remained to include *Staphylococcus aureus*, *Coagulase-negative staphylococci*, *Enterococcus species*, and *Escherichia coli* isolates. According to (Bhat *et al.*, 2021b), the increasing rate of nosocomial infections results from multidrug-resistant bacteria and methicillin-resistant *Staphylococcus aureus*.

Researchers indicate that the pathogens that cause nosocomial infections include bacteria, viruses, and fungal parasites. The pathogen distribution varies depending on medical facilities, environments, and populations (Goyal *et al.*, 2019). They cause diseases when

individual's immunity is weak. Others occur in hospital settings, such as the intensive care unit. These bacteria are known as Acinetobacter and are embedded in soil and water, contributing to over 80% of infections reported in hospital setting (Wang *et al.*, 2020). Other bacteria are found in the intestinal tract and the colon and are infectious when combined with other bacteria. This is a commensal bacteria known as *Bacteroides fragilis*. Another type of bacteria is Clostridium, which causes colon inflammation, resulting in diarrhea and colitis since the inflammation it causes eliminates the valuable bacteria. *C.difficile* transmission occurs through healthcare staff from an infected patient when hand hygiene is not observed. Other common bacteria include Enterobacteriaceae, which cause infections in the gut, where they are usually located. These bacteria constitute *Klebsiella* and *Escherichia coli* and are resistant to carbapenem (Centers for Disease Control and Prevention, 2019b).

Besides bacteria, viruses are also substantial causes of nosocomial infections, contributing to 5% of all infections. Usually transmitted through hand-to-mouth, respiratory route, and fecal-oral transmission route, viruses can cause infections in healthcare workers and staff. Common infections caused by viruses include hepatitis, HIV, Herpes simplex, and rotavirus (Center for Disease Control and Prevention, 2019b). Fungal parasites are also part of pathogens that cause nosocomial infections where the patient's immune is vulnerable. Such fungal parasites include *Aspergillus spp.*, *Candida albicans*, and *Cryptococcus neoformans*. *Aspergillus ssp.* Environmental contamination is a potential cause of nosocomial infections, whereas *Candida albicans* and *Cryptococcus neoformans* cause infections when hospital stays increase. *Candida* infections are usually endogenous and occur from the patient's microflora. In contrast, *Aspergillus* infections occur through inhalation of fungal spores contaminated by air at fieldwork in the healthcare environment and structures.

Another survey from a leading Indian intensive care hospital showed that *Staphylococcus aureus* (24.3%) was the most common isolates for surgical site infections resulting in pus, followed by *Pseudomonas aeruginosa* (21.5%), *Escherichia coli* (14.0%), *Klebsiella pneumonia* (12.2%), *Streptococcus pyogenes* (11.2%), *Staphylococcus epidermis's* (9.4%) and *Proteus* species (7.5%) (Rajkumari et al., 2014). These findings align with those in England as per surveillance by National Health Services (NHS) Hospitals in 2015. The surveillance revealed that that *Staphylococcus aureus* was the dominant from orthopedic and spinal surgeries, accounting for over 36% of all nosocomial infections. The report, however, indicated that *Coagulase-negative staphylococci* and *Enterobacteriaceae* were prevalent in colon surgery patients. Moreover, *S. aureus* was responsible for 13% of infections in the surgical ward for patients. This was attributed to a decline in *Methicilin Resistant Staphylococci Aureus (MRSA)* (Public Health of England, 2015).

In India Rao & Harsha (2014) pointed out that the most prevalent isolates was *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumonia*, *Streptococcus pyogenes*, and miscellaneous gram-negative rods with the leading dominance at 50.32% compared to the lowest 5.88%. Similarly, a report on Switzerland and Greece revealed the same order for most prevalent isolates in surgical sites. (Alexiou et al., 2017).

According to Ratemo (2014), burn wounds in Pakistan showed that *Staphylococcus aureus* (58%) was the most predominant agent in burn infections. Other causative agents, in order of their predominance, included *Pseudomonas aeruginosa* (19%), *Klebsiella* (8%), *Proteus* species (4%), *Staphylococcus epidermidis* (3%), *Escherichia coli* and *Enterobacter* (3%) each, *Citrobacter* and *Serratia* (0.84%). In contrast, however, Kehinde carried out a study in Ibadan, Nigeria regarding on burn wound infections. According to

the study, revealed *Klebsiella* was the predominant pathogen, constituting 34.4% while *Pseudomonas aeruginosa* was ranked second 29.0% and *Staphylococcus aureus* came third accounting for 26.8%

Investigations by researchers in Africa reveal *S. aureus* and *E. coli* as the most prevalent isolates causing nosocomial infections. In Ethiopia by (Gelaw *et al.*, 2014), 268 bacteria pathogens were isolated from processed specimens. The study revealed Staphylococcus species and Gram-negative rods as primary agents of surgical site infections. In Kenya, (Ratemo, 2014), *Staphylococcal aureus* was the most common isolate from surgical site infections, which are part of nosocomial infections

## **2.6 Risk Factors of Nosocomial Infections**

Although nosocomial infections are common in hospital settings, the potential of developing is hastened by various risk factors. Research-based known risk factors (Sadique *et al.*, 2016) include older age, hospitalization period, immunosuppression, invasive procedures, underlying comorbidities, and lack of infection control measures. Behar *et al.* (2017) argued that older patients have a high probability of being colonized with *C. difficile* presenting with diarrhea. This contributes to a high rate of transmission to their patients. Increased risk of colonization with *C. difficile* is associated with suppressed immunity and frailty, which are all linked to aging. Similarly, regular readmissions, hospitalization, and inpatient stays are strongly related to developing nosocomial infections. The most prevalent isolated organisms linked to SSI due to inpatient stay and readmissions or hospitalization are *S. aureus*, *anaerobic cocci*, *Enterobacteriaceae*, and *streptococci*. A study by (Isigi *et al.*, 2023) in England revealed that the most commonly isolated organisms were *S. aureus* (40.4%), anaerobic cocci (23.2%), Enterobacteriaceae (13.3%), and streptococci (7.4%)- all on skin to genital flora. On the contrary, (Chu *et al.*, 2015) study on surgical site infections during cesarean

section in Sub-Sahara Africa found that young age among women was associated with possibility of increased surgical site infections due to weak immunity.

Poor nutrition is also associated with a high risk of contracting nosocomial infections. For instance, surgical site infections are more likely to occur in people with a deficiency of micro and macronutrients like vitamins, proteins, carbohydrates, iron, zinc, and magnesium. Such deficiency lowers the healing process of the wound. (Yuwen *et al.*, 2017) and (Zewdu *et al.*, 2023) suggest that when serum levels are below 3.5 g/dl, patients may become more vulnerable to surgical site infections. According to the authors, low serum levels indicate a comorbidity that adversely affects a person's immunity.

The presence of urinary catheters is also associated with acute sepsis, especially for gram-negative multi-drug resistant *E-coli*. Inadequate Control procedures and associated intrahospital transfers are also prevalent risk factors for nosocomial infections (Blay *et al.*, 2017) showed that intrahospital transfers raised the chances of contracting diseases from the hospital (Boncea *et al.*, 2021), in their retrospective case-control investigation in the United Kingdom, supported Blay *et al.*'s finding by revealing that increased intrahospital transfer was linked with a risk of over 9% in hospital-acquired infections. Immunosuppressive conditions such as obesity and diabetes have also been discovered as contributing factors to the high risk of nosocomial infections. These conditions lower the immunity of patients, making it hard for the body to defend against infections (Gelaw & Abdela, 2018).

## **2.7 Route of Transmission of Major Hospital Acquired Infections**

The transmission of diseases in general is based on the disease transmission cycle (Wang *et al.*, 2020). Different diseases or infections are transmitted in different ways and the portal of entry in each host differs with the disease nature. Moreover, the acquisition of a

disease is dependent on various factors. Among them include the host's immunity, level of susceptibility of the host to the disease among other factors. Nosocomial infections also have its disease transmission cycle.

Microorganisms that cause Nosocomial infections have different routes of transmission. The most common route of transmission is through contact which can be direct or indirect (Bhatta *et al.*, (2021). The major organisms that are transmitted through contact are *Rota virus*, *C. difficile* and *multi-drug resistant bacteria like Vancomycin Resistant Enterococci, Methicillin Resistant Staphylococci Aureus and Extended Spectrum Beta-Lactamase-producing Gram-negative organism*. Other modes of disease transmission include droplets and airborne transmission. Transmission of droplets occurs when microorganisms are transmitted through the respiratory system through large droplets. The droplets can be from *Neisseria meningitidis, Bordetella pertussis and Influenza*. The droplets are greater than five microns in size and travel less than three feet (Weinbaum *et al.*, 2020). In contrast, airborne transmission involves organisms from the respiratory tract where droplets, less than five microns get to the environment and travel in long distances.

### **2.7.1 Central line-associated Blood Stream Infections**

Central Line- Associated Blood Stream Infections (CLABSI) affects the central venous Catheter (CVC) (Beville *et al.*, 2021). It is the most preventable form of Nosocomial infections. In the United States (US) about 55% of patients in the Intensive Care Unit (ICU) and 24% of non-ICU patients have Central Venous Catheter (Gupta *et al.*, 2020) The development of Central Line-Associated Blood Stream Infections occurs when the bacteria on the skin proliferates heading towards the intravenous part of the catheter from the external portion. Risk of the disease's occurrence happens during the process of insertion or manipulation, hematogenous seeding among other ways. Organisms causing

CLABSI and CAUTI regularly have virulence that causes biofilm production. The biofilm production leads to increased proliferation of the disease-causing organism and their adherence on external devices. A study done in the US listed the major organisms associated with the infection and their rates as *Staphylococcus aureus* (23%), *Candida species* (13%), *coagulase negative staphylococcus*, *Streptococcus* and *Enterococcus species* (12%), *Escherichia coli* (8%) and *Bacteroides species* (6%). Other studies show coagulase negative *Staphylococcus* as the most common organisms. However, the major challenge posing on all the organisms is antimicrobial resistance.

Host and catheter are the two main factors predisposing to the risk of acquiring CLABSI. Host factors mainly include bone marrow transplant, parenteral nutrition, malnutrition, extreme age and chronic cells. All these are immune-compromised status in a nut-shell. Besides, risk factors associated with catheter include prolonged hospitalization before catheterization and prolonged time of catheterization, and break of sterile barriers or failure to observe the aseptic techniques. Others include type of catheter material, multi-lumen and multiple Central Venous Catheter.

### **2.7.2 Catheter Associated Urinary Tract Infections (CAUTI)**

In the event when an internal dwelling catheter is inserted for various medical reasons the risk of acquiring CAUTI may occur (Clawson *et al.*, 2022). A study conducted in the US showed that 15 to 25% of hospitalized patients have a urinary catheter. The placement of a catheter is usually intraluminal or extraluminal. The extraluminal infections occur when bacteria manifest on the urinary tract and ascends from the urethral meatus to the bladder carried along from the surface of the catheter. On the other hand, intraluminal infections occur in the event of urinary stasis which results from blocked drainage or infection ascending from the intraluminal side of the catheter following contamination.

Bacteria and fungi depend on formation of biofilm to support growth and spread alongside the indwelling device. In addition, the bacteria that invade are from the fecal and skin micro-flora. Numerous researches have established the major bacterial organisms responsible for causing CAUTI. *Escherichia coli* is the leading causative organism which is followed by *Klebsiella oxytoca* and *pneumonia*. Other species include *Enterococcus species*, *Pseudomonas aeruginosa* and candida species. Nevertheless, there are complications that result from CAUTI and they include bacteremia, sepsis, and upper urinary tract involvement.

### **2.7.3 Skin and Soft Tissue Infection**

An approximate 2-5% of patients who have undergone surgery are prone to skin and soft tissue infections. Moreover, the infection manifests after 30 days and 90 days of surgery and implanted devices respectively (Kaminsk *et al.*, 2022). Skin and Soft Tissue Infection type depends on the location and depth of the infection. In the case of superficial Skin and Soft Tissue Infections, the infection affects the skin and subcutaneous tissue while deep SSI involves the muscles and muscle fascia and organ. Space specific SSI occupies the area where surgery has been done. Also, factors like female genital tract in ladies' cases, patient's skin and gastrointestinal tract serve as healthy flora reservoir that contaminate surgical site in regard to the location of surgery.

Duration of surgery, hypoxemia, hypovolemic and subsequent hypothermia during surgery, blood transfusion, wound class, duration of surgery, urgency of surgery, more than one intervention and type of prosthesis that is implanted. Tissue exposure to the environment based on duration of surgery is the main risk factors that determine rate of exposure to contamination. Wound class is another consideration since clean wounds do not have a high risk of contamination as compared to dirty, contaminated and clean-

contaminated wounds. Additionally, there are other risk factors revolving around SSI. They include post-operative and patient-related risk factors.

Post-operative risk factors include length of stay after surgery, poor wound hygiene, and wound drains. In contrast, patient-related risk factors include increased age, malnutrition, obesity, immunosuppression, joint disease, drug abuse especially smoking of tobacco and hyperglycemia among others. Major micro-organisms responsible for SSI include *E. coli*, *S. aureus*, *coagulase-negative Staphylococcus*, *Klebsiella species*, *Enterococcus* and *Enterobacter species*. Other but limited risk factors for acquiring SSI are exogenous sources of microorganisms such as environment and surgical instruments.

