ANTIMICROBIAL SUSCEPTIBILITY AND MANAGEMENT OF BACTERIAL SKIN AND SOFT TISSUE INFECTIONS AT MOI TEACHING AND REFERRAL HOSPITAL, ELDORET, KENYA

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A dissertation submitted to the Department of Surgery and Anesthesiology, School of Medicine, in partial fulfillment of the requirements for the award of the degree of Master of Medicine (General Surgery) of Moi University.

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DECLARATION

Student's declaration

This research is my original work and to the best of my knowledge, has not been submitted for an award of academic credit in any other university or research institution.

SIGN ……………………..   DATE ……………………………

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DEDICATION

I dedicate this work to all clinicians who seek to improve patient outcomes every day, especially those that deal with pathogenic organisms that are getting increasingly resistant to antimicrobials.

ACKNOWLEGEMENT

I acknowledge God for enabling me to carry out this research successfully. I thank my supervisors, Dr Ashraf Emarah MBBCh, MSc (Surgery), MD Surgery (Plastic Surgery) and Dr P. Kituyi Werunga MBChB, MMed (Anesthesiology) for their input in developing the proposal, its execution and preparation of this thesis. I also thank my family for their support.

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The investigator did not receive any outside funding or grants in support for this study. Neither he nor a member of his immediate family received payments or other benefits or commitment or agreement to provide such benefits from a commercial entity.

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ACRONYMS AND ABBREVIATIONS

BHS   Beta-hemolytic streptococcus

CoNS   Coagulase-negative staphylococci

ESBL   Extended Spectrum Beta Lactamase

IDSA   Infectious Diseases Society of America

IREC   Institutional Research and Ethics Committee

MRSA   Methicillin Resistant *Staphylococcus aureus*

MSSA   Methicillin Susceptible *Staphylococcus aureus*

MTRH   Moi Teaching and Referral Hospital

SIRS   Systemic Inflammatory Response Syndrome

SSI   Surgical Site Infection

SSTIs   Skin and Soft-tissue Infections.

OPERATIONAL DEFINITION OF VARIABLES AND KEY CONCEPTS

Antimicrobial- an agent that kills or inhibits the growth of pathogenic microorganisms, natural or synthetic

Appropriate antimicrobial therapy is whereby the organism(s) causing the disease is/are susceptible to the antimicrobial(s) prescribed

Definitive antimicrobial therapy refers to antimicrobial therapy given according to culture and antimicrobial sensitivity results

Empiric antimicrobial therapy refers to the therapy given on the basis of a clinical educated guess in the absence of a knowledge of the etiologic organism(s) or their antimicrobial susceptibility

Antimicrobial management refers to the antimicrobial prescription given to treat the disease (SSTI)

Methicillin Resistant *Staphylococcus aureus* (MRSA) prevalence refers to the percentage of MRSA among *Staphylococcus aureus*

Skin and soft tissue infections (SSTIs) refer to inflammatory microbial invasion of the epidermis, dermis and subcutaneous tissue including abscesses, cellulitis and wound infections

Susceptibility of a bacterial pathogen to an antimicrobial refers to the reading on disc diffusion test where the diameter of inhibition falls within the susceptible range, as opposed to intermediate or resistant range.

ABSTRACT

Background: Bacterial skin and soft tissue infections (SSTIs) are the second most common indication for antimicrobial therapy. The causative bacterial organisms are increasingly resistant, and are posing a great challenge and threat to public health, key among these being Methicillin-resistant *Staphylococcus aureus* (MRSA). There is little data on local antimicrobial susceptibility patterns in SSTIs and hence the approach to treatment remains a challenge.

Objectives: To determine the antimicrobial susceptibility of bacterial organisms causing SSTIs and the antimicrobial management of SSTIs at the Moi Teaching and Referral Hospital (MTRH).

Methods: This was a cross sectional descriptive study. Census sampling was done on patients diagnosed andadmitted with SSTIs at MTRH between January and December 2014. Pus and tissue from abscesses, cellulitis and wound infections were cultured. Bacteria were identified using gram stain and biochemical tests including indole, catalase, coagulase, oxidase, voges-proskeur, urease, bacitracin, aesculin, methyl red and citrate tests. Antimicrobial susceptibility was done using the disc diffusion method.

Results: Out of 84 bacteria cultured, *Staphylococcus aureus* constituted 47.6%. MRSA constituted 45% of *Staphylococcus aureus.* Over 80% of *Staphylococcus aureus* were susceptible to vancomycin and ceftazidime. Likewise, other gram positive bacteria, including *Enterococcus* spp., coagulase negative staphylococci and *Streptococcus pyogenes* were susceptible to vancomycin and clindamycin. Gram-negative bacteria were 25, and most were susceptible to meropenem and amikacin (>80%). However, *Acinetobacter baumanii* were not susceptible to any antimicrobial tested. Flucloxacillin and metronidazole were most used as empiric therapy, which was effective against the etiologic bacteria in 18%.

Conclusions: The most common cause of SSTIs was *Staphylococcus aureus*, which were susceptibile to vancomycin and clindamycin. Gram-negative bacteria were susceptible to meropenem and amikacin. Most empiric therapy was not appropriate.

Recommendations: Vancomycin, clindamycin, meropenem and amikacin should be used for the empiric treatment of severe SSTIs. De-escalation of this antimicrobial therapy should then be done according to the antimicrobial susceptibility results.

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CHAPTER ONE: INTRODUCTION

This chapter descibes the background information of the research, the problem statement , justification, research question, broad objective and specific objectives.

1.1 BACKGROUND

Skin and soft tissue infections (SSTIs) are the disease conditions resulting from the inflammatory microbial invasion of the epidermis, dermis and subcutaneous tissue. They are very common infections, being the second commonest indication for antimicrobial prescription. Moreover, the prevalence of SSTIs is on the increase globally. SSTIs can be life threatening if appropriate management is not instituted early (Schwartz & Lillehei, 1974; Ansari, Erntell, Goossens, Davey, & Group, 2009; Angus et al., 2001; DiNubile & Lipsky, 2004; Engel et al., 2007; Pallin et al., 2008).

The main causes of SSTIs are various bacterial species including *Staphylococcus aureus* and *Streptococcus pyogenes*. *Staphylococcus aureus* contributes up to 80%. Other causes include *Pseudomonas aeruginosa, Escherichia coli, Enterococcus* spp., *Acinetobacter* spp*. and Klebsiella* spp. Antimicrobial resistance has been documented in all these organisms (Ray, Suaya, & Baxter, 2013; Dryden, 2009a, 2010; Moet, Jones, Biedenbach, Stilwell, & Fritsche, 2007; Pendleton, Gorman, & Gilmore, 2013).

The World Health Organization (WHO) has identified antimicrobial resistance as a serious, worldwide threat to public health. The most common and serious of these multidrug resistant pathogens globally including *Enterococcus fecalis, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa* and *Enterobacter* spp., are all causes of SSTIs (Pendleton *et al.*, 2013).

Staphylococcus aureus is the commonest cause of SSTIs. Methicillin-resistant *Staphyloccus aureus* (MRSA) strains are particularly important. MRSA is increasingly being isolated in Kenya. However, the prevalence of MRSA in the Eldoret and its environs has not been reliably studied, with the most recent study at MTRH having been done in 2002. This study showed that among organisms causing surgical site infections, mainly *Staphylococcus* spp., MRSA were 80.4% (Elamenya, Nyamweya, Wafula, Okalebo, & Karimi, 2015; Maina, Kiiyukia, Wamae, Waiyaki, & Kariuki, 2013, Andhoga *et al.,* 2002).

Injudicious antimicrobial use has contributed to the emergence of resistant strains of bacteria to available drugs. This underscores the importance of antimicrobial stewardship, which is best enforced by treatment guidelines (Ansari *et al.*, 2009; Gelbrand *et al.*, 2015).

A review of the current knowledge on antimicrobial susceptibility pattern is necessary to guide antimicrobial therapy. In this regard, guidelines have been developed by various international infectious disease organizations like the Infectious Diseases Society of America (IDSA) and Surgical Infection Society to guide clinicians on appropriate antimicrobial use in SSTIs, as well as regulate antimicrobial use (Srinivasan *et al.,* 2004; May, 2011; Stevens *et al.,* 2005).

Kenya has developed a document, the 'Clinical guidelines for diagnosis and treatment of common conditions in Kenya, 2002'. This document is meant to guide caregivers at the primary level healthcare facilities. It is a summary of the management of common clinical conditions, but does not address management of the more complicated infections. For example, it does not detail the treatment of SSTIs in a hospital setting. This being a document that is well over ten years old, is also possible

that the microbial epidemiology has since changed (Kimathi, Micheni, & Muriithi, 2002).

There is need, therefore, to document the prevailing susceptibility patterns of SSTI causing bacterial organisms in Eldoret. This will aid in the development comprehensive and up to date treatment guidelines at MTRH. Such guidelines will not only guide effective treatment, but will also help in the regulation of antimicrobial prescription, which will curtail antimicrobial resistance.

1.2 PROBLEM STATEMENT

Treatment outcomes in SSTIs at MTRH have been less than optimal, with patients requiring extensive and repeated surgery as well as leading to prolonged hospital stay. This is likely to be due to bacterial organisms that are not susceptible to the antimicrobials used. The centre for disease dynamics, economics and policy has projected that in Kenya, among other African countries, the bacterial isolates are getting increasing resistant to antimicrobials, following an analysis of the susceptibility data from studies across the continent (Gelbrand *et al.,* 2015). Indeed, studies done in SSTIs in Kenya have shown evidence of high rates of resistance to antimicrobials among *Staphylococcus aureus* (Olipher *et al.,*2011, Maina *et al.,* 2015). Other etiologic bacteria have also developed resistance to antimicrobials, but this has not been studied adequately in Kenya (Pendleton *et al.*, 2013).

The spectrum of antimicrobials from which one can choose effective therapy is increasingly getting narrow. Resistant bacterial organisms have been associated with poor treatment outcomes and increased health costs (Cosgrove *et al.,* 2005). Knowledge of the local antimicrobials susceptibility of bacterial pathogens should therefore guide antimicrobial therapy (Pendleton *et al.*, 2013; Gelbrand *et al.*, 2015).

1.3 JUSTIFICATION

There is paucity of data on susceptibility patterns in SSTIs in Eldoret. This is needed in order to guide clinicians in appropriate prescription. It is also needed to develop comprehensive guidelines on the management of complicated SSTIs.

Local treatment guidelines do not adequately address the management of SSTIs. Knowledge on local antimicrobial susceptibility of pathogens should guide the development of local treatment guidelines for empirical treatment of SSTIs, and assist in updating and streamlining existing guidelines (Kimathi *et al.,* 2002).

There is need to identify the bacterial species that cause SSTIs and their susceptibility to antimicrobials. This will inform appropriate clinician prescription. It will also inform policy makers in developing treatment guidelines for SSTIs, be it institutional or national. There is also need to identify gaps in the current antimicrobial management, document the antimicrobials used as empiric therapy, and demonstrate the effectiveness of current empiric therapy at MTRH. This would set the stage for change in institutional policy regarding antimicrobial management of SSTIs.

1.4 RESEARCH QUESTION

What is the antimicrobial susceptibility and management of patients with bacterial skin and soft tissue infections at Moi Teaching and Referral Hospital (MTRH)?

1.5 BROAD OBJECTIVE

To determine the antimicrobial susceptibility of bacterial SSTIs and the antimicrobial management of patients with SSTIs at MTRH.

1.6 SPECIFIC OBJECTIVES
- To identify the bacterial etiology of SSTIs

- To determine the susceptibility of the bacterial pathogenic organisms to antimicrobials

- To determine the use of antimicrobials in the management of patients with SSTIs.

CHAPTER TWO : LITERATURE REVIEW

Based on the study objectives, this chapter reviewed the following areas: Epidemiology and etiology of SSTIs, distribution of SSTIs, risk factors for SSTIs, bacterial etiology of SSTIs, antimicrobial susceptibility of bacteria causing SSTIs including *Staphylococcus aureus, Enterococcus species, Pseudomonas aeruginosa, Escherichia coli , Klebsiella pneumonia* and *Acinetobacter baumannii*. It also reviews the specimen collection preocedures for culture and susceptibility testing, antimicrobial choice and stewardship, and the bacterial etiology and management of common SSTIs including cutaneous abscess, cellulitis, erysipelas, wound infection.

2.0 INTRODUCTION

SSTIs are a set of inflammatory conditions that result from microbial invasion of the epidermis, dermis and subcutaneous tissue, including abscesses, cellulitis and wound infections among others (Food, 2005). It is estimated that they are the second most common indication for antimicrobial therapy, which is the mainstay of therapy (Ki&Rotstein, 2008). Etiologic organisms in SSTIs have been shown to exhibit resistance to available antimicrobials, with resultant increased morbidity to patients as well as increased costs of hospitalization (Pendleton *et al.,* 2013).

SSTI is a naming modified from skin and skin structure infection. They are mainly bacterial in origin. The most common causes of hospitalization include wound infections, cellulitis and cutaneous abscess. Staphylococcal scalded skin syndrome, necrotizing fasciitis pyomyositis, and pilonidal cyst and sinus are less common, while impetigo, furuncles, carbuncle, acute lymphangitis and acute lymphadenitis are common but usually treated in the outpatient settting (Maina et al., 2013; Buck, 2015; Schwartz & Lillehei, 1974).

SSTIs may be spontaneous following hematogenous inoculation of bacteria in tissue, bacterial inoculation through hair follicles or due to breaks in the skin which allow colonization of bacteria. Most of the infections are self-limiting but others are life-threatening, requiring aggressive surgical debridement in addition to the appropriate choice of antimicrobials (Dryden, 2010; May *et al.*, 2009; Pitout *et al.*, 2008).

Complicated SSTIs include conditions that, among other criteria, are severe enough to require admission to hospital for stabilization, and require surgical treatment (Dryden, 2010; Stevens *et al.*, 2005).

2.1 EPIDEMIOLOGY AND ETIOLOGY OF SSTIS
2.1.1 DISTRIBUTION OF SSTIS

In the US, the population incidence rate of SSTIs in persons less than 65 years of age is 240-244 per 10,000 person years, with most of the infections caused by *S. aureus,* half of these being MRSA. The incidence is even higher in patients over 65 years, and those younger than 5 years. In the US, SSTIs accounted for 3.4 million hospital visits in 2005. They are the second most common indication for antimicrobial therapy, only second to chest infections (Ansari *et al,* 2009, Pallin *et al.,* 2008).

Studies have shown that most SSTIs (70-90%) are treated in the ambulatory setting. In the inpatient setting it has been estimated to account for 7-10% of all hospital admissions, but this would increase if only admissions for infections were considered. Most of them involve the lower limbs (Ray *et al.,* 2013; Ki&Rotstein, 2008).

