NOSOCOMIAL BACTERIAL PATHOGENS AND ANTIBIOTIC RESISTANCE IN JARAMOGI OGINGA ODINGA TEACHING AND REFERRAL HOSPITAL IN KISUMU COUNTY, KENYA

BY

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DECLARATION

This thesis is my original work and has not been presented for an award of a diploma or conferment of a degree in any other university or institution.

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DEDICATION

This thesis is dedicated to my family and friends.

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ABSTRACT

Hospital acquired infections are a serious public health problem worldwide affecting hundreds of millions of people every year and are difficult to treat due to the problem of antibiotic resistance. The problem has been compounded by lack of sufficient data to help understand the antimicrobial pattern and spectrum, which impedes has the fight against antimicrobial resistance. This study aimed at determining the common pathotypes causing nosocomial infection in various categories of patients including age, sex and the type of wards in which they were admitted and then determining antimicrobial resistance in the common isolates in JOOTRH. A descriptive cross-sectional study design involving bacteriological analysis of clinical samples including urine, blood, pus swab, stool, cerebrospinal fluid, and effusion was used to purposively select 111 study participants from August to December 2021. Specimens were cultured and the bacterial isolates were tested against different antibiotics by disk diffusion technique following clinical laboratory standards institute (CLSI) guidelines. Data obtained were analyzed using SPSS version 20. P values ≤ 0.05 were considered statistically significant. Tables and bar graphs were used to summarize data in percentages. More than half of the study participants 59(53.2%) were females while 52(46.8%) were males. A third, (31%) were aged less than one year while 4% were aged between six and twelve years. In total, 51(45.9%) of samples yielded bacterial growth out of which, 37(33.3%) were gram negative while 14(12.6%) were gram positive. Generally, *Klebsiella pneumoniae* was the predominant bacterial pathogen isolated from samples followed by *Escherichia coli* and *Staphylococcus aureus*; *Pseudomonas aeruginosa*, *Proteus spp.*, Enterococci and *Acinetobacter baumannii* at 16(31.4%), 13(25.5%), 12(23.5%), 4(7.8%), 3(5.9%), 2(3.9%) and 1(2%) respectively. The newborn unit had 1(7.7%) accounting for the least number of pathogens isolated. 27(52%) of the isolates were from surgical ward. *Staphylococcus aureus* was the predominant pathogen responsible for surgical site infection. This study found an association between age and the risk of hospital acquired bacterial infection ($\chi 2$, p=0.012) however, gender (p=0.338) and ward category (p=0.774) were not significantly associated with acquiring bacterial infection during hospitalization period. High prevalence of multidrug resistant bacteria was noted with many pathogens showing resistance to more than three antibiotic classes. Included in this category were ampicillin, imipenem and ceftazidime at 17(100%), 33(97.1%), and 35(92.1%) respectively. Piperacillin and penicillin G showed 27(87.1%) and 5(83.3%) resistance respectively, while sulphamethoxazole-trimethoprim resistance was at 64.7%(n=11). Low resistance was however, noted against amikacin, gentamycin and meropenem at 7.3% (n=3), 22.2% (N=8) and 24.1% (N=7) respectively. This study reported high hospital infections in surgical wards with gram-negative bacteria being most common and *Klebsiella spp.,* predominating among Enterobacteriaceae and Staphylococcus aureus among gram positive pathogens. Most bacteria isolated were resistant to multiple antibiotics. While this study recommends the use of amikacin, gentamycin and meropenem for empiric treatment particularly in resource limited areas where culture facilities are not available it is envisaged that this information will be used in clinical practice to manage patients as antibiotics are being researched on while continuously monitoring new antimicrobial threats that may emerge against them. On the other hand, the study also recommended a review on the continued use of sulphamethoxazole-rimethoprim, ampicillin, ceftazidime, piperacillin and penicillin G due to high resistance showed against them by the bacterial pathogens.

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ABBREVIATIONS

AIDS	Acquired immunodeficiency syndrome
AK	Amikacin
AMC	Amoxiclav
AMP	Ampicillin
AMR	Antimicrobial resistance
API	Analytical profile index
AZM	Azithromycin
BA	Blood agar
BSI	Blood stream infection
CAZ	Ceftazidime
CEF	Cefotaxime
CBA	Chocolate blood agar
CIP	Ciprofloxacin
CLED	Cysteine lactose electrolyte deficiency agar
CNS	Central nervous system
CXT	Ceftriaxone
ENT	Ear nose and throat
ERY	Erythromycin
ESBL	Extended spectrum beta lactamase
ETP	Ertapenem
FEP	Cefepime
GEN	Gentamycin
GIT	Gastrointestinal tract
GNB	Gram negative bacteria
HAIs	Hospital acquired infections
HCAI	Health care associated infection
HDU	High dependency unit
ICU	Intensive care unit
IMP	Imipenem
IPC	Infection prevention and control
LBW	Low birth weight
LOS	Length of stay

LRTI	Lower respiratory tract infection
MEM	Meropenem
MDGs	Millennium development goals
MDR	Multi-drug resistance
NBU	New born unit
NI	Nosocomial infection
NIs	Nosocomial infections
NAP	National action plan
OF	Ofloxacin
OTC	Over-the counter
OX	Oxacillin
PBPs	Penicillin binding proteins
Pi	Penicillin
PIP	Piperacillin
RTI	Respiratory tract infection
SDGs	Sustainable development goals
SPSS	Statistical packages for social scientists
SSI	Surgical site infection
SXT	Sulphamethoxazole-Trimethoprim
TAZ	Tazobactam
TSI	Triple sugar iron
URTI	Upper respiratory tract infection
UTI	Urinary tract infection

ACRONYMS

BD	Beckton Dickinson
BPS	Board of post graduate studies
CEO	Chief executive officer
CLSI	Clinical laboratory standards institute
CoNS	Coagulase negative *Staphylococcus aureus*
COVID	Corona virus disease
ENT	Ear nose and throat
HIV	Human immunodeficiency virus
IERC	Institutional ethics and research committee
JOOTRH	Jaramogi Oginga Odinga teaching and referral hospital
JOOUST	Jaramogi Oginga Odinga university of science and technology
KNH	Kenyatta national hospital
MDH	Mathare district hospital
MGIT	Mycobacteria growth indicator tube
MRSA	Methicillin resistant *Staphylococcus aureus*
MTRH	Moi teaching and referral hospital
NACOSTI	National commission for science technology and inovation
RSV	Respiratory syncytial virus
TSI	Triple sugar iron
U.S	United States
WHO	World health organization

CHAPTER ONE: INTRODUCTION

1.1. Background

Nosocomial (also known as hospital acquired infections-HAIs) are still a major challenge in both developed and developing countries often resulting in morbidity, mortality and increased cost of healthcare in low-, middle- and high-income countries (Sikora & Zahra, 2022). Prevalent among other causal agents are those infections caused by bacterial pathogens which often are associated with antibiotic resistance. Treatment outcomes involving HAIs therefore becomes difficult which may possibly result in death due to treatment failure or increased patient days which spirals healthcare costs because of limited antibiotic therapy options (I. Ahmed *et al.*, 2019; Peters *et al.*, 2019; Sikora & Zahra, 2022). Interestingly, bacteria among other pathogens acquired within healthcare settings are not always confined to within such set ups, but may occasionally escape their containments (healthcare set ups) and can often be carried by both human and non-human reservoirs to the community where they contribute in increasing the load of antibiotic resistant strains (I. Ahmed *et al.*, 2019; Cattoir, 2022).

Several microbial agents have been shown to cause HAIs majority of which are caused by bacteria that often complicates patient management outcomes due to their antibiotic resistance (Bryce *et al.*, 2016). Although various studies have dwelt on antibiotic characteristics of bacterial isolates from hospitalized patients, the pattern and spectra of their antibiogram to aid in the empiric treatment is still not well understood (Maina *et al.*, 2023). Pathogenic bacteria including *Pseudomonas spp.*, Coagulase negative Staphylococci, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii* and *Escherichia coli* are among other most common causal agents of HAIs (Agaba *et al.*, 2017; Bryce *et al.*, 2016; Tolera *et al.*, 2018; Walkty *et al.*, 2017).

In general, nosocomial infection can be defined as infections which are acquired during the period of hospitalization and whose clinical manifestations appear after 48hrs or more post admission or after discharge (Sikora & Zahra, 2022). Several factors often contribute to risk of acquiring infections within the hospital. These include improper management of hospital wastes, immunodeficiency of a patient, surgical procedures, presence of indwelling catheter as well as presence of underlying

conditions (Cheng *et al.*, 2020; Yallew *et al.*, 2016). Differences in prevalence rates and antibiotic resistance across the globe have been reported in previous studies. In one of the studies done in the United States (U.S) for example, a 3.2% prevalence was documented while in other European countries the prevalence was 6.5%. Another study done in Serbia revealed a prevalence of 7.1%. (Ilic & Markovic-Denic, 2017; Sikora & Zahra, 2022), while a study in Iran (Ghashghaee *et al.*, 2018) found a prevalence of 4.5%. However, due to scarcity of published reports that document prevalence rates, the true burden of nosocomial infections (NIs) which is thought to be higher that what most reports document is yet to be established (Sikora & Zahra, 2022).

Studies done on antimicrobial resistance (AMR) have also shown high levels of resistance exhibited by the bacterial isolates from hospitalized patients. For instance, in a given study done in Iranian hospitals on device associated infections revealed that 100% of *Klebsiella pneumoniae*, and *Acinetobacter baumannii* were resistant to imipenem; and 100% of *Staphylococcus aureus* causing nosocomial pneumonia and surgical site infections (SSIs) were resistant to oxacillin while respiratory tract and uropathogens isolated from catheters were resistant to imipenem (Jahani-Sherafat *et al.*, 2015); *Acinetobacter baumannii* isolated from blood stream infection due to central line catheters were all (100%) resistant to imipenem while those that caused catheter associated urinary tract infections (UTI) were 96.4% resistant to imipenem.

In Africa, some study findings of HAIs have put prevalence of HAIs to be between 2.5% and 14.8%, even though it is still thought that these figures could have been underreported (Tolera *et al.*, 2018). In a study done in Ethiopia, for example, the prevalence of culture confirmed nosocomial bacteria was 6.9%; 80% of *Staphylococcus aureus* isolates were resistant to chloramphenicol and erythromycin, 70% were resistant to cephalexin and tetracycline, 88.9% to methicillin beside being the most common bacterial isolate; while 83.7% of *Pseudomonas aeruginosa*, isolates showed resistance to cephalexin and ceftazidime, 66.7% were resistant to chloramphenicol (Tolera *et al.*, 2018). A study from hospital surveillance data in a Nigerian hospital however, documented a prevalence of laboratory confirmed HAIs to be 6.3% with a high prevalence of antimicrobial resistance in bacterial isolates (Iliyasu *et al.*, 2018)

In East Africa a metanalysis study showed that there was a high prevalence of catheter associated blood stream infections (BSIs), urinary tract infections (UTIs), surgical site infections (SSIs) and healthcare associated pneumonia which were commonly caused by *Klebsiella spp*., *Staphylococcus aureus, Escherichia coli* and *Pseudomonas spp*. Methicillin-resistant *S. aureus* and extended-spectrum beta-lactamase producing Gram-negative bacilli were the most reported antimicrobial resistant pathogens according to a study by (Feleke *et al.*, 2018). In Uganda, a similar study by (Agaba *et al.*, 2017) revealed the most prevalent bacterial pathogen causing NIs was , *Klebsiella spp*. In Kenya, studies done on HAIs have similarly found *Klebsiella pneumoniae* to be responsible for approximately 23% amongst bacterial isolates from clinical specimens and in which 82% of isolates from new born unit (NBU) were resistant to commonly used antibiotics (Patil *et al.*, 2022).

Several risk factors have been known to contribute to nosocomial bacterial infections including among others, lumping sick people together under one roof and especially when the proportion of severely ill and immunocompromised patients is high (Roohani, et al., 2018). Incidences of nosocomial infection vary in distribution depending on whether there is involvement of surgical sites, respiratory tract, urinary tract, blood stream, burn or the use of medical devices (Khan *et al.*, 2017; Taj *et al.*, 2018).

1.1.1 Potential Sources of Common Nosocomial Pathogens

Microorganisms that are potential causers of NIs may be normal microflora that occasionally gain entry in to other sites of the body causing endogenous infections (Agaba *et al.*, 2017; Johnson, 2002) such as UTI, and infection of surgical wounds as well as respiratory tract infection arising from aspiration of oral and GIT material facilitated by mechanical intubation (Agaba *et al.*, 2017). Sources of common NIs can also emanate from exogenous sources (Matinyi *et al.*, 2018).

Additionally, hospital staff working in various service delivery points have been shown to aid in the transfer of pathogens to patients (Hawkins *et al.*, 2023; Nimer, 2022). While carrying a study in one of the referral hospitals in Kenya, Obanda and coworkers demonstrated that *Staphylococcus aureus* carried in the noses of hospitalized patients pose a potential risk to transmission of opportunistic nosocomial

infections (Obanda *et al.*, 2022) which eventually complicates treatment outcomes due to multiple resistance to antibiotics (George *et al.*, 2018).

Carriage of pathogens is not just restricted to nasal passages and as has been demonstrated by (Poignant *et al.*, 2015), members of Enterobacteriaceae family have previously been transmitted to hospitalized patients (Muvunyi *et al.*, 2020) with most common encountered being *E. coli* and *Klebsiella spp*., (Magale *et al.*, 2015) which are notorious for being multidrug resistant (Maina *et al.*, 2023).

The role of environment as a source of NIs cannot be overemphasized (Suleyman *et al.*, 2018). While a number of organisms have been isolated from surfaces in hospital settings (Odoyo *et al.*, 2023), several others have been isolated from water sources making contact with inanimate areas a real risk of transmission of the pathogens to patients (Percival *et al.*, 2015; Suleyman *et al.*, 2018). And while direct transmission of organisms from contaminated environment to susceptible patients is possible, the environment can contaminate medical devices which in turn aid in the transmission of infections to patients (Percival *et al.*, 2015; Ssekitoleko *et al.*, 2020)

1.2 Problem Statement

Antimicrobial resistance is a silent pandemic that continue to be a cause of death across the globe (Kariuki *et al.*, 2022). It is estimated that about 700,000 people die globally every year and that 23.5 deaths per 100,000 were attributable to AMR in Sub-Saharan Africa compared to other regions (Kariuki *et al.*, 2022). Few studies have been done on the prevalence of antibiotic resistance in nosocomial bacteria in Western Kenya (Wangai *et al.*, 2019) which makes sources of potential pathogens causing hospital infection to be poorly understood (Sikora & Zahra, 2022). This has led to unavailability of sufficient data that ought to inform treatment of infections acquired during hospitalization particularly those which occur as a result of infection with multidrug resistant (MDR) bacteria. In resource limited rural settings, culture facilities to isolate and perform drug testing on bacteria are not often available. Clinicians therefore are forced by circumstances to resort to treating clinical suspects empirically often using trial and error to manage patients and sometimes the treatment becomes unsuccessful since the pattern and spectrum of antimicrobial resistance (AMR) is not well understood. This leaves limited treatment options that may result in

increased morbidity and mortality mostly experienced in low- and middle-income countries (Collignon *et al.*, 2018). This study therefore aims to provide data on the common bacterial pathogens causing hospital acquired infections and how they respond to antibiotics administered against them. The information will then be used to inform specific treatment particularly when empiric administration of antibiotic is inevitable which would again help in the fight against emergence of new resistant bacterial strains.

1.3 Objectives

1.3.1 Main Objective

To characterize bacterial pathogens causing hospital acquired infection and determines their antibiotic resistance at Jaramogi Oginga Odinga Teaching and Referral Hospital.

1.3.2 Specific Objectives

i. To determine whether age, sex and ward type (patient category) predispose to bacterial infection in JOOTRH
ii. To determine the most common bacterial pathogens causing hospital acquired infections in JOOTRH.
iii. To determine antimicrobial resistance of bacterial pathogens isolated from clinical specimens in JOOTRH.

1.4 Research Questions

i. Does age, sex and type of ward predispose to nosocomial infection in JOOTRH?
ii. What are the most common bacterial pathogens causing nosocomial infections in JOOTRH?
iii. Are nosocomial bacteria isolated from clinical specimens in JOOTRH resistant to commonly administered antibiotics?

1.5 Justification of the Study

JOOTRH is one of the largest among referral hospitals in the western region of Kenya. However, no study involving nosocomial bacterial and their antibiotic resistance has ever been conducted in this facility that would guide the empiric use of routinely administered antibiotics. Due to the existing problem of antimicrobial resistance, new patterns continue to emerge thereby putting more constrains on the already existing shortage of new antibiotics. It is therefore important to provide knowledge about the most common bacterial pathogens causing infection in hospitalized patients and determine if they are resistant to commonly used antibiotics. This would eventually help in the fight against emergence of MDR strains.

1.6 Significance of the Study

It is envisaged that this study has generated knowledge about common bacterial pathogen causing NIs and their antibiotic resistance commonly used antibiotics. It is therefore expected that the information contained in this study report will be used to guide policy on empiric use of antibiotics and promote stewardship that would help in the fight against prevention and control of AMR.

1.7 Scope and Limitation

1.7.1 Scope

The study was purely hospital based and will involve laboratory culture of clinical specimens obtained from patients who will have been clinically proved to be exhibiting signs and symptoms of HAIs.

1.7.2 Limitation

The study was limited to nosocomial infections caused by bacterial agents. However, a few challenges were faced because the study was done during Covid-19 pandemic and therefore direct interaction with patients was prohibited. However, engagement of selected hospital staff to help in data collection helped to surmount the challenge.

1.8 Definition of Terms

Antibiotics are medicines that kill or inhibit bacterial growth

Antimicrobials are drugs that kill or inhibit growth of a variety of microorganisms including bacteria, viruses and fungi

Antibiogram refer to a summary of susceptibility patterns of bacteria to locally available drugs

Antimicrobial resistance is the ability of a microorganism to prevent the activity of an antimicrobial drug that was once effective against it

Antimicrobial susceptibility testing is a laboratory test used to determine if a microorganism is susceptible to a particular antibiotic(s)

Antimicrobial stewardship refers to programs that are coordinated to promote the appropriate use of antimicrobials to improve patient outcomes, reduce antimicrobial resistance and to limit the spread of MDR organisms

Aerosols are fine sprays from coughs, sneezes etc. producing droplets that remain suspended in the air for some time

Appropriate use of antibiotics refers to the use of antimicrobials to treat the right conditions for the right duration on the right patient

Bacteremia: refers to the presence of un-dividing bacteria within the blood stream

Broad spectrum antibiotic is an antibiotic that works against a wide range of gram-positive and gram-negative bacteria

Cystitis is an inflammation of the urinary bladder.

Droplet nuclei are residues of solid material such as bacterial cell which is left on a surface after aerosols dry

Emerging infections are infections that have not occurred in humans before or those that have occurred throughout human history but have only recently been recognized as distinct due to an infectious agent such as Lyme disease and gastric ulcers

Empiric treatment refers to choosing of antimicrobials based on their clinical judgement and expertise in the absence of susceptibility test

Endogenous infection is an infection due to offending agents migrating from another sight within the patient body

Exogenous infection is an infection caused by a microbial agent contracted from another individual, or from the environment

Fomites are non-living objects such as bedding, towels and handkerchiefs that are contaminated by an infected person

Inappropriate use of antibiotic refers to the use of an antibiotic with no indication or for non-therapeutic purposes

Isolate refers to an organism obtained from specimen such as pus, blood and urine

Lower respiratory tract infection refers to infection involving the lungs i.e. those that occur below the voice box

Mortality refers to the number of people dying due to a particular infectious agent

Morbidity is the rate at which people get sick in a given community

Multi-drug resistance refers to resistance of microorganisms to at least one antibiotic in three or more drug classes

National action plan is a policy document that provides a comprehensive policy frame work and priority actions to contain the emergence and spread of antimicrobial resistance

Nosocomial infection is any infection that is acquired while in hospital occurring 48hrs or more after admission and up to 48hrs after discharge

Over the counter means sale of medicine at a pharmacy without a doctor's prescription

Pathogen is a disease-causing organism such as bacteria

Pathogen category refers to grouping of bacteria in to either gram-positive or gram-negative

Pathotypes refer to different bacterial species such as Klebsiella, Escherichia, Pseudomonas, Acinetobacter, Proteus, and Staphylococcus

Patient category is a grouping of hospitalized patients by their conditions (cause and severity), patient demographic characteristic and ward type.