## **2.8 Types of Bacteria**

### **2.8.1 Gram positive bacteria**

#### **2.8.1.1 *Staphylococci aureus***

*Staphylococci aureus* is non-motile, non-capsulated and gram-positive cocci. It is a common flora of the skin, upper respiratory tract, and the intestinal tract in the body; consequently, it is widely distributed in the environment. It colonizes a third of the global population (Neal & Eric Skaar, 2011). *Staphylococci* strains develop resistance to antibiotics hence posing a serious challenge in clinical management, when it escapes the natural habitat they cause osteomyelitis, bacteremia, pneumonia, endocarditis and also septic shock (Saleha *et al.*, 2021). It is the leading cause of Hospital acquired infections and bacterial pneumonia. In terms of sensitivity to antibiotics, the bacteria are categorized into *Methicillin Sensitive Staphylococci aureus* (MSSA) and *Methicillin Resistant Staphylococci aureus* (MRSA). *S aureus* majority of species produce penicillinase (beta lactamase) hence making them penicillin resistance (Athena *et al.*, 2020).

In terms of its sensitivity, research in Nigeria by Olowo-Okere and Babandina's (2018) found that *Staphylococcus aureus* was the primary contaminant in surgical sites with 42%. However, research in Morocco by Chaoui et al. (2019) disagrees as it shows that Enterobacteria (31.6%) is the primary contaminant in surgical sites.

#### **2.8.1.2 Methicillin Resistant Staphylococci aureus (MRSA)**

*Methicillin-Resistant Staphylococcus aureus (MRSA)* is a form of *staphylococci* bacteria that is antibiotic-resistant in general (Mehndiratta & Bhalla, 2014). *MRSA* can cause serious issues like bloodstream infections, pneumonia, and Skin and soft Tissue Infections in healthcare facilities like hospitals and nursing homes (Ford *et al.*, 2022). According to estimations, 53 million (2.7 percent of carriers) of the 2 billion persons who carry *S. aureus* in some form also have *MRSA* (Stokes *et al.*, 2019). *S. aureus* was identified as one of the six major infections for mortality related with resistance in 2019, and antimicrobial resistance was responsible for 100,000 deaths brought on by *MRSA* (Holmes *et al.*, 2015). Vancomycin is the drug with high efficacy in treatment of the *MRSA* (Holmes *et al.*, 2015).

#### **2.8.1.3 Clostridium difficile**

Clostridia are gram positive and they form spores. The strains are found in human, animals and some environments (Cristina *et al.*, 2018). They cause causes life-threatening diarrheal disease, hence a risk to health care management. The diarrhea occur due to prolonged antimicrobial therapy that cause imbalance to the gut normal flora. Infection has caused worldwide concern to America and Europe (Jinyu *et al.*, 2022).

#### **2.8.1.4 Enterococcus species**

Enterococcus are gram-positive cocci naturally found in the gut and the environment. The bacteria cause opportunistic infections; of major concern is *E. faecalis* and *E. faecium* strains. Its virulence includes cytolysin plus other factors. The microbe is the foremost

cause of NIs in the U.S with prevalence rate of 20%-30% and it is ranked second globally, as a key cause of such infections (Khan *et al.*, 2015). In a recent Chinese report, *E. faecium* prevalence was (74%) and *E. faecalis* at 20% , BSIs with 24% mortality rate in total (Zhang *et al.*, 2017).

## **2.8.2 Gram negative bacteria**

### **2.8.2.1 *Escherichia coli***

It is a gram-negative motile bacterium and it's found in intestinal tract of human and animals as normal flora (Marie *et al.*, 2020). The bacteria are also largely found in the environment. It is facultative anaerobe and aerobe and it acts as an opportunistic pathogen. *E. coli* is problematic to clear due to the biofilm's formation (Abdelkarim *et al.*, 2020). Biofilm is a group of microbes that coexist together and are often held by solid surfaces in a moist surrounding by polysaccharides.

### **2.8.2.2 *Klebsiella pneumonia***

Pneumonia is the inflammation of the lung parenchyma is of different types notably community acquired pneumonia and hospital acquired pneumonia. Hospital acquired pneumonia is a Nosocomial infection and occurs forty-eight hours after a patient is admitted in the hospital. Ventilator-Associated Pneumonia (VAP) occurs within 48 hours following endotracheal intubation. It is estimated that out of all patients that undergo endotracheal intubation, 5-15% of them develop VAP. Ventilator Associated Pneumonia happens following hematogenous spread, aspiration, bacterial translocation and after inhalation of bacterial aerosols. The major organisms associated with Hospital Acquired Pneumonia (HAP) and VAP consist of *S. aureus*, *P. aeruginosa*, *Candida species*, *Klebsiella pneumonia* and *Klebsiella oxytoca*.

The different strains are naturally found in the environment, gut, upper respiratory system, mouth and also in hospital moist areas. Bacteria are gram negative, capsulated

rods and non-motile. It causes Nosocomial infections pneumonia, urinary tract infection, bacteremia and life-threatening septic shock. Most strains resist penicillin with some having many antimicrobial resistances. The strain causes increased mortality when there is delay of treatment. These include UTIs (second after *E. coli*) due to prolonged catheterization and wound contamination. Virulent factors include lipopolysaccharides and O antigen, pili for adhesion to host epithelial cells (Călina *et al.*, 2017). The capsule protects from phagocytosis and siderophores help to compete for iron uptake with the host.

#### **2.8.2.3 *Klebsiella oxytoca***

*Klebsiella oxytoca* is currently in the rise rising as an opportunistic pathogen which is causing hospital acquired infections in patients. The bacteria are the second most common cause of *Klebsiella* infections in humans after *Klebsiella pneumoniae*. Its prevalence varies from 2 to 24%, outbreaks of infections due to multidrug-resistant strains can be fatal in immunocompromised individuals with comorbidities. The bacteria are responsible for a wide range of infections such as colitis to infective endocarditis, in addition to the common urinary tract infections and respiratory tract infections. Its pathogenicity has been attributed to cytotoxins' production namely Tilivalline and Tilimycin, in some intestinal disorders. The bacteria have high resistance to antibiotics.

#### **2.8.2.4 *Pseudomonas aeruginosa***

It is a gram negative, rod shaped, obligate anaerobe and it is motile. The species have their normal habitat in the environment and gut. The bacteria have high prevalence in the hospital environment (Pachoria *et al.*, 2019). Its majorly found in moist surfaces like sinks, drainages and cleaning containers. There is also evidence of it growing in various solutions of antiseptics and ointments (Evans *et al.*, 2016).

#### **2.8.2.5 *Acinetobacter baumannii***

*Acinetobacter baumannii* (*A. baumannii*) is a gram-negative bacterium that has become a nightmare for hospitals due to its ability to thrive on various surfaces and its alarming rise in antibiotic resistance (Călina *et al.*, 2017). This common contaminant lurks on equipment, bedding, and even healthcare workers' hands, posing a significant threat as it can cause a variety of healthcare-associated infections (HAIs), including pneumonia, bloodstream infections, and surgical site infections (Guh *et al.*, 2015).

What makes *A. baumannii* particularly troublesome is its tenacity in the environment. This bacterium can survive on dry surfaces for extended periods, making complete eradication a significant challenge (Guh *et al.*, 2015). Additionally, it readily acquires resistance to antibiotics, rendering many traditional treatments ineffective. This phenomenon, known as multidrug resistance (MDR), makes *A. baumannii* infections difficult to treat, often requiring the use of powerful and potentially toxic antibiotics (Goyal *et al.*, 2019).

The widespread contamination of hospitals by *A. baumannii* necessitates robust infection control practices. These practices include thorough hand hygiene for healthcare workers, regular disinfection of surfaces, and proper handling of medical equipment. Furthermore, ongoing research is crucial to develop new diagnostic tools and treatment strategies to combat Multi Drug Resistance *A. baumannii* infections.

#### **2.8.2.6 *Carbapenem Resistant Enterobacteriaceae (CRE)***

They are gram negative bacteria that do resist carbapenem antibiotics due to production of carbapenemase enzyme. *CRE* are agents that are infectious and commensals too. Health facilities are primary transmission sites for *CRE* hospital acquired infections (Guh

*et al.*, 2015). 75% of hospital admissions was due to *CRE* prolonged hospital stay or facility transfer (Guh *et al.*, 2015).

## **2.9 Antimicrobial Resistance**

It is the decline in a treatment's ability to manage or treat a condition or disease (Mehndiratta & Bhalla, 2014). Bacteria that are resistant to both single and multiple antibiotics have increased in prevalence during the past 10 years, making some infections particularly challenging to treat (Metsini *et al.*, 2018). It occurs when microbes change the ways in which they face the effects of antimicrobials (Călina *et al.*, 2017). Despite being a naturally-occurring process, AMR often results from inappropriate practice of taking drugs, or managing infections (Fatima *et al.*, 2014).

In fact, almost all-important bacterial illnesses worldwide are becoming resistant to antibiotic therapy, according to the Center for Disease Control and Prevention (2015).

Bacterial resistance may result in more doctor visits, a more serious sickness, and possibly more harmful medications for some of us. For others, it might be fatal. Nosocomial infections are a major source of death and sickness worldwide. Although there are intensively specialized interventions and policies, the infection rates stand tall due to antimicrobial-resistant bacteria. The resistance can naturally occur through genetic-mutation. Moreover, it can occur when one species acquires resistance from another (Samuel *et al.*, 2019).

Lengthy use of antimicrobials appears to hearten selection for mutations which can render antimicrobials useless (Abraham *et al.*, 2022). Global rise of Antimicrobial resistance can also be attributed to amplified recommendation and provision of antibiotic drugs It is estimated that over 700, million deaths occur annually(Sartorius *et al.*, 2024; WHO,

2023). The World Health Organization approximates 350 million deaths could arise by 2050 due to Antimicrobial resistance (Samuel *et al.*, 2021).

According to Goyal *et al.* (2019), the levels of hospital acquired infections have significantly increased. The increase is associated with complexities of resisting a plethora of antibiotic drugs' effect, including MRSA. In the United States of America, for instance, 2.8 million or more people experience antibiotic resistance annually, with over 35,000 deaths occurring. The deaths are, however, on the rise as antimicrobial resistance increases. For instance, in 2019, *Staphylococcus aureus* (61,011) and *E. coli* (41,656) were responsible for the majority of deaths due to antimicrobial resistance (AMR) (WHO, 2023). Moreover, WHO (2023) reports that antimicrobial resistance has become a concern to public health issue in Europe. From the report of European Union-European Economic Area (EU/EEA), 670,000 infections are related to bacteria resistance to antibiotic drugs, out of which 33,000 people die each due to antimicrobial resistance-related problems. This has warranted increased control measures to curb the growing resistance.

In India Bhat *et al.* (2021b), note that there has been an alarming increase in antibiotic resistance among nosocomial infections from surgical sites. Bhatt *et al.* (2021) argue that there is widespread drug resistance among bacteria isolates by over 65.4%. In Nepal, 67% of bacterial isolates were multidrug-resistant, whereas 27% resisted only one antibiotic. In the same study, 6% of the selected species were sensitive to the tested antibiotics (Călina *et al.*, 2017). The study sought to investigate prevalence, etiological agents, and drug sensitivity patterns of bacterial pathogens secluded from Skin and Soft Tissue Infections at Gondar University Teaching Hospital environment. According to standard procedures, the researcher processed the specimen for analysis and antibiotic susceptibility tests and performed a sensitivity test analysis using the disk diffusion

method. Using the three pathogenic bacteria, the researcher found that *Escherichia coli*, *Staphylococcus aureus*, *Coagulase-negative Staphylococcus*.

Bhat et al. found that different gram-negative rods and Gram-positive bacteria isolates resist broad-spectrum antibiotics like cephalosporin, chloramphenicol, and penicillin. In Africa, Antimicrobial resistance remains a considerable burden. A study by found (Sartorius *et al.*, 2024) that 1.86 million people out of 3.83 million taken for the study died of susceptible and resistant bacteria, out of which approximately 1.1 million were linked to antimicrobial resistance and 0.25 million deaths were attributable to Antimicrobial resistance. From the study, World Health Organization Africa region had the depicted the leading fatal and non-fatal antimicrobial resistance burden compared to other World Health Organizations regions. South Africa has significantly felt the blunt of antimicrobial resistance, with 9,500 deaths being attributable to antimicrobial resistance and 39,000 being associated with AMR. Similarly, 7,100 deaths were attributed to AMR, whereas 30,700 deaths were associated with it in 2019. Kenya, on the other hand, experienced 8,500 Antimicrobial resistance-attributed deaths, with 37,000 being associated with antimicrobial resistance.

#### **2.10. Conceptual framework**

Conceptual framework was sourced from prior knowledge. Independent variable for the study was bacterial contamination of the hospital environment whereas the dependent variable was the hospital acquired infections. Variables intervening included overutilization or underutilization of antibiotics, inadequate knowledge on infection prevention and control protocol and health care provider's ignorance.