2.1.2 RISK FACTORS FOR SSTIS

The presence of risk factors for SSTIs may influence the bacterial etiology, the disease progression and the response to treatment. Risk factors include comorbidities like diabetes, renal disease, liver disease, lymphedema, vascular insufficiency, neuropathy, smoking, recent hospitalization, children under 5 years or adults over 65 years, nasal carriage of *S. aureus,* central lines and other medical devices, injection drug use and low socioeconomic status (Dryden *et al,* 2009).

The risk factors for severe infection include host factors including comorbidities like diabetes, heart failure, immunosupression, renal disease and extremes of age, as well as disease factors including drug resistant organisms, virulent pathogenic strains, coinfection with multiple pathogenic organisms and area of infection. In particular, the increasing prevalence of SSTIs has been attributed to community-acquired methicillin-resistant *Staphylococcus aureus,* which is associated with significantly increased morbidity, mortality, length of hospital stay, and costs, compared with infections caused by susceptible strains (Ki& Rotstein, 2008; Dryden, 2009a.)

Complications of SSTIs include toxic shock syndrome, pyomyositis, osteomyelitis, bacteremia, necrotizing infection, limb gangrene, systemic inflammatory response syndrome, sepsis, septic shock, multiple organ dysfunction and death (Angus *et al.*, 2001; DiNubile & Lipsky, 2004; Engel *et al*., 2007; Pallin *et al.,* 2008).

2.1.3 BACTERIAL ETIOLOGY OF SSTIS

SSTIs are mainly bacterial infections, but there are also viral, fungal and protozoal causes (Dryden, 2009b). The majority of community acquired SSTIs are caused by *Staphylococcus aureus* and β-hemolytic streptococci. Others include *Pseudomonas*

aeruginosa, Escherichia coli, Enterococcus spp*., Acinetobacter* spp*. and Klebsiella* spp*.* (Dryden, 2010; Moet *et al.,* 2007).

Localized pus-producing lesions such as boils, abscesses, carbuncles and localized wound sepsis are usually staphylococcal, while rapidly spreading infections such as erysipelas, lymphangitis or cellulitis are usually caused by β-haemolytic streptococci (DiNubile & Lipsky, 2004; Dryden, 2010).

Gram-negative and anaerobic bacteria are commonly associated with surgical site infections of the abdominal wall or infections in the anal and perineal region. Polymicrobial infections occur where tissue vascular perfusion is compromised, such as diabetic foot infection or infection of ischaemic or venous ulcers, and in chronic infections, especially in patients previously treated with antimicrobials (Stevens *et al.,* 2014).

Studies done on SSTIs in Kenya have shown a similar pattern of etiology. Elamenya *et al.*, 2015 reported *Staphylococcus aureus* to be the most prevalent followed by *Pseudomonas auruginosa, Proteus mirabilis,* coagulase negative staphylococci*,* β-hemolytic streptococci*, Klebsiella* spp*.,* non lactose fermenters and *Enterococcus* spp. (Elamenya *et al.*, 2015).

2.2 ANTIMICROBIAL SUSCEPTIBILITY OF BACTERIA CAUSING SSTIs

Multidrug-resistant pathogens are a cause for serious concern. The World Health Organization (WHO) has identified antimicrobial resistance as one of the most important problems facing human health. The most virulent and resistant pathogens have been encompassed within the acronym 'ESKAPE' standing for *Enterococcus faecalis, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii,*

Pseudomonas aeruginosa and *Enterobacter* spp. They are all important causes of SSTIs (Pendleton *et al.,* 2013).

2.2.1 *STAPHYLOCOCCUS AUREUS*

Staphylococcus aureus is the commonest cause of SSTIs. It shows a very high pathogenic potential. The increased pathogenic potential in community acquired MRSA, a major cause of community acquired SSTIs, is related to Panton-Valentine leukocidin (PVL), a cytotoxin that causes leukocyte destruction and tissue necrosis. PVL is associated with necrotic lesions involving the skin or subcutaneous tissue (Lina *et al.,* 1999).

The first reports of MRSA date back to the 1960s. MRSA is now defined as any strain of *S. aureus* that has developed resistance to β-lactam antibiotics (Dulon, Haamann, Peters, Schablon, & Nienhaus, 2011). The mechanism for resistance in MRSA is the Staphylococcal Cassette Chromosome mec (SCCmec). This is a mobile genetic element that carries the central determinant for broad-spectrum beta-lactam resistance encoded by the mecA gene. The emergence of methicillin-resistant staphylococcal lineages is due to the acquisition and insertion of the SCCmec element into the chromosome of the bacteria (Al Raiy, 2007).

MRSA strains have been implicated in most outbreaks of SSTIs and cause more severe infection. They are responsible for the increase in incidence of SSTIs in Europe and America (Cosgrove *et al.*, 2005a; Dulon *et al.*, 2011; Pallin *et al.*, 2008). MRSA strains cause more severe infection. In the United States, the prevalence of MRSA is over 55% in the intensive care units, and cause significantly higher mortality (Cardo *et al.,* 2004; Cosgrove et al., 2005b).

The prevalence of MRSA in Kenya was found to be 27.7%, at the Kenyatta National Hospital (Kesah *et al.,* 2003). Subsequently, studies have shown an fluctuating trend of MRSA isolates. Maina et al. in 2012 in a study on SSTIs in Nairobi hospitals found MRSA prevalence to be 84.1%. However, Olipher *et al.* in a study on wound infections in Busia found the MRSA prevalence to be 56-82%, depending on patient's HIV status and whether the wounds were singly infected or coinfected with gram negative bacteria. Elamenya *et al.* in 2015 showed MRSA prevalence at 50.6% in pediatric wound infections (Elamenya *et al.,* 2015; Olipher *et al.,* 2013; Maina *et al.,* 2012)

Some studies have shown unexpectedly low prevalence of MRSA. The prevalence of MRSA nasal carriage among health workers was 0%, while that of methicillin-susceptible *Staphylococcus aureus* (MSSA) was 18.3% at the Aga Khan University hospital in 2011. The prevalence of MRSA among *S.aureus* isolates using automated systemsfrom clinical specimens, mostly pus swabs at the Aga Khan University Hospital and Gertrude's children's hospital showed an overall MRSA prevalence of 3.7% (Geoffrey Omuse, Kabera, & Revathi, 2014; G Omuse, Kariuki, & Revathi, 2012). This unexpectedly low prevalence could be a reflection of the better accuracy in bacterial identification using automated methods. Using conventional biochemical methods, *Staphylococcus aureus* might be mislabeled as coagulase negative staphylococci which have a higher antimicrobial resistance.

For the treatment of MRSA infections, a variety of agents have been used. Oral agents including trimethoprim-sulfamethoxazole, clindamycin, doxycycline, minocycline, linezolid, rifampin and fluoroquinolones, are used in the United States for MRSA. In Australia, the United Kingdom, and several other countries, fusidic acid in combination with rifampin are used in the outpatient setting. For more serious

infections, parenteral agents including vancomycin/ teicoplanin, daptomycin, linezolid, and tigecycline are used (Moellering, 2008).

Treatment for MRSA infections with vancomycin has led to the emergence of vancomycin-intermediate and vancomycin-resistant *S. aureus* (VISA and VRSA, respectively). VISA was first identified in Japan during the mid-1990s and has since become a global health concern. VISA strains have evolved as a result of changes in cell wall thickness and composition, trapping vancomycin and reducing permeation to the site of action. VISA is not susceptible to all but the most reserved antibacterial agents. VRSA is much less common, arising through the interspecies transfer of genetic resistance determinants from vancomycin resistant enterococci (VRE) (Pendleton *et al.*, 2013).

2.2.2 *ENTEROCOCCUS* SPECIES

Enterococci are facultative anaerobic organisms. They were previously classified as group D streptococci. They are opportunistic pathogens in SSTIs, as well as in urinary tract infections. Enterococcal infections are usually caused by *Enterococcus faecalis* or *Enterococcus faecium* (Fraser, Lim, Donskey, & Salata, 2008; Sood, Malhotra, Das, & Kapil, 2008; Wood & Murray, 2000)). *Enterococcus faecalis* has been found to account for over 80% in most studies, including studies done in Kenya where *Enterococcus faecalis* accounted for 85% (Mutuku, 2012; Wood & Murray, 2000).

Enterococci have both an intrinsic and acquired resistance to antimicrobials. They are intrinsically resistant to penicillins, cephalosporins, nalidixic acid, aztreonam, macrolides, and low levels of clindamycin, aminoglycosides and trimethoprim-sulfamethoxazole. However, combining a cell wall–active agent such as ampicillin or

vancomycin with an aminoglycoside may result in synergistic bactericidal activity against enterococci (Fraser *et al.*, 2008; Sood *et al.,* 2008; Wood & Murray, 2000).

The acquisition of vancomycin resistance by enterococci (VRE) has affected the treatment and infection control of these organisms. VRE, particularly *Enterococcus faecium* strains, are frequently resistant to most antimicrobials. Newer antibiotics for instance quinupristin-dalfopristin, linezolid, daptomycin, tigecycline have demonstrated activity against VRE strains. Beta-lactam antibiotics can increase the in vitro activity of daptomycin against vancomycin-resistant enterococci (Fraser *et al.,* 2008; Sood *et al.,* 2008; Wood & Murray, 2000).

The identification of entrococci using biochemical methods involves the use of catalase and aesculin tests carried out on gram positive cocci, whereby they are catalase negative and aesculin positive in the presence of 40% bile, 6.5% NaCl, and 0.1% methylene blue milk and at pH 9.6. They grow at 10°C and 45°C and can resist 30 min at 60°C. Species identification of enterococci by phenotypic methods is prone to the misidentification (Fraser *et al.,* 2008).

2.2.3 *PSEUDOMONAS AERUGINOSA*

This is a gram-negative, rod-shaped bacterium. It is a facultative anaerobe. Most infections occur in immune compromised hosts. The pathogenicity of these organisms is based on its ability to produce a variety of toxins and proteases and also on its ability to resist phagocytosis. It is rarely found in the normal flora of humans.

This organism has been implicated as one of the main causes of SSTIs, frequently as the leading bacterial organism among the gram negative bacteria (Dryden *et al.,* 2010,

Elamenya *et al.,* 2015). It has been reported both as a single causative organism, and as coinfection with other bacterial species (Pitout *et al.,* 2008; Olipher *et al.,* 2011).

The gram-negative outer membrane of *P. aeruginosa* makes it inherently resistant to a range of antimicrobials. However, it is normally sensitive to cephalosporins, carbenicillin, colistin, gentamicin, polymyxin, carbapenems, aminoglycosides and quinolones. Double antimicrobial therapy should be instituted in accordance with the local susceptibility patterns (Sivanmaliappan & Sevanan, 2011).

P. aeruginosa constitutively expresses β-lactamases, and has an efflux system for antimicrobials. As a result, it has become resistant to fluoroquinolones, some β-lactams and aminoglycosides. Colistin is regarded as the last line of defense (Pendleton *et al.,* 2013; Sivanmaliappan & Sevanan, 2011). In a Kenyan study across four hospitals, majority from SSTIs, 91.7% were susceptible to most antimicrobials, but among these susceptible strains, 17% were not susceptible to colistin (Matano *et al.,* 2017). Likewise, another study done at Aga Khan University Hospital revealed that 13.7% of the organisms were resistant to carbapenems, all of which tested positive for metallo-β-lactamase production (Pitout *et al.,* 2008).

2.2.4 *ESCHERICHIA COLI*

It is a gram negative bacillus. It is a leading cause of SSTIs. It is a cause of infection in immune compromised hosts, and in necrotizing infections (Dryden *et al.,* 2010). Several authors have reported *E. coli* in SSTIs including neonatal omphalitis, cellulitis, necrotizing fasciitis, and in surgical site infections (Petkovšek, 2009). A study monitoring SSTIs during a 7-year period and encompassing three continents including Europe, Latin America, and North America showed *E. coli* to be an important causative agent, being the third-most prevalent isolated species (Moet *et al.,*

2007). Olipher *et al.* in a study in Busia, Kenya, described it in wound infections, as coinfections with *S. aureus.* Elamenya *et al.* also described it in wound infections in a study done in Nairobi (Elamenya *et al.,* 2015; Olipher *et al.,* 2010).

The factors that determine its pathogenicity are varied. Susceptibility trends in the USA show that *Escherichia coli* shows the least resistance to antimicrobial drugs introduced for clinical use since 1980, such as amoxicillin/clavulanic acid, ceftriaxone and ciprofloxacin, while the most common resistance phenotypes were to older drugs such as tetracycline, sulfonamides, streptomycin and ampicillin (Tadesse *et al.,* 2012). Studies have shown an association of multidrug-resistant *Escherichia coli* especially in wounds with chronic antimicrobial drug exposure (Kibret & Abera, 2011; Moş, Micle, Zdrâncă, Mureşan, & Vicaş, 2010; Tadesse *et al.,* 2012).

In Kenya, studies have shown a similar pattern of resistance. In a study involving wound infections in Thika, the susceptibility to most aminoglycosides, penicillins and cephalosporins was low with the only antibiotics effective in over 80% of the cases being amikacin, imipenem and tazobactam (Ndung'u, Muigai, & Kariuki, 2014).

The identification of *E. coli* involves the use of standard laboratory methods including colony morphology, lactose fermentation, gram staining, oxidase, triple sugar iron, motility, and indole and citrate utilization (Mos *et al.,* 2010).

2.2.5 *KLEBSIELLA PNEUMONIA*

Several studies have listed *Klebsiella* spp. as causative agents of SSTIs. Klebsiellae are nonmotile, rod-shaped, gram-negative bacteria with a prominent polysaccharide capsule. This capsule encases the entire cell surface, accounts for the large appearance of the organism on gram stain, and provides resistance against many host defense mechanisms (Dryden *et al.,* 2010, Elamenya *et al.,* 2015).

Klebsiella organisms are resistant to multiple antimicrobials. This is thought to be a plasmid-mediated property. Agents with high intrinsic activity against *Klebsiella* spp. include third-generation cephalosporins, carbapenems, aminoglycosides and quinolones. These agents may be used as monotherapy or combination therapy. Some experts recommend using a combination of an aminoglycoside and a third-generation cephalosporin as treatment for non-ESBL-producing isolates. ESBLs are plasmid mediated, confer multidrug resistance, and are detected by in vitro resistance to ceftazidime and aztreonam. ESBL-producing isolates are treated with carbapenems (Buckland, 2016; Pendleton *et al.,* 2013).

In a Kenyan study on the antimicrobial resistance of Klebsiella pneumoniae isolates multidrug resistance was detected in 36.7%. Between 80 and 90% of the tested isolates were susceptible to all β-lactams except ampicillin, to one or more aminoglycosides, and to both of the fluoroquinolones tested. Over half were susceptible to tetracycline, but more than 60% were resistant to sulfamethoxazole-trimethoprim (Taitt, 2017).

