

VANCOMYCIN SUSCEPTIBILITY PATTERNS OF METHICILLIN RESISTANT
STAPHYLOCOCCUS AUREUS ISOLATES FROM WOUNDS AT MADINA
HOSPITAL IN MOGADISHU, SOMALIA

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W64/81480/2015

A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE
REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN TROPICAL
AND INFECTIOUS DISEASES AT UNIVERSITY OF NAIROBI-INSTITUTE OF
TROPICAL AND INFECTIOUS DISEASES (UNITID)

October 2018

DECLARATION

I, Abdullahi Adan Shaba, hereby certify that this study is my own work and it has not been done to the best of my knowledge in Somalia nor has it been presented or submitted for a degree in any other university.

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DEDICATION

First and foremost, I would like to dedicate this work to the Almighty God, by whose strength and gracious provision, I have been able to get this far. To my beloved late parents, and to my family who has supported and encouraged me throughout my education. To my colleagues, Winny Mutai, the Madina Hospital clinicians, Mr. Abdullahi Dahir Aweis microbiologist and others who have contributed a lot in making this program a wonderful experience. Without them, the experience of undertaking this program would most likely have been unsatisfactory.

ACKNOWLEDGEMENT

I would like to acknowledge, with sincere gratitude, my supervisors; Dr. Marianne Mureithi, Mrs. Susan Akinyi Odera and Mr. Ibrahim Hassan Barkhadle, for their kind guidance and continuous assistance to conduct this study.

I would also like to thank the staff from the Institute of Tropical & Infectious Diseases, University of Nairobi and the staff from Medina Hospital for giving me the opportunity to conduct my research. Last but not least, I would again like to acknowledge and thank my family for continuous moral support and their patience throughout my study period.

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ABBREVIATIONS AND ACRONYMS

CA- MRSA	Community Acquired or Associated Methicillin
CDC	Center for Disease Control and Prevention
CLSI	Clinical Laboratory Standard Institute
HA-MRSA	Hospital Acquired or Associated Methicillin Resistant
hVISA	Heterogeneous Vancomycin Intermediate <i>Staphylococcus aureus</i>
ICU	Intensive Care Unit
MRCNS	Methicillin Resistant Coagulase Negative Staphylococci
MIC	Minimum Inhibitory Concentration
MDR	Multiple Drug resistant
MRSA	Methicillin Resistant <i>Staphylococcus aureus</i>
MSA	Mannitol Salt Agar
MSSA	Methicillin Sensitive <i>Staphylococcus aureus</i>
NCCLS	National Committee for Clinical Laboratory Standard
PCR	Polymerase Chain Reaction
SCC <i>mecA</i>	Staphylococcal Cassette Chromosome <i>mecA</i>
VISA	Vancomycin Intermediate <i>Staphylococcus aureus</i>
VRSA	Vancomycin Resistant <i>Staphylococcus aureus</i>
WHO	World Health Organization.

ABSTRACT

Background: Methicillin Resistant *Staphylococcus aureus* (MRSA) is considered the main cause of all community acquired infections as well as nosocomial to susceptible individuals. Methicillin Resistant *Staphylococcus aureus* infections account for 64% of general health care setting infections and 40 % of almost all infections in the Intensive Care Unit. However, there have been records of incidences of increased prevalence of MRSA resistance to some of the glycopeptides antibiotics used in the treatment of wounds such as vancomycin.

Objective: To demonstrate the prevalence and vancomycin susceptibility pattern of MRSA isolates from patients having open wounds at Madina Hospital.

Methodology:

Study design and site: This was a cross-sectional descriptive study recruiting 151 adult patients. The study was conducted in surgical wards, dressing room and burn unit.

Study participants: Adult patients with wounds seeking treatment at Madina Hospital during the study period.

Laboratory procedure: Specimens were collected from the open wound and inoculated into Mannitol salt agar. Those with yellow colonies were aseptically transferred into Medysinal BD Chromoagar. Bacterial suspension prepared and inoculated aseptically on Mueller Hinton Agar plates. Vancomycin antibiotic discs 30 µg was then introduced. *Staphylococcus aureus* were categorized according to MIC of vancomycin; if it is ≤ 4 µg/mL, it is a VSSA. It is VISA if vancomycin drug MIC is 4-8 µg/ml and it is considered as VRSA if the minimum inhibitory concentration is ≥ 16 µg/ml.

Results: The number of the patients recruited were 151. The patients were recruited from three different wards, namely the surgery ward (n=109; 72.3%), the dressing room (n=22; 14.6%) and the burn unit (n=20; 13.3%). Patients with bullet wounds were the most frequent at the hospital representing a quarter of the patients (n=38; 25.2%) in the study, followed by explosive wounds (n=22; 14.6%) and the least frequent were chemical wounds (n=3; 2.0%). Approximately half of the patients stayed in the hospital for more than 2 weeks (n=74; 49.0%). Most of the patients had no other underlying disease (n=122; 80.8%). There was a significant number of patients with diabetes (n=14; 9.3%). Majority of the patients were put on an antibiotic drug combination therapy. The prevalence of MRSA in this population was estimated at 17.9% (n=27). All MRSA isolates were susceptible to Vancomycin.

Conclusion: The prevalence of MRSA among the patients having wounds in this study was high. All MRSA isolates were susceptible to Vancomycin. The findings of our study indicate that the three wards in which the patient was recruited, the surgical ward appeared the highest infection rate of MRSA (13.2%) followed by the burn unit (4.6%). The risk factors for MRSA that were evaluated include; hospital stay, antibiotics used for the treatment of the wound and prosthetic device. Only duration of hospital stay was found to be significantly associated with the presence of MRSA infection (p<0.001).

Recommendations: Incidence of MRSA infections could be reduced by implementing continuous surveillance of nosocomial infections, to know MRSA prevalence and antimicrobial susceptibility profile which is essential in a selection of suitable empirical treatment, MRSA screening program for patient particularly high-risk individuals and healthcare workers for infection control measures. Long stay in hospital should be minimized to reduce the risk of nosocomial infections as well as health cost.

Keywords: Methicillin resistant *Staphylococcus aureus*, Madina Hospital, Intensive Care Unit, Chromogenic agar, Clinical Laboratory Standard Institute, MIC.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

Staphylococcus aureus is found as a normal bacterial commensal in the anterior nose and the skin of a human. Asymptomatic carriers are estimated to be about one-third of the human population (Otto 2013). According to Miller, this may accidentally disseminate the organism within the healthcare setting and outside the healthcare facility (Miller et al. 2012). Infections by *Staphylococcus aureus* cause significant health problems. Currently, it is considered the major cause of community as well as nosocomial infections to susceptible individuals. These infections span across bacteremia, surgical wound infections, osteoarthritis, device related infections, as well as pneumonia (Tong et al. 2015). Almost every healthy individual has been seen to harbor asymptotically, one or more strains of *Staphylococcus aureus*. Initially, *Staphylococcus aureus* infections were treated with methicillin, though they developed resistance shortly after it was introduced (States et al. 2007).

The major isolate of a bacterial organism from surgical site infections and other open wounds are *Staphylococcus aureus*. Therefore, awareness is necessary for Methicillin Resistant *Staphylococcus aureus* (MRSA), which is commonly resistant to β -Lactam antibiotic drugs including the penicillin, cephalosporin as well as carbapenems (Bhattacharya 2016).

The primary case of methicillin resistance was identified in the year 1961 in the UK (Cdr et al. 2013) shortly after the clinical introduction of the drug. It is now a globally recognized problem in the management of staphylococcal infections. The rate of MRSA is still high in most parts of the world (Gurieva et al. 2013). In many parts of African, the hospital acquired MRSA constitute between 20 and 50% of overall *S. aureus* infection (Shuping et al. 2017).

Studies show that MRSA is acquired through hospital and community exposure. In hospital facilities, these infections often lead to a significant number of morbidity as well as mortality. The death rate of bacteremia due to *Staphylococcus aureus* is high, around 20-30% (Yilmaz et al. 2016). The bacteria are able to cause cross infections and colonize individuals for months or even years.

Duration of MRSA presence in individuals or in families depends on their interaction with a person recently infected with MRSA. It also relates to the number of times and duration of contact with infected or carrier person (Cluzet et al. 2015). MRSA infections make up 64% of all nosocomial infections caused by *Staphylococcus aureus* and almost 40% of infections in Intensive Care Units (Tang et al. 2015). MRSA infections should be promptly identified in the laboratory and infected patients treated to prevent an epidemic of MRSA in a hospital facility.

Vancomycin is considered one of the mainly effective glycopeptide antibiotics that are used against MRSA infections. A number of MRSA strains have been seen to be inhibited by vancomycin at a minimum inhibitory concentration of 0.5 - 2.0 µg/ml or ≤ 2 µg/mL (Rubinstein & Keynan 2014). Extensive use of vancomycin in the management of MRSA infections has caused the emergence of VRSA. Such strains are Vancomycin Intermediate *Staphylococcus aureus* (VISA) and Heterogeneous Vancomycin Intermediate *Staphylococcus aureus* (hVISA) (Cázares-domínguez et al. 2015). It has been assumed that the increased prevalence of MRSA resistance to vancomycin, such as VISA and hVISA is due to the prolonged course treatment of open wound with vancomycin and use of it in management of other bacterial infections (Van Hal & Fowler 2013).

