

**DRUG SENSITIVITY USING COUNTER DIFFUSION TECHNIQUE IN METHICILIN-
RESISTANT *STAPHYLOCOCCUS AUREUS* IN CLINICAL ISOLATES: A SURVEY OF
HOSPITALS PATIENTS ADMITTED WITHIN NAKURU COUNTY**

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DECLARATION AND APPROVAL

Declaration by the student

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

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DEDICATION

I dedicate this thesis project to my family who has lived to witness my academic achievements.



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My greatest appreciation and thanks goes to the Almighty God for his grace and good health.

Secondly, I wish to acknowledge valuable contributions and observations my supervisors Dr. Onyuka and Dr. Waithaka made by passionately encouraging and guiding me throughout the research project and without their help it would not have been possible. I am also indebted to my wife Christine, our girls Angel, Princess, Pretty and my late mother Adah Adoyo who encouraged and stood with me on many occasions I was absent from home to see the paper through. I also feel quite much indebted to my colleagues for their inputs as I prepared the proposal. Last but not least am indebted to Mr. Orindi for statistical advice and work.



ABSTRACT

Several infectious bacterial strains have acquired resistance towards most available antibiotics. Therefore, there is need to study drug sensitivity using counter diffusion technique for methicillin resistant *Staphylococcus aureus* (MRSA) in isolates so that combination therapy can be embraced since the core factor in using the method is the ability to give good synergistic potential among.

The specific objectives were to compare the susceptibility pattern for the MRSA organism using the Kirby Bauer disc diffusion and compare it to the counter diffusion technique; To determine which antimicrobial agent combination are sensitive to MRSA; To compare the prevalence of methicillin sensitive *Staphylococcus aureus* (MSSA) and MRSA and ; To establish the prevalence of MRSA according to the sites. The study sought to fill the literature gap by investigating drug sensitivity using counter diffusion technique in MRSA. Laboratory procedure included Sample collection, Culture, Gram staining and biochemical testing Kirby Bauer disc diffusion done following Clinical and Laboratory Standards Institute (CLSI) guidelines. Counter diffusion technique as per the protocol for the study. Data were analyzed using R version 3.2.0. Proportions were compared using Chi square or Fisher's exact test. Cohen's Kappa was used to assess the inter-drug reliability in detecting the MRSA and MSSA sensitivity of the 11 drugs on the isolates. A simple linear regression analysis was used to study the relationship between the zones inhibited and the technique used. In the model zone was the outcome variable and technique was the covariate, with the latter two being compared to the counter diffusion technique. All tests were performed at 5% significance level. Of 423 patients recruited, 344 (181 males and 163 females) had cases of *Staphylococcus species* isolated 12.5% (95% CI 17.8-16.5%) of the 344 were Methicillin resistant whereas the rest were Methicillin sensitive, *Proteus species* 30 cases, *Escherichia coli* (*E. coli*) 45 cases, *Klebsiella species* 4 cases. The proportion resistant was 14% and 11% among males and females, respectively. These two proportions were not significantly different from each other (Chi square= 0.374, df = 1, p = 0.540). The results further indicated that the prevalence of MRSA varied significantly by site (Fisher's exact test p<0.001), with wounds recording the highest at (17.9%) followed by central venous catheter (CVC) (16.7%), nasal (9.1%), and stool and urine at zero percent. It informs general public on the gains made in the drug sensitivity using the technique. Scholars will use the study as a background for their own academic papers. The study found that, MRSA is a real medical threat within Nakuru County. Proper wound management should be maintained since it was found that MRSA was higher in wound specimen more so those that were septic. The researcher recommends that, the procedure be validated and accepted as marker for testing for the drug combination therapy as it has potential to pinpoint to clinicians the way forward in patient management. Surveillance of MRSA as a means of identifying colonized or infected patients should be implemented since most of isolated cases were from patients who are admitted.

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ABBREVIATIONS AND ACRONYMS

- AIDS:** Acquired immune deficiency syndrome
- ATCC:** America Type Culture Collection
- CA:** Community-acquired
- HA-MRSA:** Hospital Acquired methicillin resistant *Staphylococcus aureus*
- HIV:** Human immunodeficiency virus
- ICAK:** Infection Control Association of Kenya
- ICU:** Intensive Care Unit
- KB:** Kirby Bauer
- KNH:** Kenyatta National Hospital
- LA MRSA:** Livestock Acquired Methicillin resistant *Staphylococcus aureus*
- MIC:** Minimum inhibition concentration
- MRSA:** Methicillin resistant *Staphylococcus aureus*
- MSSA:** Methicillin sensitive *Staphylococcus aureus*

NACOSTI: National Commission for Science Technology and Innovation

PBPs: Penicillin binding proteins

SSTIs: Skin and soft tissue infections

USA: United States of America

DEFINITION OF TERMS

Antibiotics: Is a chemical substance produced by a microorganism, which has the capacity to inhibit the growth of or to kill other microorganisms; antibiotics sufficiently nontoxic to the host are used in the treatment of infectious diseases.

CA-MRSA: Are MRSA infections in healthy people who have not been hospitalized nor had a medical procedure (such as dialysis or surgery) within the past year.

Community: Is a group of people living in the same place or having a particular characteristic in common.

Counter diffusion: A form of diffusion where two or more molecule diffuse from a region of high concentration to a region of lower concentration and in the process creates another region of extreme concentration between their point of origin.

Cross-resistance: Is immunologic resistance to the pathogenic effects of a microorganism due to previous exposure to another species or type having cross-reactive antigens.

Gram staining (or Gram's Method): Is a method of differentiating bacterial species into two large groups (gram-positive and gram-negative). Gram staining differentiates bacteria by the chemical and physical properties of their cell walls by detecting peptidoglycan, which is present

in a thick layer in gram-positive bacteria. In a Gram stain test, gram-positive bacteria retain the crystal violet dye, while a counter-stain (commonly safranin) added after the crystal violet gives all gram-negative bacteria a red or pink coloring.

McFarland standards: Are used as a reference to adjust the turbidity of bacterial suspensions so that the number of bacteria will be within a given range.

Mec A gene: It's a gene found in bacterial cell and it allows the cell to be resistant to certain antibiotic like methicillin, penicillin drugs.

Methicillin-Resistant *Staphylococcus aureus*: Is a bacterium that enters the skin through open wounds to cause septicemia and is extremely resistant to most antibiotics. It has been responsible for outbreaks of untreatable infections among patients in hospitals.

MIC: Minimum inhibition concentration it's the lowest drug concentration that prevent visible micro bacterial growth after an incubation period of overnight.

Muller-Hinton agar: Is a microbiological growth medium that is commonly used for antibiotic susceptibility testing.

Penicillin: sometimes abbreviated **PCN** or **pen**) is a group of antibiotics derived from *Penicillium* fungi, including penicillin G (IV use), penicillin V (oral use), procaine penicillin, and benzathine penicillin (intramuscular use).

PFGE: Pulsed field gel electrophoresis is a technique used for the separation of large deoxyribonucleic acid (DNA) molecules by applying to a gel matrix an electric field that periodically changes direction.

Resistance: Is the natural ability of an organism to resist microorganisms or toxins produced in disease.

SCCmec: or staphylococcal cassette chromosome *mec*. Is a mobile genetic element of *Staphylococcus* bacterial species. This genetic sequence includes the *mecA* gene coding for resistance to the antibiotic methicillin and is the only known way for *Staphylococcus* strains to spread the gene in the wild by horizontal gene transfer.



CHAPTER ONE: INTRODUCTION

1.1 Background of the Study

Resistance in methicillin resistant *Staphylococcus aureus* (MRSA) is related to a chromosomal *mecA* gene that specifies the production of an abnormal penicillin-binding protein called PBP2a or PBP21. Penicillin-binding proteins are membrane-bound enzymes, which targets for all β -lactam antibiotics (Ito *et al.*, 2012). PBP2a has a decreased affinity for binding β -lactam antibiotics resulting in resistance not only to methicillin but also to all β -lactams including penicillin and cephalosporins (Llarrul *et al.*, 2010). The *mecA* gene complex also contains insertion sites for plasmids and transposons that facilitate acquisition of resistance to other antibiotics. Thus, cross-resistance to non- β -lactam antibiotics such as erythromycin, clindamycin, gentamicin, cotrimoxazole and ciprofloxacin is common (Shenoy *et al.*, 2010).

Emily *et al.*, (2010), points out that *Staphylococcus aureus* have entered the spotlight as a globally pervasive drug-resistant pathogen. While historically associated exclusively with hospital-acquired infections in immune-compromised hosts, the methicillin-resistant form of *S. aureus* has been spreading throughout communities since the 1990s (Morrel *et al.*, 2010). Some of the populations at risk of getting *Staphylococcus aureus* are people with weak immune systems, diabetics; intravenous drug users; users of quinolone antibiotics; young children; the elderly; college students living in dormitories; people staying or working in a health care facility for an extended period of time; people who spend time in coastal waters where MRSA is present and people who spend time in confined spaces with other people.