2.2.6 *ACINETOBACTER BAUMANNII*

Acinetobacter baumannii have been associated with SSTIs in several studies. It is an opportunistic pathogen, most often encountered in intensive care units and surgical wards, where extensive antimicrobial use has enabled selection for resistance against all known antimicrobials. *Acinetobacter baumannii* is renowned for its environmental persistence, surviving for up to 5 months on inanimate surfaces (Pendleton *et al.,*2013, Karimi *et al.,* 2006).

Acinetobacter baumannii is intrinsically resistant to antimicrobials, due to protection afforded by a gram-negative outer membrane, constitutively expressed active efflux pump systems and the low-quantity expression of small-aperture outer membrane porins. Synergistic interplay between these inherent traits greatly reduces the permeability of antimicrobials. There are no antimicrobials in late-stage development for treatment of multiple drug resistant infections from *Acinetobacter baumannii*. There is need for novel antimicrobials to combat these pathogens (Buckland, 2016; Pendleton *et al.,* 2013; Seifert, Baginski, Schulze, & Pulverer, 1993).

Binglari *et al.* in a study done in Pakistan, documented multidrug resistant acinetobacter in 79%, but all were susceptible to polymyxin B. Likewise, in a study done in Nairobi, Kenya, most of the pathogens were resistant to all antimicrobials including carbapenems, where 4% were susceptible, with 16% of them being susceptible to amikacin (Binglari *et al.,* 2013; Huber *et al.,* 2014).

2.3 SPECIMEN COLLECTION FOR CULTURE AND SUSCEPTIBILITY TESTING

The rise in resistant bacteria has increased the importance of antimicrobial susceptibility testing, which should be used to promote good antimicrobial stewardship.

Specimens should be collected before the commencement of antimicrobials. Pus or tissue samples have the greatest specificity. Swabs of open, infected wounds can also provide valuable information. Whereas aspiration of the leading edge of cellulitis with a needle is often advocated by some, others consider it too invasive (Dryden, 2009b; Stevens *et al.,* 2005).

Identification of microorganisms can be based on phenotypic or genotypic methods. Phenotypic methods rely on specified culture media and incubation conditions. Genotypic methods include riboprinting, which is an automated Southern Blot apparatus, and PCR-based methods. In the hospital, phenotypic methods are utilized due to cost limitations and limited expertise. They include conventional biochemical tests like catalase, oxidase, and indole tests, urease production, citrate utilization, glucose and lactose fermentation, triple sugar iron media, and lead acetate for hydrogen sulfide production in gram-negative isolates and catalase, coagulase, and bile esculin production for gram-positive isolates (Cowan, Barrow, Steel, & Feltham, 2004).

2.4 ANTIMICROBIAL CHOICE AND STEWARDSHIP

Therapy should be guided by antimicrobial susceptibility patterns. However, little local data on susceptibility patterns has been published.

Some institutions have drafted guidelines to assist clinicians in choosing the best therapy. These guidelines are usually based on the trending susceptibility patterns. However, opinions differ between different institutions and infectious disease experts. Likewise, they remain as empiric therapy, which is the best guess. The undisputed best way to know which antimicrobial to use is by culturing the organism and subjecting it to antimicrobial susceptibility testing (Stevens *et al.,* 2005).

Patterns of antimicrobial use differ greatly between regions and countries. In Kenya, most antimicrobial use is empiric therapy. Few institutions have guidelines for antimicrobial prescription. There are no national guidelines on antimicrobial use for SSTIs. Anecdotal evidence has it that antimicrobial therapy has largely shifted from the traditional penicillins and aminoglycoides to newer agents such as cephalosporins.

Injudicious antimicrobial use has led to widespread resistance of microbes to available drugs. Demand for antimicrobials continues to rise. Between 2000 and 2010, total global antimicrobial consumption grew by more than 30 percent, the greatest increase being in lower and middle income countries, where antimicrobial use continues to rise.About 20 percent of antimicrobials are used in hospitals and other healthcare facilities, and 80 percent are used in the community, either prescribed by healthcare providers or purchased directly by consumers or caregivers without prescription. About half of community use is inappropriate (Gelbrand *et al.,* 2015).

The misuse and overuse of antimicrobials is a problem in SSTIs. Studies have demonstrated a high percentage of patients receiving treatment for SSTIs with broad-spectrum antimicrobials, with combinations of 3 or more antibacterial agents. This constitutes unnecessary health expenditure. Decreasing this use is can help in improving outcomes as well as curtailing resistance (Paydar, Hansen, Charlebois, Harris, & Young, 2006; Spellberg, 2010).

Although most clinicians view antimicrobial resistance as a serious problem, perceptions about its local importance, its causes, and possible solutions vary widely. Many clinicians have suboptimal knowledge about antimicrobials (Srinivasan *et al.*, 2004)

Common misuses of antimicrobials by physicians include: prescribing antimicrobials when no bacterial infection exists, prescribing the wrong drug, using the wrong dose, treating an infection that does not exist, prescribing antimicrobials for longer than necessary, prescribing a strong antimicrobial when a less strong one would be as effective, and choosing an expensive drug when a cheaper but equally effective one will be adequate (Srinivasan *et al.,* 2004, Shahid *et al.,* 2017).

Judicious use of antimicrobials must take into consideration their availability, and the characteristics of the established systems of medical care. Improving clinician knowledge, beliefs, and attitudes may improve antimicrobial prescribing and infection control practices (Shahid *et al.,* 2017; Srinivasan *et al.*, 2004).

2.5 ETIOLOGY AND MANAGEMENT OF COMMON SSTIS
2.5.1 CUTANEOUS ABSCESS

Cutaneous abscesses are collections of pus within the dermis and deeper skin tissues. Cutaneous abscesses may be polymicrobial, containing bacteria that constitute the normal regional skin flora. *S. aureus* is usually present, but as a single pathogen in only 25% of cutaneous abscesses (Stevens *et al.,* 2005).

Effective treatment of abscesses entails incision, thorough evacuation of the pus, and breaking up loculations. Complex abscesses and those with substantial cellulitis require adjuvant antimicrobial therapy, gram stain and culture. Inadequate response to therapy should prompt evaluation of the adequacy of drainage (Stevens *et al.,* 2005).

2.5.2 CELLULITIS, ERYSIPELAS

Erysipelas is a superficial skin infection that is described as a fiery red, tender, painful plaque with well-demarcated edges. It is usually caused by beta-hemolytic streptococci, commonly group A. Penicillin is the treatment of choice for erysipelas (Stevens *et al.,* 2005).

Cellulitis extends more deeply than erysipelas to involve the subcutaneous tissues. Offending organisms, again, are most commonly group A β-hemolytic streptococci and less commonly, *Staphylococcus aureus*. Cellulitis caused by *S. aureus* spreads from a central localized infection, such as an abscess, folliculitis, or an infected

foreign body, and it may be caused by MRSA (Nichols & Florman, 2001; Wiener, 2008).

For parenteral therapy, reasonable choices include a penicillinase-resistant penicillin such as cloxacillin, a first-generation cephalosporin such as cefazolin, or, for patients with life-threatening penicillin allergies, clindamycin or vancomycin.

Blood and wound cultures can be done (Stevens *et al.*, 2005). However, because of their very low yield and unreliability, blood cultures are not fruitful for the typical case of erysipelas or cellulitis, unless it is particularly severe (Stevens *et al.*, 2005; Wiener, 2008).

2.5.3 WOUND INFECTION

The presence of bacteria in a wound may result in contamination, colonization, contamination, critical colonization or infection. Contamination is where the bacteria do not increase in number or cause clinical problems. Colonization is where the bacteria multiply, but wound tissues are not damaged. Critical colonization is a threshold beyond which infection occurs. It arose to differentiate wounds that have delayed healing from more obvious infection. Infection is where the bacteria multiply, healing is disrupted and wound tissues are damaged (Cutting & White, 2004).

Clinical signs and symptoms for the identification of wound infection include increasing pain in the ulcer area, erythema, oedema, heat, purulent exudate, serous exudate, delayed healing of the ulcer, discolouration of the granulation tissue, friable granulation tissue, pocketing at the base of the wound, foul odour and wound breakdown accompanied by lymph node enlargement or systemic symptoms such as fever greater than or equal to 38 degrees (Food, 2005). These clinical criteria are based on a list created by Cutting and Harding (1994), see appendix 3. This list was

established from empirical data generated in a large, multidisciplinary clinical practice, and is widely accepted in wound care. Both Cutting (1998) and Gardner et al (2001) conducted validation exercises on these wound infection criteria, based on the assumption that the criteria broadly apply to most wound types (Cutting, 2004).

Etiologic bacteria in wound infections include *S.aureus, Proteus* spp*., Escherichia coli* and *Pseudomonas aeruginosa, Enterococcus fecalis* and *Klebsiella* spp. (Elamenya *et al.,* 2015, Olipher *et al.,* 2011).

CHAPTER THREE: MATERIALS AND METHODS

3.0 INTRODUCTION

This chapter presents the procedures and the methodology that was used in this study. Specifically, the chapter focusses on the study setting, study population, inclusion and exclusion criteria, study design, study procedure, data collection process, data processing, analysis and presentation and ethical considerations.

3.1 STUDY SETTING

The study was carried out at Moi Teaching and Referral Hospital (MTRH). MTRH is the second national referral hospital in Kenya, located in Eldoret town, Uasin Gishu County, Kenya, about 300 km northwest of Nairobi. The hospital has a catchment area comprising the western region of the country which cumulatively has a population of approximately 16 million. The patient clinical data and specimen collection was done at the hospital casualty/outpatient department, and theatre. The specimens collected were analyzed at the hospital microbiology laboratory.

3.2 STUDY POPULATION

The study included all patients admitted to MTRH with SSTIs including cutaneous abscesses, purulent cellulitis and wound infections.

3.3 INCLUSION AND EXCLUSION CRITERIA

3.3.1 INCLUSION CRITERIA

Participants in this study met the following criteria: consenting patients admitted at MTRH, whose primary diagnosis was a bacterial SSTI (cutaneous abscess, purulent cellulitis and wound infection). They were identified based on the clinical criteria (appendix 3).

3.3.2 EXCLUSION CRITERIA

The patients who were not included in this study were the following: patients with another infection (not an SSTI) also requiring antimicrobials. This is because it would be difficult to tell which condition the antimicrobial prescribed was meant to treat. Patients with animal or human bites were also excluded as they have been shown to have a different microbial etiology from the other SSTIs. Patients with malignant wounds were excluded. Because the focus was on community-onset SSTIs, and not on nosocomial SSTIs, chronic/decubitus ulcer, surgical site infection, post-operative wound infection and burn wound infections were not included.

3.4 STUDY DESIGN

This was a cross sectional descriptive study. Census sampling was done, with all patients presenting with the three SSTIs and getting admitted for the same over a period of one year being approached for consent to be recruited into the study. From the hospital records, there were 159 and 171 admissions with the SSTIs in question in 2012 and 2013, respectively.

3.5 STUDY PROCEDURE

3.5.1 DATA COLLECTION TOOL

An interviewer-administered structured questionnaire was used. A questionnaire was piloted in the study area (MTRH wards) before the data collection. Ten patients were randomly selected from the wards, with their data collected using the questionnaire. Amendments were made to the questionnaire before commencing the main study.

The findings from the history, physical exam, patient biodata and clinical records of antimicrobials prescribed, as well as laboratory data were summarized in the questionnaire (appendix 1).

3.5.2 PATIENT IDENTIFICATION AND RECRUITMENT

Patients with SSTIs (cutaneous abscesses, cellulitis, wound infections) were identified from the admissions book in the wards and casualty/outpatient, from which the patient numbers were recorded for subsequent retrieval of their files. Patients identified included those whose primary diagnosis was an SSTI (cutaneous abscess, purulent cellulitis and wound infection), as described in the clinical criteria (appendix 3). Eligible patients' consent was sought, and upon filling the consent form, they were recruited into the study by assigning a serial number, after which data collection began.

3.6 DATA COLLECTION PROCESS

3.6.1 HISTORY, PATIENT EXAMINATION, REVIEW OF MEDICAL RECORDS

After obtaining their biometric data, a history was taken from the patient or their relative. A physical examination was done to establish the clinical characteristics. The local site of the lesion was examined to ascertain the diagnosis. This data was recorded in the questionnaire.

The data on the antimicrobials used was obtained by reviewing the patient's file and treatment sheet, to check what was prescribed and subsequently administered as empiric therapy, and any other antimicrobial adjustments made in the course of the patient's hospital stay.

3.6.2 SPECIMEN COLLECTION FROM VARIOUS SITES

A specimen was taken by the researcher for all eligible cases. The specimen types included wound swabs, aspirated pus and tissue. For wound infections, wound swabs were taken at first contact, using levine technique. This was done before dressing change, after cleansing the wound with saline. According to the Levine technique, the part of the wound that was most symptomatic was sampled, especially the part of the wound with tissue fluid. The applicator swab was rotated and pressed down gently to express wound fluid from viable tissue. The tip of the swab was rolled on its side over the area for one full rotation.

For abscesses, patients had an aspirated pus specimen done, also at first contact, and, where possible, before the initiation of antimicrobials. These were placed in sterile bottles. For patients requiring surgery, for instance the drainage of an abscess, or in wound debridement where tissue culture was possible, the samples were taken in theatre. Tissue was placed in sterile collection bottles. The procedure for collection and transport of specimen was as follows:

Materials: sterile cotton swabs, sterile alcohol swabs, sterilized bottles, 5 ml syringe, 21 gauge needle.

1. The procedure was explained to the patient and verbal consent was obtained. It was ensured that the consent form to participate in the study was already signed.

2. Sterile cotton wool swab was used to collect a sample from the infected site after cleasing the wound with sterile saline. Any purulent exudates were expressed into the swab. The swab was placed in its original container and the bottle top was replaced tightly.

3. For aspirated pus, the overlying skin was swabbed with an alcohol swab and allowed to dry. The pus was aspirated after a single puncture where possible. The needle was removed and the pus emptied into a sterile bottle then the bottle top was replaced tightly.

4. The swab was labelled with the patient's full names, date of birth, hospital number, anatomical site as well as date and time of sample collection. Information on antimicrobial therapy, antibiotic allergy or pregnancy was included in the requisition form.

5. The swab was maintained at room temperature and transported to the laboratory immediately.

Precautions were taken to avoid cross contamination including utilization of sterile collection bottles, swabs, needles, syringes and gloves, ensuring appropriate specimen collection (taking specimen from deeper aspects of the wound, collection of tissue culture where possible and avoiding contact with skin.

All the samples were submitted to microbiology laboratory within one hour for bacteriological analysis.