The National Committee for Clinical Laboratory Standard (NCCLS) defines VISA organisms as those *Staphylococcus aureus* strains that are inhibited by Vancomycin in their growth at 8-16 µg/ml of minimum inhibitory concentration (MIC). The Center for Disease Control and Prevention (CDC) has also approved the criteria to identify VISA as *Staphylococcus aureus* strains inhibited by E-test Vancomycin at MIC of ≥ 16 µg/ml.

hVISA strain appears a stage prior to the development of VISA strain but, the criteria to categorize hVISA has not been yet fully standardized (Liu & Chambers 2003).

The more the risk of MRSA, the more likelihood of recurrent infections and frequent admissions. The remarkable increase of MRSA for the community and hospital-related infections, as well as Gram-positive cocci such as Enterococcus infections has increased the use of vancomycin globally (Spagnolo et al. 2014).

To this end, this study seeks to demonstrate MRSA prevalence and vancomycin susceptibility pattern of MRSA isolates from patients with open wounds at Madina Hospital. There is a need for investigation of the presence of MRSA in cultures from wounds and abscess as well as purulent discharges from patients on an antibiotic regimen. The investigation is also necessary for patients with recurrent open wound infections that don't respond effectively to an initial antibiotic regimen and those with extensive localized infections.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Definitions

Methicillin Resistant *Staphylococcus aureus* (MRSA) is a strain of *Staphylococcus aureus* that is genotypically different from other strains. Resistance to Methicillin is due to acquisition of the *mecA* gene which carries genetic information for PBP2a. It has a low affinity for antibiotics such as Methicillin which in turn facilitates survival and growth of a *Staphylococcus aureus* isolates in an environment or media containing Methicillin. As compared to *Penicillinase gene*, it is not located on the plasmid, but resides in the chromosome, enclosed in a *SCCmec* (Ostojić & Hukić 1959)(Santiago et al. 2015)(Features et al. 2015). It causes skin infections, pneumonia, bacteremia, and wound infections. It mostly occurs both in a person who has been ill and admitted in hospital and also healthy people outside a health facility. It is resistant to many antimicrobial chemotherapeutic agents.

2.2 Biological Characteristics

Staphylococcus aureus has different strains and MRSA strain is the most significant gram-positive cocci bacteria generally recognized as causing both Hospital Acquired or Associated Methicillin Resistant (HA-MRSA) and Community Acquired or Associated Methicillin (CA-MRSA) worldwide (Aiken et al. 2014)(Alvarez-uria & Reddy 2012). HA-MRSA infects mostly hospitalized patients with predisposing risk factors including post or pre-surgery wounds, using antibiotics, primary or secondary immunodeficiency, and presence of prosthetic devices (Chambers & Deleo 2010)(Gopal & Divya 2017). Most of the MRSA isolates is resistant to almost all available β -lactam antibiotics which limits successful treatment for severe MRSA infections (David & Daum 2010). Soon after the β -lactam antibiotics were discovered and established into clinical practice, multidrug resistance strains to β -lactam drugs developed.

In 2016, the CDC reported that MRSA strains had developed resistance to Methicillin and Oxacillin but they were sensitive to a new generation of Cephalosporin called Ceftaroline. The Ceftaroline mechanism of action in the bacterial is to inhibit Penicillin Binding Protein 2a and its anti-MRSA sensitivity demonstrates zone of inhibition of MIC 90 approximately 1-2µg/ml. It has been recommended as a chemotherapeutics drug for complicated skin and soft tissues infections (Fishovitz et al. 2014).

2.3 Clinical significance and economic burden

Methicillin Resistant *Staphylococcus aureus* is a global health problem. It causes a hospital setting and outside hospital-acquired infections. Infections from MRSA compared those from MSSA, increase morbidity and mortality as well as the costs incurred in a healthcare setting (Batina et al. 2016). The patient consequence of MRSA caused infections is a longer stay in hospitals. This increases the economic burden on patients. From 1997-2005, in the USA for example, MRSA-related infections increased the duration of hospital by more than double the time needed. In 2010, the cost of MRSA infections leading to hospitalization in the EU countries were also estimated at €380 million (Win et al. 2015).

The rapid emergence and dissemination of MRSA has created significant global public health concern particularly in developing countries. It causes multiple antibiotic resistance (Mobasherizadeh et al. 2016). MRSA still is a real challenge and increasing cause of most post-surgical wound infections. Open wound infections often have an economic burden on patients and healthcare settings. Common consequence seen include a setback of wound healing which may eventually lead to sepsis.

In 2016, the World Health Organization (WHO) reported that on the fact sheet of antimicrobial resistance, individuals who got infected with MRSA were approximated 64% more probable to die than persons with MSSA. The estimated number of patients within healthcare settings alone, who got infected with MRSA in the European Union (EU) countries, was 170,000 per year. This resulted in more than 5000 deaths. Healthcare costs related to this effect were approximately €380 million (Köck et al. 2011). In 2011 in

the EU countries, the number of MRSA strain isolated from infected patients varied among European countries from < 0.5 and to greater than 50%, in the USA, the epidemiological prevalence study of MRSA in 2003 were almost 60% (Dulon et al. 2014).

In 2011, the number of patients who contracted invasive MRSA infections in the USA was 80,461 and 11,285 deaths related to MRSA were recorded. The estimated annual economic cost of CA-MRSA management between US\$1.4 – US\$13.8 billion (Abdulgader et al. 2015). MRSA infections alone kill in the USA approximately 19,000 per year, with annual estimates of \$3-4 billion in medical costs (Antibiotics 2013).

2.4 Epidemiology and Surveillance

Globally, MRSA is particularly prevalent in the healthcare setting such as hospitals. and Asia, N. America, S. America, and Malta reported more than 50% prevalence (Sit et al. 2017). MRSA resistant strains are responsible for the acquisition of Staphylococcal Cassette Chromosome (SCC) *mec* which bear methicillin resistance gene called *mecA* gene. The gene encodes low affinity for PBP2a, which binds very weakly to β -lactam antibiotics and causes transferring resistance to most common antimicrobial penicillin and cephalosporin drugs. Pathogenic *Staphylococcus aureus* acquires *mecA* gene from nonpathogenic staphylococcus species (Proulx et al. 2016).

The emergence of epidemics of MRSA resistant strains around the world, in patients with no identifiable MRSA risk factors, is a worrying trend that is posing a challenge to the management and control of MRSA-related wound infections in health facilities. Earlier screening test for all patients seeking admission to a health care center and identification of MRSA carriers, followed by decolonization to prevent nosocomial transmission could be a powerful option for controlling intervention of hospital acquiring MRSA infections. This, however, imposes an economic burden on a healthcare setting. Another alternative health strategy, which reduces the costs, is the screening of only certain high-risk groups such as ICU admissions and patients who were historically known to be carriers (Gurieva et al. 2013).

In 2014, WHO published the global estimation of general antibiotic resistance in which they stated that the *Escherichia coli*, *Klebsiella pneumonia*, and *Staphylococcus aureus* were the microbes of most public health concern and they relate to both nosocomial and community-acquired infections.

In European hospitals, cases of MRSA was found to range from <1% to >40% (Dulon et al. 2011). MRSA is endemic in India. The incidence of epidemiological studies ranges from 25-50% in the western and southern regions of India respectively (S. et al. 2013). In Peshawar, Pakistan, the MRSA prevalence is 36.1% (Ullah et al. 2016).

In Lebanese hospitals, MRSA prevalence was reported at 72%. From that, 18% of the strains were resistant to 10-18 antimicrobial chemotherapeutic agents. The prevalence of MRSA infections in Saudi Arabia was reported to be at 61% (Dormanesh et al. 2015).

Statistics on MRSA is scarce in Africa. However, available data points towards increasing trends of MRSA infection and colonization. Epidemiological reports of MRSA prevalence vary across the countries. Madagascar, for example, has as low as 7%, whereas, Egypt has the highest number at 82%. Between, 2002 and 2007 for instance, records show that in Tunisia the prevalence of MRSA shot up from 16% to 41%. Elsewhere in Botswana, the prevalence ranged from 23% to 44%, in 2000 to 2007. MRSA prevalence in Algeria and Egypt was 45% and 52% in 2003–2005, respectively. In Nigeria, the prevalence was higher in the northern as compared to the southern part. In the same period, the prevalence in Ivory Coast and Ethiopia was at 55% and 39%, respectively (Falagas et al. 2013).

In Kenya, two publications give reports on the prevalence rate. One reports that the prevalence of MRSA is at 3.7%. This data is generated by using an automated system (Omuse et al. 2014). The other study published in 2013, reports that the MRSA prevalence was at 84.1% (Maina et al. 2017). The marked difference can be attributed to the different techniques used for identification of MRSA. The two sites that were used for the research were different in terms of population and socioeconomic status of the patients. In Africa, data collection on MRSA prevalence majorly depended on phenotypic

instead of molecular diagnostic tests. The use of molecular diagnostic assays for confirmation of MRSA isolates would result to lower figures of MRSA in prevalence studies. In addition, some research studies used *oxacillin* for identification of MRSA which is rather less precisely confirmatory than *cefoxitin* disc diffusion test (Falagas et al. 2013).

Some European health authorities have been practicing surveillance programs for MRSA screening in health professionals and patients which successfully reduced the incidence of infections associated health setting (A. S. Lee et al. 2015). This strategy is very important for controlling MRSA strains in surgical wards, ICU, and others where open wounds are treated.

The control of MRSA infections can be achieved by decontamination of surgical wards, and ICU by applying intranasal *mupirocin* and *chlorhexidine* body washing of asymptomatic carriers of MRSA in their nares. This intervention practice was found to reduce medical costs, morbidity, and mortality of those infected (Y. Lee et al. 2015).