According to Emily *et al.*, (2010), *S. aureus* has developed numerous mechanisms of virulence and strategies to evade the human immune system, including a host of surface proteins, secreted enzymes, and toxins. In hospital intensive care units, the proportion of MRSA-related *S. aureus*

infections has increased strikingly from just 2 percent in 1974 to 64 percent in 2004 (Morrel *et al.*, 2010). Its presence in the community has been rising similarly, posing a significant public health burden. The growing incidence of MRSA unfortunately has been met with dwindling efforts to develop new, more effective antibiotics. The continued emergence of resistant strains of bacteria such as MRSA demands an urgent revival of the search for new antibiotics.

In the USA, MRSA was responsible for an estimated 94,000 life-threatening infections and 18,650 deaths in 2005, which is more than the 16,268 deaths caused by HIV/AIDS in that same period (Klevens *et al.*, 2007). Recently, MRSA has been identified in food production animals and people in contact with these animals (Voss *et al.*, 2005). This involves a specific clone, Multi Locus Sequence Type 398, which seems to spread extensively among animals (Argudín *et al.*, 2015). The finding of this new zoonotic reservoir of MRSA has led to several research initiatives to investigate its implications.

In Nigeria, the widespread use of antibiotics had led to high levels of resistance among bacterial isolates from patients with nosocomial infections (Samuel *et al.*, 2010). Methicillin resistant strains that emerged by late 1980s have become increasingly present as nosocomial pathogens. The medical community was again relieved when vancomycin a glycoprotein was discovered that added effective therapy to all strains of methicillin resistant *S. aureus* (Brincat *et al.*, 2010). Nevertheless vancomycin resistant strains of coagulase–negative staphylococci were also a cause of concern (Tiwari and Sen, 2006).

At Nairobi Hospital in Kenya, the problem of MRSA was recognized in mid-year of 1996 among both in and out patients (Maina *et al.*, 2013). Swift action by the hospital infection control

committee resulted in no more cases at the end of the year. The national referral hospital, Kenyatta National Hospital also has a functional infection control committee. These serve as role models to disseminate information to smaller hospitals. As a step forward, the Infection Control Association of Kenya (ICAK) was launched in 1997 to coordinate infection control countrywide and provide an effective communication and networking channel between various target and interested groups. There is increasing concern regarding the efficacy of many disinfectants on the market. MRSA was found to be significantly less susceptible than methicillin sensitive *S. aureus* (MSSA) to chlorohexidine digluconate, 'Hibiscrub' and 'Hibisol'. Hand disinfectants containing both alcohol and chlorhexidine ("Hibisol") are more effective against MRSA than scrubs based only on chlorhexidine (Hibiscrub') and should be used in clinical practice (Hughes *et al.*, 2011). It is therefore important to select appropriate concentration of disinfectant and rationally use them for disinfection and hospital hygiene (Hughes *et al.*, 2011). There is a possibility that a significant proportion of laboratory or hospital acquired infections may partly be due to the use of ineffective or low concentration of disinfectants. A continuous monitoring of the efficacy of the commonly used disinfectants is necessary in order to minimize the risk of infection by antibiotic resistant microorganisms, which are common in the hospital (Johnston *et al.*, 2009).

1.2 Problem Statement

There is increasing resistance to available antibiotics, by the development and spread of strains resistant to the semi synthetic penicillin (methicillin, oxacillin, and nafcillin), macrolides, tetracycline, and aminoglycosides which has made the therapy of staphylococcal disease a global challenge. Thati *et al.*, (2011), said that there is no laboratory diagnosis method for testing synergy potential of different antibiotic Therefore, there is need to study drug sensitivity using counter

diffusion for methicillin-resistant *Staphylococcus aureus* in clinical isolates so that combination therapy can be embraced since the core factor in using the method is the ability to give good synergistic potential among drugs that show good minimum inhibition concentration (MIC) for the organism. Specifically, focusing on a survey of patients admitted in hospitals within Nakuru Town.

1.3 Significance of the Study

This study will be instrumental in informing the general public on the gains made in the drug sensitivity testing using counter diffusion in MRSA in clinical isolates. It will be useful in providing feedback to the government on the drug sensitivity using counter diffusion in MRSA in clinical isolates, and its importance in getting combination therapy for infections. Finally scholars will use the study as a background for their own academic papers.

1.4 Justification of the Study

The new technique will be of immense importance to the medical and scientific world since it will not only assist in helping to treat the infections caused due to MSRA but also form basis of future *in-vitro* laboratory testing for the combination therapy for other infection since the approach can also be used in other antimicrobial agents as well. The result will further assist in alleviating doctors' and other medical practitioners' dilemma in finding the right combination therapy for those infections caused by superbugs and it will further show the importance of providing evidence-based medication which is today a prerequisite in medical world.

The synergistic properties of the counter diffusion will open up the world of laboratory diagnosis more so since lots of antimicrobial agents potential have been reduced dramatically or majority are no longer effective against organisms that affect the lives of the most vulnerable in the society a fact further compounded by the fact that those that suffer most have weakened immune system

either due to lifelong medical condition or from acquired immune depressing conditions for example cancer patients, ICU patients, or those patients that have been exposed to excessive antibiotic therapy.

1.5 Objectives

1.5.1 Broad Objective

The main objective of the study was to investigate drug sensitivity using counter diffusion in methicillin-resistant *Staphylococcus aureus* in clinical isolates from patients admitted in hospitals within Nakuru County.

1.5.2 Specific Objectives

- i. To compare the susceptibility pattern for the MRSA using the Kirby Bauer disc diffusion and counter diffusion technique.
- ii. To determine which antimicrobial agent combination are sensitive to MRSA. iii.
To compare the prevalence of MSSA and MRSA. iv. To establish the prevalence of MRSA according to the sites /specimen.

1.6 Research Questions

- i. What is the susceptibility pattern for the MRSA using the Kirby Bauer disc diffusion compared to counter diffusion technique?
- ii. Which antimicrobial agent combination is sensitive to MRSA?
- iii. What is the prevalence of MSSA and MRSA?
- iv. What is prevalence of MRSA according to the sites /specimen?

1.7 Limitation of the Study

DNA analysis through PCR was not done due to the cost involved in acquiring the machine and reagents.

1.8 Delimitation of the study

DNA analysis was done by the use of MRSA screen test a rapid latex test.

1.9 Scope of the Study

To be able to detect MRSA, this study was done by swabbing the nostrils and isolating the bacteria found inside nasal nares and from clinical isolates from 423 participants from various hospitals within Nakuru Town County. Simple random sampling was used to select patients within each of the randomly selected six hospitals within Nakuru Town i.e. the study used a combination of multistage and simple random sampling.



CHAPTER TWO: LITRATURE REVIEW

2.1 Introduction

Antimicrobial resistance is a public health issue of growing concern (Wassenaar, 2005). The use of antimicrobials can lead to development of antimicrobial resistance in bacterial species. Antimicrobial use in food animal production may become a public health issue when resistant organisms or their resistance genes spread from animals to humans by in-direct contact or through the food chain (Wassenaar, 2005). Surveillance of antimicrobial resistance in human and veterinary pathogenic and indicator bacteria intends to reveal trends in the evolution of resistant organisms (Labro and Bryskier 2014). An important, traditionally human pathogen, methicillin resistant *Staphylococcus aureus* (MRSA) is currently causing a pandemic in hospitals around the world and is also emerging in the community. It is one of the common causative agents of hospital and community acquired infections, particularly skin infections, pneumonia, surgical site infections, chronic bone infections and blood stream infections. The antibiotics such as methicillin, oxacillin and nafcillin, macrolides, tetracycline and aminoglycosides which are being used to treat these infections however are getting resistant (Thati *et al.*, 2011). This chapter highlights the theoretical, empirical and the conceptualization of the study.

2.2 Methicillin-Resistant *Staphylococcus aureus*

Methicillin resistant *Staphylococcus aureus* been reported as the most common cause of hospitalacquired infection for a number of years. Methicillin-resistant *Staphylococcus aureus* is a mutated form of bacteria resistant to antibiotics, known as β -lactams, such as methicillin, oxacillin, penicillin, and amoxicillin, and the cephalosporin, such as cephalexin, and ceflaclor. It was first discovered in 1961 in the United Kingdom, (Moellering, 2011) and the first reported case of MRSA was in 1968 in the United States (Lewis *et al.*, 2014). For all MRSA infections, the annual incidence ranged between 18 and 25.7 cases per 100,000 populations, (Lewis *et al.*, 2014). The

majority of these, approximately 75%, were skin and soft tissue infections. There is only limited information on morbidity and mortality of Community Acquired Methicillin Resistant *Staphylococcus aureus* (CA-MRSA), (Barnes and Sampson 2011). *Staphylococcus aureus* is normally found on the skin or in the nose of 20-30% of healthy individuals. *Staphylococcus aureus* most commonly colonizes in the nostrils. Colonization is the state of *S. aureus* being present without causing any symptoms. When symptoms are present, it is called an infection (Barnes and Sampson 2011).

The primary symptom of staphylococcal infections, including MRSA, is a red area with a dimple appearance on the skin, which later develops into an abscess. Other symptoms of MRSA include drainage of pus or other fluids from the site, warmth around the infected area, and fever. The more serious systemic MRSA symptoms include chills, rash, shortness of breath, chest pain and muscle ache. Patients should seek medical attention as soon as the systemic symptoms appear or if the wounds do not heal. A culture from the infected site is taken to confirm MRSA infection (Stevens *et al.*, 2014).