Priority microbes are microbes that cause diseases that are associated with high rates of antimicrobial resistance, morbidity, mortality and treatment costs.

Resurging infections are infections that were once a major health problem globally or in a particular country, and then declined dramatically, but are again becoming health problems such as tuberculosis, lyme disease and group A streptococcal infections

Sample is a specimen collected for laboratory testing such as urine, pus, blood and cerebral spinal fluid

Septicemia is a condition characterized by the presence of multiplying bacteria in bloodstream with symptoms such fever, headache and shock

Surveillance is the detection and monitoring of trends and threats in antimicrobial resistance and antimicrobial use to inform strategies that reduce the risks of resistance

Upper respiratory tract infection is an infection of the nasal passages and throat

CHAPTER TWO: LITERATURE REVIEW

2.1 Introduction

Nosocomial bacterial pathogens are considered a serious threat to public health and are a major cause of mortality and morbidity worldwide (Sadeghi *et al.*, 2021), in which the very young (Schroder *et al.*, 2018) the elderly, those with underlying health conditions which put them under intensive care as well as those who have undergone surgical operation are often at risk (Huang *et al.*, 2020). Moreover, such infections ostensibly often lead to treatment failure or sometimes becomes difficult to treat due to their antimicrobial resistance resulting in death (Schroder *et al.*, 2018). Moreover, despite heightening intervention policies, infection rates continue to increase and so is the problem of antimicrobial resistance of bacteria agents which are notorious in causation of HAIs (Schroder *et al.*, 2018; Tomczyk-Warunek *et al.*, 2021).

2.1.1 Global prevalence of hospital acquired bacterial infections

Different studies have documented different global prevalence of HAIs and the patterns of antimicrobial resistance. For example, studies done in the in the United States and other European countries documented a prevalence rate of 3.2% and 6.5% respectively; but in South East Europe, particularly Serbia, a prevalence rate of 7.1% was reported (Ilic & Markovic-Denic, 2017; Sikora & Zahra, 2022). Several organisms showing varied resistance towards antibiotic therapy globally have been isolated from clinical specimens in hospitalized patients. For instance, a surveillance study on healthcare associated infections and antimicrobial resistance in an Italian hospital recorded a number of blood stream infections, several of which were due to catheterization for patients admitted in intensive care units (ICU) (Bianco *et al.*, 2018). Predominating, the isolated organisms were Gram negative bacteria including *Acinetobacter baumannii* and *Klebsiella pneumoniae*; of which 91.6% and 28.5% respectively showed resistance to carbapenem antibiotics while over 50% of the Gram-positive isolates were resistant to oxacillin (Bianco *et al.*, 2018).

A study done on MDR nosocomial pathogens in a Chinese hospital was able to identify extended spectrum beta-lactamase (ESBL) producing *Escherichia coli* as a common causal agent beside *A. baumannii* and *Pseudomonas spp*., in male patients, the elderly (above 65years), those with critical conditions and admitted in ICU; in post-surgical patients and those in neonatal units (M. Wang *et al.*, 2019b). Incidences

of antimicrobial resistance among the isolates were also reported and methicillin resistant *Staphylococcus aureus* (MRSA) were found to be 90% resistant to Penicillin G, cefoxitin, and oxacillin drugs while gram negative bacteria including ESBL *Klebsiella pneumoniae* isolates were mostly resistant to carbapenems, β-lactams, aminoglycoside and quinolones but susceptible to amikacin; while the same study revealed all isolates of *A. baumannii* were resistant to piperacillin and imipenem, resistance towards ciprofloxacin, ceftazidime and ceftriaxone was more than 90% (M. Wang *et al.*, 2019b).

Similar studies have been conducted in Iranian hospitals, in which device associated NIs rates revealed that all *Klebsiella pneumoniae* isolates, and *Acinetobacter baumannii* showed resistance to a carbapenem; and all of the *Staphylococcus aureus* isolates from lower respiratory sites causing nosocomial pneumonia and surgical site infections (SSIs) were resistant to oxacillin. Pathogens that were isolated from respiratory tract and those from urinary catheters were resistant to imipenem. *Acinetobacter baumannii* isolates from blood stream due to central line catheters were all resistant to imipenem while those that caused catheter associated UTI were 96.4% resistant to imipenem (Jahani-Sherafat *et al.*, 2015).

In a study conducted by (Golan, 2015), Enterobacteriaceae accounted to more than 60% of HAIs with ESBL producing *Escherichia coli* being most common isolate followed by Klebsiella species. Among several factors, male gender and surgical operation was found to be a risk factor for acquiring infection during time of hospitalization. A study conducting on risk factors for hospital acquired MDR uropathogens in Serbian, *Enterococcus faecalis* was most frequently isolated bacteria followed by *Klebsiella spp.*, which was a major uropathogen in patients above 65years. Generally, most infections were due to gram negative bacterial which showed multi drug resistance up to 53.8% (Milovanovic *et al.*, 2019).

Incidences of infections acquired in certain hospitals in India have in the past been linked to ICU admissions particularly in patients under care following cardiac surgery (Sahu *et al.*, 2016); findings of which revealed that 4.6% of patients developed infection and that lower respiratory tract infections (LRTI) accounted for majority of the reported cases followed by SSIs. *Acinetobacter baumannii, Klebsiella spp.*,

Escherichia coli and *Staphylococcus aureus* were the most frequent pathogens. The study further revealed that GNB isolated from different sources were highly resistant to commonly used antibiotics (Sahu *et al.*, 2016). Other global studies involving systematic reviews and meta-analysis have in the past implicated Group B Streptococcus infection of surgical site post- caesarean delivery to cause 10% post-surgical infection as well as deep organ space SSI and UTI (Collin *et al.*, 2019).
Previously, bacterial isolates from adults and neonates admitted to ICU have shown high resistance to antibiotics (Barnsteiner *et al.*, 2021). Incidentally, the most common pathogens according to this study were *Acinetobacter baumannii* and *Klebsiella spp*. In s study carried out in Madhya Pradesh Hospital, prevalence of orthopedic SSI was 7.6%, and were majorly caused by *S. aureus* of which all isolates were resistant to penicillin, 80% resistance to erythromycin and cotrimoxazole; amikacin and cefoxitin resistance was considerably high (60%). The study also revealed that amikacin was the antibiotic mostly prescribed and that sex (male) was a risk factor to developing SSI (Skender *et al.*, 2022).

2.1.2 African Perspective of Prevalence of Hospital Acquired Bacterial Infections

In Africa, studies involving infections in ICU which was done in Morocco found out that 39 % of HAIs recorded in ICU were due to pneumonia while bacteremia and catheter related BSI were 39% and 17% respectively (El Mekes *et al.*, 2020). *Acinetobacter baumannii* was most common pathogen in ICU patients (31%) followed by Enterobacteriaceae family members (30%). *Klebsiella pneumoniae* was the major pathotype causing infection and 76% of the pathogens were GNB while gram positive bacteria were (24%). Prevalence rates of MDR according to this study was (41%) with 70% of *A. baumannii* isolates (70%) showing resistance to imipenem (El Mekes *et al.*, 2020).

In a study done in Ghana, HAIs were found to be more in males as compared to females and that factors including gender, age less than 10years and more than 60 years contributes to contracting bacterial infection in the hospital. Moreover, high level of antimicrobial resistance was noted for ampicillin, sulphamethoxazole-trimethoprim as well as cefuroxime at 94.8%, 84.5% and 79% respectively while low resistance rate was documented for ertapenem, meropenem and amikacin at 1.5%, 3% and 11%, respectively. MDR was remarkably high at 89.5%. *Acinetobacter*

baumannii and *Pseudomonas spp*., were resistant to all the antibiotics tested against them (Agyepong *et al.*, 2018).

Similarly, the prevalence of hospital acquired SSI in a Nigerian teaching hospital unearthed a considerably higher infection rate among patients below 20 years and those above 50 years which classically put age and gender as risk factors to acquiring hospital infection (Olowo-Okere *et al.*, 2019). Infection acquired after surgery had a prevalence rate of 13% and were mostly caused by *S. aureus*, *E. coli* and *Klebsiella spp*. The study also noted high resistance to commonly used antibiotics and that *E. coli*, *Klebsiella spp*., *Proteus spp.*, *Pseudomonas aeruginosa* and *S. aureus* were 100% resistant to ampicillin and amoxycillin. Proteus, pseudomonas, and S. aureus isolates also showed 100% resistance to Amoxiclav (Olowo-Okere *et al.*, 2020). In Ethiopia, the patterns and spectra of hospital acquired bacterial pathogens has been studied and studies done in selected referral hospitals in Addis Ababa have shown that GNB are mostly responsible for HAIs majority of which have shown multi-drug resistance (Dessie *et al.*, 2016). The biggest problem was due to *E. coli* causing infection in surgical ward as well as *Acinetobacter baumannii*. The pathogens isolated were also highly resistant to Ampicillin, amoxycillin, penicillin, cephazolin and tetracycline while gentamycin and ciprofloxacin antibiotics were relatively effective against most isolates (Dessie *et al.*, 2016).

2.1.3 East African Perspective of Prevalence of Hospital Acquired Bacterial Infections

Comparatively, about NIs done in Rwanda have shown advanced age and longer hospital stay to significantly contribute to acquiring infection in the surgical ward (Mukagendaneza *et al.*, 2019). The study further revealed that *Klebsiella spp*., was the most common pathotype being followed by *Escherichia coli*, *Proteus spp*., *Acinetobacter baumannii* and *S. aureus*, at 15%, 12%, 9% and 6% respectively. Among the isolated bacteria, antibiotic resistance varied considerably, with resistance against amoxiclav, gentamycin, ciprofloxacin and ceftriaxone being 98.8%, 92.6% 78.1% and 53.3% respectively, however, while 50% of the isolates showed multi-drug resistance, effectivity of Amikacin and imipenem to treat bacterial infections was clearly displayed in the sense that among the tested isolates, none showed resistance to either of the two drugs (Mukagendaneza *et al.*, 2019).

Many pathogens acquired in the hospital are mostly commensals of the nose and throat. Transmission through airborne mode therefore becomes easy when infected individuals as well as carriers cough or sneeze releasing droplets in the air (Laux *et al.*, 2013; Weterings *et al.*, 2019). Presence within the respiratory tract makes transmission of the pathogens possible through aerosols to infect post-operative sites of the body (Laux *et al.*, 2013; Weterings *et al.*, 2019). As has been demonstrated by a previous study, coughing, sneezing and even laughing actively generate aerosols that settles ends up settling on surfaces and fomites leaving infectious agents inform of droplet nuclei which can remain viable for long periods of time and therefore perpetuates continuous wave of infection to susceptible individuals in the hospital (Kunkel *et al.*, 2017).

2.2 Agents of Nosocomial Infections

2.2.1 Bacteria

Bacteria are important agents responsible for NIs and various species have been implicated in majority of health care problems in developing countries with transmission being either exogenous or endogenous (Alhumaid *et al.*, 2021; Floret *et al.*, 2009; Ghasemzadeh-Moghaddam *et al.*, 2015). Several gram positive as well as gram negative bacteria have been characterized as common pathogens causing majority of HAIs (Alhumaid *et al.*, 2021). Emmergence of new pathogens causing hospital acquired blood stream infections such as *Stenotrophomonas multophilia* has also been isolated from neurologic patients in ICU (Trifonova & Strateva, 2019).

A study in Eastern Ethiopia, found the 6.9% of hospital infections to be caused by bacteria and that those bacteria that were Gram-positive were the predominant pathotypes led by *S. aureus* (Tolera *et al.*, 2018). Infections caused by *E. coli* and *S. pneumoniae* were second and third most common. SSIs were mostly caused by *S. aureus*, *P. aeruginosa*, and CoNS; *E. coli*, *Proteus* spp., and *Enterococcus* spp. were common uropathogens while URTI were predominantly caused by *S. pneumoniae* and *Klebsiella spp*., (Tolera *et al.*, 2018).

2.3 Nosocomial Infection Types Commonly Encountered

2.3.1 Urinary tract

Urinary tract infections caused by *Escherichia coli* are commonly encountered in clinical practice accounting for over 80% of cases (Alhumaid *et al.*, 2021; Folliero *et al.*, 2020). Several factors often contribute to the risk of infection of the urinary tract and according to (Odoki *et al.*, 2019), persons who are 19years and below, female gender, patients with urinary catheters and diabetes among others significantly predispose individuals to UTIs. In young healthy women however, the most presentation of UTI was uncomplicated cystitis caused by *Staphylococcus saprophyticus* while in other cases of urinary tract infections were caused by other bacteria of Enterobacteriaceae family and *Pseudomonas aeruginosa*, (Ehlers *et al.*, 2018; Karlowsky *et al.*, 2017).

The complexity of management of UTI cases in the past has been a challenge due to empiric antimicrobial therapy necessitating a need to monitor antimicrobial trends which will guide selection of an antimicrobial agents to be used in treatment (Sipahi *et al.*, 2014). Gender, contraceptive use, and presence of a debilitating disease, use of diapers, aging as well as catheterization have been shown to predispose to hospital acquired urinary tract infections (Sipahi *et al.*, 2014).

2.3.2 Wound and Surgical Site Infection

Hospital acquired infections associated with surgical site SSI is an important category of HAIs with a substantial impact on patient morbidity and mortality. Studies have suggested higher SSI rates of SSIs in sub-Saharan Africa. For example, a recent prospective study done in Kenya documented SSI in 8% of surgical patients but other estimates have been higher, including an SSI rate of 22% reported at a rural Tanzanian hospital (Nthumba *et al.*, 2010). Despite the estimates, studies that describe SSIs in sub-Saharan Africa still remain scarce (Nthumba *et al.*, 2010). Among the notorious organisms, *Providencia spp*., *Proteus spp.*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Klebsiella spp.*, *Escherichia coli*, *Enterococcus faecalis* and *Staphylococcus aureus* are still the major bacterial pathogens causing infections of surgical wounds and that they are often resistant to multiple antibiotics (Carroll *et al.*, 2016). According to (Badia *et al.*, 2017), incidences of surgical site infections have

presented with variabilities depending on the type of operation and underlying patient status.

2.3.3 Respiratory Site

Pneumonia has been and continues to be a major health problem particularly in developing countries being caused by several airborne pathogens such as *Mycobacterium tuberculosis*, *Legionella pneumophila, Streptococcus pneumoniae, Hemophilus influenza, Staphylococcus aureus, Mycoplasma pneumoniae,* enteric bacteria, Group B Streptococcus and *Chlamydia spp*. as the causal agents. Primarily, pneumococci are the predominant cause of lower respiratory tract infection in adults while both pneumococci and *Hemophillus influenzae* are commonly involved in causing pneumonia in children (de Benedictis *et al.*, 2020; H. L. Hong *et al.*, 2014).

2.3.5 Blood stream infection (BSI)

Bloodstream infections due to bacterial pathogens are a major cause of morbidity and mortality in developing countries where most patients are treated empirically based on their clinical symptoms (Syue *et al.*, 2019). Previously a raft of measures have been taken to prevent blood stream infections (Bell & O'Grady, 2017). Surprisingly these interventions have not yielded much and that there has been an increase in the number of cases of BSI whose origin appear to be both community and hospital sources (D. Ahmed *et al.*, 2017).

Meanwhile, BSIs presenting with fever of unknown origin continue to be misconstrued to be malaria in most African countries and especially in malaria endemic regions due to lack of capacity to perform blood culture to isolate bacterial agents responsible for BSI (Diekema *et al.*, 2019; Njeru *et al.*, 2016).

2.4 Antimicrobial Resistance

Antimicrobial resistance (AMR) by bacterial pathogens continues to be one of the most serious global public health threats witnessed in the current century (Nasser *et al.*, 2020). Notably, a number pathogens including *Escherichia coli, Acinetobacter baumannii* and *Klebsiella spp.*, have shown resistance to most antibiotics used to treat them posing a great concern on future of treatment caused by these pathogens (Mauldin *et al.*, 2010; Nasser *et al.*, 2020).

Continued existence of surveillance gaps continue to jeopardize the fight against AMR since it is difficult to capture newly emmerged resistance trends in a timely manner and which overrides newly discovered effective antimicrobials to prevent and treat new bacterial strains (Prestinaci *et al.*, 2015). In turn, medical procedures like organ transplantation and major surgeries like, caesarean sections and hip replacements have become very risky to perform (Garcia *et al.*, 2018; Huang *et al.*, 2020; Tomczyk-Warunek *et al.*, 2021).

Emerging MRSA, *Enterococcus faecium*, *Acinetobacter baumanii*, Enterobacteriaceae and *Pseudomonas aeruginosa* showing significantly high resistance to gentamicin, amikacin, ciprofloxacin, carbenicillin, tobramycin, amoxicillin, cefotaxime and ceftriaxone have had increased prevalence (Singh *et al.*, 2017). Moreover, the problem of antimicrobial resistance has continue to erode gains realized by the Millennium Development Goals-MDGs thereby impacting on achievement of the Sustainable Development Goals-SDGs (Jasovsky *et al.*, 2016).
Over-use of antimicrobial agents coupled with the ease of accessibility of over-the-counter, presence of counterfeit and substandard antibiotics and poor infection control measures in developing countries often antagonize the fight against AMR (Shah *et al.*, 2016). It is unfortunate that widely studied antibiotics such as methicillin together with other antibiotics administered together with it, for first line treatment such as carbapenems have also shown resistance to common pathogens causing HAIs (Chouchani *et al.*, 2011).

2.5 Mechanism of Antibacterial Resistance

2.5.1 Resistance Due to Altered Receptors

Alteration of receptors inhibit binding of the antibiotic such as penicillin to penicillin-binding proteins (PBPs) resulting in impedance of uptake process which is enzymatically controlled via methylation of the amino acid growing chain common seen in penicillin and cephalosporins, glycopeptides, macrolides as well as quinolones (Lee *et al.*, 2017; Osterman *et al.*, 2020).

2.5.2 Resistance due to Decreased Entry of a Drug

Resistance of bacteria to tetracycline develops as a result of decreased entry of the antibiotic drug inside a cell which is commonly witnessed in members of the family

Enterobacteriaceae and may occur when there is binding of tetracycline on to cell surface layers before it is passed out by diffusion as well as via a proton-motive force (Petchiappan & Chatterji, 2017). Other mechanisms of resistance can also be through modification of the antibiotic structure via acetylation, phosphorylation, or adenylation which can be exhibited by pathogens such as Pseudomonas and members of the Enterobacteriaceae which then interfere with proper binding of and hence poor uptake by the cell (Ramirez & Tolmasky, 2017).

2.5.3 Antibiotic Efflux Pump

Active pumping of antibiotics from the periplasmic space to the outer membrane of bacterial cell can occur through efflux pumps which ideally are genetically encoded proteins possessed by some bacterial pathogens. Pumping process actively extrude antibiotics out of the cell into the extracellular space, ensuring their unavailability to interfere with bacterial physiology (Jamshidi *et al.*, 2016; Mittal *et al.*, 2019).

2.5.4 Resistance Due to cell Adaptations

Bacteria can adapt to different environmental conditions capable of creating a stressful environment stressor including but not limited to antibiotics in their microcosm; of which if constantly encountered and more particularly at low doses, will enable bacteria adaption for them to survive with the end result being development of resistance (Huijbers *et al.*, 2015; Salimiyan Rizi *et al.*, 2020).

2.6 Factors Contributing to Antibacterial Resistance

Bacterial infection of animals has constantly been treated by administering antibiotics while or prophylaxis practiced by incorporating antibiotics in animal feeds and so when products from such animals such as meat, milk and milk products, poultry and poultry products and even fish are consumed, they will ultimately expose the bacteria to sub lethal doses of antibiotics which then creates room for development of antibiotic resistance (Haskell *et al.*, 2018). While antibiotic dosages are designed to eradicate entire pathogen populations, it is imperative that they must be taken in the appropriate prescribed dosages and for the prescribed duration of time, failure to which pathogenic bacteria can adapt and eventually evolve population that exhibit complete resistance to the antibiotic irrespective of the dosage administered (Bansal *et*

al., 2019). It is therefore imperative to monitor patients to ensure that there is completion of the dosage prescribed and avoid defaults.

