ANTIBACTERIAL SUSCEPTIBILITY PATTERNS AND RISK FACTORS FOR SURGICAL SITE INFECTIONS AT MOI TEACHING AND REFERRAL HOSPITAL, ELDORET-KENYA

OKELLO STEPHEN ONYANGO

A thesis submitted in partial fulfillment for the requirement of the degree of Master of Medicine (Orthopedic Surgery) of Moi University

DECLARATION

DECLARATION BY CANDIDATE:

This thesis is the candidate's original work and has not been presented for a degree in any other university. No part of this thesis may be reproduced without prior written permission of the author and/or Moi University.

Stephen Onyango Okello

SM/PGORT/01/2013

SIGN: _____ DATE: _____

DECLARATION BY THE SUPERVISORS:

This thesis has been submitted for examination with our approval as Moi university supervisors.

PROF. K. R. TENGE

Consultant Pediatric Surgeon and Professor of Surgery,
Moi University, School of Medicine, Eldoret-Kenya

Signature: _____ Date: _____

DR. B. AYUMBA

Consultant Orthopedic Surgeon and Senior Lecturer
Moi University, School of Medicine, Eldoret

Signature: _____ Date: _____

DR. E. RUTO

Lecturer, Department of Microbiology
Moi University, School of Medicine, Eldoret

Signature: _____ Date: _____

DEDICATION

This thesis is dedicated to my dear wife Lilian and to my children Michael and Myles.

ACKNOWLEDGEMENT

The candidate would like to thank Moi University School of Medicine and the Department of Orthopedic and Rehabilitation for the guidance offered throughout the development of this thesis. Thanks to Moi Teaching and Referral Hospital (MTRH) fraternity especially the administration for allowing the study to be done at MTRH, the microbiology laboratory staff for supporting the candidate in specimen examination, the surgical and orthopedic wards and clinic staff who assisted with identification of participants and supported the research process. Special thanks to my supervisors: Prof. K. R. Tenge, Dr. B.R. Ayumba and Dr. E. Ruto for their guidance. Appreciation to my colleagues for their constructive criticism. Thanks to the patients who agreed to participate in this research, without whom this study would not have been actualized. Last is to the Almighty God for giving me life and strength to develop this thesis.

ABBREVIATIONS AND ACRONYMS

AFB	Acid-Fast Bacilli
AMPATH	Academic Model Providing Access to Healthcare
ASA	American Society of Anesthesiologists
BA	Blood Agar
CBA	Chocolate Blood Agar
CDC	Centers for Disease Control and Prevention
CONS	Coagulase Negative *Staphylococci*
DRC	Democratic Republic of Congo
HIV	Human Immunodeficiency Virus
ICU	Intensive Care Unit
IREC	Institutional Research and Ethics Committee
ITC	International Tobacco Control
MAC	MacConkey Agar
MDR	Multi-Drug resistant
MIC	Minimum Inhibitory Concentration
MRSA	Methicillin Resistant *Staphylococcus aureus*
MSSA	Methicillin Sensitive *Staphylococcus aureus*
MTRH	Moi Teaching and Referral Hospital
NHSN	National Healthcare Safety Network
ORIF	Open Reduction and Internal Fixation
SOP	Standard Operating Procedures
SSI	Surgical Site Infection
THR	Total Hip Replacement
TSI	Tripple Sugar Iron
ZN	Ziehl-Neelsen Smear

OPERATIONAL DEFINITION OF TERMS

Antibacterial: An agent that kills bacteria or stops their growth.

Clean Wound: Uninfected operative wounds in which no inflammation is encountered; respiratory, gastrointestinal, genital, or urinary tract is not entered; wound is closed primarily.

Clean-contaminated wound: Operative wounds in which the respiratory, gastrointestinal, genital or urinary tract is entered under controlled conditions and without unusual contamination.

Contaminated Wound: Open, fresh accidental wounds or operations with major breaks in sterile technique, or gross spillage from a viscus and wounds with acute, purulent inflammation.

Deep incisional infection: Surgical site infection involving deep tissues including fascia and muscle layers

Dirty Wound: Old traumatic wounds with retained devitalized tissue, foreign bodies, or fecal contamination or wounds that involve existing clinical infection or perforated viscus.

Multi-drug resistant Non-susceptibility to at least 1 agent in 3 or more antibacterial categories

Organ/ space infection: Surgical site infection involving any part of the anatomy other than the incision opened or manipulated during the surgical procedure.

Risk Factor: A characteristic or condition that increases the likelihood of developing surgical site infection.

Surgical Site Infection: Infection occurring at the site of surgical operation within 30 days following the date of surgery if no implant is in place or within 1 year if an implant is in place and the infection is related to the surgical procedure.

Superficial infection: Surgical site infection involving only the skin or subcutaneous tissue

Susceptibility: A test that provides information on the response of a bacteria to an antibiotic. Reported as either susceptible, intermediate or resistant.

ABSTRACT

Background: Surgical Site Infection (SSI) poses a burden to patients and the healthcare system by increasing cost, hospital stay as well morbidity and mortality. The incidence of SSI in sub-Sahara Africa is around 10% for clean wounds and up to 60% for dirty wounds. The etiology, risk factors and antibacterial susceptibility among patients with SSI is largely unknown at the Moi Teaching and Referral Hospital (MTRH).

Objective: To determine the risk factors, bacterial etiology and antibacterial susceptibility of surgical site infections at Moi Teaching and Referral Hospital, Eldoret-Kenya.

Methods: This was a case control study involving 57 cases of SSI and 114 matched controls. Questionnaires were administered to all study patients to collect data on sociodemographic characteristics, wound class and potential risk factors for SSI. Pus swab was collected from the cases and inoculated in culture media after which antibacterial susceptibility test was done on isolated organisms using modified Kirby-Bauer disc diffusion in Mueller Hilton agar. Blood cultures were done for patients who presented with systemic features of infection including fever of 37.5^0C and above. Frequencies and proportions were determined for risk factors, etiology and antibacterial susceptibility. Predisposing factors were compared between cases and controls using the chi-square test to determine "p" values and Odds ratios. A "p" value of less than 0.05 was considered significant.

Results: Risk factors for SSI were smoking ($p<0.001$, OR=5.8), diabetes mellitus ($p=0.025$, OR=3.5) and long operation time ($p<0.001$, OR=1.5). A total of 55 bacterial organisms were isolated from 46 patients. Out of these 5 were from 12 blood cultures done. The most common isolate was *Staphylococcus aureus*, 22 (40.0%) followed by *Escherichia coli,* 11 (20.0%), *Acinetobacter baumannii- 6 (*10.9%), *Klebsiella pneumoniae* -5 (9.1%), *Pseudomonas aeruginosa*-4(7.3%), *Proteus mirabilis* -2(3.6%) and *Streptococcus pyogenes* – 1 (1.8%). *Methicillin Resistant Staphylococcus Aureus (MRSA)* comprised 59% (13) of all *Staphylococcus aureus*. Gram positive bacteria had over 50% resistance to ceftriaxone, cotrimoxazole, ciprofloxacin, azithromycin, erythromycin, cefuroxime and levofloxacin. Gram negative bacteria had more than 50% resistance to ceftriaxone, cefotaxime, ceftazidime, cefepime and levofloxacin. MRSA and *Acinetobacter baumannii* showed multidrug resistance.

Conclusion: Smoking, diabetes mellitus and prolonged operation time are risk factors for SSI. *Staphylococcus aureus* was the commonest causative agent for SSI with *MRSA* constituting 59% of *Staph aureus* infection. Organisms causing SSI were resistant to most commonly used antibacterial agents at MTRH.

Recommendations: Active surveillance for SSI causing organisms and their susceptibility patterns should be instituted at MTRH. Antibacterial use should be rationalized according to local susceptibility patterns. Patients with diabetes mellitus, history of cigarette smoking and prolonged operation time should be closely monitored for SSI.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER ONE: INTRODUCTION

1.1 Background of the Study

Surgical Site Infection (SSI) refers to infection occurring at the site of surgical operation within 30 days of surgery if no implant is in place or within one year if an implant is in place and the infection appears to be related to the surgical procedure (Stone *et al*., 2012). SSI can be classified as either incisional (superficial or deep) or organ/space infection (Berríos-Torres *et al.*, 2017). Superficial incisional infection refers to SSI involving only the skin or subcutaneous tissue while deep incisional infection refers to SSI involving deep tissues including fascia and muscle layers. Organ/ space infection is defined as SSI involving any part of the anatomy other than the incision opened or manipulated during the surgical procedure. Diagnosis features of surgical site infection include: purulent drainage from incision site or drain, organism yield from culture, pain or tenderness, localized swelling, redness as well as fever or localized heat (Henriksen *et al*., 2010). SSI increases morbidity and mortality among post-operative patients, it doubles hospital stay and significantly increases the cost of hospitalization for surgical patients (Kirkland *et al*., 2015).

Surgical wounds are classified as clean, clean-contaminated, contaminated or dirty. Clean wounds refer to uninfected operative wounds in which no inflammation is encountered; respiratory, gastrointestinal, genital, or urinary tract is not entered; and the wound is closed primarily (Berríos-Torres *et al.*, 2017). Examples include Joint replacement surgeries, mastectomy, thyroid surgery, splenectomy, herniorrhaphy as well as Open Reduction and Internal Fixation (ORIF) for closed fractures. Clean-contaminated wounds are operative wounds in which a Respiratory, Gastrointestinal, Genital or Urinary tract is entered under controlled conditions and without unusual

contamination. Contaminated wounds are open, fresh accidental wounds or operations with major breaks in sterile technique, or gross spillage from a viscus or wounds in which acute, purulent inflammation is encountered. Dirty wounds refer to old traumatic wounds with retained devitalized tissue, foreign bodies, or fecal contamination or wounds that involve existing clinical infection or perforated viscus (Stone *et al.,* 2012). The incidence of SSI is largely dependent on the wound class whereby clean surgical wounds generally report rates less than 10% in most settings (Benito *et al*., 2016; Shao *et al*., 2017). In the developing countries, infection rates following surgery have been reported variously and vary with the type of surgical wound but mostly ranges around 10% with other centers reporting rates as high as 26% (Lubega *et al.,* 2017; Mawalla *et al.,* 2011).

Patients who have any form of immune-suppression are more likely to develop SSI. This includes those with uncontrolled diabetes mellitus as well as HIV positive patients who have not achieved adequate immunological control (Mawalla *et al*., 2011). Other patient related factors that increase risk of SSI are increased age, obesity, poor ASA (American Society of Anesthesiologists) score and history of smoking. In addition, prolonged pre-operative hospital stay, prolonged operation duration, emergency as opposed to elective surgeries, use of a drain and limited surgeons experience increase risk of SSI (Blood *et al.,* 2017; Mawalla *et al*., 2011; Wang *et al*., 2017).

Most studies have shown *Staphylococcus aureus* as the most common isolate from SSI wounds (Mawalla *et al*., 2011; Mengesha *et al.,* 2014). However, others have shown a predominance of the gram negative bacteria such as *Pseudomonas aeruginosa* and *Klebsialla pneumonia* (Lubega *et al*., 2017; Manyahi *et al*., 2014).

Infection with Methicillin-resistant *Staphylococcus aureus* (MRSA) leads to increased mortality and hospital cost, high re-admission rates as well as increased hospital stay compared to methicillin-susceptible *Staphylococcus aureus* (Anderson *et al.,* 2009). The incidence of MRSA infection is reported to vary in different settings with some reporting as much as 75 -100% of all *Staphylococcus aureus* as being MRSA (Godebo *et al.,* 2013; Helal *et al.,* 2015). Most developed countries have comprehensive SSI surveillance programs and are therefore able to monitor trends of etiological agents of SSI which guides antibiotic prescription and allows for targeted interventions to control and prevent SSI (Bull *et al.,* 2017). Surveillance is however lacking in most of the developing world including the sub-Sahara region (Nejad *et al.*, 2011).

Emergence of antibacterial resistant strains has been demonstrated among SSI causing organisms (Iyamba *et al.*, 2014; Lubega *et al*., 2017; Manyahi *et al*., 2014; Pahadi *et al.*, 2014). This is mainly attributed to increased use of antibiotics in the community (Ventola, 2015) . In most of sub-Sahara Africa, there is either lack of or inadequate SSI surveillance programs thus centers are unable to update knowledge on the antibacterial resistance of SSI causing bacteria and hence lack evidence based preventive strategies (Aiken *et al.*, 2012).

1.2 Statement of the problem

Surgical Site Infection (SSI) remains one of the major post-operative complications following surgery at MTRH as is the world over. This has led to increased hospital stay, increased cost, morbidity and mortality. There is no regular surveillance program for SSI at the MTRH hence the causative organisms and their susceptibility patterns are largely unknown. Antibacterial use for both prophylaxis and treatment of SSI at

the MTRH is thus not guided by local etiology and susceptibility patterns. The effectiveness of such prophylaxis and treatment is therefore not guaranteed. Since SSI often occurs in the course of hospital stay, it is not captured as the patients primary diagnosis hence rarely ends up in the Hospital Management Information System (HMIS) at the MTRH. The burden of SSI at MTRH is therefore often underestimated when the routine HMIS data is relied upon for decision making. Little is also known on the risk factors for SSI at the MTRH hence much as there is an infection control department in the hospital, the prevention interventions are not guided by local information on risk factors.

1.3 Justification of the study

It is important to predict the likely organisms that cause surgical site infections especially where culture and sensitivity testing has limited availability. This allows optimization of the choice for antibiotic prophylaxis as well as empirical treatment of surgical site infections (Stone *et al*., 2012). In the absence of a surveillance program, regular studies are needed to inform the hospital management and clinicians on the organisms causing SSI and their susceptibility patterns. There are inadequate studies in the Kenyan context that have characterized SSIs with a view of identifying the offending organisms and their sensitivity patterns (Aiken *et al*., 2012). Most studies available in literature have focused on finding out the incidence of surgical site infection without further description of the patterns of infection such as the etiological organisms (Kigera & Gakuu, 2013).

The most recent study available on SSI organisms and their susceptibility patterns at MTRH was that from 2002 (Andhoga *et al*., 2002). It was expected that a lot must have changed regarding the etiology and susceptibility patterns. An evaluation of the

risk factors of SSI in the MTRH set up would guide infection prevention measures and patient optimization prior to surgery. The knowledge of etiological bacteria and their antibacterial susceptibility patterns would guide antibacterial use for prophylaxis and treatment of SSI. This study fills the gap in literature by describing the risk factors, bacterial etiology and antibacterial susceptibility of SSI at MTRH.

1.4 Research question

How is the antibacterial susceptibility and risk factors for bacterial surgical site infections at surgical and orthopedic department of the Moi Teaching and Referral Hospital – Eldoret, Kenya?

1.5 Objectives of the study

1.5.1 Broad Objective

To assess the antibacterial susceptibility patterns and risk factors for bacterial surgical site infections at surgical and orthopedic department of the Moi Teaching and Referral Hospital (MTRH) – Eldoret, Kenya

1.5.2 Specific objectives

i. To determine the risk factors for surgical site infections among patients at surgical and orthopedic departments.
ii. To determine the bacterial species causing surgical site infection among patients in surgical and orthopedic departments.
iii. To describe the susceptibility patterns of bacterial species causing surgical site infections in surgical and orthopedic departments.

CHAPTER TWO: LITERATURE REVIEW

2.1 Overview of Surgical Site Infection

Surgical site infection (SSI) poses a major burden to patients and the health care system. SSI doubles mortality, admission in to intensive care unit (ICU) and hospitalization cost in post-operative patients as well as increasing hospital readmission by close to five times (Kirkland *et al*., 2015). The incidence of SSI varies in different settings and is largely dependent on the wound class. Some centers have reported overally high SSI incidence of up to 26% considering all wound classes (Mawalla *et al*., 2011).

For clean surgical wounds, infection rates are mostly reported to be less than 1% in most settings in developed countries (Elgohari *et al.*, 2013). A study in Kikuyu Hospital – Kenya found an incidence of 1.5% for early post-operative infection among patients undergoing total hip replacement (Kigera & Gakuu, 2013). Infection rates following elective hernia repair (a clean elective surgery) has also been reported as approximately 1% (Rodríguez *et al*., 2013; Warwick *et al*., 2013). Some studies have however reported relatively high infection rates even in clean surgical wounds. For example, an evaluation of hospital infections after major amputations in Brazil showed that 7.3% of patients returned with amputation stump infection (de Godoy *et al.*, 2010). Other studies have shown high infection rates of more than 10% even with clean wounds (Ameh *et al*., 2008).

Clean-contaminated surgical wounds have infection rates of about 10%. For example, surgical site infection rate following emergency abdominal surgery in Kigali Rwanda was reported as 8.7% wound infection and 4.8% septicemia (Nyundo *et al.*, 2013).

Ameh *et al.,* (2008) however reported infection rate of 19.3% in clean-contaminated wounds among children.

SSI rate is generally high in contaminated wounds. Ameh *et al.,* (2008) reported incidence of SSI among this category to be 27.3%. Elgohari *et al.,* (2013) found incidence of SSI of up to 30% for contaminated wounds in hospitals in England. Dirty wounds have high infection rates of 30-60% (Ameh *et al.,* 2008). Within the various categories of wound classes, SSI incidence also varies from one center to another.