2.3 Theoretical Review

Since its first description in the early 1960s, methicillin-resistant *Staphylococcus aureus* (MRSA) has become a major public health issue because of worldwide spread of several clones. More than 20 years later, the specific genetic mechanism of its resistance has been identified as a mobile genetic element (*staphylococcal cassette chromosome mec*) integrated into the *S. aureus* chromosome, within which the *mecA* gene encodes a specific methicillin-resistant trans peptidase (penicillin-binding protein 2a) [PBP2a]. This protein has a low affinity for β -lactam antimicrobial drugs. Thus, bacteria expressing this protein are resistant to all types of these drugs. (Ito *et al.*,

2012) Resistance to methicillin, in MRSA is related to a chromosomal *mecA* gene that specifies the production of an abnormal penicillin-binding protein called PBP2a or PBP21. Penicillin-binding proteins are membrane-bound enzymes, which targets for all β -lactam antibiotics (Ito *et al.*, 2012). PBP2a has a decreased affinity for binding β -lactam antibiotics resulting in resistance not only to methicillin but also to all β -lactams including penicillin and cephalosporin (Larrul *et al.*, 2010). The *mecA* gene complex also contains insertion sites for plasmids and transposons that facilitate acquisition of resistance to other antibiotics. Thus, crossresistance to non- β -lactam antibiotics such as erythromycin, clindamycin, gentamicin, cotrimoxazole and ciprofloxacin is common (Shenoy *et al.*, 2010).

Continuous efforts to understand the changing epidemiology of *S. aureus* infection in humans and animals are therefore necessary, not only for appropriate antimicrobial treatment and effective infection control but also to monitor the evolution of the species. MRSA remains one of the principal multiply resistant bacterial pathogens causing serious healthcare-associated and community-onset infections (Li *et al.*, 2012). The molecular processes underlying epidemic waves of MRSA infection are poorly understood (Chambers and DeLeo 2009), although a major role has been attributed to the acquisition of virulence determinants by horizontal gene transfer (Holden *et al.* 2013).

Staphylococcus aureus is a versatile human pathogen that causes diseases ranging from relatively mild infections of the skin and soft tissue to life-threatening sepsis (Laurent *et al.*, 2012). The emergence of strains resistant to methicillin and other antimicrobial agents has become a major concern, especially in the hospital environment, because of the higher mortality due to systemic MRSA infections have shown significant increases in methicillin resistance in clinical strains of *S.*

aureus isolates between 1999 and 2002 in European countries, particularly Belgium, Germany, Ireland, the Netherlands and the United Kingdom (Dulon *et al.*, 2011). MRSA prevalence varied widely, from <1% in northern Europe to >40% in southern and western Europe (Laurent *et al.*, 2012). As the prevalence of healthcare-associated infections (HAIs) caused by multidrug-resistant organisms continues to increase, it seems essential to prevent MRSA transmission and reduce the number of MRSA HAIs. It is also important for healthcare workers that MRSA rates should be controlled, as a recently published review has shown that the average MRSA carrier rate in healthcare workers is 4.6%, and that about 5.1% of these carriers had symptomatic MRSA infections (Dulon *et al.*, 2011). Although most MRSA infections in healthcare workers had a mild clinical course, some infections tend to become chronic and can cause severe health problems. For healthcare facilities, surveillance is an important and generally accepted method to assess the incidence of infection due to multidrug-resistant bacteria and if necessary to improve infection control measures (Laurent *et al.*, 2012). Surveillance of MRSA is a means of identifying colonized or infected patients for whom specific control measures may be implemented. Surveillance may be passive, whereby laboratory results from clinical samples are monitored or active whereby patients are actively screened for the carrier state in order to identify the entire reservoir. The implementation of a program of active surveillance cultures beside contact precautions is recommended by different national guidelines as a way of preventing nosocomial transmission of MRSA.

Two theories exist on the molecular evolution of MRSA. The single clone theory states that all MRSAs have one common ancestor; the SCCmec element was introduced into *Staphylococcus aureus* only once (Rice 2012). The multi clone theory states that SCCmec was introduced multiple

times in different *Staphylococcus aureus* lineages, after which horizontal spread and recombination were important mechanisms of resistance transmission. Using the different techniques stated above, the theories have been tested and the following can be concluded: prevalent MSSA strains that were successful in causing disease on a global scale have evolved into MRSA on multiple, but independent occasions, horizontal transfer of the SCCmec has occurred a limited number of times compared with other bacteria and clonal spread after acquiring the SCCmec appears to be the most important mechanism of dissemination of resistance (Rice 2012).

Since the first human MRSA was isolated in 1961, at least five major clonal types (CC8, CC5, CC45, CC22 and CC30) of MRSA have been identified using PFGE according to (Nejma *et al.*, 2013). The five types predominantly harbor SCCmec type I, II or III and are often multidrugresistant. These clones are responsible for the vast majority of MRSA infections in hospitals all over the world. Besides the epidemic clones, there are also clones that occur only in single hospitals or even only in single patients (sporadic isolates) and isolates that cause infections in the community that is Community Aquired (CA) *Staplylococcus aureus* isolates (Monecke *et al.*, 2011).

The characterization of these clones revealed extensive diversity among isolates. Several studies observed strong similarities between sporadic isolates and CA-MRSA, which implies that MRSA strains described as CA may actually originate from hospitals (Monecke *et al.*, 2011). CA-MRSA isolates frequently carry SCCmec-IV or V, are susceptible to a limited number of antimicrobials and may contain additional virulence factors. SCCmec IV and V are much smaller than SCCmec I, II and III, which may lead to a more efficient transfer of the element between bacteria and less fitness cost in everyday metabolism (Monecke *et al.*, 2011). With this greater ability for

transmission and virulence, MRSA clones in the community might be an even larger threat to patients and health-care workers than hospital-acquired clones. Until now, resistance to more than just β -lactam antibiotics is relatively infrequent in these community clones, but future mutations or gene transfer may change this (Lindsay, 2014).

2.4 Empirical Review

2.4.1 The Causes of Methicillin-Resistant *Staphylococcus aureus* in Patients

Resistance of bacteria to a particular antimicrobial agent can be mediated by a pre-existing phenotype in natural bacterial populations or by acquired resistance. Two genetic mechanisms are involved in acquiring and disseminating resistance: *de novo* mutations or horizontal transmission of resistance genes between individual bacteria or between bacterial species. Resistance acquired through either mechanism is subsequently transmitted, and the frequency of resistance in populations may increase as a result of selective advantage under the pressure of antimicrobial use (Tong *et al.*, 2015).

Antimicrobial agents are widely used in humans, animal husbandry and other agricultural activities. Since any use of antimicrobial agents can result in the selection for resistance, antimicrobial usage in animals has contributed to the development of resistance in bacterial species (Tong *et al.*, 2015). To prevent outbreaks of infectious diseases, hygiene measures are being improved, management is optimized and vaccines are applied. Despite all these interventions, the use of antimicrobials in animal's husbandry is often inevitable. Antimicrobials are not only used to treat diseases, but antimicrobials are also applied strategically to prevent infections and in several parts of the world, as growth promoters (Laurent *et al.*, 2012).

Staphylococcus aureus is a major human pathogen that causes a wide range of clinical infections. It is a leading cause of bacteremia and infective endocarditis as well as osteoarticular, skin and soft

tissue, pleuropulmonary and device-related infections. The past two decades have witnessed two clear shifts in the epidemiology of *S. aureus* infections: first, a growing number of health care-associated infections, particularly seen in infective endocarditis and prosthetic device infections, and second, and an epidemic of community-associated skin and soft tissue infections driven by strains with certain virulence factors and resistance to β -lactam antibiotics (Tong *et al.*, 2015).

MRSA rates continue to increase rapidly in many regions and there is a dynamic spread of strains across the globe. Hospital acquired MRSA (HA-MRSA) is currently endemic in hospitals in certain regions (Gould *et al.*, 2012). CA-MRSA clones have been spreading rapidly in the community and also infiltrating healthcare in many regions worldwide. To date, livestock acquired MRSA (LA-MRSA) is only prevalent in certain high-risk groups of workers in direct contact with live animals. CA-MRSA and LA-MRSA have become a challenge for countries that have so far maintained low rates of MRSA. These evolutionary changes have resulted in MRSA continuing to be a major threat to public health, (Gould *et al.*, 2012).

2.4.2 The Challenges of Diagnosing *Staphylococcus aureus* in Patients

Staphylococcus aureus is a Gram-positive, coagulase-positive coccus in the family *Staphylococcaceae*. *Staphylococcal* species occur worldwide as commensal colonizers of the skin of humans and animals. They are additionally found on mucous membranes of the upper respiratory tract and lower urogenital tract and transiently in the digestive tract. It is important to note that a distinction must be drawn between colonization and infection by *S. aureus* and its methicillin resistant variant. Colonization with *S. aureus* may occur on mucous membranes of the respiratory and/or intestinal tract, or on other body surfaces, without causing disease or harming their hosts (Laurent *et al.*, 2012). Some individuals are colonized transiently and some persistently. Colonization with *S. aureus* usually precedes infection, and is mostly caused by the same subtype.