Dispensing antibiotics over the counter (OTC) without doctor's prescription often culminates in wrong administration, and furthermore other practices such as administering antibiotics for viral infection, non-adherence to hospital antibiotic policy, excessive and indiscriminate use of broad-spectrum antibiotics cumulatively results in selection pressure which in the long run culminates in development of antibiotic resistance considering that the number of new antibiotics being developed has dropped drastically in the last 40 years (Muri-Gama *et al.*, 2018).

2.7 Prevention and Control of Nosocomial Infection

In order to provide a safe hospital environment to control hospital infections a concerted effort should be put in place. Simple infection prevention and control (IPC) practices such which include regular hand washing is particularly crucial in minimizing transmission of pathogens from hands of healthcare providers to patients (Nyamogoba & Obala, 2002). If hand washing is practiced in conjunction with some other key safe handling of foods (Allerberger & Wagner, 2010).

Whereas it is important to avoid overcrowding in wards, a critical consideration regarding the nature of illnesses should be an ultimate key to infection prevention and control which therefore demands for assessment and screening of cases as a priority in order to avail isolation rooms to patients that require isolation such as the immunocompromised, neutropenic and those with immunological disorders; diarrhea, skin rashes, known communicable disease and known cases of an epidemics of bacteria (Mehta *et al.*, 2014). For prevention of airborne pathogens acquired in the hospital, prevention require observing respiratory hygiene like covering nose and mouth when sneezing, coughing on the sleeves or elbow and using disposable paper towel when wiping the nose and disposing it properly in an appropriate biohazard waste bin (Kotb *et al.*, 2020; Zayas *et al.*, 2013).

2.8 Control of Antibiotic Resistance

One of the ways through which antibiotic resistance is accelerated can be through misuse and overuse of antibiotics, as well as poor infection prevention and control practices. Steps at various levels of society can then be taken to reduce the impact and limit the spread of resistance (Scott *et al.*, 2019). Control strategies therefore need to focus attention at the following levels: -

2.8.1 Control at Individual Level

Individuals taking antibiotics can help in control of resistance by ensuring that antibiotics are taken under prescription by a certified health professional and following the prescription strictly beside regular handwashing, observing food and respiratory hygiene, following strictly the advice given by the health worker on antibiotic use as well as not sharing their drugs with other sick family members (Scott *et al.*, 2019).

2.8.2 Control at Policy Makers' Level

Policy makers also have a very distinct role to play in regards to resistance control which can be instituted by ensuring a robust national action programs (NAP) to deal with resistance, improve on surveillance systems of to detect emergence of resistant pathogens beside strengthening policies and implementing programs on IPC and also regulate the use and disposition, making available information on impact of antibiotic resistance as well as empowering healthcare industry to invest in research so as to develop new antibiotics, vaccines, diagnostics and other tools to support in prevention and control the spread of antibiotic resistant pathogens (Birgand *et al.*, 2018; Malania *et al.*, 2021; Robilotti *et al.*, 2017).

2.8.3 Health Professionals' Level

Health care professionals have a duty to fight antibiotic resistance which they can execute through prescription and dispensation of antibiotics only when they are needed and according to guidelines, reporting incidences of antibiotic resistance to surveillance teams and giving proper instructions to patients about correct use of antibiotics in line with the current guidelines ("Health workers' education and training on antimicrobial resistance: curricula guide," 2019). Coupled with improved diagnostic precision, an uncertainty would reduce leaving room to permit the more

precise prescription of antimicrobials which will reduce selection pressure by promoting the initiation or withdrawal of treatment whenever necessary (Sadiq *et al.*, 2017). Health professionals should also strive to encourage sharing of departmental data between laboratory and pharmacy on surveillance of drug resistant pathogens to help in improving mechanism towards detection, identification and monitoring of bacterial pathogens (Ajuebor *et al.*, 2019; Ashley *et al.*, 2019; Scott *et al.*, 2019).

2.8.4 The Agricultural Sector

The agricultural sector can play part in controlling antibiotic resistance by ensuring that antibiotics are only given to animals under supervision of a veterinary officer, discouraging the use of antibiotics for growth promotion to prevent diseases in healthy animals, preventing animal diseases through vaccination rather than administering antibiotics and embracing alternative remedy available; improving biosecurity on farms and preventing infection through improved hygiene and animal welfare (Braykov *et al.*, 2016; Scott *et al.*, 2019).

2.8.5 Using Combination of Therapy

Therapeutic combinations of antimicrobial use should be a routine practice in the treatment of a life-threatening infections, infections caused by mixed agents such as aerobic and anaerobic bacteria so that antibacterial activity can be enhanced; combined treatment is reasonable when the precise agents of an infection deemed serious is unknown let alone for therapy of certain chronic infections (D. J. Hong *et al.*, 2016). By using combined therapy, a lot of gains have been achieved, however amid myriads of challenges because in some occasions, the synergistic effects occasioned by combined therapy sometimes may get complicated resulting in indifference while on the contrary antagonistic effect may also reduce the activity of one or both components of the combined antibiotics (Agarwal *et al.*, 2017).

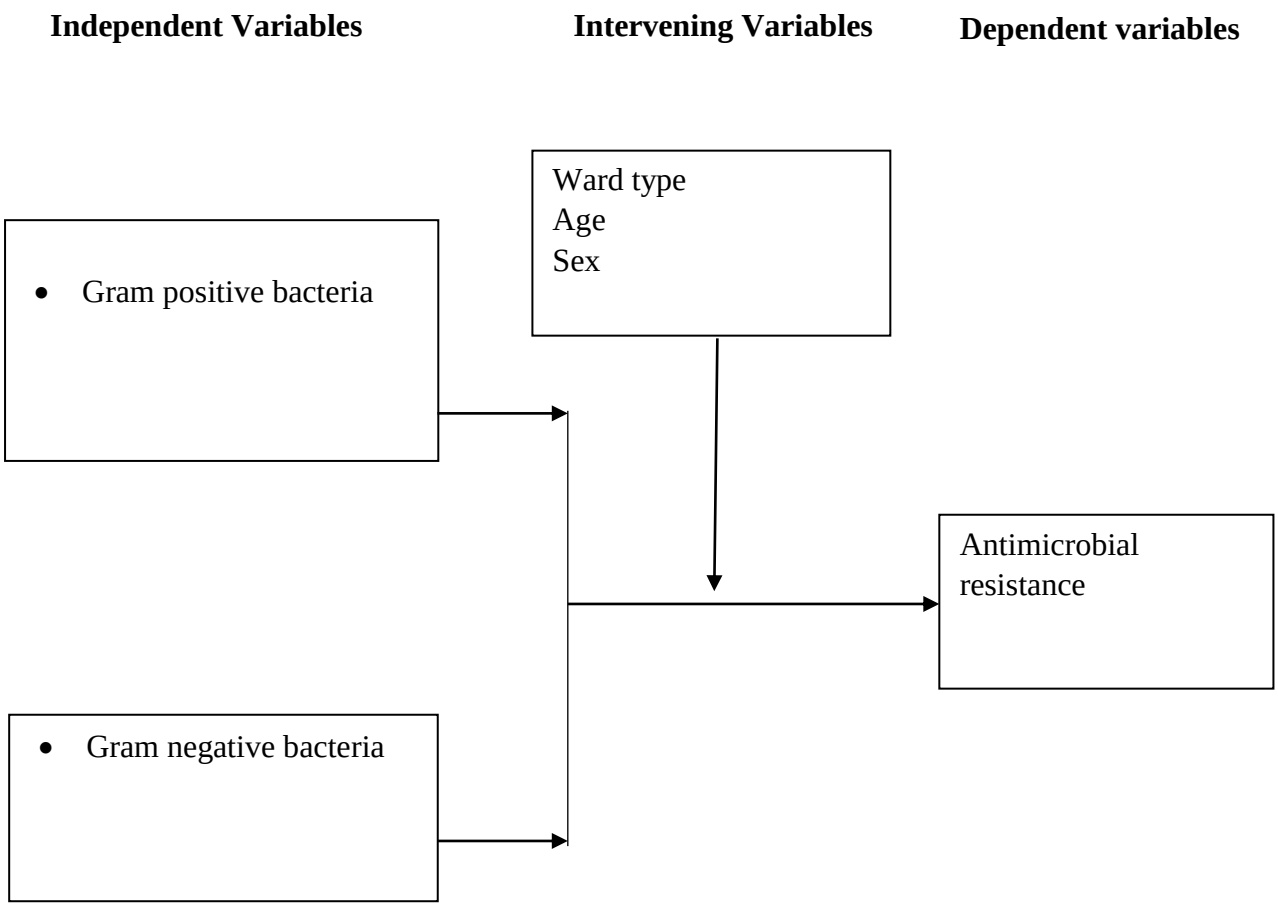


Figure 2.1: *Conceptual framework* **(Author: Arthur Aroko)**

2.9 Conceptual Framework

The above framework was conceived considering the fact that a person's health condition which will warrant admission in particular wards (ward type), age of the patient, sex and the type of pathogen in the patient environment will play a role in contracting nosocomial bacterial infection which can either gram positive or gram negative; and that bacteria contracted in the hospital settings are notorious for antimicrobial resistance.

CHAPTER THREE: METHODOLOGY

3.1 Study Site

The study was conducted in Jaramogi Oginga Odinga teaching and referral hospital (JOOTRH), which is the largest referral hospital that serve patients in Western part of Kenya. The hospital was fully expanded in the late 1960s to a district hospital serving a great number of people from western Kenya. The hospital is situated in Kisumu city between Kondele and Kibuye market along Kisumu-Kakamega highway. The hospital has many departments including: laboratory, surgery, nursing, casualty, comprehensive care center, mortuary, and physiotherapy just to mention but a few. The hospital operates in conjunction with other adjacent facilities such as regional blood transfusion center and Mitchell Obama children hospital. Being a center also for teaching and research, the hospital serves various institutions including Kenya Medical Training College campuses, Maseno University and Uzima University Schools of Medicine, among other private and faith based medical training institutions. Other research institutions which also work in collaboration with JOOTRH include Kenya Medical Research Institute and Center for Disease Control and prevention. JOOTRH has a bed capacity of 467 with bed occupancy of about 94.8%. The highest bed occupancy being in surgical wards and Gynecology, which are 148.7% and 146% respectively (available from-http://www.newnyanzapgh.com). The hospital boasts of six operating rooms. Services offered include Internal Medicine, Pediatrics medicine, General Surgery, reproductive health, Radiology, ENT, Ophthalmology, Intensive Care Medicine, Radiotherapy and Dental services

The presence of a well-equipped microbiology laboratory made it possible for this study to be conducted. Being a major referral facility in Western Kenya, the hospital serves a population in excess of 5 million and the average annual out-patient visits stands at approximately 197,200 and in-patient admissions of about 21,000, annually (available from-http://www.newnyanzapgh.com)

3.2 Study Design

Descriptive cross sectional study design involving bacteriological analysis of samples was used to carry out this research.

3.3 Study Duration/ Period

The study was conducted from August to December 2021.

3.3 Study Population

All patients admitted to surgical, medical, obstetrics and gynecology, pediatrics, NBU wards for at least 48hrs in JOOTRH or those who got discharged from the hospital, went home but developed signs and symptoms of infection after 48 hours or more, came back to the hospital and were readmitted.

3.4 Inclusion Criteria

Patient who developed clinical signs of nosocomial infection such as fever, nausea and/or vomiting, diarrhea, dysuria, discharging and discolored surgical wounds which was not present at the time of admission but had its onset 48hrs or more after admission as well as those who returned 48hrs or more after discharge and were readmitted at JOOTRH with clinical signs and symptoms of NI.

3.5 Exclusion Criteria

- Patients who presented with infection at the time of admission and getting complicated along the way due to change of pathogen and/ or symptoms
- Patients with respiratory symptoms
- Infections of burnt wounds
- Patients who were referred from other facilities

3.6 Sampling Technique

Patients admitted in JOOTRH were followed prospectively for development of NIs by the clinicians. Purposively sampling technique was used to select participants who developed signs and symptoms of NIs consecutively until the desired sample size was obtained.

3.7 Sample Size Determination

The sample size was calculated using Cochran Equation assuming 95% confidence level, 5% margin of error, 10% predicted non-response rate, and 7.1% prevalence of NIs. The final sample size was determined to be 111.

Cochrane Equation; $n_o = \frac{z^{2\rho q}}{e^2}$.

$$n_o = \frac{1.96^{2(0.071\times(1-0.071)}}{0.0025}.$$

Where;

n_0= sample size

Z= 1.96 at 95% level of confidence

P= Global percentage prevalence which is 7.1%

q= 1-p

ε = Margin of error= 5%

Therefore, the sample size = 101

A 10% adjustment to this brings a final sample size to 111

Therefore, the sample size will be 111 samples.

3.8 Data Collection Tools

Data collection tools included questionnaires, data entry forms, patient request forms, inpatient record, laboratory record, microscope, culture media, petri dishes, sterile swabs, micropipettes, water bath, autoclave, incubator, hot air oven, antimicrobial susceptibility discs, weighing balance, microscope slides, Gram stains, applicator sticks, and wire loop. The data obtained was then entered into Microsoft Excel worksheet and analyzed using SPSS version 20.

3.9 Data Collection Procedures

Patients admitted in medical, surgical, obstetrics and gynecology, pediatrics, ICU, HDU, NBU and amenity wards were followed prospectively for the development of HAIs by the clinicians and medical officers. Patients were assessed for SSI, BSI, CNS, UTI, GIT infection. Data were then collected using questionnaire by the clinicians and medical officers. Permission to obtain the relevant sample was then be sought from the patient after elaborately explaining to him/her about the exercise to be undertaken. Once consent was granted, information such as time of admission, age, sex, clinical presentations and clinical diagnosis were entered into biodata form. We relied heavily on the hospital diagnosis which was already documented in the patients' file which were extracted by the hospital staff engaged to help in data collection. Specimens were then obtained and transported to microbiology laboratory in JOOTRH for processing.

3.10 Specimen Collection

Pathological samples were collected while observing strict aseptic technique and then immediately transported to the laboratory after which they were cultured without delay. For urine specimens, at least 10ml mid-stream sample was collected in a sterile 50ml plastic tubes (falcon tubes), and transported to the laboratory for processing. In case of occasioned delay, the specimens were kept in the refrigerator until the time of

processing. In cases of catheterized patients, samples were obtained from the catheter tube and not from the collection bag. Blood samples for culture were collected during fever to increase chances of isolating the offending bacteria. The area of the skin to be punctured was sterilized using tincture of iodine and left to dry.

10ml of venous blood was drawn through venipuncture in to standard blood culture bottles and then entered in to BD™ BACTEC™ FX40 and BD™ BACTEC™ FX automated blood culture system and incubated for a maximum of five days. Specimens from surgical sites were collected using sterile swabs before dressings were changed having done several clinical assessments and noting presentations such as discoloration, bad odor and rapid separation of eschar or presence of pus emanating from the wound. Bandages were removed carefully and wounds washed using sterile physiological saline to remove top debris so as to expose the deeper wound surface. The sterile swab was dipped in sterile normal saline to moisten. The wounds were then swabbed until the swabs got saturated with the exudates. The surgical site specimens were then placed in containers and transported to microbiology laboratory for immediate processing.

3.11 Bacterial Cultivation and Isolation

Upon arrival at the laboratory, swabs from suspected SSIs samples were immediately cultured on BA, CBA and MacConkey Agar. Positive blood culture samples were removed from BACTEC machine, gram stained to identify morphotype of the bacteria before being sub-cultured on Mac CONKEY AGAR (OXOID) Lot 3132377 and Blood Agar (OXOID) and chocolate blood agar. Urine samples were cultured on CLED Agar-SIGMA-ALDRICH Lot BCC4353 while pus swab from wounds and surgical sites were inoculated on, Mac CONKEY AGAR, B.A and CBA upon arrival at the laboratory. Blood Agar and Mac CONKEY agar plates were incubated at 37°C in aerobic condition while Chocolate agar plates were incubated in anaerobic jar. Urine culture plates were incubated aerobically.

Following growth on agar plates, in vitro phenotypic characterization of the colonies like morphology (colonial), pigmentation and cellular morphology was done; further characterization was done using immunological, and biochemical tests such as catalase, urease, coagulase, citrate utilization, indole, fermentation of various sugars, bile solubility, motility and TSI tests as described by (Cheesbrough, 2005).

3.12 Gram Staining

Gram staining was the first test to be performed to help characterize bacterial isolates on the basis of cytomorphology and staining reaction as either Gram positive or Gram negative and also whether they are rods or cocci. Smears prepared from bacterial colonies were allowed to air after which they were heat fixed by passing over Bunsen flame 2-3 times. Crystal violet was then applied to the smear and left to stain for 60 seconds, washed in running tap water, flooded with iodide for 60 seconds, washed in running water and then rapidly decolorization with acetone for a few seconds. Safranin was then be used to counterstain the smear for 60 seconds after which it was washed in running water and allowed to airdry. The dried smear was examined microscopically under 100× oil immersion objective lens.

3.13 Biochemical Tests

Catalase test was done to differentiate staphylococci from streptococci. *Staphylococcus aureus* was then distinguished from other *Staphylococcus species* through serological test using Staphaurex Plus* kit. On the other hand, streptococci species were identified using Prolex™ Latex Agglutination System from PRO-LAB DIAGNOSTICS.

Gram negative rods were characterized by blending API 20E system (bioMerieux, Inc.) and conventional ability of bacteria to ferment various sugars incorporated in TRIPLE SUGAR IRON (OXOID) as well as production of various enzymes such as oxidase and urease, catalase, indole as well as citrate utilization tests. Colonial and cellular morphologies were also used to identify the isolates in the preliminary stages.

3.14 Antimicrobial Testing

Kirby-Bauer disc diffusion technique was performed to test the isolates in vitro as described by (Cheesbrough, 2005). In short a sterile 4mm deep plate of Mueller Hinton Agar(Oxoid) was prepared as per Bauer-Kirby method. For fastidious organisms, Muller Hinton Agar was enriched with 5% Blood. Colonies from an overnight culture were picked with a sterile wire loop and emulsified into 5ml of sterile 0.85% sodium chloride solution and the turbidity of the inoculum adjusted to match 0.5 McFarland equivalence turbidity standard which will have been prepared prior. A sterile Polysterene Spun Swab (SteriPack) was then dipped into the standardized inoculum and the soaked swab rotated firmly against the upper inside wall of the tube to express excess fluid. The entire agar surface of the plate was then

streaked with the swab three times, turning the plate at 60° angle between each streaking. The inoculum was then allowed to dry for 5 - 15 minutes with lid in place. Antimicrobial discs (Oxoid) including CN (10mcg), CXT(5mcg), CRO (30mcg), SXT (25mcg), P1 (iu), FOX (30mcg), AZM (15mcg), CAZ (10mcg), CFM (5mcg), ETP (10mcg), OX (1mcg), AMP (10mcg), AK (30mcg), TGL (15mcg), MEM (10mcg), CIP (5mcg), were then placed on the surface of the streaked plate using disk dispenser (OXOID). The plates were then incubated immediately at 35 ± 2°C and examine after 16-18 hours.

Zones of inhibition to the nearest millimeter were then measured using a Vernier caliper. The diameter of the area displaying no growth was then compared using interpretation guide as per the standards chart for the determination of antibiotic sensitivity and resistance status by the Disk Diffusion method (Sarker *et al.*, 2014).

3.15 Validity

The data for this report was collected using well calibrated and maintained tools and the whole process followed a well laid out standard operation procedures.

3.16 Reliability

Samples were analyzed alongside controls to ensure reliability.

3.17 Data Analysis

Prevalence of nosocomial bacterial pathogens and their antimicrobial resistance were determined using percentages. The relationship between patient category and the risk of acquiring nosocomial pathogens was determined using χ^2 (chi-square) test. P value ≤ 0.05 at 95% confidence were considered statistically significant.