2.2 Risk Factors for Surgical Site Infection

Among patients undergoing surgery, some are more likely to develop surgical site infection (SSI) more than others. This is due to both patient related and surgery/ hospital related risk factors for surgical site infection (Namba *et al.*, 2013). Among the patient related factors, cigarette smoking has been shown to be a consistent predictor of surgical wound complications including SSI (Møller *et al*., 2003). The increased incidence of SSI among tobacco users has been shown in most studies analyzing risk factors for SSI (Blood *et al*., 2017). SSI surveillance programs in the developed world have shown higher incidence among smokers compared to the nonsmokers (Elgohari *et al.,* 2013). In China, a study demonstrated an increase in SSI among smokers with 2.13 Odds ratio (Shao *et al.*, 2017). Mawalla *et al.,* (2011) in Tanzania showed that smoking increases risk of SSI with an Odds Ratio of 9.6. The prevalence of smoking seems to be rising in developing countries such as Kenya, Uganda, Gambia and Liberia (Nturibi *et al.,* 2009). Smoking lowers oxygen tension in wounds and leads to local tissues hypoxia (Sørensen *et al.,* 2009). In 2012, the prevalence of smoking among adults in Kenya was at 16% and rising (ITC Project, 2015). The smoking prevalence is even higher in particular groups of the Kenyan

population such as college students where a smoking rate of up to 42.8% has been reported (Atwoli *et al.,* 2011). It is therefore postulated that smoking will continue to play a major role in increasing the risk of SSI in developing countries such as Kenya as the prevalence of smoking increases.

Patients with pre-morbid conditions and/ or compromised immune status are more likely to develop SSI (Mawalla *et al*., 2011). Compromised immunity can be due to Human Immunodeficiency Virus (HIV) infection, chronic steroid use, Diabetes mellitus, and advanced age among other causes. Some studies have found that People Living with HIV (PLWHIV) are more likely to develop SSI than the HIV negative population (Issa *et al*., 2013). Other studies have however shown no increased risk of SSI among HIV positive patients as long as they are on stable antiretroviral therapy and have achieved immunological and virological control (Blood *et al.*, 2017; Kigera *et al.*, 2012). Since HIV remains a major burden in sub Saharan Africa, more evidence is still needed on the role of HIV status in predicting development of surgical site infection.

Diabetes mellitus has been consistently shown to increase the risk of developing surgical site infections by contributing to lowered immunity making the surgical patient more susceptible to infection (Mawalla *et al.*, 2011). Poorly controlled blood sugars also provide a favorable environment for bacterial proliferation hence increased risk of SSI (Guo & Dipietro, 2010). In a meta-analysis involving spine surgery patients, diabetes mellitus was shown to be one of the patient related risk factors for SSI alongside other factors with diabetes increasing the risk by more than threefold (Blood *et al*., 2017). Inadequate glycemic control among diabetic patients further increases the risk of SSI (Namba *et al.*, 2013). Other factors that contribute to

increased risk of hospital acquired infections include: increasing age of patient; patient's ASA (American society of anesthesiologists) score -3 or more increases risk and high body mass index (BMI) - Overweight or Obese (Elgohari *et al*., 2013; Namba *et al*., 2013).

In addition to patient related factors that predispose to surgical site infections, there are factors relating to the surgery itself or the health care system. These include prolonged operation time, prolonged hospital stay before surgery and emergency as opposed to elective surgery (Ameh *et al*., 2008; Helal *et al*., 2015). The longer the duration of operation, the higher the risk of developing SSI (Blood *et al.*, 2017;Shao *et al.*, 2017; Wang *et al*., 2017). The relationship between the surgeon's experience and surgical site infection has been reported variedly with some authors finding fewer infections with more experienced/ specialized surgeons while others have found no correlation (Wloch *et al.*, 2012). Knowledge of local risk factors for SSI coupled with an elaborate surveillance system and infection control program that include feedback of infection rates to surgeons is associated with significant reductions in surgical site infection (Astagneau *et al.*, 2009).

2.3: Bacterial pathogens causing surgical site infection

Staphylococcus aureus is the most commonly isolated bacterial species in surgical site infections in most settings (Chahoud *et al.*, 2014; Mengesha *et al.*, 2014). This is especially true in SSI following orthopedic operations where *Staphylococcus aureus* is often the predominant organism (Helal *et al.*, 2015). In a 3½ year study in West Bengal, India, *Staphylococcus aureus* was reported in 34.93% of SSI cases followed by *Escherichia coli* (20.34%), *Klebsiella pneumoniae* (18.08%), *Pseudomonas aeruginosa* (7.99%), *Acinetobacter baumannii* (7.49%) respectively (Bhattacharya *et*

al., 2016). In China *Staphylococcus aureus* was also the most commonly isolated organism causing SSI, accounting for 19.1% of all isolates though this varied with the type of surgery with SSI from chest surgery having a prevalence of *Staphylococus aureus* of 41.1% and SSI from abdominal surgery having a prevalence of 13.8% (Yang *et al.*, 2015).

Other studies have however found a predominance of gram negative organisms that commonly cause other hospital acquired infections in SSI. In hospital surveillance in England, *Enterobacteriaceae* were the predominant organisms accounting for 29% of total isolates followed by *Staphylococcus aureus* at 24% (Elgohari *et al.*, 2013). *Escherichia coli* is a common isolate in SSI following abdominal surgeries that involve entry into the gastrointestinal tract (Bhattacharya *et al.*, 2016). In Muhimbili – Tanzania, showed a predominance of *Pseudomonas aeruginosa* (16.3%) was seen in causation of SSI, followed by *Staphylococcus aureus* (12.2%) and *Klebsiella pneumoniae* (10.8%) (Manyahi *et al.*, 2014). A study in Mulago Hospital in Uganda found a predominance of *Escherichia coli* (23.7%) followed by *Staphylococcus aureus* (21.1%) as the leading agents in surgical site infections (Seni *et al.*, 2013).

Organisms that were not considered traditionally as a cause of SSI have recently been isolated in different settings. For example, *Acinetobacter baumannii* has been shown to have a prominent role and increasing in a linear pattern (Benito *et al.*, 2016; Helal *et al.*, 2015). This was also observed by Elgohari *et al* (2013) in England where 0.4% of isolates in hospital surveillance for SSI were *Acinectobacter baumanii*. They also identified other unusual bacterial organisms including Coagulase Negative *Staphylococci* (13.8%), *Enterococcus spp* (8.3%), Anaerobes (6.1%) and *Streptococcus spp* (4.0%). The bacterial diversity in SSI has also been shown in the

United States of America where two previously uncharacterized *Bacteroidales* were identified in addition to *Corynebacterium spp., Peptoniphilus spp., Staphylococcus spp., Staphylococcus aureus, Serratia marcescens, Prevotella spp*. and *Pseudomonas aeruginosa* (Wolcott *et al.*, 2009).

Among the *Staphylococcus,* Methicillin Resistant *Staphylocuccus aureus* (MRSA) has high virulence and resistance rates to most antibacterials (Bhattacharya *et al.*, 2016; Pahadi *et al.*, 2014). It has been shown that patients infected with MRSA have three times more mortality at 90 days, greater duration of hospitalization, and up to twice more hospital charges compared to patients who have Methicillin susceptible *Staphylococcus aureus* (MSSA) infection (Anderson *et al.*, 2009).

MRSA could be on the rise among SSI cases. For example, in India Bhattacharya *et al*., (2016) reported MRSA rate to be 25.45% of all *Staphylococcus aureus* while in China 41.3% of all *Staphylococcus aureus* isolates from SSI wounds were reported as MRSA (Yang *et al.*, 2015). In England, hospital surveillance for SSI in 2012/2013 showed that 18% of the *Staphylococcus aureus* isolated were Methicillin resistant (MRSA) which accounted for 4% of all bacterial isolates (Elgohari *et al.*, 2013).

MRSA prevalence in the hospital set up in Africa could be higher than most rates reported in other parts of the world. For example, a study in Kinshasa, Democratic Republic of Congo, 63.5% of all *Staphylococcus aureus* isolated among SSI cases were found to be MRSA (Iyamba *et al.*, 2014). In Egypt, MRSA rate of 100% of *Staphylococcus aureus* has been reported in SSI after orthopedic operations (Helal *et al.*, 2015). In Uganda, Ojulong *et al.*, (2009) found MRSA prevalence of 31.5% of *Staphylococcus aureus* while Seni *et al.*, (2013) found MRSA rate of 37.5%. Manyahi *et al.*, (2014) reported that 44% of *Staphylococcus aureus* isolates causing

SSI in Muhimbili, Tanzania were MRSA. In Ethiopia MRSA was found to constitute 49.7% of all *Staphylococcus aureus* isolates causing SSI (Kahsay *et al.*, 2014). In the Moi Teaching and Referral Hospital (MTRH) - Kenya, an evaluation of aerobic pathogenic bacteria in postoperative wounds in 2002 found a predominance of *Staphylococcus aureus* at 54.7% of which 80.4% were methicillin resistant (Andhoga *et al*., 2002). More recent information on etiology of SSI at the MTRH is however lacking.

Most of the studies on SSI etiology use culture of pus swabs to isolate the organisms. Pus swabs have a high positivity rate of microorganisms with about 75 - 90% of the specimens yielding organisms (Manyahi *et al.*, 2014; Mengesha *et al.*, 2014). Blood cultures generally have a low yield even for other indications with a positivity rate of 29.9% reported at the MTRH (Oduor *et al.*, 2016).

Since etiological patterns for surgical site infections keep changing with time, regular surveillance is important to identify any trends. In the absence of a comprehensive surveillance system, periodic studies on the subject would provide valuable information on the SSI causing organisms which can then guide prevention and management of SSI. There are inadequate current studies in the Kenyan and MTRH context characterizing the etiology of surgical site infections.

2.4 Antibacterial susceptibility of bacterial species associated with SSI

In the last several years, the frequency and spectrum of antibacterial-resistant infections have increased in both the hospital and the community especially in the developing world where antibacterial surveillance programs are inadequate (Mshana *et al.*, 2013). Bacterial pathogens that cause surgical site infection have been shown to have resistance to most commonly used antibacterials. In Iran, Alikhani *et al.,* (2015)

evaluated susceptibility patterns of bacterial isolates from SSI cases and found that *Staphylococcus aureus* had 30% resistance to vancomycin and amikacin as well as 23.4% resistance to teicoplanin. Iyamba *et al.,* (2014) in Kinshasha reported SSI causing bacteria especially *Staphylococcus aureus* to have resistance to ampicillin, cotrimoxazole, erythromycin, clindamycin, ciprofloxacin, cefotaxime and ceftazidime. They however found high sensitivity levels to imipenem, amoxycillin-clavulanic acid and vancomycin to which all isolated bacteria were sensitive. In Uganda, 78.3% of organisms isolated in Surgical site infections showed multi-drug resistance (Seni *et al.*, 2013).

At the Moi Teaching and Referral Hospital (MTRH), organisms isolated from blood cultures from various indications showed resistance to most commonly used antibacterial agents especially penicillins and cephalosporins (Oduor *et al.,* 2016). A high prevalence of multi-drug resistant strains for SSI have been demonstrated even where no MRSA was isolated (Godebo *et al.,* 2013; Mundhada & Tenpe, 2015). In Ethiopia, Kahsay *et al*., (2014) found that the clinical isolates from SSI showed >80% level of resistance to ampicillin, amoxicillin, penicillin G, erythromycin, gentamicin and cotrimoxazole whereas less than 50% level of resistance was observed against clindamycin, oxacillin, tetracycline and vancomycin.

An evaluation of resistance patterns for MRSA is important in order to optimize prevention strategies for this particular pathogen. In India MRSA strains were found to be 100% sensitive to linezolid and tigecycline followed by fucidin (92.51%), mupirocin (88.39%), levofloxacin (75.66%) and doxycycline (72.28%) (Bhattacharya *et al*., 2016). Whereas they found no vancomycin resistant strains 1.12% of the strains were found to be intermediately susceptible to it. In a study in Nepal 42 (100%)

MRSA isolates were resistant to ampicillin and penicillin followed by 41 (97.62%), 32 (76.19%), 31(73.81%), 29 (69.05%), 9 (21.43%) and seven (16.67%) to cefotaxime, gentamycin, cotrimoxazole, erythromycin, tetracycline and ciprofloxacin respectively (Pahadi *et al.*, 2014). They further noted that although all MRSA strains were sensitive to vancomycin on disc diffusion, four isolates were intermediates in vitro determination of MIC of vancomycin. However, in China MRSA was found to be sensitive to vancomycin (100%) and linezolid (98.9%), while 79.9% and 92.0% of MRSA was resistant to clindamycin and erythromycin respectively (Yang *et al.*, 2015).

Reduction of MRSA susceptibility to vancomycin was also reported in Turkey (Kuscu *et al.*, 2011). In Kinshasha- DRC, among the MRSA strains causing SSI , 100% were sensitive to imipenem,89% to amoxycillin-clavulanic acid and 81% to vancomycin but resistant to ampicillin, cotrimoxazole, erythromycin, clindamycin, ciprofloxacin, cefotaxime and ceftazidime (Iyamba *et al.*, 2014). In Mulago Hospital in Uganda, resistance rates of MRSA were 88.2% for trimethoprim-sulfamethoxazole, 88.2% for erythromycin, 58.8% for gentamycin, 70.6% for ciprofloxacin, and 88.2% for chloramphenicol while all isolates were found to be sensitive to vancomycin and clindamycin (Ojulong *et al.*, 2009). In Ethiopian MRSA causing SSI was found to have 5.6% resistance to Vancomycin and 100% resistance to cotrimoxazole (Kahsay *et al.*, 2014). Vancomycin, imipinem and amikacin were also shown by Seni *et al.*, (2013) to have excellent susceptibility profiles against most bacteria causing SSI.

Gram negative bacterial species in surgical site infections generally show a multi-drug resistant pattern. Helal *et al.*, (2015) in Egypt reported that 93.3% of all *Acinetobacter baumannii* isolated from SSI wounds after orthopedic operations were multi-drug

resistant. They found 14 out of the 15 *Acinetobacter baumannii* isolated to be resistant to ampicillin-sulbactam, amoxicillin-clavulinate, amikacin, gentamycin, ciprofloxacin, cefazolin, ceftriaxone, cefipime, imipinem and meropenem. In Tanzania, Manyahi *et al*., (2014) found an overall multi-drug resistance (MDR) rate of 61.4% among all gram negatives isolated in SSI with 100% MDR with *Escherichia coli* and *Acinetobacter baumannii*.

In summary, surgical site infection (SSI) remains a major burden to patients and the healthcare system causing morbidity, mortality, prolonged hospital stay and increased costs. Risk factors include smoking, diabetes mellitus, obesity, extremes of age, immune suppression, prolonged operation period. Several bacteria have been implicated in causation of SSI, most notably *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa*. Drug resistance seem to be on the rise with multidrug resistant strains often isolated in SSI especially MRSA and *Acinetobacter baumannii*.

The relationship between risk factors, etiological agents, antibacterial resistance and SSI is expressed in the conceptual framework below (Figure 2.1).

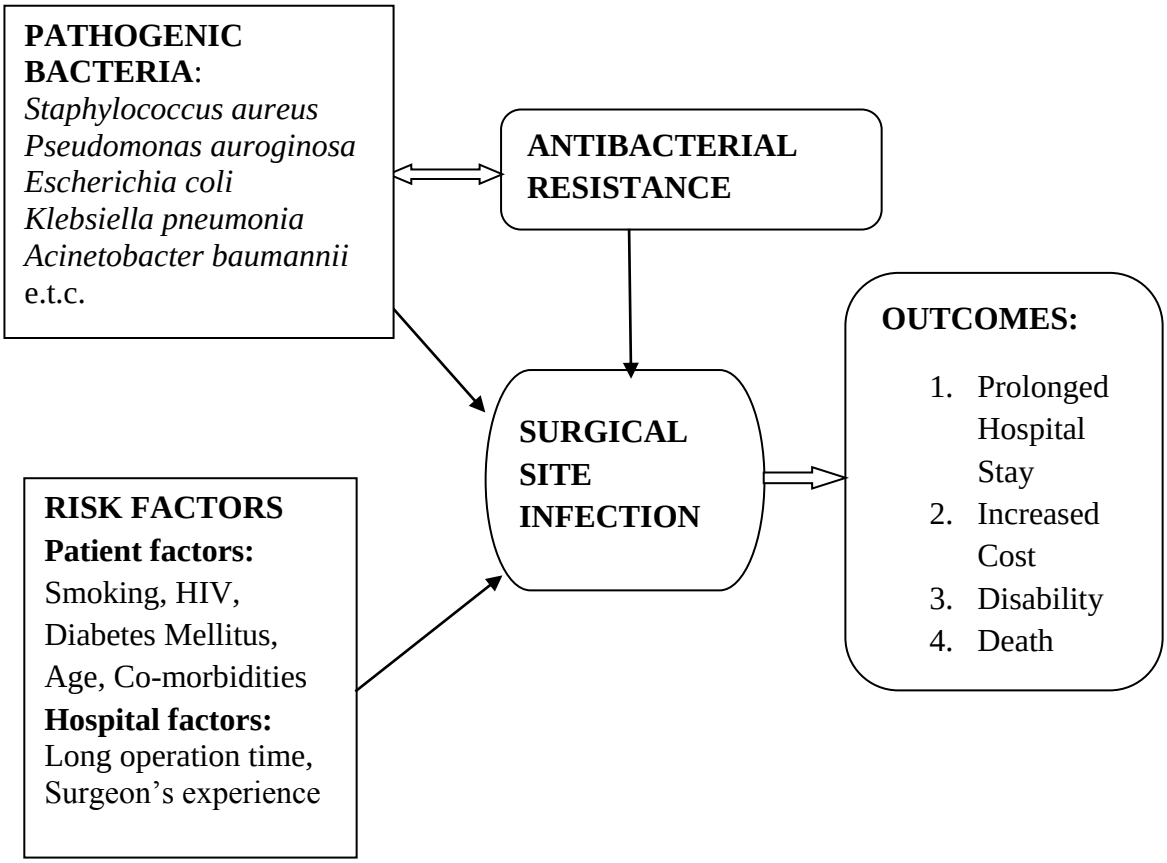

Figure 2.1: Conceptual Framework

Figure 2.1 shows a conceptual framework of the relationship between risk factors (Independent variables), surgical site infections (intervening variable) and the effects of surgical site infections (dependent variable). For surgical site infection to occur there is the interaction between causative bacterial agents and the host risk factors. Antibacterial resistance makes the bacteria unresponsive to peri-operative antibiotics. Surgical site infection in return leads to prolonged hospital stay, increased cost, disability and even death.