The prevalence of nasal colonization with *S. aureus* among the human population is relatively high >24%, while the prevalence of nasal colonization with MRSA among the same group is low <1.5% (Laurent *et al.*, 2012). When the opportunity arises, *S. aureus* can contaminate wounds, bloodstream or other tissues, causing serious and even life threatening infections (Laurent *et al.*, 2012).

2.4.3 The Challenges of Treating Patients with Methicillin-Resistant *Staphylococcus aureus* Infection

MRSA can be treated by proper wound and skin care if it is detected in its early stage. If the infection has reached its later stage, however, it may lead to complications and may require antibiotics for proper treatment (Liu *et al.*, 2011). Untreated MRSA can lead to complications including cellulites, blood poisoning, toxic shock syndrome, endocarditis, pneumonia, and even death. Antibiotics like vancomycin, trimethoprim-sulfamethoxazole, and linezolid can be used. Supplemental oxygen and intravenous medication may be required to treat severe infections. It is important to finish all doses of antibiotics. Further drug resistance can be developed in the bacteria because of unfinished doses of antibiotics (Liu *et al.*, 2011).

2.5 Summary and Research Gaps

Over the past decades, the epidemiology of MRSA has changed significantly. MRSA, traditionally a primarily nosocomial pathogen, has entered the community, causing serious infections. Therefore this study will seek to fill the literature gap by investigating drug sensitivity using counter diffusion technique in MRSA

2.6 Conceptualization

The study can be conceptualized in a conceptual framework presented in a schematic interpretation explaining the relationship. The Figure 1 shows the relationship between the dependent and independent variables:

2.6.1 Causes of methicillin-resistant *Staphylococcus aureus* - Methicillin-resistant *Staphylococcus aureus* (MRSA) is a bacterium that causes infections in different parts of the body for example boils, septic wounds amongst others. It is difficult to treat than most strains of *Staphylococcus aureus* because it is resistant to some commonly used antibiotics. Garden-variety *Staphylococcus aureus* is common bacteria that can live in human bodies. Plenty of healthy people carry *Staphylococcus aureus* as commensal or symbiotic. In fact, 25-30% of human population has *Staphylococcus aureus* bacteria colonizing nasal nares (Shenoy *et al.*, 2010).

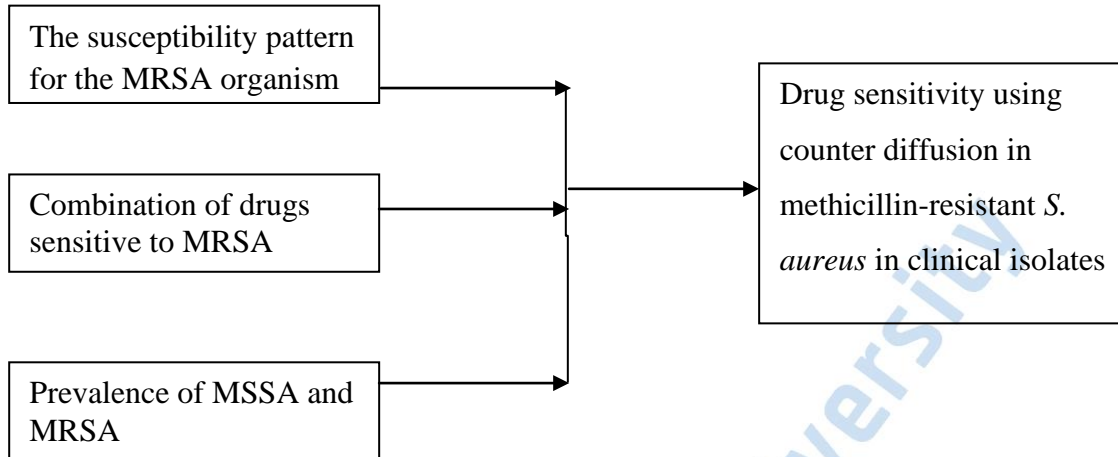
But *Staphylococcus aureus* can be a problem if it manages to get into the body, through a broken skin. Once inside the body, it can cause an infection. Less often, *Staphylococcus aureus* can cause serious problems like infected wounds or pneumonia.

2.6.2 Challenges of diagnosing *Staphylococcus aureus*- A high prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) in soft tissue infections presents a treatment challenge. The transmission of the MRSA seems to be in the community.

Challenges of treating patients - A recent increase in staphylococcal infections caused by MRSA, combined with frequent, prolonged ventilator support of an aging, often chronically ill population, has resulted in a large increase in cases of MRSA pneumonia in the health care setting. In addition, community-acquired MRSA pneumonia has become more prevalent.

(Shenoy *et al.*, 2010).

Figure 1: Conceptual Framework.



Independent variable

Dependent variable

Adapted and modified from (MacFarlane and O'Reilly 2012)

CHAPTER THREE: RESEARCH METHODOLOGY

3.0 Introduction

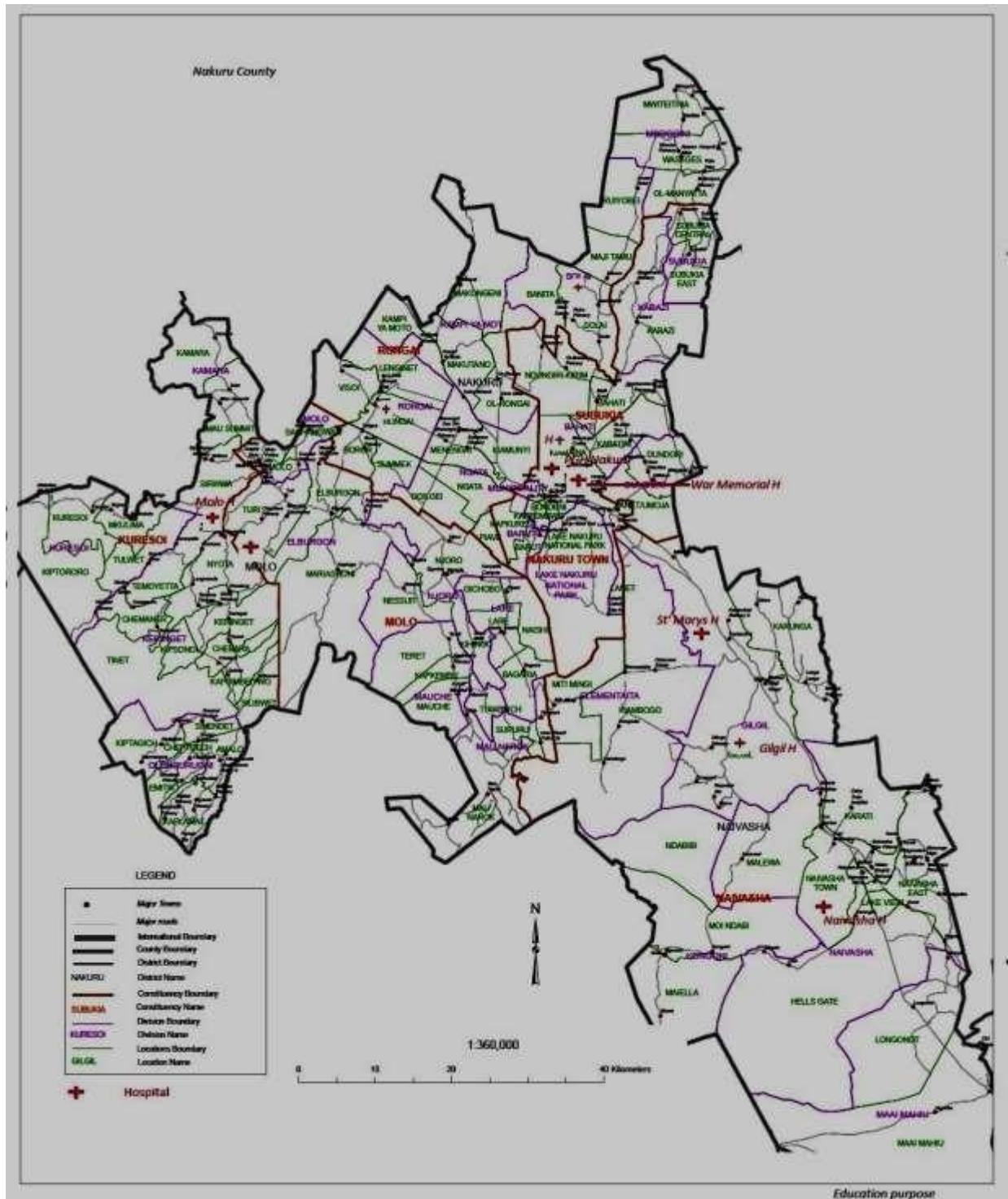
This chapter involves the methods that were used to collect the data for the study. These include research design, target population, sampling design, data collection instruments, data collection procedure and data analysis procedure.