.In Somalia, a research conducted in two major pediatric hospitals in Mogadishu, investigating nasal carriage of multi-drug resistant *Staphylococcus aureus* among health professionals and pediatric patients, found that MDRSA was detected in 10 out of 38 samples of *S. aureus* (Nur et al. 1997).

2.5 Vancomycin Susceptibility Pattern

Vancomycin has been approved as the gold standard drug for MRSA infections caused by Multiple Drug resistant MDR strains (Kumar & Chopra 2013). However, extensive use of this antibiotic for infections has ultimately led to the manifestation of reduced susceptibility strains such as vancomycin-intermediate *S. aureus* (Lai et al. 2017). So far, there is a real challenge for the management of severe infections caused by VRSA and vancomycin resistant enterococcus (VRE).

VSSA, VISA and VRSA are determined by using Etest method. Homogenous suspension is prepared and turbidity is adjusted at 0.5 McFarland Standard. The suspension is

inoculated on Mueller Hinton Agar (MHA) plates. The vancomycin Etest strips are placed on MHA and incubated at 35⁰C for 24hrs according to the manufacturer's instruction. Zones of inhibitions are measured (Bamigboye et al. 2018).

According to the present guidelines endorsed by the Clinical Laboratory Standard Institute (CLSI), *Staphylococcus aureus* are categorized according to MIC of vancomycin; if it is $\leq 4\mu\text{g/ml}$, it is a VSSA. It is VISA if vancomycin drug MIC is 4-8 $\mu\text{g/ml}$ and it is considered as VRSA if the minimum inhibitory concentration is $\geq 16\mu\text{g/ml}$ (Dhand & Sakoulas 2012). The European Committee on Antimicrobial Susceptibility Testing (EUCAST) just describe *S. aureus* strains as vancomycin sensitive (MIC $\leq 2\mu\text{g/ml}$) or resistant (MIC $> 2\mu\text{g/ml}$) (Serious et al. 2012). High prevalence numbers of vancomycin-resistant of MRSA strains are a significant problem and a public health concern. Confirmatory test for MRSA is considered a molecular diagnostic assay of either detection of *mecA* by PCR or using a commercially available slide agglutination test to diagnose the presence of PBP2a (Paterson et al. 2014).

Molecular methods using for MRSA screening are rather not cost-effective even to medical laboratories screening a high number of risk populations. Smaller laboratories that are found in low-income countries and screening a low number of risk populations can use culture-based methods (Hernandez et al. 2016). The MRSA screening program should be rapid and affordable to the patients as well as asymptomatic MRSA carriers in a health care facility to reduce MRSA infections.

Molecular methods used for detection of MRSA are more rapid and sensitive than chromogenic agars. However, there are some limitations to their accuracy. The PCR tests may give false positive when the sequence is amplified is a nonspecific sequence or the Methicillin Resistant Coagulase Negative Staphylococci (MRCNS) have *MecA* gene which erroneously could be mistaken with the MRSA strains which are coagulase positive (French 2009).

In the MRSA management or control, the laboratory result should be more rapid. Regarding this, the routine culture-based methods take a minimum of 72 hours to confirm

MRSA samples which cannot optimize the therapy and decrease the cost. So, to cover such a gap chromogenic media were developed to identify MRSA strains within 24 hours in a single step (Loo et al. 2017). In addition, processing chromogenic agar allows color based identification of MRSA and other pathogens to make unnecessary for further confirmation by using biochemical tests. Chromogenic agars are more sensitive than conventional agar and these have indicated a sensitivity of 93-99% as compared to conventional selective media but the culture still remains standard method in many parts of the world for MRSA identification (Marlowe & Bankowski 2011)(Hos- 2015).

However, there is no report indicating the prevalence and vancomycin susceptibility pattern of MRSA in open wounds in Somalia. Therefore, this study was conducted to fill the existing gap.

3.0 STATEMENT OF THE PROBLEM

With reported rising episodes of MRSA related infections across the world, there is a demand for proper surveillance to provide adequate data on specific populations. This can be achieved through proper screening of all admitted patients and decolonizing of all known infected with MRSA or asymptomatic carriers (Lawes et al. 2012).

However, in sub-Saharan Africa generally, and more specifically in Somalia, patients are rarely screened for MRSA before and during hospital admissions. This subsequently increases the likelihood of patients carrying resistant strains into the hospitals. These strains are unlikely to be isolated or identified. The use of vancomycin as an alternative antibiotic for the medical care of open wounds infected with *Staphylococcus aureus* has encountered challenges over the past years with the main one being resistance, paving way for recurrent wound infections.

This study, therefore, highlighted issues surrounding the application of vancomycin regimen in the therapy of open wound infections related to MRSA. Some of these include vancomycin dosage and monitoring, surveillance program, adequate treatment, isolation protocol designed to limit the dissemination of MRSA and the available alternative treatment for patients who end up developing vancomycin resistance leading to treatment failure. It is also necessary to find out available surveillance and testing techniques and also the current MRSA infection prevention strategies that strengthen Somali health care setting more specifically the Madina Hospital. The Madina Hospital was chosen as the study area because the hospital handles a number of trauma patients, maternity and it is the major referral center for patients with explosive and gunshot wounds.

4.0 JUSTIFICATION OF THE STUDY

Madina Hospital is a referral center, which provides health services, particularly for traumatic injuries. In the hospital, some of the patients with open wounds stay longer in the hospital due to the slow response of the wounds to the treatment. The number of the patients admitted in the different departments of the hospital per month is between 200-300 individuals. Approximately two third of whom have traumatic injuries. On average a patient with open wounds stays admitted in hospital for about 21 days, which unnecessarily increase the economic burden on both the patients and the hospital.

The myriad of challenges in dealing with infections caused by MRSA strain including increased morbidity, mortality and health cost incurred, it is necessary to do a study to know the baseline prevalence of a specific population. This will better inform policymakers in designing treatment regimens and instituting specific control measures to limit MRSA infections. Therefore, the result of this study informed the use of appropriate antibiotic therapy, which will result in a short stay in the hospital.

There is no study that has been conducted on MRSA prevalence as well as vancomycin susceptibility pattern to MRSA strains at Madina Hospital. The data generated from this study will be fundamental for antibiotic protocol and guidelines on open wounds at the hospital. The study also provided data for epidemiological prevalence studies in the country.

5.0 THEORETICAL FRAMEWORK

Antibiotic resistance has gradually increased since the antimicrobial was introduced. Gradually, several bacteria have devised mechanisms for resistance to antibiotic. The emergence and distribution of these resistant strains of microbial are associated with a number of theories. They include the wide use of antibiotics in agriculture to enhance growth in plants as well as animal production, widespread abuse of antibiotics, and the increased movements both internationally and regionally. Food such as meat, milk and plant product may act as a source of transport for transmission of antimicrobial resistant strains of bacteria to human (Verraes et al. 2013).

However, of grave concern is the evolution of multidrug-resistant Gram-positive bacteria like MRSA chiefly because of its internal virulence, its potential to cause varied life-threatening conditions, as well as the ability to withstand different conditions in the environment. Currently, *Staphylococcus aureus* is the leading causative agent of the nosocomial infections in a majority of a hospital setting. MRSA strains, that were first reported in the early 1960s, are currently endemic not just in developing economies but across the globe.

There is a limited choice of antibiotics in the treatment of infections caused by these resistant strains. Evidence of *Staphylococcus aureus* strains with complete or partial resistance to vancomycin, however minimal, calls for newer antibiotic therapies (Manuscript 2010). To this end, this paper highlighted a Somali perspective on the resistance burden with the special focus on open wound patients at Medina Hospital. In addition, it will also highlight the role of both emerging and existing antibiotics in the management MRSA caused infections.

6.0 OBJECTIVES OF THE STUDY

6.1.1 Broad objectives

To determine vancomycin susceptibility pattern of MRSA isolated from an open wound in patients admitted at Madina hospital.

6.1.2 Specific objectives

1. To determine the prevalence of MRSA isolated from open wounds in patients at Madina Hospital.
2. To profile the vancomycin susceptibility of MRSA isolated from open wounds in patients at Madina Hospital.
3. To establish factors associated with MRSA isolates from open wounds in patients admitted at Madina Hospital

6.2 Research Questions

1. What is the prevalence rate of MRSA in patients with open wounds and seeking treatment at Madina Hospital?
2. What is the vancomycin susceptibility profile of MRSA isolated from open wounds in patients at Madina Hospital?
3. What are the factors related to MRSA isolates from patients having open wounds and admitted to Madina Hospital?

7.0 CHAPTER THREE

RESEARCH METHODOLOGY

7.1 Study site

The study site was at Madina Hospital, which is referral center in the Madina district of Benadir region. The hospital is located in the western region of Mogadishu, the capital city of Somalia. In 2016, the population of the area was 76,906. The Medina Hospital offers different services such as emergency, maternity, medicine, and trauma care, receiving hundreds of war-wounded victims as well as expectant women. The hospital has admitted many patients throughout the year and has a bed capacity of 200 patients. Being the largest emergency care facility in the south and central Mogadishu, in the year 2010 for instance, the hospital treated close to 3268 war-wounded patients among them, 271 children and 875 women.



Figure 1. Google map of Madina Hospital, Mogadishu, Somalia.

7.2 Study design

A cross-sectional study was adopted with an aim of determining vancomycin susceptibility pattern of MRSA isolated from open wounds among patients at Madina Hospital.