CHAPTER THREE: MATERIALS AND METHODS

3.1 Study Site

Moi Teaching and Referral Hospital (MTRH) is the leading teaching and referral hospital in Western Kenya. It is located in Eldoret town in Uasin Gishu County, about 320 Kilometers North West of Nairobi. The hospital has a catchment population of 20 million drawn mainly from rural areas in Western Kenya region, the Rift Valley region and parts of Tanzania, Uganda, Rwanda and South Sudan. The hospital is also a leading site for surgical care with various surgical disciplines such as general surgery, orthopedic and trauma surgery, neurosurgery, cardiothoracic surgery, urology and pediatric surgery. It also serves as a teaching hospital for undergraduate medical training as well as postgraduate training in general surgery, orthopedic surgery among other disciplines. MTRH has a bed capacity of 800 distributed among the various specialties (AMPATH, 2014). The study was conducted at the MTRH orthopedic wards, surgical wards, orthopedic clinic, surgical outpatient clinic as well as accident and emergency department.

3.2 Study design

The study employed a Case-Control design. Consecutive patients (cases) presenting with surgical site infection over a 12-month period were included. For each case, 2 controls matched by age, type of operation and month of surgery were identified and included. This was as per a similar case-control study which recruited 2 controls for each case (Friedman *et al.*, 2007). The case-control design was chosen to enable comparison of the cases and controls in terms of risk factors for SSI (exposure).

3.3 Study population

The target population included patients (cases), of all ages, who had surgical site infection after undergoing either elective or emergency surgery at MTRH. The entry points for these participants were the surgical wards, orthopedic wards, surgical clinics, orthopedic clinics, outpatient as well as casualty (accident and emergency) department. For each case, two matched controls were included. Controls were matched for age, month of surgical operation and the type of surgery conducted.

3.4 Sample Size and Sampling method

The sample size was derived from the formula below for sample size determination for case control studies involving difference in proportions (Cai & Zeng, 2004).

$$n = \left(\frac{r+1}{r}\right)\frac{(\bar{p})(1-\bar{p})(Z_{\beta} + Z_{\alpha/2})^2}{(p_1 - p_2)^2}$$

Where:

n = Sample size in the case group

r = ratio of controls to cases

$\bar{p}$ = Average proportion exposed

$p_1 - p_2$ = Effect Size (the difference in proportions)

Z_{β} = standard normal variate for the desired power (0.84 for 80% power).

$Z_{\alpha/2}$ = Standard normal variate for the desired level of statistical significance (1.96)

Therefore:

For 80% power, Z_{β} = 0.84

At 0.05 significance level, $Z_{\alpha/2}$ =1.96;

r=2 (2 controls for each case)

Using the risk factor of smoking, the proportion exposed in the control group i.e. proportion of smokers in Kenya is 16% (ITC Project., 2015).

To get proportion of cases exposed:

$$p_{case\exp} = \frac{ORp_{control\exp}}{p_{control\exp}(OR-1)+1}$$

OR = Desired minimum Odds ratio. Set at 3.0 based on previous study analyzing smoking as a risk factor for surgical site infection (Møller *et al*., 2003). Therefore:

$$p_{case\exp} = \frac{3.0(0.16)}{(0.16)(3.0-1)+1} = \frac{0.48}{1.32} = 0.36$$

Average proportion exposed ($\bar{p}$) = (0.36+0.16)/2=0.26

Hence:

$$n = (\frac{r+1}{r})\frac{(\bar{p})(1-\bar{p})(Z_{\beta}+Z_{\alpha/2})^2}{(p_1-p_2)^2}$$

$$n = 1.5\frac{(0.26)(1-0.26)(0.84+1.96)^2}{(0.36-0.16)^2} = 57$$

Therefore, n=57 (57 cases and 114 controls)

Therefore a total of 57 patients who presented with surgical site infection (cases) were included. For each case, 2 controls matched by age, month of operation and indication for surgery were included hence 114 controls.

3.5 Sampling Procedure

Cases of surgical site infection that presented at the entry points (surgical wards, orthopedic wards, surgical clinics, orthopedic clinics, outpatient department and casualty) and met the inclusion criteria were enrolled until the desired sample size of 57 cases was reached. For every case enrolled into the study two appropriate matched controls were sought from the operations register at the MTRH surgical theatres, their records retrieved and contacted via phone or at the next clinic visit. Where more than two appropriate controls were identified from the registers, two were chosen using simple random sampling with the aid of a computerized table random numbers. In the event an identified potential control was not reached, replacement was done. The nurses, medical officer interns, medical officers and registrars working in the entry points were sensitized on the study and identification of SSI cases. They were asked to inform the candidate by phone, prior to initiation of antibiotic therapy, if a patient presented with the features meeting the criteria for surgical site infection. The candidate and two trained assistants also conducted daily physical checks in the eligible departments to identify participants.

3.5.1 Inclusion and Exclusion Criteria

Patients of all ages with surgical site infection, as per the standard definition, following surgery at the MTRH orthopedic, general surgery, neurosurgery, cardiothoracic, pediatric surgery or urology departments were eligible to participate as

cases. Controls were matched to the cases by age, type of surgery and month of surgery.

The study excluded patients who had evidence of infection before surgery or patients whose primary indication for surgery was an infective condition such as septic arthritis, peritonitis or osteomyelitis. Controls were excluded if they had any history suggestive of SSI or had features of SSI at the time of the study.

3.5.2 Recruitment of Controls

For each case of SSI recruited, 2 controls were sought. Once a case was identified from any of the entry points, the date of surgery was noted which was used to trace the records at the database in MTRH operative theatre. In the theatre records, potential controls were identified that were within 30 days before or 30 days after the date of surgery for the case, were within 0-5 year's age difference with the case and having undergone a similar surgical procedure. All potential controls were listed in an excel database. In the event that more potential controls were identified, two were chosen using simple random sampling via a computerized random numbers chart. The records of the controls were then retrieved from the hospital records department using the inpatient numbers after which the contacts and next date of clinic visit was noted. They were then contacted via phone and appointment made on the date of next clinic visit. At the clinic visit the controls were reviewed by the candidate to confirm that they had no history or features of SSI. If eligible, consent was sought and questionnaire administered. Potential controls that were found to be ineligible were replaced.

3.6 Data collection

Demographic and clinical characteristics from cases and controls were collected using a structured questionnaire (Appendix IV). Additional clinical information was retrieved from the patient records that were accessed from the MTRH records department. These were incorporated into the questionnaire. Laboratory procedures were performed to identify causative organisms and their susceptibility patterns. Data from the laboratory procedures was incorporated in the questionnaires. The laboratory procedures included collection of pus swab from the infected wounds on which microscopy, culture and antibiotic susceptibility testing was done. In patients with deep, organ or space SSI that were managed surgically, deep tissue samples were used. Blood cultures were done for patients with systemic features of infection and had fever of more than 37.5 degrees.

3.7 Laboratory Methods

Samples of pus swabs were collected using a standardized procedure. Intraoperative samples were collected by the operating surgeon under aseptic conditions. All tests were done at the Moi Teaching and Referral Hospital (MTRH) microbiology laboratory with the assistance of appropriately qualified and registered staff. This included specimen processing, microscopy, culture and susceptibility tests. MTRH microbiology laboratory is duly registered by the Kenya Medical Laboratory Technicians & Technologists Board (KMLTTB) which does regular inspection. The laboratory has internal and external quality assurance mechanisms and is ISO certified.

3.7.1 Procedure for Collection and Transport of Pus Swab

Pus swabs were collected by the candidate from infected post-operative wounds that had purulent discharge. The wound was first gently cleaned using sterile saline to reduce contamination by commensal flora from the skin. Sterile gloves were then worn. Using sterile cotton swab pus was then collected from as deep as possible in the wound while expressing purulent exudates into the swab. The swab was then placed back into the sterile bottle and bottle top replaced tightly. The bottle was labeled with the patient's code, date of birth, hospital number, and anatomical site swabbed as well as date and time of sample collection. Standard MTRH microbiology laboratory requisition form was then filled with all the relevant patient information. The sample was maintained at room temperature and transported to the laboratory within 15 minutes. Intraoperative samples were collected by the operating surgeon under the sterile theatre conditions by using a sterile syringe to aspirate pus from deep tissues and also collecting a sample of infected tissues themselves. These were then placed in sterile bottles, appropriately labeled and transported under room temperature to the MTRH microbiology laboratory within one hour of collection.

3.7.2 Laboratory Processing of Pus Swab Specimen

Pus swabs were subjected to primary gram staining followed by culture in various agar plates. The plates used were blood agar (BA), MacConkey agar (MAC), Chocolate Blood Agar (CBA). Once the specimen was received in the laboratory it was examined and its gross appearance described.

3.7.2.2 Primary gram staining

Primary gram staining was performed on part of the pus specimen immediately after reception in the laboratory. This was to classify any bacteria species in the specimen as either gram positive or gram negative to guide the researcher on what to expect on the culture medium. Evenly spread smears of the specimen was made on a slide and allowed to air-dry in a safe place. The slide was then fixed and stained by Gram technique (Appendix VIII). After gram staining, the smear was examined for bacteria among pus cells using 40X and 100X objectives. Any identified organism was classified as either gram positive cocci; gram positive rods, gram negative rods or gram negative cocci. Another smear of the same specimen was then examined for Acid Alcohol Fast bacilli (AAFBs). An evenly spread smear of the specimen was made on a slide and allowed to air-dry. The smear was fixed and stained by the Zeihl-Neelsen (ZN) technique (appendix IX). The slide was then examined for acid fast bacilli (AFB) using 100X objective.

3.7.2.2 Culture

Part of the pus specimen was inoculated in Blood Agar, Chocolate Blood Agar (CBA) and MacConkey Agar. This was to provide culture media for the bacteria in order to facilitate their growth and identification. The inoculated agar plates were incubated at 37^0C in Imperial III incubator with CBA being incubated in a carbon dioxide atmosphere (candle jar) while MacConkey agar plate was incubated aerobically. The plates were left to stand for 24 hours. The Imperial III incubator which was on regular maintenance and up to date calibration from the biomedical department of MTRH.

After 24 hours of incubation, the BA, CBA and MAC media were examined for any growth. In case of growths in any culture medium, the colonial morphology was

described including: shape, size, elevation, margins and surface. MAC plates were further examined to identify whether there was lactose fermentation. Gram stain was then performed on any growths obtained.

3.7.2.3 Identification of gram positive bacterial species

Gram positive bacteria were examined for morphology then subjected to a series of identification tests (Appendix X). Hemolysis test was done on BA to identify hemolytic *Streptococcus*. Catalase test was done to differentiate between *Staphylococcus* (catalase positive) and *Streptococcus* (catalase negative). All *Staphylococcus species* were then subjected to coagulase test to classify them as either *Staphylococcus aureus* (coagulase positive) or Coagulase Negative *Staphylococcus* (CONS). *Staphylococcus aureus* isolates were further subjected to susceptibility testing using oxacillin to determine whether they were Methicillin Resistant *Staphylococcus aureus* (MRSA) or Methicillin sensitive *Staphylococcus aureus* (MSSA).

3.7.2.4 Identification of gram positive bacterial species – biochemical tests

Gram negative bacteria were isolated from MAC medium. The plate was examined for any colour changes that could signify lactose fermentation. The isolated gram negative bacteria were then subjected to additional biochemical tests including Indole, Methyl Red, Voges Proskauer and Citrate as well as Tripple Sugar Iron (TSI) tests (appendix XI)

Indole, Methyl Red, Voges Proskauer and Citrate tests were reported as either positive or negative while TSI test was interpreted as either A/A (Acid slunt/acid butt), K/A (Alkaline slunt/Acid butt) or K/NC (Alkaline slunt/ No change on butt). Gas

production was also considered on TSI. The results of the biochemical tests were then used to determine the identity of the bacteria with the aid of two interpretation charts (Appendix XII). The biochemical tests were interpreted as per the charts below (Table 3.7.1 and Table 3.7.2)

Table 3.7.1: Interpretation chart for biochemical tests

Organism	**Indole**	**Methyl red**	**Voges Proskauer**	**Citrate**	**Catalase**	**Oxidase**	**Special character**
Klebsiella pnuemoniae	-	-	+	+	+	-	Mucoid at culture media
Escherichia coli	+	+	-	-	+	-	
Pseudomonas auroginosa	-	-	-	+	+	+	
Proteus spp	+	+	-	-/+			Swarming in BA
Salmonella	-	+	-	+			
Shigella	-/+	+	-	-			
Enterobacter	+	+	-	-	+	-	
Morganella	+	+	-	-			
Yersinia	-/+	+	-	-			
Acinetobacter baumannii	-	-	-	-	+	-	

Table 3.7.2 Interpretation chart for Triple Sugar Iron (TSI) test

Name of the organisms	Slant	Butt	Gas	H2S
Escherichia, Klebsiella, Enterobacter	Acid (A)	Acid (A)	Pos (+)	Neg (-)
Shigella, Serratia	Alkaline (K)	Acid (A)	Neg (-)	Neg (-)
Salmonella, Proteus	Alkaline (K)	Acid (A)	Pos (+)	Pos (+)
Pseudomonas	Alkaline (K)	Alkaline (K)	Neg (-)	Neg (-)

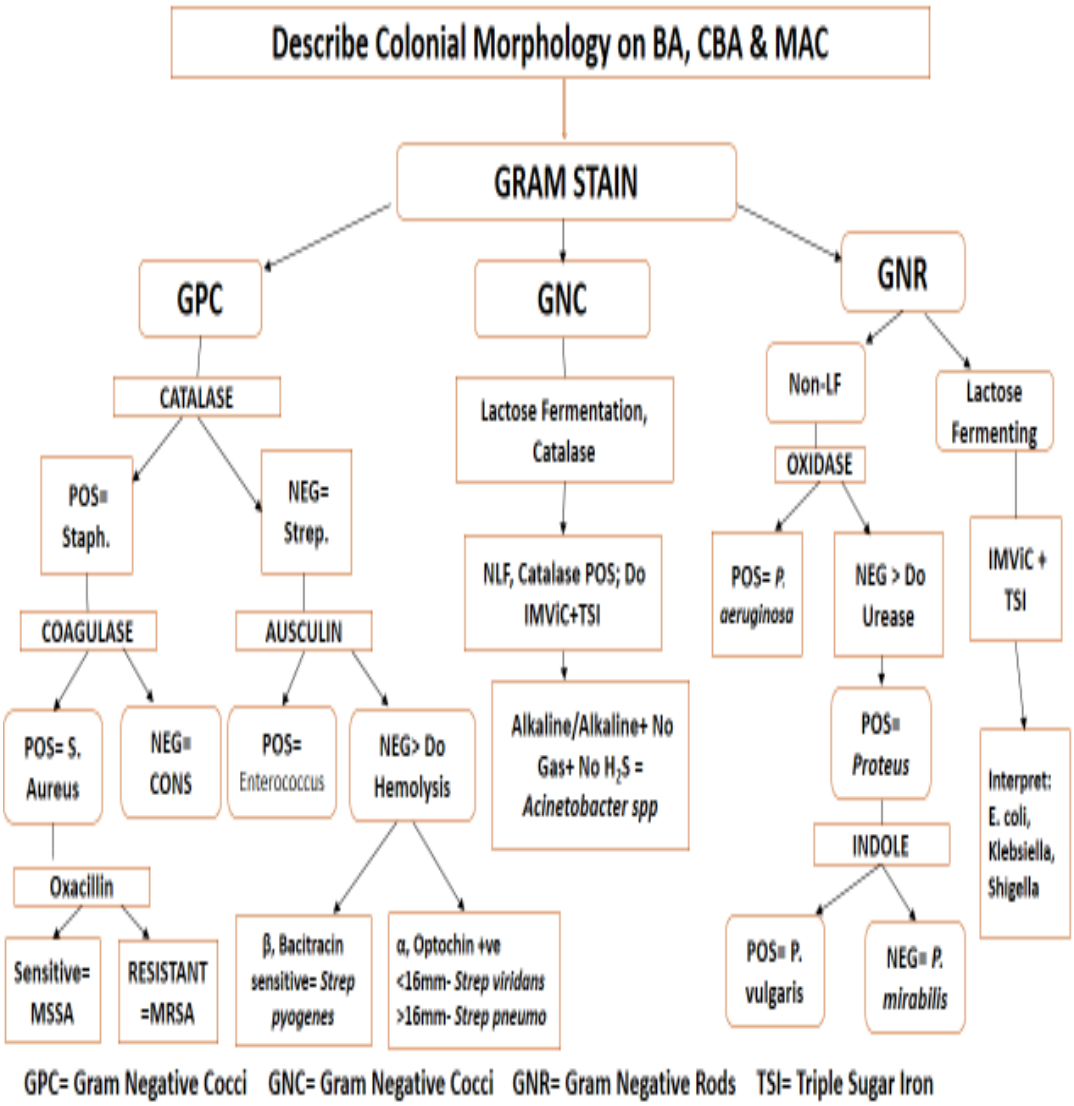

Figure 3.7.1: Algorithm for identification of Bacteria species (Adapted from MTRH SOPs).