3.6.3 LABORATORY EXAMINATION OF PUS SWABS AND TISSUE SPECIMEN

3.6.3.1 PROCEDURES FOR IDENTIFICATION OF BACTERIAL PATHOGENS

Standard operating procedures were used. The specific tests to identify the different species of bacteria were done in accordance to the Clinical and Laboratory Standards Institute (CLSI), customized for use in the hospital microbiology laboratory as Standard Operating Procedures. The procedure for conducting the specific tests are described in detail in appendix 4. Phenotypic identification of bacteria was done by

culture followed by gram staining and biochemical tests (Watts, Clinical, & Institute, 2008). The procedure followed is described briefly.

Requirements: Blood agar, MacConkey agar and Chocolate Blood Agar plates, Personal Protective Equipment and standard laboratory equipment and reagents as needed.

1. The gross appearance of the specimen was recorded

2. The culture medium was prewarmed to 37°C

3. A new clean slide was labeled and assigned a laboratory number

4. The specimen was inoculated on blood agar, Chocolate blood agar and MacConkey agar using a sterilized wire loop. The main inoculums was put and then streaked across the culture medium

5. The inoculated plates were labeled and incubated at 37°C for 18-24 hours

6. An evenly spread smear of the specimen was made on a slide and allowed to air-dry in a safe place

7. It was then fixed and stained by gram technique

8. The smear was examined for bacteria among pus cells using 100X objective under oil immersion.

9. The media was examined daily and the direct gram stain correlated with growth to identify any organisms recovered. Colonies that could be *Staphylococcus aureus, Streptococcus pyogenes, Pseudomonas aeruginosa, Proteus spp., Escherichia coli, Enterococcus* spp.and *Klebsiella spp.*were looked for.

10. All growth was quantified and reported

Reporting Results:

1. A preliminary report was generated after 48 hours.

2. All growth was quantified and listed

3. Negative cultures were held for 3 days before concluding as no growth.

An algorithm derived from the Clinical and Laboratory Standards Institute guidelines was used to identify the pathogenic bacteria. This has been adapted for use as the MTRH Standard Operating Procedures. It is summarized in Figure 3.1.
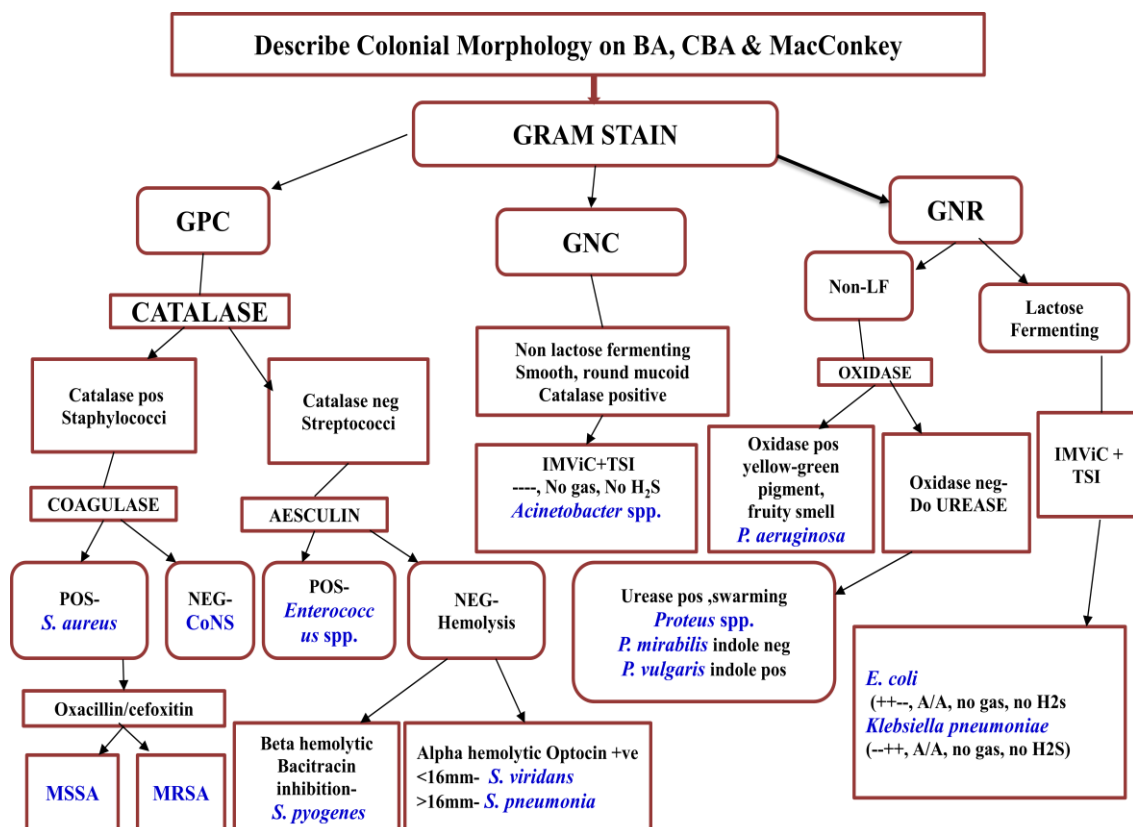
Figure 3.1 Algorithm for bacterial identification
(Adapted from MTRH Standard Operating Procedures)

3.6.3.2 IDENTIFICATION OF GRAM POSITIVE BACTERIA

Staphylococcus aureus were identified as non motile gram positive cocci. If they were positive on catalase test, then coagulase test was done, whereby the slide method was used first. Tube method was then done to confirm. The tubes were examined at 30 minutes. If it was positive within 30 minutes, they were classified as *Staphylococcus aureus* and if they were negative at 30 minutes, the tube was examined every 30 minutes for 24 hours. If it was positive at 24 hours, they were still classified as *Staphylococcus aureus.*

If, after following the procedure above using the tube method, there was no clot after 24 hours, they were classified as **coagulase negative staphylococci.** The pathogens in this group include *Staphylococcus epidermidis, Staphylococcus saprophyticus* and *Staphylococcus lugdunensis*

Gram positive organisms that were beta hemolytic on blood agar, and catalase negative had bacitracin test done. If there was any zone of inhibition, they were classified as *Streptococcus pyogenes.*

Gram positive organisms that were alpha hemolytic on blood agar had the optochin disc test done. They were classified as *Streptococcus pneumonia* if inhibition was equal to, or over 16 mm on optochin disc, if inhibition was less than 16 mm they were classified as **viridans streptococci.**

Gram positive, non hemolytic organisms on blood agar, and negative on catalase test had the aesculin test done. If there was a black color (rapid hydrolysis of aesculin to aesculetin in the presence of 40% bile), they were classified as *Enterococcus* **spp.** Further identidication of the species of Enterococcus was not possible at the laboratory.

3.6.3.3 IDENTIFICATION OF GRAM NEGATIVE BACTERIA

Growth on Maconkey indicated that the bacteria is not inhibited by bile salts and crystal violet and therefore is most likely to be a gram-negative bacterium, while pink color of the bacterial growth indicated that the bacteria is able to ferment lactose. On MacConkey agar, organisms like *Escherichia coli* and *Klebsiella* spp. ferment the lactose, producing acid which forms pink to red colonies, while *Proteus* spp. and *Pseudomonas* spp. and *Acinetobacter* spp. do not ferment the lactose, with no color change. Further identification was based on IMViC tests (indole, methyl-red, voges-proskauer and citrate), as well as TSI (Triple Sugar Iron test).

Escherichia coli were identified as gram negative rods, and lactose fermenters with IMViC tests ++-- (indole positive, methyl-red positive, voges-proskauer negative, citrate negative). On TSI, they were acid slant/acid butt (A/A), no gas and no hydrogen sulfide.

Klebsiella **spp.** were identified as gram negative rods, and lactose fermenters with IMViC --++ (indole negative, methyl-red negative, voges-proskauer positive, citrate positive). However, *Klebsiella oxytoca* are methyl red positive. On TSI, they were acid slant/acid butt (A/A), no gas and no hydrogen sulfide. *Klebsiella pneumonia* were identified on the basis of negative result on indole test.

Proteus **spp**. were identified as gram negative rods, non lactose fermenters with semen like smell, negative on oxidase test, and positive on urease test within 3 hours. Those which were indole negative were *Proteus mirabilis*, while those which were indole positive were *Proteus vulgaris*.

Pseudomonas aeruginosa were identified as gram negative rods, non lactose fermenters, non motile, producing a fluorescent yellow-green pigment and producing a fruity smell. They were also positive on oxidase test.

Suspected *Acinetobacter* **spp.** isolates were gram negative cocci, non lactose fermenters, with smooth, rounded, mucoid colonies. Catalase test was then done, where organisms which were catalase positive were classified as *Acinetobacter* spp. *Acinetobacter baumanii* were identified as citrate positive, with growth at 42 degrees celcius, and non hemolytic on Blood Agar.

3.6.3.4 ANTIMICROBIAL SUSCEPTIBILITY TESTING

Susceptibility testing was done for all pathogenic bacteria isolated by culture. Antimicrobial susceptibility test was done by use of modified Kirby-Bauer disc diffusion technique. In this technique, a pure culture of the pathogenic organism is inoculated in Mueller Hinton Agar. A disc of blotting paper impregnated with a known volume and appropriate concentration of an antimicrobial agent is then placed on a the agar plate. The antimicrobial 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. The distance of growth inhibition is measured. The predetermined zones of inhibition (Minimal Inhibitory Concentration) that would qualify the organism as susceptible, intermediate resistant or resistant are then provided. The specific distances used in this study, as provided by the manufacturer, are outlined in appendix 4M. In this study, both intermediate resistant and resistant were classified together.

Antimicrobials that are commonly used at MTRH for various bacteria were tested. The choice of which antimicrobial to do sensitivity against, was guided by the

recommendations of the Clinical and Laboratory Standards Institute (CLSI), customized to the antimicrobials used in the hospital in counsultation with an infectious disease specialist. *Staphylococcus aureus* were tested against vancomycin 30μg, erythromycin 15μg, cotrimoxazole 25μg, clindamycin 2μg and cephalosporins including ceftriaxone 30μg, ceftazidime 30μg and cefuroxime 30μg. *Enterococcus fecalis* were tested against vancomycin 30μg, aminoglycosides (amikacin 30μg, gentamicin10μg), cephalosporins (ceftriaxone 30 μg, ceftazidime 30 μg, cefuroxime 30μg), erythromycin 15μg, and penicillin 10 μg. Coagulase negative staphylococci (including *Staphylococcus epidermidis, Staphylococcus hemolyticus, Staphylococcus saprophyticus* and *Staphylococcus lugdunensis*) were tested against vancomycin 30μg, clindamycin 5μg, meropenem 10μg, amikacin 30μg and gentamicin 10μg.

Streptococcus pyogenes were tested against vancomycin 30μg, gentamicin 10μg, ceftriaxone 30μg, penicillin 10μgand clindamycin 2μg.

Escherichia coli were tested against meropenem 10 μg, amikacin 30μg and gentamicin 10μg, ceftriaxone 30μg, ceftazidime 30μg and cefuroxime 30μg. *Pseudomonas aeruginosa* were tested against meropenem 10 μg, amikacin 30μg, gentamicin 10μg, ciprofloxacin 5μg, ceftriaxone 30μg and ceftazidime 30μg. *Klebsiella pneumonia* were tested against meropenem 10 μg, amikacin 30μg and gentamicin 10μg, levofloxacin5 μg, ceftazidime 30μg and piperacillin/tazobactam 110 μg. *Acinetobacter baumanii* were tested against meropenem 10 μg, amikacin 30μg and gentamicin 10μg, ciprofloxacin 5μg and levofloxacin5 μg, and cephalosporins including ceftriaxone 30μg, cefotaxime 30μg, ceftazidime 30μg, cefepime 30μg, cefuroxime 30μg, and piperacillin/tazobactam 110 μg.

3.6.3.5 LABORATORY QUALITY CONTROL

The culture and antimicrobial susceptibility testing was done by the researcher in conjunction with a research assistant who is a duly trained and registered laboratory technologist. The MTRH microbiology laboratory is an ISO 9001:2008 certified medical microbiology laboratory that processes over twenty specimen a day for microscopy, culture and sensitivity. Both internal and external quality control mechanisms are instituted at the microbiology laboratory. Internal quality control is done once a day, while external quality control is done on quarterly basis (every four months). Laboratory examination was done by the researcher together with the research assistant, who is a certified medical laboratory technologist.

The culture media and antimicrobial discs for disc diffusion were sourced from Liofilchem, a company that manufactures microbiology products for clinical and industrial use. Liofilchem is present in more than 130 countries, offering products in clinical and industrial laboratories around the world. Prior to sourcing the products, they are validated by the Kenya Bureau of Standards and sourced through the Kenya Medical Supplies Agency.

In cases where specimen was taken on more than one occasion leading to more than one organism being isolated, only the first bacterial organism isolated was included in the analysis. In cases where more than one organism was isolated from the same specimen, the most likely organism was identified by excluding organisms that are likely to be contaminants. This was done in consultation with an infectious disease specialist.

3.7 DATA PROCESSING, ANALYSIS AND PRESENTATION

3.7.1 DATA RECORDING AND ANALYSIS OF EFFECTIVENESS OF EMPIRIC THERAPY

Patient medical records were reviewed to obtain or verify data. This data included the clinical characteristics, antimicrobials used as well as their adjustments. The laboratory results were recorded including the organisms isolated, and their susceptibility/resistance profile.

To determine the effectiveness of empiric antimicrobial therapy, the antimicrobials that had been administered as empiric therapy were included in the panels while doing susceptibility testing. In cases where it was not possible to do so, for instance when an antimicrobial that is closely related to the one used as empiric therapy had been tested, the effectiveness was judged using this antimicrobial. In particular, oxacillin disc was used in all *Staphylococcus aureus* isolates as well as other bacterial isolates to determine MRSA, so effectiveness of flucloxacillin or cloxacillin was determined as projected from oxacillin susceptibility. Susceptibility testing was not done for metronidazole as no anaerobes were identified. However, metronidazole was not used in isolation, so the other antimicrobial's effectiveness was determined. In case any information was missing, the samples were preserved and could be rerun to fill the gaps.

The data including the pathogenic bacteria, their susceptibility to antimicrobials tested, and the antimicrobials prescribed by the primary clinician, was summarized into the standardized questionnaire (appendix 1), which were kept by the researcher.

Data from the questionnaires was then coded and entered into SPSS version 21 where it was verified by two trained independent records officers. Descriptive statistics was

used including frequencies and proportions for categorical variables and means (and standard deviations) or medians (and interquartile ranges) for continuous variables.

3.7.2 DATA PRESENTATION

The data was presented as visual displays including tables, pie charts and graphs. These were used to condense information, present it in a clear format, and highlight underlying relationships and trends. The research findings were disseminated through various means including, but not limited to seminars and publications.

3.7.3 QUALITY CONTROL IN DATA HANDLING

Random checks and review after every data collection were done to check for errors or missing data and unclear parts. Cleaning of data and counter checks on data entry were done regularly. Parallel data entries were done to compare for correctness. Data was checked on regular basis to ensure consistency and that coding and entry was accurately done. This was done with the assistance of two independent records officers and a qualified statistician.