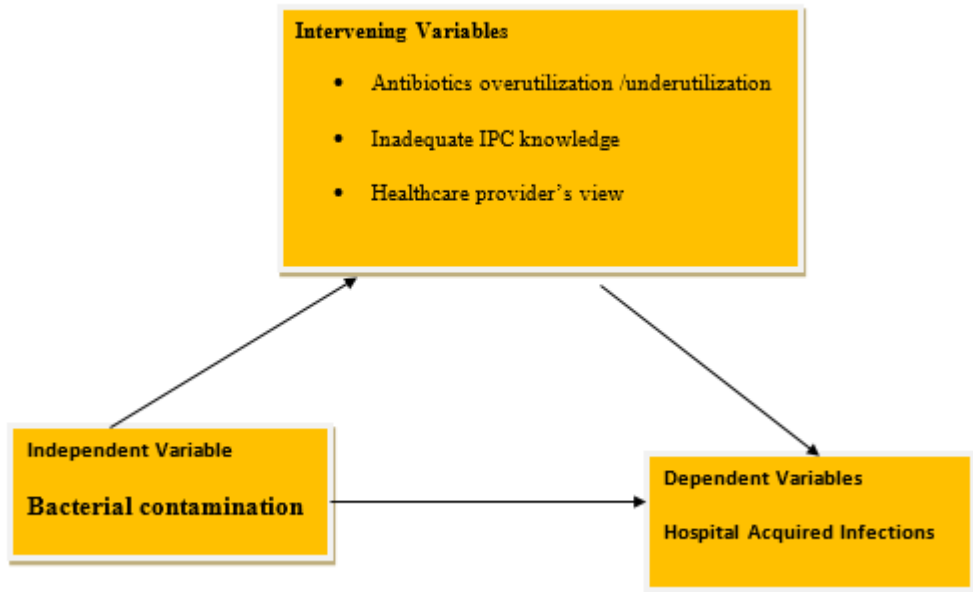


Figure 2. 1: Conceptual Framework

## **CHAPTER THREE**

### **RESEARCH METHODOLOGY**

#### **3.0 Introduction**

This chapter presents the study area, sampling techniques and laboratory pre analytical, analytical and post analytical phases of diagnostics.

#### **3.1 Study area**

The study was carried out in Kitui County Referral Hospital, Kitui County. Kitui County has a total population of 1,136,187 based on the Kenya National Bureau of Statistics (2019) where 549,003 are males and 587,151 are females. Kitui County has a total area of 30,340 km. The households' totals are 262,942 units with a population density per square kilometer of 37 people. The sample collection was done in the patient environment in medical and surgical wards of the facility. Samples were transported for analysis at Mount Kenya University in the department of Medical Laboratory Sciences in Microbiology section. Kitui County Referral Hospital is situated at Kitui Central Sub County. It offers health services to the rest of the seven Sub counties which include; Mwingi North Sub County, Mwingi West Sub-County, Mwingi Central Subcounty, Kitui West Sub County, Kitui East Sub County, Kitui South Sub County and Kitui Rural Sub County.

#### **3.2 Study design**

The researcher used cross-sectional study design and purposive sampling technique to collect samples from Kitui County Referral Hospital facility in Kitui County, Kenya. The sample collection was done between July and August 2023. Sample sources were swabbed often to ascertain the degree of contamination in the hospital. Purposive sample collection technique helped in collecting the entire sample size based on the purpose of the study.

### **3.3 Study Population**

Kitui county referral hospital has a bed capacity of 239 beds. The hospital inpatient and outpatient visits per month is approximately 800 and 500 patients respectively, Health care workers population is 650 staffs working on different shifts (morning, afternoon, straight duty and night shifts). The facility approximate number of visitors per day is 100 visitors for both patients and offices.

### **3.4 Inclusion and Exclusion Criteria**

#### **3.4.1 Inclusion Criteria**

Surgical and medical wards comprising of beds, bedside lockers, door handles, tables, chairs, sinks, drugs trolleys, infusion stands and floors

#### **3.4.2 Exclusion criteria**

Fomites from other units in the hospital other than medical and surgical wards which included outpatient areas (laboratory, pharmacy, dental, eye unit, radiology, oncology, renal, specialists' clinics), maternity, pediatric ward, amenity ward, kitchen, stores, offices and new born unit.

### **3.5 Study Variables**

Independent variable in the study was bacterial contamination of the hospital environment while Dependent variable was the hospital acquired infections. The intervening variables in the study included and not limited to antibiotics underutilization or overutilization, inadequate infection prevention and control protocols and health care providers views.

### **3.6 Sample Size Determination**

The sample size was determined using the Daniel et al formula, (2009)

Formulae:

$$n = \frac{Z^2 P(Q)}{d^2}$$

Where;

$$Q=1-P$$

n =required sample-size (target population > 10,000)

Z=number relating to degree of confidence, to be 1.96 for 95%confidence

P=the estimated prevalence to be 15%

d=proportion of error accepted in study

$$n=1.96 \times 1.96 \times 0.15(1-0.15) / 0.05 \times 0.05$$

$$n=195$$

### **3.7 Laboratory Procedures**

#### **3.7.1 Specimen Collection**

A total of 195 swabs were collected for the study from medical wards and surgical wards in the hospital. Sample collection was done on beds, bedside lockers, door handles, tables, chairs, sinks, drugs trolleys, infusion stands and floors. The selected sources were targeted because they are frequently touched by patients like beds, bedside lockers and sinks. Health care providers frequently touched the door handles, tables, chairs drugs trolleys and infusion stand. The visitors as some of them stay with the patient in the hospital wards, they frequently touch the beds, bedside lockers, sinks and door handles. Before sample collection the swabs were properly labelled with location of collection where in the study samples were collected from medical wards (ward 3 and ward 6) and surgical wards (ward 2 and ward 4), source of the samples and date of collection. Sterile swabs with amies transport media moistened with normal saline at a concentration of 0.9% to were used to collect the samples. Swabs were carefully rubbed and rotated gently on the various hospital surfaces in the patient surroundings which was the target of the required samples. Specimen collection was done 2 hours after cleaning for both morning and evening cleaning to ascertain the effectiveness of cleaning protocols.

### **3.7.2 Specimen Transportation**

Sample collection was done in sterile swabs with Amies transport media as sample analysis was to be done at Mount Kenya University hence need to preserve the quality of samples. During sample collection, once a swab was collected it was kept in a cooler box with ice packs for transporting to the Kitui County Referral Hospital laboratory fridges. The samples were later transported to Mount Kenya University for processing, identification of contaminants and susceptibility testing.

### **3.7.3 Culture**

The culturing technique entail the isolation of pathogens from pure culture for easy identification (Ogodo *et al.*, 2022). In this research, culturing techniques were used to isolate and make it possible to identify microorganisms and later performed the antimicrobial susceptibility testing to antimicrobials. The researcher used Sheep Blood agar media and MacConkey media to culture samples. Muller Hinton Agar was used to ascertain antimicrobials susceptibility testing.

#### **3.7.3.1 Culture media preparation**

The equipment and reagents used in media included the following, Media powder, weighing balance, Spatula, Hot plate, Pyrex glass volumetric flask, Distilled water, Petri dishes, and Measuring cylinder.

##### **3.7.3.1.1 MacConkey**

The media was used because it is a selective, differential, and indicator medium (Gilles *et al.*, 2022) various properties include; selective due to bile salts and crystal violet that inhibit gram-positive bacteria and selects only gram-negative bacteria, Indicator medium is due to having neutral red incorporated in it, differential medium helps to separate lactose fermenter or non-lactose fermenter bacteria.

MacConkey agar media was prepared through suspension in distilled water. The mixture was heated to boiling while stirring consistently for complete dissolution. In addition, the media was sterilized by autoclaving at 121°C for 15 minutes. Sterilization helps in killing possible contaminants. The media was then cooled to 45- 50 degrees and pour plating was done in sterile Petri dishes. The culture plates were stored at 2-8 degrees away from direct light.

#### **3.7.3.1.2 Sheep Blood agar media**

Sheep blood agar was used to grow pathogens particularly those that are more difficult to grow. The media detects and differentiates hemolytic bacteria which includes Beta, Alpha and Gamma hemolysis. It can be made selective by addition of antibiotics, chemical or dyes (Carr, 2017)

When heated cells are lysed and becomes brown hence called chocolate blood agar and can be used to grow nutritionally demanding pathogens.

#### **Preparation**

The preparation of sheep blood agar media was done as per manufactures instructions whereby 40 grams of agar was put in 1 liter of distilled water which was used to dissolve the media completely by boiling. Sterilization by autoclaving at 121°C for 15 minutes was done.

The media was cooled to 50°C and added 7% of sterile sheep blood warmed to room temperature aseptically. It was mixed well and poured into sterile Petri plates and avoided the formation of air bubbles. 15 ml was poured to sterile Petri plates aseptically. Media was labelled with the date of preparation and expiry.

The media powder was stored below 30°C in a tightly closed container and the prepared medium storage conditions at 2-8°C in a sealed plastic bags to prevent loss of moisture.

The shelf life of the prepared blood agar was up to four weeks.

#### **3.7.3.1.3 Nutrient Agar**

The Nutrient agar media was used for subculturing the harvested isolates from tryptone soya broth. It supported the growth of original bacteria. Manufactures instructions were followed in which 14 grams of powder was put in 500 milliliters of distilled water. The media was heated on hot plate and the autoclaved at 121 degrees Celsius at 15 minutes. After cooling the media was pour plated in the Petri dishes and allowed to dry. The plates were stored at 2 degrees to 8 degrees Celsius.

#### **3.7.3.2 Test Procedure**

The plates were warmed at 37°C or to room temperature for agar surface to dry before inoculating. The swabs were Inoculated and streaked with the specimen by use of sterile wire loop immediately after collection. The plates were incubated aerobically 35-37°C for 18-24 hours and colony characteristics was done.

#### **3.7.3.3 Decontamination and disposal of media plates**

Culture media plates were disposed by autoclaving at 121 °C for 30 minutes then disposed appropriately.

#### **Quality control:**

Prepared culture plates were incubated in the incubator at 37 degrees to check for any growth which could have contaminated the media during preparation.

### 3.7.4 Isolation and identification

#### Gram staining technique

Gram-positive organisms typically lack the outer membrane found in gram-negative organisms (Saka *et al.*, 2016)

Up to 90 percent of the cell wall in gram-positive bacteria is composed of peptidoglycan, with the remaining 10 percent composed of acidic substances called teichoic acids.

Teichoic acids may be covalently linked to lipids in the plasma membrane to form lipoteichoic acids. Lipoteichoic acids anchor the cell wall to the cell membrane. The gram-negative microorganisms have a thin wall of approximately 15 nm composed of toxic lipopolysaccharides (endotoxins lipid A) and lipoproteins. They contain small amount of peptidoglycan. Their cytoplasm is not acidic. Polar solvents (primary stain) disrupt lipid protein associations hence bacteria taking the counterstain color (red)

**Principle:** The gram staining technique differentiates bacteria according to their individual reaction after staining. Gram positive stains dark purple due to retaining of primary stain crystal violet, while gram negative is decolorized by the decolorizer acetone hence stains red color of neutral red counterstain. The iodine acts as a mordant for the crystal violet (Wang *et al.*, 2020)

#### Requirement:

The following were the requirements for the gram staining technique; Crystal violet stain -Primary stain, Lugol's iodine- Mordant, Acetone-Decolorizer, Neutral red -Counter stain.

#### Method:

Clean frosted slides were labelled with a pencil and sufficient material smeared. The smear was allowed to dry on a flat surface before fixation. Fixing of smear was done by use of slide warmer set at 60°C. The slides were allowed some time to cool before staining

procedure was done. Primary stain the crystal violet was flooded for 1 minutes. Decanting of crystal violet was done and running water passed through the slide to clean the crystal violet. The slide was covered with mordant Lugol's iodine for 1 minutes then rinsed gently with tap water. Decolorization was done for 30 seconds with acetone with the slide tilted till the run off is clear. The excess decolorizer was washed with gentle flow of tap water. Counterstain neutral red was flooded for 1 minute and rinsed with gentle flow of tap water. The slide was then drained and air dried on a rack in an upright cell and also gram reaction of gram positive or gram-negative bacteria cells. Examination of the smear was done first under low power objective to evaluate the general nature of the smear. The low power objective was switched to oil immersion high power objective of X100. Evaluation was done to check presence of bacteria cells (gram positive and gram negative), morphology and arrangement of cells.

#### **Gram reaction results**

Gram positive turned deep purple-rods /cocci, Gram negative was pink or red -rods /cocci, Gram variable had both gram negative and gram-positive cells

#### **Quality control**

Inoculation was done in Tryptone soya broth with colonies of standard microorganisms and incubated at 37 degrees for few hours and obtained a broth which was faintly turbid. Labelled few slides and labelled gram stain QC and preparation date. Prepared smears were air dried and fixed. The slides were kept in the slides box at room temperature.

#### **Biochemical tests (Monica Chesbrough, 2005)**

##### **Catalase**

The biochemical test was used to differentiate microorganisms that produce catalase enzyme from those that don't produce the enzyme.

**Principle:** The test shows the ability of micro-organism to produce enzyme catalase.

In this case, a drop of hydrogen peroxide was put onto clean slide. A colony was picked from culture media and stirred on hydrogen peroxide drop. Reaction was then observed immediately where effervescence indicated enzyme catalase was present and no effervescence implies catalase negative. The test was used to differentiate *Staphylococci* species which produces enzyme catalase from *Streptococci species* which are non-catalase producing bacteria.

### **Coagulase**

Coagulase biochemical test was used to differentiate *staphylococci aureus* that produces coagulase from other *Staphylococci* species like *Staphylococci epidermidis* and *Staphylococci saprophyticus* which don't produce the enzyme coagulase.

**Principle:** The coagulase test shows the ability of micro-organism to produce enzyme coagulase which causes plasma to clot by conversion of fibrinogen to fibrin.

#### **Slide method of coagulase test:**

A drop of human serum was be put on a clean glass slide. A colony was picked with pre-flamed wire loop and emulsified on the slide. Then stirring was done in a rotary motion for 2 minutes and observed macroscopically under a black background. Agglutination indicated coagulase positive and no agglutination coagulase negative.