3.17 Ethical Considerations

Board of post graduate studies of Jaramogi Oginga Odinga University of science and technology (JOOUST) gave permission to conduct this study. Approval was then obtained from Jaramogi Oginga Odinga Teaching and Referral Hospital Ethics and Review Committee Ref: IERC/JOOTRH/478/21. National Commission for Science Technology and Innovation (NACOSTI) then gave clearance to conduct the study under Ref. No: 807371. Assent forms were used to obtain data from people unable to

consent on their own and consenting process elaborately explained to this group of participants.

Confidentiality and anonymity of the participants was maintained by using codes for purposes of identification and not by names. Every information obtained was strictly used for research purpose only and data collected was stored, analyzed and reported in formats that won't allow identification of the individuals whose information have been used for purposes of this study.

3.18 Quality Control and Assurance

Quality of antimicrobial testing was ensured by following a routine internal quality control testing with a range of control strains as part of quality assurance process. This allowed monitoring of the of tests performance. Accepted zone diameters were ensured to fall within accepted ranges and any that fell outside the accepted ranges were investigated for sources of error which could have emanated from culture media, antimicrobial discs, inoculum sizes not being consistent, as well as reading of the plates. External quality assessment schemes provided an independent assessment of performance. Routine tests were repeated with identity of organisms blinded.

CHAPTER FOUR: RESULTS

4.1 Age and Gender as Risk Factors to Acquiring Nosocomial Pathogens

This study involved 111 patients. In terms of gender, over half of respondents, 59(53.2%) were female. In terms of age, about a third of participants, 35 (31.5%) were aged less than 1 year, a few respondents, 4(3.6%) were aged six to twelve years. Age was significantly associated with risk of acquiring nosocomial pathogens ($\chi 2$, p=0.012). However, this study found no association between gender and the risk of contracting nosocomial pathogens ($\chi 2$, p =0.338). More than half of samples, 60(54.1%) did not grow any pathogens. This is presented in table 4.1.

Table 4.1: *Age and Gender as Risk Factors to Acquiring Nosocomial Pathogens*

Age	n(%)	NG n(%)	Kleb n(%)	S.A n(%)	E.C n(%)	Ps n(%)	Pr n(%)	E.F n(%)	A.B N(%)	P-Value
<1	35(31.5)	29(82.9)	1(2.9)	3(8.6)	1(2.9)	1(2.9)	0 (0)	0 (0)	0 (0)	**0.012**
1-5	7(6.3)	5(71.4)	0 (0)	1(14.3)	1(14.3)	0 (0)	0 (0)	0 (0)	0 (0)	
6-12	4(3.6)	2(50)	0 (0)	1(25)	1(25)	0 (0)	0 (0)	0 (0)	0 (0)	
13-19	7(6.3)	2(28.6)	1(14.3)	3(42.9)	1(14.3)	0 (0)	0 (0)	0 (0)	0 (0)	
20-39	23(20.7)	9(39.1)	7(30.4)	2(8.7)	1(14.3)	0 (0)	2(8.7)	2(8.7)	0 (0)	
40-59	22(19.8)	9(40.9)	2(9.1)	2(9.1)	6(27.3)	1(4.5)	1(4.5)	0 (0)	1(4.5)	
60+	13(11.7)	4(30.8)	5(38.5)	0 (0)	2(15.4)	2(15.4)	0 (0)	0 (0)	0 (0)	
Gender										
Female	59(53.2)	33(55)	10(16.9)	8(13.6)	6(10.2)	0(0)	1(1.7)	1(1.7)	0 (0)	0.338
Male	52(46.8)	27(51.9)	6(11.5)	4(7.7)	7(13.5)	4(7.7)	2(3.8)	1(1.9)	1(1.9)	
Total	**111(100)**	**60(54.1)**	**16(14.4)**	**12(10.8)**	**13(11.7)**	**4(3.6)**	**3(2.7)**	**2(1.8)**	**1(0.9)**	

KEY: N.G= No growth; Kleb= *Klebsiella spp.;* S.A= *Staphylococcus aureus*; E.C= *E.coli*; Ps= *Pseudomonas aeruginosa*; Pr= *Proteus spp.;* E.F= *Enterococcus faecalis*; A.B= *Acinetobacter baumannii*

4.2: Prevalence of Nosocomial Pathogens with Respect to Ward Category

The infecting nosocomial pathogens were categorized with respect to ward category. The most common pathogens found to cause nosocomial infections was *Klebsiella spp.* followed by *E. coli* and *Staphylococcus aureus*; 16(31.4%), 13(25.5%) and 12(23.5%) respectively. Among the three most common pathogens *Klebsiella spp*., was the leading cause of infection among gram-negative bacteria while *Staphylococcus aureus* was the most frequently isolated gram-positive bacteria. *Acinetobacter baumanni*, 1(2%) was found not a common pathogen in JOOTHR hospital. Among the patient ward categories, most pathogens were isolated from surgical ward followed by amenity and gynecology at 27(52.9%), 8(15.7%) and 6(11.8%) respectively. The newborn unit had the least pathogens isolated, with only two percent of the samples, 1(2%) yielding bacterial growth. This study however,

found no significant association between the nosocomial pathogens and patient ward category ($\chi 2$, p =0.774). This is as summarized in table 4.2

Table 4.2: *Total number of Bacterial Isolates from Patients in Various Wards*

Pathogen	**n(%)**	**New n(%)**	**Sur n(%)**	**Ame n(%)**	**Gyn n(%)**	**ICU n(%)**	**HDU n(%)**	**Chil n(%)**	**Cas n(%)**	**P**
Klebsiella	16(31.4)	0(0)	6(37.5)	3(18.8)	3(18.8)	2(12.5)	1(6.3)	0(0)	1(6.3)	**0.774**
S. aureus	12(23.5)	0(0)	9(75)	1(8.3)	1(8.3)	0(0)	0(0)	1(8.3)	0(0)	
E. coli	13(25.5)	1(7.7)	5(38.5)	2(23.1)	1(7.7)	1(7.7)	0(0)	2(15.4)	0(0)	
P. aeruginosa	4(7.8)	0(0)	3(75)	1(25)	0(0)	0(0)	0(0)	0(0)	0(0)	
Proteus	3(5.9)	0(0)	3(100)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	
E. faecalis	2(3.9)	0(0)	1(50)	0(0)	1(50)	0(0)	0(0)	0(0)	0(0)	
A. baumanni	1(2.0)	0(0)	0(0)	0(0)	0(0)	1(100)	0(0)	0(0)	0(0)	
Total	**51(100)**	**1(2)**	**27(52.9)**	**8(15.7)**	**6(11.8)**	**4(7.8)**	**1(2)**	**3(5.9)**	**1(2)**	

Key: New = Newborn; Sur = Surgical; Ame = Amenity; Gyn = Gynecology; ICU = Intensive Care Unit; HDU = High Dependency Unit; Chi = Children; Cas = Casualty

Table 4.2 shows no association between the pathogens isolated ward in which the patient is admitted.

4.3: Common Bacterial Pathogens Causing Nosocomial Infections

Klebsiella spp., was the most commonly isolated pathogen causing hospital infection in JOOTRH, followed by *Escherichia coli* and *Staphylococcus aureus* at 16(31.4%, 13(25.5%) and 12(23.5%) respectively. In this study, *Acinetobacter baumannii*, 1(2%) was the least commonly isolated nosocomial pathogen. This is as summarized in figure 4.1.

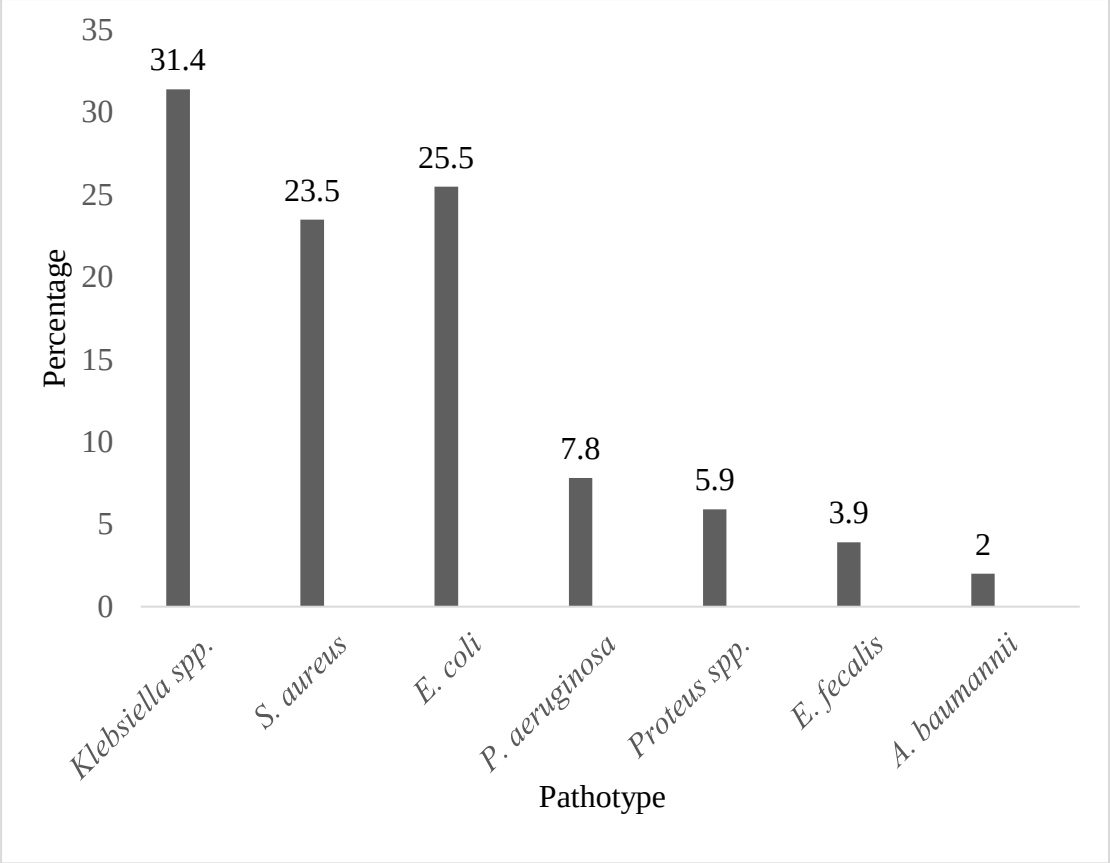


Figure 4.1: *Common Bacterial Pathogens Causing Nosocomial Infection.*

According to the figure above, *Klebsiella spp.* was the most frequent isolate followed by *E. coli* and *S. aureus*. *Acinetobacter baumannii* was the least isolated bacteria

4.3 Antibiotic Resistance Rates of the Bacterial Pathogens Isolated

The highest antimicrobial resistance rate was against Ampicillin, Imipenem and Ceftazidime at 17(100%), 33(97.1%) and 35(92.1%) respectively. The lowest rate was against Amikacin 3(7.3%), Gentamicin 8(22.2%), Meropenem 7(24.1%) and Cefepime 5(33.3%). *Acinetobacter baumannii* showed the most resistance to all antibiotics. *Staphylococcus aureus* showed the most resistance to imipenem, Ampicillin, Cefotaxime. This is as summarized in table 4.3

Table 4.3. *Antibiotic Resistance Rates of the Bacterial Pathogens Isolated*

Drug	No	Kleb (%)	Pr (%)	E.C (%)	Ps (%)	S.A (%)	E.F (%)	A.B (%)	TR (%)
OX	6	NT	NT	NT	0	60	100	NT	4(66.7)
CIP	42	53.80	0	69.20	NT	25	100	100	20(47.6)
SXT	17	100	100	100	100	25	100	NT	11(64.7)
CXT	39	61.50	0	76.90		20	100	100	24(61.5)
MEM	29	33.30	0	10	100	NT	100	0	7(24.1)
IMP	34	100	100	92.30	100	100	NT	100	33(97.1)
AK	41	13.30	0	7.70	100	0	0	0	3(7.3)
Pi	6	NT	NT	NT	NT	83.30	NT	NT	5(83.3)
GEN	36	45.50	0	22.20	0	11.10	0	0	8(22.2)
AZM	13	100	NT	NT	100	44.40	100	NT	8(61.5)
AMP	17	100	NT	NT	100	100	NT	NT	17(100)
CEF	29	60	0	75	100	100	NT	100	19(65.5)
ERY	3	NT	NT	NT	NT	50	50	NT	2(66.7)
CAZ	38	100	100	92.30	75	75	NT	100	35(92.1)
FEP	15	50	0	25	0	NT	100	NT	5(33.3)
TAZ	10	75	NT	75	0	NT	NT	100	7(70)
AMC	14	100	NT	75	0	33.00	100	100	10(71.4)
PIP	31	77.80	66.70	92.30	100	100	NT	100	27(87.1)
OF	30	20	0	58.30	0	0	NT	100	10(33.3)

KEY: OX= Oxacillin; CIP= Ciprofloxacin; SXT= Sulphamethoxazole-Trimethoprim; CXT= Ceftriaxone; MEM= Meropenem; IMP= Imipenem; AK= Amikacin; Pi= Penicillin G; AZM=Azithromycin; AMP= Ampicillin; CEF= Cefotaxime; FEP= Cefepime; ERY= Erythromycin; CAZ= Ceftazidime; FEP= Cefepime; TAZ= Tazobactam; AMC= Amoxyclav; PIP= Piperacillin; OF= Ofloxacin; No= Number of organisms; Kleb= *Klebsiella spp.;* S.A= *Staphylococcus aureus*; E.C= *E.coli*; Ps= *Pseudomonas aeruginosa*; Pr= *Proteus spp.;* E.F= *Enterococcus faecalis*; A.B= *Acinetobacter baumannii*; NT= Not tested; TR= Resistance

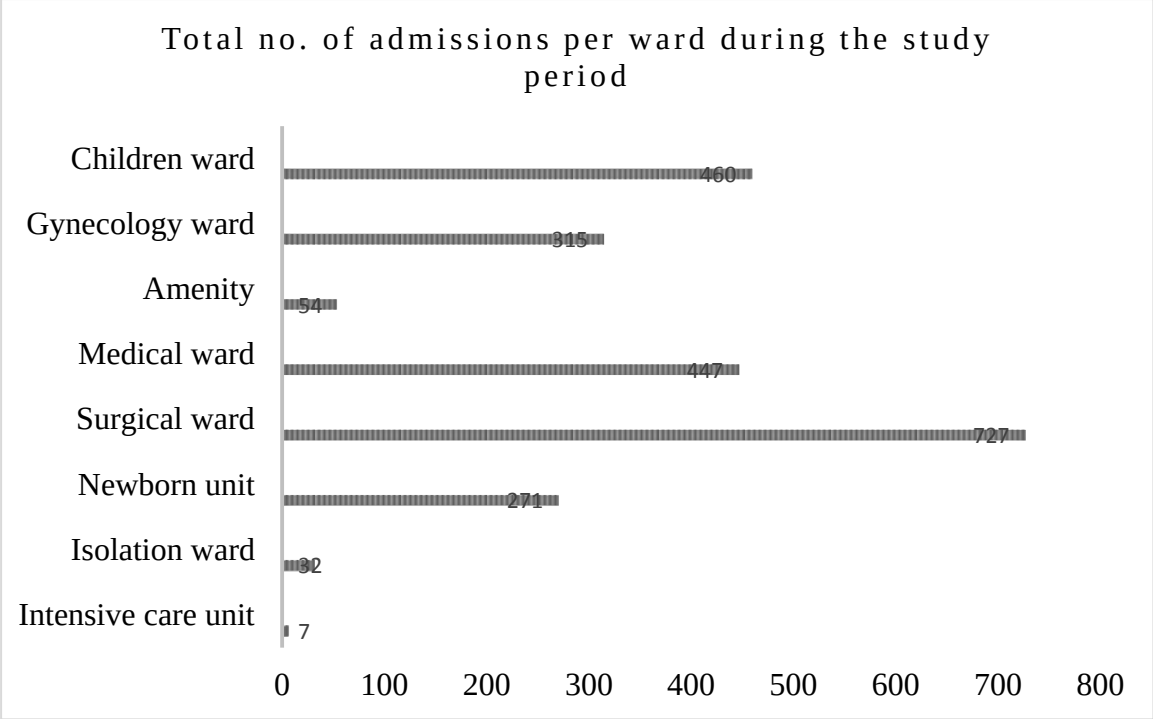


Figure 4.2: *Number of Admissions in the Wards Sampled During Study Period.*

Among the wards sampled during the study period, surgical ward had the highest number of admissions followed by children ward, medical wad, gynecology and newborn unit. Isolation ward, amenity and ICU had the lowest number of admissions.

CHAPTER FIVE: DISCUSSION

Nosocomial bacteria contracted during admission is one of the major public health threats due to difficulty in treatment because of MDR tendencies. This study was purely hospital based and was done in JOOTRH with an aim of determining prevalence of nosocomial infecting bacteria in relation to patients age, sex and ward of admission and determining the antibiotic resistance of the isolates. The study was carried out from August to December 2021. Demographic characteristics such as age, sex plus other relevant information like date and ward of admission; clinical condition for which the patient was admitted as well as other clinical histories were extracted from patient files. Clinical samples were collected from patients admitted in surgical, medical, gynecology, intensive care unit, high dependency unit, amenity, children, as well as newborn units and who presented with signs and symptoms of nosocomial infection which occurred 48hours or more post admission.

5.1 Predisposition to Infections

This study findings showed that age was significantly associated with the risk of contracting nosocomial pathogens while there was no association between gender and the risk of contracting HAIs. This study reported findings which agree with a study by (Murni *et al.*, 2022), who found that children had an increased chance of contracting nosocomial bacterial infections. Similarly, (Milovanovic *et al.*, 2019; Mukagendaneza *et al.*, 2019; M. Wang *et al.*, 2019b), in their study findings managed to find an increased risk of NIs in the elderly patients above 60 years which concurs with findings of this study. Additionally, increased predisposition to NIs can be enhanced due to weakened body defenses (Agyepong *et al.*, 2018; Olowo-Okere *et al.*, 2018) and perhaps also due to the presence of comorbidities (L. Wang *et al.*, 2019a)

As has been demonstrated by this study, surgical ward had the highest prevalence of NIs as compared to other wards. A similar study done for Sub-Saharan Africa ably demonstrated more hospital infection in surgical wards (Ngaroua *et al.*, 2016). However, findings from a study done by (Gelaw *et al.*, 2017) gave a considerably lower findings compared to the findings of this study. The discrepancy could have been due to the scope of their study which concentrated only on caesarean section cases while this study looked at surgical sites in general. Another study done in Rwanda by (Mukagendaneza *et al.*, 2019) also documented lower rates than the one posed by this study; a discrepancy which could be explained by the fact that their

results were merely based on estimates, and therefore could not capture the true burden of SSIs and that studies that would give the correct estimates still remain scarce (Badia *et al.*, 2017).

5.2 Common Bacterial Pathogens Causing Isolated

Majority of cases of NIs according to this study were caused by gram negative rods. This agrees with findings of a another study done by (El Mekes *et al.*, 2020) which found gram negative Enterobacteriaceae to be a common cause of infections in hospital settings. Among the isolates, *Klebsiella spp*., was predominant followed by *Escherichia coli*. This finding again agrees with findings of other studies by (Bianco *et al.*, 2018; Dessie *et al.*, 2016; Golan, 2015; Mukagendaneza *et al.*, 2019; Suwantarat & Carroll, 2016) which found majority of hospital infections to be dominated by *Klebsiella spp.,* and *E. coli*.

However, *Enterococcus spp.*, was found to be a common hospital acquired uropathogens in a study by (Milovanovic *et al.*, 2019) while another study by (El Mekes *et al.*, 2020) found *Acinetobacter baumannii* to be most common. Findings of the two studies are in disagreement with that of this study which found *Klebsiella spp.,* to be the most common pathogen isolated from urine. The discrepancy could have been occasioned by the study by Milovanovic *et al*., having been carried purely on patients with liver cirrhosis and that of El Mekes *et al.,* being based on patients admitted in ICU which might have narrowed the target population. This study however, included all in-patients who showed signs of nosocomial infection.

