ANTIBIOTIC SUSCEPTIBILITY PATTERNS OF BACTERIA ISOLATED FROM WARDS, OPERATING ROOM AND POST-OPERATIVE WOUND INFECTIONS AMONG PATIENTS ATTENDING MAMA LUCY HOSPITAL, KENYA.

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P150/28787/2014

A research thesis submitted in partial fulfillment of the requirements for the award of the degree of Master of Science in Infectious Diseases (Bacteriology) in the School of Medicine of Kenyatta University.

MAY, 2021
DECLARATION

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

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DEDICATION

I dedicate this work to my lovely wife and son who have been a pillar in my life.
ACKNOWLEDGEMENT

First of all I thank the almighty God. I do recognize the work of my supervisors Dr. Margaret Muturi and Dr. Scholastica Mathenge who guided me through the entire project. Their time and input was valuable in writing the whole document. My sincere gratitude go to Kenyatta university laboratory technologists Mr. Peter Mugo and Ms. Ruth Maundu for their technical support. I am grateful to the department of medical laboratory science for granting me the permission to use the Laboratory for my project.

My heartfelt thanks goes to the administration of Mama Lucy hospital for granting me access to the facility during data collection. My sincere gratitude’s also go to the staff of the hospital for creating a favorable research environment. I am really indebted to my parents and wife who prayed and encouraged me.
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ABBREVIATIONS AND ACRONYMS

AIC  Africa Inland Church
ASA  American society of anesthesiologist
ATCC American type culture collection
BMI  Body Mass Index
BSAC British Society for Antimicrobial Chemotherapy
CDC  Centre for disease prevention and control
CLSI Clinical and Laboratory Standards Institute
CoNS Coagulase negative staphylococcus
ESBL Extended spectrum beta-lactamase
EUCAST European Committee on Antimicrobial Susceptibility Testing
HPS  Health protection Scotland
IMVIC Indole, methyl red, voges proskauer in citrate test
IPC  Infection prevention and control
KUERC Kenyatta University Ethics Review Committee
MRI  Magnetic resonance imaging
MRSA Methicillin resistant Staphylococcus aureus
MTRH Moi Teaching and Referral Hospital
NHS  National Health Service
NHSN National Healthcare Safety Network
POD  Post-operative day
SPSS Statistical package for the social sciences
SSI  Surgical site infection
USA United States of America
VAC  Vacuum-assisted closure
DEFINITION OF TERMS

**Antibiotic prophylaxis** - Involves the administration of antibiotics to prevent the development of infection for example SSI.

**ASA score** - This is a classification system used to measure a patient’s pre-operative physical condition. Together with other parameters it is used to evaluate the patient’s risk of acquiring infection.

**Debridement** - This is the wide removal of unhealthy tissues from a surgical wound to promote healing of the remaining healthy tissues.

**Emplaced** - This is when something is put in place for example an implant.

**Extrinsic** - Features that are external to the patient.

**Healthcare associated infection** - This is an infection that a patient can get while receiving treatment in a hospital and the infection was not present during the admission period.

**Intermediate sensitivity** - This is a response to an antibiotic elicited by a pathogen that requires a larger dose than normal to treat the infection.

**Intrinsic** - These are features present within the patient.

**Monomicrobial** - It means that one micro-organism (bacteria) was isolated.

**Polymicrobial** - This means that more than one micro-organism (bacteria) was isolated.

**Resistant** - This is a non-response to an antibiotic by a pathogen irrespective of the dose.
Risk factor - This is a variable associated with the increased chances of getting an infection for example patients with a higher body mass index (BMI) have a greater likelihood of getting SSI.

Sensitive - This is a response to an antibiotic elicited by a pathogen when the drug is administered against it.

Surgical site infection – This is an infection of the post-operative period occurring in any part of the body following an operation.

Surgical wound - Refers to a wound created by a cutting instrument like a scalpel.

Wound classification - This is a classification scheme for categorizing wounds based on the degree of bacterial wound contamination at the time of surgery. Together with other parameters, the system is used to predict the likelihood of the patient developing surgical site infection.
ABSTRACT

Surgical site infections are a worldwide problem in the field of surgery contributing to increased mortality and morbidity. However, despite advances in the control of surgical site infections, the risk of acquiring these infections has not fully been eliminated due to the emergence and spread of resistant bacteria pathogens. In Kenya, there were scanty published reports on the antibiogram of surgical site infection pathogens lurking in the hospitals. The objective of this research was to determine the prevalence and antibiotic susceptibility patterns of bacteria isolated from the wards, operating room, and surgical site infections among patients attending Mama Lucy Hospital. This was a cross-sectional descriptive study of patients with post-operative wound infections in the hospital wards. Purposive sampling was employed and a total of 126 samples collected. Of these, 58 came from surgical site infected patients and 68 were obtained from predefined areas of the wards and operating room of the facility. The samples were processed through Gram stain, culture, and an array of biochemical tests. Subsequently, antibiotic susceptibility tests by Kirby bauer disc diffusion technique were performed on the isolated bacteria. Data collected was analyzed using a statistical package for the social sciences (SPSS) version 20 and chi-square (p<0.05). A total of 137 bacteria were isolated from the culture positive swabs, 78 of these came from the wound pus swabs while 59 were recovered from the hospital surface swabs. Among the SSI bacteria, *Staphylococcus aureus* (28.2%) was the preponderant bacteria followed by *E.coli* (15.4%). In the wards and operating room, the main bacterial contaminant was *Staphylococcus aureus*. Based on the sensitivity report, all the SSI bacteria isolates were sensitive to Chloramphenicol (69.2%). Wherease the environmental bacteria isolates had sensitivity to Ciprofloxacin (86.4%), Doxycycline (88.1%), Chloramphenicol (93.2%) and Vancomycin (100%). Majority of the environmental isolates were highly resistant to Ampicillin/oxacillin. In conclusion, the most prevalent SSI bacteria was *Staphylococcus aureus*, while Chloramphenicol was seen as the best drug for treating SSI at the hospital. The facility therefore needs to identify the most frequent bacteria associated with SSI. In addition, they need to monitor the bacteria that frequently contaminate the wards and operating room. The current antibiogram profile will help policy makers in the healthcare sector and the current setting to improve the current local guidelines on antibiotic prophylaxis for treatment of surgical site infection. The profiling will also assist in monitoring bacteria resistance trends within the institution and the country. Information generated from the hospital environment will help the infection control team at the current set-up to improve on the prevention of healthcare associated infections by carrying out active monitoring of hospital contaminants.
CHAPTER ONE

INTRODUCTION

1.1 Background of the study

A surgical site infection (SSI) is an infection that occurs within 30 days after an operation or within one year if an implant was emplaced. According to the National Healthcare Safety Network of Center for disease prevention and control (CDC), surgical site infections are classified based on depth and tissue spaces involved. These are superficial incisional, deep incisional, or organ/spaces surgical site infections (Horan et al., 2008).

This infection is known to affect people of all age groups, this is supported by a study that observed patients with as low as 15-79 years with surgical site infected wounds. The participants had a mean age of 35.95 ± 19.01 years (Mengesha et al., 2014). The high SSI incidence rates have been witnessed in males than females and vice versa (Rahman et al., 2019; Seni et al., 2013).

Surgical site infections accounts for more than one-third of post-operative deaths worldwide (Fan et al., 2014). In addition, the disease contributes to an increase in morbidity, prolonged lengths of hospital stay, and increased healthcare costs among hospitalized patients (Jenks et al., 2014; Roy et al., 2014). In the United States of America (USA) alone, the total annual expenditure for five major infections was $ 9.8 billion with SSIs and ventilator-associated pneumonia accounting for 33.7% and 31.6% of the total cost respectively (Zimlichman et al., 2013). Whereas the average cost per patient for USA hospital inpatient services was $34670 (Scott II, 2009).

In low and middle income countries SSI remains one of the leading healthcare-acquired infection with incidence rates ranging from 0.4 to 30.9 per 100 patients undergoing
surgery and a pooled incidence rate of 11.8% per 100 patients undergoing surgery, strikingly higher than proportions recorded in developed countries (World Health Organization, 2011). The situation in these countries has been augmented by the absence of guidelines on antibiotic stewardship, inadequate guidelines on infection prevention and control, and the emergence of drug resistant bacteria like Methicillin resistant *Staphylococcus aureus* and other resistant bacterial strains (Asaad and Badr, 2016; Allegranzi *et al.*, 2011; Weinshel *et al.*, 2015). Moreover, a research in Ethiopia on SSI observed that almost 100% of Gram-positive bacteria and 95.5% of Gram-negative bacteria were resistant to more than two antibiotics (Mulu *et al.*, 2012).

The prevalence rate of SSI antibiotic resistant bacterial strains in the country is unknown. Besides, there is no data on the prevalence of SSI pathogens and their antibiograms at the current hospital. This is because the facility lacks a laboratory bacteriology section for bacterial culture and relies mainly on empiric therapy. To effectively treat SSI patients, surgeons require information on SSI causative agents and their antibiograms. However this information is largely missing at the present setting, as a result, the present study was carried out in order to bridge the existing gap.

Besides intrinsic and procedure related risk factors, a contaminated hospital environment has been seen to increase the risk of surgical patients acquiring SSI (Fegio and Afriyie-Asante, 2014). This is because a contaminated environment may serve as a haven for bacteria proliferation. The consequences of this is surgical site infection in immune suppressed individuals, which can arise from bacterial wound contamination during surgery (Atata *et al.*, 2010). The problem can be complicated further by the emergence of antibiotic-resistant environmental bacterial strains making the treatment of SSI more costly and difficult (Solomon *et al.*, 2017).
To prevent this, active monitoring of environmental pathogens with positive feedbacks for the infection control team is encouraged (Nedelcheva et al., 2018). Most of these environmental pathogens vary from one setting to the other while exhibiting unique antibiotic susceptibility. Hence this project was done to determine the prevalence and antibiotic susceptibility patterns of bacteria isolated from the wards, operating room and among SSI patients attending Mama Lucy Hospital.

1.2 Statement of the problem

Despite the progress made in the field of surgery, surgical site infections remain a global problem accounting for increased deaths and morbidity among hospitalized patients. Poor infection prevention and control programs (IPC), inadequate guidelines on antibiotic stewardship, and the emergence of antibiotic-resistant bacterial strains have augmented the situation making the choice of empirical therapy more difficult and expensive. In Kenya, the infection rates of surgical site infections from Agakhan University Hospital and Kenyatta National Hospital stand at 7%, 30.8%, and 37.7% respectively (Dinda et al., 2013; Miima et al., 2016; Opanga et al., 2017). However, antiibiogram data on SSI causative pathogens which is vital in the management of surgical site infections is insufficient. This is because some facilities especially public hospitals lack the laboratory capacity to perform bacterial cultures due to limited resources.