3.8 ETHICAL CONSIDERATIONS

The approval of the Institutional Research and Ethics Committee (IREC) for Moi University and MTRH was sought before commencing the data collection. The amendments made were also cleared by the IREC.

Ethical principles were adhered to. The participants were informed appropriately on the benefits and risks of the study in a language that they fully understood. They were informed of the procedures to be conducted and consent was sought from them to participate in the study. For those below 18 years of age, consent was sought from the parent or legal guardian. In the event that the minor declined to participate in the study, consent from the parent/s or guardians was overridden and the minor

removed from being a candidate for the study. No patient was denied treatment whether s/he gave consent or not. Those who wished to withdraw from the study were free to do so without affecting their medical care.

To ensure confidentiality and privacy of the study subjects, each subject was given a serial number used on the checklist. This number was only known by the participant and the researcher. The confidentiality of data was maintained during and after the research.

CHAPTER FOUR: RESULTS

4.0 INTRODUCTION

This chapter describes the findings and results obtained from history and physical examination done to establish the diagnosis of an SSTI, the results obtained from laboratory analysis of the specimen, and the empiric antimicrobials used as obtained from the patient medical records. Specifically, the results are categorized into patient demographics, bacterial etiology of SSTIs, antimicrobial susceptibility patterns for both gram positive and gram negative isolates, and the empiric antimicrobial use in SSTIs.

4.1 PATIENT DEMOGRAPHICS

A total of 165 patients from various wards were enrolled over a one year period between January 1st, 2014 and December 31st, 2014. The patients affected by SSTIs were from a wide spectrum of ages, from 0 to 68 years. Patients with SSTIs were admitted to different wards in the hospital, but mainly to surgical wards. Female and pediatric surgical ward had 82 (49.7%), male surgical ward 48 (29.1%), adult medical ward 16 (9.7%), newborn unit 8 (4.8%), pediatric medical ward 6 (3.6%) and orthopedic ward 5 (3.0%).

The most common condition seen was abscess at 46.1% (76). Cellulitis accounted for 46 (27.9%) while wound infections were 43 (26.1%). Bacterial culture and antimicrobial susceptibility was done in 103 patients. Of these, bacteria were isolated from the first sample collection in 78 patients (76.0%) while culture was negative in 25 patients (24.0%). Coinfection with more than one bacterial pathogen was found in 6 patients, with 84 organisms being isolated.

The mean age was 31.4 years with a standard deviation of 23.8 years. The youngest patient was two months old, while the eldest was 90 years old. There were more males than females {n=91 (55.0%}, males {n=74, (45.0%)}, M:F ratio of 1.2:1.

Figures 4.1 and 4.2 show the age and gender distribution of patients with SSTIs.
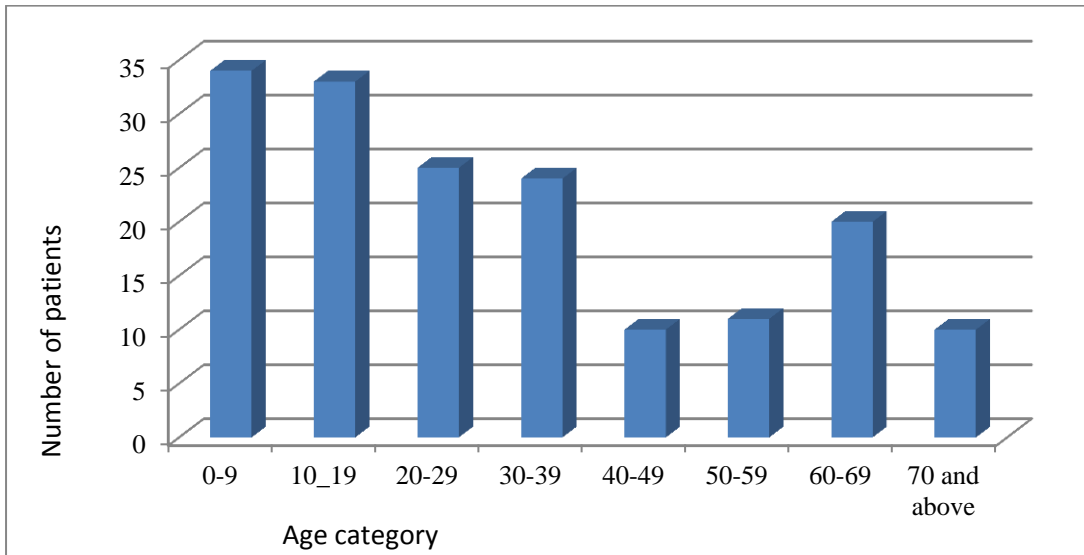
Figure 4.1: Age distribution of patients with SSTIs.
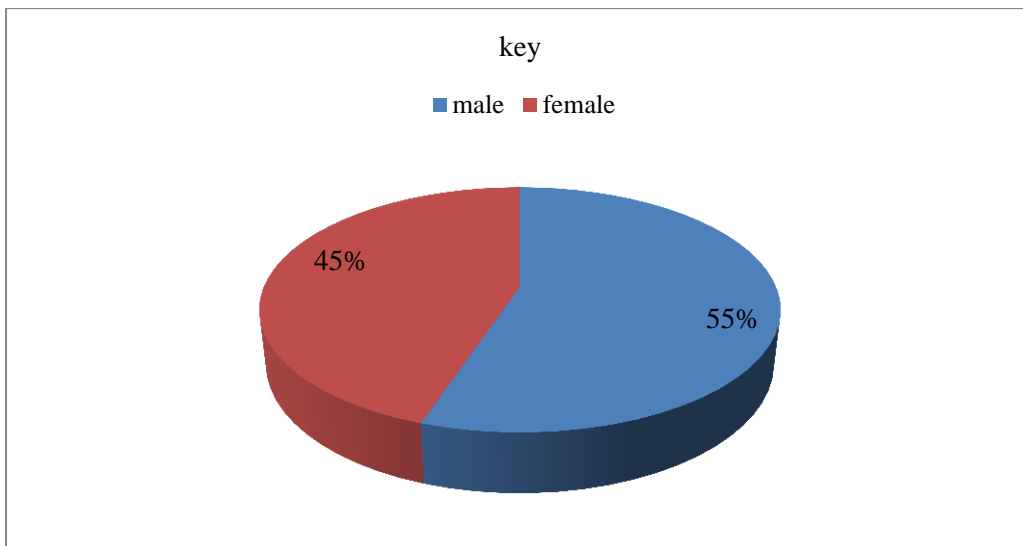
Figure 4.2: Gender distribution of patients with SSTIs.

4.2 BACTERIAL ETIOLOGY OF SSTIS

Eighty-four bacterial pathogens of various species were identified in 78 patients (abscess 33, cellulitis 14, wound infection 31). The most prevalent bacterial pathogen causing SSTIs was *Staphylococcus aureus*, while the least common was *Proteus* spp. The bacterial species identified from the samples taken are summarized in figure 4.3.

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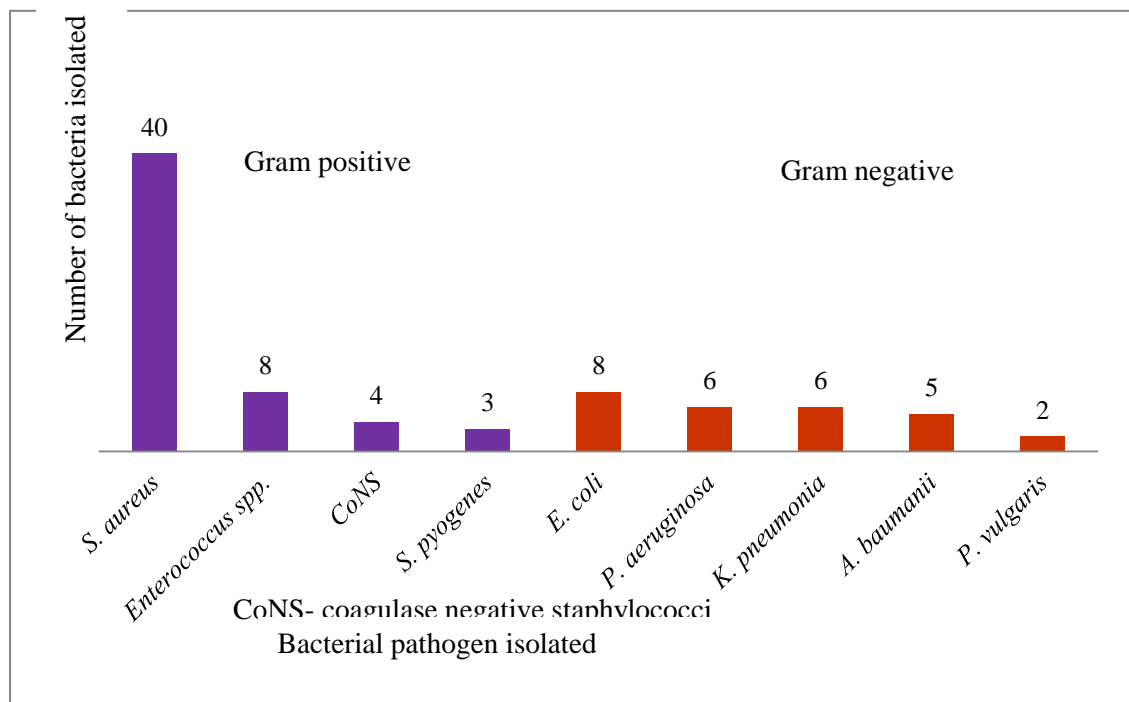
Figure 4.3: Bacterial species isolated from study participants

More gram positive bacterial pathogens were isolated from abscesses, however, this difference was not found to be statistically significant on the Pearson chi square test for independence.

The distribution of pathogens according to the diagnosis was as shown in Table 4.1.

Table 4.1: Distribution of pathogens according to the diagnosis

	wound infection	cellulitis	abscess
gram positive	19	9	28
gram negative	14	6	8
Total	33	15	36

Staphylococcus aureus were isolated from the lower limb (24), perineum (5), head/neck region and lower limb (4 each) and the trunk (3). Methicillin resistant *Staphylococcus aureus* constituted 45% of all *Staphylococcus aureus*. The occurrence MRSA was random, as determined by the Pearson chi square. The distribution of *Staphylococcus aureus* according to the diagnosis was as shown in Table 4.2

Table 4.2: Distribution of *Staphylococcus aureus* according to the diagnosis

	wound infection	cellulitis	abscess
Methicillin Susceptible *Staphylococcus Aureus*	8	3	11
Methicillin Resistant *Staphylococcus Aureus*	5	2	11
Total	13	5	22

4.3 ANTIMICROBIAL SUSCEPTIBILITY PATTERNS
4.3.1 ANTIMICROBIAL SUSCEPTIBILITY FOR GRAM POSITIVE ISOLATES

4.3.1.1 ANTIMICROBIAL SUSCEPTIBILITY FOR *STAPHYLOCOCCUS AUREUS* AND *ENTEROCOCCUS* SPP.

Staphylococcus aureus isolates were 40 (50.6%), while *Enterococcus* spp. isolates were 8 (9.5%). Their antimicrobial susceptibility is summarized in Table 4.3.

Table 4.3: Antimicrobial susceptibility for *Staphylococcus aureus* and *Enterococcus* spp.

Antimicrobial	*Staphylococcus aureus* N=40 n (%)		*Enterococcus* spp. N=8 n (%)	
	Susceptible	Not susceptible	Susceptible	Not susceptible
vancomycin	35 (87.5)	5 (12.5)	7 (87.5)	1 (12.5)
ceftazidime	34 (85.0)	6 (15.0)	2 (25.0)	6 (75.0)
ceftriaxone	29 (72.5)	11 (27.5)	2 (25.0)	6 (75.0)
cefuroxime	25 (62.5)	15 (37.5)	1 (12.5)	7 (87.5)
erythromycin	15 (37.5)	25 (62.5)	2 (25.0)	6 (75.0)
clindamycin	25 (62.5)	15 (37.5)	-	-
cotrimoxazole	13 (32.5)	27 (67.5)	-	-
gentamycin	-	-	3 (37.5)	5 (62.5)
penicillin	-	-	6 (75.0)	2 (25.0)
amikacin	-	-	6 (75.0)	2 (25.0)

4.3.1.2 ANTIMICROBIAL SUSCEPTIBILITY FOR *STREPTOCOCCUS PYOGENES*

AND COAGULASE NEGATIVE STAPHYLOCOCCI

Streptococcus pyogenes isolates were 3 (3.6%), while Coagulase negative Staphylococcus isolates were 4 (4.8%). Their antimicrobial susceptibility is summarized in Table 4.4.

Table 4.4: Antimicrobial susceptibility for *Streptococcus pyogenes* and Coagulase negative staphylococci

Antimicrobial	*Streptococcus pyogenes* N=3 n (%)		Coagulase negative staphylococci N=4 (n %)	
	susceptible	not susceptible	susceptible	not susceptible
vancomycin	100	0	100	0
clindamycin	66.7	33.3	100	0
gentamycin	100	0	50	50
penicillin	100	0	-	-
ceftriaxone	100	0	-	-
amikacin	-	-	75	25
meropenem	-	-	75	25

4.3.2 ANTIMICROBIAL SUSCEPTIBILITY FOR GRAM NEGATIVE ISOLATES

4.3.2.1 ANTIMICROBIAL SUSCEPTIBILITY **F**OR *E*SHERICHIA COLI*,*

*P*SEUDOMONAS AERUGINOSA **AND** *K*LEBSIELLA PNEUMONIA

Escherichia coli isolates were 8 (9.5%), *Pseudomonas aeruginosa* isolates were 6 (7.1%) and *Klebsiella pneumonia* isolates were 6 (7.1%). Their antimicrobial susceptibility is summarized in Table 4.5.

Table 4.5: Antimicrobial susceptibility for gram negative bacterial pathogens islolated (*Esherichia coli, Pseudomonas aeruginosa and Klebsiella pneumonia)*

Antimicrobial	*E. coli* (%) N=8 n (%)	*P. aeruginosa* N=6 n (%)	*K. pneumonia* N=6 n (%)
meropenem	8 (100)	6 (100)	6 (100)
amikacin	7 (87.5)	5 (83.3)	6 (100)
ceftriaxone	8 (100)	5 (83.3)	-
ceftazidime	2 (25.0)	2 (33.3	2 (33.3)
gentamicin	5 (62.5)	-	2 (33.3)
cefuroxime	3 (37.5)	-	1 (16.7)
ciprofloxacin	-	5 (83.3)	3 (50.0)

4.3.2.2 ANTIMICROBIAL SUSCEPTIBILITY FOR *ACINETOBACTER BAUMANII*

Acinetobacter baumanii that were isolated were 5. They were all resistant to all the antimicrobials they were tested against including meropenem amikacin, ceftriaxone, ciprofloxacin, gentamicin, levofloxacin, piperacillin/tazobactam, ceftazidime, cefepime, cefuroxime and cefotaxime.