5.3 Antibiotic Resistance

Generally, there has been a growing concern about increase in antibiotic resistance on bacterial pathogens contracted within the hospital environments exhibiting high variability in the prevalence rates; variability which could be due to differing population characteristics as well as geographical distribution that would otherwise influence seasonal variation and this has been shown to influence thriving of resistant bacteria (Erb *et al.*, 2007; Omulo *et al.*, 2021), the study design used and methods of bacterial detection, sample size involved notwithstanding. Findings of this study however, revealed an overall antibiotic resistance ranging from between 7.3 % which is the lowest rate for amikacin according to this study and 100% recorded for

ampicillin. These findings agree with that of (Erb *et al.*, 2007) which found resistance rates of between 0-100%

Furthermore, findings of this study revealed varying prevalence rates of antimicrobial resistance among the isolates with several NI agents exhibiting resistance to multiple antibiotics. For example, all *Klebsiella spp*., isolated showed high resistance to sulfamethoxazole-trimethoprim, azithromycin, and ampicillin. These findings agree with those of (Skender *et al.*, 2022), who demonstrated high level of resistance of the same antibiotics in their study. Findings of this study again revealed high prevalence of resistance against imipenem which again agrees with findings of (El Mekes *et al.*, 2020; M. Wang *et al.*, 2019b) which showed high resistance (100% and 70%) respectively. Findings by (Olowo-Okere *et al.*, 2020) that there is high resistance to ceftazidime and amoxiclav which also agrees with findings of this study. A similar study conducted in Western Kenya had findings which were coherent with the ones for this study in which *Klebsiella pneumoniae* was found to be the predominant bacterial pathogen with high resistance rates of more than 80% to drugs such as penicillin, cephalosporins, macrolides and tetracyclines (Patil *et al.*, 2022).

While notably high prevalence rates were noted in a number of antibiotics, this studies by (M. Wang *et al.*, 2019b) also revealed relatively low resistance towards meropenem which quite agrees with our finding. Another low rate of resistance was also recorded for Amikacin and gentamycin. Findings by (Critchley *et al.*, 2020; M. Wang *et al.*, 2019b) while working on *Klebsiella pneumoniae* isolates documented resistance rates against Amikacin and Meropenem respectively, which correlates well with the findings of this study.

However, the findings disagree with (Skender *et al.*, 2022) in which resistance to amikacin was 60%. This could be highly possible considering the fact that amikacin was the most prescribed antibiotic at 36% and so there could be high chances of abuse and misuse. This study therefore justifies even though with caution, the empiric administration of Amikacin and Meropenem particularly in areas where facilities for antimicrobial susceptibility testing are not readily available. High resistance against cefepime also disagrees with the finding of our study; the discrepancy of which could have resulted from the number of isolates tested against cefepime (15) in our study compared in relation to (213) in their study.

CHAPTER SIX: CONCLUSION AND RECOMMENDATION

6.1 Conclusion

This study reports that, surgical site infection was the most common type of infection acquired in JOOTRH. Furthermore, age was significantly associated with the risk of contracting infection during admission but gender was otherwise. The study also found that most cases of nosocomial infections were caused by Gram negative bacteria and *Klebsiella spp.*, was the most prevalent pathogen among gram-negative bacteria. *Staphylococcus aureus* was the most frequently isolated gram-positive bacteria and was commonly isolated from surgical sites. Generally, there was high prevalence of antimicrobial resistance with a number of pathogens showing multidrug resistance. Relatively low resistance rate was noted for meropenem, amikacin and gentamycin.

6.2 Implication of the Findings

High antibiotic resistance reported in this study is indeed an indication that antibiotics commonly used to manage infections have become ineffective and infections majority of which have been reported to occur in surgical ward might therefore be difficult to treat. The risk of disease spread, severe illness, disability and death in essence appear real.

6.3 Recommendations

1. The hospital should strive to continuously strengthen infection prevention and control in all the service delivery points and focus more in the surgical wards.
2. The hospital should carry out continuous surveillance on antimicrobials such as amikacin, gentamycin and meropenem to detect any resistance threats that would emerge.
3. This study recommends the use of amikacin, gentamycin and meropenem for empiric treatment in resource constrained settings in which culture facilities are not available.

6.4 Suggestion for Future Research

This study suggests future research to involve molecular work to isolate genes that codes for antimicrobial resistance in western Kenya.

REFERENCE

Agaba, P., Tumukunde, J., Tindimwebwa, J. V. B., & Kwizera, A. (2017). Nosocomial bacterial infections and their antimicrobial susceptibility patterns among patients in Ugandan intensive care units: a cross sectional study. *BMC Res Notes, 10*(1), 349. doi: 10.1186/s13104-017-2695-5

Agarwal, M., Dheeman, S., Dubey, R. C., Kumar, P., Maheshwari, D. K., & Bajpai, V. K. (2017). Differential antagonistic responses of Bacillus pumilus MSUA3 against Rhizoctonia solani and Fusarium oxysporum causing fungal diseases in Fagopyrum esculentum Moench. *Microbiol Res, 205*, 40-47. doi: 10.1016/j.micres.2017.08.012

Agyepong, N., Govinden, U., Owusu-Ofori, A., & Essack, S. Y. (2018). Multidrug-resistant gram-negative bacterial infections in a teaching hospital in Ghana. *Antimicrob Resist Infect Control, 7*, 37. doi: 10.1186/s13756-018-0324-2

Ahmed, D., Nahid, M. A., Sami, A. B., Halim, F., Akter, N., Sadique, T., Rana, M. S., Elahi, M. S., & Rahman, M. M. (2017). Bacterial etiology of bloodstream infections and antimicrobial resistance in Dhaka, Bangladesh, 2005-2014. *Antimicrob Resist Infect Control, 6*, 2. doi: 10.1186/s13756-016-0162-z

Ahmed, I., Rabbi, M. B., & Sultana, S. (2019). Antibiotic resistance in Bangladesh: A systematic review. *Int J Infect Dis, 80*, 54-61. doi: 10.1016/j.ijid.2018.12.017

Ajuebor, O., Shetty, N., Mah, K., & Cometto, G. (2019). Health workers' education and training to prevent antimicrobial resistance. *Bull World Health Organ, 97*(12), 791-791A. doi: 10.2471/BLT.19.241802

Alhumaid, S., Al Mutair, A., Al Alawi, Z., Alzahrani, A. J., Tobaiqy, M., Alresasi, A. M., Bu-Shehab, I., Al-Hadary, I., Alhmeed, N., Alismail, M., Aldera, A. H., AlHbabi, F., Al-Shammari, H., Rabaan, A. A., & Al-Omari, A. (2021). Antimicrobial susceptibility of gram-positive and gram-negative bacteria: a 5-year retrospective analysis at a multi-hospital healthcare system in Saudi Arabia. *Ann Clin Microbiol Antimicrob, 20*(1), 43. doi: 10.1186/s12941-021-00450-x

Allerberger, F., & Wagner, M. (2010). Listeriosis: a resurgent foodborne infection. *Clin Microbiol Infect, 16*(1), 16-23. doi: 10.1111/j.1469-0691.2009.03109.x

Ashley, E. A., Shetty, N., Patel, J., van Doorn, R., Limmathurotsakul, D., Feasey, N. A., Okeke, I. N., & Peacock, S. J. (2019). Harnessing alternative sources of antimicrobial resistance data to support surveillance in low-resource settings. *J Antimicrob Chemother, 74*(3), 541-546. doi: 10.1093/jac/dky487

Badia, J. M., Casey, A. L., Petrosillo, N., Hudson, P. M., Mitchell, S. A., & Crosby, C. (2017). Impact of surgical site infection on healthcare costs and patient outcomes: a systematic review in six European countries. *J Hosp Infect, 96*(1), 1-15. doi: 10.1016/j.jhin.2017.03.004

Bansal, R., Jain, A., Goyal, M., Singh, T., Sood, H., & Malviya, H. S. (2019). Antibiotic abuse during endodontic treatment: A contributing factor to antibiotic resistance. *J Family Med Prim Care, 8*(11), 3518-3524. doi: 10.4103/jfmpc.jfmpc_768_19

Barnsteiner, S., Baty, F., Albrich, W. C., Babouee Flury, B., Gasser, M., Pluss-Suard, C., Schlegel, M., Kronenberg, A., Kohler, P., & Swiss Centre for Antibiotic, R. (2021). Antimicrobial resistance and antibiotic consumption in intensive care units, Switzerland, 2009 to 2018. *Euro Surveill, 26*(46). doi: 10.2807/1560-7917.ES.2021.26.46.2001537

Bell, T., & O'Grady, N. P. (2017). Prevention of Central Line-Associated Bloodstream Infections. *Infect Dis Clin North Am, 31*(3), 551-559. doi: 10.1016/j.idc.2017.05.007

Bianco, A., Capano, M. S., Mascaro, V., Pileggi, C., & Pavia, M. (2018). Prospective surveillance of healthcare-associated infections and patterns of antimicrobial resistance of pathogens in an Italian intensive care unit. *Antimicrob Resist Infect Control, 7*, 48. doi: 10.1186/s13756-018-0337-x

Birgand, G., Castro-Sanchez, E., Hansen, S., Gastmeier, P., Lucet, J. C., Ferlie, E., Holmes, A., & Ahmad, R. (2018). Comparison of governance approaches for the control of antimicrobial resistance: Analysis of three European countries. *Antimicrob Resist Infect Control, 7*, 28. doi: 10.1186/s13756-018-0321-5

Braykov, N. P., Eisenberg, J. N., Grossman, M., Zhang, L., Vasco, K., Cevallos, W., Munoz, D., Acevedo, A., Moser, K. A., Marrs, C. F., Foxman, B., Trostle, J., Trueba, G., & Levy, K. (2016). Antibiotic Resistance in Animal and Environmental Samples Associated with Small-Scale Poultry Farming in Northwestern Ecuador. *mSphere, 1*(1). doi: 10.1128/mSphere.00021-15

Bryce, A., Hay, A. D., Lane, I. F., Thornton, H. V., Wootton, M., & Costelloe, C. (2016). Global prevalence of antibiotic resistance in paediatric urinary tract infections caused by Escherichia coli and association with routine use of antibiotics in primary care: systematic review and meta-analysis. *BMJ, 352*, i939. doi: 10.1136/bmj.i939

Carroll, M., Rangaiahagari, A., Musabeyezu, E., Singer, D., & Ogbuagu, O. (2016). Five-Year Antimicrobial Susceptibility Trends Among Bacterial Isolates from a Tertiary Health-Care Facility in Kigali, Rwanda. *Am J Trop Med Hyg, 95*(6), 1277-1283. doi: 10.4269/ajtmh.16-0392

Cattoir, V. (2022). The multifaceted lifestyle of enterococci: genetic diversity, ecology and risks for public health. *Curr Opin Microbiol, 65*, 73-80. doi: 10.1016/j.mib.2021.10.013

Cheesbrough, M. (2005). *District laboratory practice in tropical countries, part 2* Cambridge university press.

Cheng, K., He, M., Shu, Q., Wu, M., Chen, C., & Xue, Y. (2020). Analysis of the Risk Factors for Nosocomial Bacterial Infection in Patients with COVID-19 in a Tertiary Hospital. *Risk Manag Healthc Policy, 13*, 2593-2599. doi: 10.2147/RMHP.S277963

Chouchani, C., Marrakchi, R., & El Salabi, A. (2011). Evolution of beta-lactams resistance in Gram-negative bacteria in Tunisia. *Crit Rev Microbiol, 37*(3), 167-177. doi: 10.3109/1040841X.2011.552880

Collignon, P., Beggs, J. J., Walsh, T. R., Gandra, S., & Laxminarayan, R. (2018). Anthropological and socioeconomic factors contributing to global antimicrobial resistance: a univariate and multivariable analysis. *Lancet Planet Health, 2*(9), e398-e405. doi: 10.1016/S2542-5196(18)30186-4

Collin, S. M., Shetty, N., Guy, R., Nyaga, V. N., Bull, A., Richards, M. J., van der Kooi, T. I. I., Koek, M. B. G., De Almeida, M., Roberts, S. A., & Lamagni, T. (2019). Group B Streptococcus in surgical site and non-invasive bacterial infections worldwide: A systematic review and meta-analysis. *Int J Infect Dis, 83*, 116-129. doi: 10.1016/j.ijid.2019.04.017

Critchley, I. A., Cotroneo, N., Pucci, M. J., Jain, A., & Mendes, R. E. (2020). Resistance among urinary tract pathogens collected in Europe during 2018. *J Glob Antimicrob Resist, 23*, 439-444. doi: 10.1016/j.jgar.2020.10.020

de Benedictis, F. M., Kerem, E., Chang, A. B., Colin, A. A., Zar, H. J., & Bush, A. (2020). Complicated pneumonia in children. *Lancet, 396*(10253), 786-798. doi: 10.1016/S0140-6736(20)31550-6

Dessie, W., Mulugeta, G., Fentaw, S., Mihret, A., Hassen, M., & Abebe, E. (2016). Pattern of Bacterial Pathogens and Their Susceptibility Isolated from Surgical Site Infections at Selected Referral Hospitals, Addis Ababa, Ethiopia. *Int J Microbiol, 2016*, 2418902. doi: 10.1155/2016/2418902

Diekema, D. J., Hsueh, P. R., Mendes, R. E., Pfaller, M. A., Rolston, K. V., Sader, H. S., & Jones, R. N. (2019). The Microbiology of Bloodstream Infection: 20-Year Trends from the SENTRY Antimicrobial Surveillance Program. *Antimicrob Agents Chemother, 63*(7). doi: 10.1128/AAC.00355-19

Ehlers, M. M., Strasheim, W., Lowe, M., Ueckermann, V., & Kock, M. M. (2018). Molecular Epidemiology of Staphylococcus epidermidis Implicated in Catheter-Related Bloodstream Infections at an Academic Hospital in Pretoria, South Africa. *Front Microbiol, 9*, 417. doi: 10.3389/fmicb.2018.00417

El Mekes, A., Zahlane, K., Ait Said, L., Tadlaoui Ouafi, A., & Barakate, M. (2020). The clinical and epidemiological risk factors of infections due to multi-drug resistant bacteria in an adult intensive care unit of University Hospital Center in Marrakesh-Morocco. *J Infect Public Health, 13*(4), 637-643. doi: 10.1016/j.jiph.2019.08.012

Erb, A., Sturmer, T., Marre, R., & Brenner, H. (2007). Prevalence of antibiotic resistance in Escherichia coli: overview of geographical, temporal, and methodological variations. *Eur J Clin Microbiol Infect Dis, 26*(2), 83-90. doi: 10.1007/s10096-006-0248-2

Feleke, T., Eshetie, S., Dagnew, M., Endris, M., Abebe, W., Tiruneh, M., & Moges, F. (2018). Multidrug-resistant bacterial isolates from patients suspected of nosocomial infections at the University of Gondar Comprehensive Specialized Hospital, Northwest Ethiopia. *BMC Res Notes, 11*(1), 602. doi: 10.1186/s13104-018-3709-7

Floret, N., Bertrand, X., Thouverez, M., & Talon, D. (2009). [Nosocomial infections caused by Pseudomonas aeruginosa: Exogenous or endogenous origin of this bacterium?]. *Pathol Biol (Paris), 57*(1), 9-12. doi: 10.1016/j.patbio.2008.07.011

Folliero, V., Caputo, P., Della Rocca, M. T., Chianese, A., Galdiero, M., Iovene, M. R., Hay, C., Franci, G., & Galdiero, M. (2020). Prevalence and Antimicrobial Susceptibility Patterns of Bacterial Pathogens in Urinary Tract Infections in University Hospital of Campania "Luigi Vanvitelli" between 2017 and 2018. *Antibiotics (Basel), 9*(5). doi: 10.3390/antibiotics9050215

Garcia, H., Cervantes-Luna, B., Gonzalez-Cabello, H., & Miranda-Novales, G. (2018). Risk factors for nosocomial infections after cardiac surgery in newborns with congenital heart disease. *Pediatr Neonatol, 59*(4), 404-409. doi: 10.1016/j.pedneo.2017.11.014

Gelaw, K. A., Aweke, A. M., Astawesegn, F. H., Demissie, B. W., & Zeleke, L. B. (2017). Surgical site infection and its associated factors following cesarean section: a cross sectional study from a public hospital in Ethiopia. *Patient Saf Surg, 11*, 18. doi: 10.1186/s13037-017-0131-3

George, M., Iramiot, J. S., Muhindo, R., Olupot-Olupot, P., & Nanteza, A. (2018). Bacterial Aetiology and Antibiotic Susceptibility Profile of Post-Operative Sepsis among Surgical Patients in a Tertiary Hospital in Rural Eastern Uganda. *Microbiol Res J Int, 24*(2). doi: 10.9734/MRJI/2018/41690

Ghasemzadeh-Moghaddam, H., Neela, V., van Wamel, W., Hamat, R. A., Shamsudin, M. N., Hussin, N. S., Aziz, M. N., Haspani, M. S., Johar, A., Thevarajah, S., Vos, M., & van Belkum, A. (2015). Nasal carriers are more likely to acquire exogenous Staphylococcus aureus strains than non-carriers. *Clin Microbiol Infect, 21*(11), 998 e991-997. doi: 10.1016/j.cmi.2015.07.006

Ghashghaee, A., Behzadifar, M., Azari, S., Farhadi, Z., Luigi Bragazzi, N., Behzadifar, M., Saeedi Shahri, S. S., Ghaemmohamadi, M. S., Ebadi, F., Mohammadibakhsh, R., Seyedin, H., & Razi Moghadam, M. (2018). Prevalence of nosocomial infections in Iran: A systematic review and meta-analysis. *Med J Islam Repub Iran, 32*, 48. doi: 10.14196/mjiri.32.48

Golan, Y. (2015). Empiric therapy for hospital-acquired, Gram-negative complicated intra-abdominal infection and complicated urinary tract infections: a systematic literature review of current and emerging treatment options. *BMC Infect Dis, 15*, 313. doi: 10.1186/s12879-015-1054-1

Haskell, K. J., Schriever, S. R., Fonoimoana, K. D., Haws, B., Hair, B. B., Wienclaw, T. M., Holmstead, J. G., Barboza, A. B., Berges, E. T., Heaton, M. J., & Berges, B. K. (2018). Antibiotic resistance is lower in Staphylococcus aureus isolated from antibiotic-free raw meat as compared to conventional raw meat. *PLoS One, 13*(12), e0206712. doi: 10.1371/journal.pone.0206712

Hawkins, L. P. A., Pallett, S. J. C., Mazzella, A., Anton-Vazquez, V., Rosas, L., Jawad, S. M., Shakespeare, D., & Breathnach, A. S. (2023). Transmission dynamics and associated mortality of nosocomial COVID-19 throughout 2021: a retrospective study at a large teaching hospital in London. *J Hosp Infect, 133*, 62-69. doi: 10.1016/j.jhin.2022.12.014

Health workers' education and training on antimicrobial resistance: curricula guide. (2019). *JAC Antimicrob Resist, 1*(3), dlz073. doi: 10.1093/jacamr/dlz073

Hong, D. J., Kim, J. O., Lee, H., Yoon, E. J., Jeong, S. H., Yong, D., & Lee, K. (2016). In vitro antimicrobial synergy of colistin with rifampicin and carbapenems against colistin-resistant Acinetobacter baumannii clinical isolates. *Diagn Microbiol Infect Dis, 86*(2), 184-189. doi: 10.1016/j.diagmicrobio.2016.07.017

Hong, H. L., Hong, S. B., Ko, G. B., Huh, J. W., Sung, H., Do, K. H., Kim, S. H., Lee, S. O., Kim, M. N., Jeong, J. Y., Lim, C. M., Kim, Y. S., Woo, J. H., Koh, Y., & Choi, S. H. (2014). Viral infection is not uncommon in adult patients with severe hospital-acquired pneumonia. *PLoS One, 9*(4), e95865. doi: 10.1371/journal.pone.0095865

Huang, G., Huang, Q., Zhang, G., Jiang, H., & Lin, Z. (2020). Point-prevalence surveys of hospital-acquired infections in a Chinese cancer hospital: From 2014 to 2018. *J Infect Public Health, 13*(12), 1981-1987. doi: 10.1016/j.jiph.2020.03.003

Huijbers, P. M., Blaak, H., de Jong, M. C., Graat, E. A., Vandenbroucke-Grauls, C. M., & de Roda Husman, A. M. (2015). Role of the Environment in the Transmission of Antimicrobial Resistance to Humans: A Review. *Environ Sci Technol, 49*(20), 11993-12004. doi: 10.1021/acs.est.5b02566