#### **Tube method of coagulase test:**

Tube test for coagulase test was used as it detects free coagulase. The plasma was diluted with 0.9% physiological saline at 0.2ml and 0.8 ml respectively. In the three tubes it was put 0.5ml of the previously diluted plasma. The tube was labeled as Positive control (PC), Negative control (NC) and Test (T). 0.5 ml of diluted colony was put in the three test tubes. In the tube labelled T 5 drops of diluted plasma was put. 5 drops of standard *staphylococci aureus* broth culture were added in the tube labelled PC, while NC tube was put sterile broth. The three tubes where gently mixed and incubated at 37°C. The

tubes were examined after exactly 1 hour. The tubes were tilted to check the formation of clot. There was fibrin clot in the test and positive control tube unlike the negative control which had no fibrin clot. The results showed there was fibrin clot in the test tube and positive control hence confirmation of *staphylococci aureus* bacteria

### **Oxidase test**

The oxidase test was used in the study to identify *pseudomonas aeruginosa* as bacteria produces oxidase enzymes when subjected to oxidase test biochemical test.

**Principle:** The oxidase test uses filter paper disc with oxidase reagent when streaked with colony of test bacteria the phenylenediamine in reagent is oxidized to deep purple color. This indicated the test organism is oxidase positive and no color development shows the organism is oxidase negative.

Required for the procedure were the oxidase discs.

### **Method:**

Impregnated strips were placed on a slide and a colony picked with sterile wire loop. It was streaked across the paper and observed for color change after 10 seconds. Upon examination purple color was developed and it indicated positive reaction which concluded that oxidase was produced. No color concluded that no oxidase enzyme produced.

### **Indole**

Indole biochemical test was used to identify Enterobacteriaceae and in the study it was used to identify *Escherichia coli*.

**Principle:** Indole tests detect the ability of certain micro-organism to decompose an Amino Acid Tryptophan (media) to produce indole which accumulates into the media. Upon addition of Kovacs reagent which has 4(p)-dimethylaminobenzaldehyde the reaction occurs with indole forming a red color.

Required reagents were the Tryptone water and Kovac reagent

### **Method:**

The researcher inoculated the organism in Tryptone water media. Incubate at 37°C for overnight to allow optimum accumulation of indole. 2-4 drops of Kovacs reagent a then added. Observed the color change where red color formation was indole positive and pink color or no color indicated indole negative. The test was used to identify *Escherichia coli* and *Klebsiella oxytoca* bacteria which are positive.

### **Citrate utilization test**

Citrate utilization test biochemical test was used to identification of Enterobacteriaceae ability to use citrate as the only source of carbon and ammonia as the sole source of nitrogen.

**Principle:** It tests the ability of organism to utilize citrate due to alkaline reaction.

Required was the Simmons citrate media

### **Method:**

Saline suspension of organism was inoculated into Simmons citrate media. It was incubated at 37°C for overnight and growth was observed. Turbidity indicated positive result and no turbidity negative. The biochemical test was used in differentiating *Escherichia coli* which is negative to *Klebsiella* which utilizes citrate hence positive result.

### **Methyl Red Test**

The test was used to differentiate Enterobacteriaceae. In the study it differentiated klebsiella from *Escherichia coli*.

Requirements for the test was Glucose phosphate peptone water and Methyl red indicator

**Principle:** Methyl Red test shows the ability of micro-organism to ferment glucose

### **Method**

Test organism was inoculated into glucose phosphate peptone and incubated at 37°C overnight After incubation 5 drops of methyl red indicator solution was added and mixed. Appearance of bright red color indicated a positive test and no color while pale indicated

negative result. The test was used to differentiate *Escherichia coli* positive from *Klebsiella* which was negative.

### **Voges Proskauer**

Voges Proskauer biochemical test was used to differentiate Enterobacteriaceae. In the study it differentiated *klebsiella species* from *Escherichia coli*.

**Principle:** Test organism when cultured for 48 hours in glucose phosphate peptone and on addition of sodium hydroxide and creatine powder the acetoin produced from glucose is oxidized to diacetyl forming a red color with creatine.

The test required glucose phosphate peptone, Sodium hydroxide, Creatinine powder

### **Bacteria identification**

#### **Gram positive bacteria**

##### *Staphylococci aureus*

The bacteria upon gram staining technique-stained gram-positive cocci in clusters, singly or in pairs. This differentiated the bacteria with *streptococci* species which stains gram positive cocci in chains. *Staphylococci aureus* grew in both blood agar and chocolate blood agar media. The colonies were yellow to cream in color, slightly raised and they were easily emulsified on the slides. The other *staphylococci* species have white colonies. There was no growth in the MacConkey media as the media had bile salts and crystal violet that hindered growth of gram-positive bacteria. This was contrarily to streptococci species which are catalase negative. *Staphylococci aureus* was coagulase positive unlike other staphylococci species like *staphylococci saprophyticus* and *staphylococci epidermidis* which are coagulase negative.

#### **Gram negative bacteria**

##### *Escherichia coli*

*Escherichia coli*-stained gram-negative bacillus in the gram staining technique. The bacteria upon culturing in MacConkey they produced lactose fermenting colonies. On blood agar and chocolate blood agar the colonies had greyish white moist colonies in color. *Escherichia coli* reaction to biochemical tests was indole positive, methyl red positive, citrate utilization test positive.

### ***Klebsiella oxytoca***

*Klebsiella oxytoca* stained gram-negative rods. In MacConkey agar they were lactose fermenters. The colonies were pink and mucoid upon touch. In blood agar the colonies were also mucoid. *Klebsiella oxytoca* was indole positive unlike *Klebsiella pneumonia* which is indole negative. *Klebsiella oxytoca* was catalase positive, citrate utilization test positive, Methyl Red negative (*Klebsiella pneumonia* is Methyl Red negative), oxidase negative, urease positive and Voges Proskauer (VP) positive similar to *Klebsiella pneumonia*.

### ***Pseudomonas aeruginosa***

*Pseudomonas aeruginosa* upon gram staining technique it stained gram-negative rods. The bacteria growth in MacConkey formed round, flat and colorless colonies indicating they were lactose non fermenters. In Sheep blood agar the colonies were grey – white with metallic sheen. On chocolate blood agar the colonies appeared smooth, grey or colorless. The green color of the colonies was more pronounced in Nutrient agar during subculturing and Muller Hinton agar during sensitivity testing. When *Pseudomonas aeruginosa* colonies were subjected to biochemical tests; oxidase test was positive, citrate positive, Methyl Red negative, Voges Proskauer negative, coagulase negative, urease negative, indole negative and catalase positive.

### **3.7.5 Antimicrobial Sensitivity Testing of Isolates**

Antibiotics in the study were used by following criteria;

Broad and narrow spectrum, available drugs in the hospital pharmacy and categories of bacteria by gram staining techniques (gram positive and gram negative). Disc diffusion sensitivity technique was used to test the antimicrobial susceptibility of the isolates. The antibiotics diffused from the disc to the media. After overnight incubation the culture media was examined for zones of inhibition around the antibiotic disk. The antibiotics resistance is confirmed by growth of microorganisms up to the edge of the antibiotic discs. The bacteria sensitive to antimicrobials are inhibited at a distance from the disc. The zones of inhibition diameter breakpoints were measured in millimeters and compared to the reference guideline of Clinical Laboratory Standard Institute (CLSI M100, 2023). Muller-Hinton Agar media was used to determine antimicrobial susceptibility. Inoculum standardization was done by use of MacFarland standards adjusted to 0.5 turbidity.

#### **Muller -Hinton Agar**

Muller Hinton agar media is a microbiological growth medium that is commonly used for antibiotic susceptibility testing, disk diffusion tests specifically, it is a nonselective, non-differential medium hence almost all organisms plated on it will grow.

#### **Preparation of Muller Hinton Agar**

Mueller-Hinton agar powder was weighed following manufacturer's instructions where 16 grams of powder was weighed and put in conical flask. 500 milliliters of distilled water were measured using a measuring cylinder and added in the conical flask. The flask was swirled to disperse powder evenly. Heating was done until powder dissolved. The media was then autoclaved at 121°C for 15 minutes. The autoclave pressure was released and media allowed was allowed to cool.

The benches were carefully decontaminated and pour plating was done at 20ml in 100mm plate. Bunsen burner flame was passed to eradicate any bubbles and media was allowed to solidify. The media was stored at 2°C to 8°C in tightly sealed plastics bags for utmost 7 days.

To enhance quality control, the PH should be within 7.3 to 7.4. Prepared plates were incubated overnight and checked for growth. No growth meant the plates were sterile, while growth indicated contamination during the process of media preparation.

Muller Hinton sensitivity media was pre-warmed in the incubator before performing the sensitivity. MacFarland standard was adjusted to 0.5 turbidity and colony was streaked on the media by use of sterile swabs. The plates were incubated for 18 to 24 hours and zone of inhibitions diameters measured in millimeters. Various antibiotics were used to determine susceptibility of antibiotics to various bacteria contaminants isolated. The antibiotics used were; Clindamycin-2ug, Linezolid-30ug, Penicillin-10ug, Tazobactam piperacillin-110ug, Amoxicillin Clavulanic Acid (Augmentin)-30ug, Cefixime-5ug, Meropenem-10ug, Ciprofloxacin-5ug, Ceftazidime-30ug, Ampicillin-10ug, Vancomycin-30 ug, Oxacillin-1ug and Levofloxacin-5ug.

### **Testing of Bacteria Susceptibility**

#### ***Staphylococci aureus***

The bacteria were susceptible to Clindamycin (5.55%), Tazobactam, Ciprofloxacin, Ampicillin (38.88%), Augmentin (66.66%), and Meropenem, Linezolid and Gentamicin (88.8%).

#### ***Escherichia coli***

*E. coli* was only susceptible to Tazobactam, Ciprofloxacin, and Ceftazidime (20%), Augmentin (40%), and Meropenem (80%)

#### ***Klebsiella oxytoca***

*K. oxytoca* was 100% susceptible to Meropenem and ranked second with the most sensitivity to drugs tested: Tazobactam, Cefixime, Gentamicin, Ciprofloxacin, Ceftazidime (50%), and Augmentin, Ampicillin (25%). Vancomycin, Penicillin, and Levofloxacin had no activity on bacteria isolates.

### ***Pseudomonas aeruginosa***

*P. aeruginosa* showed high sensitivity to Meropenem (100%) but averaged 33.3 % against Piperacillin Tazobactam, Amoxicillin -clavulanic acid, and Ciprofloxacin and no sensitivity (0%) on other drugs

### **3.8 Quality control**

Quality control procedures were done and followed for validity which showed the accuracy and meaningfulness on inferences based on research and reliability as the measure of the degree unto which research instrument produces consistent results after repeated trials analysis. Standard organisms which were used in the study were; *E. coli* ATCC-25922, *Pseudomonas aeruginosa* ATCC-27853, *Staphylococci aureus* ATCC-25923, *Klebsiella Oxytoca* ATCC-70060 from Kenya Medical Research Institute (KEMRI). Antimicrobial susceptibility patterns zone of inhibition diameter breakpoints was measured in millimeters. The zone diameter breakpoints were in reference to Clinical and Laboratory Standards Institute (CLSI M100). Standard Operating Procedures, manufactures instructions were adhered to in pre-analytical, analytical and post - analytical phases. Media was adequately sterilized and positive and negative control organisms tested on the reagents. Proper storage and sterilization conditions were followed. Autoclaved samples were sub cultured to ascertain degree of sterility.

**Table 3. 1: Antibiotic Panels and Zone Diameter Breakpoints Interpretation**

<b>Antibiotic</b>	<b>Concentration (ug)</b>	<b>sensitive</b>	<b>Intermediate</b>	<b>Resistant</b>
Clindamycin	2ug	21	15-20	14
linezolid	30ug	21	15-20	14
Penicillin	1ug	29	*	17
Tazobactam				
piperacillin	110ug	21	18-20	17
Augmentin	30ug	18	14-17	13
Cefixime	5ug	21	16-20	15
Meropenem	10ug	18	15-17	14
Gentamicin	30ug	15	13-14	12
Ceftazidime	30ug	18	15-17	14
Vancomycin	30ug	17	15-16	14
Oxacillin	1ug	22	*	21
Levofloxacin	10ug	17	14-16	13
Ciprofloxacin	5ug	21	16-20	15

### **3.9 Management of Data, Analysis and Presentation**

After data was collected, it was entered into excel sheets. The researcher cleaned it and used Statistical Package for Social Sciences (SPSS) version 29 for analysis. The researcher presented the data in the form of frequency tables, bar graphs and pie charts. The analysis of the data was done in line with research objectives and questions. All data was quantitative in this study.

### **3.10 Ethical Approval**

Approvals were sought and granted from Mount Kenya University Ethical Review Committee under Reference number MKU/ISERC/2779 (appendix1) National Commission for Science, Technology and Innovation (NACOSTI)-Permit no: 946768 (Appendix 11) and Kitui County Ministry of Health, Kitui county Ministry of Education and Kitui county Ministry of Interior and National Administration (.