Surgical patients in these facilities also face the risk of acquiring infections from contaminated wards and operating room. Most of these environments have their unique bacterial flora to which patients are at risk of acquiring infections during hospitalization. These bacterial flora exhibit unique patterns of antibiotic activity during a certain period. Only when antiibiogram data is available, are the clinicians able to manage surgical site infections effectively.
1.3 Justification of the study

In spite of surgical site infection being a problem in Kenya, there is insufficient information on the implicated pathogens and their antibiotic susceptibility profiles. The few published reports have not provided a clear picture of the magnitude and extent of SSI pathogens in a whole hospital setting. Given that understanding, the prevailing antibiogram and causative agent were vital in antibiotic selection for SSI treatment. This study generated a current antibiogram report that will aid policy makers in the health sector in updating the current local guidelines on antibiotics prophylaxis for treatment of surgical site infection. This report will also assist in monitoring bacteria resistance trends in the country.

1.4 Research questions

1. What is the prevalence of bacteria isolated from patients with surgical site infections at Mama Lucy Hospital?

2. What is the prevalence of bacteria contaminating the wards and operating room of Mama Lucy Hospital?

3. What is the antibiotic sensitivity patterns of the bacteria isolated from patients with SSIs and those contaminating the wards and operating room of Mama Lucy Hospital?

1.5 Objectives

1.5.1 General objective

To determine the antibiotic susceptibility patterns of bacteria isolated from wards, operating room, and post-operative wound infections among patients attending Mama Lucy Hospital, Kenya.
1.5.2 Specific objectives

1. To determine the prevalence of bacteria isolated from patients with surgical site infections at Mama Lucy Hospital.

2. To determine the prevalence of bacteria contaminating the wards and operating room of Mama Lucy Hospital.

3. To determine the antibiotic sensitivity patterns of the bacteria isolated from patients with surgical site infections and those contaminating the wards and operating room of Mama Lucy Hospital.

1.6 Significance of the study

The present study provided the current prevalence of SSI bacteria and environmental bacteria isolates along with their antibiograms. This information will aid policy makers in the healthcare sector and the current setting to improve the current local guidelines on antibiotic prophylaxis for treatment of surgical site infection. The antibiograms will also assist in monitoring bacteria resistance trends within the institution. The information generated from the hospital environment will inform the infection control team at the current setting on areas that require improvement in terms of prevention of surgical site infections.
CHAPTER TWO
LITERATURE REVIEW

2.1 Surgical site infection global perspective

According to CDC’s National Nosocomial Infection Surveillance system, 38% of all nosocomial infections in surgical patients were surgical site infections (Goyal et al., 2015). Surgical site infections were ranked first alongside pneumonia according to a 2011 survey in USA acute care hospitals (Magill et al., 2014). They also constituted third most common healthcare-associated infection in Australia and Europe according to literature review (Mitchell et al, 2017; European Centre for Disease Prevention and Control, 2008).

The rates of surgical site infection worldwide varied greatly, with rates of between 2.6% -58% recorded (Rosenthal et al., 2013; Donal B O’Connor as part of GlobalSurg Collaborative, 2018; Apanga et al., 2014; Allegranzi, 2014; Kaur et al., 2017). The United states of America alone reported an estimated annual SSI incidence ranging from 160,000 to 300,000 cases, with an estimated SSI annual expenditure of between $3.5 to $10 billion (Loyola University Health System, 2017).

In England, SSI surveillance for the year 2017/2018 by the Public health of England reported a cumulative SSI incidence of 8.7% for large bowel surgery between (April 2013 and March 2018) with the data showing a drastic increase in SSI incidence following large and small bowel surgeries in 2017/18 reaching 8.5% and 6.5% respectively (Public Health England, 2018). Another surveillance report for the period January 2003-December 2010 by the Health Protection Scotland (HPS) revealed that 1,883 inpatient SSI cases were resulting from 192,007 procedures. This surveillance observed an SSI rate of 87.9% among discharged patients following caesarean section (Health Protection Scotland, 2011).
On the other hand, a report for the year 2013-2014 generated by the European Centre for disease prevention and control on 16 European countries showed that there were 18,364 cases of post-operative wound infection arising from 967,191 surgeries. The report revealed SSI rates of between 0.6-9.5% per 100 surgeries (European Centre for Disease Prevention and Control, 2016). A separate review of the burden of six healthcare-associated infections for the year 2011/12 on the European population recorded an annual of 2,609,911 cases of healthcare-associated infection with SSI accounting for most of the cases 799,185 per annum followed by healthcare-associated urinary tract infection at 777,639 per annum (Cassini et al., 2016).

In Southeast Asia, a meta-analysis of several records on the burden of healthcare-associated infections (HCAIs) produced a pooled overall HCAI prevalence of 9.0% and a pooled incidence of 7.8% for SSI (Ling et al., 2015). Whereas research carried out in west India, recorded an overall prevalence rate of 21.9% for HCAI in the surgical wards with SSI accounting for 10.9% of the HCAI and an incidence rate of 12.72% (PatelDisha et al., 2011).

Researchers in South Africa and across Africa reported an SSI rate of 4.60% and cumulative incidence ranging from 2.5% to 30.9% respectively (Nair et al., 2018; Nejad et al., 2011). In Kenya, the highest incidence rate (37.7%) of SSI was observed at Kenyatta National Hospital, while the lowest rate (5%) of SSI was observed at Africa Inland Church (AIC) Kijabe Hospital following a drastic decline from 9.3% after a bundle of post-interventions comprising of rationalized antibiotic prophylaxis and improved operating room activities (Opanga et al., 2017; Ntumba et al., 2015).
2.2 The burden of surgical site infections

The burden of SSI in the field of surgery is substantial and it ranges from socio-economic to physical and psychological effects on the patient and healthcare. These effects include depression, pain, loneliness, anxiety, immobility due to lack of physical activity, mortality and the huge debts accumulated by patients after spending extra days in the hospital may leave some patients in financial hardship (Tanner et al., 2012; Gelhorn et al., 2018; Dal-Paz et al., 2010; Atkinson et al., 2016). Moreover, an evaluatory study of the impact of SSI on healthcare in six European countries that is Germany, France, Britain, Italy, Netherlands, and Spain, revealed that SSI’s were associated with elevated medical expenses arising from treatment, investigations, and hospitalization charges (Badia et al., 2017).

2.3 The problem of antibiotic resistance in surgical site infections

The levels of SSIs have increased substantially due to the emergence of multi-drug resistant pathogens and Methicillin resistant \textit{Staphylococcus aureus} (Adwan et al., 2016). This increase in antibiotic resistance among SSI bacteria isolates especially in India has demonstrated that the situation warrants the attention (Dulange and Thorat, 2015; Bhardwaj et al., 2018).

Furthermore, other studies have also observed widespread drug resistance among SSI bacteria isolates in the proportions of 82.92% and 65.38% respectively (Mengesha et al., 2014; Bhatt et al., 2014). In addition, the work of another researcher in Nepal showed that 64 (66.67%) of the bacterial isolates were multi drug resistant, whilst 26 (27.08%) of the isolates were resistant to one antibiotic and only 6 (6.25%) isolates were found to be sensitive to all the antibiotics tested (Raza, et al., 2013).
Several studies also observed varied resistance with Gram-negative rods and Gram-positive bacteria isolates against broad spectrum antibiotics such as cephalosporin’s, chloramphenicol and penicillin’s respectively (Clina et al., 2017; Adegoke et al., 2010). In Tanzania, a study at Muhimbili National Hospital observed that 63% of the bacteria isolates were multi-resistant to the antibiotics tested (Manyahi et al., 2014). These widespread antibiotics resistance rates have left surgeons with fewer antibiotics for SSI treatment. Therefore this study was carried out in order to develope an antibiogram profile of the common SSI bacteria pathogens for Kenya.

2.4 Risk factors associated with surgical site infections

The factors contributing to SSIs are variable for each patient, depending on the associated morbidity. These variables are intrinsic (patient related) and extrinsic factors (procedure related). Intrinsic variables include modifiable factors such as obesity, smoking, immunosuppressive disease conditions, and non-modifiable factors for instance age (Anderson et al., 2014; Triantafyllopoulos et al., 2015). Extrinsic variables include the type of procedure performed, surgery duration, and hospital environment (Roy, 2011; Diaz V, 2015).

2.4.1 Intrinsic factors

These are patients’ characteristics and they include the following factors.

2.4.1.1 Cigarette smoking

Nicotine present in cigarette smoke is a vaso-constrictor, this substance can interfere with the wound healing process by reducing the flow of blood rich in supply to the surgical bed. The reduced flow of blood to the injured tissues can result in the impairment of the inflammatory cascade (Sørensen, 2012). These events can result in
SSI since the body may not be able to deal with bacterial invasion (Kong et al., 2017; da Costa et al., 2017; Durand et al., 2013).

### 2.4.1.2 Gender
Several studies have discovered that gender plays an important role in the development of surgical site infection among surgical patients (Langelotz et al., 2014; Abdi et al., 2018; Kikkeri et al., 2014; Takahashi et al., 2018). This factor can be linked to sex hormones for instance testosterone and estrogen hormones. These hormones can disrupt the wound healing process by modulating genes responsible for inflammation and re-epithelization (Engeland et al., 2010; Hardman and Ashcroft, 2008).

### 2.4.1.3 Age
Recent literature show that the young and the elderly are at a greater risk of acquiring SSI following surgery (Korol et al., 2013; Amoran et al., 2013; Shah et al., 2017; Chu et al., 2015). The reasons behind age predisposing surgical patients to infection has not been fully explored. However, a review of factors affecting wound healing process among the elderly discovered that altered inflammatory response was responsible for the cases of wound infections among the aged (Guo and Dipietro, 2010).

### 2.4.1.4 Immunosuppressive disease conditions
In other instances, immune compromising conditions such as diabetes mellitus and anemia were discovered as factors linked with a higher risk of SSI. This is because patients with these conditions have reduced immunity which makes it very hard for the body to clear the infection at the wound site effectively (Akoko et al., 2012; Novelia et al., 2017; Gelaw and Abdella, 2018).
2.4.1.5 Obesity

On the other hand surgical patients with a higher BMI were seen to be at a greater risk of acquiring surgical site infection (Thelwall et al., 2015; Naphade and Patole, 2017). This is due to the high tissue mass in obese patients which lowers tissue vascularity, increases the complexity of the procedure, and reduces oxygen and antibiotics permeation to the surgical bed. Hence, all these events can increase SSI susceptibility (Gupta et al., 2008).