4.4 EMPIRIC ANTIMICROBIAL USE IN SSTIS
Empiric therapy was used during the period when bacterial culture and susceptibility results were still pending. 115 patients (68.9%) received a combination of 2 antimicrobials as empiric therapy. Table 4.5 shows the antimicrobials used as empiric therapy.

Table 4.5: Empiric therapy used in patients with SSTIs

Antimicrobial	Number of patients N=165 n (%)
flucloxacillin	110 (66.7)
metronidazole	96 (58.2)
ceftriaxone	26 (15.8)
gentamicin	5 (3.0)
Others*	<5

*Others- Antimicrobials less commonly used as empiric including clindamycin 7 (3.5%), gentamicin 9 (4.5%), amikacin 4 (2.0%), augmentin 5 (2.5%), vancomycin 4 (2.0%), cefuroxime 2 (1.0%), meropenem 2 (1.0%), ciprofloxacin 4 (2.0%), cefepime 2 (1.0%), levofloxacin 2 (1.0%), and erythromycin 1 (0.5%).

The empiric therapy used, when evaluated retrospectively upon obtaining the respective antimicrobial susceptibility of the infective bacterial pathogen, was

effective against the organisms causing infection in 18.2% of the 78 patients who had the antimicrobial susceptibility studies done. All patients admitted with SSTIs received antimicrobials in hospital, with 74.2% of the patients receiving more than one antimicrobial as empiric therapy. The use of antimicrobials which are traditionally active against MRSA commonly known as MRSA active agents (Vancomycin, clindamycin, lincomycin, linezolid, cotrimoxazole and doxycycline) as empiric therapy was 6.6%.

CHAPTER FIVE: DISCUSSION

5.0 INTRODUCTION

This chapter presents the discussion of the findings. It will be based on the objectives of the study, including the etiologic pathogenic bacteria , the antimicrobial susceptibility for both gram positive and gram negative organisms and the antimicrobial management of SSTIs at MTRH including empiric therapy used in SSTIs, empiric MRSA active therapy used in SSTIs, the antimicrobial use and avoidable antimicrobial exposure.

The common SSTIs seen were abscesses and cellulitis, which together constituted over half of all the conditions. *Staphylococcus aureus* was the most prevalent bacteria. This species was found to be resistant to many commonly used antimicrobials. More males than females were affected, a finding that is corroborated by other literature. This could be because males tend to engage in risky behavior, with a resultant increase in the risk for injuries with subsequent infection (Pitout *et al.*, 2008).

Pathogenic bacteria were isolated in 76%. This was comparable to other studies done in Sub Saharan Africa, (yield ranges from 70%-86%) where the collection methods and culture media used are comparable (Azene & Beyene, 2011; Pondei, Fente, & Oladapo, 2013).

5.1 ETIOLOGIC PATHOGENIC BACTERIA

The bacterial organisms causing SSTTs in this study included *Staphylococcus aureus*, *Enterococcus* spp., *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, coagulase negative staphylococci*, Acinetobacter baumanii, Streptococcus pyogenes* and *Proteus mirabilis* (Figure 4.3). This pattern of etiology is comparable to other studies done in Kenya. In a study done in 2015 at the Kenyatta

National Hospital, the causative organisms included *Staphylococcus aureus*, *Pseudomonas aeruginosa, Proteus mirabilis,* coagulase negative staphylococci, Beta hemolytic Streptococcus, *Klebsiella* spp.*,* non lactose fermenters, and *Enterococcus* spp. (Elamenya *et al.,* 2015).

In a multicenter study on SSTIs in hospitals in Nairobi, causative bacteria included *Staphylococcus aureus,* coagulase-negative staphylococcus, *Corynebacteria, Streptococcus*spp.*, and Micrococcus* spp. (Maina *et al.,* 2013). Studies by 'SENTRY' (an Antimicrobial Surveillance Program designed to monitor the predominant pathogens and antimicrobial resistance for infections globally) have consistently shown these same organisms to be major causes of SSTIs, including *Staphylococcus aureus,Pseudomonas aeruginosa*, *Escherichia coli*, and *Enterococcus* spp. (Moet *et al.*, 2007).

The most prevalent pathogenic organism was *Staphylococcus aureus*, isolated in 47.6% (figure 2). *Staphylococcus aureus* is the commonest cause of SSTIs, a fact that is established in literature, and confirmed by studies in the country. In Elamenya *et al., Staphylococcus aureus* was the most common at 52.7%, while in Maina *et al.,* 64% were *Staphylococcus aureus,* both studies having bacteria isolated from SSTIs.

Studies done on wound infections alone have shown a slightly different etiologic pattern, where gram negative organisms sometimes predominate. In a study done at MTRH on post operative wound infections, *Staphylococcus aureus* was isolated in 54.7% while *Pseudomonas aeruginosa* and *Proteus mirabilis* followed in that order (Andhoga *et al.,* 2002). However, in a similar study done at Kenyatta National Hospital, *Staphylococcus aureus* was isolated in 33.9%, while *Pseudomonas aeruginosa* was more predominant, isolated in 42.9%. Post operative wound

infections are hospital acquired infections, which explains why *Pseudomonas aeruginosa* was more prevalent (Karimi, Maru, Bururia, Kuria, & Odhiambo, 2006). Similarly, another study done at Aga Khan University Hospital showed 30.3% of postoperative wound infections caused by *Staphylococcus aureus,* which was still the most common, but with less prevalence than other similar studies (Dinda *et al.,* 2013).

Enterococcus spp. was the second most prevalent bacteria isolated (10.1%). Recently, an increase of enterococcal infections in SSTIs has been reported (Weiner et al., 2016).

Coagulase negative staphylococci (CoNS)were isolated in 6.7% of the patients (figure 2). While CoNS are sometimes isolated in wounds that are colonized rather than infected, precaution was taken to ensure that the wounds were actually infected and necessary precautions were taken to avoid contamination. However, most of these infections are nosocomial in nature, most likely due to *Staphylococcus epidermidis* or *Staphylococcus saprophyticus*, causing infection in immune compromised hosts. The prevalence of CoNS found in this study was higher than findings in other studies in Kenya, for example at KNH where CoNS was isolated in 3.3% (Elamenya *et al.,* 2015). *Staphylococcus epidermidis* cause infection, especially in the immune compromised hosts, as well as those with indwelling devices such as orthopedic hardware and dialysis catheters. These bacteria have a high rate of resistance to multiple antimicrobials (Iyer & Jones, 2004; Piette & Verschraegen, 2009).

5.2 ANTIMICROBIAL SUSCEPTIBILITY PATTERNS

5.2.1 ANTIMICROBIAL SUSCEPTIBILITY FOR GRAM POSITIVE ORGANISMS

5.2.1.1 ANTIMICROBIAL SUSCEPTIBILITY FOR *STAPHYLOCOCCUS AUREUS*

The antimicrobials that demonstrated good activity against *Staphylococcus aureus* (over 60%) included vancomycin, ceftazidime, cefepime, ceftriaxone, cefotaxime, clindamycin and cefuroxime (Table 4.3). Most studies have shown an excellent susceptibility profile of *Staphylococcus aureus* to vancomycin. In Maina *et al.,* of the *Staphylococcus aureus* isolated, none was resistant to vancomycin. Likewise, in a Botswana study on *Staphylococcus aureus* causing SSTIs, the highest susceptibility was shown to vancomycin. The Infectious Diseases Society of America (IDSA) and the Surgical Infection Society recommend vancomycin as the first line of treatment of infections caused by *Staphylococcus aureus*. This is due to its consistent activity against the bugs, including MRSA isolates (Maina *et al.,* 2013; May, 2011; Stevens *et al.*, 2005; Truong *et al.*, 2011).

12.5% of *Staphylococcus aureus* isolated were resistant to vancomycin. This is worrying, since it suggests the possibility of vancomycin intermediate (VISA) or vancomycin resistant *Staphylococcus aureus* (VRSA). However, the determination of VRSA or VISA requires the use of the minimal inhibitory concentration, which was not used in this study.

The Infectious Diseases Society of America recommends the use of clindamycin in staphylococcal infections. In addition to the susceptibility, it is also bacteriostatic, and can prevent staphylococcal toxins. This study showed good susceptibility to this antimicrobial, at 62.5%. This was also comparable to a study by Maina *et al.,* 2013 which showed susceptibility to clindamycin of 62.2%. Likewise, in a study by Karimi

et al., 2006 on wound infections, 84% of *Staphylococcus aureus* showed susceptibility to clindamycin.However, in the use of clindamycin, there is the potential of cross-resistance of clindamycin with erythromycin. Inducible resistance to clindamycin can also emerge in MRSA isolates (Stevens, 2005; Karimi, 2006).

Staphylococcus aureus were also susceptible to most cephalosporins. This finding was consistent with findings by Elamenya *et al.,* where the only antimicrobial that had over 50% activity against *Staphylococcus aureus* was ceftriaxone. In a study by Karimi *et al.* on wound infections at KNH, the susceptibility to cefuroxime was 58%, among the highest of the antimicrobials tested. Likewise, in a study on SSTIs caused by *Staphylococcus aureus* in Botswana, the susceptibility to cephalosporins, including cephadrine, was over 60%. The Infectious Diseases Society of America recommends cephaloporins, especially first or second generation cephalosporins, for the treatment of MSSA infections (Karimi *et al.,* 2006, Truong *et al.*, 2011, Stevens *et al.,* 2005, Elamenya, 2015).

MRSA was found in 45% of all *Staphylococcus aureus*. This was comparable the prevalence observed in other studies in Kenya. In 2015, a study on wound infections at KNH revealed MRSA at 50.6% (Elamenya *et al.,* 2015). The prevalence of MRSA in studies from Kenya have been fluctuating, as shown in table 1. By the year 2012, MRSA in *Staphylococcus aureus* causing SSTIs was 84.1% in Nairobi hospitals. Likewise, by 2013, MRSA was 56-57% and 70-82% from singly infected wounds and co-infected wounds respectively, in a study done in Busia, Western Kenya (Maina *et al.*, 2013; Olipher *et al.,* 2013). Given these rates of MRSA, there needs to be a shift in the prescription pattern for SSTIs to MRSA active therapy.

One local study has shown a lower prevalence of MRSA than what has been reported in other literature in the region, at 3.7%. This study was done using automated systems, on clinical specimens, mostly pus swabs (Omuse *et al.*, 2014). This significant difference could be due to the use of automated techniques.

The susceptibility of *Staphylococcus aureus* to cotrimoxazole was found to be low (32.5%). This was also the case in the study by Maina *et al.* (24.4% susceptible). The use of cotrimoxazole is recommended for MRSA infections by the Infectious Diseases Society of America. This low susceptibility could be due to the extensive use of cotrimoxazole in the country, increasing the chances of antimicrobial resistance. In the USA, the cotrimoxazole has been found to be effective in the treatment of infections caused by *Staphylococcus aureus* (Maina *et al.,* 2013, Stevens et al.,2014).

The antimicrobial susceptibility rates found in this study were higher than findings by Elamenya et al, where susceptibility of *Staphylococcus aureus* to most antimicrobials was less than 50%, including cefoxitin, ciprofloxacin, cloxacillin, augmentin, imipenem, cefuroxime and ceftazidime (Elamenya *et al.,* 2015). The study by Elamenya *et al.* was done on wound infections, which usually have more exposure to antimicrobials. Chronic wounds, especially, have more antimicrobial resistance than other SSTIs (Howell-Jones *et al.*, 2005).

Staphylococcus aureus isolated showed decreased susceptibility to levofloxacin, erythromycin, cotrimoxazole, ciprofloxacin, linezolid and penicillin. The low susceptibility shown to erythromycin, cefuroxime and ciprofloxacin was similar to a study by Olipher *et al.* in Busia, which demonstrated that all these antimicrobials had susceptibility rates of less than 50%. Likewise, the findings were similar to a study on SSTIs by Maina *et al.,*which showed low susceptibility to co-trimoxazole,

ciprofloxacin, gentamicin and erythromycin (Maina *et al.*, 2013; Olipher *et al.,* 2013). Many of these are antimicrobials that are available locally, and have traditionally been relied on in the treatment of SSTIs. Organisms develop mechanisms of resistance with continued use, hence the need for rational antimicrobial use. It also highlights the need for antimicrobial susceptibility studies in every case.

A study by Olipher *et al.* found high susceptibility rates for penicillin (57%) and erythromycin (60%), which differed from the present study (Olipher *et al.,* 2013). There is no clear reason for this difference, but it highlights the temporal and spatial changes in antimicrobial resistance, hence the need for regular susceptibility screening.

5.2.1.2 ANTIMICROBIAL SUSCEPTIBILITY FOR *ENTEROCOCCUS* SPP.

In this study, over 70% of *Enterococcus* spp. demonstrated susceptibility to vancomycin, penicillin and amikacin (Table 4.3). This was comparable to findings by Rudy *et al.* which showed high rates of susceptibility to vancomycin. Similarly, a study by Mutuku *et al.* showed susceptibility to the glycopeptides, although there was resistance to aminoglycosides (Mutuku, 2013; Rudy, Nowakowska, Wiechuła, Zientara, & Radosz-Komoniewska, 2003).

For monotherapy of susceptible *Enterococcus* spp., penicillins are the drug of choice. For severe infections, combining a cell wall–active agent such as pencillin with an aminoglycoside results in synergistic bactericidal activity against enterococci. In addition, susceptibility to cefepime, vancomycin and amikacin was over 75%. The best treatment combination for enterococcal infections in this setting is penicillin and amikacin, or vancomycin and amikacin for severe infections.

Enterococci have a natural resistance to cephalosporins, and easily acquire resistance to many antimicrobials (Moet *et al.,* 2007). In this study, there was marked resistance to gentamicin, ceftriaxone, ceftazidime, erythromycin and cefuroxime.

Seventy-five percent of the enterococci were susceptible to penicillin. This was higher than findings by Mutuku *et al.* which found resistance to ampicillin at 11-29%. The mechanism of beta lactam resistance is usually due to beta lactamase production due to the aquisition of beta lactamase operon from *Staphylococcus aureus* (Mutuku, 2013).

The susceptibility of enterococci to erythromycin was 37.5%. This could be due to macrolide-lincosamide-streptogramin resistance (MLS). Three different mechanisms account for the acquired resistance to MLS antimicrobials in gram-positive bacteria, including modification of the drug target, inactivation of the drug, and active efflux of the antimicrobial (Mutuku, 2013).