## CHAPTER FOUR

### RESEARCH FINDINGS AND DISCUSSIONS

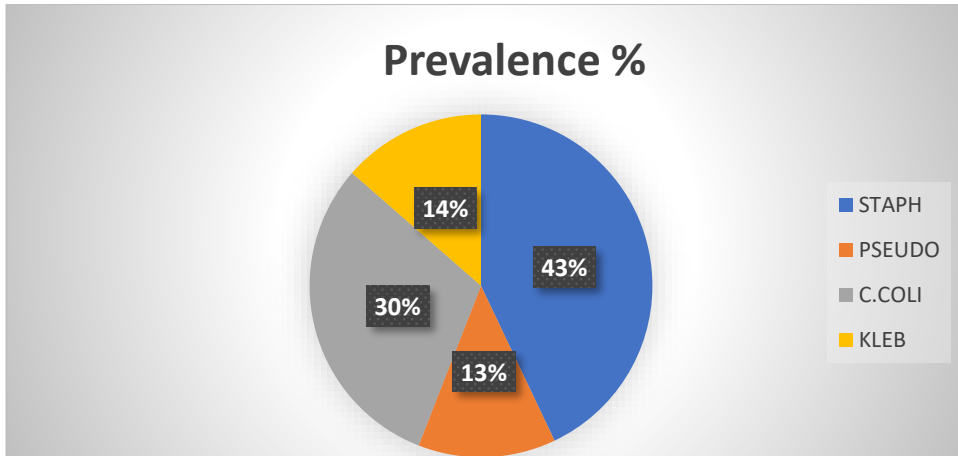
#### 4.0 Introduction

This chapter represents the study findings and results with detailed data analysis in pie charts, graphs, and tables. A total of 195 swabs were sampled where 177 swabs had bacterial contaminants. Analysis within the study was based on three objectives, which were to determine: i. Prevalence of bacteria in Kitui County Referral Hospital; ii. Major sources of bacteria in Kitui County Referral Hospital; and iii. Antibiotics susceptibility profiles of bacteria in Kitui County Referral Hospital. The study focused on surgical and medical wards.

#### 4.1 Types of bacteria

##### 4.1.1 Surgical and medical wards

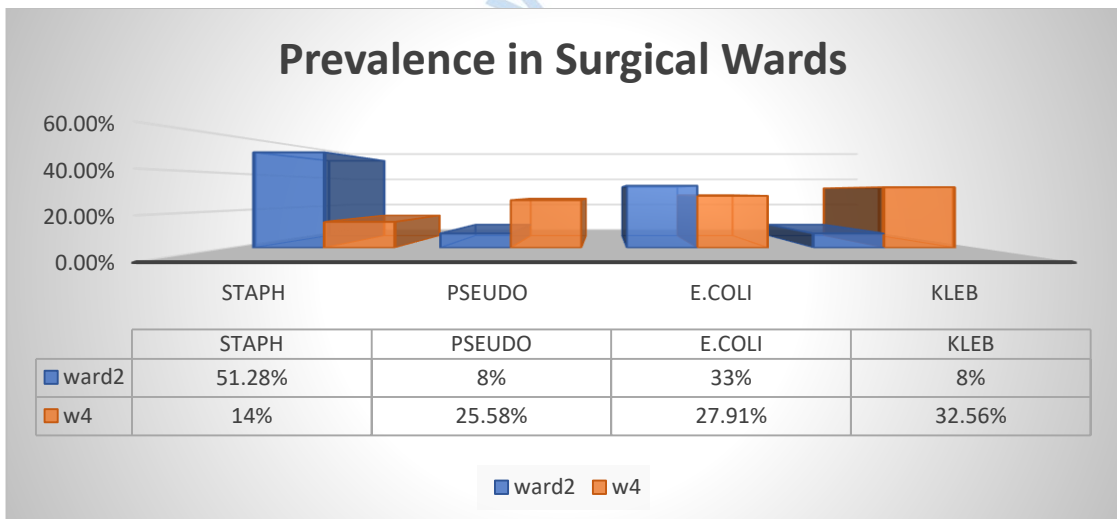
The prevalence of different types of bacteria isolated from surgical and medical wards in Kitui County Referral Hospital was determined, as shown in Figure 4.1. Out of a total of 177 isolates identified from both surgical and medical wards, 76 were *Staphylococci aureus*, 21 were *Pseudomonas aeruginosa*, 54 were *Escherichia coli*, and 24 were *Klebsiella oxytoca*; *Staphylococci aureus* had the highest prevalence at 43%; *Escherichia coli* was second highest with 30% followed by *Klebsiella oxytoca* with 14%. The isolate with the lowest prevalence was *Pseudomonas aeruginosa* with 13%. However, the prevalence of the bacteria isolates was not significantly different across the wards,  $F_{15}=0.09$ ,  $p=0.96$ ,  $\alpha=0.05$ .



**Figure 4. 1: The prevalence of different types of bacteria isolated from surgical and medical wards in Kitui County Referral Hospital**

#### 4.1.1.1 Comparison between Surgical Wards

The prevalence of different types of bacteria in two surgical wards (W2 & W4) in Kitui County Referral Hospital was determined, as shown in Figure 4.2. In ward 2, *S. aureus* had the highest prevalence at 51.3%. *Pseudomonas aeruginosa*, *E. coli*, and *K. oxytoca* had a close prevalence of Ward 4 (25.6%, 28% and 33%). However, the prevalence of the bacteria isolates was not significantly different in the surgical wards,  $t_2 < 4.30$ ,  $p > 0.05$ .

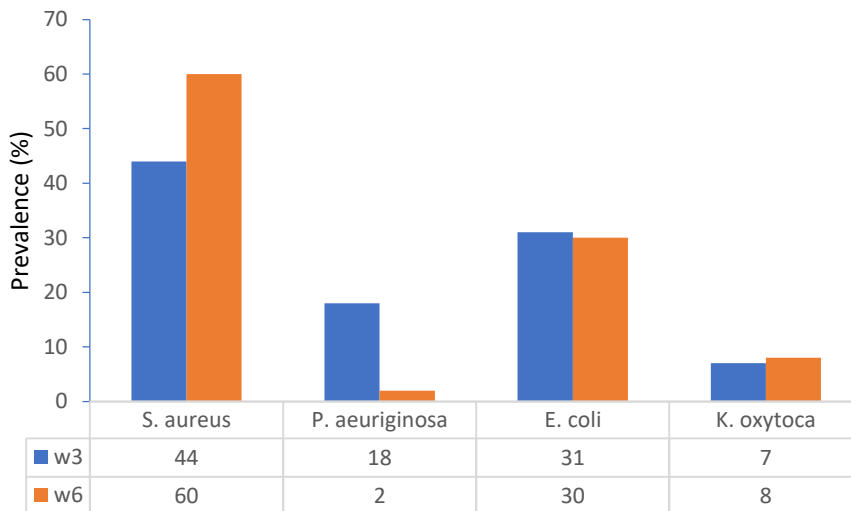


**Figure 4. 2: Comparison of prevalence between surgical wards**

#### 4.1.1.2 Comparison between Medical Wards

The prevalence of different types of bacteria in two medical wards (W3 & W6) in Kitui County Referral Hospital was determined, as shown in Figure 4.3. *S. aureus* had the

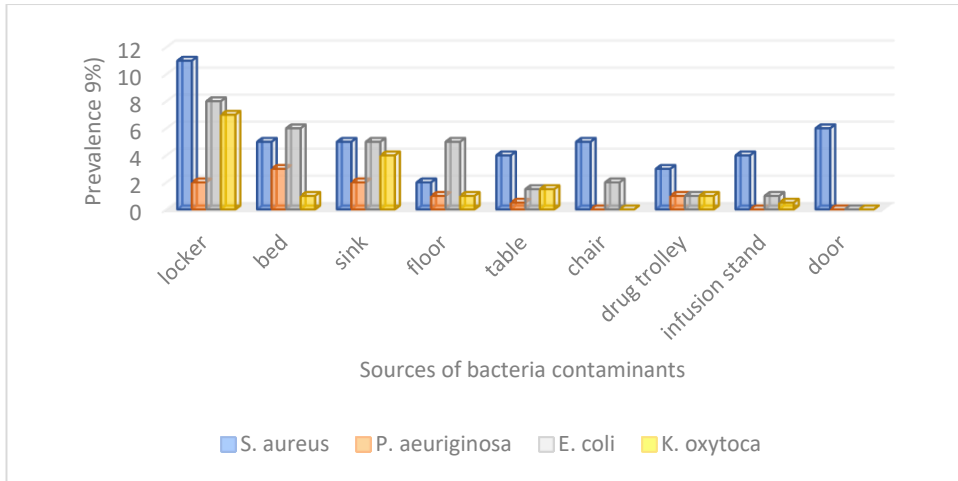
highest prevalence in wards 6 and 3 at 60% and 44%, respectively. *P. aeruginosa* had 18 % in Ward 3 and 2 % in Ward 6, whereas *E-coli* and *K. oxytoca* had 31% in Ward 3 and 30% in Ward 6; 7 % in Ward 3 and 8% in Ward 6, respectively. However, the prevalence of the bacteria isolates was not significantly different in the medical wards,  $t_2=0.99$ ,  $p>0.05$ .



**Figure 4. 3: Comparison of prevalence between medical wards**

#### 4.2 Major sources of bacterial contaminants

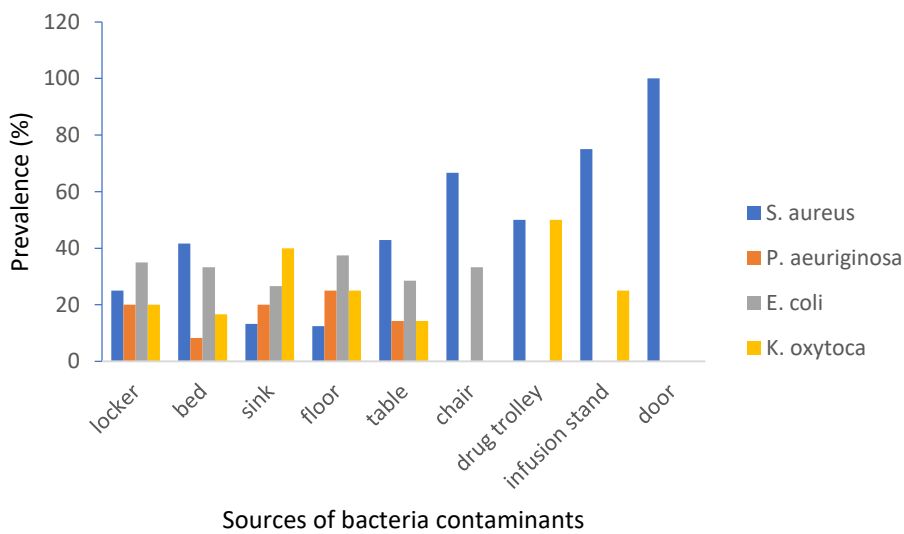
The sources of 177 different bacteria contaminants in Kitui County Referral Hospital were determined, as shown in Figure 4.4. The sources had the following contamination average n =177: locker 28%, bed 15 %, sink 16%, floor 9%, table 7.5 %, chair 7%, drug trolley 6%, infusion stand 5.5%, and door 6%. Locker had the highest bacteria contamination at an average of 28%. The infusion stand had the lowest average contaminations at 5.5%. Doors were only contaminated with *S. aureus*. More so, the sources of bacteria contaminants (isolates) differed significantly in terms of their prevalence of infection,  $F_{35}=2.73$ , CI=95%,  $p<0.05$ .



**Figure 4. 4: Sources of bacteria contaminants in Kitui County Referral Hospital**

**4.2.1 Sources of bacteria contaminants in surgical wards**

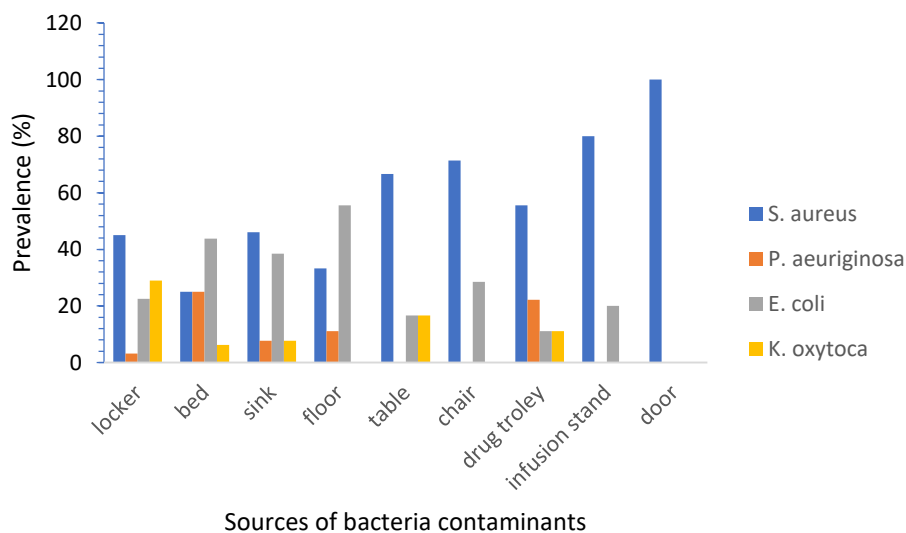
Figure 4.5 shows the determined sources of different types of bacteria in surgical wards in Kitui County Referral Hospital. Doors were the major source of *S. aureus*. The lockers, bed, sink, floors, and tables were contaminated with all bacteria isolates, with chairs, drug trolley, and infusion stand having two bacteria isolates each: drug trolley had *Klebsiella oxytoca* and *Staphylococci aureus*, chairs had *S. aureus* and *E-coli*. The prevalence of the bacteria isolates from different sources in the surgical wards was not significantly different,  $F_{35} < 2.30$ , CI=95%,  $p > 0.05$ .