2.4.1.6 Preoperative nasal colonization with Staphylococcus aureus

Recent literature show that Staphylococcus aureus colonizes the anterior nares of about 30% of the human population (Lewis, 2018). Within the hospital setting, this bacteria has the ability to cause wound infections in immune-compromised surgical patients. This is because the bacteria can be transferred from the nostril to the patient skin and later into the wound during skin incision (Savage and Anderson, 2013; Tai et al., 2013; Inoue et al., 2018).

2.4.1.7 Poor nutritional status

A deficiency of micro and macro-nutrients such as vitamins, proteins, carbohydrates, iron, zinc and magnesium can have a profound effect on the healing process of the wound after surgery (Guo and Dipietro, 2010). There is sufficient body of evidence to suggest that serum albumin levels below 3.5 g/dl can increase the susceptibility of surgical patients to SSI (Yuwen et al., 2017; Lalhruaizela et al., 2017). This is because low levels of serum albumin in surgical patients are an indicator of a wide range of comorbid conditions that can impair patient immunity (Moore et al., 2013).
2.4.1.8 Wound classification and ASA score
In other instances, a higher wound class and ASA score together with other parameters such as suppressed immunity have been shown to increase the risk of surgical patients developing SSI (Carvalho et al., 2017; Aiken et al., 2013; Olowo-okere et al., 2018). The two systems are widely used by surgeons to assess the degree of wound bacterial contamination and the pre-operative physical status of the patient during and prior to surgery (Zinn and Swofford, 2014; American society of anesthesiologist, 2014).

2.4.2 Extrinsic factors
These are factors which are not part of the patient and include the following factors.

2.4.2.1 Types of procedures
The risk of a patient developing SSI also depends on the type of procedure performed, with some studies observing higher rates of infection in abdominal surgeries such as cesarean section (Shrestha et al., 2014; Jasim, 2017). These infection rates can be ascribed to contamination of the surgical site with enteric rods after an incision has been made. Moreover, surgical procedures involving the insertion of drains and implants have been seen to put surgical patients at risk of acquiring SSIs (Yoon et al., 2018; Oliveira et al., 2018; Ikeanyi et al., 2013). This is due to tissue damage and clot formation during implant insertion which increases the likelihood of biofilm formation on the implant. These events may lead to SSIonces the host’s defenses have been breached (Veerachamy et al., 2014).

2.4.2.2 Longer procedure durations
Additionally, surgical procedures lasting for long hours increases the chances of exposed tissues being contaminated with environmental bacteria. Moreover, long surgery durations can lead to tissue desiccation with the likelihood of tissue
contamination and may also result in many technical errors as result of fatigue of the operating team (Cheng et al., 2017). Besides, there is a sufficient body of evidence that suggests that long operation times contribute to SSI susceptibility (Kasatpibal et al., 2006; Jeong et al., 2013; Shahane et al., 2012; Ameh et al., 2009).

### 2.4.2.3 Contaminated hospital surfaces

In addition to surgical procedures, the integrity of the hospital environment can also determine the outcome of surgery. Contaminated hospital surfaces can harbor pathogenic micro-organisms that have the potential to cause SSI. These grubby hospital surfaces are due to poor infection prevention and control programs, with most pathogens originating from the air, healthcare providers and can also be shed from the patient skin into the hospital environment (Gelaw et al., 2014; Alfonso-Sanchez et al., 2017; Ngaroua et al., 2016).

### 2.4.2.4 Non-sterile equipments

Likewise is the use of surgical instruments contaminated with bacteria from the theater team. There have been a few documented cases of SSI arising from contaminated surgical instruments as a result of poor instrument handling and lack of proper inspection before surgery (Dancer et al., 2012).

### 2.4.2.5 Hair removal

Hair removal is essential for ease of accessing the operative site, however shaving of the surgical site using a razor a day before an operation can result in minute skin cuts that can serve as sites for bacterial build-up and proliferation. The bacteria colonizing these sites may enter the surgical wound during skin incision and consequently cause post-operative wound infection (National Collaborating Centre for Women’s and Children’s Health(UK), 2008; Buteera and Orth, 2008). Besides, clinical trials have
shown that hair removal using a razor is associated with a higher risk of SSI (Faruquzzaman et al., 2012; Tanner et al., 2011).

2.5 Pathogenesis of surgical site infections

The development of a surgical site infection depends on bacterial contamination of a surgical site. Quantitatively, it has been shown that if a surgical site is contaminated with $>10^5$ bacteria per Gram of tissue, the risk of SSI is markedly increased (Weston et al., 2016; Greene et al., 2010). However, the dose of contaminating bacteria required to produce infection may be much lower when foreign material is present at the site (Damani, 2011).

The probability of whether bacterial contamination can lead to SSI is determined by an interplay between four factors. These are bacteria inoculum, virulence, adjuvants effects and host immunity and this can be conceptualized by the following relationship (Fry, 2013).

$$\text{Probability of SSI} = \frac{\text{bacteria inoculum} + \text{virulence} + \text{adjuvant effects}}{\text{host immunity}}$$

The infection process increases proportionally as the inoculum size and virulence of the bacteria increases. Local features of the wound like presence of blood (hemoglobin) and foreign materials like drains potentiate bacterial virulence (Fry, 2013). Most of the bacteria that cause SSI secrete toxins and possess other invasive structures such as adhesins that increase their ability to adhere to host cells, penetrate, colonize and produce damage within the host tissues (Kaye, 2011; Lachiewicz et al., 2015). Some of the bacterial species can produce an extrapolymeric substance or possess a polysaccharide capsule, which shields them from both the hosts immune response and most antimicrobial agents (Crossley et al., 2010).
In attempts to clear the invasion, the host’s pro-inflammatory cells releases tumour necrosis factor- alpha which stimulate neutrophils for bacterial phagocytosis. It also causes the release of reactive oxygen and acid hydrolases from lysosomal vacuoles resulting in lipid peroxidation, release of interleukins, evoking inflammatory response with creation of space containing pus which contain necrotic tissue, neutrophils, bacteria and proteinaceous fluid with signs of inflammations (rubor, dolor, calor and tumor) (Sriram, 2016). The micro-environment of purulent collection is relatively hypoxic and acidotic, thus most cellular and enzymatic functions are inhibited. Therefore these areas require operative drainage in addition to antibiotic therapy (Lawrence, 2013).

2.6 Sources of surgical site infections

Pathogens causing surgical site infection can originate from either exogenous or endogenous sources.

2.6.1 Endogenous sources

Most of the SSIs are due to bacterial contamination of the surgical wounds with endogenous flora of the host skin and viscera. This contamination occurs after the skin or hollow viscera has been incised (Gupta, 2006; Newman et al., 2008). These endogenous flora include skin micro-organisms for instance Staphylococcus aureus and enteric Gram-negative rods for example E.coli. These pathogens can also originate from infective sites remote from the surgical site (Lubin et al., 2013; Sarrafzadeh-Rezaei et al., 2006).
2.6.2 Exogenous sources

The sources include the operating room environment, surgical personnel-especially members of the surgical team, and all instruments brought to the operating room during an operation (Zhiqing et al., 2018; Dancer et al., 2012; Rello et al., 2007). Contamination occurs as a result of patient interaction with the surgical team or other healthcare providers colonized with pathogens and contaminated hospital surfaces with wounds serving as routes of infection. Exogenous microbiota include *Staphylococcus aureus* and *Streptococci* species (Lee et al., 2013; Singh et al., 2014).

2.7 Pathogen’s causing surgical site infections

Most studies show that the distribution of pathogens isolated from SSIs has not changed markedly during the last decade. *Staphylococcus aureus*, *Coagulase-negative staphylococci*, *Enterococcus* species and *Escherichia coli* remain the most frequently isolated bacteria. A plethora of evidence indicate that an increasing proportion of SSIs is due to multidrug-resistant bacteria, Methicillin-resistant *Staphylococcus aureus*, and ESBL producers (Seni et al., 2013; Chaudhary et al., 2017; Ramesh and Dharini, 2012; Naik and Deshpande, 2011; Bhave et al., 2016).

A report from surveillance of National Health Service (NHS) Hospitals in England for the year 2014/15 discovered that *Staphylococcus aureus* was the prevalent isolate from orthopedic and spinal operations accounting for more than 36% of the infections. However, *Coagulase negative staphylococci* and enterobacteriaceae were dominant in patients who had undergone heart bypass and colon surgical procedures respectively. *Staphylococcus aureus* also accounted for 13% of SSIs among admitted patients after a decrease between 2006 and 2007 due to a decline in MRSA (Public Health England, 2015).
In a separate report by Health protection agency (England), enterobacteriaceae accounted for 29% of the total bacteria followed by *Staphylococcus aureus* at 24% (349) with MRSA accounting for 4% of all *Staphylococci* species and 18% of *Staphylococcus aureus*. Polymicrobial was reported in procedures involving the gastro-intestinal 30.3% (314/1,036) and orthopedic 27.6% (162/588) while monomicrobial was generally high in clean surgeries (Health Protection Agency, 2012).

Additionally, several researchers observed that *Staphylococcus aureus* was the predominant SSI bacteria followed by *Pseudomonas aeruginosa, E.coli, Klebsiella* species, *Citrobacter* and *Acinetobacter* species (Biradar and Roopa, 2015; Suryawanshi et al., 2014; Subrata, 2016; Shriyan et al., 2010; Shinde and Kulkarni, 2017; Khorvash et al., 2008). However, a separate project discovered that the most frequent SSI isolate was *Pseudomonas* species (42.85%) followed by *Klebsiella* species (28.5%) (Janugade et al., 2016).

In Nigeria, India, Switzerland, and Greece, reports by different research workers reported *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Enterococcus, Pseudomonas aeruginosa, and Coagulase negative staphylococci* as the most prevalent isolates in post-operative wound infections (Akinkunmi et al., 2014; Mundhada and Tenpe, 2015; Amutha et al., 2014; Sawhney et al., 2017; Misteli et al., 2011; Alexiou et al., 2017). Investigations by other research workers in two separate countries discovered that SSIs were also caused by anaerobic bacteria such as *Bacteroides fragilis, Clostridium perfringens* and atypical pathogens for example *Mycoplasma hominis* and *Ureaplasma* species (Akhi et al., 2015; Barie, 2017).

Evaluations of ward surfaces, operating room environment, non-critical healthcare tools and bedside surfaces of different hospitals revealed that a lot of the contaminants were
Coagulase negative staphylococci, *Staphylococcus aureus*, *Klebsiella* species, *Pseudomonas* species, *Aspergillus* species, *Bacillus* species and Methicillin resistant *Staphylococcus aureus* (Getachew et al., 2018; Matinyi et al., 2018; Weldegebreal et al., 2019; Yuen et al., 2015).