The susceptibility of enterococci to vancomycin was 87.5% in the disc diffusion test. Therefore, further studies are needed using Minimal Inhibitory Concentration to document the presence of Vancomycin Resistant Enterococci (VRE). It highlights the possiblility that VRE strains may be in circulation. This also means that antimicrobials with activity against VRE like linezolid, daptomycin, tigecycline should be available at MTRH. Mechanisms of VRE include intrinsic resistance due to bacterial synthesis of modified peptidoglycan precursors with reduced affinity for the glycopeptides, or acquired resistance. There are four phenotypes of acquired resistance to the glycopeptides: van A, van B, van D and van E (Fraser *et al.*, 2008).

The susceptibility of enterococci to gentamycin was low, at 37.5%. This was in keeping with findings by Rudy et al. in Ethiopia, probably showing increasing

regional prevalence of strains with high level resistance to aminoglycosides. Further studies are needed locally to document the prevalence of resistant strains (Fraser *et al.*, 2008; Rudy *et al.,* 2003; Sood *et al.*, 2008; Wood & Murray, 2000).

5.2.1.3 ANTIMICROBIAL SUSCEPTIBILITY FOR *STREPTOCOCCUS PYOGENES*

All the *Streptococcus pyogenes* 100% were susceptible to 4 of the 5 antimicrobials tested (Table 4.4). This was comparable to other studies in the country. In the study by Elamenya *et al.,*over 80% of beta hemolytic streptococci demonstrated susceptibility to 4 of the 5 antimicrobials tested, namely, augmentin, ceftriaxone, imipenem and cloxacillin. According the Infectious Diseases Society of America, infections caused by *Streptococcus pyogenes* can be treated using penicillins or cephalosporins. This study confirms the susceptibility of this bacteria to these agents (Elamenya *et al.*, 2015; May, 2011; Stevens *et al.*, 2005).

5.2.2 ANTIMICROBIAL SUSCEPTIBILITY FOR GRAM NEGATIVE ORGANISMS

5.2.2.1 ANTIMICROBIAL SUSCEPTIBILITY FOR *PSEUDOMONAS AERUGINOSA*

This study showed the greatest antimicrobial susceptibility of *Pseudomonas aeruginosa* to meropenem at 100% (Table 4.5). The susceptibility to carbapenems was higher than, but comparable to findings from other studies in the region. Carbapenem resistance in a large tertiary-care centre in Nairobi was 13.7%, and all tested positive for metallo-β-lactamase production (Pitout et al., 2008). In a Ugandan study, 24% of *Pseudomonas aeruginosa* cultured mainly from pus swabs were carbapenem resistant. Likewise, Elamenya *et al.* demonstrated susceptibility to carbapenems (Elamenya *et al.*, 2015; Kateete *et al.*, 2016).

The susceptibility to ciprofloxacin was over 80%. This was similar to findings by Sivanmaliappan *et al.,* which showed high susceptibility to ciprofloxacin. Elamenya

et al. also demonstrated susceptibility of *Pseudomonas aeruginosa* to ciprofloxacin. Ciprofloxacin is commonly used in the treatment of infections caused by *Pseudomonas aeruginosa*. This study confirms the susceptibility of *Pseudomonas aeruginosa* to ciprofloxacin and the carbapenems (Elamenya *et al.,* 2015; Sivanmaliappan & Sevanan, 2011). Other choices include ceftriaxone and amikacin, both which demonstrated susceptibility of over 80%.

Whereas the susceptibility to amikacin was high at 83%, it was much lower for gentamycin (33%). This could be due to excessive use of gentamycin, with the exposure leading to buildup of resistance.

5.2.2.2 ANTIMICROBIAL SUSCEPTIBILITY FOR *ESCHERICHIA COLI*

Esherichia coli was the third commonest cause of SSTIs (Figure 4.3). In this study, the susceptibility was highest to meropenem, ceftriaxone and amikacin (Table 4.5). This was similar to findings from other studies in the region. Mos *et al.,* in a study done on *Esherichia coli* isolated from, among other sources, infected wounds, showed a similar susceptibility pattern, where susceptibility was high to the aminoglycosides and fluoroquinolones. Likewise, an Ethiopian study demonstrated susceptibility to aminoglycosides and fluoroquinolones (Kibret & Abera, 2011; Moş *et al.,* 2010). In a study on SSTIs done in Nepal, *Escherichia coli* demonstrated susceptibility to amikacin, gentamicin and norfloxacin, all with over 75% (Raza, Chander, & Ranabhat, 2013).

Resistance was demonstrated to most cephalosporins (cefuroxime, ceftazidime, cefotaxime and cefepime). The reduced susceptibility to cephalosporins suggests that there isa possibility of ESBL producing *Escherichia coli*. Further studies are needed to investigate this possibility. Studies have shown an association of multidrug-resistant *Escherichia coli* with chronic antimicrobial exposure (Tadesse *et al.,* 2012).

5.2.2.3 ANTIMICROBIAL SUSCEPTIBILITY FOR *KLEBSIELLA PNEUMONIA*

All the *Klebsiella pneumonia* were susceptible to meropenem and amikacin, while susceptibility to cefepime and levofloxacin was 50% (Table 4.5). *Klebsiella* spp. showed significant resistance to ceftazidime (33.3%), ciprofloxacin (16.7%) and gentamicin (16.7%). From these results, it is evident that most isolates were extended spectrum beta lactamase (ESBL)-producing. Proper treatment of Klebsiella in this setting should therefore make use of carbapenems (Pendleton *et al.,* 2013).

5.2.2.4 ANTIMICROBIAL SUSCEPTIBILITY FOR *ACINETOBACTER BAUMANII*

Most *Acinetobacter baumanii* were isolated from patients who had had prolonged hospital stay, while some had been admitted in the ICU and had chronic infection. All the strains of *Acinetobacter baumanii* showed multiple drug resistance, with 5 of them showing resistance to all antimicrobials it was tested against. This was similar to most studies, both local and international (Biglari, Hanafiah, Ramli, Rahman, & Khaithir, 2013; Gündeşlioğlu *et al.*, 2014; Huber *et al.*, 2014; Seifert *et al.*, 1993). This is a grave reality. In the study by Binglari *et al.,*all the acinetobacter were susceptible to Polymyxin B. Other options for the treatment of infections cused by these pathogens are colistin, and the use of combination therapy (Binglari *et al.,* 2014).

Acinetobacter baumanii does not typically infect or cause serious disease in the immune competent host. However, critically ill patients are particularly at risk, including most critically ill patients in intensive care. Efforts at developing new drugs should be intensified, as well as rational antimicrobial use to curtail resistance. Infection prevetion and control measures should also be intensified, to prevent cross contamination of multidrug resistant bacteria which thrive in the hospital settings where these measures are not adhered to.

5.3 ANTIMICROBIAL MANAGEMENT OF SSTIS AT MTRH

5.3.1 EMPIRIC ANTIMICROBIAL THERAPY USED IN SSTIS

Antimicrobials used as empiric therapy included flucloxacillin, metronidazole and ceftriaxone (Table 4.6). These are readily available and low cost drugs that are effective for most SSTIs. This was also in line with the national treatment guidelines. However, it is now clear that these antimicrobials have limited effectiveness in treating SSTIs given the susceptibility rates demonstrated by this study, as well other recent studies in the country. A review should be done on what antimicrobials are now the most appropriate considering the local susceptibility data (Kimathi et al., 2002); Maina *et al.,* 2013, Olipher *et al.,* 2013).

Based on the antimicrobial susceptibility done, in retrospect, empirical antimicrobial therapy was appropriate in 18.2% of the cases. Empiric therapy is necessary as susceptibility studies in the typical setup of most Kenyan hospitals frequently take about three days to become available for clinical use. This duration could be shortened by the use of automated methods, but these are not readily available for general use in resource limited settings. It is therefore necessary to invest in these

prompt methods of demonstrating susceptibility, which have the potential to improve clinical outcomes.

Antimicrobial guidelines for empiric treatment, which are developed by, among other players, infectious disease experts, can also improve the accuracy of empiric antimicrobial prescription. Studies have shown that empiric therapy by infectious disease experts are more accurate than prescription by other clinicians (Pulcini, Botelho-Nevers, Dyar, & Harbarth, 2014).

The effectiveness of empiric therapy found in this study are low compared to other studies which report rates of 50-100%, depending on the condition and whether single agent therapy or multiple agents (Hurley et al., 2013; Rodger Shortt, 2008).

5.3.2 EMPIRIC MRSA ACTIVE THERAPY USED IN SSTIS

The use of antimicrobials which are traditionally active against MRSA commonly known as MRSA active agents (Vancomycin, clindamycin, lincomycin, linezolid, cotrimoxazole and doxycycline) as empiric therapy was 6.6%. This is low compared to findings from studies done in the United States which show empiric prescription of MRSA active agents of over 60% (Hurley *et al.,* 2013; Mistry *et al.,* 2014). However, other studies in the country show even less use, at 2.0% (Elamenya *et al.,* 2015).

This is attributed to limited awareness of MRSA in the country. The increasing prevalence of MRSA needs attention, as studies in the country show consistent MRSA prevalence of over 40% (Karimi *et al.*, 2006; Ouko *et al.*, 2010; Rutare, 2013). Clinicians should be sensitized on MRSA and its increasing prevalence. When MRSA prevalence is more than 10%, studies have shown that the empiric treatment of SSTIs with MRSA active agents maximizes the probability that the antimicrobial will be active, and is recommended (Williams *et al.,* 2011).

When the chances of SSTI caused by *Staphylococcus aureus* exist, empiric treatment of SSTIs should make use of MRSA active therapy to maximize the chances of treatment success. This is in a condition where the lesion is localized and pus-producing for example boils, abscesses, carbuncles and localized wound sepsis. The best agent in such a scenario isvancomycin. Other options include ceftazidime, ceftriaxone, clindamycin and cefuroxime, all which demonstrated activity against *Staphylococcus aureus* in over 60% (table 2).

5.3.3 ANTIMICROBIAL USE AND AVOIDABLE ANTIMICROBIAL EXPOSURE

Antimicrobial use in patients admitted with SSTIs was 100%, with 74.2% of them receiving more than one antimicrobial as empiric therapy. This antimicrobial use is higher than findings from studies in other parts of the world. Antimicrobial use was 85% in the US (Mistry *et al.,* 2014). Hurley *et al.* showed antimicrobial use for abscesses after drainage to be 80% (Hurley *et al*., 2013).

The average number of antimicrobials per patient was 2.6. This was high. It was higher than similar studies in the US where the average antimicrobials used was 1.4-1.5. It has been shown that limiting the number of antimicrobials reduces the chances of antimicrobial resistance (Maki & Schuna, 1978). However, the findings in this study were comparable to a study by Hurley et al, where avoidable antimicrobial exposure occurred in 46% (Hurley *et al.*, 2013).

Antimicrobials are not indicated in some conditions like chronic ulcers. Likewise, patients with abscesses that are adequately drained and without evidence of cellulitis around the abscess do not necessarily need antimicrobials. The fact that all patients received antimicrobials points towards irrational antimicrobial use (Stevens *et al.,* 2005).

CHAPTER SIX: CONCLUSION AND RECOMMENDATIONS

CONCLUSION

The most common cause of SSTIs at MTRH was *Staphylococcus aureus*. MRSA prevalence among *Staphylococcus aureus* was 44.9%.

Staphylococcus aureus causing SSTIs were susceptibile to vancomycin and ceftazidime (over 80%), with vancomycin retaining an excellent susceptibility to other gram positive bacteria including coagulase negative staphylococci, *Enterococus fecalis* and *Streptococcus pyogenes.* In addition, all coagulase negative staphylococci were susceptible to clindamycin. Gram negative bacteria isolated, including *Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella* spp. were susceptible (over 80%) to meropenem and amikacin. *Acinetobacter baumanii* were not susceptible to any antimicrobials tested.

This study shows that only 18.2% of the patients had an empiric antimicrobial prescription that was susceptible to the aetiologic bacteria when evaluated retrospectively after antimicrobial susceptibility was done. The antimicrobials most used as empiric therapy were flucloxacillin, metronidazole and ceftriaxone.

RECOMMENDATIONS

1. Since the bacterial pathogens causing SSTIs were highly susceptible to vancomycin, clindamycin, meropenem and amikacin, these antibiotics should be used for the empiric treatment of severe SSTIs. De-escalation of this antimicrobial therapy should then be done according to the antimicrobial susceptibility results.

2. Further research should be done using modern methods of identification of resistance strains using genomic techniques.

LIMITATIONS OF THE STUDY

This study could not identify the specific resistant strains of bacteria that were prevalent, or the mechanisms of resistance to antimicrobials.

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APPENDICES

APPENDIX 1: QUESTIONNAIRE

Biodata

Serial number………   Patient number…………….   Age…………

Sex   male ☐   female   ☐

Weight…………..   Ward…………..……

Casualty diagnosis…………………………

Clinician: consultant ☐   resident   ☐ medical officer   ☐ clinical officer   ☐

 Ward diagnosis…………………….

Clinician: consultant   ☐ resident   ☐ medical officer   ☐ clinical officer   ☐

Clinical characteristics, evaluation of severity and response

Co morbidity   1)…………………………………………

                 2)…………………………………………

Etiology: trauma related   Y/N   IF YES;   a) penetrating ☐ b) not penetrating ☐

Spontaneous   ☐   Other
(specify)………………………………………………….

Site/ region of infection

Head/neck ☐ trunk ☐   perineum   ☐ upper limb   lower limb   ☐

Systemic findings:

	a		b		c	
White cell count/dl <4000	☐	4000-12000	☐	>12000	☐	
Temperature, Celsius <36	☐	36-38	☐	>38	☐	
Heart rate/min <60	☐	60-100	☐	>100	☐	
Respiratory rate/min <12	☐	12-20	☐	>20	☐	
Mean arterial pressure <70	☐	70-110	☐	>110	☐	
Hemoglobin, g/dl <10	☐	10-15	☐	>15	☐	

Other markers:	available	ordered	result
Lactate dehydrogenase	☐	☐	…………………………
C - reactive protein	☐	☐	…………………………
Procalcitonin	☐	☐	…………………………

Cellulitis/erysipelas

Size of lesion (cm2)   a) <75   ☐   b) >75   ☐

Cutaneous abscess

Redness, edema, and/or induration of from the peripheral margin of the abscess

a)Less than 5 cm   ☐   b) More than 5 cm   ☐

Wound infection
Size of lesion (cm2) :   a) <75   ☐   b) >75   ☐
Depth of lesion: fascia   ☐   muscle   ☐   bone   ☐
Management
Antimicrobials used prior yes ☐   no   ☐

Which……………………….duration………………

Source of information: patient ☐relative   ☐parent   ☐patient notes   ☐

Antimicrobials available in the ward (tick what applies)

Ampiclox ☐   cloxacillin☐   floxapen ☐   clindamycin ☐   metronidazole   ☐
ceftriaxone

cephalexin ☐   augmentin ☐   cefuroxime   ☐ xpen   gentamycin   ☐
ciprofloxacin

Other(s)………………………………………………………………………

Antimicrobial(s) prescribed and administered in hospital

1…………………………….Dose………………Frequency…………..date………

2…………………………….Dose……………….Frequency…………..date………

3…………………………….Dose……………….Frequency…………..date………

Clinician: consultant ☐   resident   ☐medical officer   clinical officer   ☐

Antimicrobial adjustment/ dose adjustment

Reason for adjustment…………………………………………………

1……………………………Dose………………Frequency…………date…………

2……………………………Dose……………….Frequency…………date………

3……………………………Dose……………….Frequency………….date………

Clinician: consultant ☐   resident   ☐ medical officer   ☐ clinical officer   ☐

Operative intervention   a) yes   b) no   If yes, how many times………………..