**Figure 4. 5: Sources of bacteria contaminants in surgical wards**

#### 4.2.2 Sources of bacteria contaminants in medical wards

The sources of different types of bacteria in medical wards in Kitui County Referral Hospital were determined, as shown in Figure 4.6. As in surgical wards, doors were the major source of *S. aureus*. Locker, bed, sink, and drug trolleys were contaminated with all bacteria isolates. Floors were contaminated with *S. aureus*, *P. aeruginosa*, and *E. coli*. Chairs and infusion stands were contaminated with *S. aureus* and *E. coli*. Alternatively, tables had *S. aureus*, *E. coli*, and *K. oxytoca* contaminants. However, the prevalence of the bacteria isolates from different sources in the medical wards was also not significantly different, CI=95%,  $p>0.05$ .



**Figure 4. 6: Sources of Bacteria Contaminants in Medical Wards**

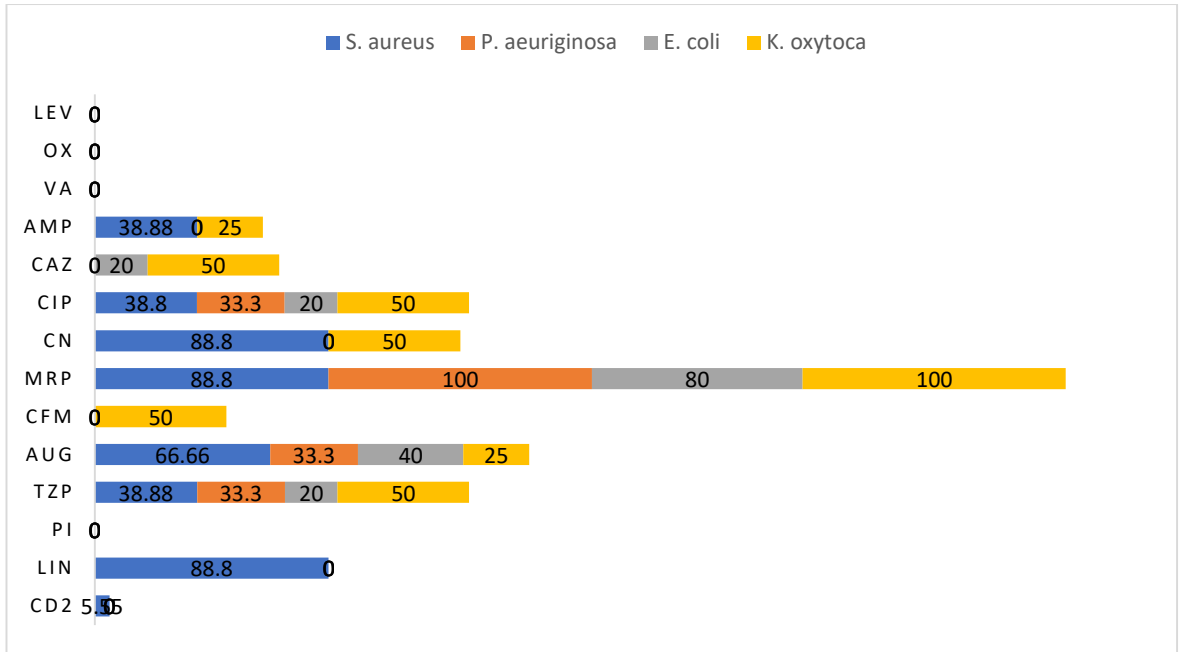
#### 4.3 Antimicrobial Susceptibility Patterns

The antimicrobial susceptibility patterns of the bacteria isolate to different antibiotics was analyzed, as shown in Table 4.1 and Figure 4.7. *Pseudomonas aeruginosa* showed high sensitivity to Meropenem (MRP) (100%) but averaged 33.3 % against Piperacillin Tazobactam (TZP), Ampicillin -clavulanic acid (AUG), and Ciprofloxacin (CIP) and no sensitivity (0%) on other drugs. *E. coli* was only susceptible to Tazobactam, Ciprofloxacin, and Ceftazidime (CAZ) (20%), Augmentin (40%), and Meropenem

(80%). Similarly, *K. oxytoca* was 100% susceptible to Meropenem and ranked second with the most sensitivity to drugs tested: Tazobactam, Cefixime (CFM), Gentamicin (CN), Ciprofloxacin, Ceftazidime (50%), and Augmentin, Ampicillin (AMP) (25%). Vancomycin (VA), Oxacillin (OX), Penicillin, and Levofloxacin (LEV) had no activity on bacteria isolates. *S. aureus* showed sensitivity to most of the drugs tested: Clindamycin (CD) (5.55%), Tazobactam, Ciprofloxacin, Ampicillin (38.88%), Augmentin (66.66%), and Meropenem, Linezolid (LN) and Gentamicin (88.8%). The susceptibility of bacteria contaminants (isolates) to the antibiotics differed significantly,  $F_{55}=5.86$ , CI=95%,  $p<0.05$ .

**Table 4. 1: Antimicrobial Susceptibility Patterns**

Bacteria isolates	CD2	LIN	PI	TZP	AUG	CFM	MRP	CN	CIP	CAZ	AMP	VA	OX	LEV
<i>S. aureus</i>	5.55	88.8	0	38.88	66.66	0	88.8	88.8	38.8	0	38.88	0	0	0
<i>P. aeruginosa</i>	0	0	0	33.3	33.3	0	100	0	33.3	0	0	0	0	0
<i>E. coli</i>	0	0	0	20	40	0	80	0	20	20	0	0	0	0
<i>K. oxytoca</i>	0	0	0	50	25	50	100	50	50	50	25	0	0	0



**Figure 4. 7: Antimicrobial Susceptibility Patterns**

**KEY:**

PI	PENICILLIN
AMP10	AMPICILLIN
MRP10	MEROPENEM
TZP	TAZOBACTUM /PIPERACILLIN
LEV	LEVOFLOXACIN
AUG30	AUGUMENTIN/GENTAMYCIN
CN30	GENTAMICIN
CAZ30	CEFTAZIDIME
LNZ30	LINSOLID
VA30	VANCOMYCIN
CFM5	CEFIXIME
CIP	CIPROFLOXACINE
OX	OXACILLIN

## CHAPTER FIVE

### SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

#### 5.0 Introduction

This chapter includes the summary of the study, conclusions made and recommendations to stake holders and further studies.

#### 5.1 Summary

##### 5.1.1 Types of Bacteria in Medical and Surgical Wards

The present study found *Staphylococcal aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Klebsiella oxytoca* in medical and surgical wards. *S. aureus* had the highest prevalence at 43%; *E. coli* was second highest at 30%, followed by *K. oxytoca* at 14%. The isolate with the lowest prevalence was *Pseudomonas*, with 13%. The prevalence varied in surgical wards (2 and 4), showing that *S. aureus* had the highest prevalence at 51.3% in Ward 2. In contrast, *Pseudomonas aeruginosa*, *E. coli*, and *K. Oxytoca* had a close prevalence in Ward 4 (25.6%, 33% and 28%).

The observation was analogous to other research work findings as it agrees with Ratemo's (2014) study, which found that *S. aureus* was the predominant isolate (29.9%), followed by *Pseudomonas* (13.7%).

However, the findings of this project were in contrast with several studies that identified *E. coli* (25.5%), *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* as the predominant bacteria isolates in surgical areas (Patel *et al.*, 2019). The disagreement was associated with differences in surgery procedures at the hospital and patient environment source sampled.

In this study, *S. aureus* had the highest prevalence in medical wards 6 and 3 at 60% and 44%, respectively. *P. aeruginosa* had 18 % in Ward 3 and 2 % in Ward 6, whereas *E-coli* and *K. oxytoca* had 31% in Ward 3 and 30% in Ward 6; 7 % in Ward 3 and 8% in Ward

6, respectively. These results were similar to those obtained by other research works in that *Staphylococcus* species, Gram positive and Gram negative were the most common isolates (Saka *et al.*, 2016). These bacteria come from the air, patients, visitors, or healthcare workers. Accordingly, Gupta (2021), asserts that their presence in these environments can be explained by their remarkable ability to survive long on poorly cleaned dry surfaces.

Most bacteria isolate from medical and surgical wards showed that *Staphylococcus aureus* was the primary contaminant. *S. aureus* had the highest prevalence at 51.3% in medical and 51% in surgical wards. Similarly, in Nigeria, Olowo-Okere and Babandina's (2018) study also found that *Staphylococcus aureus* was the primary contaminant in surgical sites with 42%. However, research in Morocco by Chaoui *et al.* (2019) disagrees as it shows that Enterobacteria (31.6%) is the primary contaminant in surgical sites. This difference could be attributed to differences in the site of wards, difficulties in cleaning, and sampling methods used.

The agree with those of Rajkumari *et al.* (2014) in India which found *Staphylococcus aureus* (24.3%) was the most common isolates for surgical site infections resulting in pus, followed by *Pseudomonas aeruginosa* (21.5%), *Escherichia coli* (14.0%), *Klebsiella pneumonia* (12.2%), *Streptococcus pyogenes* (11.2%), *Staphylococcus epidermis's* (9.4%) and *Proteus* species (7.5%) These findings also align with those in England as per surveillance by National Health Services (NHS) Hospitals in 2015. The surveillance revealed that that *Staphylococcus aureus* was the dominant from orthopedic and spinal surgeries, accounting for over 36% of all nosocomial infections.

### **5. 1.2 Distribution of Bacteria in Kitui County Referral Hospital**

The sources had the following distribution: locker 28%, bed 15 %, sink 16%, floor 9%, table 7.5 %, chair 7%, drug trolley 6%, infusion stand 5.5%, and door 6%. Locker had

the highest bacteria contamination at an average of 28%. The infusion stand had the lowest average contaminations at 5.5%. Doors were only contaminated with *S. aureus*. Lockers emerged as the most frequent harborage for bacteria, with an average contamination rate of 28%. This highlights the potential risk posed by personal belongings stored in close proximity to patients. Beds (15%), sinks (16%), floors (9%), and tables (7.5%) also exhibited significant bacterial presence, underlining the importance of thorough cleaning and disinfection of these surfaces between patient use. They align with Otter *et al.* (2011) which found that *Clostridium difficile* (C. Diff), a bacterium known for causing severe diarrhea, could survive on surfaces like doors, tables and lockers

Surgical wards displayed a wider range of bacterial contamination compared to medical wards. Lockers, beds, sinks, floors, and tables were susceptible to all four bacteria identified (*S. aureus*, *E. coli*, *Klebsiella spp.*, *Pseudomonas aeruginosa*). Chairs, drug trolleys, and infusion stands in surgical wards harbored a limited number of bacterial isolates. Notably, drug trolleys specifically contained *Klebsiella* and *S. aureus*, while chairs had *S. aureus* and *E. coli*, and infusion stands primarily yielded *S. aureus*.

Medical wards exhibited a similar pattern, with lockers, beds, sinks, and drug trolleys susceptible to all bacteria. Floors differed slightly, harboring *S. aureus*, *P. aeruginosa*, and *E. coli*. Chairs and infusion stand in medical wards shared contamination with *S. aureus* and *E. coli*, while tables uniquely presented a combination of *S. aureus*, *E. coli*, and *K. oxytoca*.

The current study's findings regarding bacterial distribution on hospital surfaces align with the observations of Ken *et al.*, (2019). Their research emphasizes how nosocomial infections can originate from various sources within healthcare facilities, including patient food, medical devices, and equipment.

Ken et al. (2019) highlights the crucial role of direct and indirect contact in spreading pathogens. Direct contact involving bacteria like *E. coli* and *S. aureus* can occur through contaminated surfaces or infected individuals. Indirect contact, often through the hands of healthcare workers, is another frequent mode of transmission within hospitals.

Bhatta *et al.*, (2021) offer explanations for the observed high bacterial distribution on surfaces and equipment. Factors such as overcrowded wards, high bed occupancy, admission of patients with diverse medical conditions, and inadequate adherence to infection control practices (ICP) likely contribute to this phenomenon. Their research emphasizes how patients, visitors, healthcare professionals, and even cleaning staff can unwittingly contaminate the hospital environment and equipment, facilitating microbial colonization.

The current study's findings resonate with Chester *et al.*, (2023), who identify surfaces, tabletops, door handles, and healthcare worker hands as key reservoirs for contaminants in hospitals. Their research suggests that most hospital-acquired infections (HAIs) stem from contact with contaminated surfaces or equipment. Notably, Chester *et al.*, (2023) found that ward surfaces harbored the highest number of bacterial isolates (58.7%), potentially due to weak disinfection protocols. As frequently touched surfaces, doors are particularly likely to harbor high bacterial loads.

The findings of this study and the referenced literature underscore the critical need for stricter infection control measures within Kitui County Referral Hospital. Implementing effective hand hygiene protocols for staff and visitors, along with thorough cleaning and disinfection procedures for surfaces and equipment, are essential steps. Additionally, addressing overcrowding and ensuring proper waste disposal practices can further minimize the risk of bacterial transmission.

In surgical wards, doors were the primary source of *S. aureus*. The lockers, bed, sink, floors, and tables were contaminated with all bacteria isolates, with chairs, drug trolley, and infusion stand having two bacteria isolates each: drug trolley had *Klebsiella* and *S. aureus* whereas chairs had *S. aureus* and *E. coli*. In medical wards, doors were the primary source of *S. aureus*. Lockers, beds, sinks, and drug trolleys were contaminated with all bacteria isolates. Floors were contaminated with *S. aureus*, *P. aeruginosa*, and *E. coli*. Chairs and infusion stands were contaminated with *S. aureus* and *E. coli*. Alternatively, tables had *S. aureus*, *E. coli*, and *K. oxytoca* contaminants. The findings align with Ken *et al.* (2019) findings that nosocomial infections can originate from patient food in healthcare facilities, medical devices, and equipment. Ken *et al* argues that microbes could be passed through direct contact (*Escherichia coli* and *Staphylococci aureus*) and droplets from infections surfaces or people. Indirect contact is a frequent way illness is spread in hospitals. The healthcare workers can spread microorganisms through their hands.