Ilic, M., & Markovic-Denic, L. (2017). Repeated prevalence studies of nosocomial infections in one university hospital in Serbia. *Turk J Med Sci, 47*(2), 563-569. doi: 10.3906/sag-1509-10

Iliyasu, G., Dayyab, F. M., Abubakar, S., Inuwa, S., Tambuwal, S. H., Tiamiyu, A. B., Habib, Z. G., Gadanya, M. A., Sheshe, A. A., Mijinyawa, M. S., Aminu, A., Adamu, M. S., Mande, K. M., & Habib, A. G. (2018). Laboratory-confirmed hospital-acquired infections: An analysis of a hospital's surveillance data in Nigeria. *Heliyon, 4*(8), e00720. doi: 10.1016/j.heliyon.2018.e00720

Jahani-Sherafat, S., Razaghi, M., Rosenthal, V. D., Tajeddin, E., Seyedjavadi, S., Rashidan, M., Alebouyeh, M., Rostampour, M., Haghi, A., Sayarbayat, M., Farazmandian, S., Yarmohammadi, T., Arshadi, F. K., Mansouri, N., Sarbazi, M. R., Vilar, M., & Zali, M. R. (2015). Device-associated infection rates and bacterial resistance in six academic teaching hospitals of Iran: Findings from the International Nocosomial Infection Control Consortium (INICC). *J Infect Public Health, 8*(6), 553-561. doi: 10.1016/j.jiph.2015.04.028

Jamshidi, S., Sutton, J. M., & Rahman, K. M. (2016). An overview of bacterial efflux pumps and computational approaches to study efflux pump inhibitors. *Future Med Chem, 8*(2), 195-210. doi: 10.4155/fmc.15.173

Jasovsky, D., Littmann, J., Zorzet, A., & Cars, O. (2016). Antimicrobial resistance-a threat to the world's sustainable development. *Ups J Med Sci, 121*(3), 159-164. doi: 10.1080/03009734.2016.1195900

Johnson, J. A. (2002). Nosocomial infections. *Vet Clin North Am Small Anim Pract, 32*(5), 1101-1126. doi: 10.1016/s0195-5616(02)00038-4

Kariuki, S., Kering, K., Wairimu, C., Onsare, R., & Mbae, C. (2022). Antimicrobial Resistance Rates and Surveillance in Sub-Saharan Africa: Where Are We Now? *Infect Drug Resist, 15*, 3589-3609. doi: 10.2147/IDR.S342753

Karlowsky, J. A., Hoban, D. J., Hackel, M. A., Lob, S. H., & Sahm, D. F. (2017). Antimicrobial susceptibility of Gram-negative ESKAPE pathogens isolated from hospitalized patients with intra-abdominal and urinary tract infections in Asia-Pacific countries: SMART 2013-2015. *J Med Microbiol, 66*(1), 61-69. doi: 10.1099/jmm.0.000421

Khan, F. Y., AbuKamar, M., & Anand, D. (2017). Nosocomial Pseudomonas putida Meningitis: A Case Report and Literature Review. *Oman Med J, 32*(2), 167-169. doi: 10.5001/omj.2017.30

Kotb, S., Lyman, M., Ismail, G., Abd El Fattah, M., Girgis, S. A., Etman, A., Hafez, S., El-Kholy, J., Zaki, M. E. S., Rashed, H. G., Khalil, G. M., Sayyouh, O., & Talaat, M. (2020). Epidemiology of Carbapenem-resistant Enterobacteriaceae in Egyptian intensive care units using National Healthcare-associated Infections Surveillance Data, 2011-2017. *Antimicrob Resist Infect Control, 9*(1), 2. doi: 10.1186/s13756-019-0639-7

Kunkel, S. A., Azimi, P., Zhao, H., Stark, B. C., & Stephens, B. (2017). Quantifying the size-resolved dynamics of indoor bioaerosol transport and control. *Indoor Air, 27*(5), 977-987. doi: 10.1111/ina.12374

Laux, R., Wirtz, S., Huggett, S., & Ilchmann, C. (2013). [Relevance of parents as source for contamination of neonates with multiresistant Gram-negative pathogens (MRGN)]. *Z Geburtshilfe Neonatol, 217*(2), 61-64. doi: 10.1055/s-0033-1341452

Lee, C. R., Lee, J. H., Park, M., Park, K. S., Bae, I. K., Kim, Y. B., Cha, C. J., Jeong, B. C., & Lee, S. H. (2017). Biology of Acinetobacter baumannii: Pathogenesis, Antibiotic Resistance Mechanisms, and Prospective Treatment Options. *Front Cell Infect Microbiol, 7*, 55. doi: 10.3389/fcimb.2017.00055

Magale, H. I., Kassim, I. A., Odera, S. A., Omolo, M. J., Jaoko, W. G., & Jolly, P. E. (2015). Antibiotic Susceptibility of Organisms Causing Urinary Tract Infection in Patients Presenting at Kenyatta National Hospital, Nairobi. *East Afr Med J, 92*(7), 333-337.

Maina, J. W., Onyambu, F. G., Kibet, P. S., & Musyoki, A. M. (2023). Multidrug-resistant Gram-negative bacterial infections and associated factors in a Kenyan intensive care unit: a cross-sectional study. *Ann Clin Microbiol Antimicrob, 22*(1), 85. doi: 10.1186/s12941-023-00636-5

Malania, L., Wagenaar, I., Karatuna, O., Tambic Andrasevic, A., Tsereteli, D., Baidauri, M., Imnadze, P., Nahrgang, S., & Ruesen, C. (2021). Setting up laboratory-based antimicrobial resistance surveillance in low- and middle-income countries: lessons learned from Georgia. *Clin Microbiol Infect, 27*(10), 1409-1413. doi: 10.1016/j.cmi.2021.05.027

Matinyi, S., Enoch, M., Akia, D., Byaruhanga, V., Masereka, E., Ekeu, I., & Atuheire, C. (2018). Contamination of microbial pathogens and their antimicrobial pattern in operating theatres of peri-urban eastern Uganda: a cross-sectional study. *BMC Infect Dis, 18*(1), 460. doi: 10.1186/s12879-018-3374-4

Mauldin, P. D., Salgado, C. D., Hansen, I. S., Durup, D. T., & Bosso, J. A. (2010). Attributable hospital cost and length of stay associated with health care-associated infections caused by antibiotic-resistant gram-negative bacteria. *Antimicrob Agents Chemother, 54*(1), 109-115. doi: 10.1128/AAC.01041-09

Mehta, Y., Gupta, A., Todi, S., Myatra, S., Samaddar, D. P., Patil, V., Bhattacharya, P. K., & Ramasubban, S. (2014). Guidelines for prevention of hospital acquired infections. *Indian J Crit Care Med, 18*(3), 149-163. doi: 10.4103/0972-5229.128705

Milovanovic, T., Dumic, I., Velickovic, J., Lalosevic, M. S., Nikolic, V., & Palibrk, I. (2019). Epidemiology and risk factors for multi-drug resistant hospital-acquired urinary tract infection in patients with liver cirrhosis: single center experience in Serbia. *BMC Infect Dis, 19*(1), 141. doi: 10.1186/s12879-019-3761-5

Mittal, R. P., Rana, A., & Jaitak, V. (2019). Essential Oils: An Impending Substitute of Synthetic Antimicrobial Agents to Overcome Antimicrobial Resistance. *Curr Drug Targets, 20*(6), 605-624. doi: 10.2174/1389450119666181031122917

Mukagendaneza, M. J., Munyaneza, E., Muhawenayo, E., Nyirasebura, D., Abahuje, E., Nyirigira, J., Harelimana, J. D., Muvunyi, T. Z., Masaisa, F., Byiringiro, J. C., Hategekimana, T., & Muvunyi, C. M. (2019). Incidence, root causes, and outcomes of surgical site infections in a tertiary care hospital in Rwanda: a prospective observational cohort study. *Patient Saf Surg, 13*, 10. doi: 10.1186/s13037-019-0190-8

Muri-Gama, A. S., Figueras, A., & Secoli, S. R. (2018). Inappropriately prescribed and over-the-counter antimicrobials in the Brazilian Amazon Basin: We need to promote more rational use even in remote places. *PLoS One, 13*(8), e0201579. doi: 10.1371/journal.pone.0201579

Murni, I. K., Duke, T., Kinney, S., Daley, A. J., Wirawan, M. T., & Soenarto, Y. (2022). Risk factors for healthcare-associated infection among children in a low-and middle-income country. *BMC Infect Dis, 22*(1), 406. doi: 10.1186/s12879-022-07387-2

Muvunyi, V., Mpirimbanyi, C., Katabogama, J. B., Cyuzuzo, T., Nkubana, T., Mugema, J. B., Musoni, E., Urimubabo, C., & Rickard, J. (2020). Community- and Hospital-Acquired Infections in Surgical patients at a Tertiary Referral Hospital in Rwanda. *World J Surg, 44*(10), 3290-3298. doi: 10.1007/s00268-020-05634-8

Nasser, M., Palwe, S., Bhargava, R. N., Feuilloley, M. G. J., & Kharat, A. S. (2020). Retrospective Analysis on Antimicrobial Resistance Trends and Prevalence of beta-lactamases in Escherichia coli and ESKAPE Pathogens Isolated from Arabian Patients during 2000-2020. *Microorganisms, 8*(10). doi: 10.3390/microorganisms8101626

Ngaroua, Ngah, J. E., Benet, T., & Djibrilla, Y. (2016). [Incidence of surgical site infections in sub-Saharan Africa: systematic review and meta-analysis]. *Pan Afr Med J, 24*, 171. doi: 10.11604/pamj.2016.24.171.9754

Nimer, N. A. (2022). Nosocomial Infection and Antibiotic-Resistant Threat in the Middle East. *Infect Drug Resist, 15*, 631-639. doi: 10.2147/IDR.S351755

Njeru, J., Henning, K., Pletz, M. W., Heller, R., Forstner, C., Kariuki, S., Fevre, E. M., & Neubauer, H. (2016). Febrile patients admitted to remote hospitals in Northeastern Kenya: seroprevalence, risk factors and a clinical prediction tool for Q-Fever. *BMC Infect Dis, 16*, 244. doi: 10.1186/s12879-016-1569-0

Nthumba, P. M., Stepita-Poenaru, E., Poenaru, D., Bird, P., Allegranzi, B., Pittet, D., & Harbarth, S. (2010). Cluster-randomized, crossover trial of the efficacy of plain soap and water versus alcohol-based rub for surgical hand preparation in a rural hospital in Kenya. *Br J Surg, 97*(11), 1621-1628. doi: 10.1002/bjs.7213

Nyamogoba, H., & Obala, A. A. (2002). Nosocomial infections in developing countries: cost effective control and prevention. *East Afr Med J, 79*(8), 435-441. doi: 10.4314/eamj.v79i8.8831

Obanda, B. A., Cook, E. A. J., Fevre, E. M., Bebora, L., Ogara, W., Wang, S. H., Gebreyes, W., Ngetich, R., Wandede, D., Muyodi, J., Blane, B., Coll, F., Harrison, E. M., Peacock, S. J., & Gitao, G. C. (2022). Characteristics of Staphylococcus aureus Isolated from Patients in Busia County Referral Hospital, Kenya. *Pathogens, 11*(12). doi: 10.3390/pathogens11121504

Odoki, M., Almustapha Aliero, A., Tibyangye, J., Nyabayo Maniga, J., Wampande, E., Drago Kato, C., Agwu, E., & Bazira, J. (2019). Prevalence of Bacterial Urinary Tract Infections and Associated Factors among Patients Attending Hospitals in Bushenyi District, Uganda. *Int J Microbiol, 2019*, 4246780. doi: 10.1155/2019/4246780

Odoyo, E., Matano, D., Tiria, F., Georges, M., Kyanya, C., Wahome, S., Mutai, W., & Musila, L. (2023). Environmental contamination across multiple hospital departments with multidrug-resistant bacteria pose an elevated risk of healthcare-associated infections in Kenyan hospitals. *Antimicrob Resist Infect Control, 12*(1), 22. doi: 10.1186/s13756-023-01227-x

Olowo-Okere, A., Ibrahim, Y. K. E., Nabti, L. Z., & Olayinka, B. O. (2020). High prevalence of multidrug-resistant Gram-negative bacterial infections in Northwest Nigeria. *Germs, 10*(4), 310-321. doi: 10.18683/germs.2020.1223

Olowo-Okere, A., Ibrahim, Y. K. E., Olayinka, B. O., & Ehinmidu, J. O. (2019). Epidemiology of surgical site infections in Nigeria: A systematic review and meta-analysis. *Niger Postgrad Med J, 26*(3), 143-151. doi: 10.4103/npmj.npmj_72_19

Olowo-Okere, A., Ibrahim, Y. K. E., Sani, A. S., & Olayinka, B. O. (2018). Occurrence of Surgical Site Infections at a Tertiary Healthcare Facility in Abuja, Nigeria: A Prospective Observational Study. *Med Sci (Basel), 6*(3). doi: 10.3390/medsci6030060

Omulo, S., Lofgren, E. T., Lockwood, S., Thumbi, S. M., Bigogo, G., Ouma, A., Verani, J. R., Juma, B., Njenga, M. K., Kariuki, S., McElwain, T. F., Palmer, G. H., & Call, D. R. (2021). Carriage of antimicrobial-resistant bacteria in a high-density informal settlement in Kenya is associated with environmental risk-factors. *Antimicrob Resist Infect Control, 10*(1), 18. doi: 10.1186/s13756-021-00886-y

Osterman, I. A., Dontsova, O. A., & Sergiev, P. V. (2020). rRNA Methylation and Antibiotic Resistance. *Biochemistry (Mosc), 85*(11), 1335-1349. doi: 10.1134/S000629792011005X

Patil, R. K., Kabera, B., Muia, C. K., & Ale, B. M. (2022). Hospital acquired infections in a private paediatric hospital in Kenya: a retrospective cross-sectional study. *Pan Afr Med J, 41*, 28. doi: 10.11604/pamj.2022.41.28.25820

Percival, S. L., Suleman, L., Vuotto, C., & Donelli, G. (2015). Healthcare-associated infections, medical devices and biofilms: risk, tolerance and control. *J Med Microbiol, 64*(Pt 4), 323-334. doi: 10.1099/jmm.0.000032

Petchiappan, A., & Chatterji, D. (2017). Antibiotic Resistance: Current Perspectives. *ACS Omega, 2*(10), 7400-7409. doi: 10.1021/acsomega.7b01368

Peters, L., Olson, L., Khu, D. T. K., Linnros, S., Le, N. K., Hanberger, H., Hoang, N. T. B., Tran, D. M., & Larsson, M. (2019). Multiple antibiotic resistance as a risk factor for mortality and prolonged hospital stay: A cohort study among neonatal intensive care patients with hospital-acquired infections caused by gram-negative bacteria in Vietnam. *PLoS One, 14*(5), e0215666. doi: 10.1371/journal.pone.0215666

Poignant, S., Guinard, J., Guigon, A., Bret, L., Poisson, D. M., Boulain, T., & Barbier, F. (2015). Risk Factors and Outcomes for Intestinal Carriage of AmpC-Hyperproducing Enterobacteriaceae in Intensive Care Unit Patients. *Antimicrob Agents Chemother, 60*(3), 1883-1887. doi: 10.1128/AAC.02101-15

Prestinaci, F., Pezzotti, P., & Pantosti, A. (2015). Antimicrobial resistance: a global multifaceted phenomenon. *Pathog Glob Health, 109*(7), 309-318. doi: 10.1179/2047773215Y.0000000030

Ramirez, M. S., & Tolmasky, M. E. (2017). Amikacin: Uses, Resistance, and Prospects for Inhibition. *Molecules, 22*(12). doi: 10.3390/molecules22122267

Robilotti, E., Holubar, M., Nahrgang, S., van de Sande-Bruinsma, N., Lo Fo Wong, D., & Deresinski, S. (2017). Educating front-line clinicians about antimicrobial resistance. *Lancet Infect Dis, 17*(3), 257-258. doi: 10.1016/S1473-3099(17)30073-7

Sadeghi, H., Khoei, S. G., Bakht, M., Rostamani, M., Rahimi, S., Ghaemi, M., & Mirzaei, B. (2021). A retrospective cross-sectional survey on nosocomial bacterial infections and their antimicrobial susceptibility patterns in hospitalized patients in northwest of Iran. *BMC Res Notes, 14*(1), 88. doi: 10.1186/s13104-021-05503-0

Sadiq, S. T., Mazzaferri, F., & Unemo, M. (2017). Rapid accurate point-of-care tests combining diagnostics and antimicrobial resistance prediction for Neisseria gonorrhoeae and Mycoplasma genitalium. *Sex Transm Infect, 93*(S4), S65-S68. doi: 10.1136/sextrans-2016-053072

Sahu, M. K., Siddharth, B., Choudhury, A., Vishnubhatla, S., Singh, S. P., Menon, R., Kapoor, P. M., Talwar, S., Choudhary, S., & Airan, B. (2016). Incidence, microbiological profile of nosocomial infections, and their antibiotic resistance patterns in a high volume Cardiac Surgical Intensive Care Unit. *Ann Card Anaesth, 19*(2), 281-287. doi: 10.4103/0971-9784.179625

Salimiyan Rizi, K., Farsiani, H., & Sasan, M. S. (2020). High rate of resistance to ceftriaxone and azithromycin among Shigella spp. isolates at three children's referral hospitals in Northeast Iran. *J Infect Chemother, 26*(9), 955-958. doi: 10.1016/j.jiac.2020.04.022

Sarker, M. M., Islam, K. N., Huri, H. Z., Rahman, M., Imam, H., Hosen, M. B., Mohammad, N., & Sarker, M. Z. (2014). Studies of the impact of occupational exposure of pharmaceutical workers on the development of antimicrobial drug resistance. *J Occup Health, 56*(4), 260-270. doi: 10.1539/joh.14-0012-oa

Schroder, C., Behnke, M., Geffers, C., & Gastmeier, P. (2018). Hospital ownership: a risk factor for nosocomial infection rates? *J Hosp Infect, 100*(1), 76-82. doi: 10.1016/j.jhin.2018.01.019

Scott, H. M., Acuff, G., Bergeron, G., Bourassa, M. W., Simjee, S., & Singer, R. S. (2019). Antimicrobial resistance in a One Health context: exploring complexities, seeking solutions, and communicating risks. *Ann N Y Acad Sci, 1441*(1), 3-7. doi: 10.1111/nyas.14057

Shah, M., Kathiiko, C., Wada, A., Odoyo, E., Bundi, M., Miringu, G., Guyo, S., Karama, M., & Ichinose, Y. (2016). Prevalence, seasonal variation, and antibiotic resistance pattern of enteric bacterial pathogens among hospitalized diarrheic children in suburban regions of central Kenya. *Trop Med Health, 44*, 39. doi: 10.1186/s41182-016-0038-1

Sikora, A., & Zahra, F. (2022). *Nosocomial Infections*. Treasure Island, USA: StatPearls Publishing.