7.3 Study population

Patients with open wound seeking treatment at Madina Hospital during the study period of March- June 2018.

7.4 Sampling technique and recruitment

Selection of the participants has followed the simple random sampling technique whereby patients were issued with identifying tags and the tags were drawn at random from a container to determine who qualifies. The patients in the wards were informed of the study information. Informed consent was obtained from those who convinced to take part in the study after full explanation of anything concerning the study. The surgical, dressing room and burn units in the facility were the areas of interest because they suit the source of a sample collection of the study. Questionnaires or checklist was provided to the patients having open wounds to collect the information relevant to MRSA risk factors such as; hospital stay duration, type of antibiotics used and immune status of the patients, being diabetic and underlying diseases.

7.5 Sample size determination

The sample size was determined using Fisher's formula 1998 and adjusted using finite correction factor (for finite population).

$$n_o = \frac{Z_{\alpha}^2 \cdot p(1 - P)}{d^2}$$

Where:

n_o = estimated sample size for infinite population

$Z_{\alpha/2}$ = Normal standard deviation 95% confidence interval ($Z = 1.96$)

P = Prevalence of the disease ($p=0.55$ based on MRSA in Ethiopia; Falagas et al., 2013)

D = margin of error (0.05)

$$n_o = \frac{(1.96)^2 \times 0.55 \times 0.45}{(0.05)^2}$$

Using the above formula the sample size $d = 381$. Since the study will be conducted in a three month period, the expected number of patients with open wounds at the Hospital was 250 (3268/12), the final sample size n will be obtained after adjusting n_o using finite population correction factor:

$$n \geq \frac{381}{1 + \frac{381}{250}}$$

The minimum sample size for this study was 151 patients.

7.6 Inclusion criteria

Patients above 18 years of age.

Patients with open wounds seeking treatment at Medina Hospital within the study period.

Patients voluntarily gave informed consent.

7.7 Exclusion criteria

Patients below 18 Years of age.

Patients with no open wounds.

Patients with mental conditions.

Patients who was unwilling to participate in the study.

7.8 Data collection methods

7.8.1 Social demographic and clinical factors of study participants

Interview-administered questionnaires (Appendix II) were used to collect information from participants and this was complemented by a checklist.

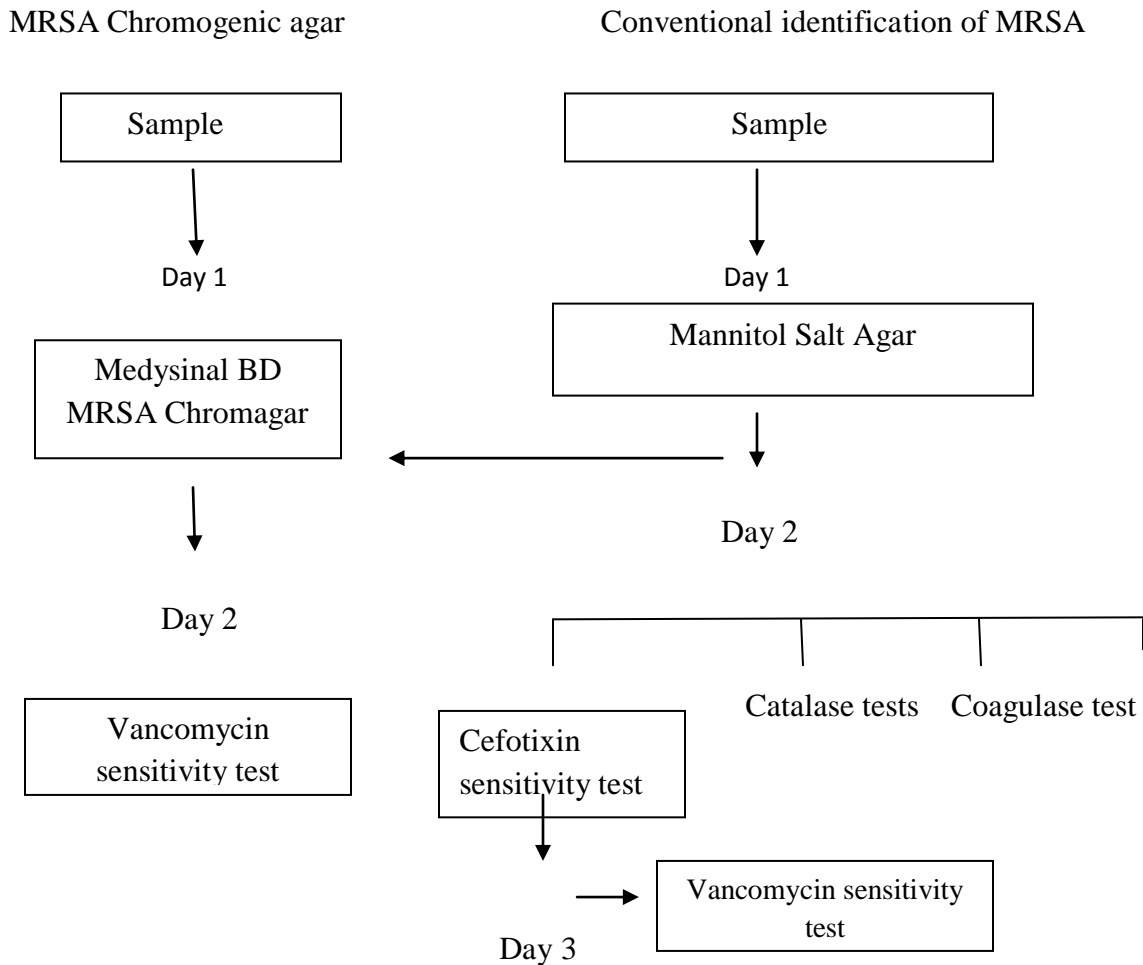
7.8.2 Specimen collection

Specimens were collected by principal investigator and research assistants from patients having open wound discharges by using the guidelines of CLSI and CDC. Sterile Cotton swabs were used for taking samples from the subject's wounds and taken to the laboratory within one hour. The area of the open wounds was cleaned by using 60% ethyl alcohol and normal saline was followed to clean the open wound and the exudates are collected with a sterilized swab and inoculated into sterile Amies Transport Media.

7.8.3 Isolation and identification of MRSA

The swabs were inoculated within one hour onto Mannitol salt agar. The media incubated at 37⁰ C for 20 and 24 hours. Those demonstrated with yellow colonies were transferred onto Medysinal BD MRSA Chromagar. The medium was incubated at 37⁰ C for 20 and to 24 hours. Conventional Culture-based procedures usually used for identification of MRSA after isolation of *Staphylococcus aureus* by using standard microbiological procedures such as inoculating the isolates into the Catalase test, Coagulase test, DNase, Cefoxitin or Oxacillin. Cefoxitin resistant, as well as positivity of other biochemical tests, indicates confirmation of MRSA. The Medysinal BD Chromagar will be used in our study to give a reasonable cost alternative for confirmation of MRSA. In general, Chromogenic agars in terms of sensitivity and speedy time of result availability are less effective than molecular methods (Buchan BW et.al, 2010).

Figure 2: Identification of MRSA by using Medysinal BD MRSA Chromagar and Conventional methods



7.8.4 Identification of Vancomycin Susceptibility

A bacterial suspension was prepared by mixing MRSA colonies and sterile normal saline. The homogeneous suspension was prepared and the turbidity of the suspension was adjusted to 0.5 McFarland standards. The suspension was inoculated aseptically on Mueller Hinton Agar plates by disc diffusion method and vancomycin antibiotic discs 30µg and control blank discs was placed on the plates. The plates was incubated at 35°C for 24 hours to observe and measure the clearance zone of inhibition to identify as sensitive, intermediate and resistant regarding the CLSI standard (Hasan et al. 2016).

Identification of Vancomycin sensitive *staphylococcus aureus* (VSSA), Vancomycin intermediate *staphylococcus aureus* (VISA) and vancomycin resistant *staphylococcus aureus* (VRSA) will be based on the isolates appear to have a vancomycin Minimum inhibition zone by using Mueller Hinton Agar plates as follows (Cdr et al. 2013).

Type of MRSA	Minimum Inhibition Zone
Vancomycin sensitive <i>staphylococcus aureus</i> (VSSA)	$\leq 2 \mu\text{g/ml}$
Vancomycin intermediate <i>staphylococcus aureus</i> (VISA)	4-8 $\mu\text{g/ml}$
vancomycin resistant <i>staphylococcus aureus</i> (VRSA)	$\geq 16 \mu\text{g/ml}$

7.9 Quality assurance

The Investigator was trained and guided by the supervisors on the questionnaire, sample collection, and sample transportation. The isolates from Medysinal BD Chromagar were evaluated by using microscope, catalase test, both tube and slide coagulase test and cefoxitin resistance. In addition, the samples were inoculated onto the traditional selective medium for *Staphylococcus aureus* such as Mannitol Salt Agar (MSA).

8.0 Data processing and analysis

The data from questionnaires were examined for errors and completeness on daily basis. The data were entered and stored using Microsoft Excel 2013 and then were imported into SPSS, coded, cleaned and analyzed. Descriptive statistics was done to explore and summarize the data. A summary was presented in tables and figures. The Histogram was plotted to show the distribution. The categorical data frequencies and proportions were reported in tables. Graphs (bar, pie) were plotted to demonstrate the distribution.

Fischer test and Chi-square were used to determine an association between risk factors and MRSA. Fischer values and corresponding p-value was reported. The study was conducted at the α -level of significance 0.05.