On the other hand, the examination of SSI pathogens in Ethiopia observed an overall bacterial prevalence of 75.6%, with *S. aureus* 33.3% and *E. coli* 14.3% as the most common bacteria isolates. (Asres et al., 2017). Separate analysis of post-operative wound infection in Ethiopia isolated 111 pathogenic bacteria from clinical swabs of which *Staphylococci* species and Gram-negative enteric rods were the predominant isolates. This analysis also saw the isolation of *Coagulase negative staphylococci* 41 (41.8%) as the prevalent isolate followed by *Staphylococcus aureus* and *Pseudomonas aeruginosa* from 75 different sites of the hospital environment. The project recorded MRSA rates of 9 (34.6%) and 2 (2.0%) from clinical and environmental samples respectively (Amare et al., 2011).

Similar work in Ethiopia isolated a total of 268 bacteria pathogens from all the processed specimens. A total of 142 (52.9%) isolates were recovered from medical devices and air, while the rest of the pathogens were from the health professionals 77 (28.8%) and patients 49 (18.3%) respectively. This analysis identified *Staphylococcus* species and Gram-negative rods as causative agents of post-operative wound infection (Gelaw et al., 2014). In Tanzania and Kenya, the preponderant bacteria isolates from SSI were Gram-negative enteric rods and *Staphylococcus aureus* (Mwambete and Rugemalila, 2015; Okello et al., 2018).
2.8 Diagnosis of surgical site infections

When diagnosing surgical site infections, the first step is physical examination of the patient for signs of infection such as erythema, localized swelling, pain, warmth, and purulent discharge (Thomas et al., 2016). The second and most important is culture and sensitivity of pus aerobically or anaerobically to establish the microbiological profile and antibiogram (Coventry, 2014). Other investigations that may be relevant include Magnetic Resonance Imaging (MRI) and C-reactive protein levels, Ultrasonography, Computed tomography scan, sedimentation rates and a complete blood count that determine whether leukocytosis and neutrophilia are present, especially when the infection becomes systemic (Kasliwal et al., 2013; Awe and Harrop, 2010).

2.9 Complications of surgical site infections

In most cases, if surgical site infection isn’t managed properly. The infection may progress from serious to life threatening condition such as, pneumonia due to prolonged hospital stay, cosmetically unacceptable scars, persistent pain, and movement restriction (Lyden, 2016; Britt et al., 2012). The patient may also experience compromised emotional well-being, necrotizing fasciitis, wound dehiscence, metastatic abscesses, septicemia, prolonged wound healing, organ failure and incisional hernia (Millar et al., 2011).

2.10 Treatment and Management of surgical site infections

Treatment strategies for SSIs are varied but they involve wound opening with debridement and drainage to remove all foreign materials, necrotic and infected tissues (Leaper et al., 2013). In incisional SSIs, the infected wounds are managed by irrigation with isotonic solutions like saline solutions to remove loose dead tissues and exudates (Cameron and Cameron, 2013). In addition, there is the use of dair protocol for treatment of patients who have undergone instrumented spinal surgery. The dair
protocol can be implemented successfully within 3 months by performing wide removal of dead tissues, implant retention, use of antibiotics, and by irrigating the wound with sterile saline solutions (Manet et al., 2018).

Moreover, the appropriate use of dressings promote wound healing by providing the wound with a balanced moist healing environment, removes wound exudates, and protect the wound from harmful germs (Keast and Swanson, 2014). Likewise, is the use of vacuum-assisted closure (VAC) dressing. In this technique, the wound surface is exposed to a controlled negative pressure, this removes localized fluid, increases blood flow, and decreases bacterial load, and stimulates the proliferation of reparative granulation tissue. This system also assists in the debridement of necrotic tissue and act as a sterile barrier (Patmo et al., 2014; Karaaslan et al., 2014).

Another treatment option is the use of citric acid, the topical application of citric acid work against *Pseudomonas aeruginosa* and *Staphylococcus aureus* both of which are SSI pathogens (Nagoba et al., 2011). Additionally, great success has been seen with the use of electrical stimulation and hyperbaric oxygen therapy in open surgical wounds (Orsted et al., 2010). For systemic features of infection such as widespread cellulitis, empiric systemic antibiotics based on sensitivity report should be administered (Townsend Jr et al., 2012).
CHAPTER THREE
MATERIALS AND METHODS

3.1 Study area
The project was carried out at Mama Lucy Hospital. This is a level 5 county government hospital located in the eastern part of Nairobi. The hospital sits on a 10 acre piece of land, with its location serving as an excellent catchment area for patients from the whole of Eastlands and eastern bypass. Since its inception, the facility has reduced the pressure on Kenyatta National Hospital in that it also serves as a referral facility for patients from areas within and outside Nairobi. The hospital’s outpatient section serves 800 patients daily and the facility has a bed capacity of 137 beds and an inpatient admission rate of 131%. This facility offers a wide range of services ranging from casualty and general outpatient, maternity, laboratory, pharmacy, nutrition, major and minor surgeries, radiology and orthopedic services.

3.2 Study design
This was a cross-sectional descriptive study.

3.3 Study population
The study included patients of all age group with post-operative wound infections following general, obstetrics and gynecologic surgeries. All the patients were drawn from the hospital’s main wards which were surgical wards, maternity wards and pediatric ward. This research work was done between October 2018 and March 2019.

3.4 Patient recruitment
All the patients who consented and met the inclusion criteria were recruited into the project. Consent was obtained by signing of the consent forms by the participants. For children, parents or guardians of legal age would sign the consent forms on their behalf.
3.4.1 **Inclusion criteria**

a) Patients with SSIs and who had consented to participate in the study

b) Patients of all age groups but who had SSIs.

3.4.2 **Exclusion criteria**

a) Those patients who didn’t develop surgical site infection during the research period.

b) Those patients who didn’t consent.

3.5 **Sample size determination**

The appropriate sample size was determined using the Fischer et al., (1998) method:

\[ N = \frac{Z^2 P(1 - P)}{d^2} \]

Where:

N = sample size required

Z = 1.96 standard error

P = the proportion of antibiotic resistant bacteria isolates from SSIs in Kenya was unknown (50% value was used)

d = corresponded to margin of error (precision), which was 5%.

\[ N = \frac{1.96^2 \times 0.5(1 - 0.5)}{0.05^2} \]

\[ N = \frac{3.8416 \times 0.5 \times 0.5}{0.0025} \]

N = 384

The sample size was reduced to 58 using the finite population correction formulae for proportions. The formulae incorporated the total number of SSI cases (68) observed at
Mama Lucy Hospital within a period of 6 months that is between July and December 2018.

\[ n = \frac{n_0}{1 + \frac{(n_0 - 1)}{N}} \]

\[ n = \frac{384}{1 + \frac{384 - 1}{68}} \quad n = 58 \]

Where by \((n)\) was the reduced sample size, \((n_0)\) was the original sample size 384 while \((N)\) was the total population of patients with surgical site infection between July and December 2018 which was 68 in total. After calculation the sample size obtained was 58 patients and in addition to 68 environmental samples, totaling to 126 samples.

### 3.6 Sampling technique

The study adopted purposive sampling, with those patients having surgical site infection characterized by purulent wound discharge recruited into the project. The patients with suspected surgical site infection were identified by surgeons during the routine daily ward rounds. The surgeons would then document the clinical signs of infection in the patient file. Identifying patients with SSI in the maternity wards was a lot easier because they were kept in one room known as the post-operative day (POD), while in the rest of the wards the doctor or clinician on duty would assist in the identification process. Participants were recruited every week and all the patients were briefed about the research and informed consents obtained prior to their inclusion as study participants. This included patients with SSI infection in the hospital wards.

Three wards that is surgical, maternity, pediatric and an operating room where patients and healthcare providers had frequent direct body contact with the surfaces, were assessed for bacterial contamination. The predefined areas were selected through risk
assessment. These areas included the floor, hospital bed handles, operating table, anaesthetic machine, surgical pack, drip stands, surgical lamp, ward screens, ward bedside tables, suction machine, sinks and tap handles.

3.7 Sample collection and analysis

3.7.1 Patients pus swabs

Asceptic collection of all the samples was done in the morning during wound dressing before the wound was cleaned with an antiseptic. To ensure the integrity of the samples, all the wound surfaces were cleaned first with sterile normal saline to prevent the introduction of skin normal flora into the pus. During sample collection, all the sterile swabs were first moistened with sterile normal saline, this was followed by collection of pus from the deep viable tissues of the wound using moistened sterile cotton wool swabs by Levine method (rotating the swab over 1 cm² area of viable tissues for 5 seconds) (Cooper, 2010). Two swabs were collected from each patient, one for Gram stain and the other one for culture. After collection, all the pus swabs were carefully labelled with patient details and transported to the laboratory of the department of medical laboratory science Kenyatta University on Stuart transport media within 2 hours of collection.

3.7.2 Wards and operating room surface swabs

The swabbing of the surfaces of the wards and operating room was carried out following guidelines by the International Organization for Standardization (ISO, 2003). The collection of the surface swabs was done by stroking the moistened swab in close parallel sweeps over the defined sampled area while rotating it slowly. For areas such as the floor which is covered by square tiles. Adequate spacing was taken into consideration to ensure that every part of the floor was covered. In total 68 surface swabs were collected for bacterial culture. Once each surface swab had been collected,
it was carefully labelled, packaged in a leak proof biohazard bag and transported to the laboratory of the department of medical laboratory science Kenyatta University on Stuart transport media within 2 hours of collection. Upon reaching the laboratory all the specimens were carefully inspected and their details entered in a book.

3.7.3 Microscopy

Smear preparation, staining and examination of the collected pus was done according to standards prescribed in bacteriology (Cheesbrough, 2006). The first step was preparation of smears on slides with frosted ends. Each slide was labelled with a patient number using a lead pencil, the slides were then air dried in a dust free environment, heat fixed by passing them three times through a flame of spirit lamp. The heat fixed slides were allowed to cool and stained using Gram staining technique (appendix IV). After staining, the backs of the slides was wiped using a clean gauze and the slides placed on a draining rack to air dry. The dried smears were examined microscopically using the oil immersion objective. Smears of discreet colonies from cultured swabs were also prepared and stained by Gram stain and observed microscopically.