Plates that had no growth at 24 hours were incubated for another 24 hours and re-examined. If any isolate was found, gram staining was done and procedure for identification of the organism followed as above. If there was no growth after 48 hours, such plates were recorded as negative but held for a total of 5 days under sterile conditions. A preliminary report was provided after 24 hours to the attending doctor. The questionnaires were updated with the laboratory results within 72 hours.

Bacterial species were judged to be laboratory contaminants if they had an unusual non uniform pattern of growth on the agar plate not in keeping with the site of inoculation, if the culture findings were not in keeping with any primary gram stain findings. Such potential contaminants were not included in the final analysis. Contaminants from outside the laboratory were minimized by optimizing sterile conditions and specimen collection techniques.

3.7.3 Blood Culture Examination

Blood for culture was collected in patients with systemic infection with a temperature of greater than 37.5^0C. The largest most accessible vein was identified and site cleaned with antiseptic solution. Sterile gloves were worn and the venepuncture site was then draped in sterile towel. The cap on the BD Bactec vial top was then flipped off and the vial inspected for cracks, contamination, excessive cloudiness and bulging or indented septum.

The septum of the vial was then swabbed with alcohol. Venepuncture was done to draw appropriate volume of blood into a 10cc syringe (10 ml of blood for an adult) after which another sterile needle was used to inoculate the blood into BD Bactec® vial that was provided by the MTRH laboratory. The volume of blood drawn from children was determined by their weight and age as was indicated in a chart available to the candidate during specimen collection (Appendix VII). The patient's code, hospital number, date of birth, sex as well as date and time of specimen collection were indicated on the request form and the culture bottle.

The inoculated BD Bactec vial was maintained at room temperature and transported to the microbiology laboratory within 15 minutes. Upon receiving the samples in the laboratory the BD Bactec vials were placed in BACTEC 9050 fluorescent series

instrument as soon as possible for incubation and monitoring. In the instrument, the vials were automatically tested for growth every 10 minutes. Positive vials were identified by the instrument through an audible alert and onscreen indication. The positive vials were then removed from the instrument and placed in the biosafety cabinet. From the biosafety cabinet 2ml of blood from the positive vial was dispensed into Ethylenediaminetetraacetic acid (EDTA) tube and mixed. Buffy coat smears were prepared from EDTA blood by adding 2ml phosphate buffered saline (PBS) and 2% fetal bovine serum (FBS) to the 2 ml blood in EDTA, sample centrifuged at room temperature and 200 x g for 10 minutes. The concentrated leukocyte band (buffy coat) and a small portion of the plasma and concentrated RBCs were removed and subjected to gram staining. The blood in the EDTA tube from positive vials was also sub-cultured into Blood Agar and MacConkey which were incubated aerobically and CBA incubated in CO_2. The incubation and progressive identification of the bacteria was then continued as in 3.7.1 above.

3.7.4 Antibacterial Susceptibility testing

Antibacterial susceptibility testing was done for all pathogenic bacteria isolated by pus and blood culture. The test was done by use of disc diffusion technique, specifically modified Kirby-Bauer disc diffusion technique in Appendix XIII (Bauer *et al.,* 1966; Boyle *et al.,* 1973). In this technique, a disc of blotting paper was impregnated with a known volume and appropriate concentration of an antibacterial agent and placed on a plate of susceptibility testing agar uniformly inoculated with the test organism. Mueller-Hinton Agar was used for the disc diffusion. In this technique the antibacterial diffuses from the disc into the medium and the growth of the test organism is inhibited at a distance from the disc that is related to the susceptibility of

the organism. Commercial discs and blotting paper were sourced through the regular hospital procurement procedure at MTRH.

The distance of inhibition was measured using a Milutoyo® caliper (Appendix XIV) and the result classified as Sensitive, Intermediate or resistant for each antibacterial depending on the distance of inhibition as per table 3.7.4.1. Antibacterial discs were selected based on the gram stain of the organism. Gram positive organisms were exposed to clindamycin, gentamicin, azithromycin, erythromycin, Ceftriaxone, levofloxacin, cotrimoxazole, meropenem, amikacin and vancomycin. Gram negative bacteria were exposed to Ceftriaxone, ciprofloxacin, ceftazidime, cefepime, gentamicin, meropenem, levofloxacin and amikacin. The antibacterial susceptibility procedures used were in conformity with the Clinical Laboratory and Standard Institute (CLSI) performance standards for antimicrobial susceptibility testing (Franklin & Cockerill, 2011).

Table 3.7.4.1: Interpretation of the Inhibition Halos on Mueller-Hilton agar

Antibacterial	Amount in disc	Diameter of zone of inhibition (mm)		
		Susceptible (≥)	Intermediate	Resistant (≤)
Amikacin	30 mcg	17	15-16	14
Azithromycin	15 mcg	18	14-17	13
Cefepime	30 mcg	18	15-17	14
Cefotaxime	30 mcg	23	15-22	14
Ceftazidime	30 mcg	18	15-17	14
Ceftriaxone	30 mcg	21	14-20	13
Cefuroxime (IV)	30 mcg	18	15-17	14
Ciprofloxacin	5 mcg	21	16-20	15
Clindamycin	2 mcg	19	16-18	15
Cotrimoxazole	1.25/23.75 mcg	19	16-18	15
Erythromycin	15 mcg	23	14-22	13
Gentamicin	10 mcg	15	13-14	12
Meropenem	10 mcg	16	14-15	13
Oxacillin	1 mcg	13	11-12	10
Levofloxacin	5 mcg	17	14-16	13

3.8 Data analysis, Storage and dissemination

Data from the questionnaire and laboratory results were coded to convert all of it into numerical data which was entered into SPSS version 20. Data cleaning was then done to confirm accuracy and consistency. Analysis was done with the aid of the SPSS. Frequencies were determined for sociodemographic characteristics among both cases and controls.

To determine the risk factors for surgical site infection, proportions of patients with the independent variables were compared between cases and controls using the $\chi 2$ and Fisher exact tests. A 2-sided P value of 0.05 or less was considered significant for all statistical tests. Odds ratios were determined to measure the strength of the association of the respective risk factors and surgical site infection. Ninety five degrees confidence intervals for the Odds ratios were determined. Multivariate analysis was done to further test the association of the specific risk factors that were statistically significant on bivariate analysis.

To determine the etiology of surgical site infection, frequencies were determined for all the bacterial species isolated. Proportions were determined and expressed in percentages within the various classes of bacteria as well as to compare different categories of patients. Frequencies and proportions were determined for antibacterial susceptibility of each species of bacteria to determine the proportions that were susceptible, intermediate and resistant to particular antibacterials tested.

The data collection tools were stored under lock and key and were only made accessible to the candidate and supervisors. Information from this study is hereby presented in tables, figures and relevant explanations. The dissemination is to be done at Moi University School of Medicine, MTRH surgical department as well as in peer reviewed journals. The information gathered will inform the MTRH and similar hospitals on risk factors of surgical site infections and the appropriate antibiotics to avail for prophylaxis and treatment of surgical site infections.

3.9 Ethical considerations

The candidate adhered to key ethical principles such as respect of autonomy, informed consent, beneficence, non-maleficence and justice. Informed consent was sought and received from all adults as well as parents or legal guardians of minors less than 18 years. In addition, assent was sought from all minors 6 years and over. Potential study patients and parents or guardians were provided with either the adult consent form (Appendix I) or the parental consent (Appendix II) as appropriate. In addition, an assent form (Appendix III) was provided form for minors who were 6 years and over. The forms contained an information sheet highlighting all the relevant details about the research process in a clear and concise manner. The form was read out and explained to the respondent in the language they would understand better between English and *Kiswahili*. The consent form contained provisions for the respondents to sign or decline to participate with potential respondents retaining the right to consent voluntarily and free from exploitation and coercion.

Forms, questionnaires and other research material that were used in this study remained under lock and key with only the candidate having access to ensure data protection. The candidate was mindful of the pain the patients could have been subjected to during collection of specimens. This was explained to the patients. Care was taken to ensure the least time possible was taken to collect the samples and that the least possible pain was caused by ensuring the candidate underwent adequate training on sample collection. In order to ensure confidentiality, the respondent's identities and responses were protected from the public domain. The identities of respondents were concealed through the assignment of codes in data analysis and throughout discussion in the study. Ethical approval for the study was sought from

and granted by the Moi University Institutional Research and Ethics Committee (IREC), approval number FAN: IREC 1312 (Appendix V). Approval was also granted by Moi Teaching and Referral Hospital to conduct the study in the facility (Appendix VI).

3.9 Study limitations

This study was conducted in hospital set up. This posed a potential limitation of not being able to capture patients who may have had surgical site infection but failed to seek care in the health facility. Study patients might not have been able to recall all medication used. This was mitigated by corroborating information given by the participant with information available in the records and any evidence that could be found such as drug packets. There were multiple entry points for the study participants hence some cases could have been missed. This was mitigated by having research assistants stationed at the entry points who alerted the candidate whenever a case of SSI was identified. Notices were also provided and staff working at the entry points sensitized on the study and need to notify the candidate upon identification of an SSI case.

CHAPTER FOUR: RESULTS

4.1: Sociodemographic characteristics of study patients

A total of 171 participants were included in the study comprising 57 cases and 114 controls matched with two controls for age, sex and type of surgery. Males were 68.4% in both groups. Age range was from 1 to 98 years with a mean of 33.3 (SD=21.3) and 33.4 (SD=20.8) for cases and controls respectively. Open Reduction and Internal Fixation (ORIF) compromised 50.9% of the surgical procedures included. Other sociodemographic characteristics of the study participants were as shown in the table 4.1.1:

Table 4.1.1: Sociodemographic characteristics of study patients

Characteristic		Cases n=57	Controls, n=114
Gender (%)	Male	68.4	68.4
	Female	31.6	31.6
Age (Years)	Mean (SD)	33.3(21.3)	33.4(20.8)
	Min, Max	1,95	1,98
Surgical Procedure (%)	ORIF	50.9	50.9
	Laparatomy	15.8	15.8
	Cranial Surgery	8.8	8.8
	Amputation	3.5	3.5
	Skin Graft/ Flaps	3.5	3.5
	Excision	3.5	3.5
	Arthroplasty	1.8	1.8
	Other	12.3	12.3

Majority of the surgical wounds among the SSI cases and controls were clean followed by clean-contaminated and contaminated as shown in Figure 4.1.1.

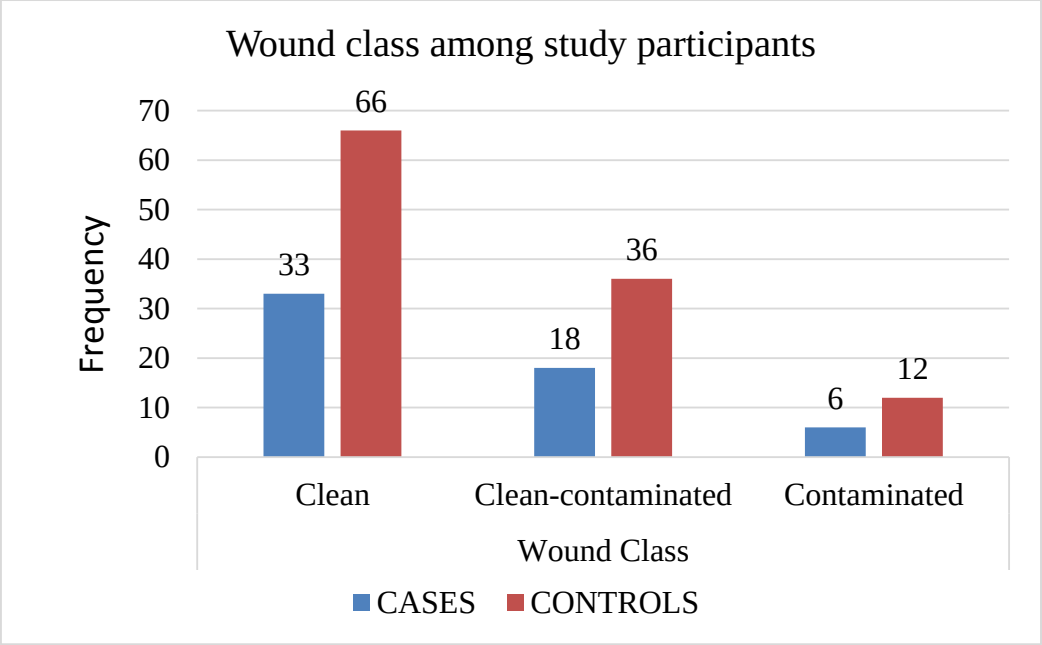

Figure 4.1.1: Wound Class among SSI cases and controls

Among the 57 cases 24 (42.1%) had deep incisional SSI, 21 (36.8%) were superficial incisional while 12 (21.1%) had organ or space SSI.

4.2: Risk factors for Surgical site Infections

Various possible risk factors were analyzed to find out their contribution to surgical site infections. Risk factors that showed a significant difference between the cases and controls included history of smoking, operation time more than 2 hours and diabetes mellitus. The mean operation time for cases was 138 minutes (SD=75.26) while that of controls was 99 minutes (SD=47.58). The difference between cases and controls was statistically significant with a p value of <0.001 (table 4.2.1).

The cases of SSI had spent on average 39 more minutes in the operating room, had more prevalence of diabetes, and were twice more likely to be smokers. HIV status,

history of alcohol intake, the lead surgeon and hypertension were found not to have any significant difference between the cases and the controls as shown in table 4.2.1.

Table 4.2.1: Risk factors for Surgical Site Infection

Variable	Surgical site infection		P value	OR	95% CI for OR
	Yes n (%) N= 57	No n (%) N= 114			
Smoking					
Yes	12 (70.6%)	5 (29.4%)	0.001	5.81	1.94-17.46
No	45 (29.2%)	109 (70.8%)			
Known diabetes					
Yes	8 (61.5%)	5 (38.5%)	0.029	3.56	1.11-11.43
No	49 (31.0%)	109 (69.0%)			
Operation time					
> 2 hours	34 (50.0%)	34 (50.0%)	< 0.001	3.49	1.79-6.76
≤ 2 hours	23 (22.3%)	80 (77.7%)			
HIV Status					
Positive	4 (44.4%)	5 (55.6%)	0.347	1.65	0.42-6.38
Negative	53 (32.7%)	109 (67.3%)			
Known hypertension					
Yes	6 (33.3%)	12 (66.7%)	0.612	1.00	0.36-2.82
No	51 (33.3%)	102 (66.7%)			
Alcohol use					
Yes	16 (32.7%)	33 (67.3%)	0.527	0.96	0.47-1.94
No	41 (33.6%)	81 (66.4%)			
Lead Surgeon					
Resident	19 (31.7%)	41 (68.3%)	0.435	1.12	0.58-2.20
Consultant	38 (34.2%)	73 (65.8%)			

On multivariate logistic regression analysis history of smoking and operation time more than 2 hours were found to be statistically significant predictors of surgical site infection (Table 4.2.2).

Table 4.2.2 Multivariate logistic regression analysis of risk factors for SSI

Risk factor for SSI	Odds Ratio	95% CI for OR	P-value
Smoking	8.78	2.18-35.33	0.002
Operation time > 2 hours	3.07	1.51-6.24	0.002
Diabetes mellitus	3.60	0.77-16.84	0.104
Positive HIV Status	1.85	0.36-9.48	0.458
Hypertension	0.43	0.11-1.69	0.224
Alcohol intake	0.41	0.15-1.08	0.070
Lead surgeon	1.29	0.61-2.73	0.500

4.3: Etiological agents for Surgical site infection

Out of the 57 cases of surgical site infections, bacterial organisms were isolated in 46 participants (80.7%). In 41 cases, organisms were isolated from culture of pus specimens alone while in 3 cases; organisms were isolated from blood culture only. Two cases had bacterial organisms isolated from both blood culture and pus specimen. Therefore, 43 out of 57 specimens of pus yielded bacterial organisms on culture (75.4%) whereas 5 out of 12 (41.7%) blood cultures had positive yield. There was a total of 55 bacterial isolates from the 46 patients who had positive cultures with 6 patients having multiple isolates. *Staphylococcus aureus* was the most common causative agent for surgical site infections with 22 isolates (40.0%) being *Staphylococcus aureus*. Other bacterial species included *Escherichia coli* (20%), *Acinetobacter baumannii* (10.9%), *Klebsiella pneumoniae* (9.1%), and *Proteus mirabilis* (3.6%) as shown in table 4.3.1.