3.1 Study Area

The study was carried out within Nakuru County (Figure 2). The County is currently the fourth largest urban centre in the country, lies about 1850 m above sea level. It is located in the Great Rift Valley and about 150 Km West of Nairobi. It has a population of 473,288 people (KNBS, 2010). It is a cosmopolitan county with people from different cultures. Nakuru is home to Lake Nakuru, one of the Rift Valley soda lakes, which forms part of the Lake Nakuru National Park. The park has large numbers of flamingoes that can be seen foraging in the shallow lake. The park also has many wild animals that can be seen during a safari. Its main economic activities are mainly manufacturing Agriculture and Tourism. Nakuru has many government, NGOs and private health facilities (KNBS, 2010)



Figure 2. Map of Nakuru county showing area of study, adapted and modified from <http://www.google.com/search?q=nakuru+county+map>



3.2 Study Site

The study was carried out in the following hospitals: Nakuru Provincial hospital, Nakuru War Memorial hospital, Bahati District hospital, Gilgil District hospital, St. Mary’s hospital and Molo

District hospital

3.3 Study Population

Hulley *et al.*, (2013) defines study population as all the members of a real or hypothetical set of people, events or objects to which the researcher wishes to generalize the results of the study. The target population were respondents from the admitted cases within the hospital consented to participate in the research study.

3.4 Study Design

The study adopted a survey research design Mugenda and Mugenda (1999). Questionnaires were used to gather information on variables of interest, Mwanukuzi, (2011).

3.5 Sample Size Determination

The minimum sample size for this study was calculated by the following formulae (Elashof and Lemeshow 2014).

$$n = \frac{Z_{\alpha/2}^2 P(1 - P)}{d^2}$$

$$= (0.5) (0.5) (1.96)^2 / (0.05)^2$$

Where n is the desired sample size, P is the proportion of patients with resistant isolates. Since this proportion is not known, an estimate of 0.5 was assumed. z^2 is the standard normal deviate and d the required level of precision taken to be 5%. This gives a minimum of 384 patients. This figure is increased by 10% to take care of the contingencies like non-responses and damaged samples, (Hawkins Jolliffe and Glickman, 2014) to get 423 patients i.e. at least 70 patients per hospital.

3.6 Sampling Design

Simple random sampling was used to select patients to be surveyed from each of the randomly selected six hospitals from a total of 396 health facilities within Nakuru County.

3.7 The Inclusion and Exclusion criteria

All consenting patients/next of kin/guardian in the out/in patient at the hospital (study sites) were included.

All non-consenting patients were not included in the study.

3.8 Instruments of Data Collection

The study used questionnaires to gather primary data. All the data collected through the questionnaire were checked to identify any anomalies/inconsistencies and instituted the necessary corrective measures. Unstructured questions allow greater depths of response and they stimulate the respondent to think about their feelings and motives while considering the best assessment of the situation.

3.9 Validity and Reliability of the Study

Questionnaires were validated prior to data collection exercise. This refers to the extent to which a research performs what it was designed for, Mugenda and Mugenda (1999) point out that validity measures the accuracy of the instruments in obtaining the anticipated data which can meet the objectives of the study. In this study, the items were considered reliable if they yielded a reliability coefficient of 0.50 and above. This figure is usually considered acceptable and desirable for consistency levels (Mugenda and Mugenda, 1999).

3.10 Sample Collection

Procedure for sample collection was done according to (Kathy, 2009). Both nostrils of the subject were sampled with a paired sterile culture swab, using the following procedure to collect the swab; Sample was also collected from wounds using aseptic technique. For patients <12 years of age,

remove and discard one swab. For all others, use the double-swab and in all cases, collect the specimen from both nostrils using one or two swabs, depending on the age of the patient (Kathy, 2009).

3.11 Laboratory Investigations

3.11.1 Culture

Swabs were kept viable in Amies transport media (Tadesse and Alem, 2006). Culturing was done by streak plate method on primary culture plates of blood agar, chocolate agar and MacConkey agar (CONDA SPAIN and BD USA). All the plates were cultured aerobically at 37⁰ for 24 hours. Suspected colonies of *S. aureus* from primary culture plates were confirmed, by Gram reaction, positive catalase, Tube coagulase and Deoxyribonucleases (DNAse) test. Sub-culturing was done on Mueller Hinton media at 37⁰ C for up to 48 hours. Methicillin disc diffusion (5µg), Oxacillin disc diffusion (5µg), were used and a growth indicated that the strain is methicillin resistant (Shrestha *et al.*, 2009). All media were prepared in the laboratory and quality control strains (*S. epidermidis* ATCC 12228, *E. coli* ATCC 25922) were used.

3.12 MRSA Screen Assay

The MRSA Screening test was performed according to the manufacturer's instructions for the kit purchased-DENKA CO JAPAN. The MRSA- screen assay is a 5 minutes slide latex agglutination test based on detection of PBP2a (Odonkor *et al.*, 2012). The method involves extraction of PBP2a from suspensions of colonies and detection by agglutination with latex particles coated with monoclonal antibodies to PBP2a. Colonies that are truly MRSA produced agglutination.

3.13 Sensitivity Testing

Drug susceptibility were conducted using counter diffusion technique and ran parallel to Kirby

Bauer disc diffusion method in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines (Bartoloni *et al.*, 2013). The sensitivity disc used were penicillin 1IU, Minocycline 30mcg, Erythromycin 15 mcg, Methicillin 5mcg, vancomycin 30mcg, Co-trimox 5mcg, Chloramphenicol 30mcg, Ampicillin 10 mcg, Lincomycin 2mcg, Gentamycin 10mcg, Bacitracin 10mcg HIMedia/Meropenem 10mcg and Azithromycin 30mcg from BIOANALYSE. Counter disc diffusion employs a technique where the edges of the drug of choice are made to touch along their margins and it is in this context that the active ingredients diffuses from each of the drugs to create a zone of high potency that deters growth of the bacteria. The concept was arrived at based on the fact that one disc has a diameter of 7mm and therefore two disc placed side by side will eventually have a diameter of 14mm from any side. The same applies to three or four disc with any of their sides being in contact with each other at any given time. It is on this principle that the lowest millimeter(mm) that could be achieved if the organism is resistant through counter diffusion being 15mm hence the researcher deduced that it's from this that final interpretation of the finding was made by measuring the zones of inhibition.

3.14 DATA ANALYSIS METHOD AND PRESENTATION

3.14.1 Data Analysis Method

Data were analyzed using R version 3.2.0 (R Core Development Team, 2015). Proportions were compared using Chi square or Fisher's exact test. Cohen's Kappa (Cohen, 1960) was used to assess the inter-drug reliability in detecting the MRSA and MSSA sensitivity of the 11 drugs on the isolates. Kappa measures the difference between the observed and expected agreements between specimen and methods in detecting pathogens (in our case). It is standardized to lie on a scale of -1 to 1, where 1 is perfect agreement, 0 is exactly what would be expected by chance, and negative values indicate agreement less than chance, i.e., potential systematic disagreement between the

methods (Viera *et al.*, 2005). A simple linear regression analysis was used to study the relationship between the zones inhibited and the technique used. In the model zone was the outcome variable and technique (with three levels: counter diffusion, lincomycin and gentamycin) was the covariate, with the latter two being compared to the counter diffusion technique. All tests were performed at 5% significance level.

3.15 Ethical Considerations

The data collection commenced once the Mount Kenya University Ethical and Review committee gave approval APPENDIX I and clearance obtained from National Commission for Science Technology and Innovation (NACOSTI) Appendix II, III, and IV permit number NACOSTI/P/15/5036/4459. Clearance was also obtained from Ministry of Medical Services Nakuru County Appendix V, VI, and VII. Hard copies of data kept under lock and key to avoid unauthorized access while all electronic copies are password protected. Data was not in any way be presented or published such that patient's identification is revealed. There was no coercion or enticement to participate in the study.

CHAPTER FOUR: RESULTS

4.0 Introduction

This chapter includes the results obtained during the study, Comparison of the Susceptibility Pattern for the MRSA using the Kirby Bauer Disc Diffusion and Counter Diffusion Technique, determining which antimicrobial agent combination are sensitive to MRSA, comparing the prevalence of MSSA and MRSA, Establishing the prevalence of MRSA according to the specimen and statistical analysis.

4.1 Comparing the Susceptibility Pattern for the MRSA using the Kirby Bauer Disc Diffusion and Counter Diffusion Technique

The Cohen's Kappa for the MRSA sensitivity analysis using KB method for the 11 drugs on 43 isolates is presented in Table 1. Except for Gentamycin, Vancomycin and Bacitracin 10 units which classified the isolates as, Intermediate sensitive and resistant, respectively, there was (almost) perfect agreement among the drugs which classified the isolates as resistant. Table 2 presents the Cohen's Kappa for the MSSA sensitivity analysis using KB method for the 11 drugs on 43 isolates. The table shows a perfect agreement among (1) Penicillin GIV, Minocycline 30 mcg and Erythromycin 15 mcg (Insensitive); (2) Co-trimox 5 mcg, chloramphenicol 30 mcg, and Ampicillin 10 mcg (resistant); and (3) Methicillin, Lincomycin 2 mcg, Gentamycin 10 mcg, and Vancomycin 30 mcg and Bacitracin 10 units (sensitive).