3.7.4 Culture

Culture of pus and surface swabs was carried out according to the set standards and procedures in bacteriology (Cheesbrough, 2006). A small amount of the specimen was applied on the agar surface of both MacConkey and Blood agar (appendix IX and X). Then using a sterile wire loop, the specimen was spread on the agar surface using the streaking method (figure 3.1). Each swab was inoculated on a separate plate and after labelling them, the plates were incubated aerobically at 35-37°C for 18-24 hours. After incubation, Individual bacteria isolates were identified from their respective plates by observation of the growth pattern which included checking the form (circular), elevation (raised, flat or convex), margin (undulate or entire), opacity (translucent,
opaque or transparent), hemolysis (beta, alpha or gamma), surface (smooth, dry or mucoid) and pigmentation (pink, golden yellow or white) of the colonies. Swarming characteristics of bacteria on blood agar surface was also used in the identification process (appendix VII). Plates with no growth after 18 hours of incubation were re-incubated while those with mixed growth were sub-cultured on separate plates until pure growth of discrete colonies was observed. Reporting of no growth was only done after the plates were incubated for 48 hours.

![Sample](image)

Figure 3.1: Streaking method

### 3.7.5 Biochemical tests

In addition to microscopy and cultural characteristics, various biochemical tests were carried out in order to identify the isolated bacteria. These tests included catalase test, coagulase test, urease test, oxidase test, indole, methyl red and voges proskauer tests.

#### 3.7.5.1 Catalase test

This test was done to differentiate catalase producing bacteria such as *Staphylococcus* species from the non-catalase producing ones like *Streptococcus* species. Catalase enzyme acts as a catalyst in the breakdown of hydrogen peroxide to oxygen and water.
The test was done by bringing a test organism into contact with a drop of 3% hydrogen peroxide. The release of oxygen bubbles were indicative of catalase producing bacteria whilst non-catalase producers were indicated by no bubbles (Apurba and Bhat, 2018).

3.7.5.2 Coagulase test

The test was carried out to identify and differentiate coagulase positive *Staphylococcus aureus* from non-coagulase staphylococcus like *Staphylococcus epidermidis* (appendix VIII). In this test, a drop of distilled water was placed on each end of the slide. Carefully selected discrete colonies of the test organism were emulsified with the drops of water on each end of the slide, with one suspension acting as a control. The formation of clumps 10 seconds after the addition of a loopful of plasma to one of the suspension demonstrated that the test was positive. All negative slides tests were subjected to a test tube coagulase test (Cheesbrough, 2006).

3.7.5.3 Urease test

This test was performed in order to identify those bacteria with the ability to hydrolyze urea using urease enzyme. In this test, the test organism was inoculated in a test tube medium containing urea and phenol red indicator. The other uninoculated tube acted as a control. After 24-48 hours of incubation at 37°C, both tubes were examined for colour change. The change in colour of phenol red from light orange to pink meant that the test was positive, whilst the lack of phenol red colour change indicated that the test was negative (appendix V). Some of urease positive bacteria include *proteus* species and *Klebsiella pneumoniae* (Hemraj *et al.*, 2013).
3.7.5.4 Oxidase test

This was carried out by picking a colony of the test organism and smearing it over a filter paper impregnated with oxidase reagent. The formation of purple colour indicated a positive test for oxidase producing bacteria like *Pseudomonas aeruginosa*, whilst no colour change demonstrated that the test was negative for bacteria such as *Escherichia coli*. (Cheesbrough, 2006).

3.7.5.5 Indole test

Indole test was carried out in order to identify those bacteria with the ability to breakdown tryptophan into indole such as *Escherichia coli*. In this test, the test organism was inoculated in tryptone broth and the tubes incubated at 37°C for 48 hours. After incubation, the indole was identified by adding 0.5ml of kovac’s reagent to the inoculated tubes. The formation of a red ring on the surface of tryptone after gentle shaking demonstrated that the test was positive, while no red layer meant that the test was negative (appendix V) (Cheesbrough, 2006).

3.7.5.6 Methyl red and Voges proskauer tests

Methyl red and voges proskauer tests were used in the identification of enterobacteriaceae such as *Escherichia coli* which is methyl red positive and voges proskauer negative. Both tests were carried out by inoculation of test organism in methyl red-voges proskauer broth. After 24 hours of incubation at 35°C, 1ml of the broth was transferred to a clean tube and the remaining broth re-incubated for another 24 hours (Hemraj et al., 2013).

The 1ml broth that was transferred to a clean test tube was later used for voges-proskauer test. To the 1ml broth, 0.6ml of 5% alpha-naphthol was added followed by 0.2ml of 40% potassium hydroxide. Following gentle shaking, the formation of a red-
pink colour on the surface of the medium after 10 minutes demonstrated that the test was positive. Whereas the development of a yellow colour on the surface of medium demonstrated that the test was negative. Examples of voges proskauer positive bacteria are enterobacter and *Klebsiella* species.

In methyl red test, 2.5ml of the 48 hour broth was transferred to a clean tube. The formation of a red colour on the surface of the medium after the addition of 5 drops of methyl red, demonstrated that the test was positive. Negative methyl red tests were demonstrated by the development of a yellow colour on the surface of the medium (Hemraj *et al*., 2013).

**3.7.5.7 Citrate test**

Citrate test was performed in order to identify those bacteria with the ability to utilize citrate as their sole carbon source. This test was performed by streaking the slope and stabbing the butt of Simmons citrate agar with the test organism using a sterile straight wire. The tubes were later incubated at 37°C for 48 hours and the change in colour of the agar from green to blue indicated that the test was positive (appendix V). The most notable bacteria that are citrate positive and citrate negative are *Klebsiella pneumoniae* and *Escherichia coli* respectively (Cheesbrough, 2006).

**3.7.6 Antibiotic susceptibility testing**

Antibiotic sensitivity tests were performed on the bacteria isolates by Kirby Bauer antibiotic testing method according to the Clinical and Laboratory Standards Institute’s guidelines (appendix XI) (Cavalieri *et al*., 2005). In this technique, 3-5 pure colonies of the test organism were emulsified in 3-4 ml of sterile physiological saline and the suspension adjusted to match that of 0.5 McFarland turbidity standard. A sterile swab was dipped in the tube with the saline suspension and pressed against the side of the
tube above the suspension to remove the excess fluid, this was followed by uniform coating of the Mueller Hinton surface with the test organism in three directions and in each instance rotating the plate at an angle of 60° to ensure even coating. The plate’s surfaces were given 3 minutes to dry with their lids in place. Then using sterile forceps, the antibiotic discs were placed uniformly on the inoculated agar surface. These plates were inverted and incubated aerobically at 35°C for 16-24 hours and after incubation the test plates and control were examined for confluent growth. When using Oxacillin, all the plates were incubated for a full 24 hrs at 35°C. The diameters of the resultant zones of inhibition on each plate were measured in millimeters and interpreted as either sensitive, intermediately sensitive or resistant based on the criteria and breakpoints set by the CLSI, EUCAST and BSAC. When interpreting Oxacillin resistance in Staphylococcus species, all Oxacillin resistant Staphylococcus aureus were taken to be methicillin resistant Staphylococcus aureus (CLSI, 2016; European Committee on Antimicrobial Susceptibility Testing, 2019; BSAC, 2013). The study also included Escherichia coli ATCC 25922 and Staphylococcus aureus ATCC 25923 as reference strains (CLSI, 2016).

3.8 Data collection tool
The questionnaire captured the demographics, admission details and clinical characteristics of the participants (appendix II). These questionnaires were administered by the principal investigator in both English and Swahili languages.

3.9 Ethical consideration
Approval for study was sought from the Kenyatta University Ethics Review Committee (KUERC) (appendix III). Authority to collect data was acquired from the research committee of Mama Lucy Kibaki Hospital (appendix XII). All the participants were briefed about the study and informed consent obtained from each of the participant
(appendix I). For minors, parents or guardians would consent on behalf of the child. The research was voluntary and those patients who didn’t take part were given the same treatment and care as those who took part. Pus was collected using a soft-sterile moistened swab minimizing any risk of contamination of the wound and subsequent pain. Details of each participant were extracted from the hospital records using serialized questionnaire, the same serial number on each questionnaire was maintained during coding therefore ensuring the anonymity of the participant details. Patient records were kept safe in lockable cabinets and password-finger print protected laptop accessed only by the principal investigator. All the study participants received treatment that was guided by sound bacteriology reports coupled by regular wound care (dressing) by the nurse on duty or intern and the attending physician.

3.10 Data analysis

The antibiogram report and data extracted from the questionnaire containing details of each patient were used to determine descriptive statistics like mean, range and percentages with the help of a Statistical Package for Social Sciences (SPSS) version 20. The association between gender and the different types of bacteria was analyzed using Chi-square (p<0.05). Results generated for each patient were presented in form of tables and charts.
CHAPTER FOUR
RESULTS

4.1 Patients demographics

In the present study, a total of 58 patients who had surgical site infection within the research period were enrolled. Out of these 19 (32.8%) were males whilst 39 (67.2%) were females, with pediatric patients accounting for 3 (5.2%) of the total cases. The disease incidence was high among patients of the age group 18-45 years, whilst low cases of SSI were observed in patients below 18 years (figure 4.1). The youngest participant was 7 years old while the eldest was 61 years and the participants had a mean age of 31.12 years.

![Figure 4.1: Patients age distribution](image)

4.2 The prevalence of bacteria isolated from surgical site infections

There were a total of 78 bacteria isolated from culture positive swabs. The prevalence of SSI isolates was as follows; Staphylococcus aureus 28.2% (n=22) was the prevalent bacteria followed by E.coli 15.4% (n=12), Acinetobacter species 14.1% (n=11), Pseudomonas aeruginosa 9.0% (n=7), Enterobacter species 9.0% (n=7), Bacillus
species 9.0% (n=7), *Coagulase negative staphylococci* 5.1% (n=4), *Proteus* species 5.1% (n=4), *Klebsiella pneumoniae* 2.6% (n=2), *Morganella morganii* 1.3% (n=1) and *Citrobacter freundii* 1.3% (n=1) (figure 4.2).

**Figure 4.2:** The prevalence of bacteria isolated from patients with surgical site infection at Mama Lucy Hospital, *Staphylococcus aureus* was the prevalent SSI bacteria isolate at the hospital.

The majority of the SSI bacteria were Gram-negative rods 57.7% (45) with Gram-positive bacteria accounting for the rest of the isolates 42.3% (33). The proportion of SSI isolates in female 73.1% (57) was more compared to male 26.9% (21) but the difference was not statistically significant ($x^2 = 2.221, p<0.136$) because the p-value was higher than the set alpha level of significance of 0.05.

### 4.3 The antibiotic sensitivity pattern of surgical site infection bacteria

Gram-positive bacteria were sensitive to Chloramphenicol 90.9% (n=30/33), Vancomycin 87.9% (n=29/33), Doxycycline 75.8% (n=25/33) and Ciprofloxacin 69.7% (n=23/33). Whereas, Gram-negative rods had sensitivity to Chloramphenicol
53.3% (n=24/45) and Amikacin 50% (n=10/20). The least sensitivity of Gram-positive and Gram-negative bacteria was observed with Ampicillin/Cloxacillin 3.0% (n=1/33) and Amoxycillin 3.7% (n=1/27) drugs respectively (Tables 4.1 and 4.2).