8. 1 Ethical considerations

To conduct this study, a letter of approval was requested from Kenyatta National Hospital and the University of Nairobi Ethics and Research Committee and then another letter of permission was requested from the administration of Medina Hospital. Prior and detailed information was availed to subjects so that they understood their role and enabled them to give an informed consent. Any information such as demographic data and patient medical status volunteered by the participants was treated with the utmost confidentiality and was used strictly for research purposes. Written informed consent was requested from each patient participant.

CHAPTER FOUR

RESULTS

4.1 Characteristics of the wound patients

4.1.1 Socio-demographic characteristics

Exudates were collected from 151 patients seeking for treatment of open wounds at Madina hospital. These patients were recruited from three different wards, namely the surgery ward (n=109; 72.3%), the dressing room (n=22; 14.6%) and the burn unit (n=20; 13.3%). Male constituted approximately three quarters (n=115; 76.2%) of the patients. More than half of the patients (n=100; 66.2%) were aged between 18 and 40 years and married (n=96; 63.6%). At least half had no formal education (n=85; 56.3%), and less than a quarter had attained at least secondary level education (n=29; 19.3%). Less than half of the patients were employed (n=64; 42.4%) whereas the rest were either unemployed (n=80; 53.0%) or retired (n=7; 4.6%). The majority were residing in the urban area (n=95; 62.9%).

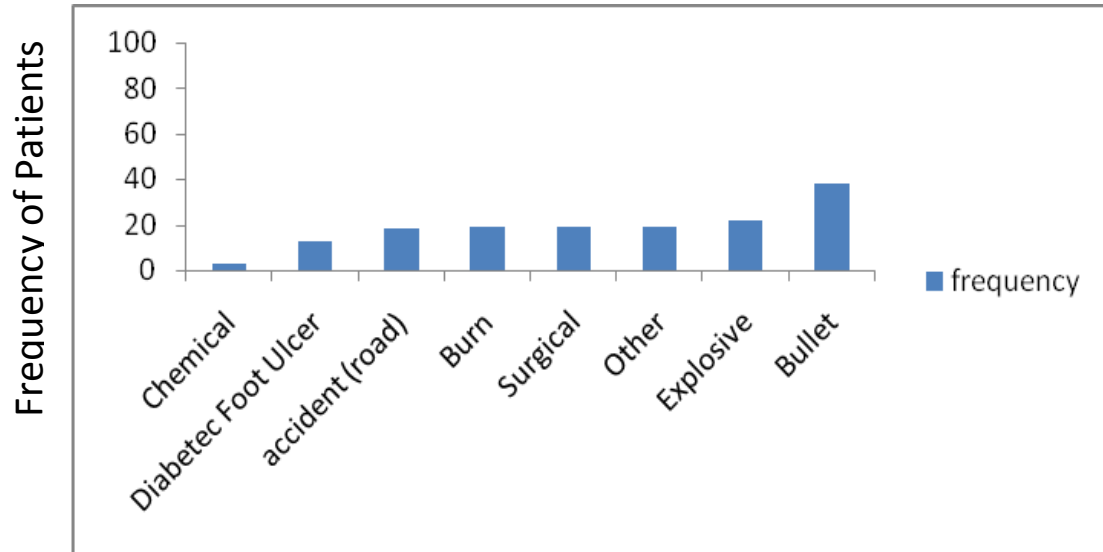
TABLE 1: SOCIO-DEMOGRAPHIC CHARACTERISTICS OF THE PATIENTS

Characteristic	Response	Frequency	Proportion (%)
Hospital ward	Surgery ward	109	72.2
	Dressing room	22	14.6
	Burn unit	20	13.2
Gender	Male	115	76.2
	Female	36	23.8
Age (years)	18-40	100	66.2
	>40	51	33.8
Marital status	Married	96	63.6
	Single	44	29.1
	Widowed/divorced	11	7.2
Highest level of Education	No formal education	85	56.3
	Primary	37	24.5
	Secondary	25	16.6
	Tertiary	4	2.6
Occupation	Employed	64	42.4
	Unemployed	80	53.0
	Retired	7	4.6
Residence	Urban	95	62.9
	Rural	56	37.1

4.1.2 Clinical profile

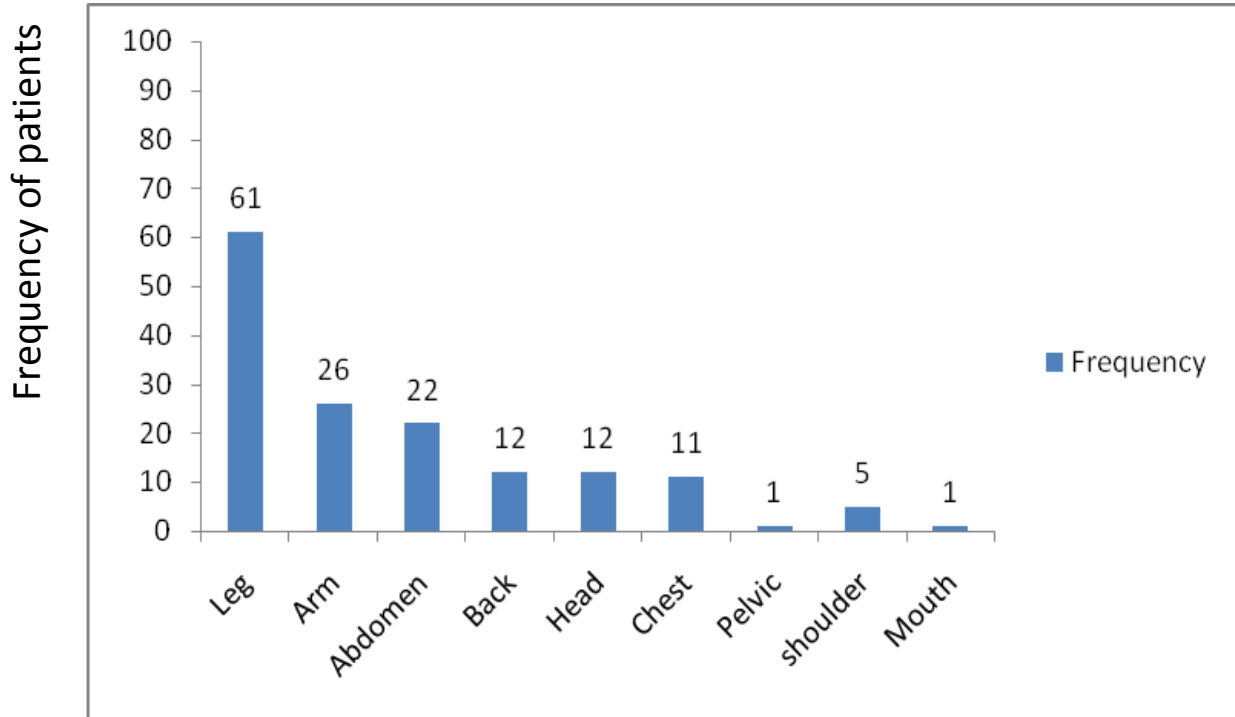
Patients with bullet wounds were the most frequent at the hospital representing a quarter of the patients (n=38; 25.2%) in the study, followed by explosive wounds (n=22; 14.6%) and the least frequent were chemical wounds (n=3; 2.0%). Figure 1 shows the distribution of the patients by type of wound.

FIGURE 2: DISTRIBUTION OF PATIENTS BY TYPE OF WOUND PRESENTED (N=151)



The specimens were collected from different parts of the body depending on the location of the open wound. Majority of the specimens were collected from the leg (n=61; 40.4%), followed by the arm (n=26; 17.2%), abdomen (n=22; 14.6%) among others. Figure 2 shows the distribution of patients by the wound location (source of the specimen).

FIGURE 3: DISTRIBUTION OF PATIENTS BY LOCATION OF WOUND/SOURCE OF SPECIMEN
(N=151)



Close to three-quarters of these patients had wounds producing discharge (n=108; 71.5%) and a few were gangrene (n=3; 2.0%). Figure 3 shows the distribution of the patients by the characteristic of the wounds.