A comparison of the pattern of sensitivity of methicillin resistant *Staphylococcus aureus* using Kirby Bauer's method and counter diffusion technique indicated that the mean zone of inhibition differed significantly using lincomycin, gentamycin and counter-diffusion techniques ($F=5171.6$, $df=2, 126$, $P<0.001$). The mean zone of inhibition using the counter diffusion technique was 27.1mm and was significantly higher than the zones of inhibition by either lincomycin (zone=8mm, $t=-87.7$, $p\text{-value}<0.0001$), or gentamycin (zone=7.8mm, $t=-88.45$, $p\text{-value}<0.0001$). The lincomycin and gentamycin gave similar results ($t=0.75$, $P=0.457$). Indeed, all the subjects were highly sensitive using the counter diffusion technique, but were all resistant using the normal method(s). The positive control gave the same results as counter diffusion technique, while the negative control gave results similar to those obtained using lincomycin and gentamycin.

Table 1. Cohen's Kappa for the MRSA sensitivity analysis using KB method for 43 isolates.

	Penicillin G IV	Minocycline 30 mcg	Erythromycin 15 mcg	Methicillin	Cotrimox 5 mcg	chloramphenico l 30 mcg	Ampicillin 10 mcg	Lincomycin 2 mcg	Gentamycin 10 mcg	Vancomycin 30mcg	Bacitracin 10 units
Penicillin GIV	1.00										
Minocycline 30 mcg	0.90	1.00									
Erythromycin 15 mcg	0.90	1.00	1.00								
Methicillin	0.93	0.97	0.97	1.00							
Co-trimox 5 mcg	0.90	1.00	1.00	0.97	1.00						
chloramphenicol 30 mcg	0.90	1.00	1.00	0.97	1.00	1.00					
Ampicillin 10 mcg	0.79	0.90	0.90	0.86	0.90	0.90	1.00				
Lincomycin 2 mcg	0.90	1.00	1.00	0.97	1.00	1.00	0.90	1.00			
Gentamycin 10 mcg	-0.47	-0.50	-0.50	-0.50	-0.50	-0.50	-0.43	-0.50	1.00		
Vancomycin	-0.50	-0.50	-0.50	-0.50	-0.50	-0.50	-0.47	-0.50	-0.33	1.00	
Bacitracin 10 units	0.20	0.09	0.09	0.13	0.09	0.09	0.06	0.09	0.30	-0.50	1.00

Table 2. Cohen's Kappa for the MSSA sensitivity analysis using KB method for 43 isolates

	Penicillin in GIV	Minocycline 30 mcg	Erythromycin 15 mcg	Methicillin	Co-trimox 5 mcg	chloramphenicol 30 mcg	Ampicillin 10 mcg	Lincomycin 2 mcg	Gentamycin 10 mcg	Vancomycin 30mcg	Bacitracin 10 units
penicillin GIV	1.00										

Minocycline 30 mcg	1.00	1.00										
Erythromycin 15 mcg	1.00	1.00	1.00									
Methicillin	-0.50	-0.50	-0.50	1.00								
Co-trimox 5 mcg	-0.50	-0.50	-0.50	-0.50	1.00							
chloramphenicol 30 mcg	-0.50	-0.50	-0.50	-0.50	1.00	1.00						
Ampicillin 10 mcg	-0.50	-0.50	-0.50	-0.50	1.00	1.00	1.00					
Lincomycin 2 mcg	-0.50	-0.50	-0.50	1.00	-0.50	-0.50	-0.50	1.00				
Gentamycin 10 mcg	-0.50	-0.50	-0.50	1.00	-0.50	-0.50	-0.50	1.00	1.00			
Vancomycin	-0.50	-0.50	-0.50	1.00	-0.50	-0.50	-0.50	1.00	1.00	1.00		
Bacitracin 10 units	-0.50	-0.50	-0.50	1.00	-0.50	-0.50	-0.50	1.00	1.00	1.00	1.00	

4.2 Determining which antimicrobial agent combination are sensitive to MRSA.

Table 3: Combination of drugs acting against MRSA

Drugs	Sensitive >23mm	Intermediate 18-22mm	Resistant 14-17mm
Gentamycin/Lincomycin,	29mm		
Gentamycin/Ciprofloxacin/Lincomycin/Vancomycin,	29mm		
Gentamycin/Ciprofloxacin/Lincomycin,	26mm.		
Gentamycin/Ciprofloxacin/Vancomycin,	28mm.		
Gentamycin/Lincomycin/Vancomycin,	28mm.		
Bacitracin/meropenem,	32mm		
Azithromycin/Meropenem,	25mm.		
Gentamycin/Vancomycin,		18mm	
Ciprofloxacin/ofloxacin/vancomycin,		18mm.	
Bacitracin/ciprofloxacin/cotrimoxazole,		18mm	
Ofloxacin/vancomycin/cotrimoxazole/azithromycin,		19mm.	
Ciprofloxacin/bacitracin,		18mm.	
Ciprofloxacin/Lincomycin/Vancomycin		18mm	
Gentamycin/Ciprofloxacin,			17mm
Ciprofloxacin/Lincomycin,			15mm.
Lincomycin/Vancomycin,			15mm.
Ofloxacin /cotrimoxazole,			15mm.
Ciprofloxacin/Vancomycin			16mm

4.3 Comparing the prevalence of MSSA and MRSA

Of 423 patients recruited, 344 (181 males and 163 females) had cases of *Staphylococcus species* 12.5% (95%CI 17.8-16.5%) of the 344 were Methicillin resistant whereas 87.5% were Methicillin sensitive. The proportion resistant was 14% and 11% among males and females, respectively. These two proportions were not significantly different from each other (Chi square= 0.374, df = 1, p = 0.540), (Table 4)

There were also other organism isolated during the study *Proteus species* 30 cases, *Esch. coli* 45 cases, *Klebsiella species* 4 cases.

Table 4: Cases of *Staphylococcus species* organism isolated

	MALES	FEMALES	TOTAL
METHICILIN RESISTANT	25(13.8%)	18(11.04%)	43(12.5%)
METHICILIN SENSITIVE	156(86.2%)	145(88.95%)	301(87.5%)
TOTAL	181	163	344

4.4 Establishing the prevalence of MRSA according to the specimen

The results further indicated that the prevalence of MRSA varied significantly by site (Fisher's exact test P<0.001), with wounds recording the highest at (17.9%) followed by intensive care patients samples (ICU PTS) (16.7%), Nasal (9.1%), and stool and urine at zero percent as shown in (figure 3) below.

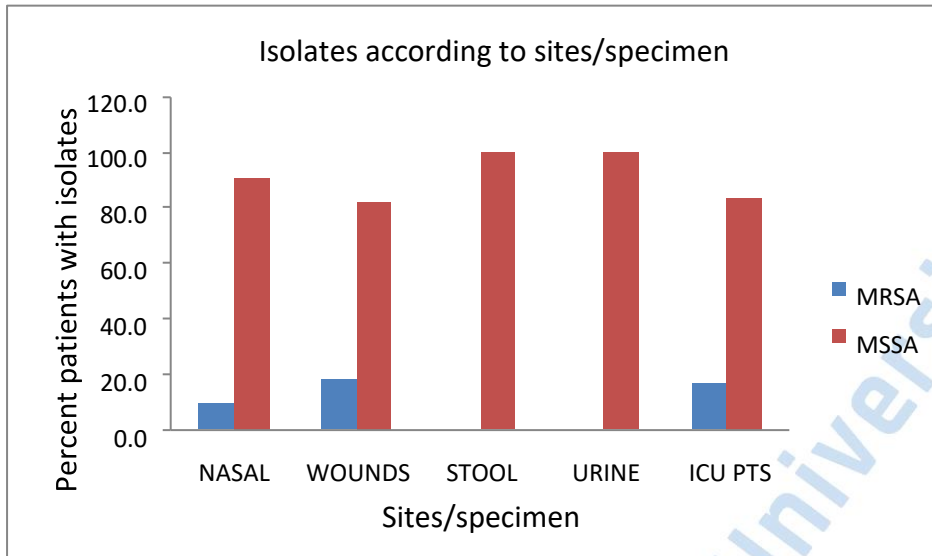


Figure 3: Prevalence of MRSA and MSSA by site of isolation

CHAPTER FIVE: DISCUSSION

5.0 Introduction

This chapter deals with discussion of the findings and their analysis under the sub-heading of; Comparison of the Susceptibility Pattern for the MRSA using the Kirby Bauer Disc Diffusion and Counter Diffusion Technique, determining which antimicrobial agent combination are sensitive to

MRSA, comparing the prevalence of MSSA and MRSA, Establishing the prevalence of MRSA according to the sites /specimen.