Table 4.3.1: Causative organisms for surgical site infections

S.No.	Bacterial species	Frequency	%
1	*Staphylococcus aureus*	22	40.0
2	*Escherichia coli*	11	20.0
3	*Acinetobacter baumannii*	6	10.9
4	*Klebsiella pneumonia*	5	9.1
5	Coagulase Negative *Staphylococci* (CoNS)	4	7.3
6	*Pseudomonas auroginosa*	4	7.3
7	*Proteus mirabilis*	2	3.6
8	*Streptococcus pyogenes*	1	1.8
	Total	55	100.0

Of the 22 *Staphylococcus aureus* isolated 13 (59.1%) were methicillin resistant *Staphylococcus aureus* (MRSA) while the rest were Methicillin Sensitive *Staphylococcus aureus*. Most bacteria were isolated from cultures of pus swabs (90.9%) whereas only 5 isolates (9.1%) were from blood cultures. There were no mycobacteria from the ZN staining. The predominant organism in blood cultures was *Pseudomonas auroginosa* while that in pus culture was MRSA (table 4.3.2).

Table 4.3.2: Isolated species from pus and blood cultures

S. No.	Bacterial Species	Source Sample		Total
		Pus culture	Blood culture	
1	MRSA	13	0	13
2	*Escherichia coli*	11	0	11
3	MSSA	8	1	9
4	*Acinetobacter baumannii*	6	0	6
5	*Klebsiella pneumoniae*	4	1	5
6	CoNS	3	1	4
7	*Pseudomonas auroginosa*	2	2	4
8	*Proteus mirabilis*	2	0	2
9	*Streptococcus pyogenes*	1	0	1
	Total	50	5	55

4.4 Antibacterial susceptibility patterns of bacterial species

Bacterial isolates showed high level of resistance to most of the commonly used antibiotics in the perioperative setting.

4.4.1 Susceptibility patterns of gram positive bacterial species

All the methicillin resistant *Staphylococcus aureus* (MRSA) were resistant to cotrimoxazole, azithromycin, ciprofloxacin, ceftriaxone and cefuroxime. There was no resistant species to vancomycin though one MRSA had intermediate susceptibility. Coagulase negative *Staphylococci* (CoNS) had more than 25% resistance to cotrimoxazole, erythromycin, azithromycin, gentamycin, ciprofloxacin, ceftriaxone and clindamycin. Methicillin sensitive *Staphylococcus aureus* (MSSA) had the most favorable susceptibility profile among the gram positive species. Refer to table 4.4.1.

Table 4.4.1: Antibacterial susceptibility in gram positive species from SSI

S/No.	Antibacterial agent	Methicillin Resistant *Staphylococcus aureus* (MRSA): n=13			Methicillin Sensitive *Staphylococcus aureus* (MSSA): n=9			Coagulase Negative *Staphylococci* (CONS): n=4		
		S= sensitive. I= intermediate. R= resistant								
		S (%)	I (%)	R (%)	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)
1	Cotrimoxazole	0	0	100	66.7	11.1	22.2	50	0	50
2	Erythromycin	15.4	0	84.6	77.8	11.1	11.1	25	0	75
3	Azithromycin	0	0	100	33.3	0	66.7	25	0	75
4	Gentamycin	38.5	0	61.5	100	0	0	0	0	100
5	Ciprofloxacin	0	0	100	33.3	0	66.7	50	0	50
6	Meropenem	30.8	0	69.2	100	0	0	75	25	0
7	Ceftriaxone	0	0	100	22.2	22.2	55.6	0	25	75
8	Cefuroxime	0	0	100	100	0	0	75	0	25
9	Vancomycin	92.3	7.7	0	100	0	0	100	0	0
10	Clindamycin	30.8	0	69.2	77.8	22.2	0	50	0	50
11	Amikacin	53.8	7.7	38.5	66.7	11.1	22.2	75	25	0
12	Levofloxacin	15.4	0	84.6	66.7	11.1	22.2	100	0	0

4.4.2 Resistance patterns of gram negative bacterial species

Gram negative bacteria showed resistance to most commonly used antibacterial agents. All the species including *Escherichia coli, Klebsiella pneumoniae, Pseudomonas auroginosa* and *Acinetobacter baumannii* had multi-drug resistant (MDR) patterns. *Acinetobacter baumannii* had more than 50% resistance to all the antibacterials tested except amikacin at 16.7%. Amikacin and meropenem had the most favorable sensitivity across all gram negative bacterial species. Refer to table 4.4.2.

Table 4.4.2: Antibacterial resistance in gram negative species from SSI

S/No	Anti**bacterial** agent	**Resistant species (%)**			
		Escherichia coli n=11	*Klebsiella pneumonia*, n=5	*Pseudomonas aeruginosa*, n=4	*Acinetobacter baumannii*, n= 6
1	Ceftriaxone	81.8	100	75	100
2	Gentamycin	36.4	40	25	66.7
3	Ciprofloxacin	27.3	60	25	100
4	Meropenem	0	0	0	50
5	Amikacin	9.1	0	0	16.7
6	Levofloxacin	72.7	80	0	66.7
7	Ceftazidime	45.5	100	50	66.7
8	Cefipime	72.7	80	0	100
9	Cefotaxime	63.6	100	75	66.7

CHAPTER FIVE: DISCUSSION

5.1 Risk factors for surgical site infection

In this study, cigarette smoking was significantly found to be associated with surgical site infection (SSI) in both bivariate and multivariate analysis. This is similar to other studies which have shown a positive correlation between history of smoking and the likelihood of developing SSI. Shao *et al.*, (2017) found an increase in SSI among smokers in China with an odds ratio of 2.13. Similar findings were also reported by Blood *et al.*, (2017) who reported increased incidence of SSI among tobacco users. As documented by Sørensen *et al.*, (2009), cigarette smoking impairs wound healing and increases surgical site infection by impairing tissue oxygenation and causing local tissue hypoxia through vasoconstriction. Møller *et al.* (2003) reported that smoking leads to lowered body immunity to infection and increases the risk of co-morbidities such as diabetes mellitus and atherosclerosis, which themselves increase the risk of SSI. The ITC project (2015) report showed a rise in smoking incidence in Kenya hence this is likely to remain a major factor in the causation of SSI.

Diabetes mellitus was found to significantly increase the risk of surgical site infection on bivariate analysis. Although the difference was not statistically significant on multivariate analysis, the odds ratio remained high at 3.6. Similar findings have been reported by Blood *et al.*, (2017) in a meta-analysis on SSI involving spine surgery patients. Mawalla *et al.*, (2011) in Tanzania also reported a similar association between diabetes mellitus and SSI. As reported by Guo and DiPietro (2010), diabetes mellitus impairs wound healing and increases risk of surgical site infection by lowering overall body immunity and reducing oxygenation at the wound. They further

noted that diabetes increases the risk of other co-morbidities such as atherosclerosis which further reduces oxygen tension in wounds and causes tissue hypoxia.

Prolonged operation time was associated with increased SSI with the cases having spent on average 38 more minutes in the operating room than the controls. Similar findings have been reported by other studies which have shown that the longer the operating room time, the higher the risk of SSI. The study findings agree with Shao *et al.,* (2017) who showed that operative time increases SSI in Open Reduction and Internal Fixation (ORIF) with an odds ratio of 2.15. It is important to note that the patients in the current study had mostly undergone ORIF procedure which is often associated with longer time in the operating room. The findings also agree with Blood *et al.,* (2017) and Wang *et al.,* (2017) both of whom have shown an increase in SSI rate with prolonged operating time especially when the time goes beyond 2 hours. In general, prolonged time in operating room seems to be an independent risk factor for SSI. Prolonging the operation time theoretically increases the likelihood of the wound being infected by increasing bacterial exposure and the extent of tissue trauma from surgery. Long operation time also leads to reduction in tissue level of any pre-operative antibacterial prophylaxis. There is need for surgeons to be conscious of operation time as a risk factor for SSI and strive to reduce the operating time.

In this study, positive HIV status was not significantly shown to increase risk of SSI in both bivariate and multivariate analysis. This agrees with Kigera *et al.,* (2012) who reported no difference in SSI incidence among HIV infected and HIV negative groups of patients. However, Mawalla *et al.,* (2011) in Tanzania reported an 11 fold increase in SSI among HIV infected patients. This could be because a significant proportion of the HIV positive patient in the Tanzania study had CD4 counts lower than 200. This

study did not assess the CD4 counts but at the time of conducting the study, Kenya had already adopted the test and start approach to HIV care where all persons diagnosed with HIV are initiated on antiretroviral therapy regardless of CD4 counts. As reported by Blood *et al.*, (2017), HIV status alone is not a significant predictor of SSI as long as the patient is on optimum care and has adequate immunological and virological control.

5.2 Bacterial pathogens causing surgical site infections

Staphylococcus aureus was the commonest etiological agent for SSI in this study followed by *Escherichia coli*. Most of the SSI patients in this study had undergone ORIF (50.9%) which could explain the predominance of *Staphylococcus aureus* since skin flora are expected to be the etiological agents in this setting. The finding agrees with those from most SSI surveillance program and studies assessing the etiology of SSI. In West Bengal- India, for example, Bhattacharya *et al*., (2016) found in a 3½ year study that *Staphylococcus aureus* was the commonest causative agent in SSI at 34.93% followed by *Escherichia coli* (20.34%), *Klebsiella spp*. (18.08%), *Pseudomonas spp*. (7.99%) and *Acinetobacter spp.* (7.49%).

As demonstrated by this study, the prevalence of *Staphylococcus aureus* in SSI varies depending on the site of surgery. This agrees with Yang *et al*., (2015) in China who despite showing an overall *Staphylococcus aureus* incidence of 19.1% of all isolates in SSI cases further found that this varied with the type of surgery with SSI from chest surgery having a prevalence of 41.1% and SSI from abdominal surgery having *Staphylococcus aureus* isolates at 13.8%. Manyahi *et al.*, (2014) in Tanzania however showed a predominance of *Pseudomonas aeruginosa* (16.3%), followed by *Staphylococcus aureus* (12.2%) and *Klebsiella pneumoniae* (10.8%) in SSI. This

could be due to the fact that the patients in Manyahi *et al.,* (2014) study had undergone mainly urological surgeries hence the predominance of gram negative organisms.

Methicillin Resistant *Staphylococcus aureus* (MRSA) should be considered due to its resistance to many bacterial agents making it difficult to treat and further increasing the cost of care and hospital stay. In this study MRSA isolates were 13 which represented 59% of all the *Staphylococcus aureus* isolated. This is consistent with other researchers who have shown a high prevalence of MRSA. For example Iyamba *et al*., (2014) showed a high MRSA rate of 63.5% of all *Staphylococcus aureus* in SSI isolates in Kinshasha, Democratic Republic of Congo (DRC). In India, Bhattacharya *et al*., (2016) reported MRSA to be 25.45% of all *Staphylococcus aureus* isolates while in Yang *et al.,* (2015) reported 41.3% MRSA in China. Other studies that have shown significant MRSA incidence in SSI include Ojulong *et al.,* (2009) and Seni *et al.,* (2013) in Uganda (31.5% and 37.5% respectively), Manyahi *et al.,* (2014) in Muhimbili, Tanzania (44%) and Kahsay *et al.,* (2014) in Ethiopian (49.7%).

The study findings of 59% *Staphylococcus aureus* being MRSA. Iyamba *et al.,* (2014) in DRC reported 63.5% MRSA rate. Kahsay *et al*., (2014) in Ethiopia and Manyahi *et al.,* (2014) in Tanzania had reported MRSA rates of 49.7% and 44.0% respectively. The study findings on MRSA rate is therefore comparable to similar studies on SSI in Eastern Africa. The prevalence of MRSA is a cause of concern and should underscore the need for more aggressive infection control and prevention in the perioperative setting. MRSA could be on the rise in the context of SSI which further underscores the need for regular SSI surveillance to detect such changes in etiological patterns. It is notable however that Andhoga *et al.,* (2002) showed an MRSA rate of 80.4% of *Staphylococcus aureus* isolated from postoperative wounds in

MTRH. It is therefore likely that MRSA prevalence in MTRH has been high hence interventions are needed to control this pathogen.

In this study 6 *Acinetobacter baumannii* were isolated representing 10.9% of all bacterial pathogens in SSI. This agrees with Benito *et al.,* (2016) who showed that despite *Staphylococcus aureus* being the commonest causative organisms, there have been reports of a linear increase of other pathogens such as *Acinetobacter baumannii* hence the need for these to be monitored. As reported by Helal *et al.,* (2015), *Acinetobacter baumannii* has a multi-drug resistant (MDR) pattern hence its identification in SSI needs to be closely monitored. The isolation of *Acinetobacter baumannii* in this study could have been contributed by very sick patients some of whom were in ICU. It is also possible that an outbreak of this pathogen could have occurred during the time of the study. An SSI surveillance program, if instituted, would enable MTRH to identify trends in such multidrug resistance organisms and institute control measures.

5.3 Antibacterial susceptibility of pathogens causing surgical site infection

The study found high levels of resistance of bacterial species to most of the commonly used antibiotics in the perioperative period at the MTRH setting. The gram positive organisms showed high levels of resistance to Ceftriaxone, cotrimoxazole, erythromycin, azithromycin and ciprofloxacin. Vancomycin was active against all gram positive isolates except one MRSA isolate which showed intermediate susceptibility. Only vancomycin and amikacin had more that 50% sensitivity to MRSA. This agrees with Seni *et al.*, (2013) in Uganda who reported all *Staphylococcus aureus* including MRSA to be susceptible to vancomycin. Resistance among *Staphylococcus aureus* isolated in SSI has also been reported in Iran by

Alikhani *et al.,* (2015) who found that *Staphylococcus aureus* from SSI cases had 30% resistance to amikacin and 23.4% resistance to teicoplanin.

Among the gram negative organisms sensitivity patterns were very variable most were resistant to ceftriaxone, ciprofloxacin, levofloxacin, cefipime and cefotaxime. All gram negatives except *Acinetobacter* were sensitive to meropenem and over 90% were sensitive to amikacin. The findings largely agree with other researchers. For example, in Kinshasa, Iyamba *et al.,* (2014) reported resistance to ampicillin, cotrimoxazole, erythromycin, clindamycin, ciprofloxacin, cefotaxime and ceftazidime among organisms causing SSI. They also found high sensitivity levels to imipenem, amoxycillin-clavulanic acid and vancomycin to which all isolated organisms were sensitive. Seni *et al.,* (2013) also showed susceptibility of gram negative bacterial species to imipinem and amikacin and resistance to other antibacterial agents. The findings also agree with Kahsay *et al.,* (2014) in Ethiopia that found that the bacterial isolates from SSI showed >80% level of resistance to ampicillin, amoxicillin, penicillin G, erythromycin, gentamicin and cotrimoxazole.

An evaluation of resistance patterns for MRSA is important in order to optimize prevention strategies for this particular pathogen. In this study, MRSA showed 100% resistance to ceftriaxone, cotrimoxazole, azithromycin, ciprofloxacin and cefuroxime as well as more than 60% resistance to levofloxacin, clindamycin, meropenem, erythromycin and gentamycin. The findings agree with Iyamba *et al.,* (2014) that showed that MRSA was resistant to ampicillin, cotrimoxazole, erythromycin, clindamycin, ciprofloxacin, cefotaxime and ceftazidime. It also agrees with Pahadi *et al.,* (2014) that found MRSA resistance to ampicillin, penicillin, cefotaxime, gentamycin, cotrimoxazole and erythromycin in Nepal. They however found 80%

sensitivity to tetracycline and ciprofloxacin. In Mulago Hospital in Uganda, resistance rates of MRSA were found by Ojulong *et al.,* (2009) to be 88.2% for trimethoprim-sulfamethoxazole, 88.2% for erythromycin, 58.8% for gentamycin, 70.6% for ciprofloxacin, and 88.2% for chloramphenicol. They however found all MRSA isolates were found to be sensitive to vancomycin and clindamycin.

Other studies have however shown relatively favourable sensitivity pattern with MRSA. For example Bhattacharya *et al.,* (2016) in India found MRSA strains to be 100% sensitive to linezolid and tigecycline followed by fucidin (92.51%), mupirocin (88.39%), levofloxacin (75.66%) and doxycycline (72.28%). Yang *et al.,* (2015) in China also showed favorable sensitivity of MRSA to vancomycin and linezolid. They however showed resistance to clindamycin and erythromycin of similar levels as this study.

There was one MRSA isolate in this study that had intermediate sensitivity to vancomycin which is considered a reserve drug for MRSA. The emerging vancomycin resistance has also been shown by Alikhani *et al.,* (2015) who found 30% of MRSA isolates to be resistant to vancomycin. As much as the current study showed over 90% sensitivity of MRSA to vancomycin, resistance could be emerging. This trend needs to be monitored and use of vancomycin needs to continue to be adequately controlled and only reserved for treatment of microbiologically confirmed MRSA. Whereas Bhattacharya *et al.,* (2016) found no vancomycin resistant strains 1.12% of the strains were found to be intermediately susceptible to it. Iyamba *et al.,* (2014) found only 81% sensitivity of MRSA strains causing SSI to vancomycin compared to 100% sensitivity to imipenem and 89% sensitivity to amoxycillin-clavulanic. Pahadi *et al.,* (2014) further noted that although all MRSA strains were

sensitive to vancomycin on disc diffusion, four isolates were intermediates in vitro determination of MIC of vancomycin. The findings also agree with Kahsay *et al.,* (2014) that showed MRSA resistance to vancomycin of 5.6%.