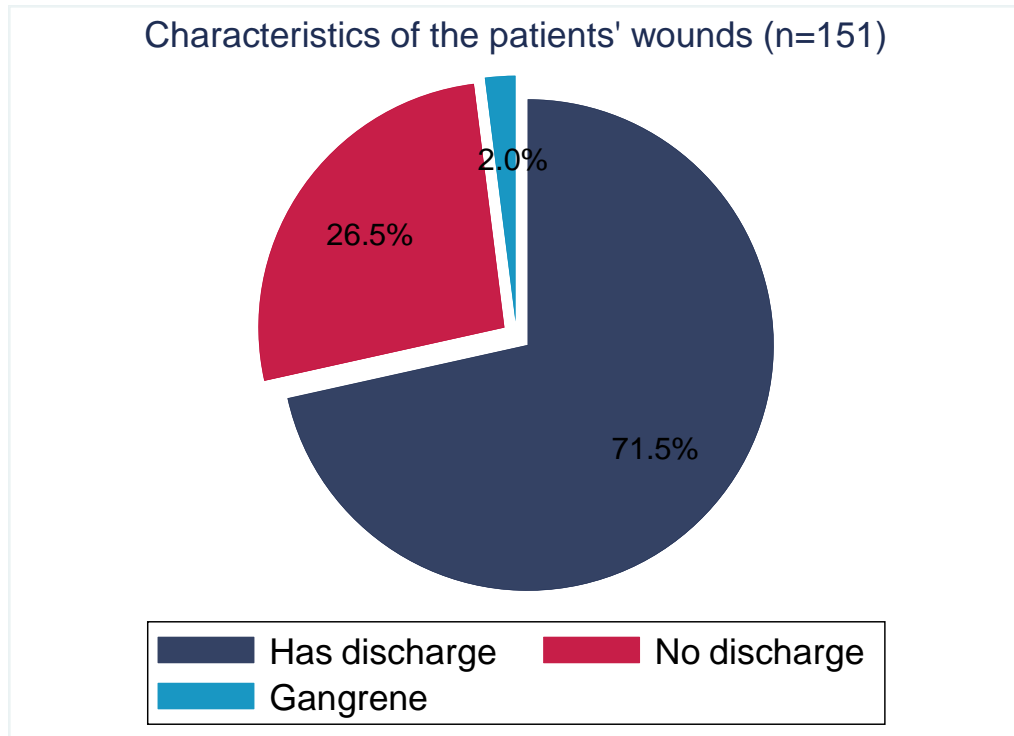


FIGURE 4: DISTRIBUTION OF THE PATIENTS BY CHARACTERISTICS OF THE WOUND BEING TREATED

4.2 PREVALENCE OF MRSA ISOLATED FROM WOUNDS AND VANCOMYCIN SUSCEPTIBILITY PATTERN

4.2.1 Prevalence of MRSA isolated from wounds

All the 151 specimens were subjected to a Culture and Sensitivity testing. The number of *staphylococcus aureus* isolated were 40 and 27 of them were MRSA. The three wards from which the patients were recruited, the surgical ward appeared the highest infection rate of MRSA (13.2%) followed by the burn unit (4.6%). The prevalence of MRSA in this population was estimated at 17.9% with 95% confidence interval [11.8%-24.0%].

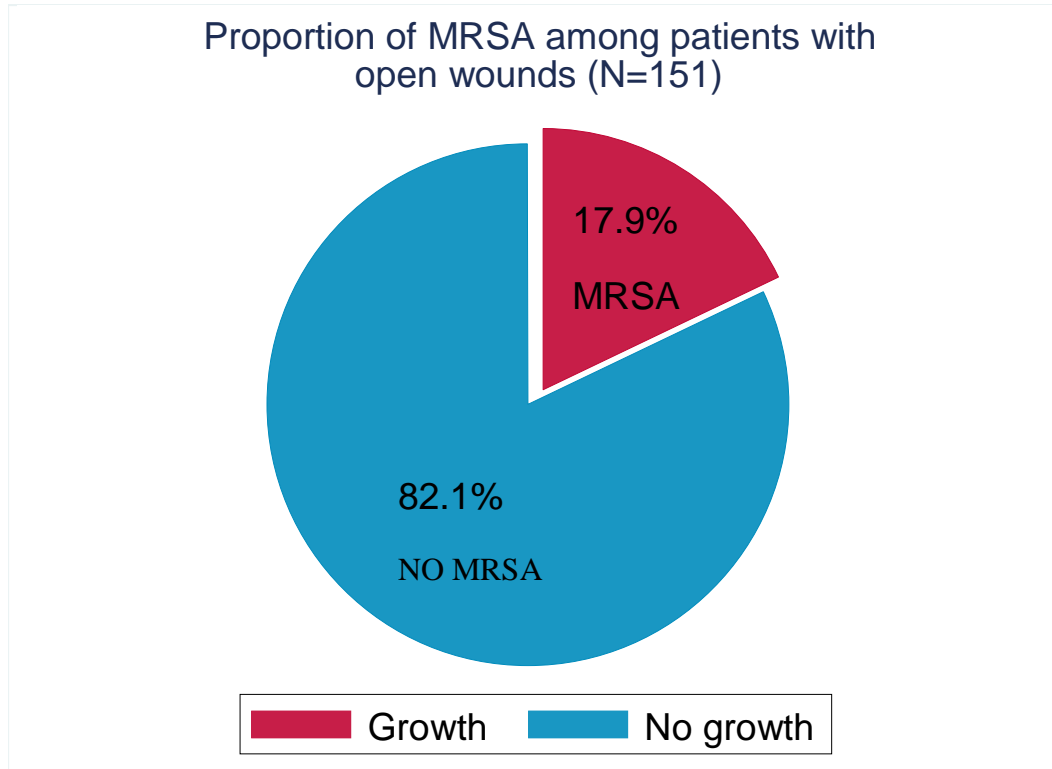


FIGURE 1: PREVALENCE OF MRSA AMONG WOUND PATIENTS

4.2.2 MRSA with gender

As shown in table 2 below, the infection rate of MRSA in males was 13.2% as compared to 4.6% in female counterpart.

Table 2. MRSA with gender

Sex	MRSA	No MRSA	Total
Male	20 (13.2%)	95(62.9%)	115
Female	07 (4.6%)	29(19.2%)	36

Table 3. MRSA with Hospital ward

Hospital Ward	MRSA		Total
	Growth	No growth	
Surgery ward	20 (18.3%)	89 (81.7%)	109
Dressing room	0 (0.0%)	22 (100%)	22
Burn unit	7 (35%)	13 (65%)	20
Total	27 (17.9%)	124 (82.1%)	151

4.2.3 Vancomycin susceptibility pattern of MRSA isolated

The number *staphylococcus aureus* isolated were 40 and 27 of them were MRSA. The 27 specimens with MRSA were analyzed for Vancomycin susceptibility. All the isolates were found to be susceptible to Vancomycin.

Table 4. Vancomycin susceptibility pattern to MRSA isolated

MRSA	Vancomycin			Total
	Sensitive	Intermediate	Resistant	
	$\leq 2 \mu\text{g/ml}$	4-8 $\mu\text{g/ml}$	$\geq 16 \mu\text{g/ml}$	
Growth	27 (17.9%)	0	0	27

4.3 FACTORS ASSOCIATED WITH MRSA INFECTIONS

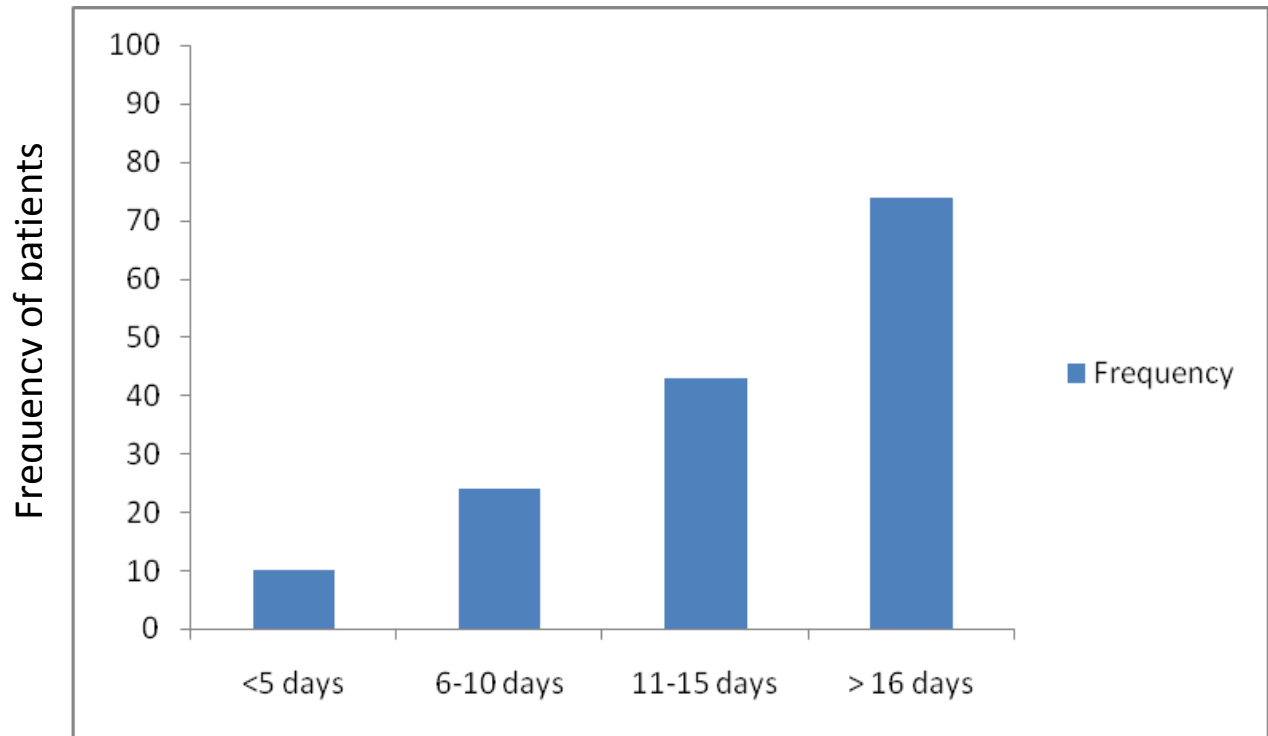
4.3.1 Potential factors associated with MRSA infection

Pearson chi-square tests were done to evaluate the factors associated with MRSA infection among patients seeking treatment for open wounds. The null hypothesis being tested was that there was no association between the patient characteristic in question and having MRSA infection. The risk factors evaluated include; irrational use of antibiotics, underlying disease, prosthetic devices, and hospital stay. Only duration of hospital stay and multiple use of antibiotics were found to be significantly associated with a patient having MRSA infection (Fischer =16.318, p-value<0.001 and 119.53, p-value<0.00001) respectively at 0.05 level of significance. Table 4 shows the findings of the Fischer tests.