5.1 Comparison of The Susceptibility Pattern for the MRSA using the Kirby Bauer Disc Diffusion and Counter Diffusion Technique

A comparison of the susceptibility pattern for the MRSA organism using the normal method and the counter diffusion technique(s) indicated that the mean zone of inhibition using the counter diffusion technique was 27.1mm. This was significantly higher than the zones of inhibition by either lincomycin (zone = 8mm, $t = -87.7$, $p = <0.0001$), or gentamycin (zone = 7.8mm, $t = -88.45$, $p\text{-value} < 0.0001$). The lincomycin and gentamycin gave similar results. Indeed, all the subjects were highly sensitive using the counter diffusion technique, but were all resistant using the normal method(s). The positive control gave the same results as counter diffusion technique, while the negative control gave results similar to those obtained using lincomycin and gentamycin.

5.2 To determine which antimicrobial agent combination are sensitive to MRSA

The current study agrees with findings of Crombé *et al.*, (2012) that MRSA strains were resistant to tetracycline and additional resistances to trimethoprim (97%), lincosamides (73%), macrolides (56%), aminoglycosides (48%), and fluoroquinolones (32%). The study is also in agreement with Maina *et al.*, (2013) who found that 82 isolates of *S. aureus* were susceptible to vancomycin and resistant in high numbers to macrolides, aminoglycosides, and quinolones.

The counter diffusion technique show immense potential to the medical and scientific world since it will not only assist in helping to treat the infections caused due to MSRA but also form basis of future in-vitro laboratory testing for the combination therapy for other infection since the approach can be used in other antimicrobial agent as well. The synergistic properties of the counter diffusion will open up the world of laboratory diagnosis more so since lots of antimicrobial agents potential have been reduced dramatically or majority are no longer effective against organisms that affect the lives of the most vulnerable in the society a fact further compounded by the fact that those that suffer most have weakened immune system either due to lifelong medical condition or from acquired immune depressing conditions for example cancer patients, ICU patients, or those patients that have been exposed to extreme antibiotics therapy.

It is a form of testing for the synergy of two or more drugs in vitro and the two drugs used gentamycin and lincomycin from aminoglycosides and another from lincosamide group respectively. Gentamycin is an aminoglycoside and it target specifically the initiation /translation complex thus inhibiting protein synthesis while lincomycin targets the enzyme peptidyl transferase involved in peptide bond formation through the addition of amino acid. It stops the process such that it cannot be trans-located into the growing chain. The method showed the effect of synergy of two kinds of drugs acting on a single sensitive part of bacterial structure the ribosome involved in protein synthesis.

The method also was effective when more than two drugs are used meaning that different drugs which ordinarily would be ineffective against the MRSA is actually capable of inhibiting the growth and show a good potential as a combination therapy for the organism. The concept was arrived at based on the fact that one disc has a diameter of 7mm and therefore two disc placed side

by side will eventually have a diameter of 14mm from any side. The same applies to three or four disc with any of their sides being in contact with each other at any given time.

5.3 Prevalence of MSSA and MRSA

The emergence of high levels of penicillin resistance followed by the development and spread of strains resistant to the semi synthetic penicillins (methicillin, oxacillin, and nafcillin), macrolides, tetracycline, and aminoglycosides has made the therapy of staphylococcal disease a global challenge. Thati *et al.*, (2011), suggests that infection caused by bacterial pathogens is a global problem. In many cases, bacterial resistance to antimicrobial agents may considerably complicate treatment. This is especially true in the case of methicillin-resistant *Staphylococcus aureus* (MRSA) which is one of the most prominent pathogens associated with hospital, community, and livestock associated infections (Maina *et al.*, 2013). From the current study of 423 patients recruited, 344 (181 males and 163 females) had cases of *Staphylococcus aureus* 12.5% (95%CI 17.8-16.5%) of the 344 were Methicillin resistant whereas 87.5 were Methicillin sensitive. The proportion resistant was 14% and 11% among males and females, respectively.

These two proportions were not significantly different from each other (Chi square= 0.374, df = 1, p = 0.540).

The study agrees with Maina *et al.*, (2013) that MRSA are significant pathogens in patients with skin and soft tissue infections presenting to hospitals in Kenya, and MRSA cases are prevalent at publicly funded health care facilities, however the study contradicts findings of Omuse *et al.*, (2012) that prevalence of MRSA carriage was 0% [95% confidence interval (CI): 0–1.5%] whereas that of methicillin-susceptible *Staphylococcus aureus* was 18.3% (95% CI: 14.0–23.6%) from study carried out at Aga Khan University Hospital Nairobi on health care workers at the

facility.

5.4 Establishing the prevalence of MRSA According to the specimen

From the study it was found that the prevalence of MRSA varied significantly by site (Fisher's exact test $P < 0.001$), with wounds recording the highest at (17.9%) followed by ICU PTS (16.7%), Nasal (9.1%), and stool and urine at zero percent. This finding agrees with study conducted by Jarvis *et al.*, 2012 in USA that found that skin and soft tissue infections (SSTs) accounted for 42.9% prevalence of MSRA similarly it agrees with study carried out in India by Indian Network for Surveillance of Antimicrobial Resistance INSAR (2013) that found that the majority of *S. aureus* isolates was obtained from patients with skin and soft tissue infections followed by those suffering from blood stream infections and respiratory infections. Susceptibility to ciprofloxacin was low in both MSSA (53%) and MRSA (21%) their study found that majority of *S. aureus* isolates were obtained from patients with skin and soft tissue infections followed by those suffering from blood stream infections and respiratory infections.

Susceptibility to ciprofloxacin was low in both MSSA (53%) and MRSA (21%). MSSA isolates showed a higher susceptibility to gentamicin, co-trimoxazole, erythromycin and clindamycin as compared to MRSA isolates. Their study also found that no isolate was found resistant to vancomycin or linezolid. Maina *et al.*, (2013) found similar outcome with wound specimens accounting for higher prevalence of MRSA, their results indicated that of 60 boil cultures, 39 (65%) grew *S. aureus*, of out of which 34 (87.2%) were MRSA. Of the 60 abscess cultures, 14 (23.3%) grew *S. aureus*, of which 10 (71.4%) were MRSA. Of 34 cellulitis cultures, 18 (52.9%) grew *S. aureus*, of which 16 (88.8%) were MRSA. Of 25 ulcer cultures, 11 (44%) grew *S. aureus*,

of which nine (81.8%) were MRSA. Omuse, *et al.*, (2014) also found that majority (79.2%) of the isolates were from pus swabs.

5.5 Conclusion

MRSA is a real medical threat within Nakuru County since the current research found that almost in every major public hospital there were cases of MRSA. The research outcome of this study are similar to that carried out in India by Indian Network for Surveillance of Antimicrobial Resistance (INSAR, 2013) that found that the majority of *S. aureus* isolates was obtained from patients with skin and soft tissue infections followed by those suffering from blood stream infections and respiratory infections therefore making MRSA a global medical threat.

The study found that counter diffusion technique give greater zone of inhibition when compared to Kirby Bauer disc diffusion technique during drug sensitivity testing. It was also found that it has the ability to show two or more drugs are able to act on MRSA when combined as opposed to single drug when used in-vitro. The study concludes that the prevalence of MSSA and MSRA is 87.5% and 12.5% respectively within Nakuru County. Furthermore it was found that wound specimen had higher MRSA isolates (17.9%) followed by central venous catheter (16.7%), nasal swabs (9.1%) being the least with no organism being isolated in stool and urine.

5.6 Recommendation

It is the opinion of the researcher that the procedure be validated and accepted as marker for testing for the drug combination therapy as it has potential to pinpoint to clinicians the way forward in patient management when it comes to superbugs. MRSA and MSSA is a threat in hospital setups within Nakuru County and surveillance as a means of identifying colonized or infected patients be implemented since most of isolated cases were from patients who are admitted.

Proper wound management should be emphasized since it was found that MRSA is higher in wound specimen. This study is in agreement with work done by Maina *et al.*, (2013) who found similar outcome with wound specimens accounting for higher prevalence of MRSA, their results indicated that of 60 boil cultures, 39 (65%) grew *S. aureus*, of out of which 34 (87.2%) were MRSA hence bringing in the need to have proper wound management in patient management so as to curb incidences of growing super bugs within the hospital environment.