Other than MRSA, *Acinetobacter baumannii* also showed multi drug resistance in this study with all the isolates being resistant to all the tested antibacterial agents except gentamycin, meropenem and amikacin which each had only 50% sensitivity. The finding agrees with other studies such as Helal *et al.,* (2015), Godebo *et al.,* (2013) and Mundhada *et al.,* (2015) which showed a high prevalence of multi-drug resistant SSI causing strains other than MRSA. This underscores the significance of other SSI causing pathogens such as *Acinetobacter baumannii* that were often considered minor contributors but have severe outcomes due to their multi-drug resistance nature. Their incidence and trends need to be monitored and adequate control measures instituted. This can be possible in a setting with functional SSI surveillance program.

CHAPTER SIX: CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Cigarette smoking, diabetes mellitus and prolonged operation time increase the risk of developing surgical site infection. *Staphylococcus aureus* was the commonest bacterial agent isolated in surgical site infection. A significant proportion of this was methicillin resistant *Staphylococcus aureus* (MRSA). *Acinetobacter baumannii* also had a prominent role in causation of surgical site infection.

The isolated bacterial agents causing SSI showed resistance to most commonly used antibiotics in the perioperative period at MTRH. MRSA and *Acinetobacter baumannii* had multiple drug resistance patterns. Gram positive bacteria including MRSA were sensitive to vancomycin though one intermediate resistant strain was detected among MRSA. Meropenem and Amikacin were active against gram negative bacteria except *Acinetobacter baumannii.*

6.2: Recommendations

Prevention strategies for SSI should include interventions to reduce operating time by exploring ways of improving efficiency. Surgical patients with history of diabetes mellitus and smoking need adequate optimization and aggressive SSI prevention strategies. They should be closely monitored for development of SSI.

MTRH should institute an SSI surveillance program in order to identify regular and emerging SSI causing organisms such as MRSA and *Acinetobacter baumannii* which should be controlled and treated appropriately. Perioperative antibiotic use should be rational and be guided by local susceptibility patterns.

REFERENCES

Aiken, A. M., Karuri, D. M., Wanyoro, A. K., & Macleod, J. (2012). Interventional studies for preventing surgical site infections in sub-Saharan Africa – A systematic review. *International Journal of Surgery (London, England), 10*(5), 242-249. doi: 10.1016/j.ijsu.2012.04.004

Alikhani, A., Babamahmoodi, F., Foroutan Alizadegan, L., Shojaeefar, A., & Babamahmoodi, A. (2015). Minimal inhibitory concentration of microorganisms causing surgical site infection in referral hospitals in North of Iran, 2011-2012. *Caspian Journal of Internal Medicine*, *6*(1), 34-39.

Ameh, E. A., Mshelbwala, P. M., Nasir, A. A., Lukong, C. S., Jabo, B. A., Anumah, M. A., & Nmadu, P. T. (2008). Surgical Site Infection in Children: Prospective Analysis of the Burden and Risk Factors in a Sub-Saharan African Setting. *Surgical Infections, 10*(2), 105-109. doi: 10.1089/sur.2007.082

AMPATH. (2014). Moi Teaching and Referral Hospital. Retrieved September 20, 2014, from http://www.ampathkenya.org/our-partners/mtrh/

Anderson, D. J., Kaye, K. S., Chen, L. F., Schmader, K. E., Choi, Y., Sloane, R., & Sexton, D. J. (2009). Clinical and financial outcomes due to methicillin resistant *Staphylococcus aureus* surgical site infection: a multi-center matched outcomes study. *PloS one*, 4(12), e8305.

Andhoga, J., Macharia, A. G., Maikuma, I. R., Wanyonyi, Z. S., Ayumba, B. R., & Kakai, R. (2002). Aerobic pathogenic bacteria in post-operative wounds at Moi Teaching and Referral Hospital. *East African Medical Journal, 79*(12), 640-644.

Astagneau, P., L'Hériteau, F., Daniel, F., Parneix, P., Venier, A. G., Malavaud, S., & Bernet, C. (2009). Reducing surgical site infection incidence through a network: results from the French ISO-RAISIN surveillance system. *Journal of Hospital Infection*, 72(2), 127-134.

Atwoli, L., Mungla, P. A., Ndung'u, M. N., Kinoti, K. C., & Ogot, E. M. (2011). Prevalence of substance use among college students in Eldoret, western Kenya. *BMC Psychiatry, 11*(1), 34. doi: 10.1186/1471-244x-11-34

Bauer, A., Kirby, W., Sherris, J. C., & Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. *American journal of clinical pathology*, 45(4), 493.

Benito, N., Franco, M., Ribera, A., Soriano, A., Rodriguez-Pardo, D., Sorlí, L., & Puente, A. (2016). Time trends in the aetiology of prosthetic joint infections: a multicentre cohort study. *Clinical Microbiology and Infection, 22*(8), 732.e731-732.e738. doi: 10.1016/j.cmi.2016.05.004

Berríos-Torres, S. I., Umscheid, C. A., Bratzler, D. W., Leas, B., Stone, E. C., Kelz, R. R., & Dellinger, E. P. (2017). Centers for disease control and prevention guideline for the prevention of surgical site infection. *JAMA surgery*, 152(8), 784-791.

Bhattacharya, S., Pal, K., Jain, S., Chatterjee, S. S., & Konar, J. (2016). Surgical Site Infection by Methicillin Resistant Staphylococcus aureus– on Decline? *Journal of Clinical and Diagnostic Research : JCDR, 10*(9), DC32-DC36. doi: 10.7860/JCDR/2016/21664.8587

Blood, A. G., Sandoval, M. F., Burger, E., & Halverson-Carpenter, K. (2017). Risk and Protective Factors Associated with Surgical Infections among Spine Patients. *Surgical Infections, 18*(3), 234-249. doi: 10.1089/sur.2016.183

Boyle, V. J., Fancher, M. E., & Ross, R. W. (1973). Rapid, modified Kirby-Bauer susceptibility test with single, high-concentration antimicrobial disks. *Antimicrobial Agents Chemotherapy*, 3(3), 418-424.

Bull, A. L., Worth, L. J., Spelman, T., & Richards, M. J. (2017). Antibiotic Prescribing Practices for Prevention of Surgical Site Infections in Australia: Increased Uptake of National Guidelines after Surveillance and Reporting and Impact on Infection Rates. *Surgical Infections, 18*(7), 834-840. doi: 10.1089/sur.2017.119

Cai, J., & Zeng, D. (2004). Sample size/power calculation for case–cohort studies. *Biometrics, 60*(4), 1015-1024.

Chahoud, J., Kanafani, Z., & Kanj, S. S. (2014). Surgical site infections following spine surgery: eliminating the controversies in the diagnosis. *Frontiers in medicine*, 1, 7.

De Godoy, J. M., Ribeiro, J. V., Caracanhas, L. A., & Godoy, M. d. (2010). Hospital infection after major amputations. *Annals of Clinical Microbiology and Antimicrobials, 9*(1), 15. doi: 10.1186/1476-0711-9-15

Elgohari, S., Mihalkova, M., & Wloch, C. (2013). Surveillance of Surgical Site Infections in NHS Hospitals in England, 2013/2014. *Public Health England. Health Protection Agency*.

Franklin, R., & Cockerill III, M. (2011). Performance Standards for Antimicrobial Susceptibility Testing, Twenty-First Informational Supplement M100-S21. *Clinical and Laboratory Standard Institute, 31*, 68-80.

Friedman, N. D., Sexton, D. J., Connelly, S. M., & Kaye, K. S. (2007). Risk factors for surgical site infection complicating laminectomy. *Infection Control Hospital Epidemiology, 28*(9), 1060-1065. doi: 10.1086/519864

Godebo, G., Kibru, G., & Tassew, H. (2013). Multidrug-resistant bacterial isolates in infected wounds at Jimma University Specialized Hospital, Ethiopia. *Annals of Clinical Microbiology and Antimicrobials, 12,* 17-17. doi: 10.1186/1476-0711-12-17

Guo, S. A., & DiPietro, L. A. (2010). Factors affecting wound healing. *Journal of dental research,* 89(3), 219-229.

Helal, S., El Anany, M., Ghaith, D., & Rabeea, S. (2015). The Role of MDR-*Acinetobacter baumannii* in Orthopedic Surgical Site Infections. *Surgical Infections, 16*(5), 518-522. doi: 10.1089/sur.2014.187

Henriksen, N. A., Meyhoff, C. S., Wetterslev, J., Wille-Jørgensen, P., Rasmussen, L. S., Jorgensen, L. N., & PROXI Trial Group. (2010). Clinical relevance of surgical site infection as defined by the criteria of the Centers for Disease Control and Prevention. *Journal of Hospital Infection,* 75(3), 173-177.

Issa, K., Naziri, Q., Rasquinha, V., Maheshwari, A. V., Delanois, R. E., & Mont, M. A. (2013). Outcomes of cementless primary THA for osteonecrosis in HIV-infected patients. *Journal of Bone & Joint Surgery*, *95*(20), 1845-1850. doi: 10.2106/JBJS.L.01583

ITC Project (December, 2015). ITC Kenya National Report. Findings from the Wave 1

(2012) Survey. University of Waterloo, Waterloo, Ontario Canada; Ministry of Health [Kenya], Kenya Medical Research Institute, International Institute for Legislative Affairs, and University of Nairobi, Nairobi, Kenya.

Iyamba, J.-M. L., Wambale, J. M., Lukukula, C. M., & Takaisi-Kikuni, N. z. B. (2014). High prevalence of methicillin resistant staphylococci strains isolated

from surgical site infections in Kinshasa. *The Pan African Medical Journal, 18*, 322. doi: 10.11604/pamj.2014.18.322.4440

Kahsay, A., Mihret, A., Abebe, T., & Andualem, T. (2014). Isolation and antimicrobial susceptibility pattern of Staphylococcus aureus in patients with surgical site infection at Debre Markos Referral Hospital, Amhara Region, Ethiopia. *Archives of Public Health, 72*(1), 16-16. doi: 10.1186/2049-3258-72-16

Kigera, J. W. M., & Gakuu, L. N. (2013). Incidence of early post operative surgical site infection after primary total hip arthroplasty in the African setting. *East African Orthopedic Journal., 7*(1), 48-53.

Kigera, J. W. M., Straetemans, M., Vuhaka, S. K., Nagel, I. M., Naddumba, E. K., & Boer, K. (2012). Is There an Increased Risk of Post-Operative Surgical Site Infection after Orthopaedic Surgery in HIV Patients? A Systematic Review and Meta-Analysis. *PLoS One, 7*(8), e42254. doi: 10.1371/journal.pone.0042254

Kirkland, K. B., Briggs, J. P., Trivette, S. L., Wilkinson, W. E., & Sexton, D. J. (2015). The Impact of Surgical-Site Infections in the 1990s: Attributable Mortality, Excess Length of Hospitalization, And Extra Costs. *Infection Control & Hospital Epidemiology, 20*(11), 725-730. doi: 10.1086/501572

Kuscu, F., Ozturk, D. B., Gurbuz, Y., Tutuncu, E. E., Sencan, I., & Gul, S. (2011). [Investigation of reduced vancomycin susceptibility in methicillin-resistant staphylococci]. *Mikrobiyol Bul, 45*(2), 248-257.

Lubega, A., Joel, B., & Justina Lucy, N. (2017). Incidence and Etiology of Surgical Site Infections among Emergency Postoperative Patients in Mbarara Regional

Referral Hospital, South Western Uganda. *Surgery Research and Practice, 2017*, 6365172. doi: 10.1155/2017/6365172

Manyahi, J., Matee, M. I., Majigo, M., Moyo, S., Mshana, S. E., & Lyamuya, E. F. (2014). Predominance of multi-drug resistant bacterial pathogens causing surgical site infections in Muhimbili National Hospital, Tanzania. *BMC Research Notes, 7*(1), 500.

Mawalla, B., Mshana, S. E., Chalya, P. L., Imirzalioglu, C., & Mahalu, W. (2011). Predictors of surgical site infections among patients undergoing major surgery at Bugando Medical Centre in Northwestern Tanzania. *BMC Surgery*, *11*, 21-21. doi: 10.1186/1471-2482-11-21

Mengesha, R. E., Kasa, B. G.-S., Saravanan, M., Berhe, D. F., & Wasihun, A. G. (2014). Aerobic bacteria in post surgical wound infections and pattern of their antimicrobial susceptibility in Ayder Teaching and Referral Hospital, Mekelle, Ethiopia. *BMC Research Notes, 7*(1), 575.

Møller, A. M., Pedersen, T., Villebro, N., & Munksgaard, A. (2003). Effect of smoking on early complications after elective orthopaedic surgery. *The Journal of bone and joint surgery*. British volume, 85(2), 178-181.

Mshana, S. E., Matee, M., & Rweyemamu, M. (2013). Antimicrobial resistance in human and animal pathogens in Zambia, Democratic Republic of Congo, Mozambique and Tanzania: an urgent need of a sustainable surveillance system. *Annals of Clinical Microbiology and Antimicrobials, 12*(1), 28.

Mundhada, A. S., & Tenpe, S. (2015). A study of organisms causing surgical site infections and their antimicrobial susceptibility in a tertiary care Government Hospital. *Indian Journal of Pathology and Microbiology, 58*(2), 195.

Namba, R. S., Inacio, M. C., & Paxton, E. W. (2013). Risk factors associated with deep surgical site infections after primary total knee arthroplasty: an analysis of 56,216 knees. *Journal of bone and joint surgery*, 95(9), 775-782.

Nejad, S. B., Allegranzi, B., Syed, S. B., Ellis, B., & Pittet, D. (2011). Health-care-associated infection in Africa: a systematic review. *Bulletin of the World Health Organization*, 89, 757-765.

Nturibi, E. M., Kolawole, A., & McCurdy, S. A. (2009). Smoking prevalence and tobacco control measures in Kenya, Uganda, the Gambia and Liberia: a review. *The International Journal of Tuberculosis and Lung Disease, 13*(2), 165-170.

Nyundo, M., Rugwizangoga, E., Ntakiyiruta, G., & Kakande, I. (2013). Outcome of emergency abdominal surgery at Kigali University Teaching Hospital: a review of 229 cases. *East and Central African Journal of Surgery, 18*(1), 31-39.

Oduor, O., Apondi, O., Gye, B., Siika, W., & Kipkoech, M. (2016). Anti-bacterial susceptibility patterns of blood culture isolates at a referral hospital in Eldoret. *East African Medical Journal, 93*(1), 3-8.

Ojulong, J., Mwambu, T., Joloba, M., Bwanga, F., & Kaddu-Mulindwa, D. (2009). Relative prevalence of methicilline resistant Staphylococcus aureus and its susceptibility pattern in Mulago Hospital, Kampala, Uganda. *Tanzania Journal of Health Research, 11*(3).

Pahadi, P. C., Shrestha, U. T., Adhikari, N., Shah, P. K., & Amatya, R. (2014). Growing resistance to vancomycin among methicillin resistant Staphylococcus aureus isolates from different clinical samples. *Journal of Nepal Medical Association, 52*(196), 977-981.

Rodríguez, P., Herrera, P., Gonzalez, O., Alonso, J., & Blanco, H. (2013). A randomized trial comparing Lichtenstein repair and no mesh Desarda repair for inguinal hernia: a study of 1382 patients. *East and Central African Journal of Surgery, 18*(2), 18-25.

Seni, J., Najjuka, C. F., Kateete, D. P., Makobore, P., Joloba, M. L., Kajumbula, H., . . . Bwanga, F. (2013). Antimicrobial resistance in hospitalized surgical patients: a silently emerging public health concern in Uganda. *BMC Research Notes, 6*(1), 298.

Shao, J., Chang, H., Zhu, Y., Chen, W., Zheng, Z., Zhang, H., & Zhang, Y. (2017). Incidence and risk factors for surgical site infection after open reduction and internal fixation of tibial plateau fractures: A meta-analysis. *International Journal of Surgery*.

Sørensen, L. T., Jørgensen, S., Petersen, L. J., Hemmingsen, U., Bülow, J., Loft, S., & Gottrup, F. (2009). Acute effects of nicotine and smoking on blood flow, tissue oxygen, and aerobe metabolism of the skin and subcutis. *Journal of Surgical Research*, 152(2), 224-230.

Stone, N. D., Ashraf, M. S., Calder, J., Crnich, C. J., Crossley, K., Drinka, P. J., & Loeb, M. (2012). Surveillance definitions of infections in long-term care facilities: revisiting the McGeer criteria. *Infection Control & Hospital Epidemiology, 33*(10), 965-977.

Ventola, C. L. (2015). The antibiotic resistance crisis: part 1: causes and threats. *Pharmacy and Therapeutics*, 40(4), 277.

Wang, T., Wang, H., Yang, D.-L., Jiang, L.-Q., Zhang, L.-J., & Ding, W.-Y. (2017). Factors predicting surgical site infection after posterior lumbar surgery: A multicenter retrospective study. *Medicine, 96*(5).

Warwick, A., Oppong, C., Boateng, D. B., & Kingsnorth, A. (2013). Inguinal hernia repair is safe in Africa. *East and Central African Journal of Surgery, 18*(2), 14-17.