Table 5. Factors Associated with MRSA infection

Characteristics	MRSA	No MRSA	Fischer Test	P-Value
Antibiotic used				
Ceftriaxone	0	12	119.53	<0.00001
Gentamycin	0	19		
Penicillin G	0	14		
Penicillin & Gentamycin	0	21		
Gentamycin & Metronidazole	0	1		
Penicillin G, Gentamycin & Metronidazole	0	6		
Ceftriaxone, Gentamycin & Metronidazole	0	1		
Penicillin G, Gentamycin, Ceftriaxone, & Vancomycin	27	0		
Ceftriaxone, Penicillin G, Gentamycin & Metronidazole	0	2		
Penicillin G, Gentamycin & Ceftriaxone	0	31		
Ceftriaxone & Gentamycin	0	17		
Prosthetic device				
Yes	1	4	0.27	1.0
No	26	120		
Underlying diseases				
None	24	98	2.090	0.88
Present	3	26		
Duration of hospital stay				
≤10 days	1	34	16.318	<0.001
11-15 days	3	39		
≥16 days	23	51		

FIGURE 6: DISTRIBUTION OF PATIENTS BY DURATION OF HOSPITAL STAY (N=151)



Approximately half of the patients stayed in the hospital for more than 2 weeks (n=74; 49.0%).

Table 6: Distribution of patients by antibiotics given and underlying diseases

Variable	Response	Freq (Percent)
	Ceftriaxone	12 (8.0)
	Gentamycin	19 (12.6)
Antibiotics Given (N=151)	Penicillin G	14 (9.3)
	Penicillin & Gentamycin	21 (13.9)
	Gentamycin & Metronidazole	1 (0.7)
	Penicillin G, Gentamycin & Metronidazole	6 (4.0)
	Ceftriaxone, Gentamycin & Metronidazole	1 (0.7)
	Penicillin G, Gentamycin, Ceftriaxone, & Vancomycin	27 (17.9)
	Ceftriaxone, Penicillin G, Gentamycin & Metronidazole	2 (1.3)
	Penicillin G, Gentamycin & Ceftriaxone	31 (20.5)
	Ceftriaxone & Gentamycin	17 (11.3)
Prosthetic Devises	Yes	5 (3.3)
	No	146 (96.7)
	None (Normal)	122 (80.8)
	Diabetes	14 (9.3)
Underlying Diseases	Viral infection	10 (6.6)
	Bacterial infection	1 (0.7)
	Immunocompromised	2 (1.3)
	Other diseases	2 (1.3)

In terms of treatment, the majority was put on an antibiotic drug combination therapy. Table 3 shows the distribution of patients by type of antibiotics given and underlying medical conditions. Overall, Gentamycin was given to majority of the patients (n=125, 82.8%), followed by Penicillin G (n=101; 66.9%), Ceftriaxone (n=90; 59.6%) with very few taking Vancomycin (n=27; 17.9%). Most of the patients had no other underlying disease (n=122; 80.8%). There was a significant number of patients with diabetes (n=14; 9.3%).

5.0 CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1 DISCUSSION

The MRSA reports are insufficient in developing countries, particularly in Africa. However, available data points towards increasing trends of MRSA infection.

The factors which most probably contributed to the emergence of MRSA in Somalia are easy availability, lack of effective guidelines of prudent use of antibiotics and weak policy of infection control measures in healthcare environment.

This study investigated vancomycin susceptibility patterns of MRSA isolates from wounds at Madina Hospital. It demonstrated the prevalence of MRSA was 17.9% among the patients who had open wounds. This study also highlighted that all MRSA isolated were sensitive to Vancomycin.

In Tunisia, the prevalence of MRSA shot up from 16% to 41% which almost similar to this study which is 17.9%. The prevalence of MRSA in Kenya is at 3.7%. This data is generated by using an automated system (Omuse et al. 2014). This study was lower than what was generated in the present study. The difference could be explained that the infrastructure of the Kenya health system is more developed than in Somalia in terms of the health budget, and quality of healthcare workers. The prevalence of MRSA in Ethiopia and Ivory Coast was reported at 55% and 39%, respectively. In Botswana, the prevalence ranged from 23% to 44%, from 2000 to 2007. MRSA prevalence in Algeria and Egypt was 45% and 52% in 2003–2005, respectively which was higher than what we found in this study (Falagas et al. 2013). This marked increase could be due to differences in duration of the study period, the sample size, availability and abuse of antibiotics.

The prevalence of MRSA in this study was higher than as reported in European HHHospitals, cases of MRSA range from <1% to >40% (Dulon et al. 2011). The

difference could be explained that the European health system has been practicing surveillance programs for MRSA screening in health professionals as well as patients and better policies of antibiotic stewardship. MRSA is endemic in India. The incidence of epidemiological studies ranges from 25-50% in the western and southern regions of India respectively (S. et al. 2013). In Peshawar, Pakistan, the MRSA prevalence is 36.1% (Ullah et al. 2016). This could be explained differences in duration of the study period, the sample size, availability and abuse of antibiotics.

In our study, none of the MRSA isolated were resistant to Vancomycin, it was not routinely available in most health care settings in Mogadishu. Furthermore, in the Madina Hospital, a limited number of physicians were authorized to prescribe vancomycin empirically to limit its use since the microbiology section of the hospital laboratory was not functioning properly.

In India, one study tested 102 of MRSA isolates and reported one VISA with minimum inhibitory concentration of 5 mg/l (Niveditha & Sujatha 2015). This study is almost similar to the finding of our present study. One of the hospitals in Pakistan reported that 139 MRSA isolated were found to susceptible to Vancomycin, linezolid & quinopristin/dalfopristin (Uddin 2010). This report is similar to the finding in our study.

Improper use of antibiotics resulted in emergence of VISA and VRSA which is currently a global concern. Continuous susceptibility testing of MRSA isolates against Vancomycin and proper surveillance could be a solution for maintaining the sensitivity of MRSA strain to Vancomycin.

Risk factors for MRSA evaluated include; irrational use of antibiotics, underlying disease, prosthetic devices, and hospital stay. Only duration of hospital stay and multiple use of antibiotics were found to be significantly associated with a patient having MRSA infection $p\text{-value} < 0.001$ and $p\text{-value} < 0.00001$ respectively at 0.05 level of significance.

Prolonged length of stay in hospitals, improper use of antibiotics, having diseases which reduce immunity, using prosthetics such as implants and catheters would be risk factors of MRSA. The findings of our study indicate that the three wards which the patient was

recruited, the surgical ward had the highest rate of MRSA isolates (13.2%) followed by the burn unit (4.6%). Approximately half of the patients recruited in this study stayed in the hospital more than 15 days which indicates that there was an association between long stay in a hospital and MRSA infection.

A study conducted in Norway showed that patients with MRSA strain stayed in hospital longer period than those with MSSA (Andreassen et al. 2017). This study is almost similar to the current study.

Irrational use of antibiotics is an important risk factor for increase of an antimicrobial resistance. About 50 % of antibiotic prescriptions are unnecessarily prescribed (Imanpour et al. 2017). Study report in the USA observed that there was a significant association between antibiotic prescriptions in outpatients and presence of HA-MRSA (P. et al. 2016). In our study, there was a correlation between multiple prescriptions of antibiotics and presences of MRSA.

Prosthetic devices are one of the important risk factors of MRSA. Implants could be contaminated with microorganisms particularly pathogens during surgery, preoperative time or hematogenous. In the USA, study report observed that over 50% of prosthetic joint infections are attributed to staphylococci and about 50% of those staphylococci are MRSA (Aslam & Darouiche 2013). In our study didn't find a significant association between prosthetic devices and presences of MRSA due to short study period and sample size.

Patients with diseases particularly immunocompromised individuals, diabetes have been found to be most probably at high risk with MRSA infections. In our study, it was found that the number of diabetes mellitus in recruited patients was 14 (9.3 %). One out of that fourteen was infected with MRSA. One study reported, patients with diabetes are at high risk for MRSA infections particularly pneumonia, wound, and other infections (Equils et al. 2016).

The current study was found that the male was more prone to get infected MRSA than the female counterpart. We found an infection rate of 13.2 % and 4.6 % respectively. Being

male and presence of MRSA is an independent association. To justify why male was more contracted MRSA than a female could be attributed the higher number (76.2 %) of male than female recruited in this study. However, this finding was not significant in regard to MRSA risk factors.

5.2 CONCLUSION

The prevalence of MRSA among the patients having wounds in this study was high, estimated at 17.9%. All MRSA isolates were susceptible to Vancomycin. The findings of our study indicate that of the three wards in which the patients were recruited, the surgical ward appeared to have the highest infection rate of MRSA (13.2%) followed by the burn unit (4.6%). Duration of hospital stay was found to be significantly associated with the presence of MRSA infection ($p < 0.001$).

5.3 RECOMMENDATIONS

1. The data obtained on the prevalence and antimicrobial susceptibility profile of MRSA isolates in this population would provide a useful guide in formulation of policies for empiric therapy.
2. Regular MRSA screening program for patient particularly high-risk individuals and healthcare workers is necessary for hospital facilities for infection control measures.
3. Long stay in hospital should be minimized to reduce the risk of nosocomial infections as well as health cost.