Table 4.1: The antibiotic sensitivity pattern of Gram-positive bacteria from patients with surgical sites infections at Mama Lucy Hospital.

<table>
<thead>
<tr>
<th>Antibiotics tested</th>
<th>Staphylococcus aureus (N=22)</th>
<th>Coagulase negative staphylococci (N=4)</th>
<th>Bacillus species (N=7)</th>
<th>Total (N=33)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vancomycin 30ug</td>
<td>20 0 2</td>
<td>3 1 0</td>
<td>6 0 1</td>
<td>29 1 3</td>
</tr>
<tr>
<td>Ciprofloxacin 30ug</td>
<td>17 2 3</td>
<td>4 0 0</td>
<td>2 3 2</td>
<td>23 5 5</td>
</tr>
<tr>
<td>Chloramphenicol 50ug</td>
<td>21 0 1</td>
<td>4 0 0</td>
<td>5 1 1</td>
<td>30 1 2</td>
</tr>
<tr>
<td>Doxycycline 30ug</td>
<td>19 2 1</td>
<td>2 1 1</td>
<td>4 1 2</td>
<td>25 4 4</td>
</tr>
<tr>
<td>Ampicillin/ Cloxacillin10ug</td>
<td>1 0 21</td>
<td>0 0 4</td>
<td>0 0 7</td>
<td>1 0 32</td>
</tr>
<tr>
<td>Azithromycin 15ug</td>
<td>11 1 10</td>
<td>1 0 3</td>
<td>0 1 6</td>
<td>12 2 19</td>
</tr>
<tr>
<td>Oxacillin 1ug</td>
<td>6 3 13</td>
<td>0 0 4</td>
<td>0 0 0</td>
<td>6 3 17</td>
</tr>
</tbody>
</table>

Key: S- Sensitive        I- Intermediately Sensitive   R- Resistant
Table 4.2: The antibiotic sensitivity pattern of Gram-negative bacteria from patients with surgical sites infections at Mama Lucy Hospital.

<table>
<thead>
<tr>
<th>Bacteria susceptibility</th>
<th>CPM 30ug</th>
<th>AK 30ug</th>
<th>CTR 30ug</th>
<th>CIP 30ug</th>
<th>C 50ug</th>
<th>DO 30ug</th>
<th>COT 25ug</th>
<th>GEN 10ug</th>
<th>AMX 30ug</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Morganella morganii</strong></td>
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<td>22</td>
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<td>26</td>
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</tbody>
</table>

CPM- Cefepime  AK- Amikacin  CTR- Ceftriaxone  CIP- Ciprofloxacin
C- Chloramphenicol  DO- Doxycycline  GEN- Gentamicin  AMX- Amoxyccillin
S- Sensitive  I- Intermediately Sensitive  R- Resistant
4.4 The resistance pattern of surgical site infection bacteria

The results presented in tables 4.3 and 4.4 show that the most resistant SSI bacteria isolates were Gram-negative rods with *Klebsiella pneumoniae* and *E.coli* recording resistance rates of 81.3% and 74% respectively. Among the Gram-positive bacteria, MRSA accounted for 65.4% (n=17/26) of all *Staphylococcus aureus* species, 59.1% (n=13/22) of the total *Staphylococcus aureus* and 100% (n=4/4) of all *Coagulase negative staphylococci* (CoNS). Overall the observed resistance rate across all isolates was 49.4%.

| Table 4.3: The resistance rate of Gram-positive bacteria isolated from surgical site infections against the drugs tested. |
|-----------------|------------|-----|-----|-----|-----|-----|-----|-----|
| Bacteria resistance | VA 30ug | CIP 30ug | C 50ug | DO 30ug | AX 10ug | AZM 15ug | OX 1ug | Total |
| *Staphylococcus aureus* | 9.1 | 13.6 | 4.5 | 4.5 | 95.5 | 45.5 | 59.1 | 33.1 |
| CoNS | 0.0 | 0.0 | 0.0 | 25.0 | 100.0 | 75.0 | 100.0 | 42.9 |
| *Bacillus* species | 14.3 | 28.6 | 14.3 | 28.6 | 100.0 | 85.7 | - | 45.2 |
| Total | 9.1 | 15.2 | 6.1 | 12.1 | 97.0 | 57.6 | 65.4 | 36.6 |

VA-Vancomycin, CIP-Ciprofloxacin, C-Chloramphenicol, DO-Doxycycline, AX-Ampicillin/cloxacillin, AZM-Azithromycin, OX-Oxacillin, CoNS-*Coagulase negative staphylococci*. 
Table 4.4: The resistance rate of Gram-negative bacteria isolated from surgical site infections against the drugs tested.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>CPM 30ug</th>
<th>AK 30ug</th>
<th>CTR 30ug</th>
<th>CIP 30ug</th>
<th>C 30ug</th>
<th>COT 25ug</th>
<th>GEN 10ug</th>
<th>AMX 30ug</th>
<th>Total resistance</th>
</tr>
</thead>
<tbody>
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<td>Morganella morganii</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>100.0</td>
<td>100.0</td>
<td>0.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Proteus species</td>
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<td>-</td>
<td>-</td>
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<td>0.0</td>
<td>100.0</td>
<td>50.0</td>
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<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
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<tr>
<td>E.coli</td>
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<td>-</td>
<td>83.3</td>
<td>58.3</td>
<td>58.3</td>
<td>66.7</td>
<td>100.0</td>
<td>50.0</td>
<td>91.7</td>
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<tr>
<td>Pseudomonas aeruginosa</td>
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<td>28.6</td>
<td>-</td>
<td>28.6</td>
<td>28.6</td>
<td>71.4</td>
<td>-</td>
<td>42.9</td>
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<td>-</td>
<td>14.3</td>
<td>14.3</td>
<td>57.1</td>
<td>57.1</td>
<td>42.9</td>
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<td>-</td>
<td>27.3</td>
<td>18.2</td>
<td>63.6</td>
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<td>63.6</td>
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<tr>
<td>Citrobacter freundii</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>100.0</td>
<td>0.0</td>
<td>0.0</td>
<td>100.0</td>
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<td>100.0</td>
</tr>
<tr>
<td>Total resistance</td>
<td>87.5</td>
<td>50.0</td>
<td>83.3</td>
<td>33.3</td>
<td>28.9</td>
<td>66.7</td>
<td>81.5</td>
<td>46.7</td>
<td>96.3</td>
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</table>

CIP- Ciprofloxacin, GEN- Gentamicin, AMX- Amoxicillin, C-Chloramphenicol, DO- Doxycycline, COT- Cotrimoxazole, CTR- Ceftriaxone, CPM- Cefepime, AK- Amikacin.

4.5 The prevalence of bacteria contaminating the wards and operating room

A total of 59 bacteria were isolated from culture positive surface swabs, with *Staphylococcus aureus* 55.9% (n=33) as the predominant bacteria followed by *Bacillus* species 30.5% (n=18), *Citrobacter* species 3.4% (n=2), *Enterobacter* species 3.4% (n=2), *Pseudomonas aeruginosa* 1.7% (n=1), *Acinetobacter* species 1.7% (n=1), *E.coli* 1.7% (n=1) and *Coagulase negative staphylococcus* 1.7% (n=1). Table 4.5 illustrates the areas where swabbing was carried out, the prevalence and the types of bacteria isolated.
### Table 4.5: The prevalence of bacteria contamining the different sections of Mama Lucy Hospital.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Surgical wards N (%)</th>
<th>Pediatric ward N (%)</th>
<th>Maternity wards N (%)</th>
<th>Operating room N (%)</th>
<th>Total N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Staphylococcus aureus</strong></td>
<td>9(56.3)</td>
<td>4(50.0)</td>
<td>16(55.2)</td>
<td>4(66.7)</td>
<td>33(55.9)</td>
</tr>
<tr>
<td><strong>Bacillus species</strong></td>
<td>6(37.5)</td>
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<td>8(27.6)</td>
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<td>18(30.5)</td>
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<td>1(3.4)</td>
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<tr>
<td><strong>Acinetobacter species</strong></td>
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<td>0(0.0)</td>
<td>1(3.4)</td>
<td>0(0.0)</td>
<td>1(1.7)</td>
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<tr>
<td><strong>Enterobacter species</strong></td>
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<td>0(0.0)</td>
<td>1(12.5)</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
<td>1(1.7)</td>
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<tr>
<td><strong>E.coli</strong></td>
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<td>0(0.0)</td>
<td>1(3.4)</td>
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<td><strong>Total no of isolates</strong></td>
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<td>8(13.6)</td>
<td>29(49.2)</td>
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<td>59(100)</td>
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Key: N- represents the number of bacteria isolated, (%) - represent the percentages

### 4.6 The antibiotic sensitivity pattern of bacteria from the wards and operating room

Majority of the isolates were resistant to Ampicillin/Cloxacillin at 96.6% (n=57/59).

However, most of them were sensitive to Ciprofloxacin 86.4% (n=51/59), Doxycycline 88.1% (n=52/59), Chloramphenicol 93.2% (n=55/59) and Vancomycin 100% (n=52/52). MRSA accounted for 61.8% (n=21/34) of the total *Staphylococci* species (Table 4.6).
Table 4.6: The antibiotic sensitivity pattern of bacteria recovered from the wards and operating room of Mama Lucy Hospital.

<table>
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<th>Bacteria susceptibility</th>
<th>VA 30ug</th>
<th>CIP 30ug</th>
<th>C 50ug</th>
<th>DO 30ug</th>
<th>AX 10ug</th>
<th>AZM 15ug</th>
<th>OX 1ug</th>
<th>CTR 30ug</th>
<th>CPM 30ug</th>
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CIP-Ciprofloxacin, AX- Ampicillin/Cloxacillin, AZM-Azithromycin, OX-Oxacillin, C-Chloramphenicol, VA-Vancomycin, DO-Doxycycline, CPM-Cefepime, CTR-Ceftriaxone, CoNS- Coagulase negative staphylococcus
CHAPTER FIVE
DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

5.1.1 The prevalence of bacteria isolated from surgical sites infections

In present study, the most prevalent SSI bacteria was *Staphylococcus aureus* 28.2% (n=22) followed by *E.coli* 15.4% (n=12). These two bacteria that is *Staphylococcus aureus* and *E.coli* are mostly skin and gastro-intestinal tract flora respectively. Therefore their high isolation rate from post-operative wounds at the current set-up was attributed to endogenous contamination of the exposed tissues with skin and gastro-intestinal tract flora during surgery.