Wloch, C., Wilson, J., Lamagni, T., Harrington, P., Charlett, A., & Sheridan, E. (2012). Risk factors for surgical site infection following caesarean section in England: results from a multicentre cohort study. *BJOG: An International Journal of Obstetrics & Gynaecology*, 119(11), 1324-1333.

Wolcott, R. D., Gontcharova, V., Sun, Y., Zischakau, A., & Dowd, S. E. (2009). Bacterial diversity in surgical site infections: not just aerobic cocci any more. *Journal of wound care*, 18(8), 317-323.

Yang, Z., Wang, J., Wang, W., Zhang, Y., Han, L., Zhang, Y., & Zhan, S. (2015). Proportions of *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* in patients with surgical site infections in mainland China: a systematic review and meta-analysis. *PLoS One, 10*(1), e0116079.

APPENDICES

Appendix I: Adult Informed Consent Form

Research Title: Antibacterial susceptibility patterns and risk factors for Surgical Site Infections at Moi Teaching and Referral Hospital, Eldoret

Investigator: Dr. Stephen Okello

Co-Investigators: Prof. K.R.Tenge, Dr. B.R.Ayumba and Dr. E. Ruto

INTRODUCTION

You are invited to join a research study to look at antibacterial susceptibility and risk factors for bacterial Surgical Site Infections at Moi Teaching and Referral Hospital, Eldoret. Please take whatever time you need to discuss the study with your family and friends, or anyone else you wish to. The decision to join or not to join is up to you. In this research, we are investigating the risk factors and the responsible bacteria for surgical site infections at MTRH. This is because surgical site infection has been recognized to be a major problem and there is need to evaluate its risk factors with a view of managing these and the responsible bacteria so as to use appropriate antibiotics.

WHAT IS INVOLVED IN THE STUDY?

If you decide to participate you will be asked to answer some questions. We think this will take about 15 minutes. In addition, blood will be drawn for laboratory testing (if need be) and a swab will be taken from your wound. These procedures may be slightly painful but will be done in the shortest time possible.

You can stop participating at any time. If you stop you will not lose any benefits.

BENEFITS TO TAKING PART IN THE STUDY

It is reasonable to expect the following benefits from this research: Appropriate antibiotic used to treat the infection following laboratory information. However, we can’t guarantee that you will personally experience benefits from participating in this study. Others may benefit in the future from the information we find in this study.

CONFIDENTIALITY

Your name will not be used when data from this study are published. Every effort will be made to keep clinical records, research records, and other personal information confidential.

We will take the following steps to keep information confidential, and to protect it from unauthorized disclosure, tampering, or damage: you will remain anonymous; data will be kept under lock and key; only the investigator, co-investigators and relevant bodies of Moi University and MTRH will have access to the data.

INCENTIVES

You will not receive anything for participating in this study.

YOUR RIGHTS AS A RESEARCH PARTICIPANT

Participation in this study is voluntary. You have the right not to participate at all or to leave the study at any time. Deciding not to participate or choosing to leave the study will not result in any penalty or loss of benefits to which you are entitled, and it will not harm your relationship with the MTRH.

CONTACTS FOR QUESTIONS OR PROBLEMS

Call Dr. Stephen Okello at +254720 869 006 or email okello.steve@gmail.com if you have questions about the study, any problems, if you/ your child experiences any unexpected physical or psychological discomforts, any injuries, or think that something unusual or unexpected is happening.

Questions about your rights as a research subject: You may contact Institutional Review Ethics Committee (IREC), Moi Teaching and Referral Hospital, 053 33471 Ext.3008. IREC is a group of people that reviews studies for safety and to protect the rights of study subjects

Consent of Participant (or Legally Authorized Representative)

Signature of Participant or Representative Date

Note: Upon signing, the participant or legal representative will receive a copy of this form, and the original will be held in the subject's research record.

Appendix II: Parental Informed Consent

Research Title: Antibacterial susceptibility patterns and risk factors for Surgical Site Infections at Moi Teaching and Referral Hospital, Eldoret

Investigator: Dr. Stephen Okello

Co-Investigators: Prof. K.R.Tenge , Dr. B.R.Ayumba and Dr. E. Ruto

INTRODUCTION

Your child has been invited to join a research study to look at Surgical Site Infections among patients in surgical departments at MTRH, Eldoret. Please take whatever time you need to discuss the study with your family and friends, or anyone else you wish to. The decision to let you child join, or not to join, is up to you.

In this research study, we are investigating the risk factors and the responsible bacteria for surgical site infections at MTRH. This is because surgical site infection has been recognized to be a major problem and there is need to evaluate its risk factors with a view of managing these and the responsible bacteria so as to use appropriate antibiotics.

WHAT IS INVOLVED IN THE STUDY

You will be asked to answer some questions on behalf of your child and your child will be asked some questions. We think this will take about 15 minutes. In addition, blood will be drawn for laboratory testing and a swab will be taken from his/her wound. These procedures may be slightly painful but will be done in the shortest time possible.

Your child can stop participating at any time. If your child stops he/she will not lose any benefits.

BENEFITS TO TAKING PART IN THE STUDY

It is reasonable to expect the following benefits from this research: Appropriate antibiotic used to treat the infection following laboratory information. However, we cannot guarantee that your child will personally experience benefits from participating in this study. Others may benefit in the future from the information we find in this study.

CONFIDENTIALITY

Your child's name will not be used when data from this study are published. Every effort will be made to keep clinical records, research records, and other personal information confidential.

We will take the following steps to keep information confidential, and to protect it from unauthorized disclosure, tampering, or damage: Your child will remain anonymous; data will be kept under lock and key; only the investigator, co-investigators and relevant bodies of Moi University and MTRH will have access to the data.

INCENTIVES

You or your child will not receive anything for participating in this study.

YOUR RIGHTS AS A RESEARCH PARTICIPANT

Participation in this study is voluntary. Your child has the right not to participate at all or to leave the study at any time. Deciding not to participate or choosing to leave the study will not result in any penalty or loss of benefits to which your child is entitled, and it will not harm his/her relationship with the MTRH.

CONTACTS FOR QUESTIONS OR PROBLEMS

Call Dr. Stephen Okello at +254720 869 006 or email okello.steve@gmail.com if you have questions about the study, any problems, if your child experiences any unexpected physical or psychological discomforts, any injuries, or think that something unusual or unexpected is happening.

Questions about your rights as a research subject: You may contact Institutional Review Ethics Committee (IREC), Moi Teaching and Referral Hospital, 053 33471 Ext.3008. IREC is a group of people that reviews studies for safety and to protect the rights of study subjects

Permission for a Child to Participate in Research

As parent or legal guardian, I authorize _____ (child's name) to become a participant in the research study described in this form.

Child's Date of Birth _____

Parent or Legal Guardian's Signature Date

_____

Note: Upon signing, the parent or legal guardian will receive a copy of this form, and the original will be held in the subject's research record.

Appendix III- Assent Form

NB: For Minors 6 years and above

Research Title: Antibacterial susceptibility patterns and risk factors for Surgical Site Infections at Moi Teaching and Referral Hospital, Eldoret

Investigator: Dr. Stephen Okello

Co-Investigators: Prof. K.R.Tenge , Dr. B.R.Ayumba and Dr. E. Ruto

We are doing a research study about Surgical Site Infections at MTRH**.** A research study is a way to learn more about people. If you decide that you want to be part of this study, you will be asked to answer a few questions for 10-15 minutes; a specimen will be taken from your wound and blood drawn (if necessary) for laboratory testing.

There are some things about this study you should know. Your blood will be taken for checking the bacteria causing your illness. The drawing of blood involves an injection which will be slightly painful but will last a few seconds. The taking of a specimen from the wound may also be slightly painful but these are important for your treatment and to inform treatment of other people in future.

Not everyone who takes part in this study will benefit. A benefit means that something good happens to you. We think these benefits might be that you will receive effective treatment for wound infection informed by the laboratory test. The society in general will also benefit by having information that will ensure they get the best treatment.

When we are finished with this study we will write a report about what was learned. This report will not include your name or that you were in the study.

You do not have to be in this study if you do not want to be. If you decide to stop after we begin, that is okay too. Your parents know about the study too.

If you decide you want to be in this study, please sign your name.

I, _____, want to be in this research study.

_____ _____

(Sign your name here) (Date)

Appendix IV: Questionnaire

SECTION A: BACKGROUND

1. Gender (circle one option): Male Female Unknown
2. Age (years):_____
3. Please circle the highest year of school completed:

(primary) (high school) (college/university) (graduate school)

4. Marital Status (check only one):
   - Married
   - Single
   - Separated
   - Divorced
   - Widowed

5. Date of Admission _____ 6. Date of Surgery _____

SECTION B: DIAGNOSIS AND CLASSIFICATION OF SURGICAL SITE INFECTION

7. Criteria for SSI (Indicate all that apply):
   - Abscess or other evidence of infection found during re-operation, by radiology or histopathology examination
   - Antibiotics prescribed by a healthcare worker for SSS (Patient reported only)
   - Aspirated pus/ swab of surgical site yields organisms and pus cells are present
   - Clinical diagnosis
   - Fever (temperature 38^0C or more)
   - Heat
   - Incision opened by surgeon or spontaneous dehisces
   - Localized pain and tenderness
   - Localized swelling
   - Purulent drainage
   - Redness

8. When was Surgical Site Infection detected?
   - During in patient follow up
   - At re-admission
   - At follow up clinic
   - Other, specify _____

9. Date of Onset of Surgical Site Infection: _____

10. SSI Type
    - Deep Incisional
    - Organ/ Space
    - Superficial Incisional

11. Specific site of organ/ space SSI
    - Bone
    - Joint or bursae
    - Intra abdominal
    - Intervertebral disc space
    - Pleural space Other. Specify_____

SECTION B: SURGERY DETAILS AND RISK FACTORS FOR SURGICAL SITE INFECTION

12. Type of Surgery
    - o Emergency
    - o Elective
13. Wound Class:
    - o 1 Clean
    - o 2 Clean-Contaminates
    - o 3 Contaminated
    - o 4 Dirty
    - o 5 Unknown
14. Primary Indication for Surgery:
    - o Trauma/Fracture
    - o Inflamatory joint disease
    - o Avascular necrosis
    - o Congenital deformity
    - o Gangrene
    - o Cancer/ Tumor
    - o Abdominal condition

Other, Specify_____

15. Category of surgical procedure:
    - o ORIF. Specify _____
    - o Cranial Surgery. Specify _____
    - o Debridement
    - o Joint replacement
    - o Amputation
    - o Abdominal Surgery, Specify_____
    - o Chest Surgery. Specify_____
    - o Other, Specify_____

16. Lead Surgeon Grade
    - o Consultant
    - o Registrar
    - o Medical Officer
    - o Other, Specify_____

17. Operation duration
Start time: _____
End time: _____
Total time (minutes): _____

18. Antibiotic prophylaxis
    - o No
    - o Yes. Specify antibiotic, route of administration and time (minutes) before surgery _____
    - o Unknown

19. Antibiotic Use after Surgery:
    - o No
    - o Yes. Specify antibiotic, route of administration and time (minutes) before surgery _____
    - o Unknown

20. a)Time Taken before surgery (Days from date of diagnosis) _____
    b) Time taken in Hospital Before Surgery _____ Days
21. Weight of the Patient:_____
22. Height of the Patient: _____
23. Body Mass Index (BMI):_____
24 Does the patient have any known chronic illness**:** Tick all that apply:
    - o Diabetes mellitus
    - o Hypertension
    - o HIV
    - o Malnutrition
    - o Other, Specify _____
25. Any history of Alcohol use:
    - o Yes Quantify (bottles per week)_____
    - o No

26. Any history of smoking:
- Yes

Quantify (Number of packs per day and duration of smoking in days)_____

- No

27. Mention all drugs used in the course of this current illness and any drugs used long term: _____

SECTION C: BACTERIAL AETIOLOGY OF SURGICAL SITE INFECTIONS

28. Bacterial organisms identified via microscopy of pus swab (Gram stain results)
Organism 1_____
Organism 2 _____
Organism 3_____

28. Bacterial organisms identified via culture of pus swab (only report those considered to be causing infection)
Organism 1_____
Organism 2 _____
Organism 3_____

28. Bacterial organisms identified via blood culture (only report those considered to be causing infection)
Organism 1_____
Organism 2 _____
Organism 3_____

SECTION D: ANTIBIOTIC SUSCEPTIBILITY PATTERNS OF IDENTIFIED BACTERIAL MICROORGANISMS

29. Indicate the antibiotic susceptibility patterns for each microorganism cultured above
For each antibiotic, indicate whether the bacteria is Sensitive, Intermediate or Resistant

Antibiotic	Organism 1: Name_____	Organism 2: Name_____	Organism 3: Name_____
Co-trimoxazole			
Erythromycin			
Azithromycin			
Gentamycin			
Ciprofloxacin			
Meropenem			
Ceftriaxone			
Cefuroxime			
Vancomycin			
Clindamycin			
Amikacin			
Levofloxacin			
Ceftazidime			
Cefipime			
Cefotaxime			

THANK YOU FOR PARTICIPATION

Appendix V: Ethical Approval Letter

INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE (IREC)

MOI TEACHING AND REFERRAL HOSPITAL
P.O. BOX 3
ELDORET
Tel: 33471//2/3

MOI UNIVERSITY
SCHOOL OF MEDICINE
P.O. BOX 4606
ELDORET

Reference: IREC/2014/155
Approval Number: 0001312

27th November, 2014

Dr. Okello Onyango,
Moi University,
School of Medicine,
P.O. Box 4606-30100,
ELDORET-KENYA.

INSTITUTIONAL RESEARCH & ETHICS COMMITTEE
27 NOV 2014
APPROVED
P.O. Box 4606-30100 ELDORET

Dear Dr. Onyango,

RE: FORMAL APPROVAL

The Institutional Research and Ethics Committee has reviewed your research proposal titled:-

"Surgical Site Infections among Patients in General Surgical and Orthopedic Departments at Moi Teaching and Referral Hospital, Eldoret."

Your proposal has been granted a Formal Approval Number: ***FAN: IREC 1312*** on 27th November, 2014. You are therefore permitted to begin your investigations.

Note that this approval is for 1 year; it will thus expire on 26th November, 2015. If it is necessary to continue with this research beyond the expiry date, a request for continuation should be made in writing to IREC Secretariat two months prior to the expiry date.

You are required to submit progress report(s) regularly as dictated by your proposal. Furthermore, you must notify the Committee of any proposal change (s) or amendment (s), serious or unexpected outcomes related to the conduct of the study, or study termination for any reason. The Committee expects to receive a final report at the end of the study.

Sincerely,

PROF. E. WERE
CHAIRMAN
INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE

cc Director - MTRH Dean - SOP Dean - SOM
Principal - CHS Dean - SON Dean - SOD

Appendix VI: Approval from Moi Teaching And Referral Hospital

MOI TEACHING AND REFERRAL HOSPITAL

Telephone: 2033471/2/3/4
Fax: 61749
Email: director@mtrh.or.ke
P. O. Box 3
ELDORET

Ref: ELD/MTRH/R.6/VOL.II/2008

27th November, 2014

Dr. Okello Onyango,
Moi University,
School of Medicine,
P.O. Box 4606-30100,
ELDORET-KENYA.

RE: APPROVAL TO CONDUCT RESEARCH AT MTRH

Upon obtaining approval from the Institutional Research and Ethics Committee (IREC) to conduct your research proposal titled:-

"Surgical Site Infections among Patients in General Surgical and Orthopedic Departments at Moi Teaching and Referral Hospital, Eldoret".

You are hereby permitted to commence your investigation at Moi Teaching and Referral Hospital.

DR. JOHN KIBOSIA
DIRECTOR
MOI TEACHING AND REFERRAL HOSPITAL

CC - Deputy Director (CS)
- Chief Nurse
- HOD, HRISM

Appendix VII: Guide on Volume of Blood Culture Samples in Children

Weight	**Approximate Age**	**Total Volume of Blood**	**Collection Procedure**
<2 kg	Neonate (less than 1 month)	1 to 2 ml	1 BACTEC PEDS (Pink)
2.1-12.7 kg	Infant (1 month – 2 years)	3 to 5 ml	1 BACTEC PEDS (Pink)
12.8-36.3 kg	Children (2 to 12 years)	5 to 10 ml (5ml per bottle)	2 BACTEC PEDS bottles collected from same venipuncture site
>36.3 kg	Adolescent (>12 years)	20ml	1 aerobic BACTEC bottle plus 1 anaerobic BACTEC bottle, collected from the same venipuncture site

Appendix VIII: Gram Stain Procedure

(Source: MTRH Microbiology Laboratory Standard Operating Procedures)

Part A: Slide Preparation:

i. Wash the slide with soap and water to remove any grease or oil, wipe with spirit or alcohol and dry the slide.
ii. Label the slide
iii. To prepare a smear for bacterial suspensions in broth, place a loop full of the broth culture on the slide using a sterile cooled loop. Spread by means of circular motion of the inoculating loop to about one centimeter in diameter. To prepare a smear from bacterial plate cultures use a sterile cooled loop to place a drop of sterile water or saline solution on the slide. Sterilize and cool the loop again and pick up a very small sample of a bacterial colony and gently stir into the drop of water/saline on the slide to create an emulsion. For primary gram staining of Swab Samples, roll the swab over the cleaned surface of a glass slide.
iv. Heat fix the smear by: Allowing the smear to air dry. After the smear has air-dried, hold the slide at one end and pass the entire slide through the flame of a Bunsen burner two to three times with the smear-side up.