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APPENDICES

APPENDICES I

INFORMED CONSENT FORM

Title of Study: Prevalence and Vancomycin Susceptibility Pattern of methicillin resistant *Staphylococcus aureus* wound Isolates at Medina Hospital in Mogadishu, Somalia

Investigator: Abdullahi Adan Shaba, MSc, Institute of Tropical & Infectious Diseases, University of Nairobi

Supervisors:

1. Dr. Marianne Wanjiru Mureithi (PhD Immunology & Microbiology), Lecturer and Postdoctoral Research Scientist, Department of Medical Microbiology, University Of Nairobi.
2. Mrs. Susan Akinyi Odera (Bsc, Msc), Tutorial Fellow, Department of Medical Microbiology, University Of Nairobi
3. Mr. IBRAHIM HASSAN BARKHADLE (BSc, MSc), Assist lecturer, Head, Department of Medical Laboratory Science at Jamhuriya University, Senior Laboratory Technologist at Medina Hospital

Introduction:

Methicillin resistant *Staphylococcus aureus* is a bacteria cause disease difficult to treat with normal antibiotics. It is mostly found in hospitals and nursing homes where high-risk groups of patients, such as people with open wounds, people with immunocompromised and patients using invasive devices like catheter are more prone to infect with these bacteria. Vancomycin is an antibiotic drug used to treat bacterial infections, particularly against infections caused by *Staphylococcus aureus* that is resistant to methicillin, antibiotic drug.

What is the purpose of the study?

The purpose I am conducting this research study is to the partial fulfilling master degree of Tropical and Infectious Diseases. In this study, will be determined the prevalence and vancomycin susceptibility to that bacteria which cause morbidity, mortality and an economic load of the admitted patients at Medina Hospital.

Feel free to inquire any question which you may require to clarify.

Why is this study important?

The result of this research will be important for the health policy makers to develop antibiotic treatment guideline and will help junior physicians to use appropriate antibiotics for the management of open wounds at Medina Hospital and also to improve MRSA infection control in the hospital. So your participation is very important.

Is there any benefit?

Once you agree to take part in this research, the benefit you may achieve is your open wound and your other colleagues will be treated with appropriate antibiotic which will make a short hospital stay and in a sense decrease hospital cost.

Risk

Apart from using a swab to collect discharge from the open wound, there will be no risk at all. Some of the patients may experience slight pain when taking sample from the open wound and that could be minimized by moisturizing the swab with normal saline.

Confidentiality

All your information will be highly treated confidential and will be used only for the purpose of the study and your name will be hidden.

Compensation

We don't do any harm to you and we don't expect to occur during your participation in the study. If any accident occurs, there will be no compensation.

How will it be if I refuse to participate?

Your participation in this study is completely free, you can refuse to participate or withdraw at any time and it will not have an effect on your care in the hospital.

Consent Form

I _____, hereby freely agree to join in the study. I have understood the potential risks and benefits and was clearly explained to me by the investigator. Therefore, I hereby consent voluntarily to be involved in this study.

Name of Participant _____

Date _____

Signature of Participant _____

Contact:

Feel free for any questions about the study. You can contact me for further clarification: The principal investigator Abdullahi Adan Shaba. Tel: 0723077959/0615430666. Email: abdullshab@gmail.com.

Supervisors:

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For more information about your rights as a research participant, you may contact the Secretary/Chairperson, Kenyatta National Hospital-University of Nairobi Ethics and Research Committee Telephone No. 2726300 Ext. 44102 email: uonknh_erc@uonbi.ac.ke.

Investigator's declaration

I, Abdullahi Adan Shaba, hereby certify that this study is my own work and it has not been done, to the best of my knowledge, neither in Somalia and nor has it been presented, or submitted for a degree to any other university.

**FOOMKA OGOLAANSHAHA KA QEYB QAADASHADA BAARITAANKA
(CONSENT FORM IN SOMALI)**

Cinwaanka Daraasada: Sida looga helo bakteeriyada iyo sida Fankomaysintu ay xasaasiga ugu tahay bakteeriyada loo yaqaano *Methicillin Resistant Staphylococcus aureus* ee ku dhacda dhaawaca furan ee Isbitaalka Madiina, Muqdisho, Soomaaliya.

Daraaseeyaha: Abdullahi Adan Shaba, Msc, Institute of Tropical & Infectious Diseases, University of Nairobi

Hordhac: *Methicillin Resistant Staphylococcus aureus* waa bakteeriya ay adag tahay in la daaweeyo maxaa yeeley waxay adkeysii u leedahay qaar badan oo ka mid ah daawooyinka antibayootikada, waxaana laga helaa isbitaalada iyo bukaaneegtooyinka, waxaana aad ugu nugul dadka qaba dhaawacyada furan, dadka difaaca jirkooda uu hooseeyo iyo dadka jirkooda la geliyo qalabka caafimaadka sida kateetarka iwm.

Sababta baaritaankaan cilmiyeed uu muhiimka u yahay waxay tahay maxsuulka ka soo baxa wuxuu wax weyn ka tari doonaa daaweynta dhaawacyada furan ee Isbitaalka Madina iyo guud ahaan isbitaalada kale, wuxuu kaloo aasaas u noqon doonaa istiraatiijayada laga sameyn doono isticmaalka daawooyinka antibayootikada oo waxtar u ah guud ahaan mujtamaceen.

Wax Qatar ah kuuma laha ka qayb qaadashadaada ee baaritaanka caafimaadka keliya dhaawacaada ayaanka qaadeyanaa dheecaansi lo baaro.

Ma jiro wax dhib ah oo kaaga imaanaaya ka qeyb qaadashaada ee baaritaanka caafimaad, hadii ay dhacdana ma jiridoonto wax qaandhab ah oo lagu sameyn doono.

Xogta kuugu saabsan ee aan kaa qoridoono waa sir aanaan la wadaagi doonin dad kale mgacaagana waan qarin doonaa markan soo bandhigaayo macluumaadka ku saabsan baaritaanka cilmiyeed.

Wax faa'iido gaar ah oo aad heli doonta ma jirto oo aan ka ahayn in dhaawacaada loo heli doono daawada ku haboon ee yareyn karta mudada aad ku jireyso isbitaalka si ay u badbaadiso dhaqaalaha kaaga bixi lahaa mudada dheer ee aad ku jiri lahayd adiga ama dadka kale ee isbitaalka ku jira.

Ka qeyb qaadashadaada ee baaritaankan cilmiyeed waa iqtiyaari hadii aad rabto waad ka qeyb qaadan kartaa hadii aad rabto waad diidi kartaa, xor baad tahay, wax dhib ah oo diidmadaada kaaga imaanaayana ma jiri doonto, waxna uma dhibi doonto daryeelkaada caafimaad ee isbitaalka.

Igala soo xiriir cinwaankeyga oo ah: Tel 0615430666/0723077959. Email: abdullshab@gmail.com. Ama suberfaysariskeyga oo kala ah:

Dr. Marianne Wanjiru Mureithi (PhD Immunology & Microbiology), Lecturer and Postdoctoral Research Scientist, Department of Medical Microbiology, University Of Nairobi. Work phone +254703704711. Email: Marianne@uonbi.ac.ke

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Hadiiaad u baahato macluumaad dheeri ah oo ku saabsan xuquuqdaada ku saabsan ka qeyb qaadashada baaritaanka cilmiyeed la xiriir madaxa ama xoghaynta gudiga akhlaakhiyaadka baaritaanka cilmiyeed ee Isbitaalka Qaran ee Kenyatta iyo Jaamacada Nayroobi cinwaankooda Telefon No. 2726300 Ext. 44102 email: uonknh_erc@uonbi.ac.ke.

Foomka Ogolaanshaha ka qeybqaataha Baaritaanka Cilmiyeed

Anigoo ah _____ waxaan halkan ku cadeynayaa inaan si xor ah uga qeyb qaadanayo baartiaankan cilmiyeed. Waan fahamsanahay qatarta iyo faaiidada ku jirta, waxaana si cad iigu sharaxay baaraha baaritaanka cilmiyeed. Sidaa daraadeed waxaan halkan ku cadeynayaa inaan si xor ah uga qeyb qaadanayo baaritaankan cilmiyeed.

Magaca ka qeyb qaataha _____

Taariikda _____

Saxiixa _____

APPENDICES II
STUDY QUESTIONNAIRE

Patient Information

Study questionnaire No. -----

Date of enrollment-----

Contact-----

Hospital ward

Surgery Dressing room Burn unit

Demographics

Gender: Male Female

Age:

Marital status: Married Single Widowed Divorced

Separat

Educational level: None Primary Secondary Tertiary

Occupational status: Employed Unemployed Retired

Area of residence: Urban Rural

Sample and wound characteristics

Type of open wound: Accident (road) Burn Explosive

Bullets Chemical Surgical

Diabetic foot ulcer Other

Specimen taken from-----

Characteristics of wound Discharge No discharge Gangrene

Risk Factors

Hospital stay: ≤ 5 days 6-10 days 11-15 days ≥ 16 days

Type of antibiotics used for your open wounds before MRSA study:

- Ceftriaxone
- Gentamycin
- Penicillin
- Vancomycin
- Linezolid
- Other-----

Prosthetic device Yes No

Underlying diseases/conditions: Diabetic Immunocompromised
Fungal infection Bacterial infection
Viral infection others

Laboratory diagnosis

Culture and sensitivity: Yes No

If no Not requested Capacity limited Not available

If yes type of organism was isolated-----

Type (s) of drug sensitivity-----

Type (s) of drug resistance-----

Type (s) of drug intermediate-----

Signature-----

Date-----