The high prevalence of *Staphylococcus aureus* observed by the current study was analogous with findings from other research works (Moses, 2016; Banashankari *et al*., 2014; Bastola *et al*., 2017; Kurhade *et al*., 2015; Das *et al*., 2015). However, the findings by this project were in contrast with several studies which identified *E.coli* (25.5%), *Acinetobacter* species (32.03%), *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* as the predominant SSI bacteria isolates (Nwankwo *et al*., 2014; Devjani *et al*., 2013; Kokate *et al*., 2017; Patel *et al*., 2019; Billoro *et al*., 2019). This difference was attributed partly on the procedures performed by the hospital, for example surgical site infection arising following procedures involving the hollow viscera or the skin could be due to enteric rods or skin commensals like *Staphylococcus* species.

This project also isolated *Bacillus* species from patient pus swabs, which proved that *Bacillus* species especially *Bacillus cereus* should not be dismissed as contaminants of clinical samples. This agrees with findings by different researchers who were able to isolate *Bacillus* species from post-operative wounds (Ako-nai *et al*., 2013; Ebert *et al*., 2019).
The majority of the SSI bacteria isolates were Gram-negative rods 57.7% (45) with Gram-positive bacteria accounting for 42.3% (33) of the total isolates. These results were linked to antibiotic resistance, because majority of the Gram-negative bacteria isolates were more resistant compared to Gram-positive isolates. These findings were congruous with observations made by other research works which observed the predominance of Gram-negative bacteria over Gram-positive bacteria 56.5% versus 43.5% and 85 versus 27 (Tuon et al., 2018; Garg and Singh, 2017). However, the results were inconsistence with the work of two researchers who observed that Gram-positives were more compared to Gram-negatives (Khyati et al., 2014; Rolston et al., 2014). The difference in the types of bacteria isolated from the current setting to those obtained by the other researchers is because different facilities have different bacteria to which patients are at risk of acquiring SSI during a given period in time (Gelaw et al., 2014; Kokate et al., 2017).

5.1.2 The prevalence of bacteria contaminating the wards and operating room

The analysis of the wards and operating room revealed *Staphylococcus aureus* 55.9% (n=33) as the preponderant contaminant followed by *Bacillus* species 30.5% (n=18), *Citrobacter* species 3.4% (n=2), *Enterobacter* species 3.4% (n=2), *Pseudomonas aeruginosa* 1.7% (n=1), *Acinetobacter* species 1.7% (n=1), *E.coli* 1.7% (n=1) and *Coagulase negative staphylococcus* 1.7% (n=1). These results were similar to those obtained by other research works in that *Staphylococcus* species, Gram-positive and Gram-negative rods were the most common isolates (Pradhan and Shrestha, 2012; Saka et al., 2016; Atata et al., 2010). These bacteria come from either the air, patients, visitors or even health care workers. Consequently, their presence in these environments can be explained by their remarkable ability to survive for long on poorly cleaned dry surfaces (Kramer et al., 2006).
In the analysis of the wards and operating room, the proportion of Gram-positive bacteria isolates (n=52) exceeded that of Gram-negative bacteria (n=7). This results were in harmony to those of a similar study done in Ethiopia (Gelaw et al., 2014). Since Gram-positive bacteria can survive for months on dry surfaces, their presence in the two environments can be ascribed to inadequate cleaning of these surfaces (Kramer et al., 2006).

Majority of the bacteria isolates were from the maternity wards 49.2% followed by surgical ward 27.1%, paediatric ward 13.6%, and operating room 10.2% and in all these areas *Staphylococcus aureus* was the main contaminant. *Staphylococcus aureus* was also seen in 42% of the bacteria that were isolated from the floors of surgical wards of a hospital in Nigeria (Olowo-okere and Babandina, 2018). These results were contrary to those from a study of the healthcare environment in Morocco that found Enterobacteria (31.6%) as the main contaminant (Chaoui et al., 2019). The variation in the isolates recorded could be ascribed to several factors, for instance, the level of shedding by patients, culturability of organisms, the difficulty of cleaning a particular area and sampling methodology (Otter et al., 2013).

### 5.1.3 The antibiotic sensitivity pattern of bacteria isolated from surgical site infection

Gram-negative isolates were resistant to Cefepime, Ceftriaxone, Cotrimoxazole, and Doxycycline. However, relative success was observed with Chloramphenicol and Amikacin. In another instance, Gram-negative bacteria showed resistance to Cefuroxime (18.5%) and sensitivity to Lomefloxacin (70.3%) and Netillin (61.1%).

The same research noted that Gram-positive bacteria were resistant to Ciprofloxacin (52.5%) but sensitive to Ampicillin+Salbutam (87.5%) and Linezolid (85%) (Insan et al., 2013). The variation in sensitivity of Gram-positive bacteria to Ciprofloxacin
between the present and aforementioned study may be attributed to bacteria mutation which led to bacteria isolates becoming less sensitive to these antibiotics (eLife, 2017).

This project discovered that a majority of the SSI isolates were highly resistant to Ampicillin and Amoxicillin proving that the two drugs were no longer very effective in the treatment of surgical site infection at the hospital. A similar trend of wide spread resistance had been reported by a number of studies including (Al-Awaysheh, 2018; Kahsay et al., 2014). However, the relative success of Ampicillin and Amoxicillin has been documented in India (Gangania et al., 2015). Although the two drugs performed relatively well. The sensitivity of SSI bacteria isolates to these drugs was actually low, meaning that bacteria have developed a very high resistance against the two drugs.

The current research discovered that the most resistant SSI bacteria isolates were Gram-negative rods, with *Klebsiella pneumoniae* and *E.coli* recording resistance rates of 81.2% and 74% respectively. This observation was comparable with the work of a separate worker who reported a resistance rate of 75% among Gram-negative rods (Dessie et al., 2016). These high resistance rates recorded by this work can be attributed to the indiscriminate use of antibiotics at the hospital which encouraged bacteria to evolve into highly resistant pathogens. The facility also lacked mechanisms in place to identify ESBL producers like *Klebsiella pneumoniae* and *E.coli* which may prove to be difficult to treat for the clinicians.

This study recorded a Methicillin resistant *Staphylococcus aureus* rate of 65.4% which was high compared to the rate of 8.6% reported in India by (Bhave et al., 2016). Nevertheless the results by this work were similar to a study conducted in Uganda that reported a MRSA rate of 65.9% (George et al., 2018). These high levels of MRSA recorded in the current set-up may be attributed to the lack of mechanisms for MRSA
active surveillance. Moreover, the current setting lacked a sound diagnostic microbiology laboratory for bacteria culture and relied mainly on empiric therapy. This lack of antibiogram profiles and poor antibiotic stewardship, may have contributed to the emergence of bacteria resistance especially with Methicillin resistant \textit{Staphylococcus aureus} and other bacteria isolates.

5.1.4 The antibiotic sensitivity pattern of bacteria from the wards and operating room

Gram-positive bacteria had 100% sensitivity to Vancomycin. Whereas both Gram-positive and Gram-negative bacteria showed sensitivity to Chloramphenicol 93.2%, Doxycycline 88.1% and Ciprofloxacin 86.4%. The resistance of all isolates to Ampicillin/cloxacillin 96.6% was generally high. These findings were in harmony with a study done in Uganda, of which environmental bacteria isolates were 100% and 80% susceptible to Vancomycin and Ciprofloxacin respectively (Sserwadda \textit{et al.}, 2018). The two studies showed that environmental bacteria were more sensitive to the drugs tested against them. However, not all isolates were susceptible to these drugs, as a result more work is needed to identify population of resistant bacteria within the hospital environment.

The MRSA rate of 61.8% (n=21/34) for all staphylococci species observed in the current set-up was different to the work of another research worker who recorded a MRSA rate of 73.7% with \textit{Staphylococcus aureus} and 11.4% with \textit{Coagulase negative staphylococcus} (Worku \textit{et al.}, 2018). These high levels of MRSA in the current setting may be attributed to the failure by the hospital to institute mechanisms for active surveillance of MRSA, which meant that surgical patients were at risk of acquiring MRSA infection.
5.2 Conclusions

i. Among SSI bacteria isolates, *Staphylococcus aureus* was identified as the leading cause of SSI among surgical patients attending Mama Lucy Hospital.

ii. In the wards and operating room of Mama Lucy Hospital, the most prevalent bacteria contaminant was *Staphylococcus aureus*.

iii. All SSI bacteria isolates were sensitive to Chloramphenicol, but a majority of the Gram-positive and Gram-negative SSI bacteria were resistant to Ampicillin and Amoxycillin respectively.

iv. Almost all environmental bacteria isolates were resistant to Ampicillin but had sensitivity to Vancomycin, Chloramphenicol, Ciprofloxacin and Doxycycline.

5.3 Recommendations

i. The facility needs to identify the most frequent bacteria isolates associated with SSI together with their antibiograms.

ii. The hospital needs to monitor bacteria isolates that frequently contaminate the wards and operating room of Mama Lucy Hospital.

iii. The facility should consider using Chloramphenicol in the treatment of surgical site infections at the hospital.

5.4 Recommendations for further research

i. Studies should be done on Molecular characteristics of Methicillin resistant *Staphylococcus aureus* in surgical site infections.

ii. There should be an elaborate analysis of extended spectrum beta-lactamase producers (Gram-negative enterics) and constitutive or inducible Clindamycin resistance in *Staphylococcus aureus*.
REFERENCES


Asres, G., Legese, M., & Woldearegay, G. (2017). Prevalence of multidrug resistant bacteria in postoperative wound infections at Tikur Anbessa Specialized Hospital,
Addis Ababa, Ethiopia. *Archives of Medicine, 9*(4), 12.


Faruquzzaman, S., & Mazumder, S. (2012). Surgical Site Infections in Relation to the


Nedelcheva, G., Tsankova, G. S., Ermenlieva, N. M., Todorova, T. T., & Tsankova, D.


Infection, 21(11), 1008.e1-1008.e8. https://doi.org/10.1016/j.cmi.2015.07.003


APPENDICES

Appendix I-Consent form

Study title: Antibiotic susceptibility patterns of bacteria isolated from wards, operating room and post-operative wound infections among patients attending Mama Lucy Hospital, Kenya.

Principal investigator: Amulioto Johnstone Auna, Bsc. (MLS), Post-graduate Student Kenyatta University.

Co-investigators: Dr. Margaret Muturi (PHD), Dr. Scholastica Mathenge (PHD)

Aim of the study: To determine the prevalence and antibiotic susceptibility patterns of bacteria isolated from wards, operating room and surgical sites infections among patients attending Mama Lucy Hospital.