According to Bhatta *et al.* (2021), the high distribution of bacteria on surfaces and equipment observed may be due to overcrowded wards, high bed occupancy for surgical areas, patients admitted with different clinical conditions from other health facilities, and lack of compliance to infection control practices (ICP). Bhatta *et al.* assert that patients, visitors, health professionals, and workers could contaminate the hospital environment and equipment, thus leading to microbial colonization.

These findings agree with Chester *et al.*'s (2023) research that asserts that surfaces, table tops, door handles, and hands of healthcare staff, among others, act as catchments for contaminants in the hospital setting. According to Chester *et al.*, most Hospital acquired infections are acquired from contact with hospital surfaces or interaction with contaminated equipment. Chester *et al.*'s research shows that the ward surfaces had the

highest number of isolates at 58.7% (n=27). Weak guidelines for disinfection of surfaces and equipment could explain this. As the most touched surfaces, doors are likely to carry more contaminants.

### 5.1.3 Susceptibility patterns of bacteria in Kitui County Referral Hospital

Gram-positive isolates showed sensitivity to several drugs tested, whereas *S. aureus* showed sensitivity to most of the medicines tested: Clindamycin (CD) (5.55%), Tazobactam, Ciprofloxacin, Ampicillin (38.88%), Augmentin (66.66%), and Meropenem, Linezolid (LN) and Gentamicin (88.8%). These findings are analogous to Ratemo (2014) findings, where *S. aureus*, *P. aeruginosa* and *K. oxytoca* showed high sensitivity to Meropenem (MRP) (100%). This study's findings agree with Goyal et al. (2019) and Ratemo's study, which found that *Pseudomonas spp* was highly sensitive to meropenem (81.1%), amikacin (86.7%), piperacillin (80%), ciprofloxacin (83.3%) and levofloxacin (77.4%).

Vancomycin (VA), Oxacillin (OX), Penicillin, and Levofloxacin (LEV) had no activity on the four bacteria isolates. *S. aureus* showed sensitivity to most drugs tested: Clindamycin (CD) (5.55%), Tazobactam, Ciprofloxacin, and Ampicillin resistance was 38.88%. *E. coli* was lowly sensitive to Tazobactam, Ciprofloxacin, and Ceftazidime (CAZ) (20%), In this study, only *Klebsiella*, of all the gram-negative isolates, was more sensitive, with 50% or more sensitivity to Tazobactam, Cefixime, Ciprofloxacin, and Ceftazidime. Other gram-negative isolates were susceptible to MRP, whereas *P. aeruginosa*, *E. Coli*, and *K. oxytoca* had 100%, 80%, and 100% sensitivity to MRP, respectively. This agrees with the Ratemo (2014) study.

*P. aeruginosa* showed high sensitivity to Meropenem (MRP) (100%) but averaged 33.3% against Tazobactam (TZP), Ampicillin-clavulanic acid (AUG), and Ciprofloxacin (CIP) and no sensitivity (0%) on other drugs. These findings were similar to studies

that showed *Pseudomonas spp* were most sensitive to carbapenems and aminoglycosides (Ratemo, 2014).

Regarding *Staphylococcus aureus* (*S. aureus*), the study revealed encouraging sensitivity towards several antibiotics, including Clindamycin (5.55%), Tazobactam, Ciprofloxacin, Ampicillin (38.88%), Augmentin (66.66%), Meropenem, Linezolid (LN), and Gentamicin (88.8%). These findings align with Ratemo (2014) who reported high sensitivity of *S. aureus*, *P. aeruginosa*, and *K. oxytoca* to Meropenem (MRP) (100%).

Similarly, *Escherichia coli* (*E. coli*) bacterium exhibited lower sensitivity compared to *S. aureus*. Moderate sensitivity was observed against Tazobactam, Ciprofloxacin, and Ceftazidime (CAZ) (20%). Interestingly, *Klebsiella* isolates displayed the highest overall sensitivity among gram-negative bacteria. They exhibited 50% or more sensitivity to Tazobactam, Cefixime, Ciprofloxacin, and Ceftazidime. This finding highlights the potential effectiveness of these antibiotics against *Klebsiella* infections within the hospital setting.

*Pseudomonas aeruginosa* (*P. aeruginosa*): This bacterium demonstrated high sensitivity to Meropenem (MRP) (100%) but displayed an average of 33.3% sensitivity to Tazobactam (TZP), Ampicillin-clavulanic acid (AUG), and Ciprofloxacin (CIP). Notably, *P. aeruginosa* was resistant to all other antibiotics tested in this study. These findings echo previous research, with studies like Ratemo (2014) reporting *Pseudomonas spp.* to be most susceptible to carbapenems and aminoglycosides.

In respect to resistance, the study identified levels of antibiotic resistance, particularly among gram-negative bacteria. This resistance was most pronounced against Clindamycin, Linezolid, Penicillin, Ampicillin, Vancomycin, Oxacillin, and Levofloxacin. The potential reasons for this resistance may include:

Indiscriminate Use of Antibiotics, Overuse and prolonged administration of antibiotics can exert selective pressure, allowing resistant bacterial strains to thrive. Impermeable Outer Membrane: Gram-negative bacteria possess an outer membrane that restricts the penetration of certain antibiotics, such as penicillin, hindering their effectiveness. Understanding the susceptibility patterns of isolated bacteria is crucial for selecting appropriate antibiotic therapy. The observed resistance trends highlight the need for: Antibiotic Stewardship Programs: Implementing hospital-wide programs to promote judicious antibiotic use can help curb the emergence of resistant bacterial strains. Regular Susceptibility Testing: Conducting regular susceptibility testing of isolated bacteria allows healthcare professionals to tailor treatment strategies based on the most effective antibiotics against prevalent strains. Alternative Treatment Options: Exploring alternative therapies for infections caused by multidrug-resistant bacteria becomes increasingly important as resistance patterns evolve.

## 5.2 Conclusion

Based on the research objectives, the researcher sought to investigate the types of bacteria contaminants at the Kitui County Referral Hospital. *S. aureus*, *P. aeruginosa*, *E. Coli*, and *K. oxytoca* were the predominant bacteria isolated from the surgical and medical wards surfaces and equipment. Secondly, the researcher investigated the major sources of bacterial isolates in the hospital. The study identified hospital surfaces, doors, lockers, beds, sinks, medical equipment and trolleys as the major sources of bacterial contaminants. Finally, the susceptibility patterns of the isolates were investigated. Of all the four isolates, *S. aureus* was the most prevalent in medical and surgical wards (51%). All the isolated bacteria were sensitive to meropenem, while *S. aureus* showed relatively high resistance to Linezolid (LIN), Ampicillin -Clavulanic Acid (AUG), and Gentamicin (CN). *Klebsiella* was reasonably prudent to Piperacillin Tazobactam (TZP), Cefixime

(CFM), Gentamicin (CN), CIP, and Ceftazidime (CAZ) (50%), with the remaining isolates showing low sensitivity to the antibiotics. By addressing these concerns, the hospital can work towards minimizing antibiotic resistance and ensuring effective treatment options remain available for patients.

### **5.3 Recommendations**

*Staphylococci aureus*, *Pseudomonas aeruginosa*, *Escherichia Coli*, and *Klebsiella oxytoca* are the key types of bacterial contaminants, with door surfaces, lockers, medical equipment, and the environment in the hospital being the major sources that are a threat to increased transmission of nosocomial infections. This study recommends the following to minimize the spread of Hospital Acquired Infections (HAIs) in surgical and medical wards:

Hospital management in support from the ministry to have a multidisciplinary team of infection prevention and control to counter hospital acquired infections. Compliance with infection prevention practices by increasing the frequency and effective cleaning of all patient items during hospital stay and surfaces. Appropriate use of disinfectants in decontaminating highly-touched surfaces and make policies on handling contaminated linen of the patient or after discharge or upon new admission. Improvement of bed occupancy ratio and control of the number of visitors to the wards visiting patients. Infection prevention and control tool to be launched in the Hospital to monitor probable contamination in patient environment and continuous training and education to health care workers. Monitor periodically trend of nosocomial infections in the facility via laboratory procedures and performing antimicrobial susceptibility testing to study prevalence of resistance. This study used phenotypic techniques; thus, molecular techniques are recommended for further studies.

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## APPENDICES

### Appendix I: Observation Checklist

#### 1. Bacterial contaminants on hospital surfaces

BACTERIA TYPE	PRESENT (√)	NOT PRESENT (√)
<i>S.aureus</i>		
<i>E. coli</i>		
<i>K. oxytoca</i>		
<i>K. pneumonia</i>		
<i>Proteus mirabilis</i>		
<i>Clostridium difficile</i>		
<i>Pseudomonas aeruginosa</i>		
<i>Acinetobacter baumannii,</i>		
<i>Burkholderia cepacian</i>		
<i>Streptococcus pyogenes</i>		
<i>Staphylococcus epidermis's</i>		

#### 2. Major sources of bacterial contaminants

Floor	
Sink	
Beds	
Infusion stands	
Locker	
Door	
Tables	
Chairs	
Drug trolleys	
Curtains	
Electronic devises	
Patient monitoring tools	

## Appendix II: Ethical Clearance Letter



# Mount Kenya University

REF: MKU/ISERC/2779  
TO: CHARITY MUTAVE KIMWELE  
REG: MMLS/2021/41118

Date: 19 May 2023

Dear Sir/Madam,

**RE: BACTERIA PROFILE, DISTRIBUTION AND SUSCEPTIBILITY TO ANTIBIOTICS IN PATIENT CARE ENVIRONMENT AT KITUI COUNTY REFERRAL HOSPITAL: SURGICAL AND MEDICAL WARDS**

This is to inform you that **Mount Kenya University** has reviewed and approved your above research proposal. Your application approval number is **1776**. The approval period is **19/05/2023 - 18/05/2024**.

This approval is subject to compliance with the following requirements:

- i. Only approved documents including informed consents, study instruments, MTA will be used
- ii. All changes including amendments, deviations and violations are submitted for review and approval by **Mount Kenya University**
- iii. Death and life-threatening problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to **Mount Kenya University** within 72 hours of notification
- iv. Any changes, anticipated or otherwise that may increase the risks or affect the safety or welfare of study participants and others or affect the integrity of the research must be reported to **Mount Kenya University** within 72 hours
- v. Clearance for export of biological specimens must be obtained from relevant institutions
- vi. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. Attach a comprehensive progress report to support the renewal
- vii. Submission of an executive summary report within 90 days upon completion of the study to **Mount Kenya University**

Prior to commencing your study, you will be expected to obtain a research license from National Commission for Science, Technology and Innovation (NACOSTI) <https://research-portal.nacosti.go.ke> and also obtain other clearances needed.

Yours sincerely,

  
The Chairman  
Mount Kenya University  
Ethics Review Comm.  
P.O. Box 212 - 0100, Thika

**Dr. Peter G. Kirira**  
Chairman, Mount Kenya University ISERC

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Main Campus, General Kago Road, P.O. Box 342-01000 Thika.  
Tel: 020-2875 000, Cell: +254 709 153 000  
Email: info@mku.ac.ke Web: www.mku.ac.ke

## Appendix III: Introduction Letter

  
**Mount Kenya University**

**DIRECTORATE OF GRADUATE STUDIES**

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MMLS/2021/41118

19<sup>th</sup> May, 2023

*National Commission for Science Technology & Innovation (NACOSTI)*  
*Off Waiyaki, Upper Kabete*  
*P.O Box 30623- 00100*  
*NAIROBI, KENYA*

Dear Sir/Madam,

**RE: CHARITY MUTAVE KIMWELE - REGISTRATION NO. MMLS/2021/41118**


The purpose of this letter is to introduce the above named student who is pursuing **Master of Science in Medical Laboratory Science Degree** in the Department of **Medical Laboratory Science** in Medical School.

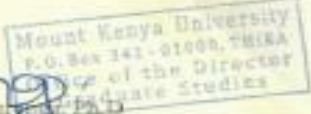
The title of the research is *"Bacteria Profile Distribution and Susceptibility to Antibiotics in Patient Care Environment at Kilifi County Referral Hospital Surgical and Medical Wards."*

It has been cleared by the University's Ethics Review Committee (Certificate attached) and now has to proceed to the field to collect data between April, 2023 and June, 2023.

Any assistance accorded to the student will be highly appreciated.

Thank you.


  
Dr. Samuel M. Karani, PhD  
Director, Graduate Studies

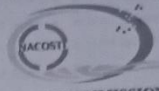
  
Mount Kenya University  
P.O. Box 342-01000, THIKA  
Office of the Director  
Graduate Studies

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Main Campus, General Kago Road, P.O. Box 342-01000 Thika.  
Tel: 020-2878 000, Cell: +254 709 153 000  
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## Appendix IV: Nacosti Research Permits

  
REPUBLIC OF KENYA

  
NATIONAL COMMISSION FOR  
SCIENCE, TECHNOLOGY & INNOVATION

Ref No: 946768 Date of Issue: 15/June/2023

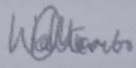
**RESEARCH LICENSE**




This is to Certify that Ms. Charly Mutave Kimwele of Mount Kenya University, has been licensed to conduct research as per the provision of the Science, Technology and Innovation Act, 2013 (Rev.2014) in Kitui on the topic: BACTERIA PROFILE, DISTRIBUTION AND SUSCEPTIBILITY TO ANTIBIOTICS IN PATIENT CARE ENVIRONMENT AT KITUI COUNTY REFERRAL HOSPITAL: SURGICAL AND MEDICAL WARDS. for the period ending : 15/June/2024.