Part B: Gram Stain Procedure

i. Place slide with heat fixed smear on staining tray.
ii. Gently flood smear with crystal violet and let stand for 1 minute.
iii. Tilt the slide slightly and gently rinse with tap water or distilled water using a wash bottle.
iv. Gently flood the smear with Gram's iodine and let stand for 1 minute
v. Tilt the slide slightly and gently rinse with tap water or distilled water using a wash bottle. The smear will appear as a purple circle on the slide.
vi. Decolorize using 95% ethyl alcohol or acetone. Tilt the slide slightly and apply the alcohol drop by drop for 5 to 10 seconds until the alcohol runs almost clear. Be careful not to over-decolorize.
vii. Immediately rinse with water
viii. Gently flood with safranin to counter-stain and let stand for 45 seconds.
ix. Tilt the slide slightly and gently rinse with tap water or distilled water using a wash bottle.
x. Blot dry the slide with bibulous paper.
xi. View the smear using a light-microscope under oil-immersion.

Appendix IX: Zn Staining Procedure
(Source: MTRH Microbiology Laboratory Standard Operating Procedures)

Reagents

i. Carbol fuschin (basic dye)
ii. Mordant (heat)
iii. 20% sulphuric acid (decolorizer)
iv. Methylene blue (counter stain) or Malachite green

Procedure

i. Fix the smear of the specimen over the glass slide, either by heating or alcohol fixation.
ii. Pour carbol fuschin over smear and heat gently until fumes appear. Do not overheat and allow it to stand for 5 minutes, then wash it off with water.
iii. Pour 20% sulphuric acid, wait for one minute and keep on repeating this step until the slide appears light pink in color. Wash off with water.
iv. Pour methylene blue, wait for two minutes, again wash with water
v. Allow it to air dry and examine under oil immersion lens.

Result

Acid fast bacilli stain pink, straight or slightly curved rods, at times having beaded appearance. The background appears blue due to methylene blue.

Appendix X- Identification Tests For Gram Positive Bacteria: Procedure
(Source: MTRH Microbiology Laboratory Standard Operating Procedures)

X a. Catalase test

This is used to differentiate the bacteria that produce the enzyme catalase such as *Staphylococci* from non-catalase producing bacteria such as *Streptococci.*

Procedure:

i. 2-3ml of hydrogen peroxide solution is poured into a test tube
ii. Using a wooden stick or a glass rod several colonies of the test organism are removed and immersed in the hydrogen peroxide solution
iii. Active bubbling indicates a positive catalase test.

X b. Coagulase test

This test is used to identify *Staphylococcus aureus* which produces coagulase. Both tube test and slide test were employed.

Slide test procedure (detects bound coagulase):

i. A drop of distilled water is placed on each end of a slide or on two separate slides
ii. A colony of the test organism is emulsified in each of the drops to make two thick suspensions
iii. A drop of plasma is added to one of the suspensions and mixed gently
iv. Clumping of the organisms will occur within 10 seconds if the organism is *Staphylococcus aureus*
v. No plasma is added to the second suspension. This is used to differentiate any granular appearance of the organism from true coagulase clumping.

Tube test procedure (detects free coagulase):

i. Plasma is diluted in the ratio of 1:10.
ii. Three small test tubes are labeled; test organism, positive control and negative control.
iii. 0.5ml of the diluted plasma are pipetted into each tube.
iv. Five drops (about 0.1ml) of the test organism are added into the labeled positive, 5drops of the *Staphylococcus aureus* culture to the tube labeled positiveand 5 drops of sterile broth to the tube labeled negative.
v. The tubes are incubated at 35-37 degrees celcius after mixing gently. Clotting should occur within 1hr, if not, the examination is repeated every 30 minutes for up to 6hours.
vi. Clotting is indicative of *Staphylococcus aureus.*

X c. Bacitracin test

This test was used to identify *Streptococcus pyogenes.*

Procedure

i. Bacitracin disk is placed on a culture plate inoculated with the organism and incubated at 35-37^{o}C overnight.
ii. A zone of inhibition around the disc is indicative of *Streptococcus pyogenes.*

X d. Aesculin test

Principle: Bile-esculin test is used to differentiate *Enterococci* and *nonenterococcus* group D *streptococci*, which are bile tolerant and can hydrolyze esculin to esculetin, from non-group D viridans group *streptococci*, which grow poorly on bile. It has good sensitivity and specificity (>90%).

Procedure:

i. With an inoculating wire or loop, touch two or three morphologically similar streptococcal colonies and inoculate the slant of the bile esculin medium with an S-shaped motion, or streak the surface of a bile esculin plate for isolation.
ii. The inoculated tube is incubated at 35-37 degree Celsius for 24 hours and the results are determined.
iii. All group D *streptococci* will be bile-esculin positive within 48 hours.

X e. Hemolysis test

Principle: Done on Blood agar to determine the type of hemolysis (destruction of red blood cell walls) an organism produces. Blood agar is a rich medium that has been supplemented with fresh 5-10% blood.

Test Procedure:

i. Streak a plate of blood agar for isolation. Do your last streak with a needle and poke into the agar. This usually gives clear, reliable zones of beta hemolysis and is especially important to see the effects of streptolysin O which is oxygen labile
ii. Incubate the plates at 37°C for 24-48 hours. Strep organisms should be incubated in the CO2 incubator.
iii. The plate will be a brownish red color after 48hours.

Results

Four types of hemolysis can be differentiated by the appearance of the agar:

Beta hemolysis is indicated by a clear colorless zone surrounding the colonies. There has been total lysis of the red blood cells.

Alpha hemolysis is indicated by a small zone of greenish to brownish discoloration of the media. This is caused by the reduction of hemoglobin to methemoglobin and its subsequent diffusion into the surrounding medium.

Alpha prime hemolysis is indicated by a zone of complete hemolysis, surrounded by a zone of partial hemolysis, a pink halo. This pattern can be easier to see if you scrape off the colony.

Gamma hemolysis is indicated by no change in the media.

Appendix XI- Identification Tests For Gram Negative Bacteria: Procedure
(Source: MTRH Microbiology Laboratory Standard Operating Procedures)

XI a. Indole test

Principle: Used to identify enterobacteria. Most strains of enterobacteria break down the aminoacid tryptophan with the release of indole.

Procedure:

Using a sterile straight wire, inoculate 5ml of sterile medium with test organism. Place an indole paper strip in the neck of the tube and put a stopper. Incubation is done at 35-37^{o}C overnight.Indole production will be exhibited by reddening of the lower part of the strip.

XI b. Methyl red test

Principle: Methyl Red (MR) test determines whether the microbe performs mixed acid fermentation when supplied with glucose. In mixed acid fermentation, three acids (acetic, lactic and succinic) are formed in significant amounts. These large amounts of acid results in a significant decrease in the pH of the medium below 4.4. This is visualized by using pH indicator, methyl red (p-dimethylaminoaeobenzene-O-carboxylic acid), which is yellow above pH 5.1 and red at pH 4.4.MR-VP broth is used for both MR Test and VP test.

Procedure:

i. Inoculate two tubes containing MR-VP Broth with a pure culture of the microorganisms under investigation.
ii. Incubate at 35 °C for up to 4 days.
iii. Add about 5 drops of the methyl red indicator solution to the first tube (for Voges-Proskauer test, Barrit's reagent is added to another tube).
iv. A positive reaction is indicated, if the colour of the medium changes to a stable red within a few minutes.

XI c. Voges-proskeur (VP) test

Procedure

i. 2ml of sterile glucose phosphate peptone water is inoculated with the test organism and incubated at 35-37^{o}C for 48hours.
ii. A small amount of creatinine will be added and mixed well.
iii. 3ml of sodium hydroxide will be added and mixed well.
iv. The bottle cap will be removed and left for one hour at room temperature.
v. Development of pink colour will be indicative of *Klebsiella pneumoniae.*

XI d. Citrate test

Principle: Citrate utlilization test is used to determine the ability of bacteria to utilize sodium citrate as its only carbon source and inorganic (NH4H2PO4) is the sole fixed nitrogen source. Upon uptake by the cell, citrate is cleaved by citrate lyase to oxaloacetate and acetate. The oxaloacetate is then metabolized to pyruvate and CO2.The carbon dioxide that is released will subsequently react with water and the

sodium ion in the medium to produce sodium carbonate, an alkaline compound that will raise the pH. In addition, ammonium hydroxide is produced when the ammonium salts in the medium are used as the sole nitrogen source.

Growth usually results in the bromothymol blue indicator, turning from green to blue. The bromothymol blue pH indicator is a deep forest green at neutral pH. With an increase in medium pH to above 7.6, bromothymol blue changes to blue

Procedure

i. Inoculate simmons citrate agar lightly on the slant by touching the tip of a needle to a colony that is 18 to 24 hours old.
ii. Incubate at 35oC to 37oC for 18 to 24 hours. Some organisms may require up to 7 days of incubation due to their limited rate of growth on citrate medium.
iii. Observe the development of blue color; denoting alkalinization.

Expected results in citrate utilization test:

- Citrate positive: growth will be visible on the slant surface and the medium will be an intense Prussian blue. The alkaline carbonates and bicarbonates produced as by-products of citrate catabolism raise the pH of the medium to above 7.6, causing the bromothymol blue to change from the original green color to blue .
- Citrate negative: trace or no growth will be visible. No color change will occur; the medium will remain the deep forest green color of the uninoculated agar. Only bacteria that can utilize citrate as the sole carbon and energy source will be able to grow on the Simmons citrate medium, thus a citrate-negative test culture will be virtually indistinguishable from an uninoculated slant

XI e. Oxidase test

This test was used to identify *Pseudomonas* spp.

Procedure

i. Apiece of filter paper is placed in a petri dish and soaked with 2-3 drops of freshly prepared oxidase reagents.
ii. Using a piece of stick or glass rod, a colony of the test organism is then smeared on the filter paper.
iii. Development of blue- purple colour within a few seconds indicates positive oxidase test.

XI f. Urease test

This test was used to identify *Proteus spp.*

Procedure

i. A straight wire is used to inoculate a tube of MIU with a colony of the test organism.
ii. An indole paper strip is placed in the neck of the tube above the medium.

iii. The tube is stoppered and incubated at 35-37^{o}C overnight.
iv. Production of urease will change the colour of the paper strip to pink.

XI g. Triple Sugar Iron (TSI) Agar test

Principle: The has three sugar (Lactose, Sucrose, and Glucose) and also iron; and it contains Agar Agar as solidifying agent (TSI is a semi solid media having slant and butt).

Composition of Triple Sugar Iron Agar (TSI)

Lactose, Sucrose and Glucose in the concentration of 10:10:1 (i.e. 10 part Lactose (1%), 10 part Sucrose (1%) and 1 part Glucose (0.1%)).

- 0.1% Glucose: If only glucose is fermented, only enough acid is produced to turn the butt yellow. The slant will remain red
- 1.0 % lactose/1.0% sucrose: a large amount of acid turns both butt and slant yellow, thus indicating the ability of the culture to ferment either lactose or sucrose.
- Iron: Ferrous sulfate: Indicator of H2S formation
- Phenol red: Indicator of acidification (It is yellow in acidic condition and red under alkaline conditions).
- It also contains Peptone which acts as source of nitrogen- produces ammonia.

Procedure:

i. With a sterilized straight inoculation needle touch the top of a well-isolated colony
ii. Inoculate TSI Agar by first stabbing through the center of the medium to the bottom of the tube and then streaking on the surface of the agar slant.
iii. Leave the cap on loosely and incubate the tube at 35°C in ambient air for 18 to 24 hours.

Interpretation of Triple Sugar Iron Agar Test

i. If lactose (or sucrose) is fermented, a large amount of acid is produced, which turns the phenol red indicator yellow both in butt and in the slant. Some organisms generate gases, which produces bubbles/cracks on the medium.
ii. If lactose is not fermented but the small amount of glucose is, the oxygen deficient butt will be yellow but on the slant the acid will be oxidized to carbondioxide and water by the organism and the slant will be red(alkaline or neutral pH).
iii. If neither lactose/sucrose nor glucose is fermented, both the butt and the slant will be red. The slant can become a deeper red-purple (more alkaline) as a result of production of ammonia from the oxidative deamination of amino acids
iv. If H2S is produced, the black color of ferrous sulfide is seen.

Appendix XII: Antibacterial Susceptibility Testing Procedure

Principle of Disc diffusion technique:

i. A disc of blotting paper is impregnated with known volume and appropriate concentration of an antibiotic
ii. The disc is placed on a plate of susceptibility testing agar uniformly inoculated with the test organism
iii. The antibiotic diffuses from the disc into the medium and the growth of the test organism is inhibited at a distance from the disc that is related to the susceptibility of the organism.
iv. Strains susceptible to the antibiotic are inhibited at a distance from the disc whereas resistant strains have smaller zones of inhibition or grow up to the edge of the disc.

Specifically, Modified Kirby-Bauer diffusion technique was used.

Modified Kirby-Bauer disc diffusion Technique:

i. A sterile medium is prepared according to the manufacturer's instructions. The pH of the medium is set at 7.2-7.4.
ii. The media is poured into a 90mm sterile petri-dish to a depth of 4mm (about 25ml per plate). This is done on a level surface so that the depth of the medium is uniform. If the media is too thin the inhibition zone will be falsely large and if it is too thick the zones will be falsely small.
iii. Each new batch of agar is controlled using *E. faecalis* (ATCC 29212 or 33186) and cotrimoxazole disc. The zone of inhibition should be 20mm or more in diameter.
iv. The plates are stored at 2-8^{o}C in sealed plastic bags. Before use the plates are dried with their lids slightly raised in 35-37^{o}C incubator for about 30minutes.
v. About one hour before use, the working stock of the discs are allowed to warm to room temperature, protected from direct sunlight.

Modified Kirby-Bauer Disc diffusion Procedure:

i. Using a sterile wire loop, touch 3-5 well isolated colonies of similar appearance to the test organism and emulsify in 3-4ml of sterile physiological saline or nutrient broth.
ii. In good light, match the turbidity of the suspension to the turbidity of the standard (mix the standard immediately before use). When comparing turbidities it is easier to view against a printed card or sheet of paper
iii. Using a sterile swab, inoculate a plate of Mueller Hinton agar. Remove excess fluid by rotating and pressing the swab against the side of the tube above the level of the suspension. Streak the swab evenly over the surface of the medium in three directions, rotating the plate approximately 60^{o}C to ensure even distribution
iv. With the petri dish lid in place, allow 3-5 minutes (no longer than 15minutes) for the surface of the agar to dry
v. Using sterile forceps, needle mounted in a holder, or multidisc dispenser, place appropriate antibiotic discs, evenly distributed on the inoculated plate. The discs should be 15mm from the edge of the plate and no closer than about 25mm from disc to disc. No more than eight discs are applied on each petri dish. Each disc is lightly pressed down to ensure its contact with the agar. It should not be moved in one place

vi. Within 30minutes of applying the discs, invert the plate and incubate it aerobically at35^{o}C for 16-18 hours

vii. After overnight incubation, examine the control and the test plates to ensure the growth is confluent or near confluent. Using a ruler on the underside of the plate measure the diameter of each zone of inhibition in millimeters. The endpoint of inhibition is where growth starts.

Interpretation of zone sizes

Using the interpretative chart, the zones of each antibacterial were interpreted and reported for each organism as Resistant, Intermediate susceptibility or Susceptible for every antibacterial tested.

Appendix XIII: Caliper for Measuring Zone Of Inhibition (Milutoyo^{R})

Appendix XIV: Project Work Plan

No.	ACTIVITY	TIME DURATION	RESPONSIBLE PERSON
1.	Development of the research proposal	January – June 2014	Candidate and supervisors
2.	Defending the proposal at the Department of Orthopedics and pretesting tools	June 2014	Candidate
3.	Submission of Research Proposal for review by IREC	July 2014	Candidate
3.	Data Collection including Laboratory Tests	December 2014 to November 2015	Candidate, and Laboratory Assistants
4.	Data Analysis	January - February 2016	Candidate and Biostatistician
5.	Preparing the manuscript	March 2016	Candidate and Supervisors
6.	Presentation of Manuscript	April - May 2016	Candidate
7.	Writing the thesis	June- July 2016	Candidate
8.	Defending the thesis	August- September 2016	Candidate
9.	Making Corrections and Submitting Final Thesis	October- December 2016	Candidate and Supervisors

Appendix XV: Project Budget

No.	DESCRIPTION	UNIT OF MEASURE	QUANTITY	@ (Ksh)	COST (Ksh)
1.	Printing & Photocopying (Proposal, questionnaires)	Pages	500	2	1,000
2.	Culture and Sensitivity Tests- Pus swabs	Samples	60	500	30,000
3.	Culture and Sensitivity Tests – Blood (Patients with Deep/ Organ-Space Infection, estimated at 35% of all SSIs)	Samples	20	1200	24,000
4.	Biostatistician Allowance	Days	2	5000	10,000
5.	Laboratory Assistant (Part time)	Months	12	3000	36,000
6.	Preparation of Manuscript and Thesis (Printing, Binding, Photocopy)	Number of documents	20	300	6,000
7.	Communication (Including airtime for internet modem)	-	-	3000	3,000
8.	Miscellaneous				10,000
	TOTAL				**120,000**