Singh, N. P., Rani, M., Gupta, K., Sagar, T., & Kaur, I. R. (2017). Changing trends in antimicrobial susceptibility pattern of bacterial isolates in a burn unit. *Burns, 43*(5), 1083-1087. doi: 10.1016/j.burns.2017.01.016

Sipahi, O. R., Arda, B., Nazli-Zeka, A., Pullukcu, H., Tasbakan, M., Yamazhan, T., Ozkoren-Calik, S., Sipahi, H., & Ulusoy, S. (2014). Piperacillin/tazobactam vs. cefoperazone/sulbactam in adult low-risk febrile neutropenia cases. *Int J Clin Pract, 68*(2), 230-235. doi: 10.1111/ijcp.12279

Skender, K., Machowska, A., Singh, V., Goel, V., Marothi, Y., Lundborg, C. S., & Sharma, M. (2022). Antibiotic Use, Incidence and Risk Factors for Orthopedic Surgical Site Infections in a Teaching Hospital in Madhya Pradesh, India. *Antibiotics (Basel), 11*(6). doi: 10.3390/antibiotics11060748

Ssekitoleko, R. T., Oshabaheebwa, S., Munabi, I. G., Tusabe, M. S., Namayega, C., Ngabirano, B. A., Matovu, B., Mugaga, J., Reichert, W. M., & Joloba, M. L. (2020). The role of medical equipment in the spread of nosocomial infections: a cross-sectional study in four tertiary public health facilities in Uganda. *BMC Public Health, 20*(1), 1561. doi: 10.1186/s12889-020-09662-w

Suleyman, G., Alangaden, G., & Bardossy, A. C. (2018). The Role of Environmental Contamination in the Transmission of Nosocomial Pathogens and Healthcare-Associated Infections. *Curr Infect Dis Rep, 20*(6), 12. doi: 10.1007/s11908-018-0620-2

Suwantarat, N., & Carroll, K. C. (2016). Epidemiology and molecular characterization of multidrug-resistant Gram-negative bacteria in Southeast Asia. *Antimicrob Resist Infect Control, 5*, 15. doi: 10.1186/s13756-016-0115-6

Syue, L. S., Tang, H. J., Hung, Y. P., Chen, P. L., Li, C. W., Li, M. C., Tsai, P. F., Liu, C. C., Lee, N. Y., & Ko, W. C. (2019). Bloodstream infections in hospitalized adults with dengue fever: Clinical characteristics and recommended empirical therapy. *J Microbiol Immunol Infect, 52*(2), 225-232. doi: 10.1016/j.jmii.2018.11.003

Taj, A., Shamim, A., Khanday, S. B., & Ommid, M. (2018). Prevalence of common nosocomial organisms in surgical Intensive Care Unit in North India: A hospital-based study. *Int J Crit Illn Inj Sci, 8*(2), 78-82. doi: 10.4103/IJCIIS.IJCIIS_8_18

Tolera, M., Abate, D., Dheresa, M., & Marami, D. (2018). Bacterial Nosocomial Infections and Antimicrobial Susceptibility Pattern among Patients Admitted at Hiwot Fana Specialized University Hospital, Eastern Ethiopia. *Adv Med, 2018*, 2127814. doi: 10.1155/2018/2127814

Tomczyk-Warunek, A., Blicharski, T., Blicharski, R., Pluta, R., Dobrowolski, P., Muszynski, S., Tomaszewska, E., & Jablonski, M. (2021). Retrospective Study of Nosocomial Infections in the Orthopaedic and Rehabilitation Clinic of the Medical University of Lublin in the Years 2018-2020. *J Clin Med, 10*(14). doi: 10.3390/jcm10143179

Trifonova, A., & Strateva, T. (2019). Stenotrophomonas maltophilia - a low-grade pathogen with numerous virulence factors. *Infect Dis (Lond), 51*(3), 168-178. doi: 10.1080/23744235.2018.1531145

Walkty, A., Lagace-Wiens, P., Adam, H., Baxter, M., Karlowsky, J., Mulvey, M. R., McCracken, M., & Zhanel, G. G. (2017). Antimicrobial susceptibility of 2906 Pseudomonasaeruginosa clinical isolates obtained from patients in Canadian hospitals over a period of 8 years: Results of the Canadian Ward surveillance study (CANWARD), 2008-2015. *Diagn Microbiol Infect Dis, 87*(1), 60-63. doi: 10.1016/j.diagmicrobio.2016.10.003

Wang, L., Zhou, K. H., Chen, W., Yu, Y., & Feng, S. F. (2019a). Epidemiology and risk factors for nosocomial infection in the respiratory intensive care unit of a teaching hospital in China: A prospective surveillance during 2013 and 2015. *BMC Infect Dis, 19*(1), 145. doi: 10.1186/s12879-019-3772-2

Wang, M., Wei, H., Zhao, Y., Shang, L., Di, L., Lyu, C., & Liu, J. (2019b). Analysis of multidrug-resistant bacteria in 3223 patients with hospital-acquired infections (HAI) from a tertiary general hospital in China. *Bosn J Basic Med Sci, 19*(1), 86-93. doi: 10.17305/bjbms.2018.3826

Wangai, F. K., Masika, M. M., Lule, G. N., Karari, E. M., Maritim, M. C., Jaoko, W. G., Museve, B., & Kuria, A. (2019). Bridging antimicrobial resistance knowledge gaps: The East African perspective on a global problem. *PLoS One, 14*(2), e0212131. doi: 10.1371/journal.pone.0212131

Weterings, V., Veenemans, J., van Rijen, M., & Kluytmans, J. (2019). Prevalence of nasal carriage of methicillin-resistant Staphylococcus aureus in patients at hospital admission in The Netherlands, 2010-2017: an observational study. *Clin Microbiol Infect, 25*(11), 1428 e1421-1428 e1425. doi: 10.1016/j.cmi.2019.03.012

Yallew, W. W., Kumie, A., & Yehuala, F. M. (2016). Point prevalence of hospital-acquired infections in two teaching hospitals of Amhara region in Ethiopia. *Drug Healthc Patient Saf, 8*, 71-76. doi: 10.2147/DHPS.S107344

Zayas, G., Chiang, M. C., Wong, E., MacDonald, F., Lange, C. F., Senthilselvan, A., & King, M. (2013). Effectiveness of cough etiquette maneuvers in disrupting the chain of transmission of infectious respiratory diseases. *BMC Public Health, 13*, 811. doi: 10.1186/1471-2458-13-811

APPENDICES

APPENDIX I: CONSENT FORM

Title of the proposal

Characterization of antibiotic resistant nosocomial bacterial isolates from clinical specimens at Jaramogi Oginga Odinga Teaching and Referral Hospital.

Respondent's name:……Age: …… Sex……

Address……Tel…… E-mail…….....

Purpose of the study:

The aim is to determine and characterize antibiotic resistant nosocomial bacterial pathogens isolated from clinical specimens at JOOTRH

Procedure to be followed

The data collector will introduce himself/herself to the respondent (patient) and explain the procedure he/she intends to perform after which personal information about the patient such as age, sex, date and time of admission, treatment administered will be entered into the data collection sheet. The data collector will then put on gloves after hand washing and then he/she will collect the requisite specimen following strict aseptic technique after the respondent will have consented.

Risks

The study will not expose you to unusual risks.

Benefits

The study will help in provision of information about antibiotic resistance of commonly isolated nosocomial bacterial pathogens that cause nosocomial infections hence enabling timely management and treatment of such infections. This will reduce length of hospital stay, reduce cost of healthcare as well as preventing disabilities and deaths.

Confidentiality of records

Codes will be used to number the data collection sheets to protect confidentiality of the patients. No identity of the participants will be disclosed to the public in any reports or publications

Basis of participation

It is important for you to know that you have the freedom to decline to participate in the study and this will not affect your relationship with the hospital or the investigator

Signature

I have the above information and have had an opportunity to ask questions and all my questions have been answered. I have consented to taking part in the study and have allowed the investigator to take my sample(s). I fully understand there are no risks associated with participating in this study.

Signature……..... Date……

I, the undersigned, have fully explained the relevant details of this study to the respondent named above.

Investigator's Name:..... Signature:……
Date:……

APPENDIX II: PARENTAL INFORMED CONSENT

Brief Description of Research Study

The purpose of this research is to isolate nosocomial bacterial pathogens from clinical specimens and determine their antibiotic resistance. During this study specimens will be collected from patients with signs and symptoms of nosocomial infection and then cultured to isolate bacterial pathogens after which they will be tested against antibiotics. Please read the rest of this form before deciding if you will allow your child to be in this research study.

My name is **Arthur Aroko** and I am a student at Jaramogi Oginga Odinga University of Science and Technology. Because you are the parent or legally authorized representative of the hospitalized child, I am seeking your permission to let your child participate in this research study. Involvement in the study is voluntary, so you may decide whether to let your child participate or not. I will also ask your child if he or she wants to be in the study, and I will only collect information if both you and your child agree. Before making your decision, please read the information below and ask me any questions that you have about the research; I will be happy to explain anything in greater detail. Duration of data collection is envisaged to take three months however, your direct involvement or that of your child will take at most thirty minutes.

Even if your child does not take part in my study, he or she will still be able to get services from of this hospital and therefore you should feel free to either participate or decline to do so. However, to ensure confidentiality I will not reveal any private information about your child to anyone, unless required by law to do so. My records will be in my possession at all times, and only I will know which records belongs to which child. In any reports I make about this study, I will not use your child's name or any other information that could be used to identify him or her directly or indirectly. When my study is complete, I will destroy all of the information I collected from individual patients.

Risks and Benefits of Participation

Risks that may be experienced by children may be minimal and would vary from child to child. The mild Some children may experience mild pain for a few minutes particularly when samples such as venous blood is being collected. Otherwise, the study does not pose grave danger to your child. There are no rewards for taking part in this study, and no penalties of any kind if they do not take part. However, being in

this research study might provide important data that could be used to improve management of hospital acquired bacterial infections.

Participant Rights

You have the right to ask any questions you have before, during or after the study, and I encourage you to do so. If you do not want your child to be in this study, there will be no penalties or loss of benefits that he or she is entitled to. If you agree to let your child, be in this study and later change your mind, you have the right to take him or her out simply by contacting me at the email address below, and I will destroy any research data collected about your child. This research has been approved by the JOOUST board of post graduate studies as well as JOOTRH Ethics Review Committee (JOOTRH ERC, which is responsible for ensuring that the safety and rights of research participants are protected. For information about your participation in this study, please, feel free to contact me using below contact mobile number as email address: -

Name: Arthur Aroko Tel. no 0721463866; E-mail: arthuraroko@gmail.com; Or you can contact my university supervisors,

Name: Dr. Daniel Onguru Tel. 0721818368; E-mail: danonguru@yahoo.com

Name: Dr. Daud Ibrahim Tel. no. 0726487199; E-mail: ibrayed@gmail.com

Before signing this form, please ask me any questions you have about participation in this study.

To be completed by participant

I have read all of the information on this form, and all of my questions and concerns about the research described above have been addressed. I choose, voluntarily, to permit my child to take part in this research study. I certify that I am at least 18 years of age.

Signature of parent or legally authorized representative---------- Date----------

To be completed by Researcher

I confirm that the legally authorized representative of the child whose signature appears above has been given an opportunity to ask questions about the study, and all the questions asked have been answered to the best of my knowledge and ability. A copy of this Consent Form has been provided to the child's legally authorized representative, and I will keep the original for a minimum period of 1 year.

Name of researcher……..Signature……Date…….

APPENDIX III: CHILD ASSENT FORM

Dear Mr/Ms……., My name is Arthur Aroko, and the reason for this letter is to ask if you want to be in a research study I am doing. By "research" I mean that I am trying to find out more about something. In this study I am trying to find out more about bacteria (germs) that cause diseases and are contracted within hospital settings. We will also look at how they behave towards the medicine used in treating them when they infect. These germs can be termed nosocomial bacterial pathogens. They are usually isolated from clinical specimens such as urine, sputum, blood, pus and stool among others. I have already asked your parent/ guardian if they will permit you to be in this study. If they did not agree, you will not be asked to sign this form. If they did agree, it is still your choice to make, and I am now going to describe what you will do if you agree to be in this study. I am going to read this information to you, so listen carefully and ask any questions you have before you decide whether to be in the study or not.

What will you do if you are in this study?

During the next thirty minutes if you agree to be in my study, you will allow me to ask you a few questions which you may choose to answer or not as I fill them in the forms that I have with me here, after which I will carry out a simple procedure on you to obtain a specimen which will be taken to the laboratory for testing.

What will you do if you are not in this study?

Nothing bad will happen to you if you do not want to be in the study, and it will not hurt your relationship with me as a researcher or other staff of this hospital. You will still enjoy the health services provision. Also, if you are not in the study, I will not obtain any specimens from you.

Will anything bad happen to you in this study?

If you decide to be in this stud will not expose you to risks that may endanger your life, however you may experience mild discomfort during specimen collection which is normal. But should you feel that the procedure is extremely discomforting to you, then you are free to tell the researcher to stop. I will ensure that special steps are taken to make sure that you feel okay during and after the procedure in question. You do not have to answer any questions during the interview that you do not want to answer. I

will also make sure that you do not miss any instruction that you need to be given at any given time.

Will anything good happen to you in this study?

You will not receive any special rewards or services for agreeing to be in this study.

Will anyone else know what was done or what you said in this study?

In my study, I will not use your names or any form of identification that will infringe on your privacy. If you decide to be in this study, I will not tell anyone else about what you said during your individual meetings with me, unless I have to for legal reasons or if you give me an okay to do so.

What if you have any questions?

Be sure to ask me any questions you have before deciding whether to be in this study or not. Even if you don't have questions now, you can ask me about this study at any time later. If you would like time to discuss it with your parents before making your decision, please tell me.

What if you change your mind?

If you decide to be in this study and later change your mind, just tell me that you want to stop. I will stop collecting information about you for my study and will take out all of the information I already have about you. I will finish my study on June 2022, so that is the deadline when you should tell me if you want your information taken out of the study.

Name of researcher…….Signature……. Date……

To the patient (child)

Your signature below indicates that you have read the information on this form [or that I have read the information on this form aloud to you], and that all of your questions about this research study have been answered. Please put an X next to your decision:

__ I agree to take part in this research and I will let you make a recording of what I say

__I DO NOT want to have any information about me used in this research

Signature of patient……Date……

APPENDIX IV: DATA ENTRY FORM

Serial. No.	Age	Sex	Adm. Date	Ward	Specimen type	Date of collection	Organism isolated	Antibiotic resistance

APPENDIX V: DATA COLLECTION FORM

TITLE: Characterization of antibiotic resistant nosocomial bacterial isolates from clinical specimens at JOTRH

Serial No…….

Age …… Gender ☐ M F ☐

Date of admission----------

Condition for which admitted/ Diagnosis ----------

Degree of severity of the condition Mild ☐ Moderate ☐ Severe ☐

Other comorbidities (specify)----------

Is the patient on any antibiotics? Yes ☐ No ☐

If yes then specify----------

Type of nosocomial infection----------

Date of onset of NI signs and symptoms----------

Treatment given to alleviate NI symptoms----------

Sample type---------- Sample collection date----------

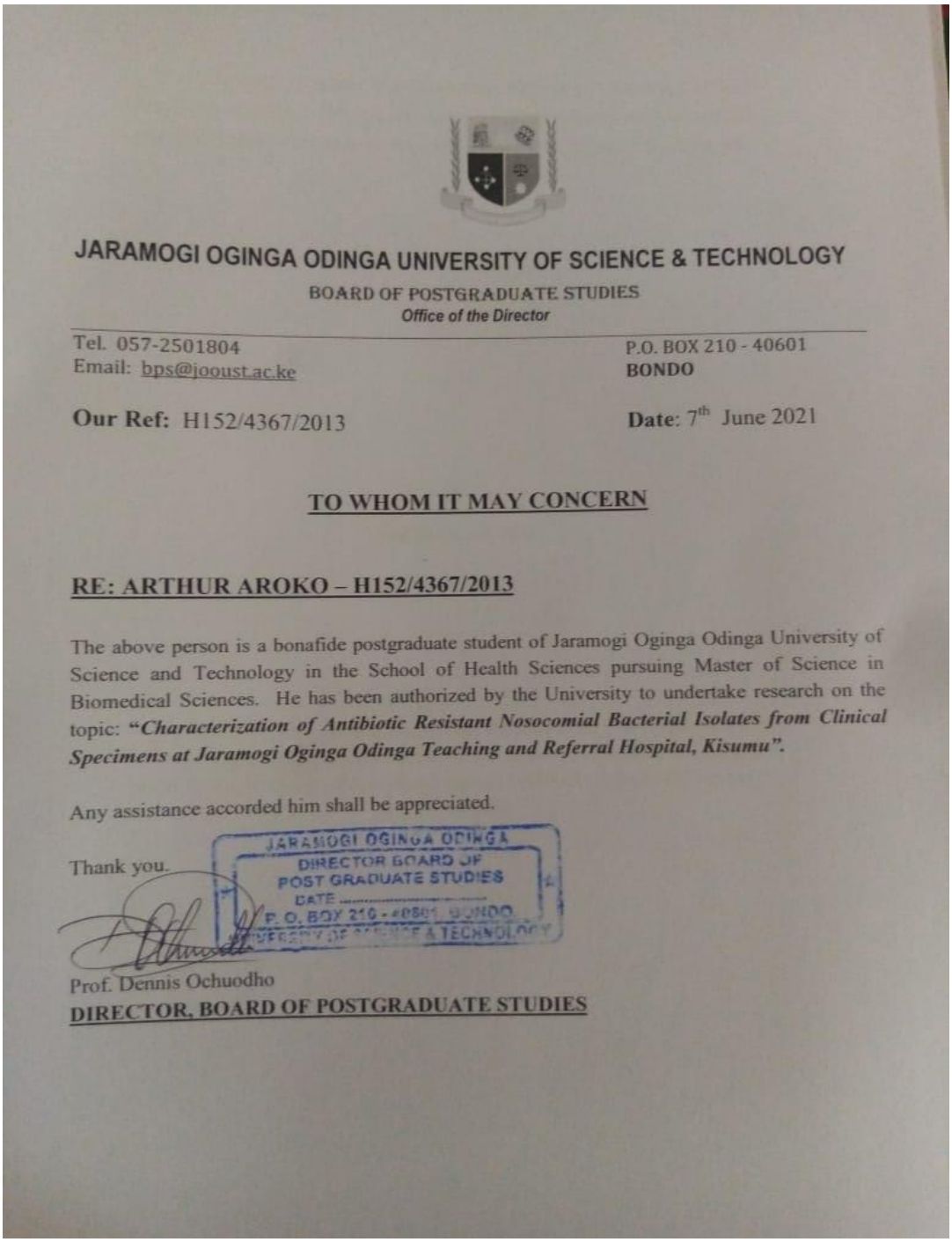
Sample culture date----------

Bacteria isolated----------

Antibiotic susceptibility results

	Antibiotic tested	Sensitive (S)	Intermediate (I)	Resistant (R)
1.				
2.				
3.				
4.				
5.				
6.				
7.				
8.				
9.				
10.				
11.				
12.				
13.				
14.				
15.				
16.				

APPENDIX VI: BOARD OF POST GRADUATE STUDIES APPROVAL LETTER



JARAMOGI OGINGA ODINGA UNIVERSITY OF SCIENCE & TECHNOLOGY

BOARD OF POSTGRADUATE STUDIES

Office of the Director

Tel. 057-2501804
Email: bps@jooust.ac.ke

P.O. BOX 210 - 40601
BONDO

Our Ref: H152/4367/2013

Date: 7th June 2021

TO WHOM IT MAY CONCERN

RE: ARTHUR AROKO – H152/4367/2013

The above person is a bonafide postgraduate student of Jaramogi Oginga Odinga University of Science and Technology in the School of Health Sciences pursuing Master of Science in Biomedical Sciences. He has been authorized by the University to undertake research on the topic: ***"Characterization of Antibiotic Resistant Nosocomial Bacterial Isolates from Clinical Specimens at Jaramogi Oginga Odinga Teaching and Referral Hospital, Kisumu".***

Any assistance accorded him shall be appreciated.

Thank you.

JARAMOGI OGINGA ODINGA
DIRECTOR BOARD OF
POST GRADUATE STUDIES
DATE .....
P. O. BOX 210 - 40601 BONDO
UNIVERSITY OF SCIENCE & TECHNOLOGY

Prof. Dennis Ochuodho
DIRECTOR, BOARD OF POSTGRADUATE STUDIES

APPENDIX VII: ERC APPROVAL LETTER

COUNTY GOVERNMENT OF KISUMU
DEPARTMENT OF HEALTH

Telephone: 057-2020801/2020803/2020321
Fax: 057-2024337
E-mail: ercjoorth@gmail.com

When replying please quote

JARAMOGI OGINGA ODINGA TEACHING & REFERRAL HOSPITAL
P.O. BOX 849
KISUMU

Ref: IERC/JOOTRH/478/21

Date: 26th August, 2021

To: Arthur Aroko

Dear Arthur,

RE: REQUEST FOR ETHICAL APPROVAL TO UNDERTAKE A STUDY TITLED: NOSOCOMIAL BACTERIAL PATHOGENS AND THEIR ANTIBIOTIC RESISTANCE AT JARAMOGI OGINGA ODINGA TEACHING AND REFERRAL HOSPITAL, KISUMU.