Investigations: microscopy, culture and sensitivity

Sample sent   yes   ☐   no   ☐

 Sample back   yes   ☐   no   ☐

Sample sent prior to ward antimicrobial initiation   yes   ☐   no   ☐

Culture positive   ☐   negative   ☐

Date sent ………………………..   Date back………………………

Culture/sensitivity guided antimicrobial adjustment   yes   no   ☐

Antimicrobial

1 ………………………Dose……………Frequency…………………….

Antimicrobial
2………………………..Dose……………Frequency…………………….

Antimicrobial
3………………………..Dose…………….Frequency…………………….

Organism(s) cultured

      1)…………………………….   2)…………………………………

 Susceptible/   resistant   to   a)……………………………
b)…………………………………

APPENDIX 2: CONSENT FORM

ANTIMICROBIAL SUSCEPTIBILITY AND MANAGEMENT OF BACTERIAL SKIN AND SOFT TISSUE INFECTIONS AT MOI TEACHING AND REFERRAL HOSPITAL

INVESTIGATOR – CALEB KIPKOECH LANGAT, P.O. BOX 95, KAPKATET

I …………………………………… of P.O Box…………………………………

hereby give informed consent to participate in this study in MTRH. The study has been explained to me clearly by Caleb Kipkoech Langat (or his appointed assistant).

I have understood that to participate in this study, I shall volunteer information regarding my condition, and undergo medical examination. I am aware that I can withdraw from this study any time without prejudice to my right of treatment at MTRH now or in the future. I have been assured that no injury shall be inflicted on me from my participation in this study. I have also been assured that all information shall be treated and managed in confidence. I have not been induced or coerced by the investigator (or his appointed assistant) to cause my signature to be appended in this form and by extension participate in this study.

Name of participant (initials)………………………………………………………

Signature………………………………….. Date…………………………

Name of witness………………………………………………………………….

Signature…………………………………. Date……………………………

APPENDIX 3: CLINICAL CRITERIA FOR PATIENT IDENTIFICATION

Wound infection

These clinical criteria are based on a list created by Cutting and Harding (1994). They shall include patients with a break in skin who also have:

- increasing pain in the ulcer area
- erythema, oedema, heat
- purulent exudate or serous exudate
- delayed healing of the ulcer
- discolouration of the granulation tissue or friable granulation tissue
- pocketing at the base of the wound
- foul odour
- wound breakdown
- lymph node enlargement, or systemic symptoms such as fever (Cutting et al., 2004).

Cellulitis

Non necrotizing inflammation of the skin and subcutaneous tissues with the signs of inflammation as described by Celcus including erythema, pain, swelling and warmth, without evidence of a deeper infection like osteomyelitis (Rather, 1971)

Cutaneous abscess

A localized collection of pus, with inflammation of the skin and subcutaneous tissuessigns of inflammation as described by Celcus including erythema, pain, swelling and warmth, without evidence of bone involvement (Rather, 1971)

APPENDIX 4: IDENTIFICATION AND SUSCEPTIBILITY TESTING
A) GRAM STAIN PROCEDURE

This is used to differentiate gram positive bacteria (appear purple) and gram negative bacteria (appear pink). The following steps were followed:

a) Label a new clean slide and assign a laboratory number

b) Fix the dried smear by passing over a flame three times

c) Cover the fixed smear with crystal violet for 60 seconds

d) Rinse the smear with clean water

e) Cover the smear with grams iodine for 30 seconds

f) Rinse the iodine with clean water

g) Decolorize the smear with acetone until no blue color comes out

h) Cover the smear with neutral red stain for one minute

i) Rinse the smear with clean water

j) Wipe the back of the slide clear and place it in a draining rack for the smear to air dry

k) Examine the smear microscopically in oil immersion under 100 objective

l) Interpret and report the results.

B) INDOLE TEST

This is used to identify enterobactericiae. Most strains of enterobactericiae break down the aminoacid tryptophan with the release of indole in which when the indicator Kovac is added it produces a red or pink layer or ring.

Using a sterile straight wire, inoculate 2 ml of sterile tryptophan brothwith test organism.Incubate at 37°C overnight then add 0.5ml of kovac's reagent. Indole production will be exhibited by reddening of the lower part of the strip.

C) 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.

a) A drop of hydrogen peroxide solution is poured into a slide

b) Using a wooden stick or a glass rod 2-3 colonies of the test organism are removedand immersed in the hydrogen peroxide solution

c) Active bubbling with effervescent sound indicates a positive catalase test.

D) COAGULASE TEST

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

SLIDE METHOD (DETECTS BOUND COAGULASE)

a) A drop of normal saline is placed on each end of a slide

b) A colony of the test organism is emulsified in each of the drops to make suspensions

c) A drop of plasma is added to one of the suspensions and mixed gently

d) Clumping of the organisms will occur within 2 seconds if the organism is *Staphylococcus aureus*

e) No plasma is added to the second suspension. This is used to differentiate any granularappearance of the organism from true coagulase clumping.

TUBE METHOD (DETECTS FREE COAGULASE)

a) Plasma is diluted in the ratio of 1:10.

b) Three small test tubes are labeled; test organism, positive control and negative control.

c) 0.5ml of the diluted plasma are pipetted into each tube.

d) 2-3 colonies of the test organism are added into the respective tubes

e) The tubes are incubated at 37 degrees Celcius after mixing gently. Clotting should occur within 3hr, if not, the examination is repeated every30minutes for up to 24hours.

f) Clotting is indicative of *Staphylococcus aureus.*

D) OXIDASE TEST

This test was used to identify *Pseudomonas* spp.

a) Apiece of filter paper impregnated with oxidase is placed in a slide

b) Using a piece of stick or glass rod, a colony of the test organism is then smeared on the filter paper

c) Development of blue- purple colour within 3 seconds indicates positive oxidase test.

E) VOGES-PROSKEUR (V-P) TEST

a) 2ml of MR-VP broth is inoculated with the test organism and incubated at 37°C for 18hours.

b) Add an equal volume of Barrits A (alpha napthol) and Barrits B (KOH) reagents, mix and leavefor 15 min at room temperature small amount of creatinine will be added and mixed well.

c) 3ml of sodium hydroxide will be added and mixed well.

d) The bottle cap will be removed and left for one hour at room temperature.

e) Development of pink colour will be indicative of *Klebsiella pneumoniae.*

F) UREASE TEST

This test was used to identify *Proteus* spp.

a) A straight wire is used to inoculate a tube of urea broth with a colony of the test organism.

b) It is incubated at 37°C

c) Production of urease will change the colour of the paper strip to pink.*Proteus* spp. will turn positive within 3hrs

G) BACITRACIN TEST

This test was used to identify *Streptococcus pyogenes.*

a) Bacitracin disk is placed on a culture plate inoculated with the bacteria and incubated at 35-37°C overnight.

b) A zone of inhibition around the disc is indicative of *Streptococcus pyogenes.*

H) AESCULIN TEST

Bile-esculin test is used to differentiate 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.

a) With a straight wire, touch 2-3 pure colonies and inoculate into the bile esculin medium

b) The inoculated tube is incubated at 37 degree Celsius overnight.

c) Report and interprete the results, where *Enterococcus* spp. will be positive, indicated by black color change

I) METHYL RED TEST

Methyl Red (MR) test determines whether the microbe performs mixed acid fermentation when supplied with glucose. MR-VP broth is used for both MR Test and VP test.

Method

a) Inoculate a tube containing MR broth with a pure culture of the test organism
b) Incubate at 37 °C overnight.
c) Add 2 drops of the methyl red indicator solution
d) A positive reaction is colour change to a stable red within 3 minutes.

J) CITRATE TEST

Citrate utilization is used to distinguish between coliforms such as *Klebsiella* spp. (+ve) and fecal coliforms such as *Escherichia coli* (-ve).

a) Stab simmons citrate agar using sterile straight wire inoculated with test organism
b) Incubate at 37 °C overnight
c) Observe the development of blue color; denoting alkalinization.

K) ANTIMICROBIAL SUSCEPTIBILITY TESTING

K-1) PREPARATION OF MULLER-HINTON CULTURE MEDIA

a) A sterile medium was prepared according to the manufacturer's instructions. The pH of the medium was set at 7.2-7.4.

b) The media was poured into a 90mm sterile petri-dish to a depth of 3mm (about 20ml per plate). This was done on a level surface so that the depth of the medium is uniform.Each new batch of agar was controlled using *E. faecalis* (ATCC 29212 or 33186) and cotrimoxazole disc. The zone of inhibition should be 20mm or more in diameter.

c) The plates are stored at 2-8°C in sealed plastic bags. Before use the plates were dried with their lids slightly raised in 37°C incubator for about 30minutes.

d) About one hour before use, the working stock of the discs are allowed to warm to room temperature, protected from direct sunlight.

K-2) MODIFIED KIRBY-BAUER DISC DIFFUSION METHOD

A disc of blotting paper impregnated with known volume and concentration of an antimicrobial is placed on a plate of susceptibility testing agar uniformly inoculated with the test organism. The antimicrobial 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. Strains susceptible to the antimicrobial 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.

a) Using a sterile wire loop, touch 3-5 pure colonies and emulsify in 3-4ml of sterile physiological saline or nutrient broth

b) Using a colorimeter, measure the turbidity of the suspension to 0.5 McFarland

c) Using a sterile swab, inoculate a plate of Mueller Hinton agar. Remove excess fluid by rotating and pressing the swab against 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° to ensure even distribution

d) With the petri dish lid in place, allow 3-5 minutes (no longer than 30 minutes) for the surface of the agar to dry

e) Using sterile forceps, place appropriate antimicrobial 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

f) Within 30 minutes of applying the discs, invert the plate and incubate aerobically at37°C overnight

g) Examine the control and the test plates to ensure the growth isconfluent or near confluent.

h) 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.

M) INTERPRETATION OF ZONE SIZES

Using the interpretative chart, the zones of each antimicrobial are interpreted reporting eachorganism as Resistant, Intermediate susceptible or Susceptible.

Interpretation of inhibition halos on disc diffusion test

Antimicrobial	Zone diameter			organism
	Susceptible >	intermediate	Resistant<	
vancomycin 30 µg	17	15-16	14	enterococci
	12	10-11	9	other gram positive
	17	-	-	streptococci
erythromycin 15 µg	23	14-22	13	all
cotrimoxazole 25 µg	16	11-15	10	gram negative bacteria
	19	16-18	15	*Streptococcus pneumonia*
clindamycin 2 µg	19	16-18	15	all
ceftriaxone 30 µg	21	14-20	13	all
ceftazidime 30 µg	18	15-17	14	all
cefuroxime 30 µg	23	15-22	14	all
cefotaxime 30 µg	23	15-22	14	gram negative
	24	-	-	beta hemolytic streptococcus
	28	26-27	25	Streptococcus viridans
cefepime 30 µg	18	15-17	14	all
meropenem 10 µg	16	14-15	13	all
amikacin 30 µg	17	15-16	14	all
gentamicin 10 µg	16	13-14	12	all
ciprofloxacin 5 µg	21	16-20	15	all
levofloxacin 5 µg	17	14-16	13	all
penicillin 10 µg	29	-	28	staphylococci
	15	-	14	enterococci
	28	20-27	19	Nonenterococcal streptococci
	20	-	19	*Listeria monocytogenes*

APPENDIX 5:IREC APPROVAL

INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE (IREC)

MOI TEACHING AND REFERRAL HOSPITAL
P.O. BOX 3
ELDORET
Tel: 33471//2/3
Reference: IREC/2013/137
Approval Number: 0001049

MOI UNIVERSITY
SCHOOL OF MEDICINE
P.O. BOX 4606
ELDORET
29th August, 2013

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

Dear Dr. Langat,

RE: FORMAL APPROVAL

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

"Antibiotic Management of Skin and Soft Tissue Infections (SSTI) at the Moi Teaching and Referral Hospital".

Your proposal has been granted a Formal Approval Number: *FAN: IREC 1049* on 29th August, 2013. You are therefore permitted to begin your investigations.

Note that this approval is for 1 year; it will thus expire on 28th August, 2014. 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,

DR. W. ARUASA
DEPUTY - CHAIRMAN
INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE

APPROVED
29 AUG 2013

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

INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE (IREC)

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

Reference: IREC/2013/137
Approval Number: 0001049

MOI UNIVERSITY
SCHOOL OF MEDICINE
P.O. BOX 4606
ELDORET
Tel: 33471/2/3

26th July, 2016

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

INSTITUTIONAL RESEARCH &
ETHICS COMMITTEE
2 6 JUL 2016
APPROVED
P. O. Box 4606-30100 ELDORET

Dear Dr. Langat,

<u>RE: APPROVAL OF AMENDMENT</u>

The Institutional Research and Ethics Committee has reviewed the amendment made to your proposal titled:-

"Antimicrobial Susceptibility and Antimicrobial Management of Skin and Soft Tissue Infections at the Moi Teaching and Referral Hospital".

We note that you are seeking to make an amendment as follows:-

1.   To change the title as above from: *"Pattern of Antibiotic Management of Inpatients with Skin and Soft Tissue Infections at the Moi Teaching and Referral Hospital".*

The amendment has been approved on 26th July, 2016 according to SOP's of IREC. You are therefore permitted to continue with your research.

You are required to submit progress(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
<u>INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE</u>

cc: CEO   -   MTRH   Dean   -   SPH   Dean   -   SOM
        Principal -   CHS   Dean   -   SOD   Dean   -   SON

APPENDIX 6:HOSPITAL APPROVAL

MOI TEACHING AND REFERRAL HOSPITAL

Telephone: 2033471/2/3/4
Fax: 61749
Email: director@mtrh.or.ke
Ref: ELD/MTRH/R.6/VOL.II/2008

P. O. Box 3
ELDORET

29th August, 2013

Dr. Caleb Langat,
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:-

"Antibiotic Management of Skin and Soft Tissue Infections (SSTI) at the Moi Teaching and Referral Hospital".

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

DR. J. KIBOSIA
DIRECTOR
MOI TEACHING AND REFERRAL HOSPITAL

CC   -   Deputy Director (CS)

      -    Chief Nurse

      -    HOD, HRISM