Procedure: Participation in this study will require that I ask you a few questions before obtaining a swab from your wound area using a sterile moistened cotton swab for testing. But please remember that this study is voluntary and your decision to refuse or take part in the study will not in any way change the care and treatment you will receive from this hospital today or in future.

Risks and benefits: The study involves collection of wound pus using a painless sterile moistened cotton swab, although you might experience minimal discomfort during maneuvering of the skin areas, I guarantee you that it is safe and there will be no harm. The findings generated from positive samples will help the clinician to administer appropriate antibiotics for the treatment of your infection.

Cost: This study is free to all patients who are willing to participate, no fee is charged for participation.
Confidentiality: Participants information will be collected on a serialized questionnaire and the same serial number will be used in coding and this will ensure that your details remain anonymous. Therefore at no point in time will the name of the participant appear anywhere in the study. The participants’ records will also be kept in secure lockable cabinets only accessible by the principal investigator.

Participant:
I have read and understood fully the contents of this form. My questions have been answered by the investigator. I also understand that the study is voluntary and whether I decide to participate in the study or not, will not in any way affect my care and treatment at this hospital. I____________________________ voluntarily agree to take part in the study mentioned above.

Participant’s Name______________________________

Signature_____________ Date__________

The investigator: I confirm that I have answered all your questions regarding this study and that you have voluntarily consented to participate in the study

Investigator’s name ______________________________

Signature_________________________ Date__________

If you have any queries about this study, feel free to contact the principal investigator Amulioto Johnstone Auna, P.o box 43844-00100, Nairobi, Tel 0738815499 or Dr. Margret Muturi, Kenyatta University, Tel 0722758523. Concerns about your rights as participants have been addressed by Kenyatta University Ethics Review Committee.
Appendix II-Questionnaire

Serial number___________

Demographics

Patient name_______________________ Sex________________________
Age________________________ Patient number____________________

Clinical history

Date of admission_______________ Date of discharge______________
Length of hospital stay____________
Appendix III- KUERC approval letter

KENYATTA UNIVERSITY
ETHICS REVIEW COMMITTEE

Fax: 8711242/8711575
Email: kuerc.chairman@ku.ac.ke
        kuerc.secretary@ku.ac.ke
Website: www.ku.ac.ke

P. O. Box 43844,
Nairobi, 00100
Tel: 8710901/12

Our Ref: KU/ERC/ APPROVAL/VOL.I (157) Date: 13th July, 2018

AMULIOTO JOHNSTONE AUNA
P. O. Box 625 – 00100,
Nairobi

Dear Johnstone,

APPLICATION NUMBER: PKU/700/1772 “DETERMINATION OF ANTIBIOTIC
SUSCEPTIBILITY PATTERNS OF ISOLATES IN POST-OPERATIVE WOUND
INFECTIONS AMONG PATIENTS AT MAMA LUCY KIBAKI HOSPITAL”

1. IDENTIFICATION OF PROTOCOL
The application before the committee is with a research topic “Determination of Antibiotic
Susceptibility Patterns of Isolates in Post-Operative Wound Infections among Patients at
Mama Lucy Kibaki Hospital” received on 18th July 2017 and discussed on 12th June, 2018

2. APPLICANT
Amulioto Johnstone Auna

3. SITE
Mama Lucy Kibaki Hospital

4. DECISION
The committee has considered the research protocol in accordance with the Kenyatta University
Research Policy (section 7.2.1.3) and the Kenyatta University Ethics Review Committee
Guidelines and APPROVED that the research may proceed for a period of ONE year from
12th June, 2018.
5. ADVISE/CONDITIONS

i. Progress reports are submitted to the KU-ERC every six months and a full report is submitted at the end of the study.

ii. Serious and unexpected adverse events related to the conduct of the study are reported to this committee immediately they occur.

iii. Notify the Kenyatta University Ethics Committee of any amendments to the protocol.

iv. Submit an electronic copy of the protocol to KUERC.

When replying, kindly quote the application number above.
If you accept the decision reached and advice and conditions given please sign in the space provided below and return to KU-ERC a copy of the letter.

PROF. JUDITH KIMIYWE
CHAIRPERSON. ETHICS REVIEW COMMITTEE

I \underline{\text{\textsc{\textbf{I accept the advice given and will fulfill the conditions therein.}}}}

Signature \underline{\text{\textsc{\textbf{\textday}}} \text{\textbf{\text{17 JUL 2018}}}}

\underline{\text{\textbf{\textday}}} \text{\textbf{\text{17 07 18}}}

cc. DVC-Research Innovation and Outreach
Appendix IV- Gram staining technique

Principle

Gram staining technique is used for differentiating bacteria into two groups which are Gram-negative and Gram-positive based on the physical and chemical properties of their cell walls. Gram-positive bacteria have a thick cell wall which can resist decolourization therefore they retain the colour of the initial stain which is crystal violet. Whereas Gram-negative bacteria appear pink in colour because of their thin cell wall which is easily washed away by a slight alcohol wash permitting the escape of the initial stain and allowing the cell to take the colour of the counterstain.

Procedure

i. The heat fixed smears were flooded with crystal violet stain for 1 minute
ii. The excess stain washed off with clean water
iii. The slides were then covered with lugol’s iodine to fix the stain for 1 minute
iv. The excess iodine washed off with clean water
v. Followed by rapid decolourization using acetone until the acetone running from the slides was clear.
vi. Washed off the acetone with clean water and counterstained the slides with neutral red stain for 2 minutes.
vii. Washed off the excess counter stain with clean water, wiped the backs of the slides with a clean gauze and left the slides to air dry on a draining rack.
viii. Applied immersion oil and observed the slides microscopically x100

Results interpretation

Gram-positive bacteria ………. appear purple example *Staphylococcus aureus*

Gram-negative bacteria……….. appear pink example *Pseudomonas aeruginosa*
Appendix V - Biochemical tests

Indole test

Citrate test
Urease test
Antibiotic susceptibility patterns of bacteria isolates from post-operative wound infections among patients attending Mama Lucy Kibaki Hospital, Kenya

Johnstone Amulioto1*, Margaret W. Muturi1, Scholastica Mathenge1 and Gideon M. Mutua2

1 School of Medicine, Kenyatta University, P. O Box 43844-00100 Nairobi, Kenya. 2 Department of Obstetrics and Gynecology, Mama Lucy Kibaki Hospital, P. O Box 1278-00515 Nairobi, Kenya.

Received 11 May, 2020; Accepted 10 June, 2020

Surgical site infections account for high mortality, morbidity, and elevated costs of treatment for surgical patients. The study sought to determine the prevalence and antibiotic susceptibility patterns of bacterial isolates from postoperative wound infections among patients attending Mama Lucy Kibaki Hospital. A cross-sectional descriptive study was carried out between October 2018 and March 2019. It included patients of all age groups with surgical site infections following general, obstetrics, and gynecological surgeries. Pus swabs were obtained aseptically from 58 consented patients with clinical evidence of surgical site infections. Gram stain, culture, biochemical tests, and antibiotic susceptibility tests were done for each pus swab. The preponderant isolate was Staphylococcus aureus (28.2%) followed by Escherichia coli (15.4%). Whereas Methicillin-resistant S. aureus accounted for 65.4% (n=17) of the total Staphylococcus species. Chloramphenicol was the most sensitive drug to all the bacteria isolates. Ampicillin and amoxycillin recorded resistance rates >90% against Gram-positive and Gram-negative bacteria. The majority of the Gram-negative rods were highly resistant. Hence, this calls for continuous monitoring of the susceptibility patterns to determine the profile of surgical site infections bacteria isolates found in the hospitals.
Appendix VII- Culture plates

Bacteria swarming on blood agar

Lactose fermenter on MacConkey agar
Appendix VIII- An algorithm for identifying Gram-positive bacteria

- **Gram-positive**
  - Cocci
    - Aerobes
      - Catalase test
        - Catalase positive examples
          - Staphylococcus species
        - Catalase negative examples
          - Streptococcus species
      - Coagulase test
        - Coagulase positive examples
          - S. aureus
        - Coagulase negative examples
          - S. epidermidis
          - S. saprophyticus
      - Novobiocin test
        - Novobiocin sensitive examples
          - Staphylococcus epidermidis
        - Novobiocin resistant examples
          - Staphylococcus saprophyticus
  - Rods
    - Anaerobes
      - Peptostreptococcus
    - Aerobes
      - Bacillus species
    - Anaerobes
      - Clostridium species

- Coagulase test
  - Beta hemolysis
  - Alpha hemolysis
  - Gamma hemolysis
  - S. pyogenes
  - S. pneumoniae
  - S. viridans

- Novobiocin test
  - Optochin test
  - Optochin sensitive examples
    - S. pneumoniae
    - S. viridans
Appendix IX - Preparation of MacConkey agar

a) Suspend 49.53 grams of the MacConkey powder in 1000 mls of distilled water

b) Heat to boiling to completely dissolve the medium

c) Autoclave the medium at 121°C for 15 minutes

d) Cool the medium to 45-50°C, mix well and then pour it into sterile petri dishes
Appendix X- Preparation of Blood agar

a) Suspend 40 grams of the powder in 1000mls of distilled water

b) Heat to boiling to completely dissolve the medium

c) Autoclave the medium at 121°C for 15 minutes

d) Cool the medium to 45-50°C and aseptically add 5% sterile defibrinated blood

e) Mix well and pour the medium into sterile petri dishes
Appendix XI- Preparation of Mueller Hinton

a) Suspend 38 grams of the powder in 1000 mls of distilled water

b) Heat to boiling to completely dissolve the medium

c) Autoclave the medium at 121\(^\circ\)C for 15 minutes

d) Cool the medium to 45-50\(^\circ\)C, mix well and then pour it into sterile petri dishes
Appendix XII - Mama Lucy hospital research approval letter

Telephone: Nairobi
020 - 2297000

E-mail: medsupmedh@yahoo.com

When replying please quote

OUR REF: MLKH/ADM/RES/1/4( )

DATE: 19th September 2018

AMULIOYO JOHNSTONE AUNA
P.O. BOX 625-00100
NAIROBI

RE: TEMPORARY PERMISSION TO COLLECT DATA

TITLE: “DETERMINATION OF ANTIBIOTIC SUSCEPTIBILITY PATTERNS OF ISOLATES IN POST-OPERATIVE WOUND INFECTIONS AMONG PATIENTS AT MAMA LUCY KIBAKI HOSPITAL”

Refer to your application to collect data on the above research in this institution.

This is to inform you that the hospital has given you temporary permission to allow you collect data which expires after the next Research Committee Meeting.

Dr. MUSA MOHAMMED
MEDICAL SUPERINTENDENT