This is to inform you that ***JOOTRH IERC*** has reviewed and approved your above research proposal. Your application approval number is ***IERC/JOOTRH/478/21.*** The approval period is ***26th August, 2021 – 26th August, 2022.*** 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 ***JOOTRH - IERC.***
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 ***JOOTRH - IERC*** within 72 hours of notification
iv. Any changes, anticipated or otherwise that may increase the risks or affected safety or welfare of study participants and others or affect the integrity of the research must be reported to ***JOOTRH - IERC*** 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 JOOTRH - ***IERC.***

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

In case the study site is JOOTRH, kindly report to Chief Executive Officer before commencement of data collection.

Yours sincerely,

26 AUG 2021

NANCY MAKUNDA-SECRETARY
JOOTRH-IERC
KISUMU

APPENDIX VIII: NACOSTI LICENCE

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: **807371**

Date of Issue: **06/September/2021**

RESEARCH LICENSE

This is to Certify that Mr.. ARTHUR AROKO of Jaramogi Oginga Odinga University of Science and Technology, has been licensed to conduct research in Kisumu on the topic: NOSOCOMIAL BACTERIAL PATHOGENS AND THEIR ANTIBIOTIC RESISTANCE AT JARAMOGI OGINGA ODINGA TEACHING AND REFERRAL HOSPITAL, KISUMU for the period ending : 06/September/2022.

License No: **NACOSTI/P/21/12695**

807371

Applicant Identification Number

Director General
NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Verification QR Code

NOTE: This is a computer generated License. To verify the authenticity of this document, Scan the QR Code using QR scanner application.

APPENDIX IX: MAP OF THE STUDY AREA

https://www.google.com/search?jaramogi+oginga+odinga+teaching+and+referral+hospital

APPENDIX X: BIOCHEMICAL TEST PROCEDURES

Catalase (Slide Test) assay

i. A small amount of bacterial colony was transferred to the surface of a clean, dry glass slide using sterile wooden stick.

ii. A drop of 3% H_2O_2 was then placed on to the slide and mixed.

iii. Rapid evolution of oxygen evidenced by evolution of bubbles (within 5-10 sec.) was regarded as positive result.

iv. A negative result was evidenced by absence of air bubbles.

Slide Coagulase Test assay

i. A staphylococcal colony will be emulsified in a drop of sterile physiological saline on a clean, dry and grease-free glass slide. A similar suspension of control positive and negative strains was set to confirm the proper reactivity of the plasma.

ii. A flamed and cooled straight inoculating wire was dipped into the undiluted plasma at room temperature, withdrawn, and the adhering traces of plasma (not a loopful) stirred into the staphylococcal suspension on the slide. The wire loop was flamed again and the procedure repeated for the control suspensions.

iii. A positive reaction was manifested by the presence of characteristic coarse clumping of cocci visible to the naked eye within 10 seconds.

iv. Absence of clumping or any reaction taking more than 10 seconds to develop was interpreted as a negative reaction.

v. Slow reacting strains were examined by the tube coagulase test.

Tube coagulase test procedure

i. A 1-in-6 dilution of the plasma in physiological saline was prepared. 1 ml volumes of the diluted plasma put placed in small tubes.

ii. Several isolated colonies of test organism were emulsified in 1 ml of diluted plasma to give a milky suspension.

iii. The tubes were incubated at 35°C in a water bath for 4 hours and examine at intervals of 1, 2 and 4 hours for clot formation by tilting the tube through 90°.

iv. Negative tubes were left at room temperature overnight and re-examine to capture some strains of *S. aureus*, including many MRSA, produce a delayed clot which is rapidly lysed at 37°C by staphylokinase.

Procedure of Bacitracin test

i. Two to three colonies of a pure culture of suspected organism were streaked onto a blood agar plate using a sterile inoculation loop.
ii. Using heated forceps, a bacitracin disk was placed in the first quadrant (area of heaviest growth).
iii. The plate was incubated for 18 to 24 hours at 37^{o}C in ambient air.
iv. Zone of inhibition around disk signified a positive test result.

Procedure for Triple Sugar Iron Agar (TSI) Test

i. TRIPLE SUGAR IRON (CM0277) OXOID slants were prepared in tubes
ii. The top of an isolated colony was touched by sterilized inoculation wire.
iii. TSI agar was inoculated by first stabbing through the center of the medium to the bottom of the tube and then streaking on the surface of the agar slant.
iv. The cap of the tube was left loose and the tube incubated at 37°C in ambient air for 18 to 24 hours.

Interpretation of Triple Sugar Iron Agar Test

i. Lactose (or sucrose) fermentation was characterized by a large amount of acid being produced, which turns the phenol red indicator yellow both in the butt and in the slant. Some organisms generate gases, which produces bubbles and/or cracks on the medium.
ii. None-lactose fermentation and small amount of glucose fermentation results in the oxygen-deficient butt turning into a yellow color butt has comparatively more glucose than slant i.e., more media more glucose), but on the slant the acid produced is oxidized to carbon dioxide and water by the organism making the slant red.
iii. If neither lactose/sucrose nor glucose is fermented, both the butt and the slant turn red. The slant can become a deeper red-purple (more alkaline) as a result

of the production of ammonia from the oxidative deamination of amino acids provided for by peptone.

iv. If hydrogen sulfide (H_2S) is produced, the black color of ferrous sulfide is usually apparent.

Procedure of Oxidase test:

i. A filter paper soaked with the Oxidase reagent (B02D008M) Becton Dickinson

ii. The colony to be tested was picked with sterile wooden applicator stick and smeared on the filter paper.

iii. Inoculated areas of the oxidase paper strips were observed for development of a deep blue or purple color change within 10-30 seconds signifying a positive result

Procedure for Indole Test

Pure bacterial culture was grown in sterile Peptone Water-HIMEDIA (M028-500G) for 18-24 hours at 37°. Five drops of Kovac's reagent were then added to the culture broth. The presence of a red or red-violet or orange (due to presence of skatole) color in the surface alcohol layer of the broth indicated a positive reaction while a negative result appeared yellow.

Procedure for urease test

Pure colony of suspected bacteria was inoculated in tubes containing Urea Broth Base-HIMEDIA (M111-500G). The tubes were then incubated at 37°C in ambient air for 18-24hrs loosely capped and examine for the development of a pink color denoting a positive result.

Procedure for citrate Utilization test

Pure colonies from suspected bacteria were inoculated into tubes containing sterile Koser Citrate Medium-HIMEDIA (M069-500G) and incubated aerobically at 370C for 18-24hours. A positive test indicating utilization of citrate was shown by turbidity of the medium; a sign of bacterial growth.

Antimicrobial testing procedure

Kirby-Bauer disc diffusion technique was performed to test isolates in vitro for antimicrobial susceptibility following clinical and laboratory standards institute CLSI criteria as follows:

i. A sterile 4mm deep plate of Mueller Hinton Agar from (CM0337) from OXOID was prepared as per Bauer-Kirby method. For fastidious organisms, Muller Hinton Agar was enriched with 5% Blood.
ii. Colonies from an overnight culture were picked with a sterile wire loop and emulsified into 5ml of sterile 0.85% sodium chloride solution and the turbidity of the inoculum adjusted to match 0.5 McFarland equivalence turbidity standard which will have been prepared prior.
iii. A sterile Polysterene Spun Swab-SteriPack (60566RevA) was then dipped into the standardized inoculum and the soaked swab rotated firmly against the upper inside wall of the tube to express excess fluid.
iv. The entire agar surface of the plate was then streaked with the swab three times, turning the plate at 60° angle between each streaking.
v. The inoculum was then be allowed to dry for 5 - 15 minutes with lid in place.
vi. Antimicrobial discs were then placed on the surface of the streaked plate using disk dispenser (OXOID).
vii. The plates were then incubated immediately at 35 ± 2°C and examine after 16-18 hours.
viii. Zones of inhibition to the nearest millimeter were then measured using a Vernier caliper.

Result interpretation

The diameter of the area displaying no growth were measured and then compared with the interpretation guide as per the standards chart for the determination of antibiotic sensitivity and resistance status by the Disk Diffusion method (Sarker et al, 2014).

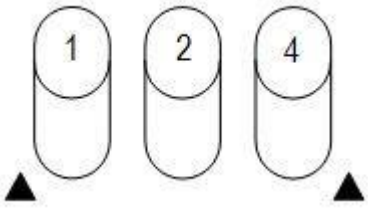
Analytical Profile Index (API 20E) system Assay

API (Analytical Profile Index) 20E-bioMerieux, Inc. was employed in the identification and differentiation of members of the family *Enterobacteriaceae*.

1. A single isolated colony (from a pure culture) was picked and used to make a suspension in sterile API® Nacl 0.85% medium.
2. The API20E biochemical test strip which containing dehydrated bacterial media/biochemical reagents in 20 separate compartments was placed on a clean bench.
3. A sterile Pasteur pipette was then used to fill up to the compartments with the bacterial suspension.
4. Sterile oil was added into the ADH, LDC, ODC, H2S, and URE compartments.
5. Some drops of water were placed in the tray before the API Test strip was put and the tray closed with a lid.
6. The set tray was then marked with an identification initial.
7. The tray was incubated at 37^{o}C for 18 to 24 hours.

Results interpretation

1. Before interpretation, the following reagents were added to specific compartments as follows:
    1. One drop of ferric chloride was added to TDA compartment
    2. One drop of Kovacs reagent was added to IND compartment
    3. VP: Put one drop of 40 % KOH (VP reagent 1) & One drop of VP Reagent 2 (α-Naphthol) were added to VP compartment and allowed to react for 10 minutes before it a negative interpretation is given.
2. The API reading scale (color chart) was obtained
    1. Each test was marked as positive or negative on the lid of the tray
    2. The wells were marked off into triplets by black triangles, for which scores were allocated as follows:



The scores were added up for the positive wells only in each triplet with the highest score possible for a triplet being 7 (the sum of 1, 2 and 4) and the lowest being 0.

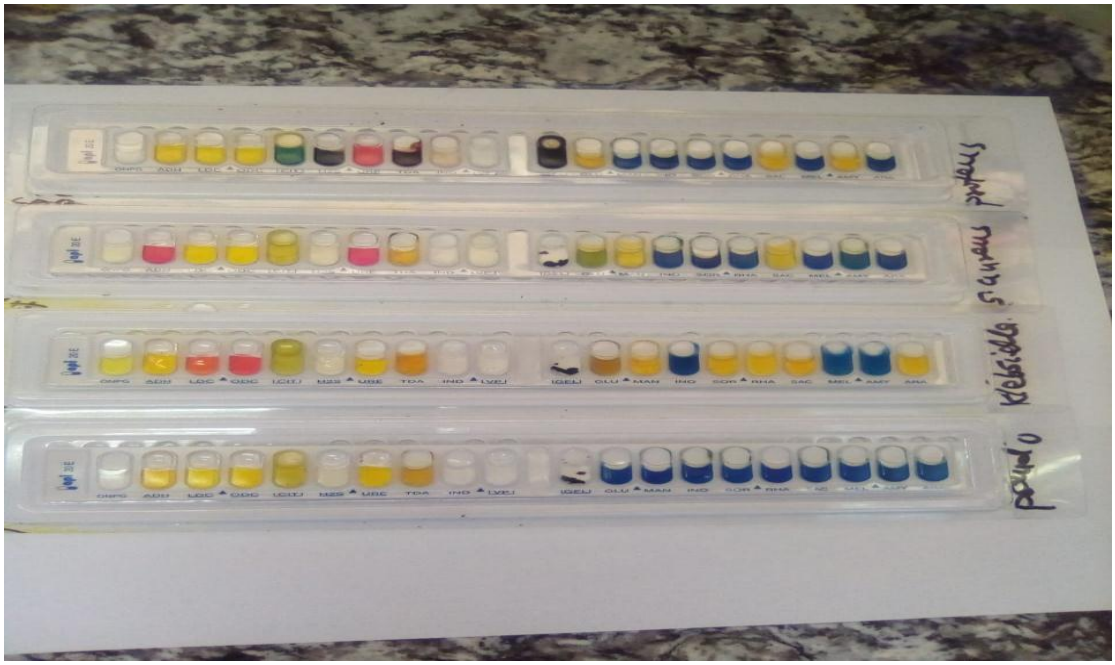
Triad	I			II			III			IV			V			VI			VII		
Tube	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	oxidase
Reaction	+	+	+	-	-	-	+	+	-	+	-	-	-	+	+	-	-	+	+	-	+
Point	1	2	4	0	0	0	1	2	0	1	0	0	0	2	4	0	0	4	1	0	4
Add	7			0			3			1			6			4			5		
7-digital Code	7031645																				

The figure shows triplet codes in API chart: Available from https://microbeonline.com/api-20e-test-system-introduction-procedure-results-interpretations/

Numbering in API 20E Test Strip

1. The profile for the combination of reactions in figure above is therefore 7031645 (7 digit code), which was then interpreted by using API catalog from apiweb

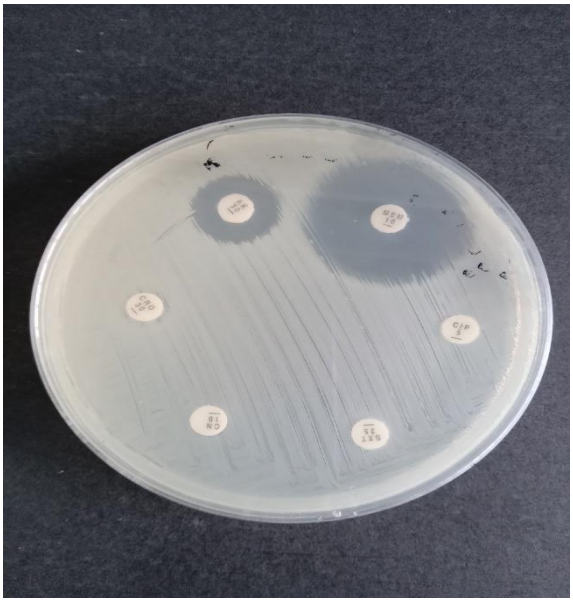
Sample of Api 20E identification results



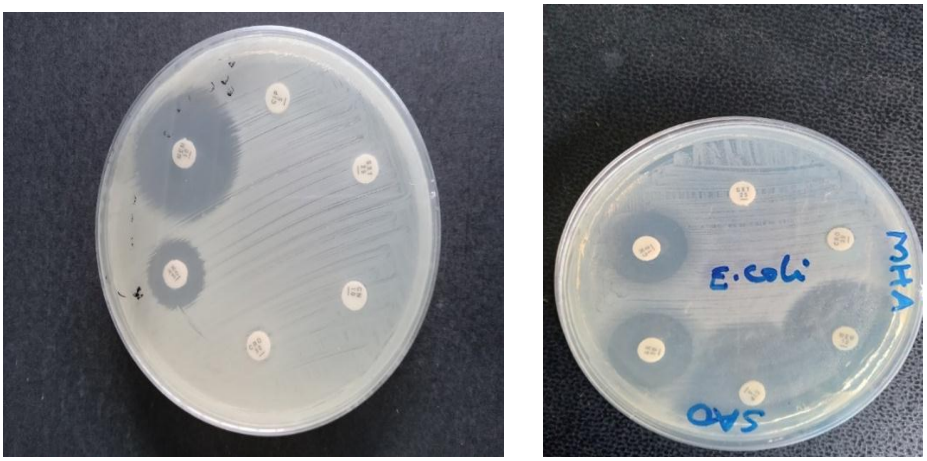
APPENDIX XI: ANTIMICROBIAL SUSCEPTIBILITY PLATES

The above image is a sensitivity plate showing inhibition zones of various antibiotic discs against *Klebsiella spp.*

The above image is a sensitivity plate showing inhibition zones of various antibiotic discs against *Proteus spp*.



Above photo shows a drug sensitivity test of *Acinetobacter baumannii*.

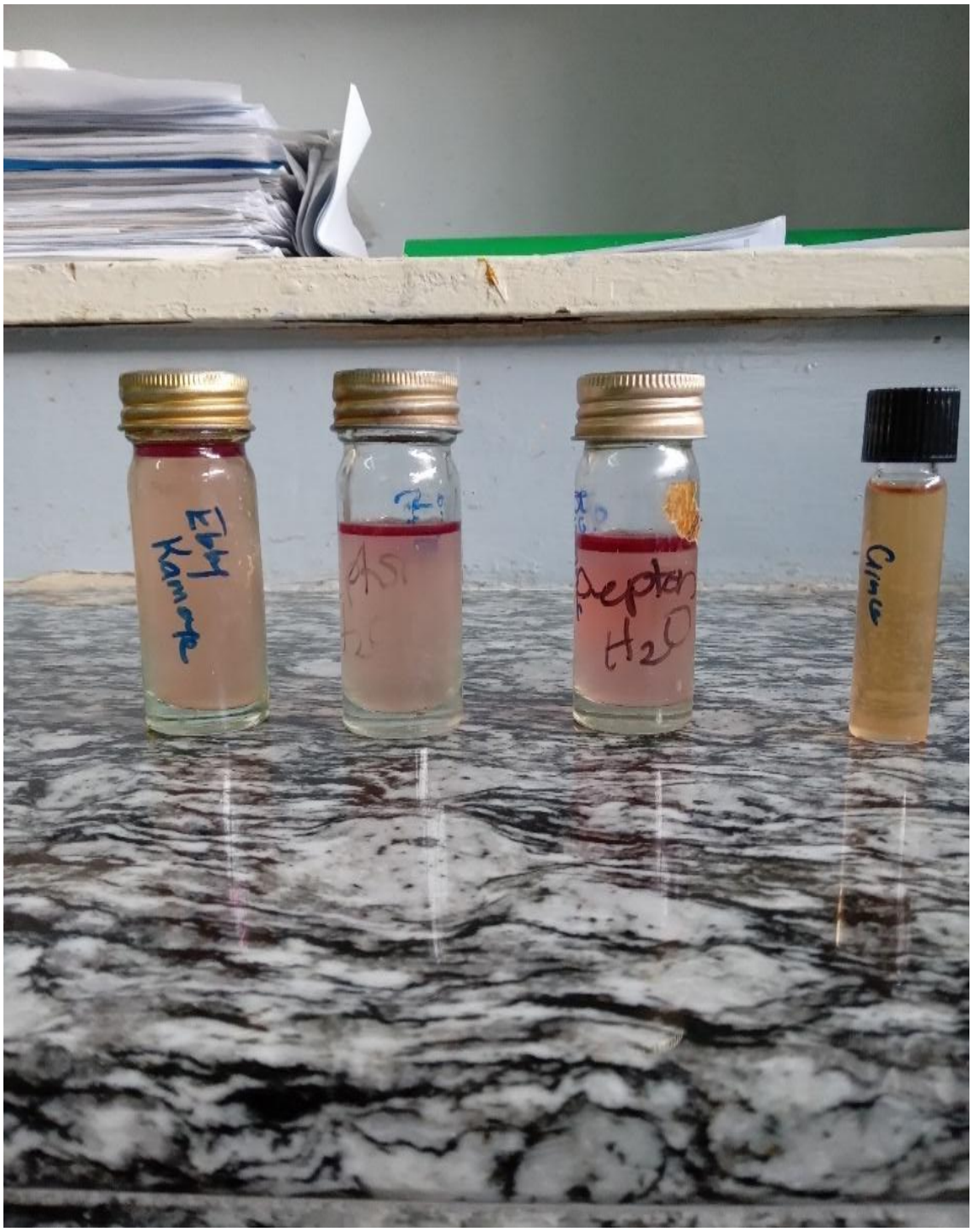


The above image is a sensitivity plate showing inhibition zones of various antibiotic discs against *E. coli*.

(A) (B)

The image labelled (A) is Staphylococcus aureus colonies showing golden yellow appearance on TSA while (B) is the same organism grown on MacCONKEYagar. Below is a plate of lactose fermenting colonies on MacCONKEY agar.

APPENDIX XII: INDOLE TEST BOTTLES



The above image shows three bottles on the left displaying positive indole test denoted by presence of pink color, while on on far right is indole negative test (pale yellow) color.

APPENDIX XIII: BIOSAFETY CABINET

The above photo shows a technologist in the process of media preparation inside a biosfaety cabinet

APPENDIX XIV: BACTEC INSTRUMENT FOR BLOOD CULTURE

APPENDIX XIV: AN INCUBATOR