License No: NACOSTI/P/23/26490

946768  
Applicant Identification Number

  
Director General  
NATIONAL COMMISSION FOR  
SCIENCE, TECHNOLOGY &  
INNOVATION

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See overleaf for conditions

## Appendix V: Kitui County Referral Hospital Research Approval

**COUNTY GOVERNMENT OF KITUI**

Email: health@kitui.go.ke



Office of the Chief Officer,  
Medical Services  
P.O. Box 460-90200  
KITUI

**MINISTRY OF HEALTH AND SANITATION**

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**Ref:** CGKTI/NOH/HRM/8/1(145) **Date:** 21<sup>st</sup> June, 2023

Charity Mutave Kimwele  
Reg No. NMLS/2021/41118  
Mount Kenya University

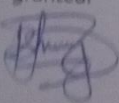
**RE: RESEARCH AUTHORISATION**


The above subject matter refers.

Reference is made to the letter dated 19<sup>th</sup> May 2023 from Mount Kenya University for your authorization to conduct research titled " Bacterial profile, distribution and susceptibility to antibiotics in patient care environment at Kitui County Referral Hospital: Surgical and Medical wards."

Further reference is made to research approval Ref. No. 1776 dated 15<sup>th</sup> June 2023 by the Director-General NACOSTI for you to conduct the research for the period ending 18<sup>th</sup> June 2024.

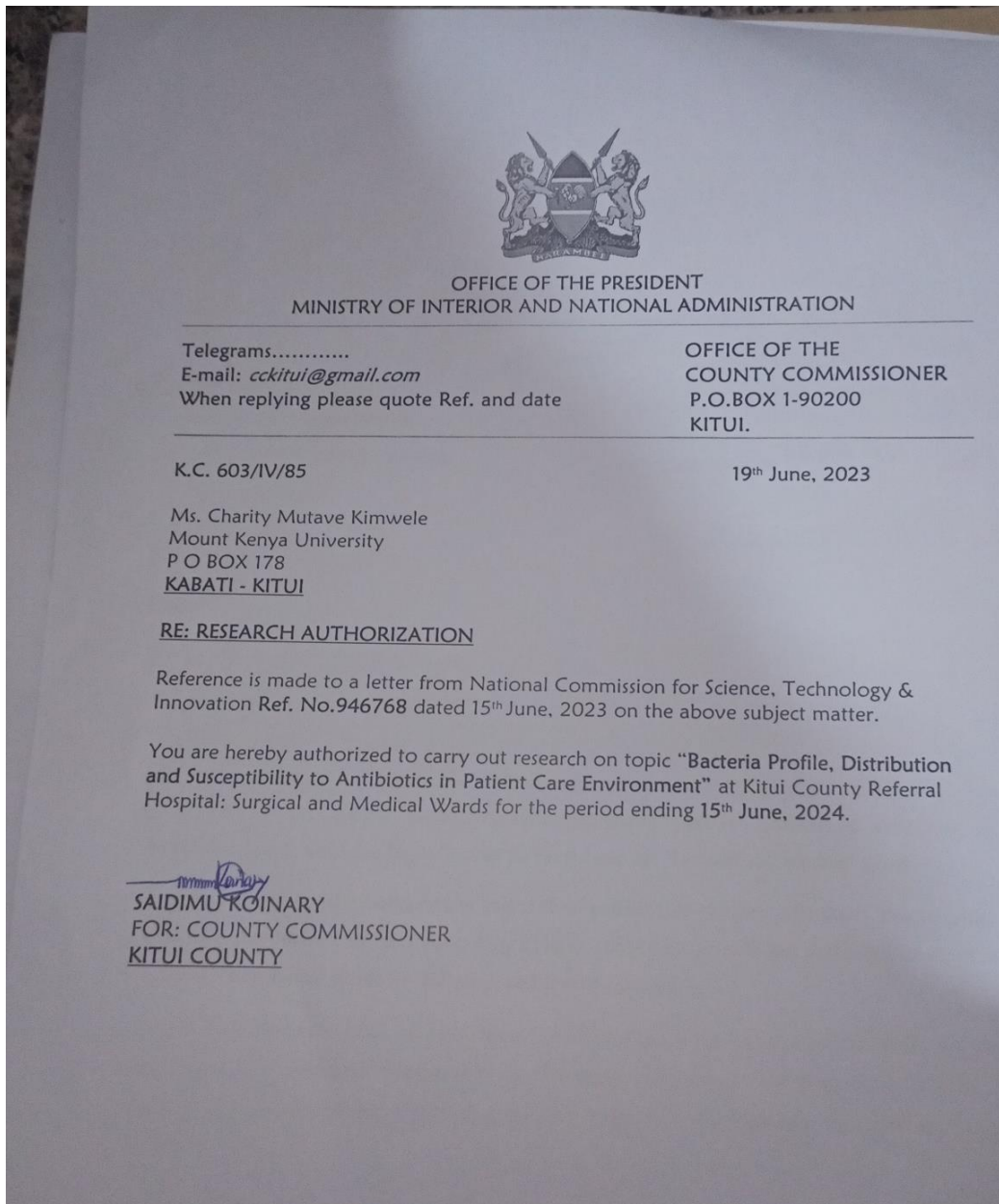
Authority to carry out the research in Kitui County in the selected facility is hereby granted.

  
Dr. Benson Musyoka  
Chief Officer Medical Service  
**Ministry of Health and Sanitation**

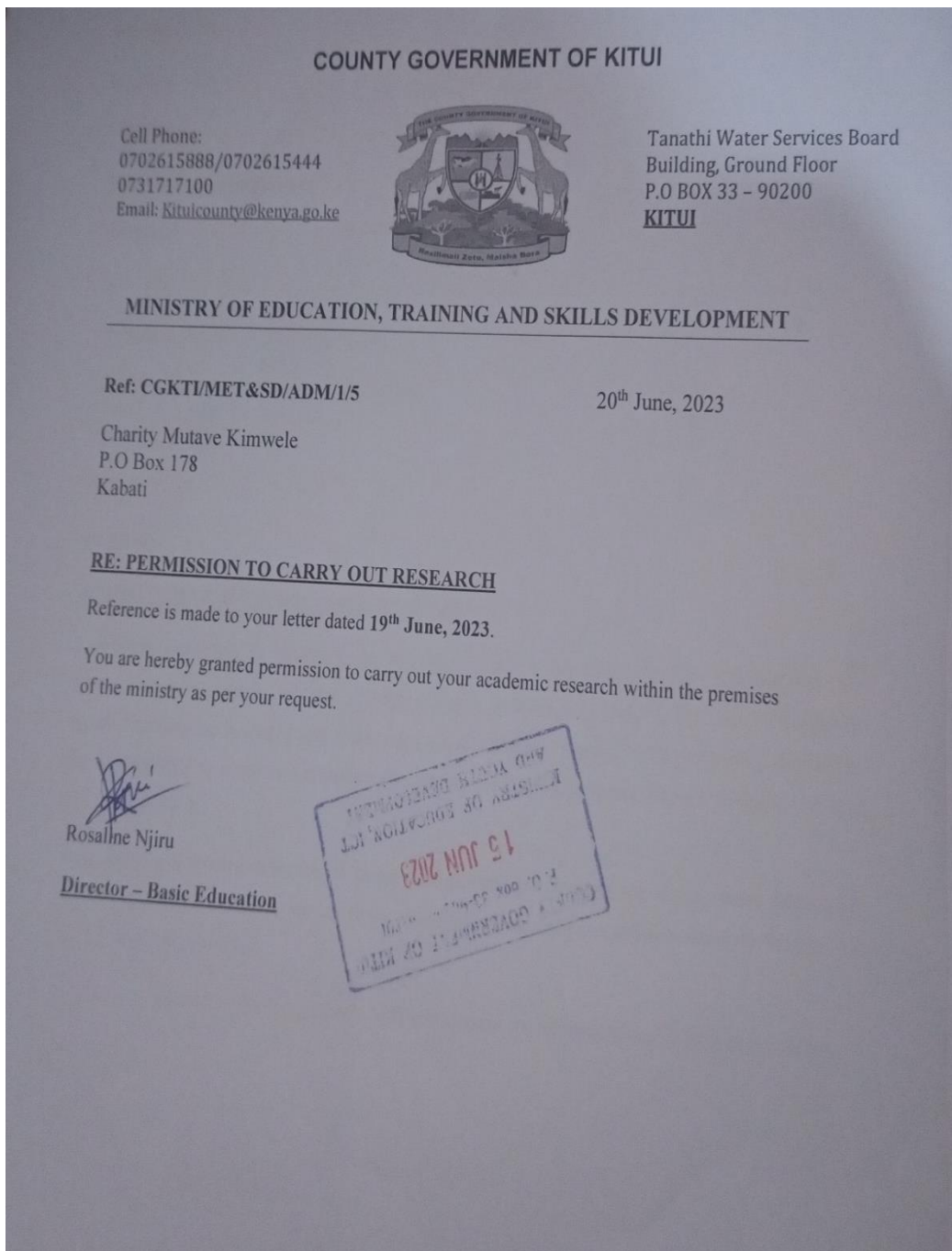


CC: CECM, Health and Sanitation

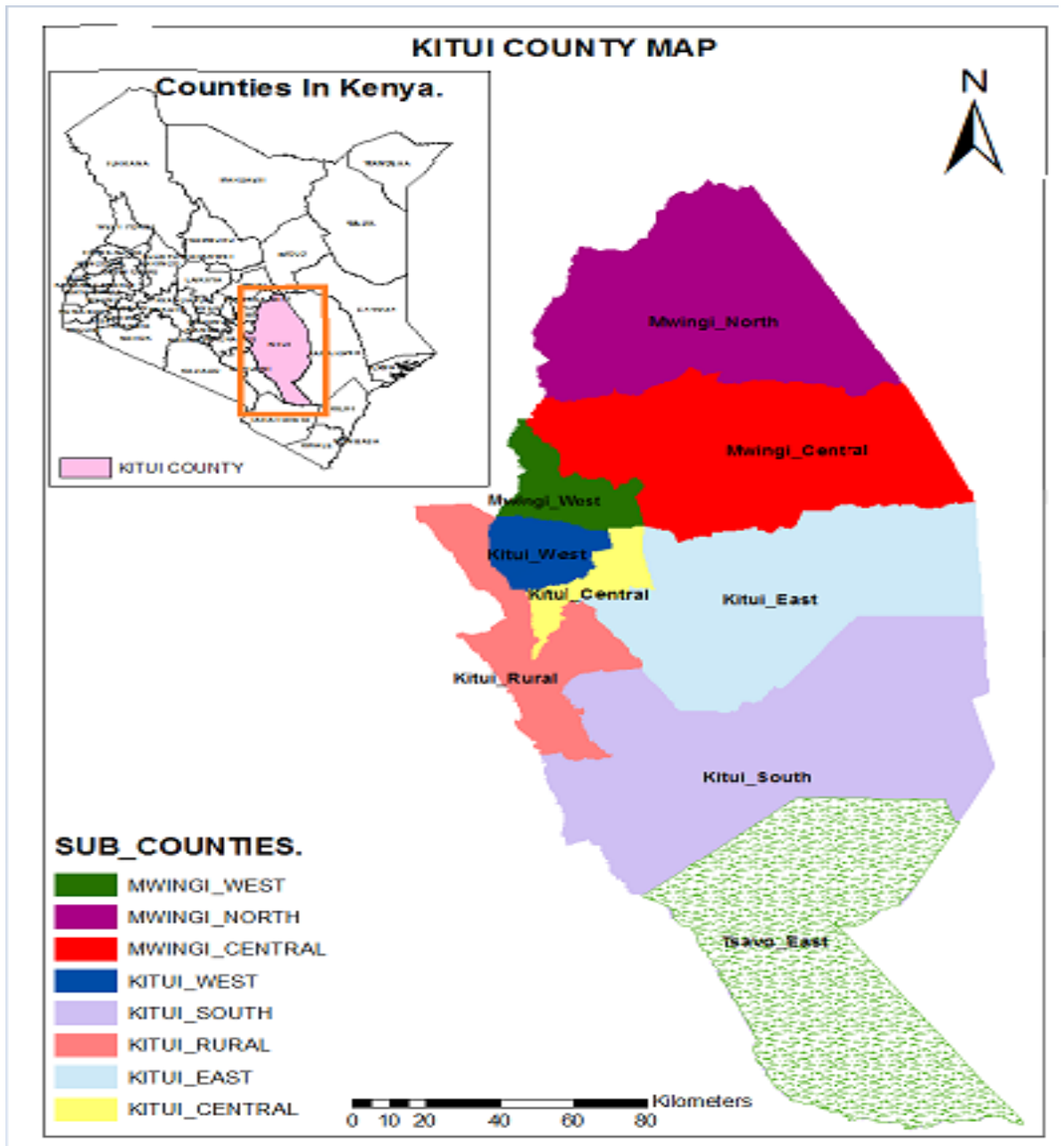
**Appendix VI: Kitui Approval from Ministry of Interior**



**Appendix VII: Approval from Letter Ministry of Education Kitui County**



Appendix VIII: Kitui County Map



# Appendix IX : Similarity Report



## Charity Mutave Kimwele

### PREVALENCE OF BACTERIAL CONTAMINANTS AND THEIR ANTIBIOTIC SUSCEPTIBILITY PROFILES AT KITUI COUNTY ...

- Quick Submit
- Quick Submit
- Mount Kenya University

#### Document Details

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