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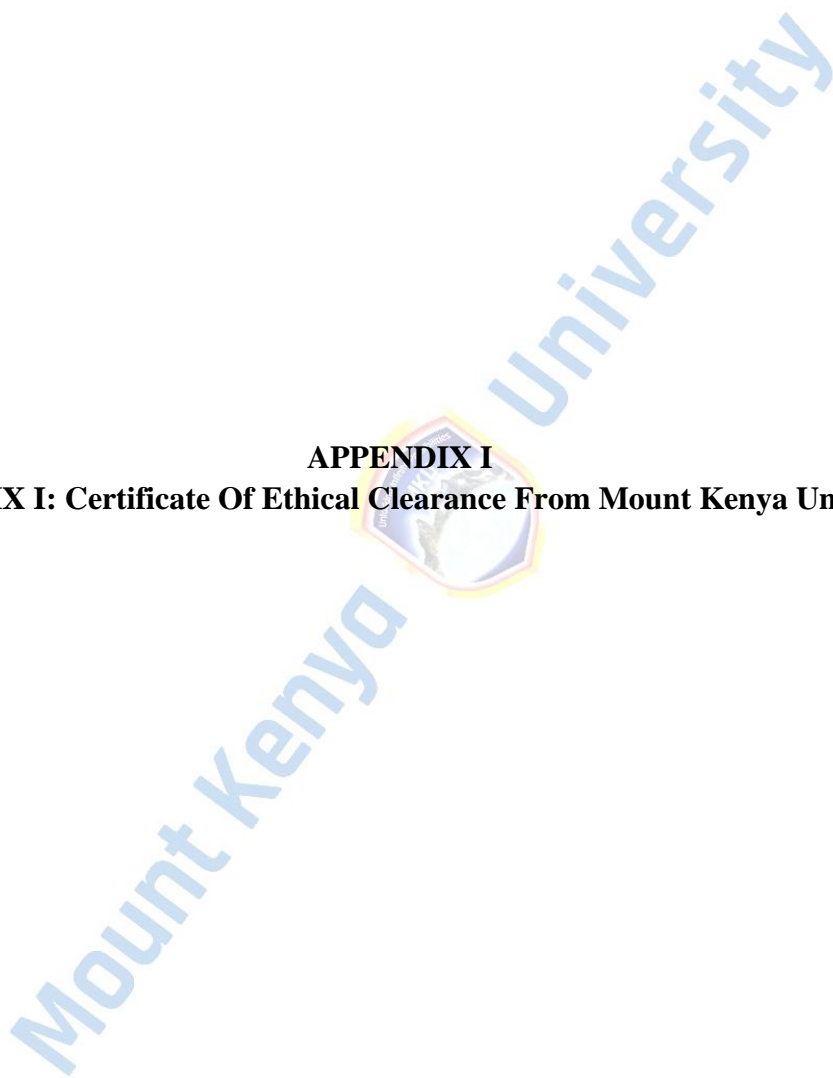
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- www.google.com/search?q=nakuru+county+map



APPENDIX I

APPENDIX I: Certificate Of Ethical Clearance From Mount Kenya University

Mount Kenya University



March 3, 2015


Ref. No. MKU/ERC/0013

CERTIFICATE OF ETHICAL CLEARANCE

This is to certify that the proposal titled “ DRUG SENSITIVITY USING COUNTER DIFFUSION TECHNIQUE IN METHICILIN-RESISTANT STAPHYLOCOCCUS AUREUS IN CLINICAL ISOLATES-A SURVEY OF HOSPITALS PATIENTS ADMITTED WITHIN NAKURU COUNTY”, whose Principal Investigator is Mr Joseph Odhiambo Owino (MMLS/000202/113/23607) has been reviewed by Mount Kenya University Ethics Review Committee (ERC), and found to adequately address all ethical concerns.

Prof. Mbaruk Suleiman

Chairman, Mount Kenya University ERC

Date: 
03/03/2015

Dr. Francis W. Muregi

Secretary, Mount Kenya University ERC

Date: 
~~Mount Kenya University~~
Director, Research & Development
& Development
P. O. Box 342 - 01000, Thika

Main Campus, General Kago Road, P.O Box 342-01000 Thika. Tel +254 020 208 83 10, +254 020 2 338 143/6/8, Fax: +254 020 20 503 15,
Cell: +254 720 790 796, +254 789 126 571 Email: info@mku.ac.ke, Web: www.mku.ac.ke
ISO 9001 : 2008 Certified

APPENDIX II: Research Authorization From NACOSTI



**NATIONAL COMMISSION FOR SCIENCE,
TECHNOLOGY AND INNOVATION**

Telephone: +254-20-2213471,
2241349, 310571, 2219420
Fax: +254-20-338245, 318249
Email: secretary@nacosti.go.ke
Website: www.nacosti.go.ke
When replying please quote:

9th Floor, Utalii House
Uhuru Highway
P.O. Box 30623-00100
NAIROBI-KENYA

Ref. No.

Date:

30th March, 2015

NACOSTI/P/15/5036/4459

Joseph Odhiambo Owino
Mount Kenya University
P.O. Box 342-01000
THIKA.

RE: RESEARCH AUTHORIZATION

Following your application for authority to carry out research on "*Drug sensitivity using counter diffusion in methicilin resistant staphylococcus aureus in clinical isolates. A survey of hospital patients admitted within Nakuru County.*" I am pleased to inform you that you have been authorized to undertake research in **Nakuru County** for a period ending **30th September, 2015.**

You are advised to report to **the County Commissioner, the County Director of Education and the County Coordinator of Health**, Nakuru County before embarking on the research project.

On completion of the research, you are required to submit **two hard copies and one soft copy in pdf** of the research report/thesis to our office.


DR. S. K. LANGAT, OGW
FOR: DIRECTOR GENERAL/CEO

Copy to:

The County Commissioner
Nakuru County,

National Commission for Science, Technology and Innovation. Is ISO 9001:2008 Certified.

APPENDIX III: Research License From NACOSTI



THIS IS TO CERTIFY THAT:
MR. JOSEPH ODHIAMBO OWINO
of **MOUNT KENYA UNIVERSITY THIKA**
CAMPUS, 240-2100 nakuru, has been
permitted to conduct research in
Nakuru County

Permit No : **NACOSTI/P/15/5036/4459**
Date Of Issue : **30th March, 2015**
Fee Received : **Ksh 1,000**

on the topic: **DRUG SENSITIVITY USING**
COUNTER DIFFUSION IN METHICILIN
RESISTANT STAPHYLOCOCCUS AUREUS
IN CLINICAL ISOLATES. A SURVEY OF
HOSPITAL PATIENTS ADMITTED WITHIN
NAKURU COUNTY.



for the period ending:
30th September, 2015

[Signature]
Applicant's
Signature

[Signature]
Director General
National Commission for Science,
Technology & Innovation

Moun

APPENDIX IV: NACOSTI permit



APPENDIX V: Research Authorization From Ministry Of Interior And Coordination Of National Government Nakuru County



**OFFICE OF THE PRESIDENT
Ministry of Interior and Coordination of
National Government**

Telegram: "DISTRICTER" Nakuru
Telephone: Nakuru 051-2212515
When replying please quote

SUB COUNTY COMMISSIONER
NAKURU SUB COUNTY
P.O. BOX 81
NAKURU

Ref No ED.12/10 Vol.VIII/157

10th April 2015

TO WHOM IT MAY CONCERN

**RE:- RESEARCH AUTHORIZATION
JOSEPH ODHIAMBO OWINO**

The above named student has been authorized to carryout research on *"drug sensitivity using counter diffusion in methicilin resistant staphylococcus aureus in clinical isolates"*. A survey of hospital patients admitted at War Memorial and Provincial General Hospital Nakuru Sub County.

Please accord him all the necessary support.

**C. W. NJOROGE
FOR DEPUTY COUNTY COMMISSIONER
NAKURU SUB COUNTY**

APPENDIX VI: Research Authorization From The Ministry Of Health Nakuru County

MINISTRY OF HEALTH
NAKURU COUNTY GOVERNMENT

Telegrams "PROVMED" Nakuru
Tele: Nakuru 2216710
Fax 2210350
Email: cohalth.nakuru@gmail.com



COUNTY DIRECTOR
MEDICAL SERVICES
P O BOX 2060
NAKURU COUNTY

When replying please quote

Ref No: NCG/CDMS/2015/006

Date: 13th April, 2015.

TO:

Medical Superintendent,
Rift Valley Provincial General Hospital
Molo Sub-County Hospital
Gilgil Sub-County Hospital
Bahati Sub-County Hospital
War Memorial Hospital
St. Mary's Mission Hospital

Dear Sir/Madam

RE: RESEARCH SURVEY ON METHICILIN RESISTANT STAPHYLOCOCCUS AUREUS

Mr. Joseph Odhiambo Owino of Mount Kenya University has been granted permission by the National Commission for Science Technology and Innovation to undertake a Survey on the subject mentioned above from 30th September, 2015.

Please allow him to conduct the Survey in your facility then share the findings with the Hospital Management Team at the end of the exercise.

Please accord him full support.

Thanks in advance.

Yours faithfully,

Dr. S. K. Sirma
County Director Medical Services
NAKURU COUNTY

APPENDIX VII: Research Authorization From Office Of The President Ministry Of Internal Coordination Of National Government To Deputy County Commissioners Nakuru County



OFFICE OF THE PRESIDENT
MINISTRY OF INTERIOR AND
CO-ORDINATION OF NATIONAL GOVERNMENT

Telegrams: "DISTRICTER", Nakuru
Telephone: Nakuru 051-2212515
When replying please quote

COUNTY COMMISSIONER
NAKURU COUNTY
P.O. BOX 83
NAKURU

Ref. No. **C.C.JR.EDU 12/1/2 VOL.1/154**

9th April, 2015

The Deputy County Commissioners,
Nakuru
Nakuru North
Gilgil
Molo

RE: RESEARCH AUTHORIZATION - JOSEPH ODHIAMBO OWINO

The above named student has been given permission to carryout research on
"Drug sensitivity using counter diffusion in methicilin resistant staphylococcus aureus in clinical isolates. A survey of hospital patients admitted" in your Sub Counties.

Kindly give him the necessary assistance.

C.W. NJOROGE
FOR: COUNTY COMMISSIONER
NAKURU COUNTY